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# Chapter 10

## Adaptive divergence in coral-dwelling gall crabs: signature of host driven evolution

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## Abstract

Intimate interactions between host organisms and their symbionts can, on a long time scale, lead to impact on the evolution of the partner. Within the theoretical context of host-parasite evolution, coevolution is only considered appropriate for a given host-symbiont assemblage if the hosts and their symbionts show similar patterns of phylogenetic differentiation. Many studies on coevolutionary relationships focus on terrestrial organisms and involve vertebrates as hosts. The present research on the association between stony corals (Scleractinia) and gall crabs (Cryptochiridae) concerns an invertebrate-invertebrate association in the marine realm. For the Cryptochiridae the phylogenetic relationships within the family were reconstructed based on 16S, COI and H3 markers, whereas information on the phylogenetic relationships within the Scleractinia was already largely available in the literature. The congruence between both phylogeny reconstructions was tested using the programme Jane 4.0, which tests for the occurrence of coevolutionary events. The phylogram of the Cryptochiridae shows three large clades and multiple paraphyletic genera. Further taxonomic work is needed to find out whether some genera are monophyletic. The test for congruency resulted in 20 cospeciation events, three duplication events, 14 duplication - host switching events, eight losses and 10 failures between the gall crab phylogeny and coral phylogeny. The statistics show that coevolution is the most likely scenario for the observed congruence, as the outcome is significantly higher than it would have been as expected by chance alone. The observed events should most probably be ascribed to sequential evolution, which indicates that the phylogeny of the Cryptochiridae has been directed by the evolution of the Scleractinia.

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## Introduction

Evolutionary diversification among close associations between heterospecific species (symbiosis), as an alternative to direct competition between associated species for the same host, is an important strategy for survival in biotic communities. Symbioses include a broad category of heterospecific associations embracing various degrees of adaptive interactions that involve intimate physiological and ecological interactions (Castro, 1988). If interactions between species are close enough, the organisms involved may have speciated synchronously, so a reconstruction of their evolutionary histories would show congruent events of speciation (Paterson and Banks, 2001). Nonetheless, the impact of these interactions on the evolution of each partner depends on the time-scale considered. Only macroevolutionary patterns will be considered here, i.e., the long-term evolutionary dynamics of speciation following host shifts. These are differentiated from studies at a shorter time scale (e.g. changes in allele frequencies over successive generations, Red Queen driven processes) (Desdevises, 2007; de Vienne *et al.*, 2013).

Many studies on coevolutionary relationships focus on mammal, bird and (to a lesser extent) fish hosts and their parasites but cophylogenetic analyses have also been carried out in a diverse range of other systems, including non-symbiotic ones such as plants – pollinator and vertebrate – virus systems (for overviews see Lanterbecq *et al.*, 2010; Duchene *et al.*, 2013). A well-known symbiotic coevolution example is that of gophers and lice (Hafner and Nadler, 1988; Hafner *et al.*, 1994), but studies of intimate evolutionary associations between hosts and parasites started with avian hosts and their parasites (Hoberg *et al.*, 1997).

Parasite speciation and specificity is based on their host group, hence the phylogenies of parasites are considered to have great predictive value in elucidating the associated host phylogeny (Eichler, 1942). A series of parasitological rules were developed of which Fahernholz's rule – *parasite phylogeny mirrors host phylogeny* – is the most well-known. Indeed, phylogenetic studies of interacting organisms often reveal congruence between the phylogenies of the interacting taxa. Congruence between host and parasite phylogenies is seen as evidence for coevolution (e.g. Hafner and Nadler, 1988; Hafner *et al.*, 1994; Patterson and Banks, 2001). Within a theoretical context of host-parasite evolution, coevolution is only considered appropriate for a given host-parasite assemblage if the hosts and their parasites show identical patterns of phylogenetic differentiation. In contrast, identical patterns in host organisms and their parasites are only rarely observed and certain levels of discordance between host and parasite phylogenies are considered the norm (Hafner and Nadler, 1990). Moreover, parasites can vary in their host specificity. Groups of parasites occupy a spectrum from highly host-specific to host generalist. There is a general tendency among parasites that infect more than one host species to infect hosts that are phylogenetically closely related - that is, usually species within the same genus or family – which appears to be an important factor in speciation (Norton and Carpenter, 1998).

*Coevolution* is the universally accepted term for the process involving two or more lineages that reciprocally influence each other's evolution. This is, however, a general term that encompasses strict coevolution and sequential coevolution. Strict coevolution implies that two separate taxa mutually influence the evolution of the other, the two taxa tending to i) change together (coadaptation), or ii) speciate together (cospeciation) (Ridley, 1996). It has been assumed that coadaptation favours cospeciation, but it appears that the critical factor may be the rate at which the symbiont or parasite encounters potential new host species (Ronquist, 1997). Sequential evolution is a particular case of coevolution where the changes (morphological, physiological or behavioural) and the phylogeny of the symbionts are influenced by the host evolution, but it is not reciprocal (Ridley, 1996).

