The evolutionary history of parasitic gastropods and their coral hosts in the Indo-Pacific

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A molecular analysis of the evolutionary history of mushroom corals (Scleractinia: Fungiidae) and its consequences for taxonomic classification

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Abstract

DNA samples from fungiid corals were used to reconstruct the phylogeny of the Fungiidae (Scleractinia), based on the markers COI and ITS I & II. In some cases coral DNA was isolated and sequenced from parasitic gastropods that have eaten from their host corals, by using fungiid-specific primers. Even though the present molecular phylogeny reconstructions largely reflect the one based on morphological characters by Hoeksema (1989), there are some distinct differences. Most of these are probably linked to parallel or convergent evolution. Most fungiid coral species live fixed to the substrate in juvenile stage and become detached afterwards. A loss of this ability to become free-living, appears to have induced similar reversals independently in two fungiid species. These species express ancestral, plesiomorphic character states, known from the closest relatives of the Fungiidae, like encrusting and multistomatous growth forms. Consequently, they were both placed in the genus *Lythophyllon* by Hoeksema (1989). However, the present molecular analysis indicates that these species are not even closely related. Another discrepancy is formed by the separate positions of *Ctenactis crassa*, away from its congeners, in various cladograms that were based on either of the two markers. This may have been caused by one or more bottleneck events in the evolutionary history of that species, which resulted in a much faster average DNA mutation rate in *Ctenactis crassa* as compared to the other fungiid species. Furthermore, it was investigated whether the exclusion of intraspecifically variable base positions from molecular data sets might improve the phylogeny reconstruction. For COI and ITS I&II in fungiid corals this has three positive effects: (1) it raised the support values of most branches in the MrBayes, Parsimony and Neighbor Joining consensus trees, (2) it lowered the number of most parsimonious trees, and (3) it resulted in phylogeny reconstructions that more closely resemble the morphology-based cladograms. Apparently, the exclusion of intraspecific variation may give a more reliable result. Therefore, the present hypotheses about the evolutionary history of the fungiid corals are based on analyses of both the data sets with and without intraspecific variation.

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Introduction

Most coral species (Scleractinia) show much ecophenotypical variation. Because of this and the low number of plesiomorph characters states, phylogeny reconstructions based on morphology are troublesome. Molecular analyses have helped to

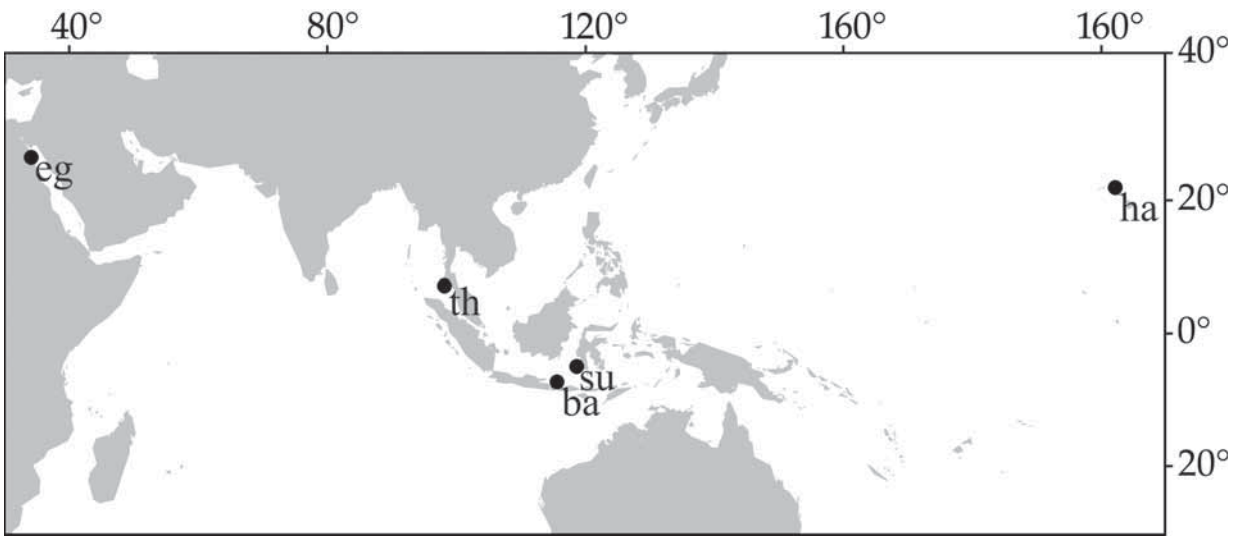

Fig. 1. The Indo-Pacific region, from the Red Sea to the Hawaiian Archipelago, illustrating the localities of the material used in this study (table 1). Abbreviations: ba, Bali, Indonesia [3]; ha, Oahu, Hawaii [5]; eg, Egypt (Red Sea) [1]; su, Sulawesi, Indonesia [4]; th, Phiphi Islands, Thailand [2].

shed more light upon their evolutionary history. Discrepancies between coral phylogeny reconstructions based on either morphological or molecular data are frequently found (Fukami et al., 2004). Even though such incompatible results have been found in various animal taxa, so-called reticulate evolution has been used most predominantly as the most likely explanation in corals (Diekmann et al., 2001). Other evolutionary history scenarios, like homeostasis, parallel or convergent evolution, and bottleneck events are considered less frequently. Such scenarios may at least partly be the cause of different mutation speeds in sister taxa or data saturation in general. The possibility of misidentifications because of e.g. the presence of cryptic species is usually also neglected.

Characters that are variable within species and within populations are commonly used in molecular phylogeny reconstructions. Even characters varying within individuals are usually included, like the base positions varying between the copies of ITS sequenced from one specimen. Such characters are often excluded in morphology-based phylogeny reconstructions. Therefore we have analysed the data sets both with and without intraspecifically variable base positions.

Material and methods

Sampling

The fungiid corals of which a DNA-sample was analysed, were collected during various expeditions in the Indo-Pacific conducted over the last thirty years by either the National Museum of Natural History Naturalis or by affiliated institutes. To get a good representation of intraspecific molecular variation, the specimens that were included for each species were preferably taken from populations far apart (fig. 1), i.e. Egypt (Red Sea), Thailand (Indian Ocean), Indonesia (Sulawesi and Bali: border of Indian and Pacific Oceans) and Hawaii (Pacific Ocean). The coral samples were preserved on ethanol 70% or 96%. All corals were identified twice, after photographs and/or specimens, independently by B.W. Hoeksema and A. Gittenberger.

DNA extraction and sequencing

Small pieces of coral tissue and skeleton were scraped off each specimen with a sterile scalpel to fill about

half a 1.5 ml tube. A mixture of 0.003 ml proteinase K (20 mg/ml) 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 pH8, was added to the tube 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'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' 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'. After centrifugation for 10' at 8000 rpm, this solution was discarded. The pellet was dried in a vacuum centrifuge and than dissolved in 0.020 ml 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 Spacer I & II) and 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 on the basis of fungiid sequences that were retrieved with Folmer Universal COI primers. The fungiid specific primer sequences were checked against the COI sequences (A. Gittenberger and E. Gittenberger, 2005; A. Gittenberger et al., chapter 8) of their epitoniid ecto-parasites (Mollusca: Gastropoda: Epitoniidae) and their coralliophilid endo-parasites (Mollusca: Gastropoda: Coralliophilidae) to make sure that they would not fit on the COI region of these gastropods. Although the DNA-extract of fungiids was used for most sequences, we also successfully sequenced the fungiid COI region using the DNA-extract of their parasitic gastropods. This was done to get data from localities where only the gastropods could be collected and no fungiid DNA material was available. Knowing the fungiid species with which the snails are associated, the retrieved sequences were checked with those of the same fungiid species from other localities. The PCR was performed in a Peltier Thermal Cycler PTC-200, using the following PCR- program: 1 cycle of 94°C for 4' and 60 cycles of 94°C for 5''; AT (Annealing Temperature; table 2) for 1'; 0.5°C/s to 60°C; 72°C for 1'. The optimalized PCR reaction mix consisted of 0.0025 ml PCR buffer (10x), 0.0005 ml MgCl2 (50 mM), 0.0010 ml forward primer (10 pM), 0.0010 ml reverse primer (10 pM), 0.0005 ml dNTP's (10 mM), 0.0003 ml Taq polymerase (5 units / 0.001 ml), 0.0132 ml MilliQ and 0.0010 ml 1:10 DNA stock-solution (= c. 100 ng DNA). For amplifying the ITS region, 0.0020 ml Qsolution (QIAGEN) was used instead of the 0.0020 ml 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'', 50°C for 5'' and 60°C for 4'. The reaction mix used contained 0.0020 ml Ready Reaction Mix (Big DyeTM by PE Biosystems), 0.0020 ml Sequence Dilution-buffer, 0.0005 ml primer (5 pM forward or reverse primer solution) and 0.0055 ml amplified DNA (= half the PCR-product, evaporated to 0.0055 ml by vacuum centrifugation). 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. Because MacClade 4. ClustalW had some difficulties aligning the ITS data set due to multiple gaps, some manual insertions, manual modifications were made in the resulting alignment. Afterwards the COI alignment was checked for stopcodons with MacClade 4.0 (Maddison and Maddison, 2000). Alignments are available from the authors.

