Contributions to Zoology, 80 (2) 107-132 (2011)

A molecularly based phylogeny reconstruction of mushroom corals (Scleractinia: Fungiidae) with taxonomic consequences and evolutionary implications for life history traits

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Key words: COI, evolutionary history, ITS 1 & 2, maximum coral size, polystomatism, reproduction strategy

Abstract

The phylogenetic relationships of the Fungiidae, a family of predominantly free-living, zooxanthellate, reef corals, were studied by sequencing a part of the mitochondrial Cytochrome Oxidase I (COI) and the complete ribosomal Internal Transcribed Spacers (ITS) I & II of specimens from various locations in the Indo-West Pacific. Some sequences were retrieved by using fungiid-specific primers on DNA-extracts from parasitic gastropods living with these corals. The analyses were performed both including and excluding intraspecific variation to investigate the potential effect of saturation. Even though the present molecular phylogeny reconstructions largely reflect those based on morphological characters, there are some distinct differences. Three major clades are distinguished, one of which consists of species with relatively long tentacles. The two other major clades cannot yet be clearly separated from each other morphologically. Several polyphyletic taxa were detected and some genera and species that previously were considered closely related to each other, appear not to be so. Proposed nomenclatorial changes include amongst others the upgrading of subgenera in *Fungia* to genus level. A few species moved from one genus to another. Among all Fungiidae, the loss of the ability to become free-living appears to have evolved independently as reversals in four separate clades, including two that were previously assumed to be sister groups. The evolution of corals with additional (secondary) mouths leading to polystomatous growth forms from corals with only a single primary mouth (monostomatous growth form) appears to have occurred independently ten times: seven times by extrastomatal budding and three times by intrastomatal budding. In two clades, *Herpolitha* and *Polyphyllia*, both mechanisms co-evolved. In general there is no clear relationship between the loss of a free-living phase and the evolution of multiple mouths.

Contents

Introduction

The taxonomy of stony corals (Scleractinia) used to be based on their skeleton morphology, which was convenient because it enabled the inclusion of extinct taxa that are exclusively represented in the fossil record (Vaughan and Wells, 1943; Wells, 1956; Chevalier and Beauvais, 1987; Stanley, 2003). On the other hand, the distinction at species level has remained problematic because Scleractinia show much phenotypic variation due to genetically determined polymorphism and to plastic skeleton shapes reacting to

various environmental conditions, such as light and water movement (Hoeksema and Moka, 1989; Gittenberger and Hoeksema, 2006; Todd, 2008). In cases where inter-specific boundaries were obscured by intraspecific variation and homoplasy, molecular methods have become helpful in determining species boundaries (Oppen *et al*., 2000; Diekmann *et al*., 2001, Gittenberger and Hoeksema, 2006), along with subsequent newly examined microstructural skeleton characters (Benzoni *et al*., 2007, 2010; Budd and Stolarski, 2009; Budd *et al*., 2010).

Molecular analyses have also shown that the traditional higher-level taxonomy of Scleractinia is not taken for granted anymore, dividing this order into two major clades and various para- and polyphyletic genera and families (Romano and Palumbi, 1996; Romano and Cairns, 2000, Chen *et al*., 2002; Fukami *et al*., 2004, 2008; Le Goff-Vitry *et al*., 2004; Kerr, 2005; Nunes *et al*., 2008, Dai and Horng, 2009a, b; Kitahara *et al*., 2010; Huang *et al*., 2011). The new phylogenetic models are not reflected in traditional classifications but they are also not complete yet, as more taxa need to be included with the help of additional genetic markers before the scleractinian taxonomy becomes optimally resolved.

Within some scleractinian families that appear monophyletic based on morphological criteria, molecular data have corrected and supported old taxonomic views or have lead to new insights that are also supported by re-examined morphological characters (Fukami *et al*., 2000; Stolarski and Roniewicz, 2001; Benzoni *et al*., 2007, 2010; Wallace *et al*., 2007; Huang *et al*., 2009). The Indo-Pacific coral family Fungiidae, which also appears to be monophyletic, consists of many charismatic species that show relatively complex life history strategies. Many species have large free-living polyps (Hoeksema, 1989, 1991a), they occur abundantly in mixed assemblages on shallow reefs (Claereboudt, 1988; Hoeksema and Moka, 1989; Hoeksema, 1991b; Goffredo and Chadwick-Furman, 2000; Elahi, 2008; Hoeksema and Koh, 2009; Hoeksema and Matthews, 2011), or they may even occur in aggregations as a result of asexual reproduction, either by budding or fragmentation (Krupp *et al*., 1993; Kramarsky-Winter and Loya, 1996, 1998; Gilmour, 2002, 2004b; Hoeksema, 2004; Hoeksema and Gittenberger, 2010). Maybe owing to these traits, the Fungiidae have received much attention with regard to their evolutionary history, which so far has been tracked by analyses of morphological characters (Wells, 1966; Cairns, 1984; Hoeksema 1989, 1991a, 1993b).

The last phylogeny reconstruction based on morphological characters (Hoeksema, 1989) resulted in various taxonomic changes in the Fungiidae in relation to previous classifications. These changes were supported with the help of cladistic arguments, *e.g.* the separation of *Lobactis* from *Pleuractis*, which were considered similar by other authors (*e.g.* Veron and Pichon, 1979). However, Hoeksema (1989) distinguished various clades that he considered subgenera in *Fungia*,

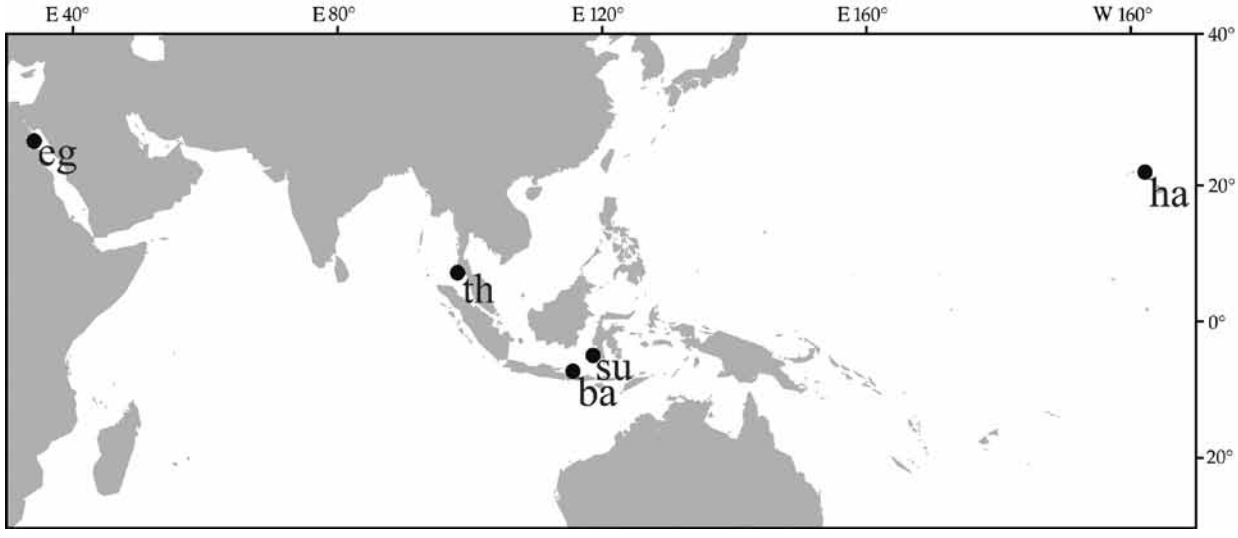


Fig. 1. The Indo-Pacific region, from the Red Sea to the Hawaiian Archipelago, illustrating the localities of the material used in this study (Table 1). Abbreviations: ba, Bali, Indonesia [3]; ha, Oahu, Hawaii [5]; eg, Egypt (Red Sea) [1]; su, Sulawesi, Indonesia [4]; th, Phiphi Islands, West Thailand [2].

which was maintained as a paraphyletic group. A nomenclature strictly based on a morphological cladistic analysis was premature at that time because many phylogenetic relationships were still unclear.

The present study gives results derived from molecular analyses providing additional support for the use of cladistic models as a basis for the taxonomy of the Fungiidae. This is the first time that an extensive molecular phylogeny of the Fungiidae is published, since previous studies in which mushroom corals were included in molecular analyses concerned only a few species that served as representatives of the whole family Fungiidae (*e.g.* Benzoni *et al*., 2007; Fukami *et al*., 2008; Barbeitos *et al*., 2010). Phylogenetic models may give insight in the evolution of morphological, ecological, and life history traits (Hoeksema, 1991a; Collin and Cipriano, 2003; Pagel, 2004; Kohlsdorf and Wagner, 2006; Baird *et al*., 2009; Galis *et al*., 2010; Kerr *et al*., 2011). When morphological characters are excluded in phylogeny reconstructions, independent molecular methods enable us to focus on the evolution of important morphological and ecological (life history) traits, such as corallum size, the development of multiple mouths (monostomatous or solitary vs. polystomatous or modular) and local mobility and dispersal in adult phase (free vs. attached mode of life). Furthermore it also facilitates us to discern the possible role of character reversal in these traits, such as a return to permanent coral attachment and thereby losing the free-living mode of life.

Most coral species (Scleractinia) are renowned for their confusing ecophenotypical variation (Wijsman-Best, 1974; Best *et al*., 1984; Hoeksema and Moka, 1989; Gittenberger and Hoeksema, 2006; Todd, 2008; Forsman *et al*., 2009; Ow and Todd, 2010). Therefore and because of parallelism (homoplasy) or convergent evolution, phylogeny reconstructions only based on morphological data are troublesome (Hoeksema, 1989). Molecular analyses have helped to shed more light upon their evolutionary history (Romano and Cairns, 2000). Discrepancies between coral phylogeny reconstructions based on either morphological or molecular data are not rare (Fukami *et al*., 2004). Such incompatible results have been found in various animal taxa. In corals this has often been referred to as reticulate evolution, but so far there is no indication from Fungiidae (Kenyon, 1997; Veron, 2001; Willis *et al*., 2006; Stefani *et al*., 2007). Other evolutionary history scenarios, like homeostasis, parallel or convergent evolution, and bottleneck events are considered less frequently. The possibility of misidentifications is usually also neglected. Characters that are variable within populations or species are commonly used in molecular phylogeny reconstructions (Fukami *et al*., 2008). These intraspecifically variable characters can hinder analyses however, because they may represent a saturated part of the data set. Therefore, unstable characters like polyp colour in corals are often excluded in morphology-based phylogeny reconstructions (Hoeksema, 1989). To study the effect of excluding these characters in molecular data sets, similar to the exclusion of 3rd base positions in coding DNA markers, the phylogenetic relationships of mushroom corals have been studied based on the genetic data sets with and without intraspecifically variable base positions. This has not been done before in molecular phylogeny reconstructions of corals. The resulting molecular phylogeny reconstructions are compared with the last previous one based on morphology (Hoeksema, 1989) to investigate the evolutionary history of the Fungiidae, with a focus on the potential role of parallelism and convergent evolution.

Materials and methods

Sampling

DNA-samples of mushroom corals were collected at various Indo-Pacific regions that are far apart from each other (Fig. 1), *i.e.* the Red Sea, eastern Indian Ocean (Andaman Sea), Central Indo-Pacific (Indonesian Throughflow) and Central Pacific (Hawaii). The coral samples were preserved in ethanol 70% or 96%. All samples were identified twice independently by BWH and AG based on voucher specimens themselves or their photographs. The identifications are based on a taxonomic revision of the Fungiidae (Hoeksema, 1989) and subsequent descriptions of new species (Hoeksema, 1993a, b, 2009; Veron, 1990, 2000, 2002; Hoeksema and Dai, 1991; Ditlev, 2003). Coral specimens that were collected have been deposited in the collection (RMNH Coel.) of NCB Naturalis. The present species names and their authorities are listed in Table 5.