Documentation of widespread coevolution in a host-parasite assemblages requires statistical evidence that the congruence observed between the host and parasite phylogenies exceeds that expected by chance (Huelsenbeck *et al.*, 1997; Hafner and Nadler, 1990). Two kinds of evidence are necessary to document coevolution in a host-parasite assemblage: evidence that the host and parasite phylogenies are derived independently and statistical evidence that the topological similarity of the host and parasite trees exceeds chance expectations (Hafner and Nadler, 1990). By comparing the phylogenies of host species and their associates, it is possible to detect if a statistically significant cophylogenetic signal is present and estimate the role played by the different historical events (Paterson and Gray, 1997). Analyses of coevolutionary relationships, however, are obstructed by the complex interplay of coevolutionary events. Four types of basic coevolutionary events were defined, here applied to parasitic relationships (Page, 1994; Page and Charleston, 1998): cospeciation (concomitant host and parasite speciation), host switching (colonization of a new host by a parasite), duplication (parasite speciation on a single host lineage), and sorting event (disappearance of a parasite lineage from a host). Some authors define more types of events (e.g. Paterson and Banks, 2001; Johnson *et al.*, 2003), but they broadly fall into the four basic categories described above (Desdevises, 2007). These coevolutionary events may all produce incongruence between host and parasite phylogenies (Patterson and Banks, 2001). Speciation of the symbiont can occur independently of host speciation, often through host shifts as the symbiont comes to occupy a new host environment in isolation from the ancestral lineage (de Vienne *et al.*, 2013).

Only few taxa received much of the attention in studies on cophylogenies. Marine models have not been extensively studied, especially not models in which marine invertebrates are involved, yet their difference compared to more known terrestrial systems may shed light on processes concerning the generation of cophylogenetic patterns (Desdevises, 2007; Duchene *et al.*, 2013). This chapter studies the relationship between gall crabs (Cryptochiridae) and their stony coral hosts (Scleractinia). Cryptochiridae is a family of coral-inhabiting crabs occurring on reefs worldwide. These crabs depend on their hosts for food and shelter (Kropp, 1986, 1990a). The observed host-specificity patterns of gall crabs (e.g. Fize and Serène, 1957; van der Meij, 2015a) triggers questions about the nature of the association. The relatively small size and worldwide occurrence of the Cryptochiridae (approx. 50 described species – Davie, 2014) allows to study coevolutionary patterns between a monophyletic family (van der Meij and Schubart, 2014) and their scleractinian hosts across the whole family, as well as between oceanic basins. Cophylogenetic approaches in coevolution and biogeography studies ask for a whole new set of analytical methods (Ronquist, 1997). The combination of a high species diversity in certain crab genera, biogeographic patterns, host specificity, and (presumably) millions of years of association, prompts many questions about the underlying mechanisms causing diversification. In order to study these mechanisms the following questions need to be answered first. 1. Does the phylogeny of the Cryptochiridae mirror the phylogeny of the corals (Fahernholz's rule) or are there incongruences between the two? 2. Is there coevolution (in the broad sense) between the crabs and their hosts, and if so, i) which type of coevolution can be distinguished, and ii) which coevolutionary events are expected to have occurred? To study these questions the phylogenetic relationships within the Cryptochiridae are reconstructed and compared with a phylogeny reconstruction of the Scleractinia.

## Material and methods

The material used in this study has been collected from 2007 to 2013 in Indonesia, Malaysia and the Saudi Arabian part of the Red Sea in the Indo-Pacific and in Curaçao, Dutch Caribbean, in the Atlantic. Corals from many different families were searched for galls and pits, and subsequently split with hammer and chisel. The gall crabs were preserved in 80% ethanol, after being photographed with a digital SLR camera equipped with a 50 mm macro lens. The crab specimens are deposited in the collections of Naturalis Biodiversity Center in Leiden, The Netherlands (formerly Rijksmuseum van Natuurlijke Historie), collection-coded as RMNH.Crus.D).

### *Molecular analyses*

For the reconstruction of relationships within the Cryptochiridae, 38 shallow-water species belonging to 17 genera were selected. The type species of each genus was included. Material from the Atlantic (ATL) and Indo-Pacific (IP) was used. Unfortunately, deep sea gall crab species were not available for molecular study. The *Hemigrapsus pennicilatus* (Varunidae) was selected as an outgroup (van der Meij and Schubart, 2014).

Gall crabs were sequenced for three markers (16S, COI mtDNA, H3 nDNA). DNA extraction was performed following the protocols specified in Van der Meij (2015a). For each marker, sequences were trimmed to be of equal length and aligned in Guidance using the Prank algorithm (Penn *et al.*, 2010a, b), resulting in scores of 0.98 for 16S (minimally adjusted by eye in BioEdit (Hall, 1999)), 0.99 for COI, and 1.0 for H3. The 16S dataset contained 383 constant, 169 parsimony-informative and 33 uninformative characters. The COI dataset contained 396 constant, 238 parsimony informative and nine uninformative variable characters. The H3 dataset contained 203 constant, 75 parsimony-informative and eight uninformative characters.

