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

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CHAPTER 1

1

Introduction

Tao Xu

1. Symbiosis

‘Living together of dissimilarity named organisms’

-By Anton de Bary (1879)

Symbiosis refers to the living together of two or more organisms in a close association (Margulis, 1971). Within a symbiosis, the involved organisms, known as symbionts, interact with each other in a way that may be beneficial, neutral, or detrimental to one or both parties. There are three main types of symbiotic interactions: mutualism, where both parties benefit; commensalism, where one benefits without affecting the other; and parasitism, where one benefits at the expense of the other (Leung and Poulin, 2008). These interactions can occur among a diverse range of organisms, including plants, animals, fungi and even different kingdoms of organisms (Paracer and Ahmadjian, 2000).

Symbiosis is a very common and important biological interaction within coral reef ecosystems (Blackall et al., 2015). However, many coral-associated symbionts remain understudied and incompletely described (Reaka-Kudla, 1997), especially inconspicuous, and infaunal symbiotic organisms tend to be largely overlooked. These include small-sized crustaceans, worms, molluscs, and barnacles (Achituv and Newman, 2002; Bouchet et al., 2002; Stella et al., 2011; Navarro-Barranco and Guerra-García, 2016). Moreover, existing research on coral-associated organisms is heavily biased towards the most conspicuous reef species like fishes (Stier and Leray 2014), thereby neglecting other associates. These understudied taxa dominate the biodiversity of coral reefs as they account for the vast majority of animal species on coral reefs (Stella et al., 2011), and these organisms play a vital role in maintaining the ecosystem’s diversity (Stier and Leray 2014; Montano et al., 2017). This thesis aims to explore the evolution and diversification of coral-dwelling gall crabs, and reveal the factors influencing the formation of symbiont assemblages (including gall crabs) on coral reefs. Understanding these factors will be beneficial for comprehending the biodiversity of coral reef ecosystems and providing scientific support for conservation management.

1.1 The influence of symbiosis on coral reef ecosystems

Coral reefs provide habitats for at least an estimated 25% of known marine life (Hoegh-

Guldberg et al., 2017), with this high biodiversity within coral reef ecosystems being dominated by invertebrates (Stella et al., 2011). Many of these invertebrates live in symbiotic associations ranging from mutualism to commensalism and parasitism (Stella et al., 2011; Barton et al., 2020), relying on corals for food and shelter. These intricate relationships have far-reaching effects on the health and stability of coral reefs ecosystems (Rouzé et al., 2014; McKeon and Moore, 2014; Glynn, 1976; Stewart et al. 2006; Stier and Leray 2013; Montano et al., 2017).

Although these invertebrates depend on the coral hosts for survival, numerous studies have shown that symbiotic invertebrates play a key role in enhancing coral resiliency by providing services beneficial to the health of the coral host (Pratchett, 2001; Stewart et al., 2006; McKeon and Moore, 2014; Rouzé et al., 2014). A well-known example is the obligate coral-dwelling crabs from the family Trapeziidae, which employ various ways to protect coral hosts. For example, the presence of these crabs can reduce the potential harm from coral-feeding organisms, such as crown-of-thorns starfish, by actively defending their host coral from these larger and potentially devastating predators (Pratchett, 2001). Additionally, these obligate crabs contribute to the removal of excess sediment, significantly reducing mortality rate of coral hosts (Stewart et al., 2006). Other benefits include preventing excessive algal overgrowth and enriching nutrient concentration, which have proved to have significant benefits for coral reef ecosystems (Stella et al., 2011; Spadaro and Butler, 2021).