Table 1. Specimens of which the COI and/or ITS marker was successfully sequenced. Locality data and availability of voucher specimen or photo is indicated.

Sequenced specimens	**Locality [locality nr. fig.1]**	**Voucher Specimen or Photo**	**COI**	**ITS**
Ctenactis albitentaculata	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Ctenactis crassa	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Ctanactis crassa	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Ctenactis echinata	Egypte, Red Sea, Marsa Nakari [1]	Photo	X	X
Ctenactis echinata	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Cycloseris*) *costulata*	Egypte, Red Sea, Marsa Nakari [1]	Photo	X	X
Fungia (*Cycloseris*) *costulata*	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Fungia (*Cycloseris*) *costulata*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Cycloseris*) *cyclolites*	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Fungia (*Cycloseris*) *fragilis*	Thailand, Krabi, Phiphi Islands [2]	Photo	X	
Fungia (*Cycloseris*) *fragilis*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Fungia (*Cycloseris*) *sinensis*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Cycloseris*) *tenuis*	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Fungia (*Cycloseris*) *tenuis*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Fungia (*Cycloseris*) *vaughani*	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Fungia (*Cycloseris*) *vaughani*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Fungia (*Danafungia*) *fralinae*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Danafungia*) *scruposa*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Danafungia*) *horrida*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec		X
Fungia (*Fungia*) *fungites*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Lobactis*) *scutaria*	Egypte, Red Sea, Marsa Nakari [1]	Photo	X	X
Fungia (*Lobactis*) *scutaria*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Fungia (*Lobactis*) *scutaria*	United States of America, Hawaii, Kaneohe Bay [5]	Spec	X	X
Fungia (*Lobactis*) *scutaria*	United States of America, Hawaii, Kaneohe Bay [5]	Spec	X	
Fungia (*Pleuractis*) sp. A (see A. & E. Gittenberger, 2005)	Egypte, Red Sea, Marsa Nakari [1]	Photo	X	X
Fungia (*Pleuractis*) *gravis*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Pleuractis*) *moluccensis*	Thailand, Krabi, Phiphi Islands [2]	Photo	X*	
Fungia (*Pleuractis*) *moluccensis*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec		X
Fungia (*Pleuractis*) *paumotensis*	Thailand, Krabi, Phiphi Islands [2]	Photo	X*	
Fungia (*Pleuractis*) *paumotensis*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Pleuractis*) *taiwanensis*	Indonesia, Bali, Tanjung Benoa [3]	Spec		X
Fungia (*Verrillofungia*) *concinna*	Thailand, Krabi, Phiphi Islands [2]	Photo	X*	

Fungia (*Verrillofungia*) *concinna*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Verrillofungia*) *repanda*	Thailand, Krabi, Phiphi Islands [2]	Photo	X*	
Fungia (*Verrillofungia*) *scabra*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Verrillofungia*) *scabra*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Fungia (*Verrillofungia*) *scabra*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Fungia (*Verrillofungia*) *spinifer*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Fungia (*Wellsofungia*) *granulosa*	Egypte, Red Sea, Marsa Nakari [1]	Photo		X
Fungia (*Wellsofungia*) *granulosa*	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Halomitra clavator	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Halomitra pileus	Thailand, Krabi, Phiphi Islands [2]	Photo	X*	
Halomitra pileus	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Halomitra pileus	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	.
Heliofungia actiniformis	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Heliofungia actiniformis	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Heliofungia actiniformis	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Heliofungia actiniformis	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Photo	X**	
Herpolitha limax	Egypte, Red Sea, Marsa Nakari [1]	Photo	X*	
Herpolitha limax	Thailand, Krabi, Phiphi Islands [2]	Spec	X	X
Herpolitha limax	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Lithophyllon undulatum	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Lithophyllon undulatum	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Lithophyllon mokai	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Lithophyllon mokai	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Lithophyllon mokai	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Podabacia sp. A	Thailand, Krabi, Phiphi Islands [2]	Photo	X	
Podabacia sp. B	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Podabacia crustacea	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Podabacia crustacea	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Podabacia motuporensis	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Podabacia motuporensis	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	
Polyphyllia talpina	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Sandalolitha dentata	Thailand, Krabi, Phiphi Islands [2]	Photo	X	X
Sandalolitha dentata	Indonesia, Bali, Tanjung Benoa [3]	Spec		X
Sandalolitha dentata	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Sandalolitha robusta	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X
Zoopilus echinatus	Indonesia, South Sulawesi, Spermonde Archipelago [4]	Spec	X	X

* Sequence obtained from DNA extract of *Epitonium* spec. ** Sequence obtained from DNA extract of *Leptoconchus* spec.

Table 2. Primer sequences, annealing temperatures and sources.

Primer	Annealing temp.	Primer seq.	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 AAA ATC A-3'	25-mer	Folmer *et al*., 1994
COI mod F (FungCO1for1)	53	5'-CTG CTC TTA GTA TGC TTG TA-3'	20-mer	Newly developed primer
COI mod R (FungCO1rev2)	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	Our lab

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, we included conspecific samples from distant localities like e.g. Indonesia and the Red Sea (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 we performed the G1 skewness statistic 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 done with 1000 random addition replicates, TBR algorithm and steepest descent. In addition a non-parametric parsimony bootstrap analysis was done 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 done with 10,000 bootstrap replicates. Bayesian inference was performed in MrBayes 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 loglikelihoods of saved trees were plotted in a Microsoft Excel graph to see from where on they become stationary.

Results

The COI data set (table 1) consist of 63 sequences of 500 bases each. The data set does not include any gaps or stopcodons. The ITS data set (table 1) consists of 45 sequences with lengths varying between 604 and 618 bases. The length varies due to multiple gaps. Results from the statistical analyses are represented in the tables 3-4. The parsimony analyses are

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	176	105	0.705	0.518	29
COI & ITS with intraspecific variation	791	377	0.589	0.439	95
COI & ITS without intraspecific variation	36	220	0.695	0.583	61

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 Mr Modeltest. We use the result from the likelihood ratio test, because it is 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 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). This supports the positive effect of [1] excluding intraspecific variation and [2] including more than one marker in the analysis. The found lower tree-scores do not necessary have anything to do with a false or good phylogenetic signal in the excluded positions, because one expects them to be lower in any data set with fewer characters.

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 figures 2-7. Here, we only present the results of the MrBayes analyses. Neighbor joining, maximum parsimony and parsimony bootstrap analyses gave similar results, which will be provided by the authors on request.

General discussion

Our discussion starts from the six molecular phylogeny reconstructions that result from the Bayesian analysis (figs 2-7). Because the maximum parsimony and neighbor joining analyses gave similar results, they support the conclusions that are made below. In this study we have focussed on the following questions:

[1] can a gastropod parasite successfully be used as a source for both its own DNA and that of its coral host;

[2] what is the effect of excluding all intraspecifically variable base positions when reconstructing a molecular phylogeny;

Table 4. Results of Chi-square-, G1 skewness- and permutation- tests to check for phylogenetic signal and consistency of the analysed data sets.

	Chi square test				
Type of data set	**X^2**	**df**	**P**	**G1 skewness test**	**Permutation test**
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	-*

* We were not able to obtain this result due to extremely long calculation times.

