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

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Abstract

The phylogenetic relationships of the Fungiidae, a family of pre-dominantly 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 intrastomatral 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.

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,
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 (Wijsmann-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 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 |
| Ctenactis 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] | EU207219 | 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 tenais     | Thailand, Andaman Sea, Phiphi Island [2] | EU149891 | EU149823 |
| Cycloseris tenais     | 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] | EU149872 | EU149827 |
| Danafungia scruposa   | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149892 | EU149829 |
| Fungia fungites       | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149904 | EU149837 |
| Halomitra clavator    | 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 fraliniae  | 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 MgCl\(_2\) (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\(^{-1}\)), 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\textsuperscript{TM} 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-

| Sequenced specimens          | Locality [locality nr. in Fig. 1]             | COI            | ITS            |
|------------------------------|---------------------------------------------|----------------|----------------|
| Lithophyllon concinna        | Thailand, Andaman Sea, Phipi Islands [2]    | EU202721*     |                |
| Lithophyllon concinna        | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149893     | EU149832       |
| Lithophyllon repanda         | Thailand, Andaman Sea, Phipi Islands [2]    | EU149883*     |                |
| Lithophyllon scabra          | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149903     | EU149833       |
| Lithophyllon scabra          | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149874     |                |
| Lithophyllon spinifer        | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149894     |                |
| Lithophyllon undulatum       | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149864     | EU149834       |
| 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 gravis            | Egypt, Red Sea, Marsa Nakari [1]            | EU149913     | EU149851       |
| Pleuractis granulosa         | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149884     | EU149835       |
| Pleuractis granulosa         | Egypt, Red Sea, Marsa Nakari [1]            | EU149867     | EU149843       |
| Pleuractis gravis            | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149873     | EU149844       |
| Pleuractis moluccensis       | Thailand, Andaman Sea, Phipi Islands [2]    | EU149909*     | EU149849       |
| Pleuractis moluccensis       | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149918     |                |
| Pleuractis paumotensis       | Thailand, Andaman Sea, Phipi Islands [2]    | EU149812*     | EU149849       |
| Pleuractis paumotensis       | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149911     | EU149850       |
| Pleuractis sinai             | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149916     | EU149858       |
| Podabacia crustacea          | Indonesia, Bali, Tanjung Benoa [3]         | EU149907     | EU149845       |
| Podabacia crustacea          | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149878     |                |
| Podabacia kunzmanni          | Thailand, Andaman Sea, Phipi 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, Phipi Islands [2]    | EU149888     |                |
| Polyphylia talpina           | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149915     | EU149853       |
| Sandalolitha dentata         | Indonesia, Bali, Tanjung Benoa [3]         | EU149914     | EU149854       |
| Sandalolitha dentata         | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149918     | EU149856       |
| Sandalolitha robusta         | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149917     | EU149857       |
| Zoopilus echinatus           | Indonesia, S Sulawesi, Spermonde Archipelago [4] | EU149916     | EU149858       |
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, 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, 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, TBR algorithm, steepest descent and 1000 random addition replicates per replicate. PAUP 4.0b10 was used for maximum parsimony and neighbor joining analyses.

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     |

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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 with intraspecific variation| 791                               | 337        | 0.589             | 0.439                       | 95                                  |
| COI & ITS without intraspecific 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 |
|-----------------------------------|-----------------|------------------|-----------------|
|                                   | χ²   | df  | P    | P    |
| COI with intraspecific variation  | 4.0  | 75  | 1.00 | P<0.01 |
| COI without intraspecific variation| 3.7  | 63  | 1.00 | P<0.01 |
| ITS with intraspecific variation  | 12.5 | 141 | 1.00 | -*    |
| ITS without intraspecific variation| 4.5  | 105 | 1.00 | -*    |
| COI & ITS with intraspecific variation| 7.1  | 123 | 1.00 | -*    |
| COI & ITS without intraspecific variation| 4.0  | 99  | 1.00 | -*    |
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).

