Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of Western Australia

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ABSTRACT: The identity and diversity of algal symbionts (*Symbiodinium* spp.) in reef corals is thought to influence to the resilience of reef ecosystems to climate change, and varies depending on coral species, environmental conditions, and biogeography. We examined these factors by surveying corals along a latitudinal gradient in Western Australia on reefs connected north to south by the warm-water Leeuwin Current. We used the internal transcribed spacer-2 (ITS-2) region of ribosomal DNA to characterize *Symbiodinium* communities in 19 species of reef coral (in 16 genera) from tropical Dampier (20.5° S) to temperate Dunsborough (33.5° S). We documented a high number of novel ITS-2 types in *Symbiodinium* clades B (1 type) and C (14 types) as well as 7 previously reported ITS-2 types in clades B, C, and D. In addition, we compared symbiont distributions with Giovanni's OBIG MODIS-Aqua satellite temperature data set and found that the putatively thermotolerant *Symbiodinium* ITS-2 type D1a was more frequently detected in 'chronically warm' tropical sites than at 'chronically cool' temperate sites, while clade B symbiont types showed the reverse pattern, being found in certain corals at the southernmost sites. *Symbiodinium* type D1a was generally most abundant at Dampier, where bleaching had occurred 1 mo prior to sampling, although some variation by host taxa was observed. The diverse and novel *Symbiodinium* communities documented here may be a result of (1) the variable environmental histories of the study sites, (2) the apparent genetic divergence within this genus resulting from the relative isolation of Western Australian reefs, and/or (3) the frequent transport of symbionts (*in hospite* or free-living) from Indo-Malay reefs to Western Australia via the Leeuwin Current. These results indicate that understudied reefs in remote and isolated areas may contain *Symbiodinium* diversity that has not been reported previously.

KEY WORDS: Corals · Scleractinia · Denaturing gradient gel electrophoresis · DGGE · Internal transcribed spacer-2 region · ITS-2 · Latitudinal gradient · *Symbiodinium* · Western Australia · Reefs

INTRODUCTION

Symbiotic associations between corals and dinoflagellate algae (genus *Symbiodinium*) underpin the high levels of productivity characterizing coral reefs (Muscatine & Porter 1977). However, when exposed to stressful environmental conditions, coral-algal symbioses can break down, resulting in 'bleaching' (the loss of algal cells and/or pigments; reviewed in Glynn 1996). If bleaching is prolonged or severe, colony mortality can result (Brown 1997, Goldberg & Wilkinson 2004). Biogeographic patterns in coral distribution cor-

relate with prevailing temperature regimes, indicating that this environmental factor strongly influences coral-algal mutualisms (Veron 1995) and their susceptibility to bleaching (Coles et al. 1976). In addition, high sea-surface temperatures (SSTs) have been implicated in all episodes of mass bleaching documented to date (Glynn 1996, Baker et al. 2008). Given that climate change is expected to increase the severity and frequency of thermal anomalies in the coming decades (Hoegh-Guldberg 1999, Hughes et al. 2003, Donner et al. 2005), there is considerable uncertainty regarding the capacity of corals to adapt or acclimatize at pace with projected rates of climate change (e.g. Hoegh-Guldberg et al. 2007), and the potential role(s) of algal symbiont diversity in this process (e.g. Baker et al. 2004, Berkelmans & van Oppen 2006, Sampayo et al. 2008, Correa & Baker 2011).

Scleractinian corals typically associate with *Symbiodinium* in 4 sub-generic clades, A, B, C, and D (reviewed in Baker 2003), while symbionts in clades F and G are only rarely harbored by stony corals (Rodriguez-Lanetty et al. 2004, LaJeunesse et al. 2010a). Numerous sub-clade 'types' have also been identified within each *Symbiodinium* clade, most frequently using the internal transcribed spacer-2 (ITS-2) region of a multi-copy ribosomal DNA (rDNA) gene family (e.g. LaJeunesse 2002). Studies have shown that functional traits can differ among symbiont types both *in hospite* and in culture, particularly with respect to their temperature optima (Iglesias-Prieto et al. 1992, Warner et al. 1999, Sampayo et al. 2008; Thornhill et al. 2008, Hennige et al. 2009), response to thermal stress, and influence on the relative bleaching susceptibility/ resistance of their scleractinian hosts (Rowan et al. 1997, Glynn et al. 2001, Rowan 2004, Goulet et al. 2005, Berkelmans & van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2010b).

Coral species vary in the potential and realized diversity of symbionts that they associate with. In a given coral host, local environmental conditions (e.g. temperature, irradiance), symbiont availability, biogeography, and/or stress history (reviewed in Baker 2003) can all influence which *Symbiodinium* taxa numerically dominate a colony. Corals living at latitudinal extremes are particularly interesting in this respect, because they experience intense seasonal environmental changes which may promote diverse symbiont communities. Western Australian reefs span transitional (tropical to temperate) reef coral environments, and are further extended into high latitudes by the warm-water Leeuwin Current (an offshoot of the Indonesian Throughflow), allowing coral colonies to survive in areas where low temperature might otherwise be prohibitive (Cresswell 1991, Caputi et al. 1996, Wijffels et al. 1996). Scleractinian coral generic and

species richness decreases with latitude from 57 genera (216 species) at Dampier, to 4 genera (7 species) at Dunsborough (Veron & Marsh 1988, our Table 1). A latitudinal sampling strategy is therefore more likely to capture important intermediate or high *Symbiodinium* diversity zones along Australia's western coast (if they exist). Surveys of coral-algal symbioses from Western Australia can also be compared with similar latitudinal comparisons from other geographic regions (e.g. Baker 1999, Loh et al. 2001, Rodriguez-Lanetty et al. 2001, van Oppen et al. 2001, 2005, Savage et al. 2002, Macdonald et al. 2008, LaJeunesse et al. 2010a) to corroborate hypothesized biogeographic patterns in the distribution of *Symbiodinium* diversity, and to determine where exceptions to these patterns arise, and why.