[3] what is the most likely phylogeny of the fungiid corals, taking all kinds of data into account;
[4] do all the genera and subgenera of the Fungiidae that are recognized in the literature represent monophyletic taxa;
[5] what classification of the Fungiidae represents the phylogeny of that family best and how should the nomenclature be adapted to reflect this?
One source for two sequences

By using specific primers, DNA of the coral and that of its parasite could be amplified successfully with certainty (table 1). Since the entire body of the snails were used, it remains unclear whether the coral DNA was isolated from the stomach of the snail, or from other parts of the parasite that are in frequent intensive contact with the coral.

Excluding intraspecific variation

There are differences in the phylogeny reconstructions based on the COI and ITS data sets with intraspecifically variable base positions (figs 2, 4) in comparison to those constructed with these positions excluded (figs 3, 5). The "better" phylogeny reconstruction is here assumed to be the one that is most similar to the phylogenies that were based on other, unrelated data sets, e.g. on another marker or on morphology.

In phylogenies resulting from the molecular analyses of the ITS data sets and the combined COI+ITS data sets, the sequence of *Verrillofungia concinna* clusters far away from the sequences of the other *Verrillofungia* species and *Lithophyllon undulatum* when intraspecifically variable base positions are included (figs 2, 6). When these are excluded, all *Verrillofungia* and *Lithophyllon undulatum* form a monophyletic group, with support values of 51 and 100, based on respectively the ITS (fig. 3) and the combined COI+ITS data set (fig. 7). This result is also supported by the analyses of the COI data set (figs 4-5) and gives an indication of what error may happen when intraspecifically variable base positions are included in molecular analyses.

A similar phenomenon seems to have influenced the position of *Heliofungia fralinae* in the phylogeny reconstruction based on the ITS data set with intraspecifically variable base positions included. There this species clusters with a significant support value of 65 (fig. 2) as the sister species of *Verrillofungia concinna*. In the analysis of the ITS data set without these base positions (fig. 3), it clusters much more closely to the *Heliofungia actiniformis* sequence, with which it forms a strongly supported monophyletic group in the other molecular analyses (figs 4-7), i.e. with support values of 64, 74, 96 and 100, respectively.

A final example of the misleading effect of the use of intraspecifically variable base positions in phylogeny reconstruction is the position of the clade with *Pleuractis granulosa*, *P. paumotensis*, *P. taiwanensis* and *P. moluccensis*. These species seem to be distantly related to *Pleuractis gravis*, *P.* spec. A and the *Cycloseris* in the phylogeny based on ITS including the intraspecific variation (fig. 2), while it forms a significantly supported monophyletic group with these species in all other analyses (figs 3-7).

Table 5. Proposed classification of the Fungiidae.

Genus	**Species**
Fungia	*F. fungites*
Cycloseris [used to be *Fungia* (*Cycloseris*)]	*C. costulata; C. cyclolites; C. curvata; C. distorta; C. fragilis; C. hexagonalis; C. mokai* [used to be *Lithophyllon mokai*]; *C. sinensis; C. tenuis; C. somervillei; C. vaughani*
Danafungia [used to be *Fungia* (*Danafungia*)]	*D. horrida; D. scruposa*
Lobactis [used to be *Fungia* (*Lobactis*)]	*L. scutaria*
Pleuractis [used to be *Fungia* (*Pleuractis*)]	*P. granulosa* [used to be *Fungia* (*Wellsofungia*) *granulosa*]; *P. gravis*; *P. moluccensis; P. paumotensis*
Verrillofungia [used to be *Fungia* (*Verrillofungia*)]	*V. concinna; V. repanda; V. spinifer; V. scabra*
Cantharellus	*C. doederleini; C. noumeae*
Ctenactis	*C. albitentaculata; C. crassa; C. echinata*
Halomitra	*H. clavator; H. pileus*
Heliofungia	*H. actiniformis; H. fralinae* [used to be *Fungia (Danafungia) fralinae*]
Herpolitha	*H. limax*
Lithophyllon	*L. undulatum*
Podabacia	*P. crustacea*
Polyphyllia	*P. novaehiberniae; P. talpina*
Sandalolitha	*S. dentata; S. robusta*
Zoopilus	*Z. echinatus*

Even though the COI data set has less intraspecifically variable base positions than the ITS data set, these positions do seem to induce a similar error (figs 4-5). Most monophyletic groups that are strongly supported by the analyses of the other data sets (see the genus discussions for details) have higher support values, or are only present in the COI based phylogeny reconstruction, when the intraspecific variation is excluded (fig. 5). Excluding characters with a good phylogenetic signal would logically result in lower bootstrap values and a more random final tree, which, because of the many possible trees, is very unlikely to become more similar to the morphological phylogeny only by chance.This is shown for the clades [1] *Halomitra* spp. and *Danafungia scruposa*, [2] *Heliofungia actiniformis* and *Heliofungia fralinae*, and [3] *Cycloseris* spp., *Lithophyllon undulatum*, and *Pleuractis* spp., which are supported by values of 74, 64 and 74, respectively, in figure 4, and by 82, 74 and 81 in figure 5. In one case, a clade that is supported by the other data sets, has a distinctly lower support value in figure 4 in comparison to figure 3. This concerns the clade with *Verrillofungia* spp. and *Lithophyllon undulatum*, of which the support value of 71 (fig. 4) drops to 37 when intraspecific variation is excluded (fig. 5).

Even though the support values are low, there are two clades in figure 5 that are absent in figure 4, which are strongly supported by the analysis of the morphological data set (fig. 8; Hoeksema, 1989) and/or the other molecular data sets (figs 2-3, 6-7). This concerns the clade in figure 4 where *Halomitra clavator* is more closely related to *Danafungia scruposa* than to *Halomitra pileus*, making *Halomitra* paraphyletic. In figure 5 and in all other molecular and morphological analyses *Halomitra* is monophyletic. A second case is the clade with *Herpolitha limax*, *Ctenactis albitentaculata* and *C. echinata*, which does not form a monophyletic group with the clade containing *Polyphyllia talpina* and *Ctenactis crassa* in figure 4, while it forms a monophyletic group in figure 5. Even though *C. crassa* is not even closely related to *Ctenactis albiten-*

