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Title: Evolutionary diversification of coral-dwelling gall crabs (Cryptochiridae)
Issue Date: 2015-06-03

Chapter 6

Origin and diversification of coral-dwelling gall crabs (Decapoda: Cryptochiridae)

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Abstract

Coral-dwelling gall crabs (Cryptochiridae) belong to the subsection Thoracotremata, which is estimated to have originated around 108 [± 11] Mya. The age of their most recent common ancestor is, however, unknown. A selection of 38 shallow-water gall crab species belonging to 17 of the 21 currently recognised genera, including type species for all genera and representatives from the Atlantic and Indo-Pacific oceans, was therefore used in this study to estimate their origin. Divergence time estimation was performed using a Bayesian relaxed molecular clock approach in BEAST with external brachyuran substitution rates. The analysis gave total support for the monophyly of the Cryptochiridae. The age of the most recent common ancestor was estimated at 50-23 Mya (early Eocene – early Miocene). Within the Cryptochiridae three large clades could be identified, which is in congruence with the phylogeny reconstruction of their scleractinian hosts. The short branches leading to these clades suggest an accelerated radiation during the last 10-2 Mya. The estimated origin and diversification of the Cryptochiridae corresponds with a general Cenozoic diversification of reef-associated taxa in the Tethys Ocean, with representatives in the Atlantic – Indo-Pacific divergence within the genus *Opecarcinus* most likely corresponding with the Pliocene closure of the Isthmus of Panama.

Manuscript in preparation

Introduction

Cryptochiridae, commonly known as gall crabs, are obligate symbionts of stony corals (Scleractinia). They live in dwellings (galls, pits or depressions) within corals and are fully dependent on their hosts for food and protection (Potts, 1915; Kropp, 1986). The relationship between the corals and crabs is tight with a high degree of host specificity (e.g. Fize and Serène, 1957; Kropp, 1990A; van der Meij, 2015a, b). There is a striking congruence between the phylogenetic reconstructions of Scleractinia (Fukami *et al*., 2008; Kitahara *et al*., 2010) and Cryptochiridae (van der Meij and Reijnen, 2014; van der Meij, chapter 10). This association even appears to be so tight that gall crabs can be used as phylogenetic indicators of scleractinian evolution (van der Meij, chapter 10).

The family Cryptochiridae is considered to be monophyletic, but their position within the brachyuran subsection Thoracotremata remains unclear (Guinot *et al*., 2013; van der Meij and Schubart, 2014). Within the Thoracotremata a wide variety of habitats occurs, as for example is observed among: 1) intertidal or shore crabs (e.g. Grapsidae, Sesarmidae), 2) specialised mangrove and mudflat dwellers (Camptandriidae, most Ocypodidae), 3) freshwater-dependent crabs (Glyptograpsidae, certain Varunidae), 4) hydrothermal vent specialists (Xenograpsidae), and 5) permanently symbiotic crabs (Cryptochiridae, Pinnotheridae). The superficial resemblance between the latter two families (small size, large brood pouches) and their host dependency lead previous authors to believe that they are closely related (e.g. Fize and Serene, 1957), however, based on molecular analyses this appears not to be correct (Tsang *et al*., 2014; van der Meij and Schubart, 2014).

In a multi-marker paper by Tsang *et al.* (2014) the age of the most recent common ancestor (tMRCA) of Thoracotremata is estimated at 108 [± 11] Mya, and the divergence of the Cryptochiridae from the Xenograpsidae is placed into the Cretaceous (83 [± 11] Mya). As their clade has no statistical support, the exact position of the Cryptochiridae within the Thoracotremata still remains enigmatic. The question of the origin of the gall crabs is interesting in the light of their obligate relationship with corals. In this study we aim to estimate the age of the MRCA of the Cryptochiridae and that of the clades within the Cryptochiridae, based on a dataset containing species from the Atlantic and Indo-Pacific ocean. This will allow a comparison of the diversification within the Cryptochiridae with the diversification times of their host corals. The placement of Atlantic and Indo-Pacific species will shed light on biogeographical patterns seen in the gall crabs and their host taxa.

