

# **Title: Diversity and distribution of algal symbionts (*Symbiodinium* spp.) in reef-building corals in the Gilbert Islands**

**Authors: Stephen Manley, Simon Donner, Andrew C. Baker**

## **1. Summary**

In 2004, the Gilbert Islands of the Republic of Kiribati experienced a mass coral reef bleaching event. This event drastically altered the reef communities in the Gilbert Islands, by increasing the relative abundance of thermally tolerant coral species, and decreasing the abundance of intolerant species. In 2009, the diversity of algal symbionts (genus *Symbiodinium*) in reef corals from these reefs was assessed to provide a baseline to monitor future changes in these communities as a result of projected climate change. Several novel types of *Symbiodinium* in clade C were found, as well as relatively frequent occurrence of clade A, which is usually absent from the equatorial western Pacific. The symbiont community structure in coral taxa from these reefs may also help explain the patterns of mortality documented during the 2004 mass bleaching event, and will be of use in interpreting regional patterns of symbiont distribution.

## **2. Introduction**

Cnidarians are host to obligate dinoflagellate algae of the genus *Symbiodinium*. These symbionts are critical to the survival of scleractinian corals, as they provide a major source of energy for their hosts (Muscatine and Porter 1977). However, environmental stress, particularly elevated temperatures, can cause the coral to expel their *Symbiodinium* in a process known as “bleaching” (Hoegh-Guldberg 1999). If the stress is widespread, it can lead to mass mortality of the coral hosts and collapse of reef ecosystems (Baker 2003).

To date, most research on this issue has focused on the ability of corals to associate with different algal symbionts, whose different physiologies may impart resistance to thermal stress (Buddemeier and Fautin 1993; Rowan *et al.* 1997; Baker 2003; Baker 2004; Berkelmans and van Oppen 2006; LaJeunesse *et al.* 2009; LaJeunesse *et al.* 2010). In particular, members of *Symbiodinium* clade D (principally D1 and D1a) have been shown to confer resistance to increased temperatures (Baker *et al.* 2004). In contrast, members of the *Symbiodinium* clade A are thought to be more tolerant of cold, due to the fact that they are commonly found at higher latitudes (Baker 2003). However, there is increasing evidence that some members of clade A (particularly type A1) may also have the ability to withstand increased temperatures (Suggett *et al.* 2008).

The reefs around the equatorial island atoll nation of Kiribati lie in the isolated and relatively understudied central Pacific. In 2004, the Gilbert Islands experienced a mass bleaching event, which greatly reduced the coral cover and diversity in this region (Donner *et al.* 2010). Subsequent surveys have found that thermally tolerant coral species have begun to dominate recovering reefs in the area (Donner *et al.* 2010). As recovery from this event continues, monitoring algal symbiont as well as coral communities of these reefs will help us understand their survival trajectories under future climate change conditions.

## **3. Methods**

### *3.1 Field Collections*

Replicate (n=5) tissue samples of common coral species were collected in May, 2009 from Abaiang and Tarawa Atolls during benthic monitoring activities. All samples were collected haphazardly at between 5 – 10 m depth via random swims (using dive gear) or repeated free dives (when gear was not available). Fragments of roughly 2 cm length were broken from living coral colonies using a chisel with branching colonies or a hammer and chisel on massive and encrusting colonies. The samples were brought onto the boat and placed in 2 ml labelled vials and preserved with saline DMSO.

### 3.2 Molecular Methods

*Symbiodinium* DNA was extracted from preserved samples using an organic extraction protocol (Baker *et al.* 1997). The Internal Transcribed Spacer-2 (ITS-2) region of ribosomal DNA (rDNA) was PCR-amplified using the *Symbiodinium* specific primers ITSfor2 and ITS2revclamp (LaJeunesse 2001). The following PCR profile was used: 94°C for 3 min to denature the DNA, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 74°C. The program finished with a single cycle of 74°C for 7 min. Amplification success was determined using agarose gel electrophoresis. After successful amplification, the samples were separated using denaturing gradient gel electrophoresis (DGGE) with a gradient of 35% to 70% (Sunnucks *et al.* 2000). Both prominent and unique bands were excised from gels and re-amplified using ITS2for2 and ITS2rev primers, using the same program as described above. Amplified bands were then sequenced using the Big Dye Terminator version 3.1 protocol. The resulting sequences were first identified to the clade level using BLAST searches on GenBank. The sequences were then assembled, arranged, and aligned by clade using Geneious 4.8.5. Pairwise alignments were performed using MUSCLE with a maximum of 8 iterations. Multiple alignment parameters included a Geneious alignment of 65% similarity (5.0/-4.0), with a gap penalty of 16, a gap extension penalty of 3, and a maximum of 8 iterations. The alignments were then trimmed to the shortest sequence and checked against a database of known clade types (Correa and Baker 2009). Finally, TCS version 1.21 was used to group the samples according to type, with 95% confidence (Clement *et al.* 2000).