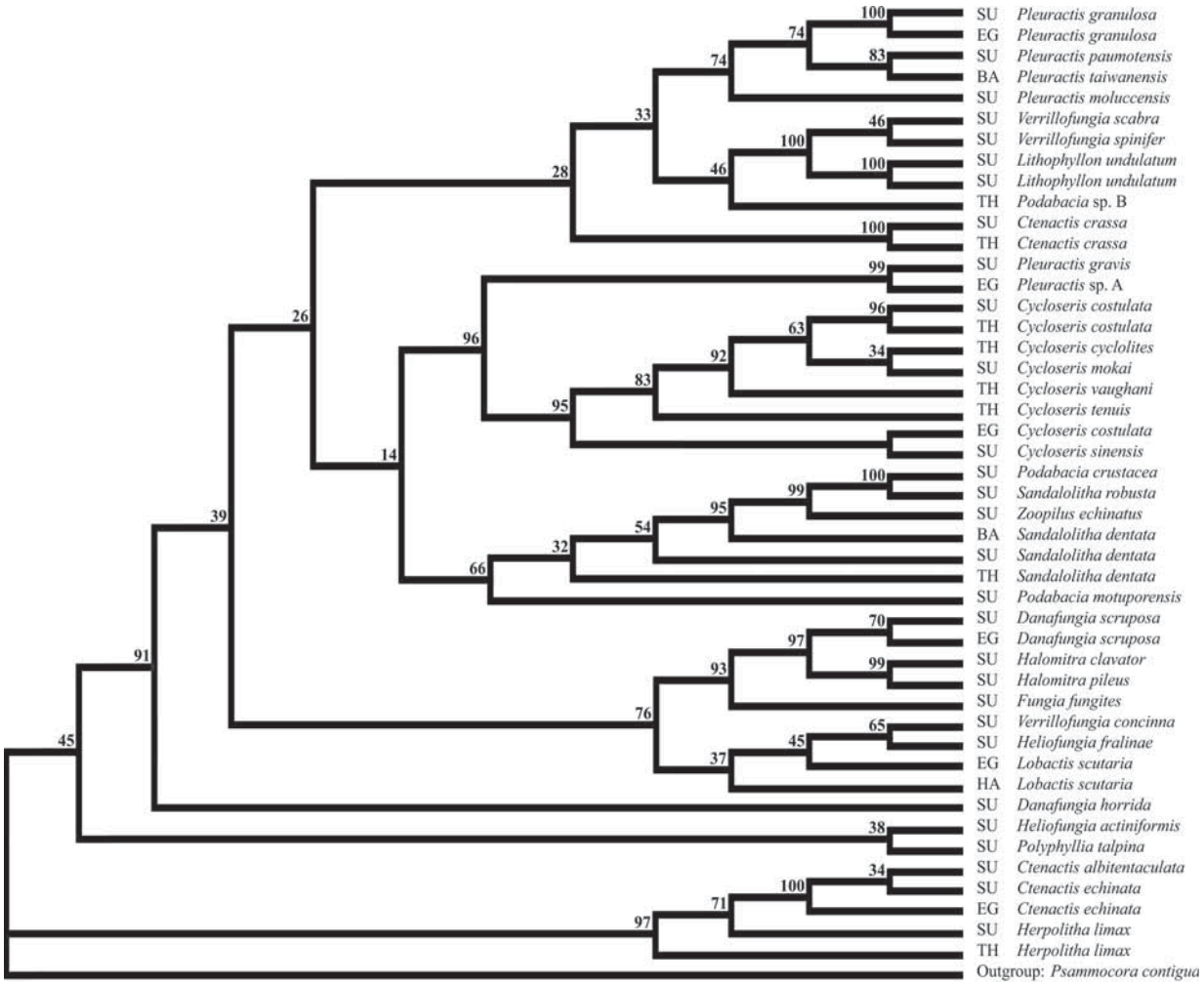

Fig. 2. Bayesian analysis of ITS data set with intraspecific variation: 50% majority rule consensus tree with compatible groupings. Locality abbreviations (fig. 1): ba, Bali, Indonesia; ha, Oahu, Hawaii; eg, Egypt (Red Sea); su, Sulawesi, Indonesia; th, Phiphi Islands, Thailand. Taxonomy as in proposed classification (table 5).

taculata and *C. echinata* in all other molecular phylogenies, it forms a sister clade (together with *Polyphyllia talpina*) of the clade with *C. albitentaculata* and *C. echinata* in figure 5. As is discussed in its genus description, *C. crassa* seems to have gone through a period with an accelerated mutation rate in comparison to the other fungiid species, resulting in its inconsistent position in the molecular phylogeny reconstructions.

Some of the above mentioned "errors" were resolved when the COI and ITS data sets were combined before analysing them (figs 6-7). One could expect this effect because autapomorphic character states, which are often present in saturated base positions, have more influence in small data sets than in large ones. In the latter case they may be neutralized while supporting incongruent results. Characters or base positions that support a similar hierarchy will than automatically gain influence.

Even though the molecular phylogeny reconstructions of the Fungiidae calculated without intraspecific variation seem to be more reliable in general, excluding this variation may also have disadvantages. It is advisable to analyse molecular data sets both with and without intraspecifically variable base positions to acquire the optimal informative contents. Furthermore the analysis of a data set that includes two markers instead of a single one, may result in a phylogeny reconstruction that has higher support

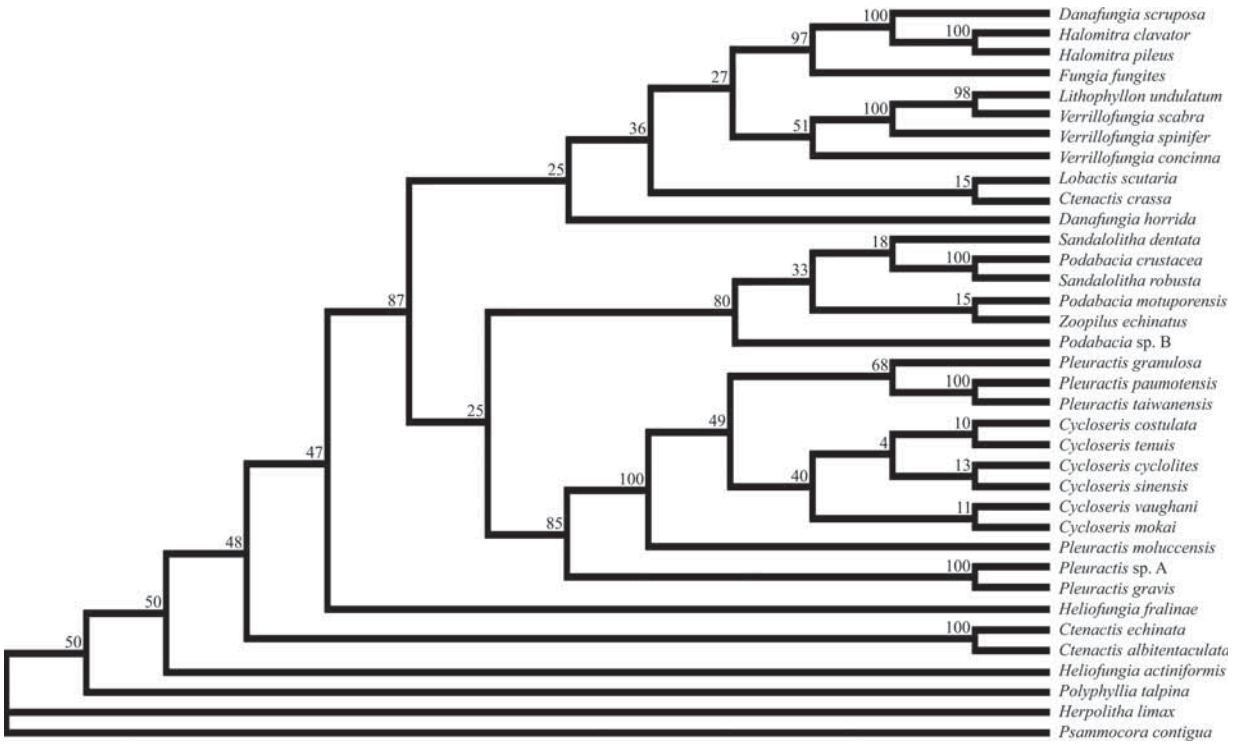

Fig. 3. Bayesian analysis of ITS data set without intraspecific variation: 50% majority rule consensus tree with compatible groupings. Taxonomy as in proposed classification (table 5).

values and has relatively more in common with a phylogeny based on morphology.

A classification of the Fungiidae

None of the taxa of the genus group that were accepted by Hoeksema (1989), i.e. *Ctenactis*, *Fungia*, *Halomitra*, *Lithophyllon*, *Podabacia*, and subgenera, i.e. *Cycloseris*, *Danafungia*, *Verrillofungia*, *Pleuractis*, comes out as monophyletic in all phylogeny reconstructions when more than one species was included in the analyses (table 1) (figs 2-7). This can be explained by a misinterpretation of morphological data, a misinterpretation of molecular data, or by the low amount of interspecific genetic variation in the studied markers. Here we discuss all the redefined (sub)genera on the basis of the newly acquired molecular data and the morphological analyses published by Hoeksema (1989). We focus on those nominal taxa that turn out as paraphyletic in one or more of the reconstructed phylogenies. The taxonomical revisions that are necessary to make the taxa in the Fungiidae monophyletic are summarized in table 5. Each of these revisions is discussed in the following paragraphs.

Genus *Cantharellus* Hoeksema and Best, 1984

Type species: *Cantharellus noumeae* Hoeksema and Best, 1984.

Molecular analysis: No specimens were available for DNA-analyses.

Genus description: The description of Hoeksema (1989: 209) remains sufficient.

Genus *Ctenactis* Verrill, 1864

Type species (by original designation): *Madrepora echinata* Pallas, 1766.