Material and methods

Species selection

In this study, the same species selection was used as in the study of Van der Meij (chapter 10) on cospeciation, namely 38 shallow-water species belonging to 17 genera. The type species of each genus was included. The dataset includes three species from the West Atlantic, one endemic of waters surrounding the Arabian peninsula, and various species that are widespread in the Indo-Pacific. The Atlantic *Opecarcinus hypostegus* (Shaw & Hopkins, 1977) belongs to a genus that is otherwise exclusively Indo-Pacific. Unfortunately, deep sea gall crabs of, e.g., the genera *Cecidocarcinus* and *Zibrovia*, were not available. *Hemigrapsus pennicilatus* (de Haan, 1835) (Varunidae) was selected as the outgroup (van der Meij and Schubart, 2014).

Gall crabs were sequenced for three markers (mtDNA: 585 bp 16S rRNA gene, 643 bp COI; nDNA: 286 bp Histone H3). DNA extraction was performed following the protocols specified in Van der Meij (2015a). The total alignment length was 1514 bp.

Divergence time analyses

Divergence time estimation was performed using a Bayesian approach in BEAST 1.7.5 (Drummond *et al.,* 2012). One chain was ran for 50 × 10^6 iterations sampling every 10,000 iterations. Convergence of sampled parameters and potential autocorrelation (effective sampling size for all parameters >100) was investigated in Tracer 1.6 (Rambaut *et al.,* 2014). The first 500 trees were discarded as burn-in, keeping 4500 trees. The maximum credibility tree was calculated and parameter values annotated with TreeAnnotator (part of the BEAST package). The GTR+Γ substitution model was applied for all partitions as suggested by PartitionFinder v1.1.1 (Lanfear *et al.,* 2012) using the Bayesian Information Criterion and considering GTR, TrN, HKY and JC models with and without gamma distributed substitution frequencies. A Yule tree prior was used and the nucleotide exchange rates for the 16S rRNA mitochondrial gene partition were adjusted after initial test runs. Unfortunately, there are no fossil gall crabs or trace fossils of their dwellings in corals available for calibration of a molecular clock, hence we estimated divergence times using external substitution rates using an uncorrelated lognormal relaxed molecular clock approach. In detail, these are:

(1) A mean rate of 1.09% per Ma (normal distribution) for the 16S mitochondrial rRNAs (SD = 0.239% per Ma; 5-95% interquantile range = 0.63-1.4% per Ma) was applied, that resulted from a phylogeny of the Old World freshwater crab family Gecarcinucidae that was dated with three fossil calibration points (for the calibration scheme, see Klaus *et al.,* 2010; for chronostratigraphy of the fossils, see Klaus and Gross, 2010). These are the MRCA of the genus *Potamon* (divergence *P. fluviatile* and *P. persicum*) calibrated with fossil *P. quenstedti*; the MRCA of the gecarcinucid genus *Sartoriana* based on fossil claws from the South Asian Siwalik formation; and the MRCA of *Potamonautes niloticus* and *Platythelphusa armata* based on Late Miocene *P.* aff. *niloticus*. The taxonomy and chronostratigraphy of the potamid and gecarcinucid fossils was recently assessed (Klaus and Gross 2010), and associated uncertainty was modelled conservatively in the study of Klaus *et al.* (2010).