## 4. Results

Reef corals in the Gilbert Islands most commonly associated with algal symbionts belonging to *Symbiodinium* clade C, which dominated 87% of colonies. Clades D and A were also present, although uncommon, dominating 8% and 1% of colonies, respectively. A small proportion of colonies contained communities of *Symbiodinium* in clade C mixed with either clade A (2%) or clade D (2%). At the level of resolution provided by DGGE, no colonies contained both A and D. The two islands sampled (Tarawa and Abaiang) had a very similar clade distribution (Fig. 3). However, the island of Tarawa had the most diverse assemblage of symbiont types and was the most heavily sampled island (Fig. 4).

More than one quarter of the samples (all corals in the family Poritidae, as well as some Acroporidae) contained only type C15 (at the level of resolution provided by DGGE). Consequently, this was the most common *Symbiodinium* type found in the Gilbert Islands (Fig. 1). Three closely-related variants of C15 were also found, none of which have been reported from elsewhere (Fig. 1). Other relatively common *Symbiodinium* types included C1, C3, C40, and C1c. No novel types of clades A or D were found. The members of the coral family Acroporidae hosted the most diverse assemblage of symbiont types, and was the only family to contain symbionts from clade A, which were all type A1 (Fig. 2).

## 5. Discussion

Three novel types of *Symbiodinium* were found in the Gilbert Islands. While all of these types are previously undescribed, they were all clearly closely related to the common type C15. One of these novel types (C15 $\alpha$ ) has not been found anywhere else, while the other two types (C15 $\beta$  and C15 $\epsilon$ ), have also been found in the Phoenix Islands), where they appear to be more common (Manley, Obura, Baker, unpubl. data). The presence of C15 $\beta$  and C15 $\epsilon$  in both the Gilbert and Phoenix Islands, which are separated by over 1,000 km, suggests these islands are not completely isolated from each other. However, there were five other novel symbiont types seen in the Phoenix Islands that were not found in the Gilbert Islands, indicating that contact between these two systems is minimal (Manley, Obura, Baker, unpubl. data).

Four samples of corals in the family Acroporidae hosted symbiont type A1. *Symbiodinium* from clade A are usually found at high latitudes, and this is the first time that this symbiont type has been found close to the equator. This type is usually only found in the Caribbean, but it has been reported in the Pacific at higher latitude reefs such as Hawaii (~20°N, Stat and Gates 2008) and Johnston Atoll (16°N, Stat *et al.* 2009). Since this type is considered to be more thermally tolerant, it may be able to persist in the reefs of the Gilbert Islands (Suggett *et al.* 2008). However, the low abundance of this symbiont type may suggest that it is not well suited to lower latitude reefs. Further studies over successive years may be able to determine how common this symbiont type is on the reefs of the Gilbert Islands.

The proportion of *Symbiodinium* belonging to clade D was low. Three of the coral families sampled (Acroporidae, Agariciidae, and Poritidae) did not contain any symbionts in this clade. This is surprising given the relatively warm temperatures in the region, as well as recent bleaching (Donner *et al.* 2010). However, this may help explain why the acroporid corals suffered a disproportionate amount of mortality in the 2004 bleaching event (Donner *et al.* 2010). One hypothesis why this clade is not as common as expected, is that the low SST variability of SSTs does not favor long-term persistence of D in these corals, although availability of D might also be a limiting factor.

## **6. Further Research**

The novel symbionts found in this study suggest the relative isolation of the Gilbert Islands may have resulted in a number of endemic symbiont types. Further research may establish what role these symbionts play in the local reef ecosystems. The low abundance of thermally tolerant clade D in the Gilberts, as well as the discovery of *Symbiodinium* type A1 in the Gilbert Islands can also be addressed in future studies. Finally, additional surveys of these reefs are needed to monitor *Symbiodinium* communities and track changes that may occur as climate change continues.