Molecular analysis: In all molecular phylogeny reconstructions (figs 2-7) *Ctenactis echinata* and *C. albi-*

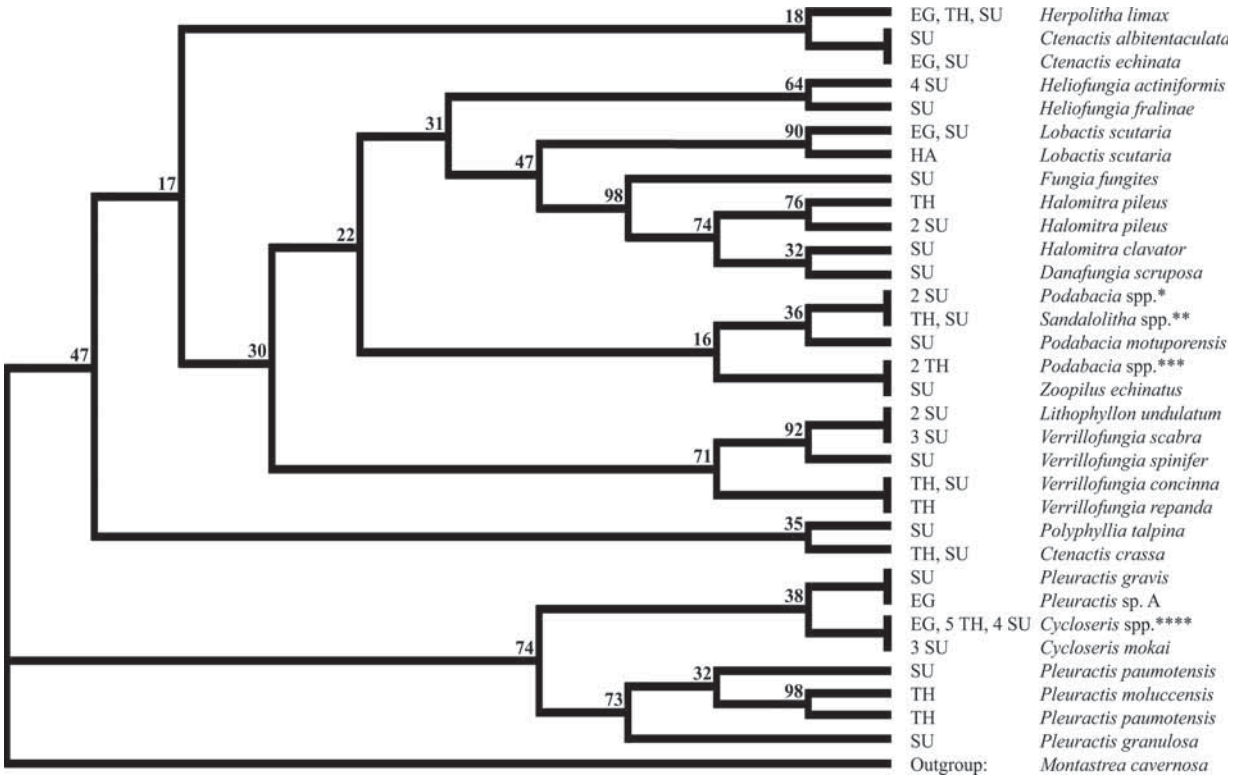

Fig. 4. Bayesian analysis of COI data set with intraspecific variation: 50% majority rule consensus tree with compatible groupings. Locality abbreviations (fig. 1): ba, Bali, Indonesia; ha, Oahu, Hawaii; eg, Egypt (Red Sea); su, Sulawesi, Indonesia; th, Phiphi Islands, Thailand. Taxonomy as in proposed classification (table 5).
Numbers with localities refer to the number of identical sequences.
* *Podabacia crustacea* (su), *P. motuporensis* (su)
** *Sandalolitha dentata* (th & su), *S. robusta* (su)
*** *Podabacia* sp. A (th), *P.* sp. B (th)
**** *Fungia* (*Cycloseris*) *costulata* (eg, th), *F.* (*C.*) *cyclolites* (th), *F.* (*C.*) *fragilis* (th, su), *F.* (*C.*) *sinensis* (th), *F.* (*C.*) *tenuis* (th, su), *F.* (*C.*) *vaughani* (th, su)

tentaculata cluster together with strong support values. In no case these two species form a monophyletic group with *Ctenactis crassa*. These results do not necessarily indicate that *Ctenactis* is paraphyletic however. The position of *C. crassa* in the molecular phylogenies is much less consistent and poorly supported than the position of any of the other fungiid species that were included. These inconsistencies in the results of the analyses of the COI and ITS data sets may be related to the fact that much more mutations have occurred in the *C. crassa* clade than in any of the other clades (the data and alignments that illustrate these high mutation numbers can be obtained from the authors). The average mutation rate in the *C. crassa* clade is much higher than in all other clades and may have caused the inconsistencies. Because the DNA of the studied markers of *C. crassa* has evolved distinctly different from the DNA in the other fungiid species, the position of *C. crassa* in these phylogenies is unreliable. Therefore and on the basis of the morphology of the three species (Hoeksema, 1989: 154-166) we here conclude that the nominal genus *Ctenactis* refers to a monophyletic group. Possibly the *C. crassa* population has gone through one or more bottleneck events, which could explain the relatively high number of mutations in the COI and ITS regions.

Except for the sequences of *Ctenactis crassa*, the sequences of the genera *Ctenactis*, *Herpolitha* and *Polyphyllia* 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

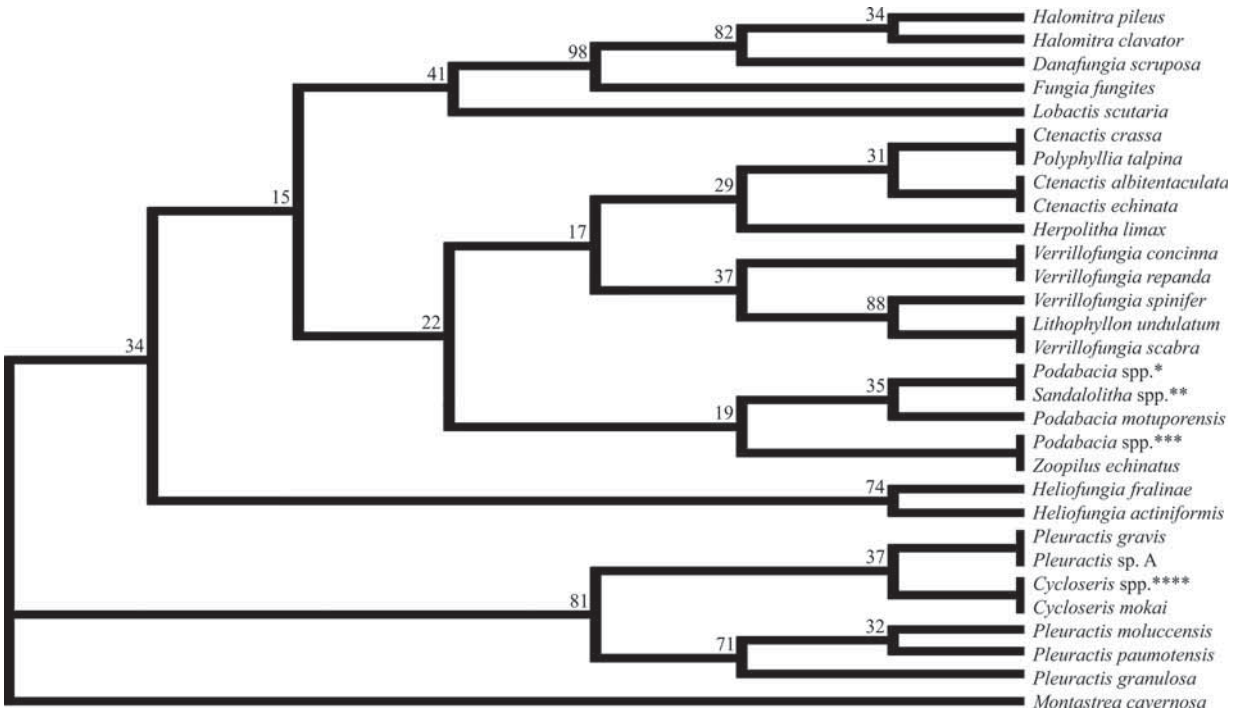

Fig. 5. Bayesian analysis of COI data set without intraspecific variation: 50% majority rule consensus tree with compatible groupings. Taxonomy as in proposed classification (table 5).
* *Podabacia crustacea, P. motuporensis*
** *Sandalolitha dentata, S. robusta*
*** *Podabacia* sp. A, *P.* sp. B
**** *Fungia* (*Cycloseris*) *costulata, F.* (*C.*) *cyclolites, F.* (*C.*) *fragilis, F.* (*C.*) *sinensis, F.* (*C.*) *tenuis, F.* (*C.*) *vaughani*

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) by Hoeksema (1989), with *Herpolitha* and *Polyphyllia* forming a clade to which *Ctenactis* is only very distantly related.

