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## Diversity and evolution of coral-dwelling gall crabs (Cryptochiridae: Opecarcinus)

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# CHAPTER 6

# 6

## Synthesis

Tao Xu

## 1. Diversity and evolution of coral-dwelling gall crabs

This thesis aims to reveal the history of diversification and evolution of coral-dwelling gall crabs. The species-rich genus *Opecarcinus*, associated with Agariciidae corals, was used as a model group across all chapters. Through collaborations and over a decade of data accumulation, more than 400 lots of *Opecarcinus* were collected from the West Atlantic and Indo-Pacific oceans. Chapter 1 served as a brief introduction to symbiosis on coral reef ecosystems and provided background information on symbiotic gall crabs. Chapter 2 revealed the hidden biodiversity within *Opecarcinus*, historical biogeography and their colonization route from the Indo-Pacific to the West Atlantic. Chapter 3, subsequently, described 16 species as new to science and examines the factors driving the fluctuation of diversification rates. From a broader perspective, Chapter 4 focused on the evolutionary history of several symbiotic lineages (Cryptochiridae, Pinnotheridae, certain Varunidae) within the group of ‘derived’ crabs, the Thoracotremata, employing a phylomitogenomic approach. Lastly, Chapter 5 investigated the effects of coral colony size and depth on the symbiont assemblage (including gall crabs), based on fieldwork data from Curaçao.

The main results of this thesis:

- Time to the Most Recent Common Ancestor (tMRCA) of *Opecarcinus* was estimated to be between 15–6 million years ago (Mya), spanning the middle to late Miocene. The Indo-West Pacific was inferred as the most probable ancestral area. From there *Opecarcinus* colonized the West Atlantic by crossing the East Pacific Barrier before the complete closure of the Isthmus of Panama, and subsequently speciated into *O. hypostegus* (Chapter 2).
- *Opecarcinus* harbored a high number of previously uncovered biodiversity. The genus was revised, and 16 species were described as new to science. Additionally, *Opecarcinus* experienced adaptive radiation during the Pleistocene, which might be related to glacial cycles (Chapters 2, 3).
- Symbiotic lineages within thoracotreme crabs evolved independently on multiple occasions. Additionally, signals of positive selection were identified in the mitochondrial protein-coding genes of these symbiotic crabs, possibly related to the presumably higher energetic demands of a symbiotic lifestyle (Chapter 4).

- In the non-branching Caribbean coral species *Porites astreoides*, *Siderastrea siderea*, and *Agaricia agaricites*, symbiont assemblage was not correlated with coral colony size. A significant decrease in symbiont assemblage with increasing depth was observed in *P. astreoides* and *S. siderea*, but not in *A. agaricites*. The factors driving the composition of infaunal assemblages remain poorly understood, especially in non-branching corals (Chapter 5).

### 1.1 Biodiversity of gall crabs

Numerous studies have estimated the number of species associated with coral reefs, ranging from 550,000 to 1,330,000 (Fisher et al., 2015). This variability is largely due to the diverse invertebrates that are often understudied and incompletely described (Reaka-Kudla, 1997). While research has predominantly focused on more visible reef organisms like corals and fish, less conspicuous and small invertebrates taxa, including worms, barnacles, boring mussels, gall crabs and other cryptic fauna, contribute significantly to coral reef biodiversity yet have received comparatively little attention (Stella et al., 2011). Our findings strongly support the notion that a substantial number of coral-associated invertebrates remain undescribed. In this thesis, we described 16 new species within the genus *Opecarcinus*. Furthermore, two of them form a putative species complex, but an insufficient number of specimens restricts further species-level identification (Chapters 2 and 3; Bähr et al., 2021; Van der Meij, 2023; Wong et al., 2023).

### 1.2 Historical biogeography of gall crabs

Multiple potential colonization routes exist for marine species to disperse from the Indo-Pacific to the Atlantic. These routes include traversing the Tethyan seaway before its closure (12.8 Mya; Sun et al., 2021), around the Cape of Good Hope (southern tip of South Africa), or crossing the Isthmus of Panama before its complete closure approximately 2.8 Mya (O’Dea et al., 2016). In this thesis, we inferred that *Opecarcinus* originated in the Indo-West Pacific and subsequently likely crossed the East Pacific Barrier (EPB), traversed the Isthmus of Panama before its full closure, and eventually reached the West Atlantic (Chapter 2). The closure of the Isthmus of Panama effectively halted gene flow between the East Pacific and the West Atlantic, which might have contributed to the subsequent speciation into *Opecarcinus hypostegus*.