## **Acknowledgements**

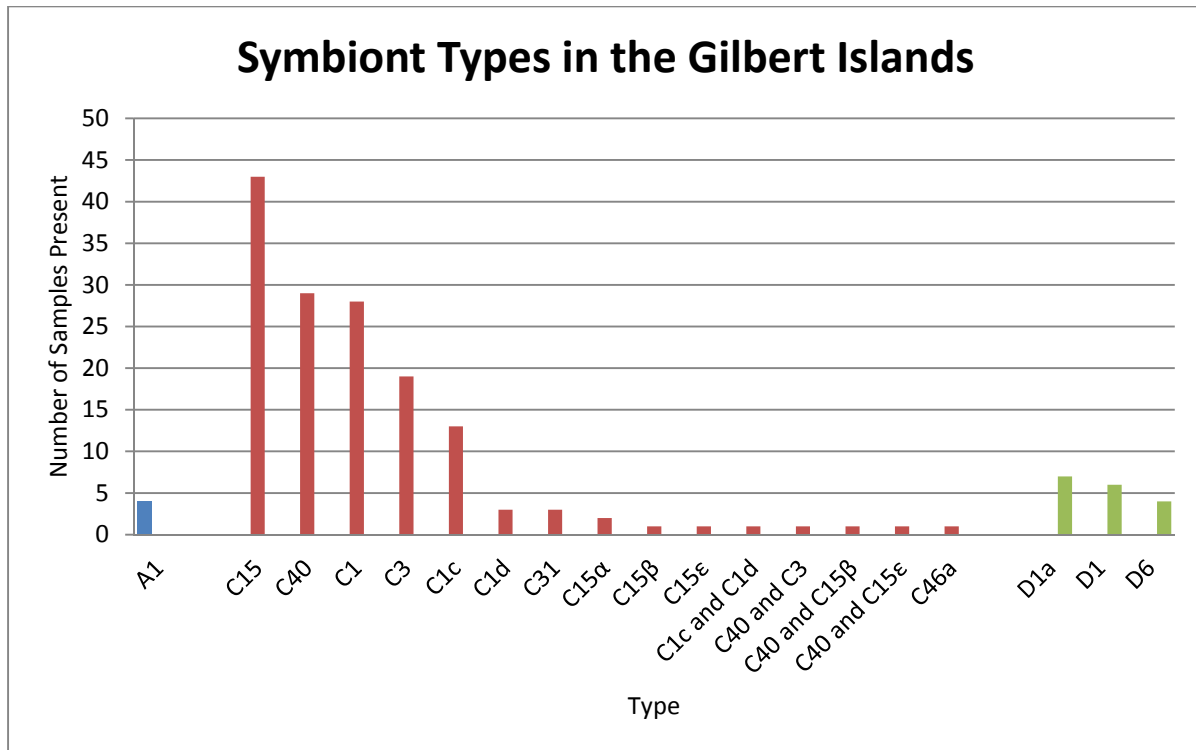
This research would not have been possible without the support of the government of the Republic of Kiribati. The authors thank H.H. Wirshing and R.K. Boonstra for their assistance and contributions in the lab, and the Lenfest Ocean Program for funding the laboratory component of the work.