Genus description: The description of Hoeksema (1989: 153-154) remains sufficient.

Genus *Fungia* Lamarck, 1801

Type species: *Fungia fungites* (Linnaeus, 1758)

Molecular analysis: In all molecular phylogenies (figs 2-7) *Fungia fungites* clusters as a sister taxon of a clade with *Halomitra pileus, H. clavator* and *Fungia* (*Danafungia*) *scruposa*, making *Fungia* paraphyletic.

The molecular results also consistently imply that *Fungia* is more closely related to the genera *Lithophyllon, Podabacia, Sandalolitha* and *Zoopilus*, than to its alleged subgenera *Wellsofungia, Pleuractis* and *Cycloseris*, making *Fungia* polyphyletic. These molecular results are fully supported by morphology (fig. 8; Hoeksema, 1989). To make *Fungia* monophyletic we suggest that its so-called subgenera are upgraded to the genus level.

Genus description: The description of this genus is similar to that of its type species (see Hoeksema, 1989: 116).

Genus *Cycloseris* Milne Edwards and Haime, 1849 (= upgraded subgenus; see the molecular analysis of the "Genus *Fungia*")

Type species: *Fungia cyclolites* Lamarck, 1815.

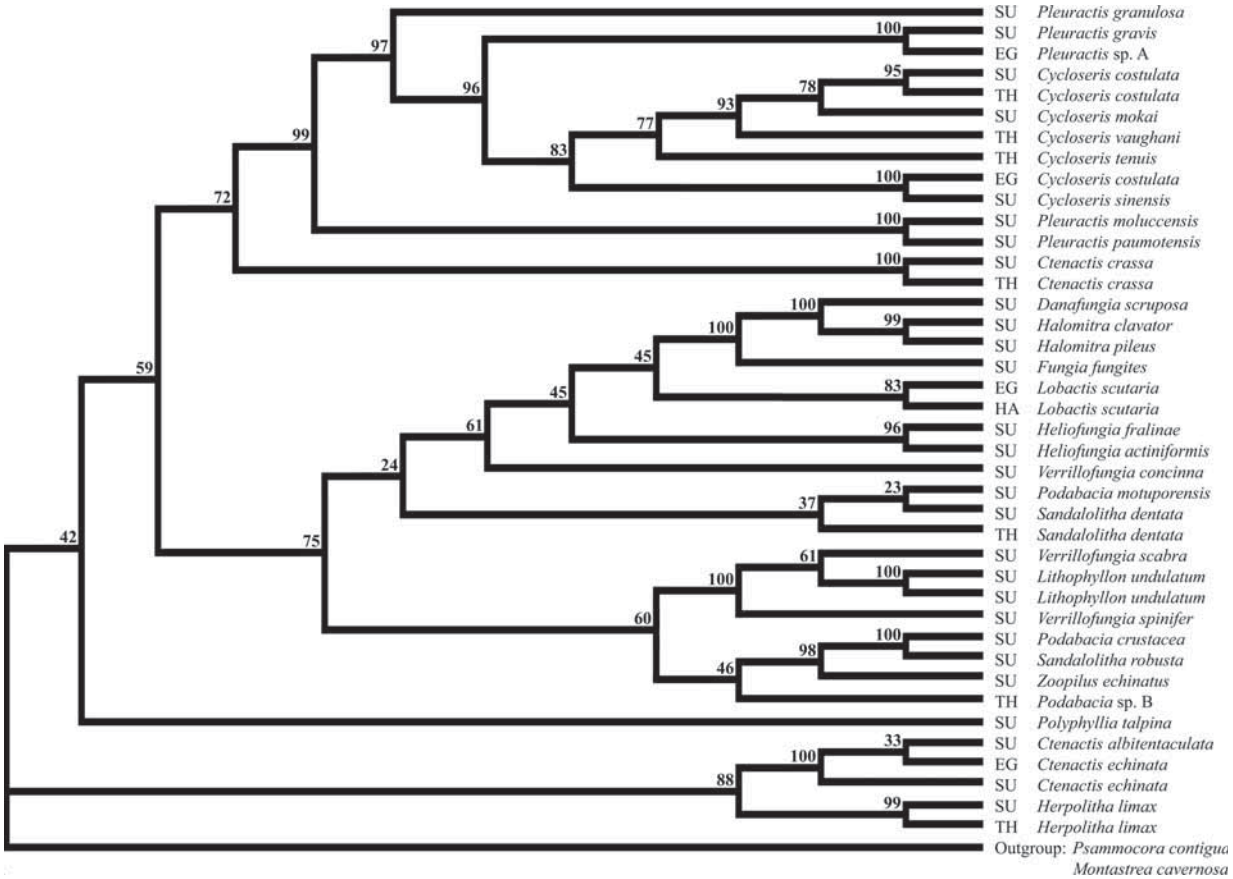

Fig. 6. Bayesian analysis of the combined ITS & COI data set with intraspecific variation: 50% majority rule consensus tree with compatible groupings. Locality abbreviations (fig. 1): ba, Bali, Indonesia; ha, Oahu, Hawaii; eg, Egypt (Red Sea); su, Sulawesi, Indonesia; th, Phiphi Islands, Thailand. Taxonomy as in proposed classification (table 5).

Molecular analysis: In all molecular phylogenies (figs 2-7) the *Cycloseris* sequences cluster together with the sequences of *Lithophyllon mokai*. Analyses based on the ITS and the combined data sets of COI and ITS (figs 2-3, 6-7) indicate that *L. mokai* is not a basal lineage in the *Cycloseris* clade. It may even be the sister species of the type species of *Cycloseris*, i.e. *Fungia* (*Cycloseris*) *cyclolithes*. We therefore conclude that *Lithophyllon mokai* Hoeksema, 1989 should be named *Cycloseris mokai* (Hoeksema, 1989).

Specimens of the species *Cycloseris mokai* have a stronger stem than the other species in the genus and therefore do not break loose from the substrate. This may have resulted in encrusting specimens which are poly-stomatous, instead of free-living and monostomatous as in all other *Cycloseris* species. This hypothesis is supported by the morphology of *Lithophyllon undulatum*, another fungiid species with encrusting, polystomatous specimens, similar to those in *Cycloseris mokai*. The sister species of *L. undulatum* (figs 2-7), viz. *Verrillofungia* species, also have free-living, monostomatous specimens. This is a classic example of convergent evolution. In both cases, becoming sessile may have caused the corals to become encrusting and polystomatous. Hoeksema (1989: 258) already predicted for Fungiidae on the basis of morphology, that reversals like species that loose their ability to detach themselves from the substrate, may be difficult to recognize because they represent a multistate character (i.e. a series of successive character states) in which the final state resembles the initial one. This seems to have happened independently in the species "*Lithophyllon*" *mokai* and *Lithophyllon undulatum*. The