In addition to the positive impacts that symbiotic invertebrates have on corals, there are many instances where symbiotic organisms can exert detrimental effects (Scott and Risk, 1988; Shima et al., 2010; Barton et al., 2020). Symbiotic organisms from various taxa, including acoels (Xenacoelomorpha: Acoela), digeneans (Trematoda: Digenea), polyclads (Rhabditophora: Polycladida), gastropods (Mollusca: Gastropoda), decapods (Malacostraca: Decapoda), copepods (Hexanauplia: Copepoda), and pyrgomatids (Cirripedia: Pyrgomatidae), have been reported to harm corals (Barton et al., 2020). They impact coral health through activities such as light-shading, mucus removal, and disruption of heterotrophic feeding (Brown and Bythell, 2005; Barneah et al., 2007; Wijgerde et al., 2012), potentially leading to coral reef degradation, and a decline in biodiversity and productivity (Sano 2000; Pratchett 2001; Berumen and Pratchett 2006; Kayal et al., 2012). However, research exploring the impact of coral-associated

invertebrates on their coral hosts remains scarce, especially given the extensive variety of such invertebrates.

1.2 Threats to symbiosis in coral reef ecosystems

Coral reefs worldwide are facing serious survival challenges at the local, regional, and global scales due to climate change (Hoegh-Guldberg et al., 2007), overfishing (Jackson et al., 2001), pollution (Dubinsky and Stambler, 1996), and coral diseases (Harvell et al., 2007; Haapkylä et al., 2013). These threats lead to the degradation and destruction of reef habitats in many places (Munday 2004; Bravo et al., 2021). Since 2009, there has been a global decline in coral cover, resulting in a loss of 14% of corals (Souter et al., 2021). The high degree of habitat specialization among coral-associated symbionts may expose them to a great risk of extinction due to these serious threats to coral reefs (Hughes et al., 2017). Existing research suggests that the degradation of corals or alteration of reef structures can result in a substantial decline in the diversity of coral-associated organisms and symbiont assemblages (Stella et al., 2014). This decline further negatively impacts the functionality of coral reef ecosystems, rendering symbiotic associations more vulnerable. For example, the coral-defending crab *Trapezia cymodoce* (Herbst, 1801) has shown increased mortality in response to elevated temperatures and reduced coral-host availability (Stella et al., 2014).

Even though climate change-induced challenges such as elevated sea-surface temperature, ocean acidification, and rising sea levels threaten the survival of coral hosts and symbionts, the effects of climate change on these organisms still remain largely unexplored. Many marine invertebrates undergo a pelagic larvae state, during which they disperse as plankton for hours, weeks, or even months, covering great distances (Bilodeau and Hay, 2022). Rising sea temperature could influence larval dispersal distance and survival rates, suggesting that changes in ocean temperature could directly and predictably affect population connectivity, community structure, and biodiversity patterns on a regional to global scale (O'Connor et al., 2007). During dispersal, marine organisms rely on environmental chemical cues for settlement and habitat selection, but ocean acidification may impair their olfactory abilities to detect these cues, ultimately affecting recruitment success (Durant et al., 2023). Additionally, species associated with coral reefs, whether in obligate or facultative associations, face varying levels of threat due to climate change. Obligate symbionts, which cannot

survive without their hosts, exhibit more severe vulnerabilities to these threats (Glynn et al., 1985; Bravo et al., 2021).

1.3 Coral-associated symbiont assemblage

In coral reef ecosystems, the influence on corals extends beyond individual invertebrates; the assemblages of these associates also affect corals (McWilliam et al., 2018). A decline in symbiont numbers may lead to diminished health and widespread mortality of coral hosts, particularly during bleaching events (Hughes et al., 2018). For example, increasing the number of decapod symbionts can enhance the removal of sediment from a coral host (Stier et al., 2012). Another example is the involvement of two or more coral guard-crab species (*Trapezia* spp.), which results in notably superior performance in safeguarding their coral hosts from seastar predators compared to a single one (Mckeon et al. 2012; Mckeon and Moore, 2014). Despite the crucial role that symbiont associations play in benefiting corals, so far, a significant proportion of research has focused on the influence of the assemblages of coral endosymbiotic algae on corals (Ziegler et al., 2018; Swain et al., 2020; Brown et al., 2022).