DNA extraction and sequencing

Small pieces of tissue were scraped off each specimen with a sterile scalpel to fill about half of a 1.5 ml vial. A mixture of 3 μl proteinase K (20 mg ml^{-1}) and 0.5 ml CTAB buffer, *i.e.* 2% CTAB, 1.4 M NaCl,

0.2% mercapto-ethanol, 20 mM EDTA and 100 mM TRIS-HCl pH 8, was added to the vial for incubation at 60° C, for c. 15 hours. After incubation the solution was mixed with 0.5 ml chloroform / isoamyl alcohol, and centrifuged for 10 minutes at 8000 rpm. The supernatant was extracted, mixed with 0.35 ml isopropanol, put aside for c. 15 hours at 4° C and finally centrifuged for 10 minutes at 8000 rpm to precipitate the DNA. The supernatant was discarded and the remaining DNA-pellet was washed at room temperature with 0.5 ml of an ethanol/ammonium-acetate solution for 30 minutes. After centrifugation for 10 minutes at 8000 rpm, this solution was discarded. The pellet was dried in a vacuum centrifuge and then dissolved in 20 μl MilliQ. The DNA quality and quantity were tested by electrophoresis of the stock-solution through an agarose gel and by analysing a 1:10 dilution of the stock in a spectrophotometer. The ITS (Internal Transcribed Spacers I & II) and a part (from the 3'-end) of the COI (Cytochrome Oxidase I) regions of the samples in Table 1 were amplified using the primers and annealing temperatures (AT) as specified in Table 2.

Fungiid DNA-specific COI primers were made by developing internal primers based on the fungiid sequences that were retrieved with Folmer Universal COI primers (Folmer *et al*., 1994). Although DNA-extract directly taken from the fungiid corals was used for the majority of sequences, the fungiid COI region was also successfully sequenced with the fungiid specific primers from DNA-extracts of parasitic gastropods. Many corallivorous gastropods are specialised in only one or a few coral species (Gittenberger, 2003,

Table 1. List of sequenced samples and Genbank accession numbers. * sequence obtained from DNA extract of *Epifungium* spec. ** sequence obtained from DNA extract of *Leptoconchus* spec. *** *Pleuractis* sp. 1 as indicated in Gittenberger and Gittenberger (2005: 181).

Sequenced specimens	Locality [locality nr. in Fig. 1]	COI	ITS
Ctenactis albitentaculata	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149869	EU149813
Ctanactis crassa	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149889	EU149814
Ctenactis crassa	Thailand, Andaman Sea, Phiphi Islands [2]	EU149859	EU149815
Ctenactis echinata	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149879	EU149816
Ctenactis echinata	Egypt, Red Sea, Marsa Nakari [1]	EU149899	EU149817
Cycloseris costulata	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU202718	EU149818
Cycloseris costulata	Egypt, Red Sea, Marsa Nakari [1]	EU149870	EU149819
Cycloseris costulata	Thailand, Andaman Sea, Phiphi Islands [2]	EU149890	EU149820
Cycloseris cyclolites	Thailand, Andaman Sea, Phiphi Islands [2]	EU202719	EU149821
Cycloseris fragilis	Thailand, Andaman Sea, Phiphi Islands [2]	EU149880	
Cycloseris fragilis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149860	
Cycloseris mokai	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149906	EU149842
Cycloseris mokai	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149877	
Cycloseris mokai	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149897	
Cycloseris sinensis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149900	EU149822
Cycloseris tenuis	Thailand, Andaman Sea, Phiphi Island [2]	EU149891	EU149823
Cycloseris tenuis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149871	
Cycloseris vaughani	Thailand, Andaman Sea, Phiphi Islands [2]	EU149881	EU149824
Cycloseris vaughani	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149861	
Danafungia horrida	Indonesia, S Sulawesi, Spermonde Archipelago [4]		EU149826
Danafungia scruposa	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149872	EU149827
Fungia fungites	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149892	EU149829
Halomitra clavator	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149904	EU149837
Halomitra pileus	Thailand, Andaman Sea, Phiphi Islands [2]	EU149865*	
Halomitra pileus	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149875	EU149838
Halomitra pileus	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149895	
Heliofungia actiniformis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149885	EU149839
Heliofungia actiniformis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149905	
Heliofungia actiniformis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149876	
Heliofungia actiniformis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU202720**	
Heliofungia fralinae	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149901	EU149825
Herpolitha limax	Egypt, Red Sea, Marsa Nakari [1]	EU149866*	
Herpolitha limax	Thailand, Andaman Sea, Phiphi Islands [2]	EU149886	EU149841
Herpolitha limax	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149896	EU149840

2008; Gittenberger *et al*., 2000; Gittenberger and Gittenberger, 2005), among which almost all fungiid species, which makes them valuable sources of coral DNA. This method was used to obtain data from localities where corals could not be collected. Knowing the fungiid host species, the retrieved sequences were checked with those of identical coral species from other localities. The PCR (Polymerase Chain Reaction) was performed in a Peltier Thermal Cycler PTC-200, using the following PCR- program: 1 cycle of 94°C for 4 minutes and 60 cycles of 94°C for 5 seconds; AT (Annealing Temperature; Table 2) for 1 minute; 0.5°C s^{-1} to 60°C; 72°C for 1 minute. The optimised PCR reaction mix consisted of 2.5 μl PCR buffer (10x), 0.5 μl MgCl2 (50 mM), 1.0 μl forward primer (10 pM), 1.0 μl reverse primer (10 pM), 0.5 μl dNTP's (10 mM), 0.3 μl Taq polymerase (5 units ml^{-3}), 13.2 μl MilliQ and 1.0 μl 1:10 DNA stock-solution (= c. 0.1 μg DNA). For amplifying the ITS region, 2.0 μl Qsolution (QIAGEN) was used instead of the 2.0 μl MilliQ. After the PCR, the samples were kept on 4°C until purification by gel extraction using the QIAquick Gel Extraction Kit (QIAGEN). The samples were kept on 4°C until cycle sequencing. Cycle sequencing was done in both directions of the amplified region, with a program consisting of 45 cycles of 96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes. The reaction mix used contained 2.0 μl Ready Reaction Mix (Big Dye™ by PE Biosystems), 2.0 μl Sequence Dilution-buffer, 0.5 μl primer (5 pM forward or reverse primer solution) and 5.5 μl amplified DNA (= half the PCR-product, evaporated to 5.5 μl by vacuum centrifuga-

Table 1. Continued.

Sequenced specimens	Locality [locality nr. in Fig. 1]	COI	ITS
Lithophyllon concinna	Thailand, Andaman Sea, Phiphi Islands [2]	EU202721*	
Lithophyllon concinna	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149893	EU149832
Lithophyllon repanda	Thailand, Andaman Sea, Phiphi Islands [2]	EU149883*	
Lithophyllon scabra	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149903	EU149833
Lithophyllon scabra	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149874	
Lithophyllon scabra	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149894	
Lithophyllon spinifer	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149864	EU149834
Lithophyllon undulatum	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149867	EU149843
Lithophyllon undulatum	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149887	EU149844
Lobactis scutaria	United States of America, Hawaii, Kaneohe Bay [5]	EU149862	EU149830
Lobactis scutaria	United States of America, Hawaii, Kaneohe Bay [5]	EU149882	
Lobactis scutaria	Egypt, Red Sea, Marsa Nakari [1]	EU149873	EU149831
Lobactis scutaria	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149902	
Pleuractis sp. 1***	Egypt, Red Sea, Marsa Nakari [1]	EU149913	EU149851
Pleuractis granulosa	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149884	EU149835
Pleuractis granulosa	Egypte, Red Sea, Marsa Nakari [1]		EU149836
Pleuractis gravis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149910	EU149848
Pleuractis moluccensis	Thailand, Andaman Sea, Phiphi Islands [2]	EU149909*	
Pleuractis moluccensis	Indonesia, S Sulawesi, Spermonde Archipelago [4]		EU149849
Pleuractis paumotensis	Thailand, Andaman Sea, Phiphi Islands[2]	EU149812*	
Pleuractis paumotensis	Indonesia, S Sulawesi, Spermonde Archipelago[4]	EU149911	EU149850
Pleuractis taiwanensis	Indonesia, Bali, Tanjung Benoa [3]		EU149852
Podabacia crustacea	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149907	EU149845
Podabacia crustacea	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149878	
Podabacia kunzmanni	Thailand, Andaman Sea, Phiphi Islands [2]	EU149908	EU149847
Podabacia motuporensis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149898	EU149846
Podabacia motuporensis	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149868	
Podabacia sinai	Thailand, Andaman Sea, Phiphi Islands [2]	EU149888	
Polyphyllia talpina	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149915	EU149853
Sandalolitha dentata	Indonesia, Bali, Tanjung Benoa [3]		EU149854
Sandalolitha dentata	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149914	EU149855
Sandalolitha dentata	Thailand, Andaman Sea, Phiphi Islands [2]	EU149918	EU149856
Sandalolitha robusta	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149917	EU149857
Zoopilus echinatus	Indonesia, S Sulawesi, Spermonde Archipelago [4]	EU149916	EU149858

Table 2. Primer sequences, annealing temperatures and sources.

Primer	Annealing temperature	Primer sequence	Primer length	Reference
COI Folmer Universal primer (LCO-1490)	53	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	25-mer	Folmer *et al*., 1994
COI Folmer Universal primer (HCO-2198)	53	5'-TAA ACT TCA GGG TGA CCA AAAA ATC A-3'	25-mer	Folmer *et al*., 1994
COI mod F (FungCOIfor1)	53	5'-CTG CTC TTA GTA TGC TTG TA-3'	20-mer	Newly developed primer
COI mod R (FungCOIrev2)	53	5'-TTG CAC CCG CTA ATA CAG -3'	18-mer	Newly developed primer
TW5 (ITS F)	45	5'-CTT AAA GGA ATT GAC GGA AG-3'	20-mer	White *et al*., 1990
JO6 (ITS R)	45	5' -ATA TGC TTA AGT TCA GCG GGT-3'	21-mer	Diekmann *et al*., 2001
ITS mod F (ITS-F-Bastian)	45	5'-AGA GGA AGT AAA AGT CGT AAC AAG-3'	24-mer	Newly developed primer

tion). The cycle sequence products were purified with Autoseq G50 columns (Amersham Pharmacia Biotech) and kept on 4°C until they were run on an ABI 377 automated sequencer (Gene Codes Corp.), using the water run-in protocol as described in the User Bulletin of the ABI Prism 377 DNA Sequencer (PE Biosystems, December 7, 1999). The consensus sequences that were used in further analyses were retrieved by combining the forward and reverse sequences in Sequencher 4.05 (Genes Codes Corp.). The consensus sequences were checked against sequences from GenBank, *i.e*. the National Centre for Biotechnology Information (NCBI), as a check for contamination.

Sequence alignment and phylogenetic analyses

The COI and ITS sequences were aligned with ClustalW Multiple alignment, which is implemented in BioEdit 7.0.1 (Hall, 1999). The default parameters of these programs were used. Since ClustalW had difficulties aligning the ITS data set due to multiple gaps, manual modifications were made in the resulting alignment. Afterwards the COI alignment was checked for stop codons with MacClade 4.0 (Maddison and Maddison, 2000). Alignments are available from the authors.

The phylogenetic analyses were performed on six data sets, *i.e*. the full COI data set, the ITS data set and the combined COI+ITS data set, and finally these three data sets without the intraspecifically varying base positions. The latter three data sets were included to get an idea of the amount of 'false' versus 'good' phylogenetic signal that may be present in relatively fast mutating base-positions. To get a better idea of which positions vary intraspecifically, conspecific samples were included from distant regions like the Red Sea and the Central-Indo Pacific (Table 1, Fig. 1). The data sets were analysed with Paup 4.0b10 (Swofford, 2002). The homogeneity of base frequencies in the sequences was tested with chi-square for the full data sets of ITS and COI, and additionally for COI for the first, second and third codon positions separately. To test for the presence of phylogenetic signal the G1-skewness statistic was performed based on 1000 random trees (Hillis and Huelsenbeck, 1992) and the permutation test (Archie, 1989; Faith and Cranston, 1991) with 100 replicates, a full heuristic search, TBR algorithm, steepest descent and 1000 random addition replicates per replicate. PAUP 4.0b10 was used for maximum parsimony and neighbor joining analyses. MrBayes 3.0B4 (Ronquist and Huelsenbeck, 2003) was used for a Bayesian inference analysis. To find the most parsimonious tree(s), a full heuristic search was performed with 1000 random addition replicates, TBR algorithm and steepest descent. In addition a non-parametric parsimony bootstrap analysis was performed with a full heuristic search, 1000 bootstrap replicates, a maximum duration of one hour per replicate, one random addition per replicate and TBR algorithm. A neighbor joining bootstrap analysis was performed with 10,000 bootstrap replicates. Bayesian inference was performed in Mr-Bayes 3.0B4 with five incrementally (T=0.20) heated Markov chains and a cold one, which were run 4,000,000 generations and sampled once every 50 generations, using the best-fit model for nucleotide substitution, *i.e*. HKY+I+G. The best-fit model was calculated by both the likelihood ratio test

and the Akaike information criterion in MrModeltest 2.1 (Nylander, 2004) based on the calculated likelihood scores of 24 models of nucleotide substitution. To determine the burnin, the log likelihoods of saved trees were plotted in a Microsoft Excel graph to see from where on they become stationary.