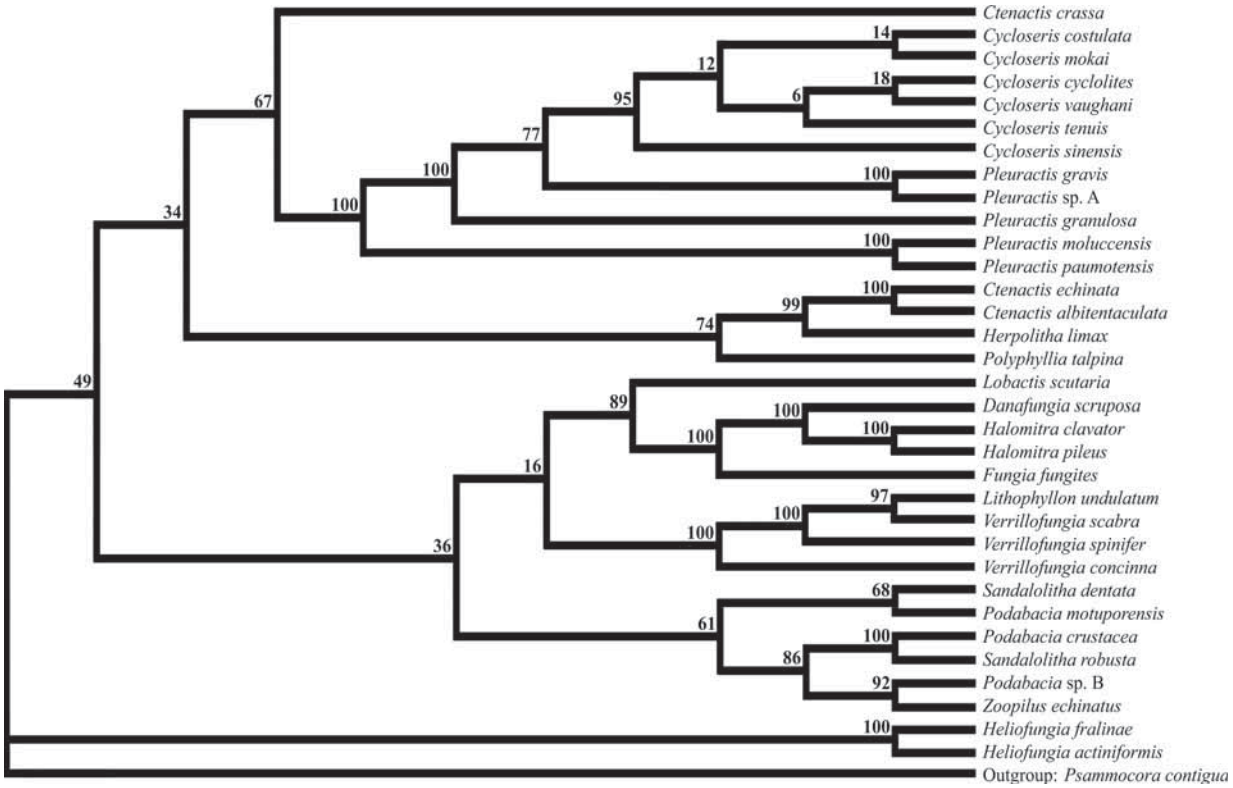

Fig. 7. Bayesian analysis of the combined ITS & COI data set without intraspecific variation: 50% majority rule consensus tree with compatible groupings. Taxonomy as in proposed classification (table 5).

resulting autapomorphies are inappropriate for phylogeny reconstruction, which may at least partly explain the conflicting views that were published by Wells (1966: fig. 3), Cairns (1984: fig. 3) and Hoeksema (1989)(fig. 8) when constructing a Fungiidae phylogeny based on morphology. See also the remarks on the molecular analyses of *Lithophyllon* and *Verrillofungia*.

Genus description: The following should be added to the description of *Cycloseris* by Hoeksema (1989: 30): One species, i.e. *Cycloseris mokai* (Hoeksema, 1989), differs from the other *Cycloseris* species in being encrusting, polystomatous, and irregularly shaped instead of free-living, monostomatous and circular to oval.

Genus *Danafungia* Wells, 1966
(= upgraded subgenus; see the molecular analysis of the "Genus *Fungia*")

Type species: *Fungia danai* Milne Edwards and Haime, 1851, sensu Wells, 1966 [= *Fungia scruposa* Klunzinger, 1879].

Molecular analysis: The phylogenies based on the COI data sets support that *Heliofungia actiniformis* and *Danafungia fralinae* are sister species with values of 64 and 74 respectively in figures 4 and 5. Even though the ITS data sets do not seem to support this result when analysed separately from the COI data sets (figs 1-2), the support values for this relationship become very high when the COI and ITS data sets are combined, i.e. 96 and 100 respectively in figures 6 and 7. All molecular phylogenies (figs 2-7) strongly support that *Danafungia fralinae* does not form a monophyletic group with the type species of *Danafungia*, *D. scruposa*. We therefore conclude that *Danafungia fralinae* Nemenzo, 1955, should be named *Heliofungia fralinae* (Nemenzo, 1955). In the analyses of the ITS data sets *Danafungia horrida* does not cluster together with the

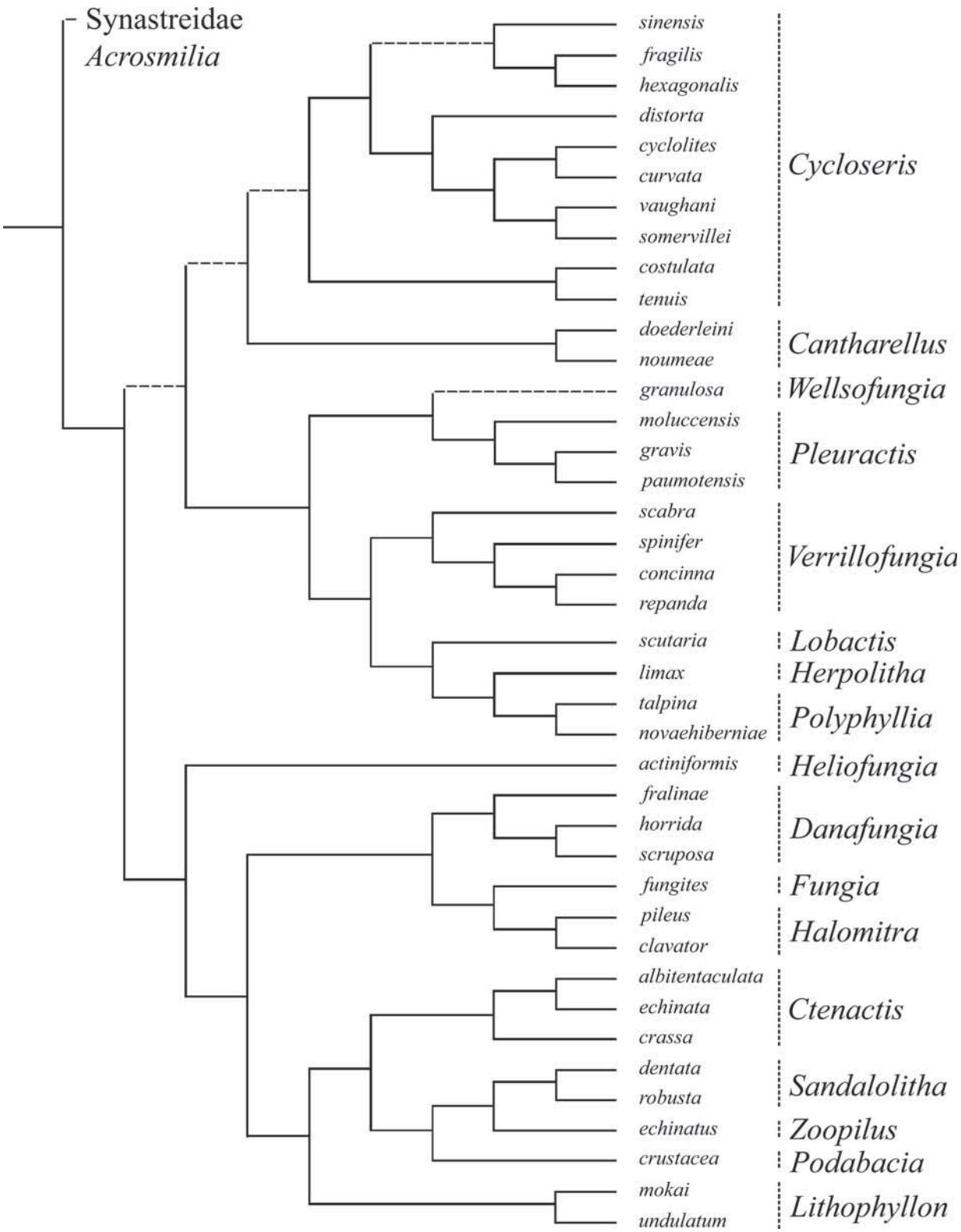

Fig. 8. A cladogram of the Fungiidae based on morphological characters; after Hoeksema (1989: 256).

type species *D. scruposa* (figs 2-3). This result is not strongly supported however, because it is based on a single ITS sequence of *D. horrida* that clusters at two totally different places in the two reconstructed phylogenies (figs 2-3). Therefore and on the basis of the morphology of the two species (Hoeksema 1989: 101-115), we conclude that *D. horrida* should remain in the nominal genus *Danafungia*.

Genus description: The description of Hoeksema (1989: 96-97) remains sufficient with the adjustment that two instead of three species are recognized within this genus.