A single coral colony generally hosts multiple associated invertebrates, including both epifauna and infauna (Baker and Romanski, 2007). However, the mechanisms driving the occurrence of these symbiont assemblages remain largely unknown. Only a few studies have focused on the co-occurrence pattern through both inter- and intraspecific interactions among these coral-associated symbionts (Stier et al., 2012; McWilliam et al., 2022). Additionally, some research has explored the correlation between coral colony size and symbiont assemblage in branching corals (Abele and Patton, 1976; Leray et al., 2012). Considering the high complexity of habitat and niches provided by corals, as well as the high biodiversity of symbionts, existing research has been conducted within a relatively narrow scope.

Climate change poses existential threats not only to coral reefs but also their symbiotic organisms, which in turn affects the assemblage structure of these associates. These external factors can disrupt the healthy and robust symbiotic relationships between hosts and symbionts (Tsuchiya 1999; Tremblay et al., 2014). For example, coral bleaching can impact the physiological state of symbiotic fish and invertebrates, increase intra- and interspecific competition, and decrease the population density of

these coral-associated symbionts (Stella et al., 2014; Froehlich et al., 2021). Consequently, the symbiont assemblage faces underlying super existence challenges. The critical importance of and threats of symbiont assemblage in coral reef ecosystems highlight the urgent need for future related studies.

2. Coral-associated crabs

The brachyuran crabs constitute one of the most diverse groups of invertebrates within coral reef ecosystems, with hundreds of species belonging to numerous families (Glynn and Enochs, 2010). Many of these crabs are associated with scleractinian corals; some are free-living, while others maintain symbiotic relationships with living corals (Casto 1976). These symbiotic brachyurans display specialized forms and functions to varying degrees. Some are known as obligate symbionts, exclusively associated with a specific group of coral hosts and rarely found on non-living substrates. To date, several crab lineages have been identified in obligate association with scleractinian corals. Four families of brachyuran crabs (Cryptochiridae, Domeciidae, Tetraliidae, Trapeziidae) are, with few exceptions, exclusively obligate symbionts. Additionally, some other species from other families, such as Portunidae, Tanaochelidae, and Xanthidae, are also living in obligate association with corals (Ho and Ng, 2005; Spiridonov and Neumann, 2008; Castro, 2015). Cryptochiridae and Domeciidae (excluding *Maldivia* Borradaile, 1902, which associates with gorgonians) are the only two lineages with representatives found in both the Atlantic and Indo-Pacific oceans (Lai et al., 2009; Castro, 2015). This thesis utilized the obligate symbiont Cryptochiridae (gall crabs) as a model taxon to study its evolution and diversification.

2.1 Coral-associated gall crabs

The family Cryptochiridae lives in obligate symbiosis with scleractinian coral hosts, and they cause the formation of galls in the coral skeleton as a result of a modification in coral growth (Simon-Blecher and Achituv 1997; Simon-Blecher et al., 1999). Consequently, these coral-dwelling crabs were named as coral gall crabs (Verrill, 1867). The first species, *Hapalocarcinus marsupialis*, was described by Stimpson in 1859 from Hawaii (Stimpson, 1859). To date, over 50 species of gall crabs, distributed across 21 genera, have been described, with no known fossil species (Bähr et al., 2021; Xu et al., 2022). Cryptochiridae exhibit sexual dimorphism, with adult females generally

surpassing males in size across most species (Castro 1976; Bähr et al., 2021). Adult female cryptochirid crabs spend their entire life within a gall, while males are more mobile, moving around to mate (Castro, 1976; Van der Meij 2014; Vehof et al., 2014). Gall crabs are characterized by their diminutive size, typically ranging from 1 to 6 mm in carapace length (Bähr et al., 2021), and the size of the galls they inhabit is positively correlated with the size of the inhabiting crabs (Simon-Blecher and Achituv 1997). Research has shown that some gall crabs have their preference for consuming coral mucus and tissue (Kropp, 1986; Bravo et al., 2024), but *Hapalocarcinus marsupialis* was observed to be filter feeding (Abelson et al., 1991).