The EPB stands as the widest marine biogeographic barrier globally, spanning

approximately 5,400 km of uninterrupted deep water between the central and eastern Pacific (Grigg and Hey, 1992). This barrier constitutes the greatest marine obstacle for the dispersal of shallow-water organisms. However, numerous marine species have been recorded from both sides of the EPB, suggesting remarkable dispersal capabilities (Chapter 2 and 3; Glynn and Ault, 2000; Lessios et al., 1998; Lessios and Robertson, 2006). *Opecaercinus* spp. has also been reported from the Indo-West Pacific and the East Pacific (Kropp, 1989). The wide distribution of these species raised questions about their dispersal ability acrossing the EPB.

How is it possible for marine species to cross such an extensive barrier without any islands serving as stepping stones in migration? In marine systems, adults are commonly less mobile, while larvae can spend hours to weeks, or even months, as plankton, potentially dispersing over great distances (Bilodeau and Hay, 2022). However, it has been suggested that larvae are unlikely to survive long enough for currents to transport them across the EPB (Lessios et al., 1998). The average speeds of surface currents across the EPB are likely too slow for timely larval transport, with estimated conveyance times spanning years (Robertson et al., 2004). Some studies have attributed the most recent migrations to El Niño events (Lessios et al., 1998; Lessios and Robertson 2006), as these events could significantly increase flow speeds, resulting in random success of migration for long-lived larvae. The *Opecaercinus* lineage arrived in the East Pacific millions of years ago (Xu et al., 2022), while El Niño events only have been occurring for nearly thousands of years. Other perspectives attribute the historical success of dispersal to Pleistocene glacial cycles and geological uplift (Grigg and Hey, 1992; Robertson and Allen, 1996; Chabbarria and Pezold 2013). During the Pleistocene glacials, sea levels experienced a protracted decline lasting over ten thousand years. Simultaneously, the Line Islands were repositioned into the path of the North Equatorial Counter-Current, which is presumed to have facilitated larval transport (Lessios et al., 1998). Even though this scenario is consistent with the extended migration time and route of the *Opecaercinus* lineage, the successful dispersal of *Opecaercinus* from the Indo-West Pacific to East Pacific still remains unclear.

In this thesis, a large number of speciation events of *Opecaercinus* occurred during the Pleistocene (Chapter 3). Glacial cycles during the Pleistocene are widely considered as drivers shaping species diversification and distribution patterns for coastal marine life such as species associated with coral reefs (Shen et al., 2011; Hoareau et al., 2012; Ludt

and Rocha 2015). Pleistocene glacial cycles reduced global sea level by up to 130 m below the current average, leading to a decrease in the availability of coastal habitats and the fragmentation of populations across various taxa, thereby contributing to speciation (Clark et al., 2009). The results of a Lineage-through-Time (LTT) plot suggested that *Opecarcinus* underwent rapid diversification during the Pleistocene. Therefore, the speciation of coral-dwelling gall crabs in the Indo-Pacific can likely be attributed, at least to a certain extent, to the direct or indirect effects of glacial cycles, in addition to types of speciation such as allopatric speciation or host switching.

### 1.3 Evolutionary history of symbiotic lineages within Thoracotremata

‘True’ crabs within the clade/subsection Thoracotremata exhibit a wide range of lifestyles across (semi)terrestrial, freshwater, and marine habitats (Castro 2015; Tsang et al., 2022). Some crabs, such as land crabs, live on land as adults while their larvae undergo development in the sea (Green et al., 2008; Ryu and Kim, 2020). Other lineages, such as gall crabs (Cryptochiroidea), pea crabs (Pinnotheroidea), and certain Varunidae species, live in symbiotic associations with invertebrates (stony corals, bivalves, echinoderms, worms, etc), where these invertebrate hosts provide benefits for shelter, reproduction, and food. The phylogenetic relationships of these symbiotic lineages have been under debate. Our findings, based on full mitogenomic data, consisting of concatenated sequences from 13 protein-coding genes and two rRNA genes, revealed that symbiotic crab lineages in Thoracotremata evolved multiple times, suggesting that the evolution from a free-living to a symbiotic lifestyle appears to be relatively common in thoracotreme crabs.