Genus *Lobactis* Verrill, 1864
(= upgraded subgenus; see the molecular analysis of the "Genus *Fungia*")

Type species: *Fungia dentigera* Leuckart, 1841 (= *Fungia scutaria* Lamarck, 1801).

Molecular analysis: In most of the phylogenies (figs 3-7) and especially in the analyses of the combined COI + ITS data sets (figs 6-7), the sequences of the type and only species in the genus, i.e. *Lobactis scutaria* (Lamarck, 1801), cluster with low support at the basis of a clade with the genera *Danafungia*, *Fungia* and *Heliofungia*. In the phylogeny based on morphology by Hoeksema (1989) (fig. 8) it is situated basally from *Herpolitha* and *Polyphyllia*, however. This difference can be explained by parallel or convergent evolution by which the oval coral form that placed *Lobactis* basally to a clade with *Herpolitha* and *Polyphyllia*, has evolved twice.

Genus description: The description of Hoeksema (1989: 129) remains sufficient.

Genus *Pleuractis* Verrill, 1864
(= upgraded subgenus; see the molecular analysis of the "Genus *Fungia*")

Type species: *Fungia scutaria* Lamarck, 1801, sensu Verrill, 1864 [= *Fungia paumotensis* Stutchbury, 1833].

Molecular analysis: In all phylogeny reconstructions (figs 2-7) the *Pleuractis* sequences cluster together with the sequences of *Wellsofungia granulosa*, the type and only species of *Wellsofungia*. The analyses furthermore strongly indicate that *Wellsofungia granulosa* is more closely related to *Pleuractis moluccensis* and *P. paumotensis*, than the latter two species are related to *P. gravis* and *P.* spec. A. Hoeksema (1989: 255), when describing the subgenus *Wellsofungia* on the basis of morphology, stated: "*Wellsofungia* is separated from *Pleuractis* because it does not contain species that show an oval corallum outline (apomorph character state 28). Phylogenetically such groups of which the monophyly cannot be demonstrated by the presence of synapomorphies are of a reduced interest". Based on this statement, the morphology of the species, and the molecular data presented here, we conclude that *Wellsofungia granulosa* should be called *Pleuractis granulosa*. The nominal genus *Wellsofungia* has hereby become a synonym of *Pleuractis*.

A clade with *Cycloseris* sequences clusters within the clade with the *Pleuractis* sequences in all molecular phylogenies (figs 2-7) indicating that the latter genus may be paraphyletic. Some of these reconstructions support that *P. moluccensis*, *P. paumotensis* and *P. granulosa* are more closely related to the *Cycloseris* species than *P. gravis* and *P.* spec. A (figs 3, 6-7), while other data (figs 2, 4-5) indicate that *P. gravis* and *P.* spec. A are more closely related to *Cycloseris* spp. Because of these inconsistent results it cannot be said which of the two hypotheses is more likely and therefore it also remains uncertain whether *Pleuractis* is paraphyletic in the first place. Based on these inconsistent results and the morphological analyses in Hoeksema (1989), we keep on considering *Pleuractis* to be monophyletic.

Genus description: Adult animals are free-living and monostomatous. Their outline varies from oval to elongate. The corallum wall is perforated in adults. The blunt costal spines are either simple and granular or fused and laterally compressed. The septal dentations vary from fine and granular to coarse and angular. The septa are usually solid, but in some species they are perforated. The granulations on the septal sides are either irregularly arranged or they form rows or ridges parallel or perpendicular to the septal margins.

Genus *Verrillofungia* Wells, 1966
(= upgraded subgenus; see the molecular analysis of the "Genus *Fungia*")

Type species: *Fungia repanda* Dana, 1846.

Molecular analysis: In the paragraph "Excluding intraspecific variation" (p. 44) the *Verrillofungia concinna* sequence 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) appears to be incorrect because it differs strongly from its position in the other phylogenies (figs 3-5, 7). In all molecular phylogenies (figs 2-7) *Verrillofungia* sequences cluster with the sequences of *Lithophyllon undulatum*, the type species of the genus *Lithophyllon*. All analyses furthermore strongly indicate that *L. undulatum* is not on a basal lineage in the *Verrillofungia* clade. Based on these results, and the fact that *Lithophyllon* Rehberg, 1892, has priority over *Verrillofungia* Wells, 1966, one may suggest to consider *Verrillofungia* simply a junior synonym of *Lithophyllon*. This would cause much confusion however, because the generic name *Lithophyllon* is generally known as referring to species, which are encrusting and polystomatous, and all *Verrillofungia* species are free-living and mono-stomatous. In this exceptional case we therefore accept a paraphyletic genus, *Verrillofungia*, with species of which the individuals are free-living and monostomatous. See also the remarks on the molecular analysis of *Cycloseris* and *Lithophyllon*.

Genus *Halomitra* Dana, 1846

Type species: *Fungia pileus* Lamarck, 1801 [= *Halomitra pileus* (Linnaeus, 1758)].

Molecular analysis: In five out of the six molecular phylogenies (figs 2-3, 5-7), the *Halomitra* species *H. clavator* and *H. pileus* form a monophyletic group. Even though the COI data set with intraspecifically variable base positions indicates that *Halomitra clavator* clusters with *Danafungia scruposa* (fig. 4), the support value of this clade is very low, i.e. 32. In contrast, the support values for the *H. clavator* and *H. pileus* clades in the phylogenies based on the ITS and the combined data sets are very high, i.e. 99, 100, 99 and 100, respectively (figs 2-3, 6-7). Therefore we conclude that *Halomitra* is a monophyletic taxon.

Genus description: The description of Hoeksema (1989: 199-200) remains sufficient.

Genus *Heliofungia* Wells, 1966

Type species: *Fungia actiniformis* Quoy and Gaimard, 1833.

Molecular analysis: See the remarks on the molecular analysis of *Danafungia*.

Genus description: Adult animals are free-living and monostomatous. Their outline varies from circular to slightly oval. The corallum wall is solid and granulated. The polyps are fleshy, with extended tentacles that are relatively long, i.e. up to at least 2 cm.

Genus *Herpolitha* Eschcholtz, 1825

Type species: *Herpolitha limacina* (Lamarck) (= *Madrepora limax* Esper, 1797). Designated by Milne Edwards and Haime, 1850.

Molecular analysis: See the remarks on the molecular analysis of *Ctenactis*.

Genus description: The description of Hoeksema (1989: 167-168) remains sufficient.

Genus *Lithophyllon* Rehberg, 1892

Type species: *Lithophyllon undulatum* Rehberg, 1892.

Molecular analysis: See the remarks on the molecular analysis of *Cycloseris* and *Verrillofungia*.

Genus description: The description of this genus is similar to that of this type species (see Hoeksema 1989: 216).

Genus *Podabacia* Milne Edwards and Haime, 1849

Type species: *Agaricia cyathoides* Valenciennes, ms., Milne Edwards and Haime, 1849 [= *Podabacia crustacean* (Pallas, 1766)].

Molecular analysis: In the phylogeny based on morphology (fig. 8) by Hoeksema (1989), and in all molecular phylogenies, the sequences of *Podabacia*, *Sandalolitha* and *Zoopilus* cluster as a monophyletic group or at least close to each other. We can only conclude on the basis of morphology that these three nominal genera are separate entities. The individual *Sandalolitha*, *Podabacia* and *Zoopilus* sequences vary too little to distinguish these taxa. The support values within the clades are generally low and, whenever they are higher, give conflicting results in the various analyses.

Genus description: The description of Hoeksema (1989: 226) remains sufficient.

Genus *Polyphyllia* Blainville, 1830

Type species: *Fungia talpa* Lamarck, 1815 [=*Polyphyllia talpina* (Lamarck, 1815)].

Molecular analysis: See the remarks on the molecular analysis of *Ctenactis* and *Podabacia*.

Genus description: The description of Hoeksema (1989: 176) remains sufficient.

Genus *Sandalolitha* Quelch, 1884

Type species: *Sandalolitha dentata* Quelch, 1884.

Molecular analysis: See the discussion on the molecular results of *Podabacia*.

Genus description: The description of Hoeksema (1989: 186) remains sufficient.

Genus *Zoopilus* Dana, 1846

Type species: *Zoopilus echinatus* Dana, 1846.

Molecular analysis: See the discussion on the molecular results of *Podabacia*.

Genus description: The description of Hoeksema (1989: 195) remains sufficient.

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