Evolution of life history traits

By the projection of morphological characters and life history traits on cladograms, it may become clear how frequently a particular morphological character results from synapomorphy or from homoplasy (convergence), and also character reversals may be detected (Hoeksema, 1991a). The following traits have been listed for 50 mushroom corals species, including those that are not included in the molecular analyses: Loss of detachment, growth of large corallum size (> 25 cm), and the presence of secondary mouths by either instrastomatal or extrastomatal budding (Hoeksema, 1989, 1991a, 1993a, b, 2009; Veron, 1990, 2000, 2002; Hoeksema and Dai, 1991; Ditlev, 2003). These characters have been projected on a cladogram that differentiates between lineages with and without molecular support.

Results

Molecular phylogeny reconstructions

The COI data set (Table 1) consist of 63 sequences of 500 bases each, *i.e*. NCBI GenBank accession numbers EU149859-EU149918, EU202718-EU202721. The data set does not include any gaps or stop codons. The ITS data set (Table 1) consists of 45 sequences with lengths varying between 604 and 618 bases, *i.e*. NCBI GenBank accession numbers EU149813-EU149858. The length varies due to multiple gaps. Results from the statistical analyses are represented in Tables 3-4. The parsimony analyses are presented in Table 3 together with the number of informative base positions for both kinds of data sets (with and without intraspecifically varying base positions). For the ITS alignment without intraspecific variation, the likelihood ratio test and the Akaike information test resulted in different substitution models when analysed by MrModeltest. The result from the likelihood ratio test was used, since it was more in congruence with the result obtained by both the likelihood ratio test and the Akaike information test on the data set without intraspecific variation. Base frequencies in the

Table 3. Results from parsimony analyses (heuristic search, 1000 random addition sequences, TBR swapping algorithm with steepest descent) for the data sets that were analysed.

Data set	Number of most parsimonious trees	Tree score	Consistency index	Rescaled consistency index	Parsimony informative base positions
COI with intraspecific variation	226	92	0.783	0.652	23
COI without intraspecific variation	112	83	0.807	0.652	18
ITS with intraspecific variation	241	300	0.530	0.367	77
ITS without intraspecific variation	276	105	0.705	0.518	29
COI & ITS withintraspecific variation	791	337	0.589	0.439	95
COI & ITS withoutintraspecific variation	36	220	0.695	0.583	61

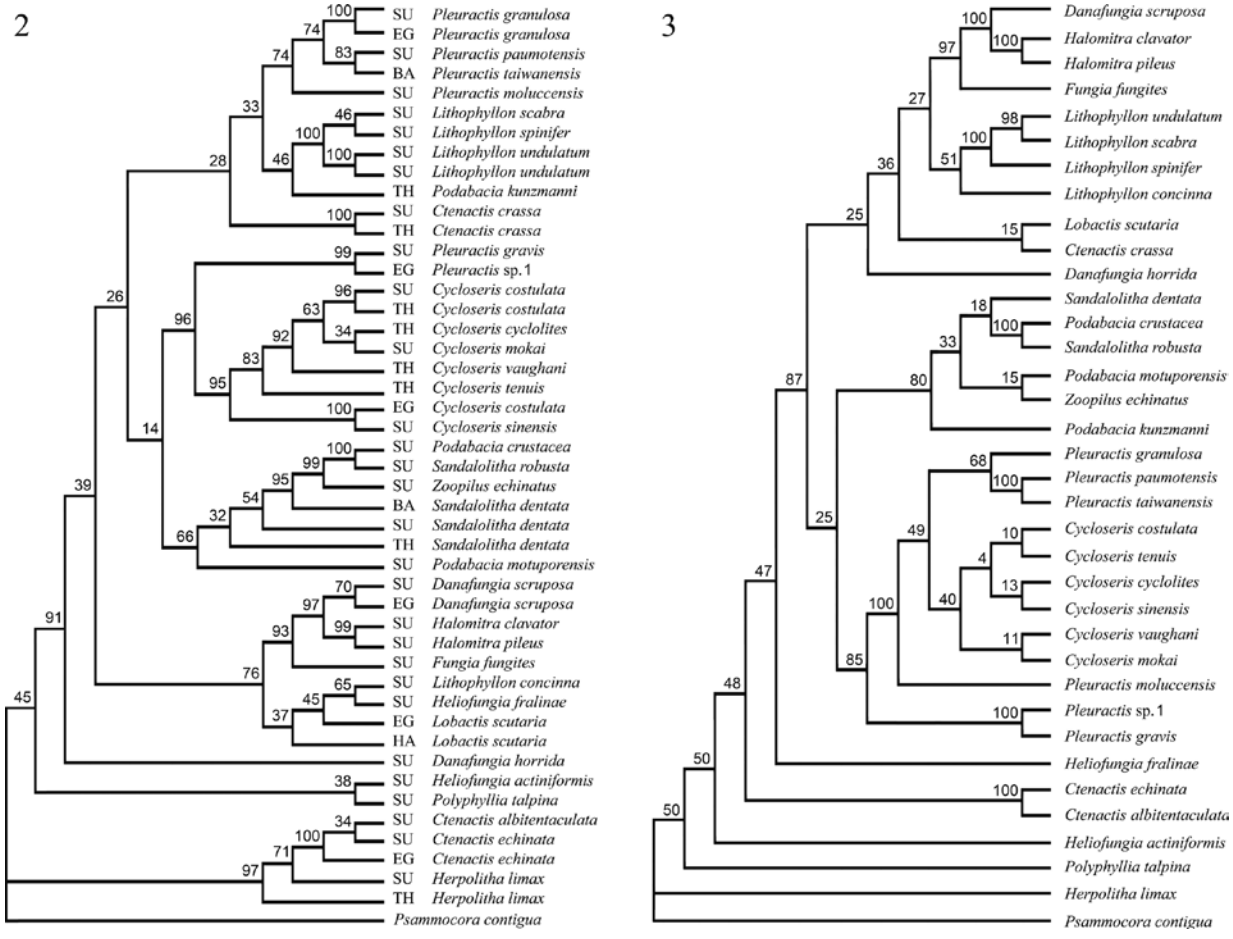
Table 4. Results of Chi-square, G1-skewness and permutation tests to check for phylogenetic signal and consistency of the analysed data sets. * These P-values were not obtained because of extremely long calculation times.

Type of data set	Chi-square test			G1-skewness test	Permutation test
	χ^2	df	P		
COI with intraspecific variation	4.0	75	1.00	-0.627	P<0.01
COI without intraspecific variation	3.7	63	1.00	-0.761	P<0.01
ITS with intraspecific variation	12.5	141	1.00	-0.529	-*
ITS without intraspecific variation	4.5	105	1.00	-0.372	-*
COI & ITS with intraspecific variation	7.1	123	1.00	-0.536	-*
COI & ITS without intraspecific variation	4.0	99	1.00	-0.570	-*

complete data set and in the first, second and third codon positions separately, are not significantly inhomogeneous across taxa, *i.e.* P = 1.00 in all cases. In all cases the consistency index of the most parsimonious trees was higher for the data set without the intraspecifically variable base positions (Table 3). The data sets without these positions resulted in less most parsimonious trees than the data sets with intraspecifically variable base positions included. The combined COI+ITS data set without intraspecific variation results in the lowest number of most parsimonious trees, *i.e.* 36 instead of 791 when intraspecific variation is included (Table 3). The phylogeny reconstructions based on the six data sets, *i.e.* the full COI data set, the ITS data set and the combined COI+ITS data set, and these three data sets without the intraspecifically varying base positions, are illustrated in Figs 2-7. Here, only the results of the MrBayes analyses are presented. Neighbor joining, maximum parsimony and parsimony bootstrap analyses gave similar results, which will be provided on request.

Evolutionary trends in morphology and life history traits

Even though the present molecular phylogeny reconstructions largely reflect those based on morphological characters, there are some distinct differences (Figs 2-9).

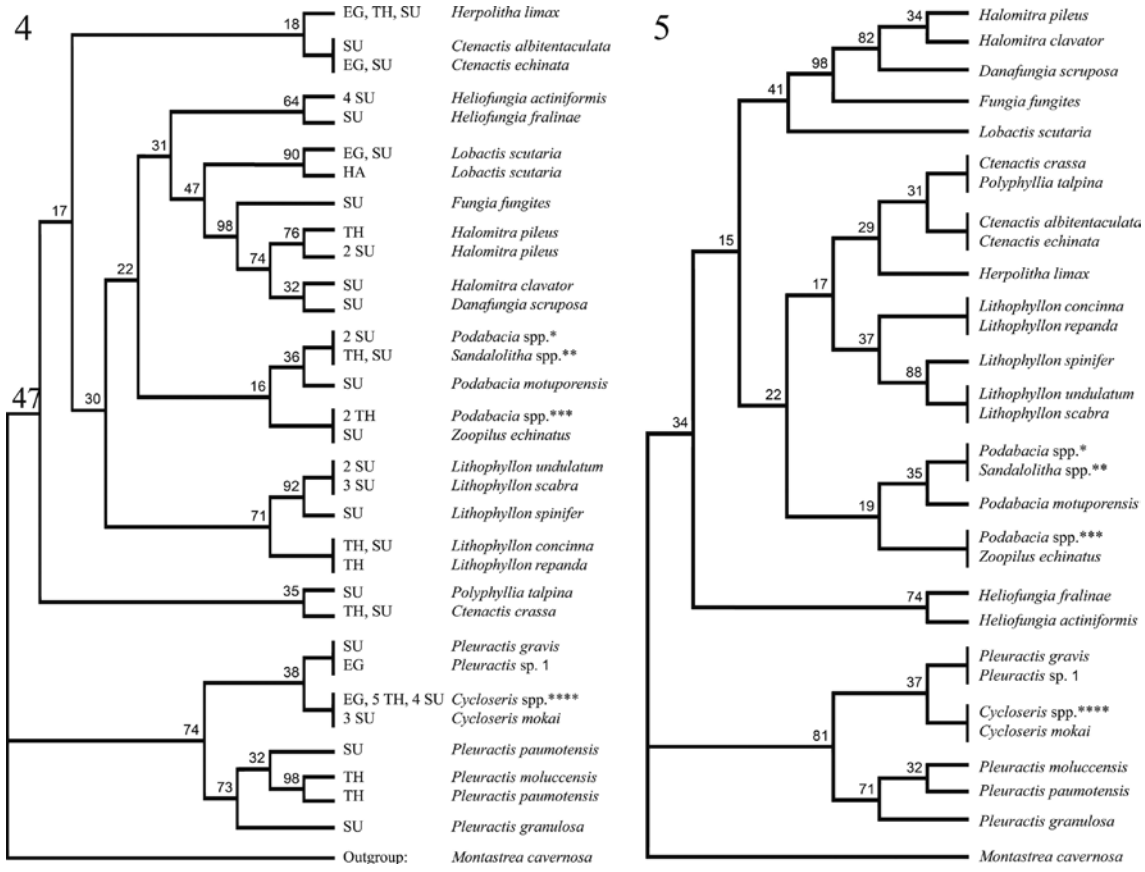


Figs 2-3. Bayesian analysis of ITS data set: 50% majority rule consensus tree with compatible groupings. Values at the nodes represent Bayesian probabilities. Taxonomy as in the proposed classification (Tables 6-7). 2, analysis of data set with intraspecific variation; Locality abbreviations (Fig. 1): ba, Bali, Indonesia; ha, Oahu, Hawaii; eg, Egypt (Red Sea); su, Sulawesi, Indonesia; th, Phiphi Islands, West Thailand. 3, analysis of data set without intraspecific variation.

Three major clades are distinguished, one of which consists of monostomatous species with relatively long tentacles (Fig. 10A-C). The two other major clades cannot be clearly separated from each other morphologically. Several polyphyletic taxa were detected and some genera and species that previously were considered to be closely related, appear not to be so.

The present phylogeny reconstruction has been used to study the evolution of morphological characters and life history traits. Although most mushroom coral species become free-living by detachment, some of them have lost the ability to detach themselves and remain fixed to the substratum (Hoeksema, 1989). The ability of coral detachment is considered an ancestral trait separating the earliest Fungiidae from their sister group (Hoeksema, 1989). The loss of the ability to become free-living appears to have evolved independently as reversals in four clades (Fig. 9), occurring in ten species (Table 5), including two that were previously assumed to be sister species within *Lithophyllon* (Fig. 8), but presently are separated from each other, *i.e. Lithophyllon undulatum* and *Cycloseris mokai*.