The appropriate model of evolution was determined using jModeltest 2.1.3 (Darriba *et al.*, 2012) using the Akaike Information Criterion (AIC). For COI this resulted in TrN+I+G (Tamura and Nei, 1993), for 16S in TIM2+I+G (Posada, 2008), and for H3 in GTR+I+G (Tavaré, 1986). Sequences were concatenated in Sequence Matrix (Vaidya *et al.*, 2011), converted to nexus and partitioned as follows: 16S bp 1-585, COI bp 586-1228, H3 bp 1229-1514.

### *Phylogeny reconstructions*

Bayesian inferences were estimated in MrBayes (Ronquist and Huelsenbeck, 2003). The programme was run for 5,000,000 generations using the most complex GTR+I+G model. The analysis stabilized at 0.004865, burnin was set to 25%. Maximum Likelihood (ML) analyses were carried out in Garli 2.0 (Zwickl, 2006) on the partitioned dataset, with the evolutionary models as specified earlier. Two search replicates were carried out with 250 bootstrap replicates. The bootstrap consensus tree was visualised with the SumTrees 3.3.1 package of the DendroPy 3.12.0 package in the Phyton library (Sukumaran and Holder 2010). Scleractinian phylogeny, for the coevolutionary analyses, was reconstructed based on literature. The main groupings were based on Fukami *et al.* (2008), supplemented by data from Budd *et al.* (2012) and Huang *et al.* (2014).

### *Coevolutionary analyses*

The congruence between coral and gall crab phylogenies was tested by using the programme Jane 4.0 (Conow *et al.*, 2010). The programme is based on an event-based model which considers cospeciation as the most parsimonious explanation for congruence between host and parasite trees. Coevolutionary relationships are obstructed by the complex interplay of cospeciation, duplication (intrahost speciation), host switching, sorting (extinction) and inertia (lack of parasite speciation). For definitions see Paterson and Banks (2001) and Conow *et al.* (2010). The evolutionary events are used to superimpose phylogeny reconstruction of the associated taxon on that of the host taxon. Jane 4.0 assigns a cost to each evolutionary event, after which it seeks to find mappings minimizing the total cost. The default costs settings of Jane were used, as follows:

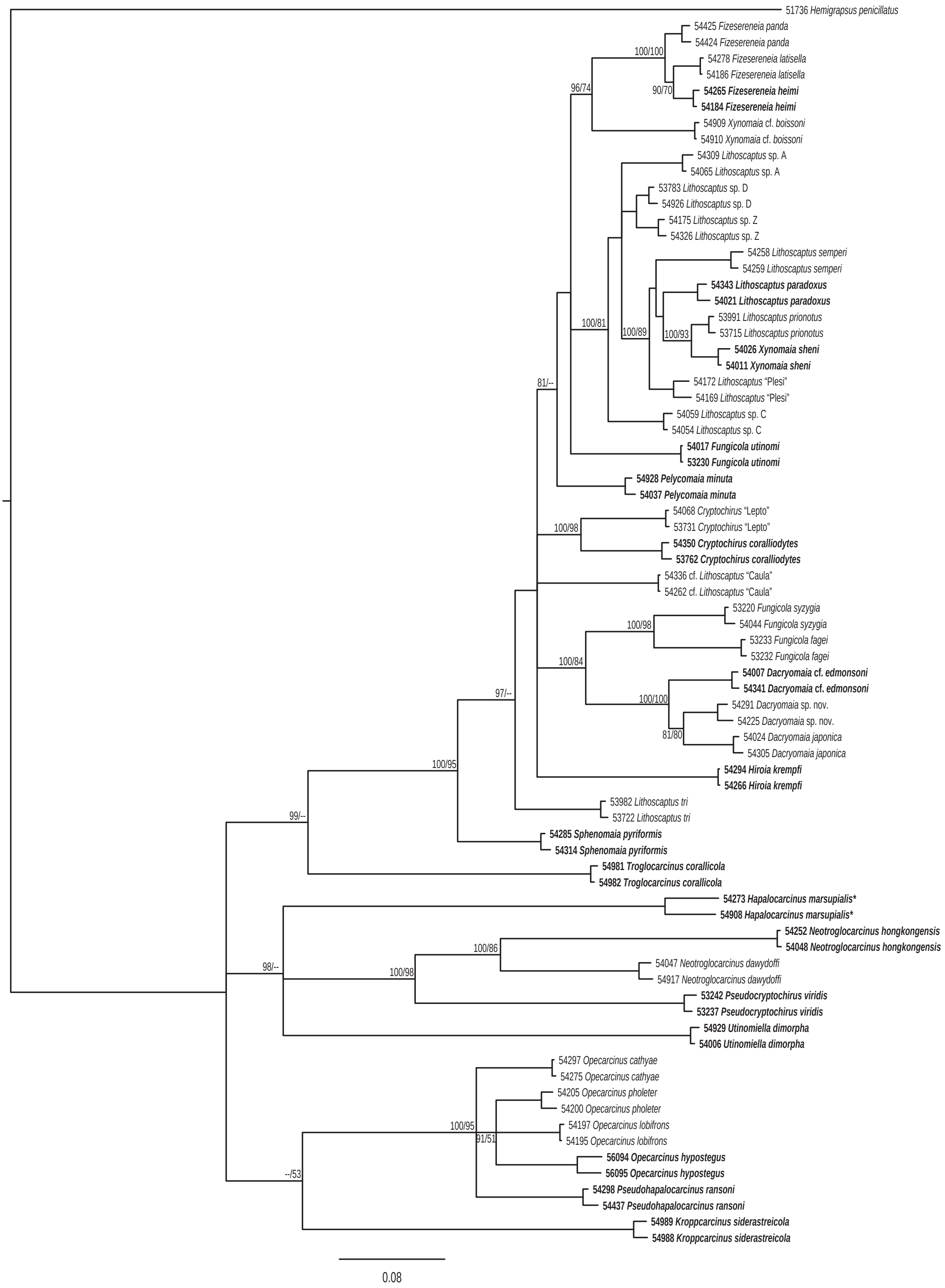


**Fig. 1.** Bayesian inference (BI) tree based on the concatenated dataset of 16S, COI and H3, with the varunid *Hemigrapsus penicillatus* as outgroup. Maximum likelihood (ML) values resulting from the Garli run are plotted on the BI tree. BI values <80 and ML values < 50 are not provided. Type species are printed in bold, * represents a species complex.

cospeciation (0), duplication (1), duplication – host switching (2), loss (1) and failure to diverge (1). Statistical analyses are performed by comparing the best (minimum) costs found for the host parasite data set against randomized data sets (Cruaud *et al.*, 2012). The following settings were used in stats mode: generations 500, population size 2500, sample size 100. All other settings were left unchanged.