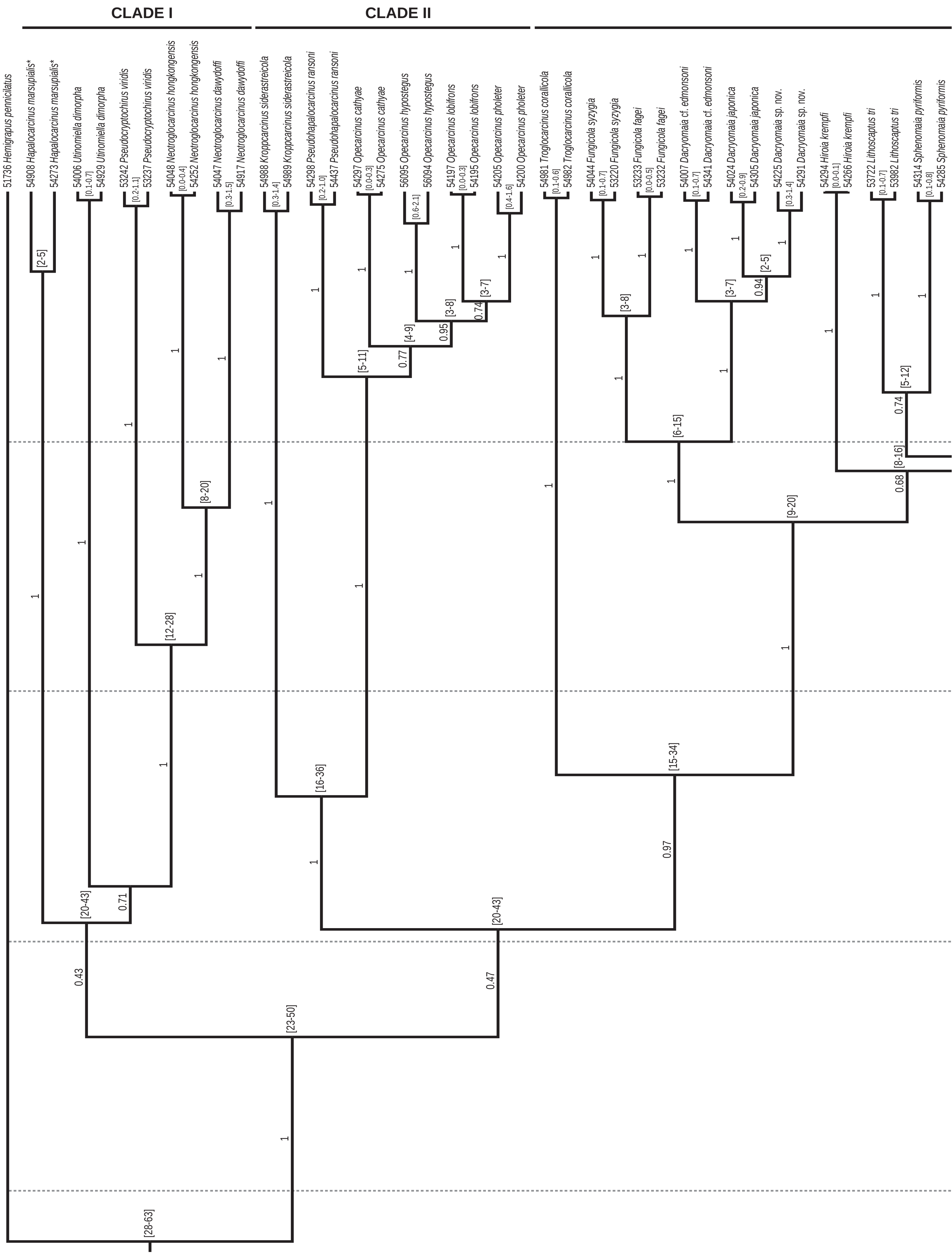
(2) 0.19% per Ma for the H3 gene (SD = 0.04% per Ma; 5-95% interquantile range = 0.12-0.26% per Ma). This rate is also derived from the study on gecarcinucid freshwater crabs of Klaus *et al.* (2010; see above).

(3) For the COI locus a substitution rate of 1.165% per Ma (SD = 0.90% per Ma with 0.00% and 2% as hard lower and upper bounds; 5-95% interquantile range = 0.20-2.69% per Ma) was used as inferred for Jamaican sesarmid freshwater crabs based on the Pliocene closure of the Isthmus of Panama (Schubart *et al.,* 1998). Similar values for the COI substitution rate have been obtained for other arthropod taxa using biogeographical calibration (Papadopoulou *et al.,* 2010; and references therein).

Results

The gall crabs are shown to be monophyletic with total support. The age of the MRCA was estimated at 50-23 Mya (Early Eocene – Early Miocene; credibility interval). Short branches at the base of the clades suggest an accelerated radiation in the last 10-2 Mya (Fig. 1).