A large corallum (diameter > 25 cm) is recorded for at least 19 species (Table 5) distributed over five independent lineages (Fig. 9). Some of the lineages include smaller species (*i.e. Danafungia horrida* and *Podabacia kunzmanni*), which may be due to character reversal. However, the position of these two species in the

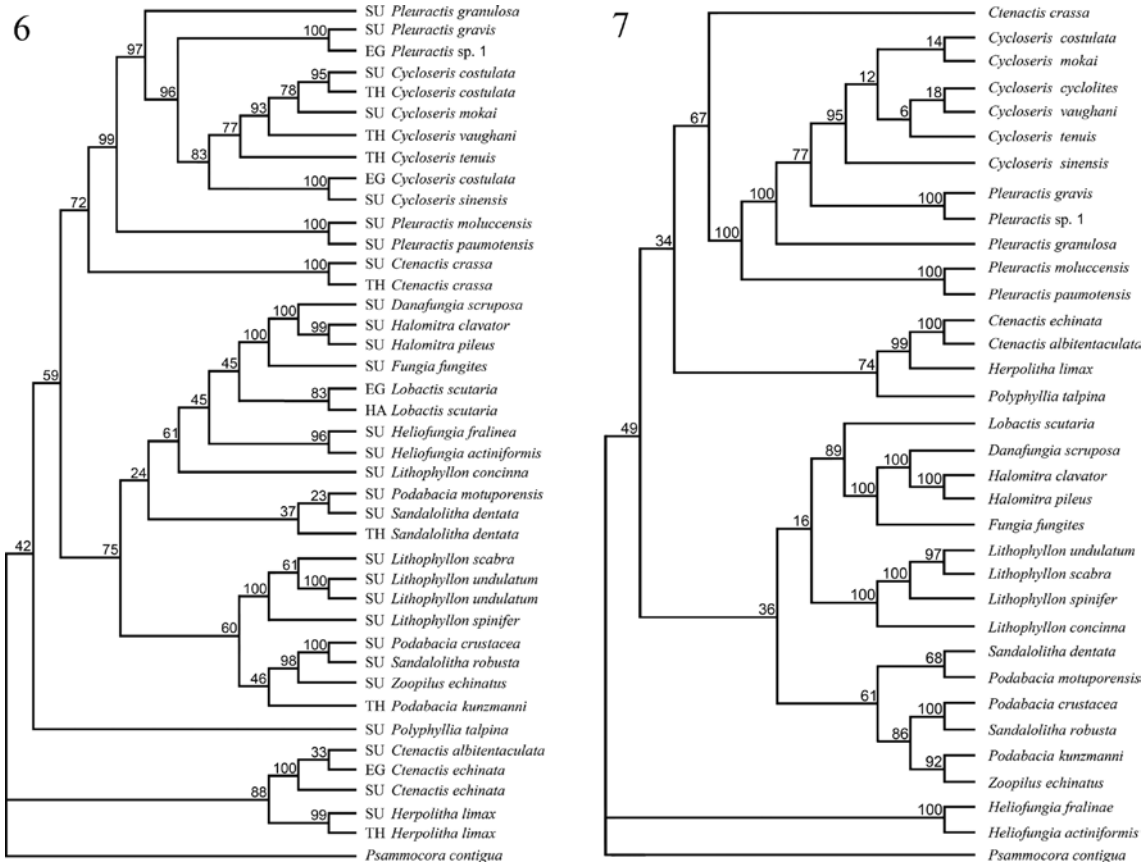


Figs 4-5. Bayesian analysis of COI data set: 50% majority rule consensus tree with compatible groupings. Values at the nodes represent Bayesian probabilities. Taxonomy as in proposed classification (Tables 6-7). 4, analysis of data set with intraspecific variation. Locality abbreviations (Fig. 1): ba, Bali, Indonesia; ha, Oahu, Hawaii; eg, Egypt (Red Sea); su, Sulawesi, Indonesia; th, Phiphi Islands, West Thailand; * *Podabacia crustacea* (su), *P. motuporensis* (su); ** *Sandalolitha dentata* (th, su), *S. robusta* (su); *** *Podabacia kunzmanni* (th), *P. sinai* (th); **** *Cycloseris costulata* (eg, th), *C. cyclolites* (th), *C. fragilis* (th, su), *C. sinensis* (th), *C. tenuis* (th, su), *C. vaughani* (th, su). 5, analysis of data set without intraspecific variation; * *Podabacia crustacea*, *P. motuporensis*; ** *Sandalolitha dentata*, *S. robusta*; *** *Podabacia kunzmanni*, *P. sinai*; **** *Cycloseris costulata*, *C. cyclolites*, *C. fragilis*, *C. sinensis*, *C. tenuis*, *C. vaughani*.

cladogram (Fig. 9) needs to be confirmed by additional analyses.

A total of 17 species has secondary mouths by either intrastomatal and/or extrastomatal budding (Table 5). The evolution of polystomatous corals (with multiple mouths) from monostomatous corals (with a single mouth) appears to have occurred ten times: seven times by extrastomatal budding and three times by intrastomatal budding (Fig. 9). In two clades, *Herpolitha* and *Polyphyllia*, both mechanisms co-occurred. There appears to be a relation between corallum size and polystomatism, with 15 out of the 18 larger species showing secondary mouths (Table 5). In three lineages a large corallum appears to have co-evolved with the development of additional mouths, whereas in two lineages the production of more mouths was preceded by an evolutionary size increase (Fig. 9). *Cycloseris mokai* is the only polystomatous species showing a small corallum; its encrusting growth form makes it dependent on available substratum surface, which may be size-restricting.

Some morphological characters like the size, density and form of the costae are hard to describe objectively. However, especially microstructural features, such as the patterns of granulation on the costal spines (explained in detail by Hoeksema (1989)), seem to support the results of the molecular analyses concerning the apparently closely related species *Heliofungia actiniformis* and *H. fralinae* (Fig. 11A-B), *Pleuractis granulosa* and *P. paumotensis* (Fig. 11C-D), *Cycloseris sinensis* and *C. mokai* (Fig. 11E-F), and *Lithophyllon scabra* and *L. undulatum* (Fig. 11G-H).



Figs 6-7. Bayesian analysis of the combined ITS & COI data set: 50% majority rule consensus tree with compatible groupings. Values at the nodes represent Bayesian probabilities. Taxonomy as in proposed classification (Tables 6-7). 6, analysis of data set with intraspecific variation; Locality abbreviations (Fig. 1): ba, Bali, Indonesia; ha, Oahu, Hawaii; eg, Egypt (Red Sea); su, Sulawesi, Indonesia; th, Phiphi Islands, West Thailand. 7, analysis of data set without intraspecific variation.

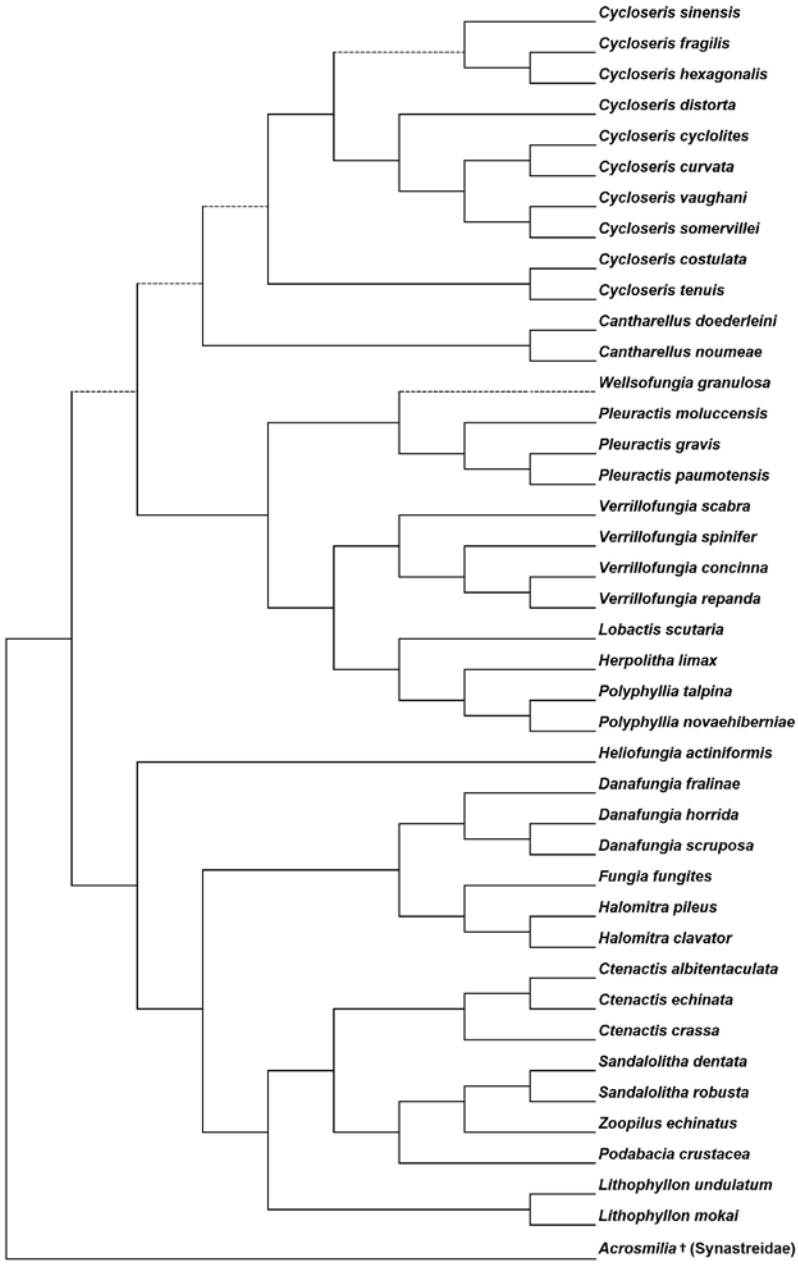
Table 5. Morphological character states within the Fungiidae evolution with ecological implications (order as in Fig. 9): Loss of detachment (remaining fixed instead of becoming free-living in adult phase), growth of large corallum size (> 25 cm), formation of secondary mouths by instrastomatal and extrastomatal budding. For sources, see text.

Species	Loss of free-living	∅ >25 cm	Budding intrastomatal	Budding extrastomatal
Cycloseris mokai (Hoeksema, 1989)	+	-	-	+
Cycloseris costulata (Ortmann, 1889)	-	-	-	-
Cycloseris cyclolites (Lamarck, 1815)	-	-	-	-
Cycloseris vaughani (Boschma, 1923)	-	-	-	-
Cycloseris tenuis (Dana, 1846)	-	-	-	-
Cycloseris sinensis Milne Edwards & Haime, 1851	-	-	-	-
Cycloseris curvata (Hoeksema, 1989)	-	-	-	-
Cycloseris distorta (Michelin, 1842)	-	-	-	-
Cycloseris fragilis (Alcock, 1893)	-	-	-	-
Cycloseris hexagonalis (Milne Edwards & Haime, 1848)	-	-	-	-
Cycloseris somervillei (Gardiner, 1909)	-	-	-	-
Cycloseris sp. 1	-	-	-	-
Cantharellus doederleini (Von Marenzeller, 1907)	+	-	-	-
Cantharellus noumeae Hoeksema & Best, 1984	+	-	-	-
Cantharellus jebbi Hoeksema, 1993	+	-	-	-
Pleuractis sp. 1	-	?	-	-
Pleuractis gravis (Nemenzo, 1955)	-	-	-	-
Pleuractis granulosa (Klunzinger, 1879)	-	-	-	-
Pleuractis moluccensis (Van der Horst, 1919)	-	-	-	-
Pleuractis paumotensis (Stutchbury, 1833)	-	-	-	-
Pleuractis taiwanensis Hoeksema & Dai, 1991	-	+	-	+
Pleuractis seychellensis Hoeksema, 1993	-	-	-	-
Ctenactis echinata (Pallas, 1766)	-	+	-	-
Ctenactis albitentaculata Hoeksema, 1989	-	+	-	-
Ctenactis crassa (Dana, 1846)	-	+	+	-
Herpolitha limax (Esper, 1797)	-	+	+	+
Polyphyllia talpina (Lamarck, 1801)	-	+	+	+
Polyphyllia novaehiberniae (Lesson, 1831)	-	+	+	+
Lobactis scutaria (Lamarck, 1801)	-	-	-	-
Danafungia horrida (Dana, 1846)	-	-	-	-
Danafungia scruposa (Klunzinger, 1879)	-	+	-	-
Halomitra clavator Hoeksema, 1989	-	+	-	+
Halomitra pileus (Linnaeus, 1758)	-	+	-	+
Fungia fungites (Linnaeus, 1758)	-	+	-	-
Lithophyllon ranjithi Ditlev, 2003	+	+	-	+
Lithophyllon undulatum Rehberg, 1892	+	+	-	+
Lithophyllon scabra (Döderlein, 1901)	-	-	-	-
Lithophyllon spinifer (Claereboudt & Hoeksema, 1987)	-	-	-	-
Lithophyllon concinna (Verrill, 1864)	-	-	-	-
Lithophyllon repanda (Dana, 1846)	-	-	-	-
Sandalolitha dentata Quelch, 1884	-	+	-	+
Sandalolitha sp. 1	-	?	-	+
Sandalolitha robusta (Quelch, 1886)	-	+	-	+
Zoopilus echinatus Dana, 1846	-	+	-	+
Podabacia crustacea (Pallas, 1766)	+	+	-	+
Podabacia kunzmanni Hoeksema, 2009	+	-	-	+
Podabacia motuporensis Veron, 1990	+	+	-	+
Podabacia sinai Veron, 2000	+	+	-	+
Heliofungia fralinae (Nemenzo, 1955)	-	-	-	-
Heliofungia actiniformis (Quoy & Gaimard, 1833)	-	-	-	-