The ongoing debate regarding the evolutionary history of symbiotic lineages stems from the lack of convergence in phylogenetic analyses using different target genes (Chapter 4; Tsang et al., 2014; Sun et al., 2022; Tsang et al., 2022; Kobayashi et al., 2023). In this thesis, we utilized full mitogenomes for phylogenetic analyses, incorporating additional information provided by mitochondrial gene order (MGO). Our findings revealed that these symbiotic lineages formed distant, independent clades with their own unique MGOs, thereby ruling out the possibility of a single origin for these symbiotic crabs. However, two other studies using a similar mitogenome-based dataset yielded disparate results (Sun et al., 2022; Kobayashi et al., 2023). These studies were limited by including only a single gall crab mitogenome, which restricted the

robustness of the phylogeny. Moreover, Tsang et al. (2014) used eight molecular markers (six nuclear protein-coding genes and two mitochondrial rRNA genes), indicating separate clades for gall crabs and pea crabs and suggesting they do not share a common ancestor. Subsequently, another study using ten molecular markers (two mitochondrial markers and eight nuclear protein-coding genes) led to different conclusions (Tsang et al., 2022). However, the same limitation, datasets with only one gall crab species, persisted in these studies. This thesis introduced three new mitogenomes, bringing the total to four including one previously published, into the analysis, thereby enhancing the credibility of our results. Thus, obtaining more comprehensive data, both in terms of more taxa and more robust genetic data, may overcome the limitations of current phylogenetic analyses involving gall crabs.

The evolution of lifestyles or habitats among Thoracotremata crabs has involved morphological, behavioral, and physiological adaptations. These adaptations enable organisms to effectively cope with the challenges presented by their changing environments (Lü et al., 2023). Moreover, these transitions also result in genetic variations. Many studies have highlighted genetic variability that facilitate better adaptation to diverse habitats (Hui et al., 2018; Shen et al., 2019; Lü et al., 2023), especially in mitochondrial protein-coding genes. In this thesis, some of the protein-coding genes in symbiotic crabs were found to have experienced positive selection. This suggests that these mitochondrial genes may have evolved to enhance the efficiency of ATP production, meeting the increased energy requirements since all 13 PCGs in a mitogenome are involved in aerobic metabolism (Wang et al., 2016). Although we detected the presence of positive selection across these symbiotic lineages, the specific reasons for this selection remain unknown. The observed positive selection in symbiotic crabs may be caused by differences in reproduction, body size, mobility, or a combination of these (and other) factors, compared with free-living species. These factors could influence oxygen or energy usage, associated with a secluded lifestyle.

#### **1.4 Symbiont assemblage patterns within coral reef ecosystems**

Studies on how symbiont assemblages affect corals are relatively limited compared with studies of specific taxa in coral reef ecosystems. Nevertheless, these often-overlooked symbiont assemblages, much like individual organisms, can play a beneficial role in maintaining the health of coral hosts (Stier et al., 2012; Mckeon et al.,



2012; Mckeon and Moore, 2014; McWilliam et al., 2018), even though some studies have also reported that symbiont assemblages can bring detrimental effects to corals (Stella et al., 2011; Hoeksema et al., 2022). Given their significant importance, and contribution to the diversity on reefs, understanding the factors that drive the formation of these coral symbiont assemblages is both beneficial and necessary for developing protective strategies against potential threats. So far, only a few studies have illustrated the factors influencing symbiont assemblage in branching corals (López-Pérez et al., 2017; Smallhorn-West et al., 2017; Britayev et al., 2023). In contrast, the dynamics within non-branching corals remain largely unexplored, and as a result are poorly understood.

A study on the impact of coral colony size and depth on symbiont assemblage reveals that non-branching corals exhibit distinct patterns (Chapter 5). In branching corals a positive correlation between coral colony size and symbiont assemblage was observed (Abele and Patton, 1976; Leray et al., 2012), but this correlation was not observed in the three non-branching coral species examined in this thesis (Chapter 5). The discrepancy may be attributed to differences in composition of symbiont assemblages, linked to the differences in available habitat niches between branching and non-branching corals. Specifically, branching corals, such as acroporids and pocilloporids, demonstrate a positive correlation between their structural complexity and colony size (Britayev et al., 2023), which is positively related to the availability of habitat niches (Tews et al., 2004). In contrast, non-branching corals, characterized by plate-like and massive forms with simple macromorphology, offer fewer structural benefits for symbionts (Kerry and Bellwood, 2012). However, different symbionts may have a certain preference for the habitats provided by corals. For example, *Trapezia* spp. in branching corals, which require more space for shelter, food, and mating, are more frequently found among branches (Castro, 1978; Canizales-Flores et al., 2021). Conversely, infaunal symbionts, such as boring mussels, may inhabit coral skeletons, necessitating more extensive coral reef substrates. Thus, the specific symbionts targeted in the study could also influence the interpretation of the results.