## Results

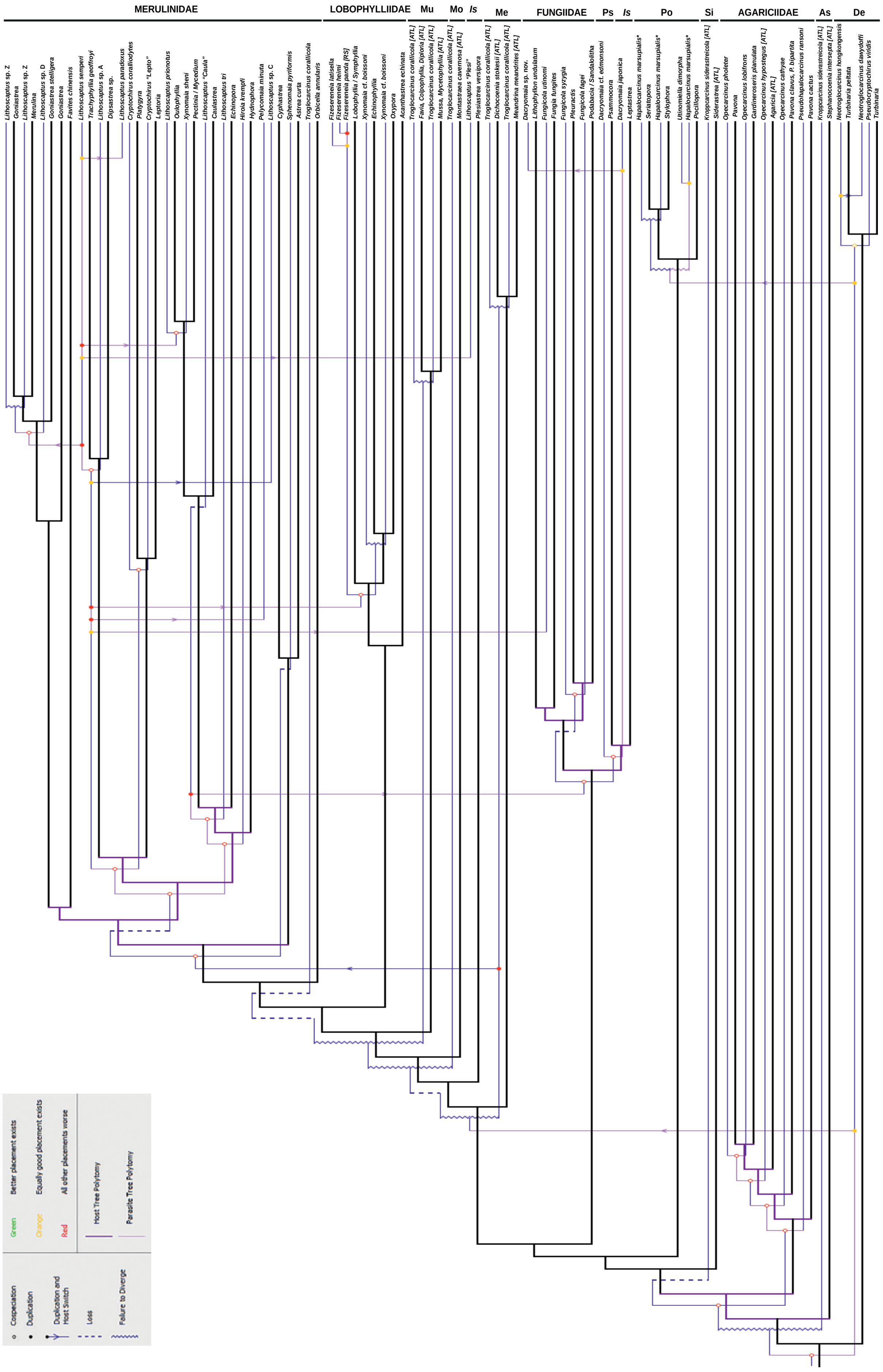
*Phylogenetic tree*

The topology of the phylogeny reconstruction (Fig. 1) is derived from the Bayesian inference 50% majority rule consensus of the trees remaining after the burnin, with high support values in the basal part as well as in the distal phylogenetic branches. The outgroup is separated by a long branch. Within the Cryptochiridae, three major clades can be distinguished, but the relationships between these clades are unclear. The first large clade has *Troglocarcinus corallicola* (ATL) as the most basal clade (not supported by the ML analysis), followed by *Sphenomaia pyriformis* (IP) and *Lithoscaptus tri* (IP). Several subclades can be discerned within this clade; 1) *Fungicola fagei* and *F. syzygia* are closely related to the genus *Dacryomaia*. The type species of the genus *Fungicola* does not cluster in the same subclade. *Cryptochirus coralliodytes* is closely related to a presumably undescribed species associated with the coral genus *Leptoria*. A larger clade is formed by several species (including undescribed species) of *Lithoscaptus*, including the type species *L. paradoxus*. This clade also contains the type species of *Xynomaia*. Another clade is formed by *Fizesereneia*, with another *Xynomaia* species clustering basally. A second clade is formed by the Indo-Pacific genera *Hapalocarcinus*, *Utinomiella*, *Neotroglocarcinus* and *Pseudocryptochirus*, however, this clade is not supported by the ML analysis. The latter two genera form a well-supported subclade within this clade. The third clade is formed by the genera *Opecarcinus* (IP+ATL) and *Pseudohapalocarcinus* (IP), with *Kroppcarcinus* (ATL) in a basal position (albeit with low support and long branch length).

*Coevolution analyses*

Based on the analysis in Jane 4.0, the following events can be discerned: 20 cospeciation events, three duplication events, 14 duplication – host switching events, eight losses, and 10 failures to diverge between Cryptochiridae and Scleractinia (Fig. 2). The majority of the cospeciation events were recorded in associations of gall crabs and hosts species belonging to the Agariciidae, Dendrophyllidae, Fungiidae and Merulinidae. The results of the stats run show that the costs of the random sample solutions are higher than the optimal [= coevolution] solution, for which the costs are 49 (Fig. 3). For all the isomorphic optimal solutions provided by Jane 4.0 the costs and number of estimated coevolutionary events were the same.

**Fig. 2.** Tree resulting from analysis in Jane 4.0 showing the different coevolutionary events between Scleractinia (black lines) and Cryptochiridae (blue lines). ATL = Atlantic, RS = Red Sea, all other species are from the Indo-Malay region. * indicates species complex. Letters in bold refer to the host coral family of the gall crabs specimens: As = Astrocoeniidae, De = Dendrophylliidae, *Is* = Insertae sedis, Me = Meandrinidae, Mo = Montastreidae, Mu = Mussidae, Po = Pocilloporidae, Ps = Psammocoridae, Si = Siderastreidae (classification after Budd *et al.*, 2012; Huang *et al.*, 2014). ▶