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**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, PhiiPhii 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
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 polystatism, 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), *Pleuroctis 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 | $\emptyset$ >25 cm | Budding instrastomatal | Budding extrastomatal |
|----------------------------------|---------------------|---------------------|------------------------|-----------------------|
| *Cycloseris mokai* (Hoeksema, 1989) | +                   | -                   | -                      | +                     |
| *Cycloseris costulata* (Ortmann, 1889) | -                   | -                   | -                      | -                     |
| *Cycloseris cycloites* (Lamarck, 1815) | -                   | -                   | -                      | -                     |
| *Cycloseris vaughani* (Boschma, 1923) | -                   | -                   | -                      | -                     |
| *Cycloseris tenais* (Dana, 1846) | -                   | -                   | -                      | -                     |
| *Cycloseris sinensis* Milne Edwards & Haime, 1851 | -                   | -                   | -                      | -                     |
| *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.](image)

![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 intrastomatinal and extrastomatinal budding.](image)
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 intraspecific variation included (Figs 3, 7). 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*    | *Cantharellus noumeae* Hoeksema and Best, 1984    |
| *Cycloseris*      | *Fungia cyclolites* Lamarck, 1815                  |
| *Ctenactis*       | *Madrepora echinata* Pallas, 1766                  |
| *Danafungia*      | *Fungia danai* Milne Edwards and Haime, 1851, sensu Wells, 1966 (= *F. scruposa* Klunzinger, 1879) |
| *Fungia*          | *Fungia agariciformis* Lamarck, 1801 (= *Madrepora fungites* Linnaeus, 1758) |
| *Halomitra*       | *Fungia pileus* sensu Lamarck, 1801 (= *Madrepora pileus* Linnaeus, 1758) |
| *Heliofungia*     | *Fungia actiniformis* Quoy and Gaimard, 1833       |
| *Herpolithia*     | *Hericolium limacina* Lamarck, 1801 (= *Madrepora limacina* Esper, 1797) |
| *Lithophyllon*    | *Lithophyllon undulatum* Rehberg, 1892             |
| *Lobactis*        | *Fungia dentigera* Leuckart, 1841 (= *F. scutaria* Lamarck, 1801) |
| *Pleuractis*      | *Fungia scutaria* Lamarck, 1801, sensu Verrill, 1864 (= *F. paumotensis* Stutchbury, 1833) |
| *Podabacia*       | *Agaricia cyathoides* Valenciennes, ms (= *Podabacia cristacea* (Pallas, 1766)) |
| *Polyphylia*      | *Fungia talpa* Lamarck, 1815 (= *Polyphylia talpina* (Lamarck, 1801)) |
| *Sandalolitha*    | *Sandalolitha dentata* Quelch, 1884                |
| * Zoopolipus*     | *Zoopolipus 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).
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
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-

| 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 scuposa* (Klunzinger, 1879) | *Fungia (Danafungia) scuposa* 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 trilinae* (Nemenzo, 1955) | *Fungia (Danafungia) trilinae* Nemenzo, 1955 |
| *Herpolitha limax* (Esper, 1797) | - |
| *Lithophyllum concinna* (Verrill, 1864) | *Fungia (Verrillofungia) concinna* Verrill, 1864 |
| *Lithophyllum ranjathi* Ditlev, 2003 | - |
| *Lithophyllum repanda* (Dana, 1846) | *Fungia (Verrillofungia) repanda* Dana, 1846 |
| *Lithophyllum scabra* (Döderlein, 1901) | *Fungia (Verrillofungia) scabra* Döderlein, 1901 |
| *Lithophyllum spinifer* (Claereboudt and Hoeksema, 1987) | *Fungia (Verrillofungia) spinifer* Claereboudt and Hoeksema, 1987 |
| *Lithophyllum 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 | - |
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 nomenclatural 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* Eschscholtz, 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 plesiomorphic characteristic 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 Zoopolis, than to its alleged subgenera Wellsofungia, Pleuractis and Cycloseris, which would make Fungia polyphyletic if these taxa would have been maintained as subgenaera. These results are fully supported by the morphological analysis (Fig. 8), in which the consequent nomenclatorial changes were not yet introduced. To retain monophyly for Fungia, its previous subgenaera 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 congenic 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 Wellsofungia.

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 (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 (homoplasys).

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 an 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. frallinae (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 intrastomal budding, five times by extrastomal 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 (Yamashiro and Yamazato, 1987a, b, 1996; Yamashiro and Samata, 1996; Vizel et al., 2009). In some of the attached mushroom coral species, i.e. Cyclloseris 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 reacquisition of digits in lizards and the coiling of 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*, *Zoopolis* 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.

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