Three distinct clades could be observed, albeit some with low support: 1) clade I (tMRCA 43-20 Mya) is comprised of the Pocilloporidae-inhabiting genera *Hapalocarcinus* and *Utinomiella*, together with the Dendrophylliidae-inhabiting genera *Neotroglocarcinus* and *Pseudocryptochirus*, however, support for this clade is low. No recent radiation was observed in this clade; 2) the well-supported clade II (tMRCA 36-16 Mya) consists of the Atlantic species *Kroppcarcinus siderastreicola*, which is the sister genus of a clade (tMRCA 11-5 Mya) containing the


CLADE I
CLADE II
51736 Hemigrapus penniciliatus
54908 Hapalocarcinus marsupialis*
54273 Hapalocarcinus marsupialis*
54006 Utinomiella dimorpha
[0.1-0.7]
54929 Utinomiella dimorpha
53242 Pseudocryptochirus viridis
[0.2-1.1]
53237 Pseudocryptochirus viridis
54048 Neotroglocarcinus hongkongensis
[0.0-0.4]
54252 Neotroglocarcinus hongkongensis
54047 Neotroglocarcinus dawydoffi
[0.3-1.5]
54917 Neotroglocarcinus dawydoffi
54988 Kroppcarcinus siderastreicola
[0.3-1.4]
54989 Kroppcarcinus siderastreicola
54298 Pseudohapalocarcinus ransoni
[0.2-1.0]
54437 Pseudohapalocarcinus ransoni
54297 Opecarcinus cathyae
[0.0-0.3]
54275 Opecarcinus cathyae
56095 Opecarcinus hypostegus
[0.6-2.1]
56094 Opecarcinus hypostegus
54197 Opecarcinus lobifrons
[0.0-0.3]
54195 Opecarcinus lobifrons
54205 Opecarcinus pholeter
[0.4-1.6]
54200 Opecarcinus pholeter
54981 Troglocarcinus corallicola
[0.1-0.6]
54982 Troglocarcinus corallicola
54044 Fungicola syzygia
[0.1-0.7]
53220 Fungicola syzygia
53233 Fungicola fagei
[0.0-0.5]
53232 Fungicola fagei
54007 Dacryomaia cf. edmonsoni
[0.1-0.7]
54341 Dacryomaia cf. edmonsoni
54024 Dacryomaia japonica
[0.2-0.9]
54305 Dacryomaia japonica
54225 Dacryomaia sp. nov.
[0.3-1.4]
54291 Dacryomaia sp. nov.
54294 Hiroia krempfi
[0.0-0.1]
54266 Hiroia krempfi
53722 Lithoscaptus tri
[0.1-0.7]
53982 Lithoscaptus tri
54314 Sphenomaia pyriformis
[0.1-0.8]
54285 Sphenomaia pyriformis
[2-5]
1
1
1
1
1
1
1
1
[8-20]
[12-28]
0.71
[20-43]
0.43
1
1
1
1
1
1
1
1
1
0.77
[4-9]
0.95
[3-8]
0.74
[3-7]
[5-11]
1
[16-36]
1
[20-43]
0.47
[23-50]
1
[28-63]
1
1
1
1
1
1
1
1
1
1
[3-8]
[3-7]
0.94
[2-5]
1
[6-15]
[5-12]
0.74
[8-16]
0.68
[9-20]
1
[15-34]
0.97