MERULINIDAE
LOBOPHYLLIIDAE
Mu
Mo
Is
Me
FUNGIIDAE
Ps
Is
Po
Si
AGARICIIDAE
As
De
Lithoscaptus sp. Z
Goniastrea
Lithoscaptus sp. Z
Merulina
Lithoscaptus sp. D
Goniastrea stelligera
Goniastrea
Favites chinensis
Lithoscaptus semperi
Trachyphyllia geoffroyi
Lithoscaptus sp. A
Dipsastrea sp.
Lithoscaptus paradoxus
Cryptochirus coralliodytes
Platygyra
Cryptochirus "Lepto"
Leptoria
Lithoscaptus prionotus
Oulophyllia
Xynomaia sheni
Pectinia / Mycedium
Lithoscaptus "Caula"
Caulastrea
Lithoscaptus tri
Echinopora
Hiroia krempfi
Hydnophora
Pelycomaia minuta
Lithoscaptus sp. C
Cyphastrea
Sphenomaia pyriformis
Astrea curta
Troglocarcinus corallicola
Orbicella annularis
Fizesereneia latisella
Fizesereneia heimi
Fizesereneia panda [RS]
Lobophyllia / Symphyllia
Xynomaia cf. boissoni
Echinophyllia
Xynomaia cf. boissoni
Oxypora
Acanthastrea echinata
Troglocarcinus corallicola [ATL]
Favia, Colpophyllia, Diploria [ATL]
Troglocarcinus corallicola [ATL]
Mussa, Mycetophyllia [ATL]
Troglocarcinus corallicola [ATL]
Montastraea cavernosa [ATL]
Lithoscaptus "Plesi"
Plesiastrea versipora
Troglocarcinus corallicola [ATL]
Dichocoenia stokesii [ATL]
Troglocarcinus corallicola [ATL]
Meandrina meandrites [ATL]
Dacryomaia sp. nov.
Lithophyllon undulatum
Fungicola utinomi
Fungia fungites
Fungicola syzygia
Pleuractis
Fungicola fagei
Podabacia / Sandalolitha
Dacryomaia cf. edmonsoni
Psammocora
Dacryomaia japonica
Leptastrea
Hapalocarcinus marsupialis*
Seriatopora
Hapalocarcinus marsupialis*
Stylophora
Utinomiella dimorpha
Hapalocarcinus marsupialis*
Pocillopora
Kroppcarcinus siderastreicola [ATL]
Siderastrea [ATL]
Opecarcinus pholeter
Pavona
Opecarcinus lobifrons
Gardineroseris planulata
Opecarcinus hypostegus [ATL]
Agaricia [ATL]
Opecarcinus cathyae
Pavona clavus, P. bipartita
Pseudohapalocarcinus ransoni
Pavona cactus
Kroppcarcinus siderastreicola [ATL]
Stephanocoenia intersepta [ATL]
Neotroglocarcinus hongkongensis
Turbinaria peltata
Neotroglocarcinus dawydoffi
Pseudocryptochirus viridis
Turbinaria
Cospeciation
Duplication
Duplication and Host Switch
Loss
Failure to Diverge
Green
Orange
Red
Better placement exists
Equally good placement exists
All other placements worse
Host Tree Polytomy
Parasite Tree Polytomy

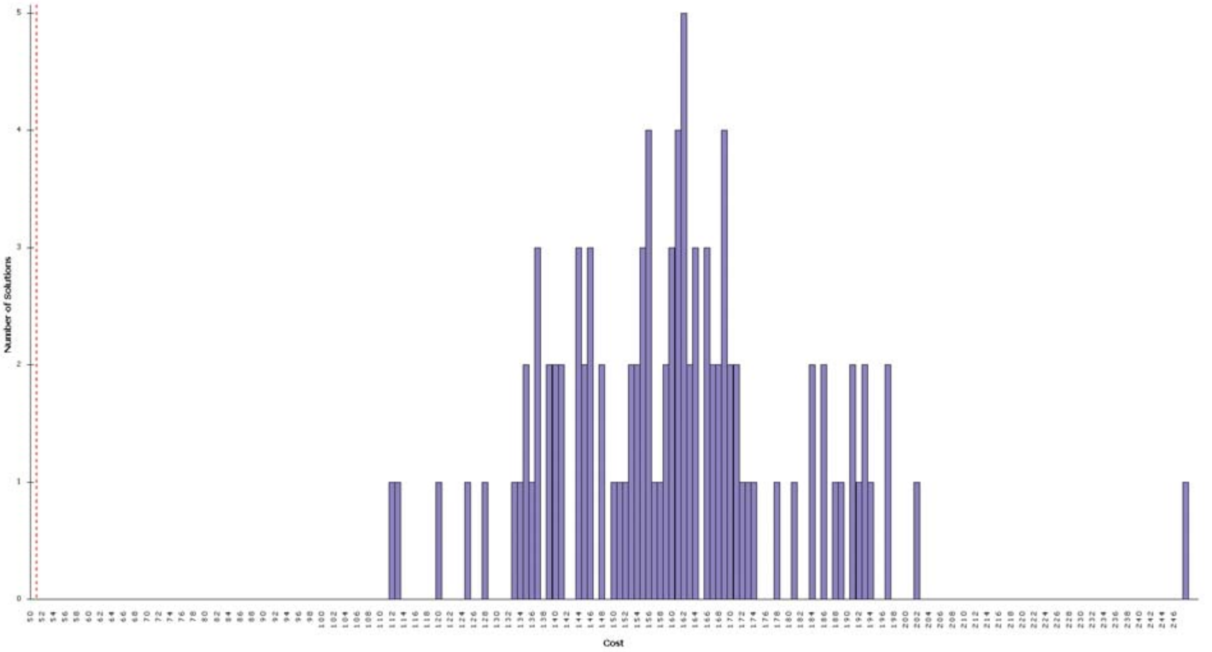


**Fig. 3.** Histogram resulting from a stats run in Jane 4.0, showing the distributions of costs of the random sample solutions. The costs of the optimal [= coevolution] solution is indicated by the red dotted line.