MATERIALS AND METHODS

Field methods. Mixed samples of coral tissue, skeleton, and resident symbiont communities were collected from 19 species of scleractinian coral host (in 16 genera) during the austral winter season between 30 May and 11 June 2005 (with the exception of the Houtman-Abrolhos Islands, which were sampled in July 2005) at 5 locations along the coast of Western Australia. Sampled locations were tropical (Dampier: 20° 32′ S, 116° 38′ E; Ningaloo Reef: 23° 08′ S, 113° 46′ E), or temperate (Abrolhos Islands: 28°16′S, 113° 35′ E; Rottnest Island: 32° 00′ S, 115° 31′ E; Dunsborough: 33° 36′ S, 115° 05′ E) (see Fig. 3, Table 1). Average monthly SSTs for each site between July 2002 and June 2005 were obtained from the GES-DISC Interactive Online Visualization ANd aNalysis Infrastructure (Giovanni; http://disc.sci.gsfc.nasa.gov/giovanni), which is part of NASA's Goddard Earth Sciences (GES) Data and Information Services Center (DISC). The averages for each month were then averaged between the same month for 2002, 2003, 2004 and 2005. Average yearly temperature ranges as well as average temperatures for the months immediately prior to sampling (March through June 2005) are reported in Table 1. Specifically, SSTs were acquired from Giovanni's OBIG MODIS-Aqua Monthly Global standard mapping images (9 km resolution), through the Ocean Biology Processing Group. Although the temperatures reported here represent those at the sea surface rather than at the depth of sampling, satellite-derived SSTs are common proxies for temperatures experienced by corals at relatively shallow depths, and have been used to accurately predict bleaching events (e.g. Jokiel & Coles 1990, Goreau & Hayes 1994, Strong et al. 1997, Berkelmans 2002, McClanahan et al. 2007).

All sample collections were made using SCUBA at <7 m depth, except in Dunsborough, where the maxi-

Table 1. General information on study sites including geographic coordinates, number of scleractinian coral genera and species previously documented (Veron & Marsh 1988), average (avg.) sea surface temperature (SST) (averaged from March to June 2005), average yearly SST range (monthly average SSTs, averaged over 2002–2005), and known bleaching history (from 2002 to 2005; www.reefbase.org)

Site	Coordinates	No. of genera and spp.	Avg. SST (°C)	Avg. SST range (°C)	Bleaching
Dampier	20° 32′ S, 116° 38′ E	57 and 216	30	24–30	Moderate
Ningaloo	23° 08′ S, 113° 46′ E	54 and 217	27	22–27	None
Abrolhos	28° 16′ S, 113° 35′ E	42 and 184	23	19–23	None
Rottnest	32° 00′ S, 115° 31′E	16 and 25	22	18–22	None
Dunsborough	33° 36′ S, 115° 05′ E	4 and 7	21	17–21	None

mum collection depth was 15 m. Samples were collected using a hammer and hollow steel punch (<1 cm^2 diameter) penetrating the coral skeleton deeply enough to capture all tissue layers (~3 to 6 mm deep). Samples were taken only from the tops of healthy colonies, including at Dampier, where moderate bleaching (11 to 30% of corals affected; www.reefbase. org) was reported approximately 1 mo prior to sampling (www.reefbase.org, D. Blakeway pers. comm.). Corals that were heavily shaded or overtopped by other colonies were not sampled, nor were colonies at Dampier that appeared to have experienced bleaching (i.e. showed signs of abnormal pigmentation) in June 2005. Colonies were identified based on the taxonomy of Veron (2000). To minimize the likelihood of repeatedly sampling corals that were the same genetic individual, only conspecifics separated by >10 m were sampled. Collected tissue was preserved for DNA analysis in either 95% ethanol or saline DMSO buffer (20% (v/v) DMSO, 250 mM EDTA, saturated NaCl) (Seutin et al. 1991).

Molecular methods. DNA was extracted from each sample using an organic extraction protocol (Baker et al. 1997). The ITS-2 region was amplified from each sample using the *Symbiodinium*-specific primers 'ITSintfor2' and 'ITS2clamp' (LaJeunesse & Trench 2000) with the following profile: an initial denaturing step of 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 74°C, followed by a single cycle of 7 min at 74°C. Products were electrophoresced on 1.2% agarose gels to check for amplification success. *Symbiodinium* amplicons were then separated using denaturing gradient gel electrophoresis (DGGE, 35 to 75% gradient; Sunnucks et al. 2000) on a CBS Scientific system. Prominent bands characteristic of unique profiles (as described by LaJeunesse 2002) were excised, bead-beaten, and re-amplified using the same primer set (without the GC clamp) under the conditions described above. Sequencing

was performed using a Big Dye Terminator v. 3.1 cycle sequencing kit and an Applied Biosystems 3730xl DNA Analyzer.

Sequences were assembled and edited using the Vector NTI™ Advance 10 software (Invitrogen) and then identified using BLAST searches of GenBank to determine the most similar known *Symbiodinium* type, cf. LaJeunesse (2001). To verify GenBank identifications, sequences were grouped according to clade, and aligned with a database of known *Symbiodinium* diversity for that clade using ClustalX version 2.0 (Thompson et al. 1997), as described in Correa & Baker (2009). Pairwise alignment parameters included a gap opening of 10.00 and a gap extension of 0.10. Multiple alignment parameters included a gap opening of 15.00 and a gap extension of 0.30 (Hall 2001). Clade-level alignments were corrected manually and trimmed to the length of the shortest sequence. Final alignments (including gaps) for *Symbiodinium* clades B, C, and D were 322, 340, and 246 base pairs long, respectively. TCS version 1.21 (Clement et al. 2000) was then used to group identical sequences within each clade-level alignment and to produce statistical parsimony networks with 95% certainty. Gaps were treated as a fifth character state. Sequences within the same TCS-defined 'haplotype' were all scored as the same *Symbiodinium* type; each 'haplotype' took the name of the lowest alphanumeric *Symbiodinium* type (cf. LaJeunesse 2001) that it contained.