## **References**

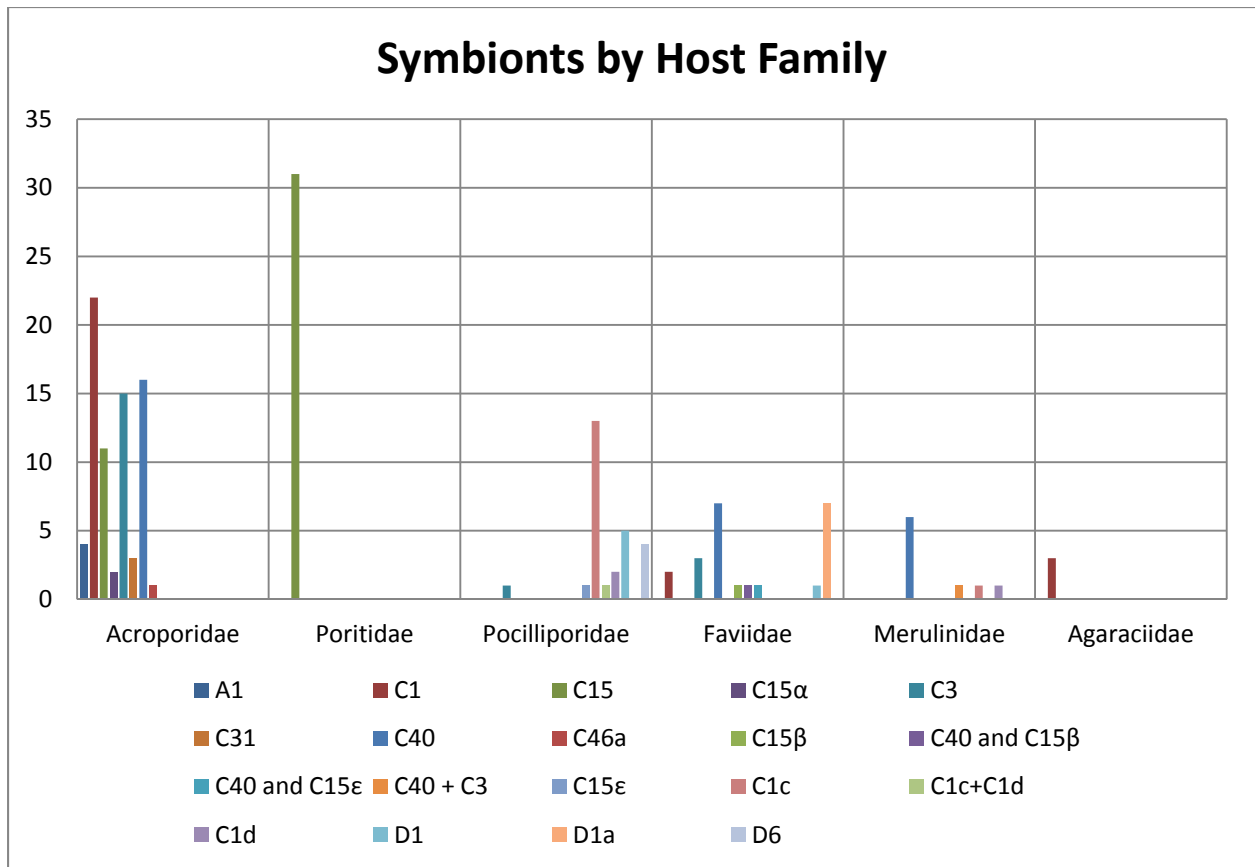
Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: Diversity, ecology and biogeography of *Symbiodinium*. *Ann. Rev. Ecol. Evol. Syst.* 34: 661-689.

- Baker AC (2004) Symbiont diversity on coral reefs and its relationship to bleaching resistance and resilience. In: Rosenberg E, Loya Y (eds) Coral Health and Disease. Springer-Verlag, Berlin, New York, pp 177-194.
- Baker AC, Rowan R, and Knowlton N (1997) Symbiosis ecology of two Caribbean acroporid corals. Proceedings of the 8th International Coral Reef Symposium, Panama 2: 1295-1300.
- Baker AC, Starger CJ, McClanahan TR, and Glynn PW (2004) Corals' adaptive response to climate change. Nature 430: 741-741.
- Berkelmans R and van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. Proceedings of the Royal Society B-Biological Sciences 273: 2305-2312.
- Buddemeier RW and Fautin DG (1993) Coral bleaching as an adaptive mechanism - a testable hypothesis. Bioscience 43: 320-326.
- Clement M, Posada D and Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657-1659.
- Correa AMS and Baker AC (2009) Understanding diversity in coral-algal symbiosis: a cluster-based approach to interpreting fine-scale genetic variation in the genus *Symbiodinium*. Coral Reefs 28: 81-93.
- Donner SD (2009) Coping with Commitment: Projected thermal stress on coral reefs under different future scenarios. PLoS One 4: e5712.
- Donner SD, Kiriata T and Vieux C (2010) Recovery from the 2004 coral bleaching event in the Gilbert Islands, Kiribati. Atoll Res. Bull. 587: 1-25.
- Hoegh-Guldberg, O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research 50: 839-866.
- LaJeunesse TC, Smith RT, Finney J and Oxenford H (2009) Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral 'bleaching' event. Proceedings of the Royal Society B-Biological Sciences 276: 4139-4148.
- LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, Obura DO, Hoegh-Guldberg O and Fitt WK (2010) Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. Journal of Biogeography 37: 785-800.
- LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the internal transcribed spacer region: in search of a "species" level marker. J Phycol 37: 866-880
- Muscantine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27: 454-460.
- Rowan R, Knowlton N, Baker AC and Jara J (1997) Landscape ecology of algal symbiont communities explains variation in episodes of coral bleaching. Nature 388: 265-269.
- Stat M and Gate RD (2008) Vectored introductions of marine endosymbiotic dinoflagellates into Hawaii. Biol Invasions 10: 579-583.
- Stat M, Pochon X and Cowie ROM, Gates R D (2009) Specificity in communities of *Symbiodinium* in corals from Johnston Atoll. Mar. Ecol. Prog. Ser. 389: 83-96.
- Suggett DJ, Warner ME, Smith DJ, Davey P, Hennige S and Baker NR (2008) Photosynthesis and production of hydrogen peroxide by *Symbiodinium* (Pyrrophyta) phylotypes with different thermal tolerances. J Phycol 44: 948-956.
- Sunnucks P, Wilson ACC, Beheregaray LB, Zenger K, French J and Taylor AC (2000) SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Molecular Ecology 9: 1699-1710.