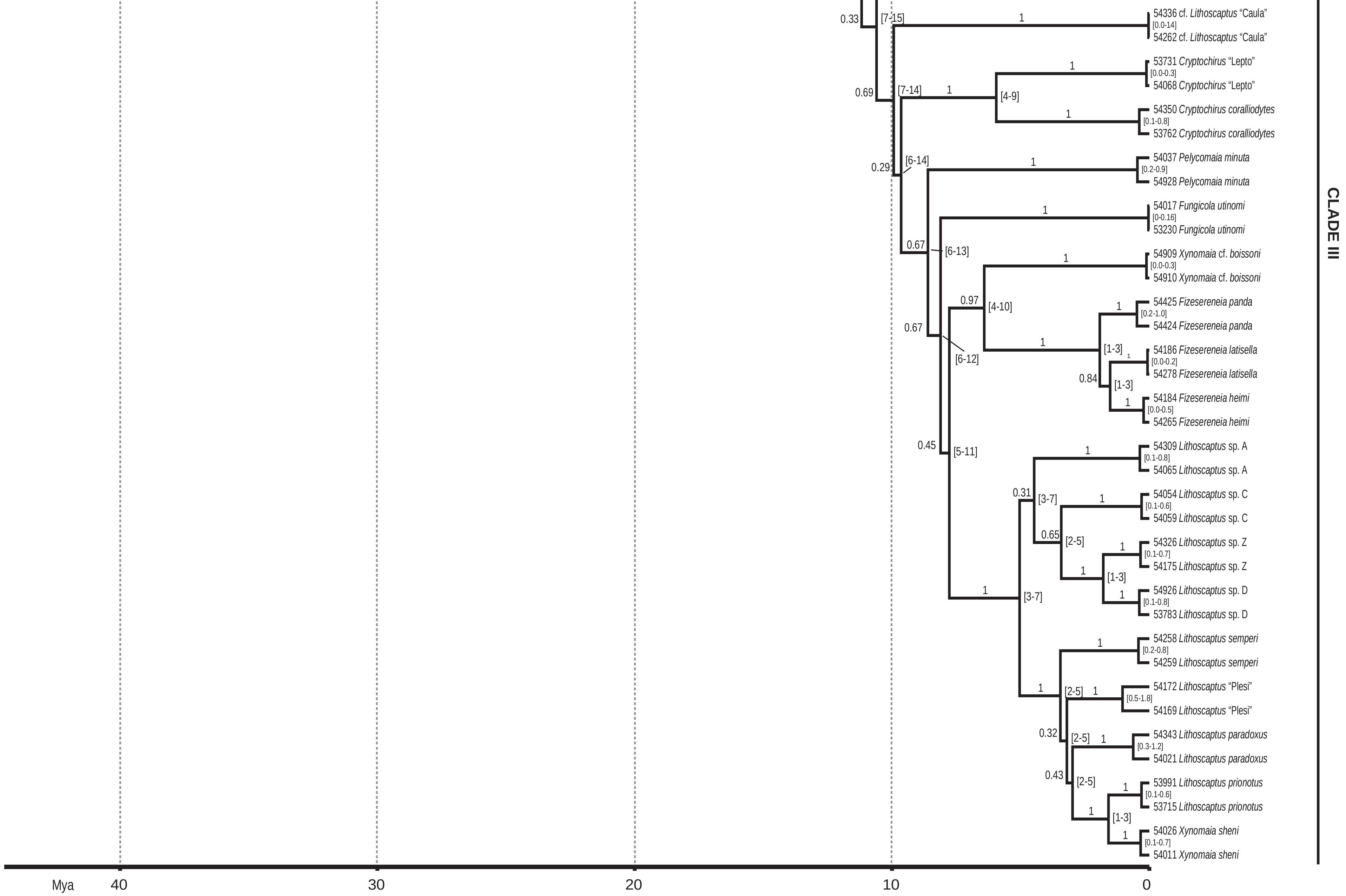


Fig. 1. Chronogram (maximum credibility tree of the Bayesian divergence time analysis in BEAST 1.7.5) with 95% highest posterior density intervals (HPD) of divergence events in million years ago (Mya). Values on branches represent their posterior probabilities, branches without values have full support. ATL = Atlantic, RS = Red Sea (Arabia, endemic), * species complex.

Agariciidae-inhabiting genera *Opecarcinus* and *Pseudohapalocarcinus*. The genus *Opecarcinus* is the only monophyletic cryptochirid genus consisting of Atlantic and Indo-Pacific species; 3) the well-supported clade III (tMRCA 34-15 Mya) comprised all remaining genera. Within clade III the Atlantic species *Troglocarcinus corallicola* diverged early from its relatives, the latter being divided in two subclades; one containing the crab species inhabiting Fungiidae, Siderastreidae, Psammocoridae and *Leptastrea*. The clades at generic level are generally well-supported (Fig. 1).