The genus *Opecarcinus* Kropp and Manning, 1987 was established for several species inhabiting Agariciidae corals (Kropp and Manning 1987). To date, ten species have been described from the Indo-Pacific (from the Red Sea to the Pacific coast of Central America) and the Atlantic (Kropp 1989; Van der Meij 2014). It is the only genus that occurs in the tropical and subtropical shallow waters of both the Atlantic and Indo-Pacific. with *Opecarcinus hypostegus* (Shaw and Hopkins, 1977) as the only Atlantic representative (Van der Meij 2014). The extensive distribution of this genus renders it an excellent model taxon for studying global phylogeographic patterns.

2.2 Ecological interaction of gall crabs and coral hosts

Gall crabs are obligate symbionts with scleractinian corals, yet the ecological interaction between them still remains controversial. Existing research on identifying the nature of symbiotic relationship has primarily relied on the settlement behavior and feeding habitats of gall crabs. Based on the results of settlement behavior, gall crabs have been considered as parasites (Simon-Blecher and Achituv, 1997; Simon-Blecher et al., 1999). For example, observations of postlarval and adults of *Cryptochirus coralliodytes* Heller, 1860 [= *Lithoscaptus* sp., identification incorrect, Van der Meij, in prep] settling on the living polyps lead to the death of the polyps. Furthermore, this settlement would modify coral morphology by affecting growth (Simon-Blecher and Achituv, 1997; Simon-Blecher et al., 1999). Additionally, gall crabs may indirectly facilitate the abrasion of the coral skeleton by enhancing the growth of algae occurring around the pits/galls with metabolic excretions, which contain ammonium (Simon-Bleche et al., 1999), suggesting that gall crabs are parasites.

Another reference factor is feeding habits, which also form the basis of the assumption

that gall crabs are parasites. Gall crabs are considered to feed on coral mucus and tissue (Kropp, 1986), rather than filter feed as was suggested by Potts (1915). He suggested that *Hapalocarcinus* feeds on nanoplankton, and later Castro (1976) reported on a reduction in certain mouthparts and the stomach in *Hapalocarcinus*. The third maxillipeds are equipped with abundant setae which characterize filter feeding brachyurans, suggesting that species in this genus are filter feeders. However, Kropp (1986) provided an alternative explanation, suggesting that the fanning movement of the maxillipeds resembling fanning could have been mistakenly interpreted as filter feeding, which might be linked to the unique gall shapes observed in *Hapalocarcinus* (Castro, 1976). Moreover, Kropp (1986) reported that gall crabs from three genera (*Hapalocarcinus*, *Utinomia*, *Cryptochirus*) collect mucus and tissue from the outer surface of the host. Simon-Blecher et al. (1999) presented stronger evidence that *Cryptochirus coralliodytes* (corrected as *Lithoscaptus* sp.) feed on coral products through histochemical examination of the stomach and gut. More recently, Bravo et al. (2024) explored the feeding habits of *Opecarcinus* using isotope analysis and found evidence supporting their consumption of coral mucus. However, corals continuously synthesize and secrete mucus, while these mucus serve as a sediment rejection system. Thus, the consumption of this mucus by cryptochirids does not impose metabolic burden on the host (Kropp 1986; Bravo et al., 2024). To date, many other genera have not been reported, which needs further study.

3. Thesis Outline

So far, four PhD theses have focused on Cryptochiridae as a model taxon, including Fize (1956), Kropp (1988), Zayasu (2014), and Van der Meij (2015). This thesis represents the fifth since these coral-dwelling crabs have been known for over 100 years.