GenBank and TCS-derived identifications of sequenced samples were further checked through comparisons of their DGGE banding patterns with those of *Symbiodinium* ITS-2 types in the published literature. Finally, the *Symbiodinium* ITS-2 types within non-sequenced samples were identified by matching their DGGE banding patterns to those of sequenced samples on the same gel. All sequences deemed 'novel' had a distinct and reproducible DGGE banding pattern with a clearly dominant band and associated minor banding fingerprint.

Statistical analyses. Average monthly temperatures (and their standard deviations) were calculated for each site based on temperature data for 2002 to 2005 downloaded from the Giovanni online data system. Since these data passed normality and equal-variance tests, a 1-way ANOVA was used to determine whether sites differed in their average monthly temperatures, using SigmaStat 3.0 (www.spss.com). The Holm-Sidak method was used to perform multiple pairwise comparisons.

The abundance of *Symbiodinium* in clade D was compared (within a given host species) between sites

and latitudes using Fisher's exact tests. These comparisons involved either (1) specific host species between individual sites, (2) multiple host species in sites that were collectively 'chronically warm' (Dampier and Ningaloo Reef; mean temperature range: 27 to 30°C) versus 'chronically cool' (Abrolhos Islands, Rottnest Island, and Dunsborough; mean temperature range: 21 to 23°C) (see Fig. 4), or (3) multiple host species in sites with different bleaching histories.

Since few of these host taxa were observed at multiple sites, analyses of the distribution of clade D necessitated comparisons of different host taxa (from different sites). A caveat of this approach is that it assumes that all host taxa associate with *Symbiodinium* in clade D with equal facility. To help correct for this, Fisher's exact tests were therefore conducted for each location using only coral species that were known to be capable of hosting clade D symbionts, based on detections from conspecifics in the present study and (as reviewed in Baker & Romanski 2007) Baker (1999), Fabricius et al. (2004), Chen et al. (2005), Visram & Douglas (2006), Mostafavi et al. (2007), and Abrego et al. (2009). To be conservative, only clade D detections based on conventional molecular techniques were considered for these comparisons; real-time PCR detections were excluded. Coral species included in these tests were therefore *Acropora latistella*, *Echinopora lamellosa*, *Favites abdita*, *Galaxea fascicularis*, *Goniastrea aspera*, *Goniastrea australiensis*, *Hydnophora rigida*, *Lobophyllia hemprichii*, *Pocillopora damicornis*, *Stylophora pistillata*, *Turbinaria mesenterina*, and *T. reniformis*.

RESULTS

DGGE banding patterns

Many of the DGGE banding patterns produced by Western Australian coral samples were complex (i.e. contained many minor bands), particularly within clade C (Fig. 1a–c). From such samples, numerous bands were excised in order to determine the number of types present, and which bands were intragenomic variants, heteroduplexes, or non-target amplifications (i.e. host sequence). Complex profiles were especially common for *Symbiodinium* ITS-2 type C1 and co-dominant paralogs (i.e. C1c–ff, C1gg, C1gg–hh). Frequently, bands within C1 profiles were sequenced and were found to have associated band sequences that differed by a few base pairs. To capture the full breadth of sequence variation present in this region, therefore, these sequences were submitted to GenBank and differentiated by lower case letters (denoting co-dominant paralogs) according to LaJeunesse (2002) (our Table 3).

Symbiodinium diversity and distribution

Distribution of *Symbiodinium* ITS-2 types in Western Australia

Of the 174 samples analyzed, 24 distinct *Symbiodinium* ITS-2 types were identified from clades B, C, and D within 19 different coral species, representing 16 genera (Table 2). Sixteen novel *Symbiodinium* ITS-2 types (1 in clade B and 14 in clade C) were identified based on their distinct DGGE banding profiles and sequences, including 1 variant profile representing D1a (Fig. 1, Table 3). Of these novel sequences, *Symbiodinium* ITS-2 types C124 and C128 appear most divergent: they could not be connected to the statistical parsimony network with 95% certainty (Fig. 2b), although C124 could be connected with 93% certainty (with 93% certainty, this type was 8 base-pair changes from both the C3 and C1ff types; data not shown). Novel types C125 and C126 harbored by *Pocillopora damicornis* colonies appear to be closely related to one another, yet are highly divergent from the ancestral C1 type and most other known types. Novel types C123 and C127 also differ from other C-types by at least 5 fixed base-pair changes each (Fig. 2b). In the clade B statistical parsimony network, B40 could not be connected to other known types with 95% statistical support (Fig. 2a), nor with as low as 90% statistical support (data not shown). In a separate maximum parsimony clade B tree generated from a similar sequence data set, type B40 was also placed in its own branch (data not shown).

The most frequently detected algal types in all of the coral communities sampled were in *Symbiodinium* clade C (Fig. 3, Table 2). ITS-2 variants of type C1 (i.e. C1 and accompanying diagnostic paralogs) were found in 50% of all samples from the region, when all host species are considered (n = 87 of 174; Fig. 3, Table 2). *Symbiodinium* ITS-2 types B18 and B40 were restricted to temperate regions (Rottnest Island and Dunsborough; n = 17 of 63 colonies, 26.7%; Fig. 3, Table 2), and were mainly hosted by corals observed only at these temperate sites: B40 associated with *Coscinaraea marshae*, *C. mcneilli*, and B18 associated with *Plesiastrea versipora* (Table 2). While B18 has been documented previously (A. C. Baker & T. C. LaJeunesse unpubl. data; GenBank accession number: AY258471.1), the present study is the first published report in which it appears. ITS-2 type D1a was the only clade D symbiont detected from Western Australian reefs, although an unusual variant D1a profile was detected from 1 colony of *Pocillopora damicornis* from Dampier (Fig. 1d). D1a was primarily found in the tropics, at Dampier and Ningaloo Reef, and most commonly in *Galaxea fasci-*

cularis, *Favites abdita*, and *Goniastrea aspera* (Table 2).