All three known West Atlantic species are included in the present analysis. They could be retrieved in two different clades. As stated above, *Kroppcarcinus siderastreicola* and *T. corallicola* diverged early within their clades. *Opecarcinus hypostegus* was retrieved as part of the (monophyletic) genus *Opecarcinus*. The only Red Sea – Arabia endemic clustered within the large clade III, together with its Indo-Malayan congeners (Fig. 1).

Discussion

Origin of the Cryptochiridae

The most recent common ancestor of the Cryptochiridae appears to have originated between 50-23 Mya, whereas Tsang *et al. (*2014) traced the divergence of Cryptochiridae from its sister group (albeit without support) into the Cretaceous. The origin of the Thoracotremata was well-supported and is estimated to have originated around 108 [± 11] Mya, which makes it the most recently originated subsection within the Brachyura (Tsang *et al*., 2014). Paulay and Starmer (2011) postulated that Thoracotremata evolved in ‘safe places’, such as intertidal, non-marine, deep water and endo-symbiotic habitats. Survival and diversification of thoracotreme crabs might therefore be related to their adaptability to new environments. Several other thoracotreme families – all with different lifestyles – appear to have originated around the same time as the Cryptochiridae (e.g. Sesarmidae and Glyptograpsidae) whereas other families originated earlier (Dotillidae) or later (Percnidae) (Tsang *et al.,* 2014).

Comparison with the evolution of Scleractinia

Scleractinia are much older than Cryptochiridae. The most recent common ancestor of the Scleractinia is estimated to have originated in the Triassic (ca. 250 to 200 Mya; Park *et al*., 2012). There are two main clades in the Scleractinia: the “complex” clade and the “robust” clade (Fukami *et al*., 2008; Kitahara *et al*., 2010). These clades diverged in the Triassic and the most recent common ancestor for each clade originated in the middle of the Cretaceous (ca. 145 ± 4 to 66 Mya) (Park *et al*., 2012). The phylogenetic topology of the Cryptochiridae (but not the divergence times) follows this pattern; the host corals of the gall crabs in clades I and II belong to the complex clade, whereas the host corals of the gall crabs in clade III belong to the robust clade (Fig. 1).

Clade I consists of Pocilloporidae- and Dendrophylliidae-inhabiting crabs, all of which are restricted to the Indo-Pacific (IP). West Atlantic (ATL) gall crab species are retrieved in two out of the three main clades (Fig. 1). This suggests that there have been multiple exchanges of gall crab species between what is currently recognised as the Atlantic and the Indo-Pacific. The strictly Atlantic genus *Kroppcarcinus* clusters as a sister genus to *Opecarcinus* (IP + ATL) and *Pseudohapalocarcinus* (IP) (clade II). The recovery of the Agariciidae-inhabiting genus *Opecarcinus* (with one Atlantic and several Indo-Pacific species) as monophyletic and recently divergent surprising, yet corresponds with the monophyletic family Agariciidae occurring in both basins. The origin of the West Atlantic crab species *O. hypostegus,* estimated at 8-3 Mya (Fig.1), fits the closure of the Panamanian Isthmus. The timing of vicariance of transisthmian sister species varies among taxa, with many falling around 3.1 Mya (Malay and Paulay, 2010; and references therein). The Merulinidae is the only other coral family to host gall crabs in both oceanic basins, yet there is no evidence for a close relationship between Atlantic and Indo-Pacific Merulinidae-inhabiting gall crabs (clade III in Fig. 1). *Troglocarcinus corallicola*, like *Kroppcarcinus*, is strictly Atlantic and clusters as a sister genus to the remaining genera and species in clade III. The position of *Detocarcinus balssi,* an East Atlantic species recorded from between ca. 3 and 98 meters depth, has not yet been assessed using molecular methods, but this species appears to be closest to the Indo-Pacific species *Utinomiella dimorpha* (clade I) (Kropp and Manning, 1987; Kropp, 1988; van der Meij and Nieman, unpubl.). Analyses of this species and deep-water gall crab species could shed more light on these results, especially given the results by Kitahara *et al*. (2010) who showed that shallow-water corals originated from deep-water species.