Discussion

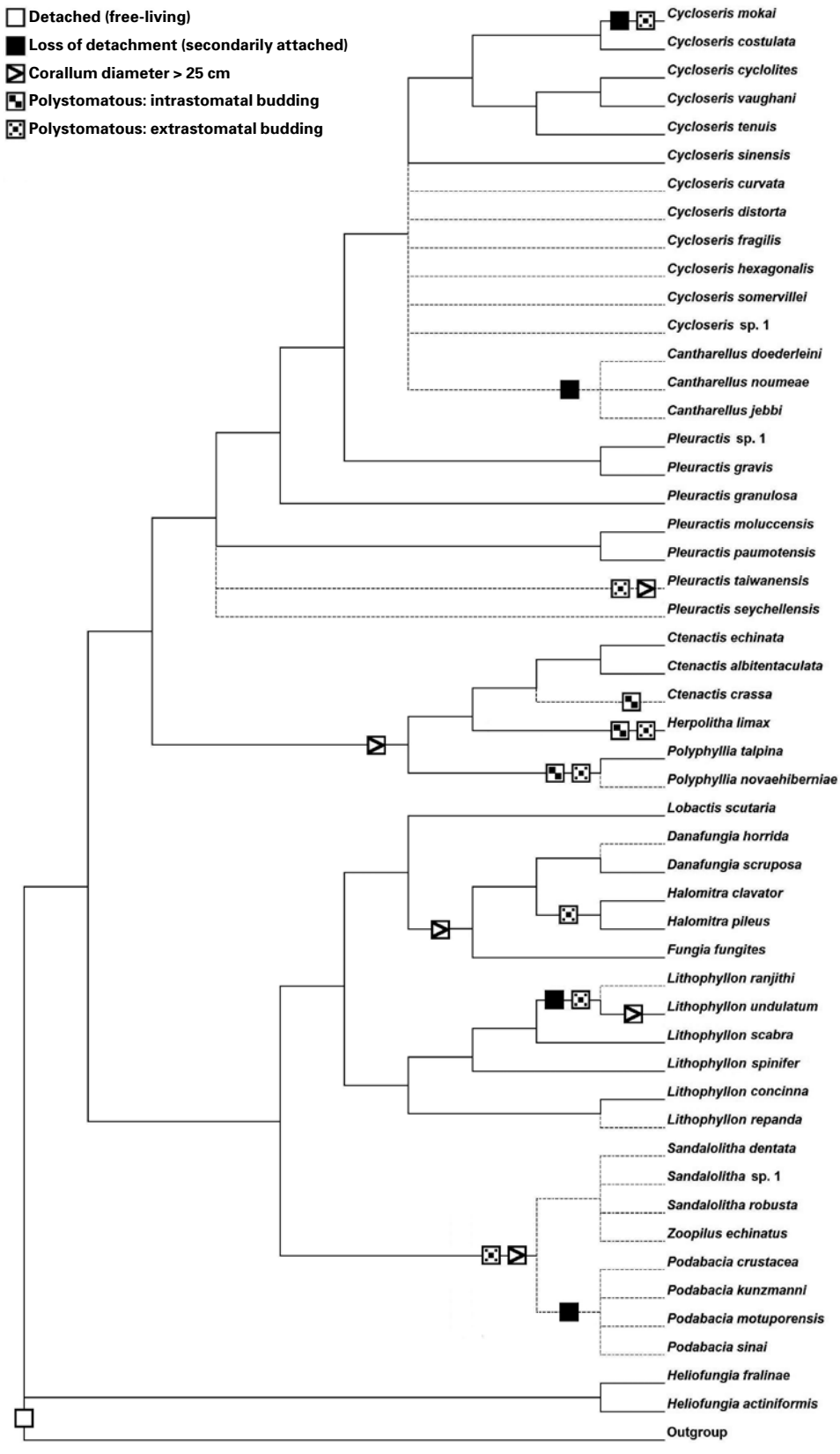
Coral DNA sequenced from corallivorous gastropods

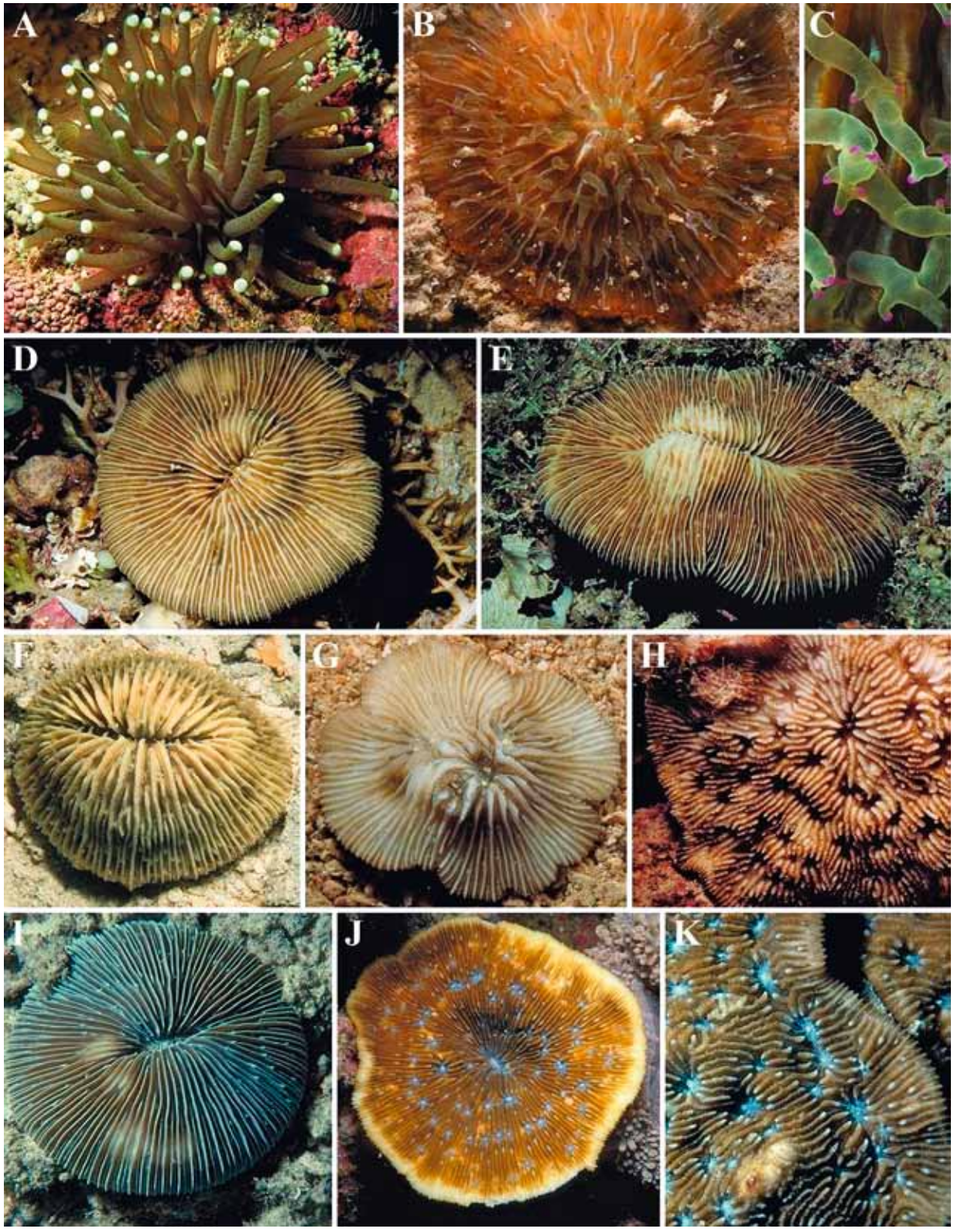
By using specific primers, the DNA from corals was isolated from coral parasites (Table 1). Since the entire body of the parasitic snails was used, it remains unclear whether the coral DNA was isolated from the stomachs or from other parts that were in contact with the coral. This methodological result is useful for coral research in general since it indicates a way to skirt around the problems with permits related to the transport of coral material for DNA linked research. Moreover, it also gives insight in the associations of corallivorous molluscs when their host species is unknown (Gittenberger *et al*., 2006; Gittenberger, 2008; Oliverio *et al*., 2009; Reijnen *et al*., 2010).



◀ *Fig. 8*. The first cladogram of the Fungiidae at species level based on morphological character state transformations after Hoeksema (1989). Outgroup comparison (main criterion), fossil character precedence (after Wells, 1966), and the correlation of transformation series were used, indicated by synapomorph (commonly inherited derived) character states as compared to plesiomorph (ancestral character states). The cladogram is based on 55 characters and 40 species known at the time of the analysis, in which a maximum parsimony was sought without character weighing.

▶ *Fig. 9*. A cladogram of the Fungiidae based on the present molecular analysis (solid lines). Species lineages that are not supported by the molecular analyses or have remained uncertain are indicated by a broken line. This model is based on the molecular analysis represented by Fig. 7. In case no reliable molecular data were available for a species, which is indicated by the absence of a solid line, its position has been derived from Fig. 8. Morphological life history traits indicated in Table 5 have been superimposed on the cladogram and based on the sharing of these traits by the species within the species lineages, their appearance in the phylogeny have been reconstructed accordingly: loss of detachment (remaining attached), growing large corolla (> 25 cm in diameter), polystomatism by instrastomatal and extrastomatal budding.


Detached (free-living)
Loss of detachment (secondarily attached)
Corallum diameter > 25 cm
Polystomatous: intrastomatal budding
Polystomatous: extrastomatal budding
Cycloseris mokai
Cycloseris costulata
Cycloseris cyclolites
Cycloseris vaughani
Cycloseris tenuis
Cycloseris sinensis
Cycloseris curvata
Cycloseris distorta
Cycloseris fragilis
Cycloseris hexagonalis
Cycloseris somervillei
Cycloseris sp. 1
Cantharellus doederleini
Cantharellus noumeae
Cantharellus jebbi
Pleuractis sp. 1
Pleuractis gravis
Pleuractis granulosa
Pleuractis moluccensis
Pleuractis paumotensis
Pleuractis taiwanensis
Pleuractis seychellensis
Ctenactis echinata
Ctenactis albitentaculata
Ctenactis crassa
Herpolitha limax
Polyphyllia talpina
Polyphyllia novaehiberniae
Lobactis scutaria
Danafungia horrida
Danafungia scruposa
Halomitra clavator
Halomitra pileus
Fungia fungites
Lithophyllon ranjithi
Lithophyllon undulatum
Lithophyllon scabra
Lithophyllon spinifer
Lithophyllon concinna
Lithophyllon repanda
Sandalolitha dentata
Sandalolitha sp. 1
Sandalolitha robusta
Zoopilus echinatus
Podabacia crustacea
Podabacia kunzmanni
Podabacia motuporensis
Podabacia sinai
Heliofungia fralinae
Heliofungia actiniformis
Outgroup


A
B
C
D
E
F
G
H
I
J
K

Exclusion of intraspecific variation

There are distinct differences between the phylogenies with intraspecifically variable base positions included (Figs 2, 4) and excluded (Figs 3, 5). In phylogeny reconstructions based on analyses of the ITS data sets and the combined COI+ITS data sets, *Lithophyllon concinna* clusters far away from the other species of *Lithophyllon*, but only so when intraspecifically variable base positions are included (Figs 2, 6). When these are excluded, all *Lithophyllon* species form a monophyletic group with support values of 51 and 100, based on the ITS data set (Fig. 3) and the combined COI+ITS data set (Fig. 7), respectively. This result is supported by the analyses of the COI data set (Figs 4-5). Similarly, *Heliofungia fralinae* clusters with a significant support value of 65 (Fig. 2) as the sister species of *Lithophyllon concinna* in the reconstruction based on the ITS data set with intraspecifically variable base positions included. When excluded (Fig. 3), *H. fralinae* clusters much more closely to *H. actiniformis*, with which it forms a strongly supported (64, 74, 96 and 100) monophyletic group in the other molecular analyses (Figs 4-7). With inclusion of the intraspecifically variable positions in the analysis (Fig. 2), the clade with *Pleuractis granulosa*, *P. paumotensis*, *P. taiwanensis* and *P. moluccensis* seems to be only distantly related to *P. gravis*, *P.* spec. 1 and all *Cycloseris* species, while these species combined form a monophyletic group in all other analyses (Figs 3-7).