The present paper represents the first report of *Symbiodinium* type C7 from the Indo-Pacific; however, sequence analysis of the hyper-variable non-coding region of the chloroplast *psbA* minicircle indicates that this may be due to homoplasy, with the C7 in Western Australia having a distinct evolutionary history from the Caribbean C7 (data not shown). ITS-2 types C7 and C7d were only identified in Dunsborough, suggesting that these may be low-temperature or low-irradiance specialists, although several of the host species from which these types were identified were only encountered during sampling in Dunsborough (*Coscinarea mcneilli* [n = 1] and *Turbinaria reniformis* [n = 2]; Fig. 3, Table 2). However, *Symphillia wilsoni* hosted C7 at Dunsborough (n = 3) but contained the novel C type C1gg at Rottnest Island (n = 4) (Fig. 3, Table 2), supporting the notion that C7 and its variant C7b may be optimal in lower-latitude environments.

Similar to *Symphillia wilsoni*, *Pocillopora damicornis* also hosted different clade C symbionts at different sites. In northern locations (Dampier and the Ningaloo Reef), sampled colonies hosted C1h (n = 25), while only novel C-types were observed at the more southerly Abrolhos and Rottnest Islands (Abrolhos Islands: C125 [n = 2], C126 [n = 5]; Rottnest Island: C1c-ff [n = 11]; Table 2).
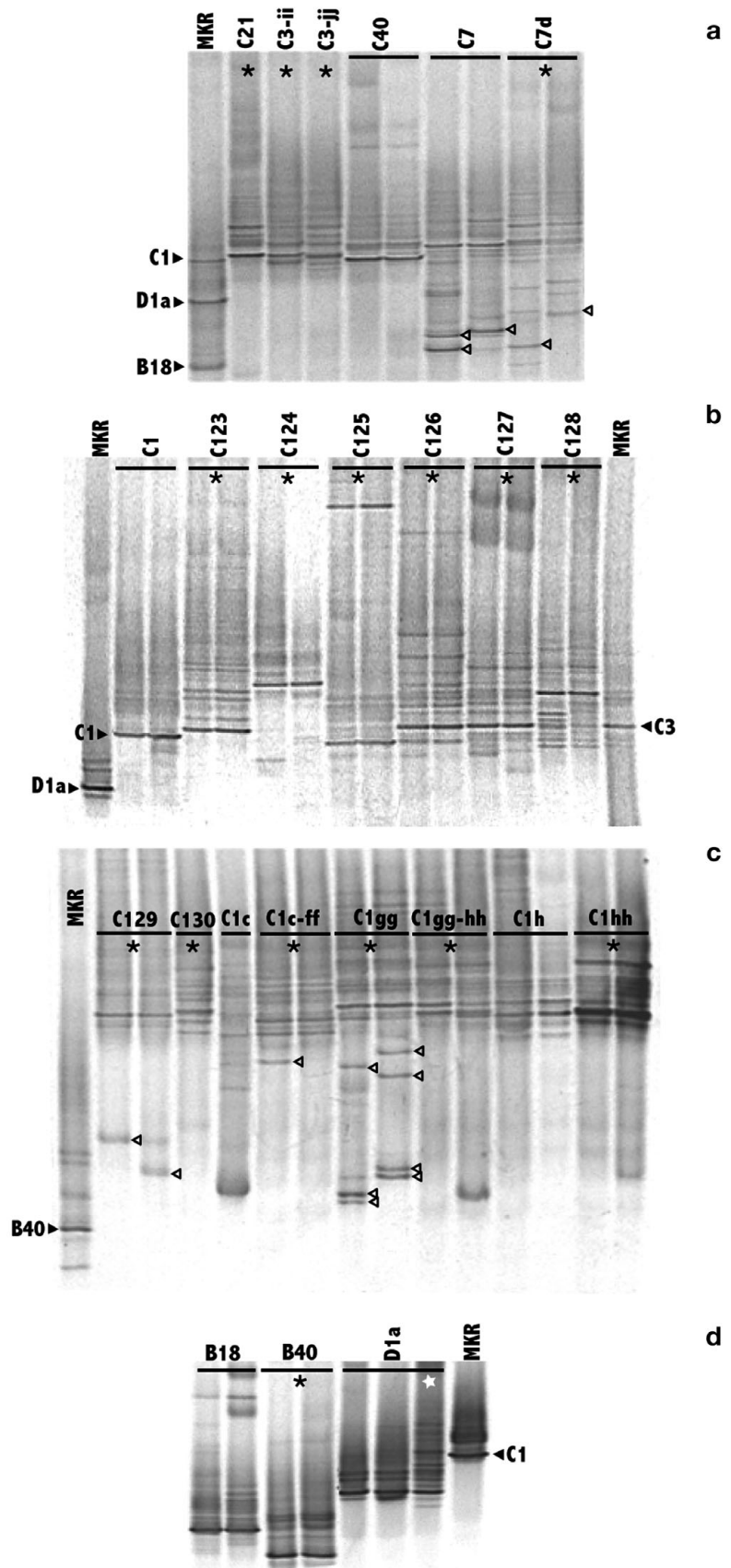

Fig. 1. Negative images of denaturing gradient gel electrophoresis (DGGE) profiles for the *Symbiodinium* internal transcribed spacer-2 (ITS-2) types collected in Western Australia. Alphanumeric designation for each symbiont type is given above the corresponding fingerprint profile: uppercase letters indicate clade, numbers represent ITS-2 type, and lowercase letters (if present) denote the presence of an rDNA paralog (i.e. co-dominant intragenomic variants that are a diagnostic part of the fingerprint). ✱: Types that are novel (i.e. previously unreported). Representative banding patterns for detected *Symbiodinium* types in (a–c) clade C and (d) clades B and D. Open arrowheads indicate bands that sequenced as coral host. Solid arrowheads indicate known ITS2 type marker sequences. The white star denotes an unusual D1a banding pattern identified in 1 sample of *Pocillopora damicornis* from Dampier

Table 2. Site, coral host species, and *Symbiodinium* identity for samples collected from Western Australia, as well as the number of times a particular coral-*Symbiodinium* combination was observed. *Coral species from which algal symbionts have not previously been identified, or coral-*Symbiodinium* combinations that have not been reported previously (based on Visram & Douglas 2006 and data in Baker & Romanski 2007)