The Red Sea – Arabia endemic *Fizesereneia panda* van der Meij, 2015 was retrieved within the large overall clade, otherwise containing Indo-Malayan species. It appears that gall crabs diversified in the Indo-Pacific Ocean and radiated from there to secondary biodiversity areas such as the Red Sea, however, the position of a single species is not enough to reach a conclusion. Such radiation is shown in a study on hermit crabs, which indicated that allopatrically distributed sister species pairs were significantly younger than sympatric sister species (Malay and Paulay, 2010). These results are also in agreement with a study on coral-dwelling gobies which diversified mostly in the last 5 Mya, supporting a hypothesis in which they diversified in the Indo-Pacific Ocean and then radiated recently, with multiple new variants found in the Red Sea (Duchene *et al*., 2013).

Van der Meij (chapter 10) suggested that the evolutionary development of the association between corals and gall crabs should be seen as sequential evolution. Sequential evolution is defined as a particular case of coevolution where the changes and the phylogeny of the symbionts are influenced by the host evolution without reciprocity. The discrepancy between the origin of the Scleractinia and Cryptochiridae supports this hypothesis. Fossil-calibrated phylogenies of reef-building corals are, however, only sparsely becoming available (Santodomingo *et al*., 2014). The first results show that within “robust” clade of Scleractinia a high diversification is observed between ca. 20 to 2 Mya among species of the families Merulinidae, Diploastreidae, Montastreidae and Lobophyllidae. Many of the gall crab species in clade III (Fig. 1), inhabit corals belonging to the Merulinidae and diversification in the Cryptochiridae is highest between approx. 10 to 2 Mya. Cenozoic climate change and tectonic events likely shaped the strong diversification in reef-associated taxa (e.g. Budd, 2000; Williams *et al*., 2013). Further analyses are needed to study the temporal pattern of diversification of both coral and gall crab species in these recently diverging clades. If the origins of taxa within these clades turn out to be synchronous, the strict coevolution vs sequential evolution paradigm needs to be revisited.

Acknowledgements

The fieldwork in Indonesia was organized by Naturalis and the Indonesian Institute of Sciences (LIPI), under the umbrella of Ekspedisi Widya Nusantara (E-Win). Fieldwork in Lembeh Strait in 2012 took place during a Marine Biodiversity Workshop based at the Bitung Field Station (LIPI), co-organized by Universitas Sam Ratulangi in Manado, N Sulawesi (Indonesia). I am grateful to LIPI and RISTEK for granting research permits. Bert Hoeksema (Naturalis) and Yosephine Tuti Hermanlimianto (RCO-LIPI) are acknowledged for all their efforts in organizing the various expeditions in Indonesia. The 2010 Semporna Marine Ecological Expedition was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah's Borneo Marine Research Institute, Universiti Malaya's Institute of Biological Sciences and Naturalis, and was funded through WWF-Malaysia. The research permits for Malaysia were granted by the Economic Planning Unit, Prime Minister's Department, Sabah Parks and Department of Fisheries Sabah. The Tun Mustapha Park Expedition (TMPE) 2012 was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah (UMS), Sabah Parks and Naturalis. TMPE was funded by the Ministry of Science, Technology and Innovation (MOSTI) and USAID Coral Triangle Support Partnership (CTSP). The research permits were granted by the Economic Planning Unit, Prime Minister's Department and Sabah Biodiversity Centre. Fieldwork in the Red Sea was supported by the King Abdullah University of Science and Technology under the Biodiversity in the Saudi Arabian Red Sea program, award number CRG-1-BER-002 to Michael Berumen. The fieldwork in Curaçao was supported by the CARMABI Research Station and DiveVersity Curaçao. Sequences were produced as part of a Naturalis Barcoding project. Charles Fransen and Bert Hoeksema provided helpful comments on the manuscript. Funding for the several fieldwork trips was provided by the AM Buitendijkfonds, LB Holthuisfonds, JJ ter Pelkwijkfonds (all Naturalis), Schure-Beijerinck-Poppingfonds (KNAW), Stichting Fonds Dr C van Tussenbroek (N Ongerboerfonds), LUF International Study Fund (Leiden University), TREUBmaatschappij (Society for the Advancement of Research in the Tropics) and the Van Tienhoven Foundation for International Nature Protection.