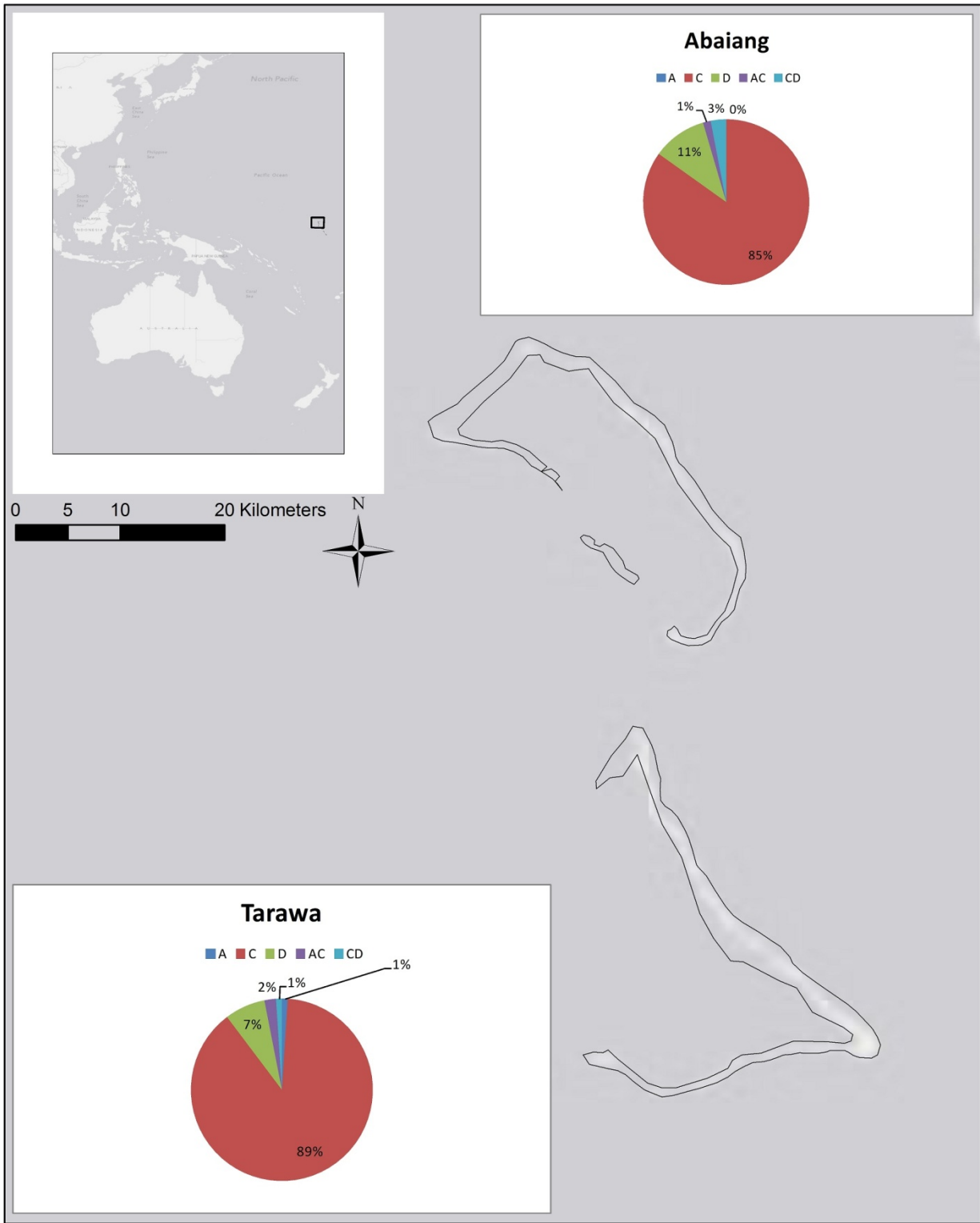
## Figures



**Figure 1:** A summary of the types and amount of symbionts found in coral samples from the Gilbert Islands.



**Figure 2:** The types of symbionts found in different host coral families from the Gilbert Islands.



**Figure 3:** Distribution of algal symbionts (at the level of *Symbiodinium* clade) in reef corals from Tarawa and Abaiang in the Gilbert Islands. Mixed clades of A+C and C+D are also shown.