## Discussion

*Relationships within the Cryptochiridae*

There are three major clades within the Cryptochiridae, similar to the results of van der Meij and Reijnen (2014), which was based on 16S and COI mtDNA, and the results of Wei *et al.* (2013) that were based on the morphological data of Kropp (1988). The first large clade shows the Atlantic genus *Troglocarcinus* in a basal position, which is not supported by the ML analysis. The remainder of the clade consists of Indo-Pacific species, of which one species (*Fizesereneia panda*) is endemic to the Red Sea and to other waters around the Arabian peninsula (van der Meij *et al.* in press). The genera *Fungicola*, *Lithoscaptus* and *Xynomaia* appear to be paraphyletic. Based on their host specificity (Fungiidae) and overall morphology this result is especially surprising for the genus *Fungicola*. The type species, *F. utinomi* clusters in a subclade with four other genera, whereas *F. fagei* and *F. syzygia* cluster with the genus *Dacryomaia*. The second clade, which is formed by Dendrophylliidae-associated genera *Neotroglocarcinus* and *Pseudocryptochirus,* is very well supported, whereas the clustering of *Hapalocarcinus* and *Utinomiella* with this clade is only supported by Bayesian inference. The clade containing *Opecarcinus* and *Pseudohapalocarcinus*, two genera associated with Agariciidae, is very well supported. *Kroppcarcinus* clusters weakly with this clade. This genus is strictly Atlantic, whereas *Opecarcinus* occurs in the Atlantic and the Indo-Pacific (Kropp, 1989) and *Pseudohapalocarcinus* only in the Indo-Pacific (Kropp, 1990a). The position of *Hapalocarcinus* and *Utinomiella* is so far not consistent, and with low support (see Van der Meij and Reijnen, 2014). Again their position (Fig. 1) is only supported by the Bayesian analysis, in the ML analysis the resulting tree ended in a polytomy. Interestingly, these genera are both associated with Pocilloporidae corals.

More species need to be added for certain genera, especially for *Lithoscaptus*, to understand the relationships within the paraphyletic genera. It is however clear that taxonomic revisions of certain genera are needed in order to become monophyletic genera.

*Coevolution*

Two kinds of evidence are necessary (and sufficient) to document widespread cospeciation in a host-parasite assemblage: evidence that the host and parasite phylogenies are derived independently and statistical evidence that the topological similarity of the host and parasite trees exceeds chance expectations (Hafner and Nadler, 1990). They furthermore warn that the taxonomy of either host or parasite may have been influenced, explicitly or implicitly, by knowledge of relationships within the other. They further their statement by mentioning that systematic investigations of parasites generally postdate systematic studies of their hosts. The latter is not true for gall crabs. The recent overhaul in scleractinian systematics (e.g. Gittenberger *et al.*, 2011; Arrigoni *et al.,* 2014a; Huang *et al.,* 2014) will have undone any implicit influence of scleractinian systematics on gall crab systematics, in addition to a molecular approach to reconstruct the Cryptochiridae relationships. The present analysis supports the hypothesis that the topological congruence between the gall crab and coral trees is not due to chance alone, hence speciation of stony corals may have induced speciation in gall crabs. The Cryptochiridae and corals, however, do not have strict parallel phylogenies and evolutionary events other than cospeciation are needed to explain the topological incongruence found in the gall crab-coral tree pairs. Sorting events, host-switches, losses and, to a lower degree, duplications, were present all along the twin history of these organisms.

An important aspect in determining whether there are mutual events between the crabs and hosts is the origin of the Cryptochiridae compared to the origin of the Scleractinia. The most recent common ancestor of the gall crabs appeared between 48-23 Ma, with a strong diversification roughly around 10 Ma (van der Meij and Klaus, chapter 6). This preliminary data shows that gall crabs likely diversified in a later stage than their host corals (Budd, 2000; Duchene *et al.*, 2013; Santodomingo *et al.*, 2014). Also, the common ancestor of the gall crabs does not necessarily have the same symbiotic lifestyle of the extant Cryptochiridae (i.e. this ancestor may not have constructed clear pits and may not have shown a strict host specificity). It appears that the observed coevolutionary event should be ascribed to sequential evolution – the phylogeny of the symbionts are influenced by the host evolution, but it is not reciprocal.