The COI data set has less intraspecifically variable base positions than the ITS data set, but when the analyses are performed both with and without these positions, a similar pattern is observed (Figs 4-5). Most monophyletic groups that are strongly supported by the analyses of the other data sets have higher support values at least, or are even only present in the COI based phylogeny reconstruction, when intraspecific variation is excluded (Fig. 5). This is exemplified by clades [1] *Halomitra* spp. and *Danafungia scruposa*, [2] *Heliofungia actiniformis* and *H. fralinae*, and [3] *Cycloseris* spp., *Lithophyllon undulatum*, and *Pleuractis*

Table 6. Revised mushroom coral genera (Fungiidae) based on molecular analyses. For type species designations, their nomenclature and synonymies, see Hoeksema (1989).

Genus	Type species
Cantharellus Hoeksema and Best, 1984	*Cantharellus noumeae* Hoeksema and Best, 1984
Cycloseris Milne Edwards and Haime, 1849	*Fungia cyclolites* Lamarck, 1815
Ctenactis Verrill, 1864	*Madrepora echinata* Pallas, 1766
Danafungia Wells, 1966	*Fungia danai* Milne Edwards and Haime, 1851, *sensu* Wells, 1966 (= *F. scruposa* Klunzinger, 1879)
Fungia Lamarck, 1801	*Fungia agariciformis* Lamarck, 1801 (= *Madrepora fungites* Linnaeus, 1758)
Halomitra Dana, 1846	*Fungia pileus* sensu Lamarck, 1801 (= *Madrepora pileus* Linnaeus, 1758)
Heliofungia Wells, 1966	*Fungia actiniformis* Quoy and Gaimard, 1833
Herpolitha Eschscholtz, 1825	*Herpolitha limacina* Lamarck, 1801 (= *Madrepora limax* Esper, 1797)
Lithophyllon Rehberg, 1892	*Lithophyllon undulatum* Rehberg, 1892
Lobactis Verrill, 1864	*Fungia dentigera* Leuckart, 1841 (= *F. scutaria* Lamarck, 1801)
Pleuractis Verrill, 1864	*Fungia scutaria* Lamarck, 1801, *sensu* Verrill, 1864 (= *F. paumotensis* Stutchbury, 1833)
Podabacia Milne Edwards and Haime, 1849	*Agaricia cyathoides* Valenciennes, ms (= *Podabacia crustacea* (Pallas, 1766))
Polyphyllia Blainville, 1830	*Fungia talpa* Lamarck, 1815 (= *Polyphyllia talpina* (Lamarck, 1801))
Sandalolitha Quelch, 1884	*Sandalolitha dentata* Quelch, 1884
Zoopilus Dana, 1846	*Zoopilus echinatus* Dana, 1846

◀ *Fig. 10*. A-C. Monostomatous coralla with long tentacles: A. *Heliofungia actiniformis*, monostomatous with very long tentacles showing white acrospheres (Philippines); B. *H. fralinae*, monostomatous with long tentacles (Indonesia); C. *H. fralinae* tentacles showing violet acrospheres (Indonesia); D. *Pleuractis granulosa*, monostomatous and circular (Indonesia); E. *Pleuractis paumotensis*, monostomatous and oval (Papua New Guinea); F. *Cycloseris cyclolites*, monostomatous, free-living and oval (Philippines); G. *C. fragilis*, fragmented and regenerated, *Diaseris* form (Indonesia); H. *C. mokai*, polystomatous and encrusting, resembling *Lithophyllon* (Indonesia); I. *Lithophyllon scabra*, monostomatous, free-living and circular (Indonesia); J. *L. undulatum*, polystomatous, attached and foliaceous (Indonesia); K. *L. undulatum*, multiple stomata (Indonesia). Size ranges of each species are given by Hoeksema (1989).

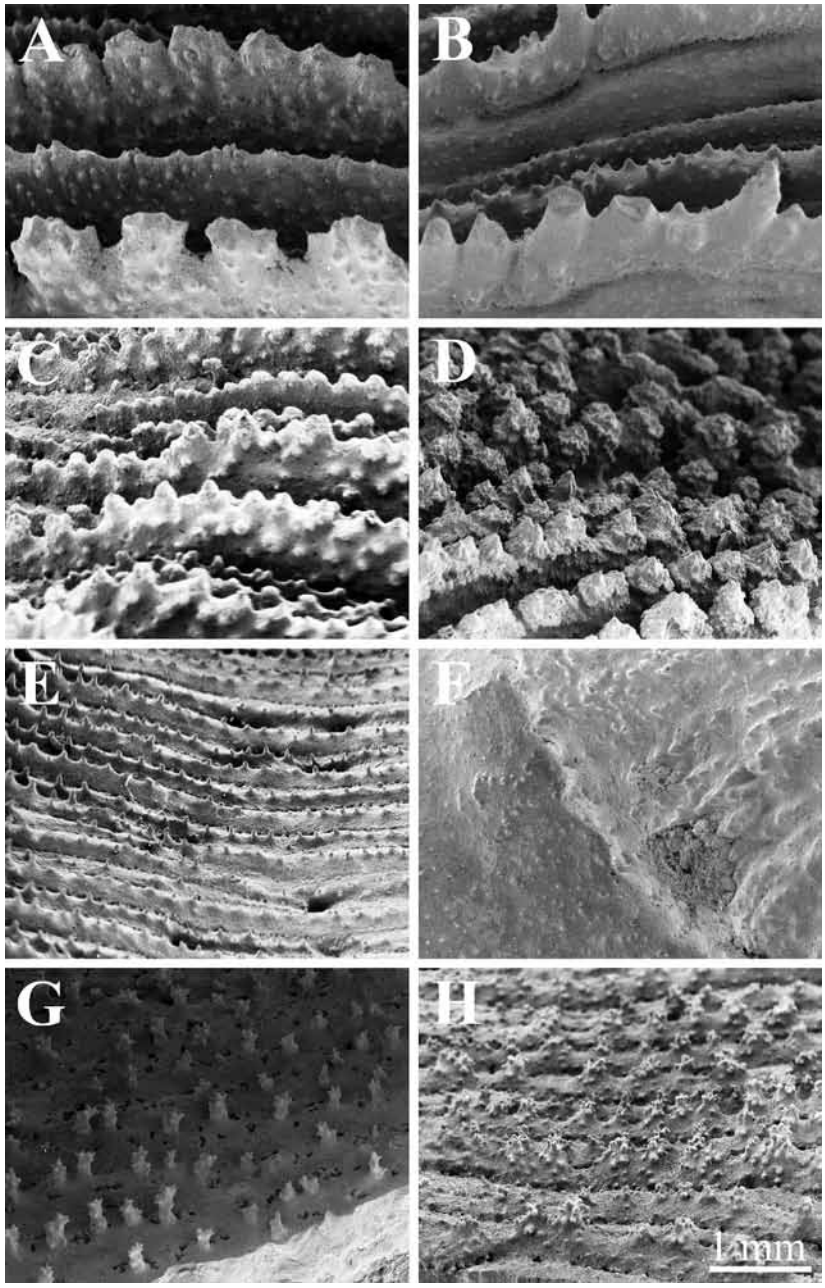


Fig. 11. SEM photographs of costae showing similarity of ornamentations (granulation patterns) within four genera (AB = *Heliofungia*, CD = *Pleuractis*, EF = *Cycloseris*, GH = *Lithophyllon*) of species that previously were not consider congeneric (compare Hoeksema, 1989). A. *Heliofungia actiniformis*; B. *H. fralinae* (ex *Danafungia*); C. *Pleuractis granulosa* (ex *Wellsofungia*); D. *P. paumotensis*; E. *Cycloseris sinensis*; F. *C. mokai* (ex *Lithophyllon*); G. *Lithophyllon scabra* (ex *Verrillofungia*); H. *L. undulatum*. Scale bar for all photographs: 1 mm.

spp., which are supported by values of 74, 64 and 74, respectively, when intraspecific variation is included (Fig. 4), and by 82, 74 and 81 when the alternative approach was followed (Fig. 5). The opposite is seen in only one case; the clade with *Lithophyllon* spp. has a support value of 71 in the analysis with intraspecific variation included (Fig. 4), which drops down to 37 when those positions are excluded (Fig. 5).

Two clades that are strongly supported by the analysis of the morphological data set (Fig. 8) and/or the other molecular data sets (Figs 2-3, 6-7) appear with low support values in only the COI-based phylogeny reconstruction with intraspecific variation excluded (Fig. 5) and are absent where intraspecific variation is included (Fig. 4). The genus *Halomitra* is shown as monophyletic (Fig. 5) or *H. clavator* is seen as more closely

Table 7. Mushroom coral species (Fungiidae) in the revised classification based on molecular analyses and their previously used names based on morphological characters (Hoeksema, 1989)**.**

Revised name	Previous name if different (Hoeksema, 1989)
Cantharellus doederleini (Von Marenzeller, 1907)	(not analysed)
Cantharellus jebbi Hoeksema, 1993	(not analysed)
Cantharellus noumeae Hoeksema and Best, 1984	(not analysed)
Ctenactis albitentaculata Hoeksema, 1989	-
Ctenactis crassa (Dana, 1846)	-
Ctenactis echinata (Pallas, 1766)	-
Cycloseris costulata (Ortmann, 1889)	*Fungia (Cycloseris) costulata* Ortmann, 1889
Cycloseris curvata (Hoeksema, 1989)	*Fungia (Cycloseris) curvata* Hoeksema, 1989
Cycloseris cyclolites (Lamarck, 1815)	*Fungia (Cycloseris) cyclolites* Lamarck, 1815
Cycloseris distorta (Michelin, 1842)	*Fungia (Cycloseris) distorta* Michelin, 1842
Cycloseris fragilis (Alcock, 1893)	*Fungia (Cycloseris) fragilis* (Alcock, 1893)
Cycloseris hexagonalis (Milne Edwards and Haime, 1848)	*Fungia (Cycloseris) hexagonalis* Milne Edwards and Haime, 1848
Cycloseris mokai (Hoeksema, 1989)	*Lithophyllon mokai* Hoeksema, 1989
Cycloseris sinensis Milne Edwards and Haime, 1851	*Fungia (Cycloseris) sinensis* (Milne Edwards and Haime, 1851)
Cycloseris somervillei (Gardiner, 1909)	*Fungia (Cycloseris) somervillei* Gardiner, 1909
Cycloseris tenuis (Dana, 1846)	*Fungia (Cycloseris) tenuis* Dana, 1846
Cycloseris vaughani (Boschma, 1923)	*Fungia (Cycloseris) vaughani* Boschma, 1923
Danafungia horrida (Dana, 1846)	*Fungia (Danafungia) horrida* Dana, 1846
Danafungia scruposa (Klunzinger, 1879)	*Fungia (Danafungia) scruposa* Klunzinger, 1879
Fungia fungites (Linnaeus, 1758)	*Fungia (Fungia) fungites* (Linnaeus, 1758)
Halomitra clavator Hoeksema, 1989	-
Halomitra pileus (Linnaeus, 1758)	-
Heliofungia actiniformis (Quoy and Gaimard, 1833)	-
Heliofungia fralinae (Nemenzo, 1955)	*Fungia (Danafungia) fralinae* Nemenzo, 1955
Herpolitha limax (Esper, 1797)	-
Lithophyllon concinna (Verrill, 1864)	*Fungia (Verrillofungia) concinna* Verrill, 1864
Lithophyllon ranjathi Ditlev, 2003	-
Lithophyllon repanda (Dana, 1846)	*Fungia (Verrillofungia) repanda* Dana, 1846
Lithophyllon scabra (Döderlein, 1901)	*Fungia (Verrillofungia) scabra* Döderlein, 1901
Lithophyllon spinifer (Claereboudt and Hoeksema, 1987)	*Fungia (Verrillofungia) spinifer* Claereboudt and Hoeksema, 1987
Lithophyllon undulatum Rehberg, 1892	-
Lobactis scutaria (Lamarck, 1801)	*Fungia(Lobactis) scutaria* Lamarck, 1801
Pleuractis granulosa (Klunzinger, 1879)	*Fungia (Wellsofungia) granulosa* Klunzinger, 1879
Pleuractis gravis (Nemenzo, 1955)	*Fungia (Pleuractis) gravis* Nemenzo, 1955
Pleuractis moluccensis (Van der Horst, 1919)	*Fungia (Pleuractis) moluccensis* Van der Horst, 1919
Pleuractis paumotensis (Stutchbury, 1833)	*Fungia (Pleuractis) paumotensis* Stutchbury, 1833
Pleuractis seychellensis (Hoeksema, 1993)	*Fungia (Pleuractis) seychellensis* Hoeksema, 1993
Pleuractis taiwanensis Hoeksema and Dai, 1991	*Fungia (Pleuractis) taiwanensis* Hoeksema and Dai, 1991
Podabacia crustacea (Pallas, 1766)	-
Podabacia kunzmanni Hoeksema, 2009	-
Podabacia motuporensis Veron, 1990	-
Podabacia sinai Veron, 2002	
Polyphyllia novaehiberniae (Lesson, 1831)	-
Polyphyllia talpina (Lamarck, 1801)	-
Sandalolitha dentata Quelch, 1884	-
Sandalolitha robusta (Quelch, 1886)	-
Zoopilus echinatus Dana, 1846	-