Depth was another factor examined in this thesis, significantly influencing symbiont assemblages in *Porites astreoides* and *Siderastrea siderea*, but not in *Agaricia agaricites*. A similar pattern of decline with increased depth has been reported in branching coral *Acropora* species (Patton, 1994). The distinct pattern observed in *A.*

*agaricites* may need further investigation, which may be attributed to this species's preference for deeper depths than those studied in this research (Chapter 5; Bongaerts et al., 2013; Van Tienderen and Van der Meij, 2016).

In addition to size and depth, several other factors, including geographic location (López-Pérez et al., 2017), depth (Smallhorn-West et al., 2017), density of host corals (Britayev et al., 2023), wave energy (Depczynski and Bellwood, 2005), and water flow (McWilliam et al., 2018), have been shown to significantly affect the composition of symbiont communities in branching corals (López-Pérez et al., 2017; Smallhorn-West et al., 2017; McWilliam et al., 2018; Britayev et al., 2023). However, similar research on non-branching corals has yet to be conducted.

In Chapter 5 we showed that the symbiont assemblages on each single coral colony, the majority of symbionts are rare, with only a few organisms, such as barnacles (*Ceratoconcha* sp.) and Christmas tree worms (*Spirobranchus* sp.), being dominant in this thesis. A similar pattern has been observed in branching corals (Austin et al., 1980), where most coral colonies host a simple assemblage - typically no more than two symbiotic species. Additionally, we also observed that this trend in three non-branching corals becomes more pronounced with increasing depths (Chapter 5). The symbiont occurrence (0, 1, and 2) was not correlated with colony size. Consequently, we proposed that this pattern is unrelated to colony age since colony size has been established to positively correlate with colony age. We further suggested that settlement of symbiont also seems unrelated to colony age.

## **2. Limitations and future perspectives**

### **2.1 Insufficient sampling**

Few samples of *Opecarcinus* have been collected from the East Pacific (Kropp 1989), and no additional material has been collected in the last three decades. Chapter 2 suggested that the *Opecarcinus* lineage likely crossed the EPB from the Indo-West Pacific to the East Pacific. This inference was partly based on the current distribution patterns of *Opecarcinus* and its coral hosts. More samples from the East Pacific would significantly enhance the robustness of this inference by allowing for a comparison of genetic connections between populations on both sides of the EPB.

Chapter 3 highlighted the presence of potential species complexes within *Opecarcinus*

*esmaraldus* **sp. nov.** and *O. hypostegus*. These two species exhibited high levels of intraspecific genetic diversity based on the Cytochrome c oxidase I (COI) gene. The diversity of the Atlantic species *O. hypostegus* has been demonstrated to likely result from adaptive divergence over *Agaricia* coral hosts (Van Tienderen and Van der Meij, 2017). However, the limited number of available specimens for *O. esmaraldus* **sp. nov.** restricts further work on a possibly (pseudocryptic) species complex or for studies similar to the one on *O. hypostegus*.

*Opeparcinus* has a flexible depth distribution, ranging from the intertidal zone to mesophotic depths (Van der Meij et al., 2015, 2017); however, samples from the mesophotic zones are relatively rare. To date, only two records of this genus have been collected from mesophotic coral reef ecosystems (Van der Meij et al., 2015; Vimercati et al., 2023). Coral hosts such as *Leptoseris* and *Agaricia* dominate mesophotic coral ecosystems (Kahng et al., 2010; Terraneo et al., 2017), suggesting the potential for discovering additional *Opeparcinus* samples in these zones given their obligate association with coral hosts (Vimercati et al., 2023). Coral-dwelling organisms associated with mesophotic reefs have been largely overlooked and need further investigation.

To uncover the historical biogeography of the gall crab family Cryptochiridae, conducting ancestral area reconstruction would shed light on the global dispersal patterns of the family spanning the Indo-Pacific and Atlantic Oceans. Such insights in the dispersal patterns of a coral-dwelling crab family would significantly contribute to our understanding of historical biogeography and provide valuable support and evidence for the field of paleogeology (Cowman and Bellwood, 2013). So far, the gall crab *Detocarcinus balssi* (Monod, 1956) has only been recorded in the East Atlantic (Kropp and Manning 1987), indicating that this species may have undertaken a different dispersal route from the Indo-Pacific to the Atlantic, considering the multiple potential dispersal routes. Moreover, the four gall crab species found in the Atlantic at shallow depths—*Kropparcinus siderastreicola*, *Troglocarcinus corallicola*, *O. hypostegus*, and *D. balssi*—do not cluster together in a partial phylogenetic reconstruction of the family (Van der Meij and Klaus, 2015), suggesting that gall crabs might have invaded the Atlantic from the Indo-Pacific on multiple occasions. Therefore, further research involving ancestral area reconstruction could reveal the dispersal patterns and evolutionary history of the Cryptochiridae family on a global scale.