Based on the present results, it appears that the coral-cryptochirid system is a good model of marine cophylogeny involving symbionts. It is difficult to compare the present results with those presented in literature, which exclusively involve either parasites or mutualists, because (i) the number of hosts and symbionts used in the various existing studies is extremely variable, and (ii) the taxonomical range of symbionts and hosts is also extremely different from one study to another. Only one study is known that deals with such coevolutionary relationships in the marine environment, i.e., by looking at the relationship between crinoids and their myzostomid commensals (Lanterbecq *et al.*, 2010). This study showed a minimum of eight cospeciation events between 16 Myzostomida worms and their Crinoidea hosts. This is comparable with the gall crabs, which showed 20 events between 38 Cryptochiridae and their coral hosts. However, the study of Lanterbecq *et al.* (2010) only comprised a small subset of the known associations between myzostomids and crinoids, whereas the present study includes about half the number of known associations between gall crabs and corals (van der Meij *et al.*, chapter 12; van der Meij, unpublished data). The importance of one evolutionary event on another within a host-symbiont system can vary from case to case, based on the type of association (parasitism, commensalism, mutualism) (Lanterbecq *et al.*, 2010). The association between Cryptochiridae and Scleractinia is mostly considered to be a symbiotic relationship (Kropp, 1986; Castro, 1988).

### *Limitations of this study*

Since information on the host specificity of certain gall crabs is now limited to genus level, the resolution of the test would be improved by adding more specific data on their hosts. Also the addition of more species, especially for species rich genera such as *Lithoscaptus*, and the inclusion of known cryptic species would shed more light on coevolutionary events in these associations. The coevolutionary analysis used in this paper is an event-based method, which would ideally be supplemented by a topology- and distance-based methods (de Vienne *et al.* 2013). For the majority of the programmes that can perform such analyses the Scleractinia phylogeny has to be reconstructed based on molecular data, an exercise that is now hampered by large datasets, a lack of suitable markers and missing species. Preferably additional testing would also include a test of biogeography.

### *Gall crabs as phylogenetic indicators of scleractinian evolution*

The relationship between corals and gall crabs is a tight one, with at least 20 cospeciation events according to Jane 4.0. Also when comparing the phylogenies by eye, several similarities between the large overall clades become apparent. Within the Scleractinia two main clades are recognized: a ‘complex’ clade and a ‘robust’ clade (Fukami *et al.* 2008). A third basal clade (containing the Gardineriidae and Micrabaciidae) can be recognized, representatives of the most basal lineage of modern scleractinians (Kitahara *et al.* 2010). No gall crabs have so far been recorded from this basal clade. Within the ‘complex’ and ‘robust’ clades several main clades can be distinguished. In the complex clade we find the gall crab hosting families Dendrophylliidae, Agariciidae, and Pocilloporidae, whereas the robust clade is comprised of a subclade containing the Fungiidae, Psammocoridae and *Leptastrea*, and a large subclade (again with several subclades) consisting of Merulinidae, Lobophylliidae and several smaller families. Several Atlantic species cluster basal to this large subclade.

The Cryptochiridae show a similar pattern with the Dendrophylliidae and Agariciidae associated gall crabs in separate clades. Two gall crab genera inhabit corals of the Pocilloporidae. The position of these genera within the Cryptochiridae is somewhat equivocal. Support for the position of these genera is low and so far they have ‘jumped’ through the different trees resulting from phylogeny reconstructions. Two *Fungicola* species and *Dacryomaia* inhabit corals from the Fungiidae, Psammocoridae and *Leptastrea* which perfectly matches the coral phylogeny. The types species of *Fungicola*, however, clusters in a different clade. Like with the corals, the remaining gall crabs, associated mostly with Merulinidae and Lobophylliidae, form a large clade, and, like the corals, the Atlantic species *Troglocarcinus corallicola* clusters basally to this clade. In a more narrow framework of one family, gall crabs have shown to be good indicators of their host relationships, especially at generic level (van der Meij, 2015). Recent results from recent molecular studies on Lobophyllidae and Merulinidae, such as the close relationship between the coral genera *Lobophyllia* and *Symphyllia*, and *Oxypora* or between *Oulophyllia* and *Mycedium* are mirrored in the gall crab phylogeny (Arrigoni *et al.*, 2014b; Huang *et al.*, 2014). The presence of deep-water species in the Cryptochiridae allows for future studies on the relationship between deep-water corals and shallow-water reef corals (Kitahara *et al.*, 2010).

There are other groups of symbionts ‘predicting‘ systematic relationships, in the case of cryptic sympatric sponges the food preferences of predatory starfish proved to be a good indicator of the different species (Wulff, 2006). Similarly, based on the results of this study, gall crabs could serve as phylogenetic indicators of scleractinian relationships. Especially for scleractinian species and genera that are currently classified as *insertae cedis*, for example *Leptastrea* spp. or *Plesiastrea versipora*, gall crabs could provide an indication of their closest coral relatives. This could be somewhat weakened by apparent host shifts.

*Concluding remarks*

The two kinds of evidence as required according to Hafner and Nadler (1990) are met. The host and parasite phylogeny reconstructions were derived independently and the cospeciation analysis in Jane 4.0 showed that the topological similarity of the trees exceeds chance expectations, and thus the observed coevolutionary events should be ascribed to sequential evolution. The relationship between Scleractinia and Cryptochiridae appears to be so tight that gall crabs can be used as phylogenetic indicators of scleractinian evolution.

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