Site (no. colonies sampled site^{-1})	Coral species	*Symbiodinium* Clade	ITS-2 type	No. observed
Dampier (n = 27)	*Acropora latistella*	C	C3ii	1
		C	C40	2
		C+D	C40+D1a	1
	Favites abdita	D	D1a	5
	Goniastrea aspera	C	C1c	1
		D	D1a	6
	Goniastrea australiensis	D	D1a	1
	Pocillopora damicornis	C	C1h	9
		D	D1a	1
Ningaloo Reef (n = 61)	*Cyphastrea chalcidicum*	C	C40	2
	Echinopora lamellosa	C	C21	1
		C	C40	7
	Galaxea fascicularis	C	C40	3
		C+D	C1+D1a	1
		C+D	C40+D1a	2
	Hydnophora rigida	C	C40	2
	Lobophyllia hemprichii	C	C40	3
	Pocillopora damicornis	C	C1h	16
	*Seriatopora caliendrum**	C	C123	7
	Stylophora pistillata	C	C1	15
	Turbinaria mesenterina	C	C130	1
		C	C3jj	1
Abrolhos Islands (n = 23)	*Favites abdita*	C	C1	5
		C	C129	2
	Goniastrea aspera	C	C1gg-hh	6
	Goniastrea australiensis	C	C1gg	3
	Pocillopora damicornis	C	C125	2
		C	C126	5
Rottnest Island (n = 45)	*Coscinaraea marshae**	B	B40	9
	Goniastrea australiensis	C	C1gg	13
	Montipora mollis	C	C124	4
		C	C128	2
	Plesiastrea versipora	B	B18	2
	Pocillopora damicornis	C	C1c-ff	11
Dunsborough (n = 18)	*Coscinaraea mcneilli**	C	C7d	1
		B	B18	1
	Goniastrea australiensis	C	C1ii	4
	Plesiastrea versipora	B	B18	5
	*Symphyllia wilsoni**	C	C7	3
	Turbinaria mesenterina	C	C127	2
	Turbinaria reniformis	C	C7d	2
Grand total				174

Temperature and the distribution of *Symbiodinium* ITS-2 types

Average monthly temperatures from 2002 to 2005 were significantly different between all sites, except for Rottnest Island, which was not significantly different from the Abrolhos Islands or Dunsborough (df = 4, p < 0.001 for all site comparisons except the Abrolhos Islands vs. Dunsborough, where p < 0.05; Fig. 4). Based

on average monthly temperatures from 2002 to 2005 and the results of the 1-way ANOVA, for subsequent statistical analyses, Dampier and Ningaloo were designated as 'chronically warm' sites, while the Abrolhos Islands, Rottnest Island, and Dunsborough were classified as 'chronically cool' (Fig. 4).

Within the coral taxa known to host *Symbiodinium* in clade D, ITS-2 type D1a was collectively identified more frequently in the 'chronically warm' sites (Dampier and Ningaloo; n = 17 of 83, 20.4%) than in the 'chronically cool' sites (Abrolhos Islands, Rottnest Island, Dunsborough; n = 0 of 55, 0%; Fisher's exact test: p < 0.0001), as respective groups. Moreover, mean SSTs were significantly higher at Dampier (where bleaching was reported 1 mo prior to sampling) than at the other 'chronically warm' site, Ningaloo (Fig. 4) (1-way ANOVA, df = 4, p < 0.001). *Symbiodinium* ITS-2 type D1a was also detected more commonly at Dampier (also within coral taxa known to be capable of hosting *Symbiodinium* in clade D; n = 14 of 27, 51.8%), than at Ningaloo Reef (n = 3 of 52, 5.7%; Fisher's exact test: p < 0.0001) or the Abrolhos Islands, a chronically cool site (n = 0 of 23, 0%; Fisher's exact test: p < 0.0001).

However, overall patterns in the distribution of type D1a varied between coral host species. For example, significantly more *Goniastrea aspera* colonies in Dampier hosted clade D (n = 6 of 7 colonies, 86%), than did conspecifics in the Abrolhos Islands (n = 0 of 6 colonies, 0%; Fisher's exact test: p < 0.01). Similarly, *Favites abdita* hosted more clade D *Symbiodinium* in Dampier (n = 5 of 5 colonies, 100%) than in the Abrolhos Islands (n = 0 of 7 colonies, 0%; Fisher's exact test: p < 0.01). In contrast, in *Pocillopora damicornis* colonies, *Symbiodinium* ITS-2 type D1a abundance was low at Dampier (n = 1 of 10, 10%), Ningaloo Reef (n = 0 of 16, 0%), and the Abrolhos Islands (n = 0 of 7, 0%; no significant differences between pairwise comparisons by Fisher's exact test). *Symbiodinium* D1a was also observed within colonies of *Turbinaria mesenterina* and *Goniastrea australiensis* at multiple sites, but statistical comparisons could not be performed for these
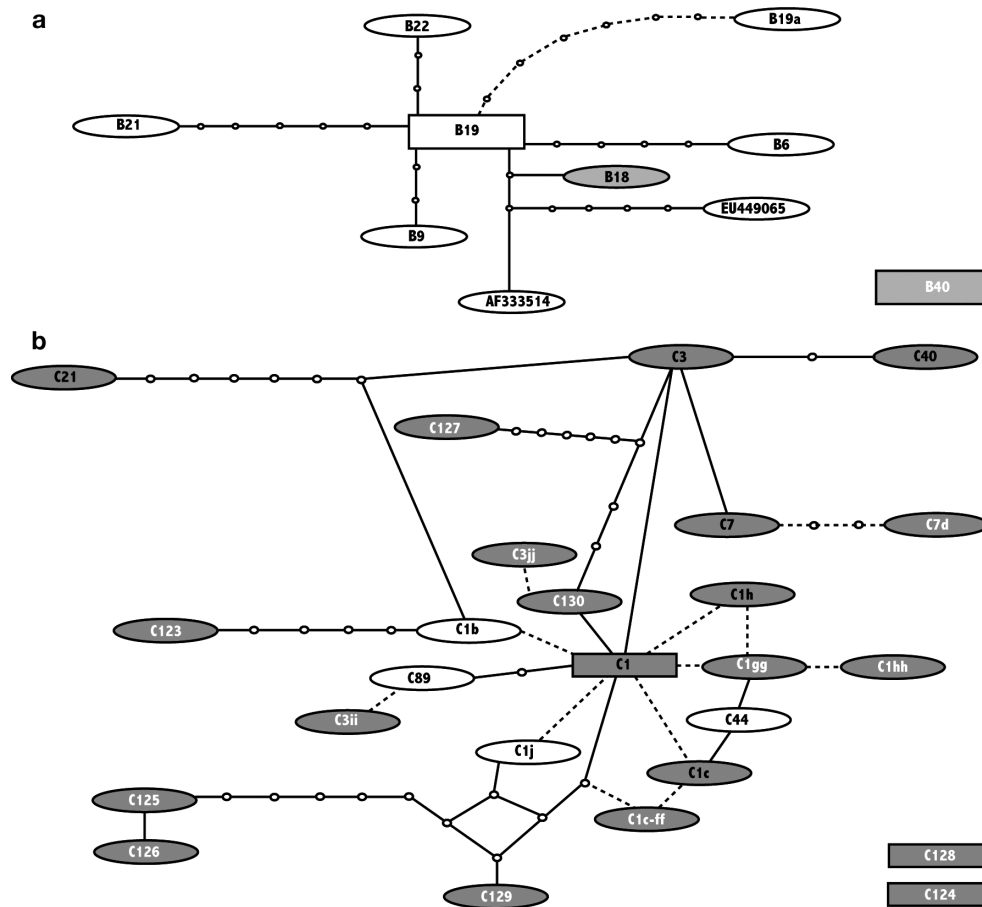
Fig. 2. Statistical parsimony networks depicting the novel *Symbiodinium* (a) clade B and (b) clade C internal transcribed spacer-2 (ITS-2) types identified in the present study and their relationships to related, previously described types, cf. LaJeunesse (2001). The estimated root for each network is represented by a rectangle. Lines represent single base-pair changes, while the dots between some lines represent hypothetical intermediate sequences. Dashed lines depict potential paralogs. Types with shaded backgrounds represent types identified in the present study, while types with white backgrounds represent ancestral or closely related types to those identified in this study. Types written in white letters denote novel (i.e. previously unreported) *Symbiodinium* types recovered in the present study