## 2.2 Challenges in coral taxonomy

*Opearcinus* is obligately associated with agariciid corals, but the taxonomic resolution of these coral hosts remains partly unresolved. For example, *Pavona* spp. display strikingly similar interspecific skeletal characteristics, often leading to misidentification using macromorphological characters (Glynn and Ault, 2000). This highlights the importance of combining morphological and molecular tools in taxonomy. However, Luck et al. (2013) demonstrated that molecular results do not always align with morphological characteristics in *Pavona* and *Leptoseris*, introducing further challenges to these taxonomic studies. Moreover, the discovery of species complexes complicates the matter further. For example, a phylogenetic reconstruction using mitochondrial regions and the ribosomal ITS1 region showed that 15 agariciid species had unclear boundaries, with three forming species complexes (Terraneo et al., 2017). These taxonomic challenges not only hinder the precise identification of coral hosts, but also impede research on coral-associated symbionts and potentially cophylogenetic patterns. Therefore, the limitations of relying solely on morphological or molecular tools underscore the need for an integrative approach to taxonomy in future studies.

The taxonomic limitations of coral hosts pose constraints on the robustness of phylogeny reconstruction. However, constructing a robust phylogenetic tree for hosts is essential to uncover ecological speciation by host switching and/or adaptive radiation for symbionts (Beverley-Burton and Klassen, 1990). Detecting the main processes underlying speciation in marine symbiosis is crucial for a more general understanding of the origins and distribution of biodiversity (Faria et al., 2021). Since the 1970s, an increasing number of studies have been conducted to illustrate the relationship between the cophylogeny of hosts and their associates in plants and animals (Brooks et al., 1977; Beverley-Burton and Klassen 1990; Weckstein, 2004), emphasizing the crucial role of phylogenetic reconstructions in explaining the process of adaptive radiation. In contrast, studies on marine symbiosis remain relatively scarce (Lanterbecq et al., 2010). The obligate association between *Opearcinus* and their Agariciidae hosts, and at a larger scale between Cryptochiridae and Scleractinia, and global distribution patterns, make them a valuable model taxon for coevolutionary studies in marine ecosystems.

## 2.3 The nature of symbiotic relationships

Even though gall crabs have been known for over a century, the type of symbiosis with

their host is still under debate. Numerous studies have proposed that gall crabs may function as parasites, examining different perspectives such as feeding habitat and settlement behaviors (Simon-Blecher and Aчитuv, 1997; Simon-Blecher et al., 1999), but the energetic drain on the coral (if any) is unclear (Kropp, 1986; Bravo et al., 2014). Moreover, the impact of gall formations on corals remains poorly understood. Boring organisms could weaken coral skeletons by increasing porosity (Chamberlain, 1978), and higher skeletal porosity, along with reduced bulk density and stiffness, may contribute to an increased susceptibility to damage under low pH conditions (Fantazzini et al., 2015). Gall crabs have the ability to modify the morphology of coral around the galls, yet the specific effects of gall crabs on coral skeletons have only received scant attention. Therefore, more research in the future is imperative to gain a conclusive understanding of the ecological interactions between gall crabs and their coral hosts.

#### **2.4 Conservation of marine symbiosis**

Climate change contributes not only to the degradation of coral reefs but, as a result, can also lead to the breakdown of symbiotic relationships (Goulet and Goulet, 2021), posing significant survival challenges for obligate coral symbionts. Understanding the mechanisms by which climate change impacts these symbiotic relationships could help to develop adaptive conservation strategies. Many marine organisms rely on chemical cues for communication throughout their life, facilitating activities such as settlement, displacement, and partner recognition, from early development to adult stages (Bilodeau et al., 2022; Doll et al., 2022). Numerous studies have highlighted the significance of environmental chemical cues in influencing the behavior of marine species (Solari et al., 2019; Wolfe et al., 2022; Caulier et al., 2023). Additionally, Durant et al. (2023) have shown that ocean acidification can impair the olfactory pathways in crabs, affecting their ability to detect food. Therefore, the factors influencing coral hosts will likely also affect stable symbiotic relationships between the coral and their associated taxa. Implementing adaptation strategies, such as reducing the emission of greenhouse gasses, primarily carbon dioxide, is crucial to mitigating the effects of climate change on coral reefs and their associated fauna.