related to *Danafungia scruposa*, making *Halomitra* paraphyletic (Fig. 4). Similarly, the clade with *Herpolitha limax*, *Ctenactis albitentaculata* and *C. echinata* does not form a monophyletic group with the clade containing *Polyphyllia talpina* and *Ctenactis crassa* when intraspecific variation is used in the analysis (Fig. 4), while it does so when those data are excluded (Fig. 5). The relatively high number of unique base positions in *C. crassa* suggests that this species has gone through a process with an accelerated mutation rate or high selec-

tion pressure in comparison to the other fungiid species, or passed a genetic bottleneck.

Referring to the principle of reciprocal illumination (Wägele, 2005), the preferential phylogeny reconstruction may be defined as the one that is most similar to the reconstructions that were based on other, unrelated data sets, another marker or morphology. Such a preferential phylogeny reconstruction was only found when intraspecifically variable base positions were not used in the molecular analyses. When the COI and ITS data sets were combined prior to the analysis, the phenomenon was less obvious (Figs 6-7). Therefore, especially when only small data sets are available, the identification of the species themselves is not problematic, and the number of markers cannot be increased, it seems to be preferable to analyse the data sets both with and without intraspecifically variable base positions to acquire the optimal informative contents.

Taxonomic consequences

The previous major phylogeny reconstructions of the Fungiidae have shown what the taxonomic consequences might be, if the classification would be based on phylogenetic relations (Wells, 1966; Cairns, 1984; Hoeksema, 1989). The taxonomic changes introduced in the latest taxonomic revision of the Fungiidae were supported by a cladogram based on morphological character transformations (Hoeksema, 1989). Although the cladogram already indicated how a completely amended classification would look like (Fig. 8), a nomenclature totally based on that cladistic analysis was considered premature because many phylogenetic affinities were not clear enough. More support was needed from molecular data to accomplish such taxonomic changes. In the present study, such additional data have been produced and enabled the construction of a more reliable and complete cladogram, involving all species that were included in the molecular analysis. Gaps in the information were filled in with morphological data but without further subsequent taxonomic changes (Fig. 9). The present taxonomic changes in the classification are supposed to represent the fungiid phylogeny optimally without unnecessarily frustrating the basic 'principles of stability and universality' (ICZN 1999: 2).

Various genera that were accepted by Hoeksema (1989), *i.e. Ctenactis*, *Fungia*, *Halomitra*, *Lithophyllon*, *Podabacia*, *Sandalolitha*, and alleged subgenera, *i.e. Cycloseris*, *Danafungia*, *Verrillofungia*, *Pleuractis*, come out as monophyletic in the present phylogeny reconstructions when more than one species is included in the analyses (Table 1, Figs 2-7, 9). By combining the newly acquired molecular data and the morphological analyses as published by Hoeksema (1989), the various taxa are redefined in such a way that polyphyletic entities are avoided. All genera and subgenera may be considered monophyletic groups now based on the presently available information. The nomenclatorial consequences of this revision, which are mainly changes in taxonomic rank and some generic shifts of species, are summarized in Tables 6-7.

The genus Cantharellus

During the present study no specimens of *Cantharellus* Hoeksema and Best, 1984 could be collected and preserved for DNA analyses. This genus was therefore not included in the analyses. However, the earlier phylogeny reconstruction based on morphological characters (Fig. 8) indicates that two *Cantharellus* species included in the earlier study (Hoeksema, 1989) form the sister group of *Cycloseris*. A third species, *Cantharellus jebbi*, is polystomatous and encrusting (Hoeksema, 1993a), like *Cycloseris mokai*, which has just been included in *Cycloseris* (Figs 6-7; see also the remarks on *Cycloseris*). Therefore, it is very likely that future molecular studies indicate that one or more *Cantharellus* species will have to merge with *Cycloseris*.

The genera Ctenactis, Herpolitha, *and* Polyphyllia

In all molecular phylogeny reconstructions (Figs 2-7) *Ctenactis echinata* and *C. albitentaculata* cluster together with strong support values. In no case these two species form a monophyletic group with *C. crassa*. The position in the cladogram of both *C. crassa* specimens, which are from Phiphi Islands, West Thailand and Makassar (SW Sulawesi), Indonesia is much less consistent however, and more poorly supported than the position of any other fungiid species. Maybe, the *C. crassa* population went through one or more genetic bottleneck events or an increased selection pressure. With these considerations in mind and because of the morphology of the three species, Hoeksema (1989) is followed in accepting *Ctenactis* as a monophyletic group. Except for the sequences of *Ctenactis crassa*, the sequences of species of *Ctenactis* Verrill, 1864, *Herpolitha* Eschcholtz, 1825, and *Polyphyllia* Blainville, 1830 cluster in one monophyletic group or relatively close to each other (Fig. 7). In general, they cluster as the most basal lineages of the Fungiidae. These results suggest that the elongated form, the relatively long central burrow and the potential to

form several stomata in this burrow, are plesiomorph character states. These character states are considered to be autapomorphies in the phylogeny based on morphology (Fig. 8), with *Herpolitha* and *Polyphyllia* forming a clade to which *Ctenactis* is distantly related.

The genus Fungia

In all molecular phylogeny reconstructions (Figs 2-7) *Fungia fungites* figures as the sister taxon of a clade with *Halomitra pileus*, *H. clavator* and *Danafungia scruposa*, resulting in *Fungia* being paraphyletic. The molecular analyses also consistently indicate that *Fungia* Lamarck, 1801 is more closely related to the genera *Lithophyllon*, *Podabacia*, *Sandalolitha* and *Zoopilus*, than to its alleged subgenera *Wellsofungia*, *Pleuractis* and *Cycloseris*, which would make *Fungia* polyphyletic if these taxa would have been maintained as subgenenera. These results are fully supported by the morphological analysis (Fig. 8), in which the consequential nomenclatorial changes were not yet introduced. To retain monophyly for *Fungia*, its previous subgenera are upgraded to genus level in the present study.

The genera Cycloseris *and* Lithophyllon

In all molecular phylogeny reconstructions (Figs 2-7) *Cycloseris* Milne Edwards and Haime, 1849, clusters with *C. mokai*, which was previously classified with *Lithophyllon* Rehberg, 1892 (Hoeksema, 1989). The analyses based on both ITS and the combined data sets of COI and ITS (Figs 2-3, 6-7) indicate that *L. mokai*, despite its aberrant shape among the fungiid corals, does not represent a basal lineage in the *Cycloseris* clade. Therefore, instead of introducing a new generic name, and accepting a paraphyletic *Cycloseris* by doing so, the species in question is here transferred to *Cycloseris*. *Cycloseris mokai* differs from congeneric species in being encrusting, polystomatous, and irregularly shaped instead of free-living, monostomatous and circular to oval, or instead of consisting of regenerated fragments (Fig. 10F-H). See also the remarks on *Lithophyllon* and *Verrillofungia*.

The genera Danafungia *and* Heliofungia

The phylogeny reconstructions based on the COI data sets indicate that *Heliofungia actiniformis* and *Danafungia fralinae* are sister species, with values of 64 and 74, respectively (Figs 4-5). The ITS data sets do not support this when analysed separately (Figs 1-2), but the support values become very high when the COI and ITS data sets are combined, *i.e.* 96 and 100, respectively (Figs 6-7). All phylogeny reconstructions (Figs 2-7) clearly indicate that *Heliofungia fralinae*, previously classified as *Fungia (Danafungia) fralinae*, does not form a monophyletic group with the type species of *Danafungia* Wells, 1966, *i.e. D. scruposa*. It is therefore concluded that this species should presently be classified as *Heliofungia fralinae*, and that *Heliofungia* Wells, 1966, which previously was considered monotypic, now consists of two species. This new classification is supported by morphological and life-history traits, since both *Heliofungia* species show similar skeletal micro structures (Fig. 11A-B), relatively long tentacles with acrospheres, inflatable polyps, consequently a well-developed mobility, and the capacity to reproduce asexually by budding (Hoeksema, 1989, 2004).

In the analyses of the ITS data sets *Danafungia horrida* does not cluster with *D. scruposa* (Figs 2-3), but this result is not strongly supported. It is based on a single ITS sequence of *D. horrida* that clusters at two different places in the two reconstructed phylogenies (Figs 2-3). Therefore and because of the morphology of the two species (Hoeksema, 1989), *D. horrida* is still classified with *Danafungia*.

The genus Lobactis

In most of the phylogeny reconstructions (Figs 3-7) and especially in the analyses of the combined COI+ITS data sets (Figs 6-7), *Lobactis* Verrill, 1864**,** represented by the single species *L. scutaria*, clusters with low support at the basis of a clade with *Danafungia*, *Fungia* and *Heliofungia*. However, in the phylogeny reconstruction based on morphological data (Fig. 8) the species is basal to *Herpolitha* and *Polyphyllia*. This difference can be explained by accepting that the oval coral form, which placed the monotypic genus *Lobactis* basally to a clade with *Herpolitha* and *Polyphyllia*, was not a synapomorphy but evolved twice independently (homoplasy).

The genus Pleuractis

In all phylogeny reconstructions (Figs 2-7) *Pleuractis* Verrill, 1864 clusters with the monotypic genus *Wellsofungia* Hoeksema, 1989. The analyses strongly support that *Wellsofungia* is more closely related to *Pleuractis moluccensis* and *P. paumotensis*, than the latter two species are related to *P. gravis* and *P.* spec. 1. Hoeksema (1989: 255), when describing *Wellsofungia* as a subgenus of *Fungia*, stated: '*Wellsofungia* is separated from *Pleuractis* because it does not contain species that show an oval corallum outline (apomorph character

state 28; Fig. 10D-E). Phylogenetically such groups of which the monophyly cannot be demonstrated by the presence of synapomorphies are of a reduced interest'. It is concluded that *Wellsofungia* should be considered a junior synonym of *Pleuractis*, so that *W. granulosa* should be classified as *Pleuractis granulosa*.

A clade with the *Cycloseris* species clusters amidst the *Pleuractis* sequences in all molecular phylogenies (Figs 2-7), indicating that the latter genus may be paraphyletic. The results are inconsistent however (see Figs 3, 6-7 versus Figs 2, 4-5) and, therefore, it remains uncertain whether *Pleuractis* is really paraphyletic. Based on both these inconsistencies and the morphological analyses (Hoeksema, 1989), the generic status of *Pleuractis* is maintained for now.