hosts due to the low number of colonies sampled at some sites.

Members of clade B, analyzed using all coral species, were found only at higher latitudes and appear restricted to temperate locations (Rottnest Island and Dunsborough; 17 of 63 colonies, 27 %).

DISCUSSION

Symbiodinium ITS-2 diversity and novel types in Western Australia

Despite a relatively limited sampling effort, a high diversity of *Symbiodinium* ITS-2 types, including various novel (i.e. previously unreported) types, were detected in reef corals from sites along the coast of

Western Australia (Figs. 1 & 3, Tables 2 & 3). Although some *Symbiodinium* ITS-2 types are common to Western Australia, eastern Australia (LaJeunesse et al. 2004a), and the northeastern and western Indian Ocean (LaJeunesse et al. 2010a), overall Western Australia can be differentiated from other nearby reefs based on its *Symbiodinium* diversity.

What factors might have contributed to *Symbiodinium* ITS-2 diversity and the high frequency of previously unreported symbionts in Western Australia? We suggest that the warm-water Leeuwin Current, which influences the coastal waters of Western Australia (Cresswell & Golding 1980, Legeckis & Cresswell 1981, Maxwell & Cresswell 1981, Caputi et al. 1996) from the surface to 150–300 m depth (Thompson 1987), may help to supply Western Australian reefs with symbionts that are commonly found in tropical waters fur-

Table 3. Novel *Symbiodinium* internal transcribed spacer-2 (ITS-2) types identified in the present study

Type cf. LaJeunesse (2001)	GenBank accession no.
C123	EU099826
C124	EU099827
C125	EU597011
C126	EU597012
C127	HQ328056
C128	EU099821
C129	HQ385805
C130	HQ385807
C1c-ff (the 'ff' band)	HQ385810
C1gg (the 'gg' band)	HQ328057
C1gg-hh (the 'hh' band)	HQ328055
C3ii (the 'ii' band)	HQ385806
C3jj (the 'jj' band)	HQ385809
C7d (the 'd' band)	HQ385808
B40	EU099825

ther north. This could occur via the dispersal of free-living *Symbiodinium*, or the transport of coral larvae containing symbionts *in hospite* from the SE Asian Coral Triangle and the Timor Sea. The Leeuwin Current has previously been identified as being responsible for bringing tropical species (e.g. plankton, invertebrates, pollen) south of their expected principal distributions (i.e. the Indo-Malay region) to Australia's west coast as far as the Great Australian Bight (Blackburn 1942, Markina 1976, Marsh 1976, Maxwell & Cresswell 1981, van der Kaars & De Deckker 2003). It has also been identified as contributing to regions of biotic diversity that exhibit characteristics of both tropical and temperate areas (Markina 1976, Maxwell & Cresswell 1981, Caputi et al. 1996).

Long-distance dispersal (e.g. 100s of km) of *Symbiodinum* by ocean currents has not been extensively studied. However, Howells et al. (2009) suggest that currents are the primary dispersal mechanism for *Symbiodinium* in horizontally transmitting coral species which do not pass *Symbiodinium* to their larvae (see also Magalon et al. 2006). Although ITS-2 *Symbiodinium* diversity has yet to be well described from the Indo-Malay region (but see Loh et al. 2001), we hypothesize that reefs in Western Australia are potentially 'sinks' for Indo-Malay *Symbiodinium* diversity. To test this hypothesis, a combination of phylogenetic and population genetic analyses of *Symbiodinium* (e.g. Santos et al. 2004, Magalon et al. 2006, Kirk et al. 2009, Pettay & LaJeunesse 2009) in the same species of coral from the Indo-Malay region and Western Australia will be necessary to characterize symbiont population structure over this broad geographic area.