This thesis aims to reveal the diversity and evolutionary history of obligate coral-dwelling gall crabs, with a focus on the genus *Opecarcinus*, and to explore the factors affecting the distribution of symbiont assemblages consisting of gall crabs. Chapter 2 illustrates the biogeographic history and dispersal pathway from the Indo-Pacific to the Atlantic, and identifies a species complex based on species delimitation tests. Chapter 3 further extends the discovery of new species from Chapter 2 by describing 16 new species and further explores the factors that may drive diversification rates. In chapter 4, we assemble and characterize the complete mitochondrial genomes of three gall crab

species. Phylomitogenomics is conducted to demonstrate that symbiotic crabs have evolved independently multiple times, and their symbiotic lifestyle has exerted positive selection pressure on protein-coding genes. Chapter 5 focuses on *Opecarcinus hypostegus* and data from at least 15 other coral-dwelling infaunal symbionts collected during visual surveys off Curaçao. It reveals that the size of non-branching coral does not impact the symbiont assemblage. However, depth plays a significant role in shaping the symbiont assemblage for certain non-branching corals, which differs from the findings observed in branching corals.

In Chapter 2, ‘Diversification and distribution of gall crabs (Brachyura: Cryptochiridae: *Opecarcinus*) associated with Agariciidae corals’, a concatenated gene dataset combining two mitochondrial and one nuclear DNA gene fragments of *Opecarcinus* specimens sampled from the Indo-Pacific and Atlantic was applied in the analysis of this study. Three species delimitation tests were performed to detect putative species. Maximum likelihood (ML) and Bayesian Inference (BI) were employed to construct the phylogenetic tree, alongside a molecular clock to estimate divergence time. The Dispersal-Extinction Cladogenesis model (DEC) was utilized to infer the ancestral area. At least 15 new species were detected, as well as several potential species complexes within *Opecarcinus*. This genus was inferred to have dispersed crossing the Isthmus of Panama to the Atlantic before its complete closure.

In Chapter 3, ‘A global phylogeny and revised taxonomy shed light on the radiation of coral-dwelling *Opecarcinus* crabs (Brachyura: Cryptochiridae)’, integrative taxonomy was employed based on the discoveries in Chapter 2, to describe these new species to science. The Lineages through time (LTT) plots were utilized to assess the patterns of diversity of *Opecarcinus* over time. The Pleistocene glacial cycles were hypothesized to be the factor shaping the current pattern of distribution and diversity. Additionally, this chapter provided the most comprehensive information available on this genus from variable perspectives, including distribution, depth records, and coral hosts.

In Chapter 4, ‘Phylomitogenomics elucidates the evolution of symbiosis in Thoracotremata (Decapoda: Cryptochiridae, Pinnotheridae, Varunidae)’, we assembled and characterized three new mitogenomes of gall crabs. Phylomitogenomics was employed to infer the evolutionary relationships among these symbiotic lineages within Thoracotremata, based on the mitogenomes. We also conducted analyses on selection

pressure to test whether adaptation to a symbiotic lifestyle exerted positive selection on protein-coding genes.

In Chapter 5, ‘Coral colony size and depth as drivers of coral-dwelling symbiont assemblages in the Caribbean’, field surveys were conducted in Curaçao by performing visual assessments on three non-branching corals (*Porites astreoides*, *Siderastrea siderea*, and *Agaricia agaricites*) at two depths (6 m and 15 m) along the leeward side of the island. The species and abundance of symbionts were documented for each colony, with a total of 4,969 symbionts (>15 species) recorded from 585 coral colonies. Analytical results indicated that the abundance of coral-associated fauna was not correlated with colony size in non-branching corals; however, depth significantly influenced their distribution in certain corals.

Chapter 6 summarized the main findings of the thesis and discussed these results within the context of the diversification and diversity of coral-dwelling crabs. Future research directions have been outlined to elucidate the mechanisms driving the robust symbiotic relationship between coral hosts and gall crabs.