The genus Lithophyllon

While dealing with the exclusion of intraspecific variation in molecular analyses, the position of the species *Lithophyllon concinna*, which was previously classified with *Verrillofungia* Wells, 1966 (Hoeksema, 1989) is discussed in detail. Its position in the phylogenies that were based on the ITS data set with intraspecifically variable base positions (Figs 2, 6) differs strikingly from that in the other reconstructions (Figs 3-5, 7). In all reconstructions (Figs 2-7) the species that were previously classified within *Verrillofungia*, *i.e.* *Lithophyllon concinna*, *L. repanda*, *L. scabra* and *L. spinifer*, cluster with *L. undulatum*, the type species of *Lithophyllon* Rehberg, 1892. All analyses furthermore strongly support that *L. undulatum* is not the basal lineage in this clade. Accordingly, because paraphyletic nominal taxa are not accepted, we consider *Lithophyllon* Rehberg, 1892, a senior synonym of *Verrillofungia* Wells, 1966. We accept that this may at first cause some confusion because the generic name *Lithophyllon* is generally known as referring to coral species that have foliaceous and polystomatous coralla, whereas all *Verrillofungia* species are free-living and monostomatous (Fig. 10I-K). See also the remarks on the molecular analysis of *Cycloseris* and *Lithophyllon*.

The genus Halomitra

In five out of the six molecular phylogeny reconstructions (Figs 2-3, 5-7), *Halomitra clavator* and *H. pileus* form a monophyletic group. The COI data set with intraspecifically variable base positions indicates that *Halomitra clavator* clusters with *Danafungia scruposa* (Fig. 4), but the support value of this clade is only 32. In contrast, the values for a *H. clavator - H. pileus* clade in the reconstructions based on both the ITS and the combined data sets are very high, *i.e.* 99, 100, 99 and 100, respectively (Figs 2-3, 6-7). Therefore the generic status of *Halomitra* Dana, 1846 remains unchanged.

The genera Podabacia, Sandalolitha, *and* Zoopilus

In the phylogeny reconstruction based on morphology (Fig. 8), and in all molecular reconstructions, the sequences of *Podabacia* Milne Edwards and Haime, 1849, *Sandalolitha* Quelch, 1884, and *Zoopilus* Dana, 1846, cluster as a monophyletic group or at least close to each other. It can only be concluded referring to morphology that these three nominal genera are separate entities. The individual *Sandalolitha*, *Podabacia* and *Zoopilus* sequences vary too little to consider these distinct taxa. The support values are generally low and, whenever they are higher, they give conflicting results in the various analyses. The morphological differences between these three taxa are very distinctive and therefore they are maintained as separate genera.

Ecomorphological consequences

Molecular phylogeny reconstructions may have as disadvantage that not all known species can be included due to lack of specimens or because the analyses did not give clear results for all species. By dealing with as many species as possible, molecular phylogeny reconstructions help to construct phylogenetic models that are independent of morphological character state transformations. As such, they are ideal to detect evolutionary trends in morphology and life history traits.

The development of additional mouths over the upper surface of mushroom corals has assisted the growth of larger coralla because food does not need to be transported to only the central mouth anymore and the coral has a larger chance of survival during sedimentation (Hoeksema, 1991a). The present analysis indicates that in most species lineages additional mouths evolved after the coralla grew larger, rather than the other way around. By the addition of secondary mouths, growth has become practically indeterminate, especially in free-living mushroom corals because they are the least restricted to available space. This trait, which ontogenetically can be considered a mechanism of intratentacular budding is usually not combined with asexual reproduction by extratentacular budding, as commonly demonstrated by a few species of solitary fungiids, such as *Fungia fungites*, *Heliofungia actiniformis* and *H. fralinae* (Hoeksema, 1989, 2004). Thanks to their mobility and apparent re-

sistance to toxins secreted by various sessile organisms, they usually survive when they happen to come in close contact with other competitors for space, either by growth or by bumping into them (Sheppard, 1979; Chadwick, 1988; Hoeksema, 1988; Chadwick-Furman and Loya, 1992; Yamashiro and Nishira, 1995; Abelson and Loya, 1999; Voogd *et al*., 2005). In free-living polystomatous corals, fragmentation in combination with regeneration and mobility facilitates continuous growth and may result in large surface areas of reef bottom to become covered by one or only a few species (Pichon, 1974; Littler *et al*., 1997; Hoeksema and Gittenberger, 2010), whereas monostomatous species clearly show determinate growth (Chadwick-Furman *et al*., 2000; Goffredo and Chadwick-Furman, 2003; Gilmour, 2004a; Knittweis *et al*., 2009). From an evolutionary perspective, polystomatism is probably not much constrained, since even in monostomatous mushroom coral species the production of secondary mouths can be induced artificially (Boschma, 1923; Jacoby *et al*., 2004), and as such it appears to be a plastic character in some fungiid species (Hoeksema, 1989).

The present study and its predecessors (Hoeksema 1989, 1991a) show that ecological benefits of evolutionary traits in the Fungiidae as a monophyletic taxon are best understood when the whole family is analysed. The present reconstruction involving corallum size and the development of secondary mouths among 50 species (Fig. 9) indicates that polystomatism has developed independently in eight lineages: once by intrastomatal budding, five times by extrastomatal budding and twice by a combination of both mechanisms. When only a subset of species is analysed, entirely opposite patterns may occur. Barbeitos *et al*. (2010) include only seven fungiid species in their study of coloniality in the Scleractinia. Their cladogram suggests that coloniality is an ancestral trait and that reversal toward a solitary state (loss of coloniality) has evolved twice in the Fungiidae. They restricted their study to species for which molecular data were available. Only two of these are monostomatous (*i.e*. *Heliofungia actiniformis* and *Lobactis scutaria*) and the five other ones are polystomatous, which they classified as *Halomitra* sp., *Herpolitha* sp., *Polyphyllia* sp., *Sandalolitha* sp., and *Zoopilus echinatus*. In case they would also have referred to phylogenetic models of Fungiidae based on morphological data (Hoeksema 1989, 1991a, 1993b; Fig. 8), they could have concluded that the evolution from a solitary (monostomatous) to a colonial (polystomatous) is generally not a reversal and that it has occurred more frequently than according to the phylogeny reconstruction based on the molecular data available to them. Our present study demonstrates that it is necessary to include as many species as possible in quantitative studies dealing with evolutionary trends, preferably all known species within a monophyletic clade (Fig. 9), or to avoid statements that indicate the occurrence frequency of such trends when only few species are included.

Mobility

Since mobility appears to be an advantageous trait in mushroom corals, there is no clear reason why in some lineages there is a loss of the capacity to become free-living. Corallum detachment is an active process in which part of the coral skeleton is weakened by partial dissolution of the stalk where it borders with the corallum disc (Yamishiro and Yamazato, 1987a, b, 1996; Yamashiro and Samata, 1996; Vizel *et al*., 2009). In some of the attached mushroom coral species, *i.e*. *Cycloseris mokai* and *Cantharellus jebbi*, attachment by a stalk has even developed further by development of an encrusting growth form (Hoeksema, 1989; 1993a). Furthermore, some of the free-living mushroom coral species (*e.g*. *Pleuractis moluccensis* and *Sandalolitha* spp.) show a large detachment scar and their juveniles remain relatively long in the attached anthocaulus phase. A possible reason for postponed detachment is that the juvenile coral will not be buried in the sediment. In a fixed position, especially if the coral remains more vertically oriented, sediment can more easily be shed by the coral than in a horizontal position (Chadwick-Furman and Loya, 1992). In some fungiid species the timing of detachment appears to be variable and its postponement may easily lead to misidentifications by causing confusion with species that usually remain fixed, such as specimens that have been named *Cantharellus noumeae* but actually are attached specimens of *Fungia fungites* (Veron, 2000: 252). A by-product of coral detachment is that several new polyps may regenerate from the empty stalks, even simultaneously (Hoeksema, 1989). Some of the specimens shown by Veron (2000: 252), show clusters of newly regenerated polyps, which is characteristic for species that may detach themselves but not by attached specimens of *C. noumeae*. Eventually in mushroom corals, there appears to be a trade-off between the advantages and risks of being mobile and of remaining attached. Loss of detachment by prolonged attachment can be seen as a way in which mushroom

corals remain secondarily attached. This phase in the life history of mushroom corals can be considered an evolutionary reversal, whereas there is a rule, Dollo's Law, which states that evolution is irreversible, especially when it concerns complex structures. Examples of Dollo's Law concern the loss and reacquirement of digits in lizards and the coiling in shells (Collin and Cipriano, 2003; Pagel, 2004; Kohlsdorf and Wagner, 2006; Collin and Miglietta, 2008; Galis *et al*., 2010). Prolonged attachment and eventual loss of detachment is a matter of paedomorphic (retarded) heterochrony, which also has impact on the overall coral growth form (Hoeksema, 1991a). Since it has become a fixed character in some species and occasionally a plastic one in others, the re-evolution of an attached mode of life can also be seen as a mushroom coral's failure to execute the physiological detachment process for which the trigger for onset in the timing is not yet understood. As such, a secondary attached mode of life, after the loss of a free-living phase cannot be seen as re-evolution of a complex trait.

Polystomastism

There is no clear relationship between the loss of a free-living phase and the evolution of multiple mouths. In the clade consisting of *Sandalolitha*, *Zoopilus* and *Podabacia*, loss of detachment as shown by *Podabacia* appears to have evolved after the coralla in its lineage became polystomatous. Additional mouths enable the corals to grow larger, whereas the loss of detachment in combination with an encrusting growth form may restrict growth. In the previous ecomorphological study on mushroom corals (Hoeksema, 1991a) it was hypothesized that polystomatism in the relatively small species *Cycloseris mokai* can be explained because its ancestors had acquired multiple mouths and the encrusting growth form limited its size. In the present analysis it is clear that *C. mokai* has evolved in a clade consisting of small species (Fig. 8).

Conclusion

Thanks to the present study, molecular information on fungiid species has become available, and additional species will be included in future research, among which some that were previously classified with the Siderastreidae but show stronger affinities with the Fungiidae (Benzoni *et al*., 2007). Although molecular methods have become helpful in clarifying phylogenetic relationships at lower taxonomic levels, analyses combining molecular with microstructural and micromorphological characters prove to be more helpful in our understanding of the scleractinian evolution (Stolarski and Roniewicz, 2001, Benzoni *et al*., 2007, 2010; Budd and Stolarski, 2009; Budd *et al*., 2010, present study). A similar conclusion has also been reached in the octocoral genus *Sinularia* (McFadden *et al*., 2009).

The phylogeny reconstruction of the Fungiidae in the present study has been used to introduce changes in the taxonomy of mushroom corals. Some subgenera have been raised to genus level and some species show different affinities than previously assumed and have moved to other genera. The present evolution model also proves to be useful as a tool to show innovations in life history traits, such as the loss of mobility and the development of additional mouths, which enables mushroom corals to grow larger and to reproduce asexually by continuous fragmentation. In future research, the phylogeny reconstruction of the Fungiidae and its successors will be used to study the evolution of ecological traits, such as the distribution of species along environmental gradients, or the evolution of inter-specific associations in which mushroom corals act as hosts for symbionts.

Recent phylogeny reconstructions using only a few representative species of particular genera and families showed that the taxonomy of the Scleractinia needs to be overhauled (*e.g.* Fukami *et al*., 2004, 2008; Kerr, 2005; Kitahara *et al*., 2010). As shown in the present study and other ones (Benzoni *et al*., 2007, 2010; Wallace *et al*., 2007; Huang *et al*., 2009), the most straightforward way to get a better insight in the evolution of the Scleractinia is by simply adding more species to the scleractinian evolution model, one family or one genus at a time.

Acknowledgements

We are very grateful to Drs A.F. Budd, F. Benzoni, C.A. Chen, and E. Gittenberger for their helpful comments on the manuscript. Dr. C. Hunter is thanked for providing material from Hawaii. Prof. dr. A. Noor (Hasanuddin University, Makassar) gave support during the fieldwork in South Sulawesi. This research was financially supported by WOTRO (grant W82-249), the Schure-Beijerinck-Poppingfonds (KNAW), the Alida Buitendijkfonds and the Jan Joost ter Pelkwijkfonds (both at NCB Naturalis). The research permit was issued by the Indonesian Institute of Sciences (LIPI).

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Received: 10 August 2010
Revised and accepted: 16 November 2010
Published online: 23 March 2011
Editor: R.W.M. van Soest