Adaptive radiations, particularly of the ancestral C1 type, could also be responsible for the high levels of *Symbiodinium* diversity identified from Western Australia. Further investigations of the *Symbiodinum* diversity found in this region using multiple genetic markers (e.g. ITS-1, *psbA* mitochondrial minicircles, and microsatellite flanking regions) and high-sensitivity techniques, such as quantitative PCR, will better illustrate how evolutionarily disparate these Western Australian symbionts are from symbionts in other locations. Ultimately, the high diversity detected in this region may be the result of both the influx of tropical *Symbiodinium* types, and their subsequent isolation and divergence under the varied and changing environmental conditions found along this continent's coastline.

Biogeographic and environmental patterns in *Symbiodinium* ITS-2 type diversity

Symbiodinium ITS-2 diversity detected from Western Australian stony corals corroborated previously observed biogeographic and latitudinal patterns, while also increasing the total known diversity of coral-*Symbiodinium* associations and *Symbiodinium* ITS-2 types (Tables 2 & 3). The clade C dominance reported here agrees with the only other study of *Symbiodinium* from Western Australia: van Oppen et al. (2001) found only clade C symbionts in 3 *Acropora* species (n = 10 colonies total) from Ningaloo Reef in 1998 using single-stranded conformation polymorphism analysis of the ITS-1 region.

Our results also complement previous studies of *Symbiodinium* diversity at high latitudes (e.g. Rodriguez-Lanetty et al. 2001, Savage et al. 2002, Macdonald et al. 2008, Thornhill et al. 2008) and in isolated locations (e.g. LaJeunesse et al. 2004a) and studies done on the host species sampled in the present study throughout the Indo-Pacific (e.g. Loh et al. 2001, LaJeunesse et al. 2003, 2004b, 2008, 2010a, Chen et al. 2005, McClanahan et al. 2005). The symbiont type C1h harbored by most of the *Pocillopora damicornis* sampled in the present study, for example, was also the most common symbiont in these corals sampled from Zanzibar, Tanzania, in the Western Indian Ocean (LaJeunesse et al. 2010a), indicating that some of the partner combinations found in Western Australia occur in other regions of the Indian Ocean.

Furthermore, Baker (1999) and Rodriguez-Lanetty et al. (2001) conducted latitudinal surveys of *Plesiastrea versipora* along the eastern coast of Australia and documented that symbionts in clade C-dominated colonies in tropical areas, while *Symbiodinium* clade B associated with colonies from temperate waters. *P. versipora* and *Coscinaraea marshae* in the present study also harbored clade B representatives B18 and B40, respec-
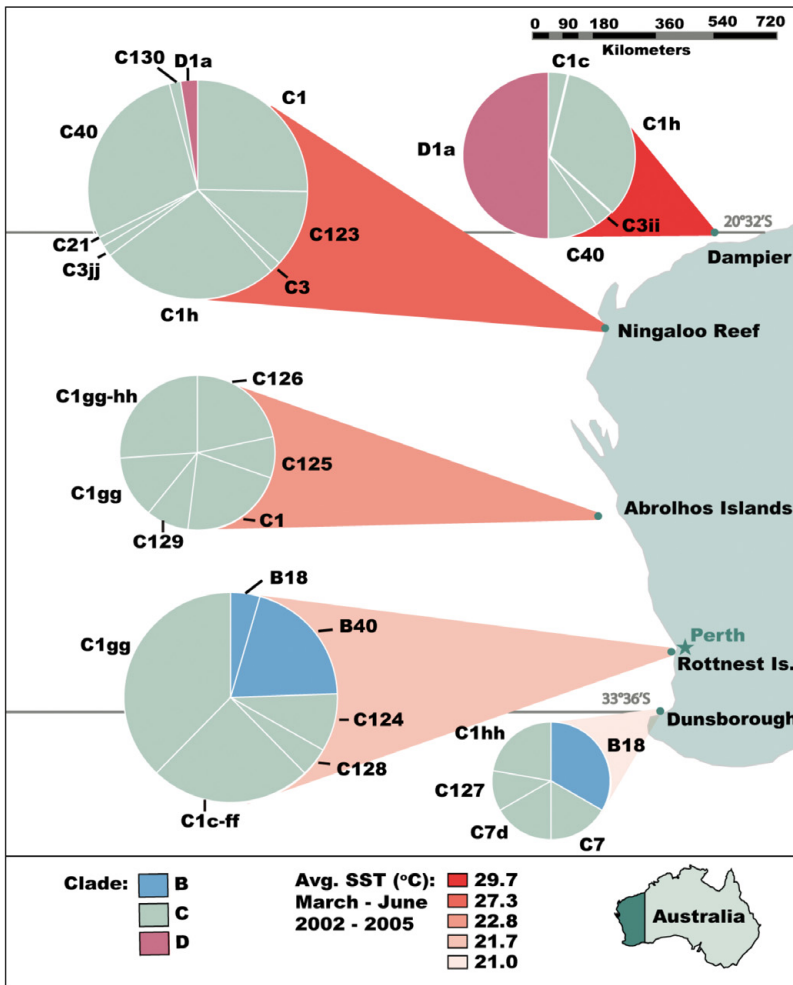
Fig. 3. Observed distribution of *Symbiodinium* internal transcribed spacer-2 (ITS-2) types across a latitudinal gradient along the coast of Western Australia. Pie charts are scaled by area according to the number of samples analyzed: Dampier (n = 27), Ningaloo Reef (n = 61), Abrolhos Islands (n = 23), Rottnest Island (n = 45), Dunsborough (n = 18). Total symbiont diversity detected from each sampled colony was scaled to equal one. For colonies hosting multiple types, each type was therefore scored as a fraction (e.g. for a colony hosting 2 types, each type was scored as 0.5). Color of pie wedge indicates *Symbiodinium* clade. Red-shaded cones correspond to the average March–June sea surface temperatures (SST) at each site from 2002 to 2005
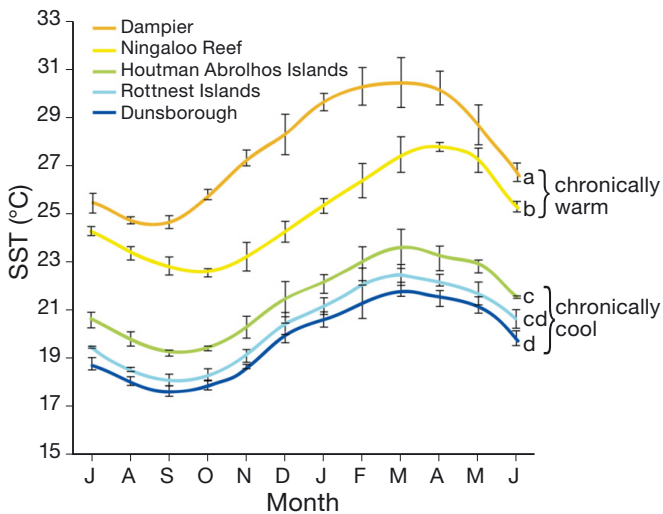
Fig. 4. Average monthly temperatures at 5 sites in Western Australia from 2002 to 2005. Sites with significantly different average monthly temperatures are indicated by 'a','b','c','cd', and 'd' (1-way ANOVA, df = 4, p < 0.001 for all site comparisons except for the Abrolhos Islands vs. Dunsborough, where p < 0.05). Error bars represent SD

tively, in temperate areas; however, we were unable to locate (and therefore, to sample) tropical conspecifics to determine whether Western Australian populations of these corals also experience a latitudinal transition in their symbioses. The *P. versipora* in temperate eastern Australia, however, appear to associate with a geographic variant of B18 (B18a), indicating that eastern and western populations of this coral associate with the same lineage of symbiont (A. C. Baker & T. C. LaJeunesse unpubl. data). Samples collected in Dunsborough were also several meters deeper than at the other sites; this could have influenced the *Symbiodinium* types detected from this site. Our results nonetheless support the growing body of evidence suggesting that some *Symbiodinium* ITS-2 B types (e.g. B2) appear to be adapted to cooler temperate environments with greater seasonal fluctuations in temperature and irradiance (e.g. Thornhill et al. 2008).

Similarly, C7 and C7d were found only at temperate sites, while C40 appears to occur only in hosts from tropical locations. In previous studies, C40 occurred in several scleractinian genera from the central Great Barrier Reef (GBR), but was absent from coral populations in the cooler and more seasonably variable southern GBR (LaJeunesse et al. 2003, 2004a). The restricted sampling of coral diversity and low sample sizes in the present survey limit our ability to draw broad conclusions about the importance of temperature in restricting the range of these symbionts. Ideally, physiological examination would be required to understand the environmental tolerances of these symbiont types.

A single clade D symbiont type, D1a, was detected (from colonies known to be capable of hosting *Symbiodinium* in clade D, see 'Materials and methods') with higher frequency at 'chronically warm' sites than at 'chronically cool' sites. This pattern was particularly pronounced in populations of *Goniastrea aspera* and *Favites abdita*; colonies of these species harbored D1a at significantly higher frequencies at Dampier than in the Abrolhos Islands. In contrast, *Pocillopora damicornis* did not show a high incidence of clade D symbionts at any of the sites examined. *P. damicornis* in this region does not appear to readily host symbionts in clade D, as has been observed in other regions where conditions are highly turbid, extremely warm, and/or undergo wide seasonal fluctuations in temperature (e.g. Glynn et al. 2001, Baker et al. 2004, Correa 2009, LaJeunesse et al. 2010a,b). Between tropical and temperate sites, however, different C types dominated colonies of *P. damicornis*. The physiologies of these different types should be compared to determine whether they exhibit different temperature optima.

A growing body of evidence indicates that some symbionts in clade D can (1) increase the thermotolerance of some host taxa (Rowan 2004, Berkelmans & van Oppen 2006, but see Abrego et al. 2008), and (2) become more abundant in coral populations after episodes of severe bleaching and mortality (Baker et al. 2004, Jones et al. 2008, LaJeunesse et al. 2009, 2010b). Hosting these symbionts, however, likely represents a trade-off in terms of growth rate and/or reduced carbon uptake in juveniles (Little et al. 2004, Cantin et al. 2009) or other factors. Since moderate bleaching occurred in Dampier (but not in the Abrolhos Islands or the Ningaloo Reef) approximately 1 mo prior to sampling, this may have contributed to the higher relative abundance of *Symbiodinium* in clade D detected in *Goniastrea aspera* and *Favites abdita* sampled from Dampier reefs during May and June of 2005. Higher abundance of D1a may have resulted from (1) changes in symbiont communities as a result of severe physiological stress resulting in bleaching (e.g. Baker et al. 2004, Jones et al. 2008, LaJeunesse et al. 2009), (2) differential survival and/or reproduction (e.g. LaJeunesse et al. 2010b), or (3) both mechanisms. However, since individual colonies were not monitored through time during the present study, and non-bleached colonies were selectively sampled from Dampier, the mechanism(s) that contributed to the observed distribution patterns of ITS-2 type D1a cannot be teased out from the effects of chronically higher temperatures in the Dampier region compared to Ningaloo or the Abrolhos Islands. Further sampling of bleached and unbleached conspecific hosts in Dampier and Ningaloo before, during, and after bleaching events would improve our ability to tease out the potential effects of temperature and/or bleaching history on the abundance of *Symbiodinium* D1a.

DGGE resolution of variation in the *Symbiodinium* ITS-2 region

Although DGGE analysis of *Symbiodinium* ITS-2 rDNA has been suggested as a method for identifying *Symbiodinium* species (LaJeunesse 2005, Sampayo et al. 2008, 2009, Finney et al. 2010, LaJeunesse et al. 2010a), the ecological relevance of some *Symbiodinium* ITS-2 types has not been rigorously established (Correa & Baker 2009). Regardless of whether some variation within the *Symbiodinium* ITS-2 is actually intra-specific (rather than species-level), it is widely agreed that the clade-level is too coarse for generalizations regarding the ecological and physiological attributes of *Symbiodinium* (Iglesias-Prieto & Trench 1997a,b, Savage et al. 2002, LaJeunesse et al. 2003, 2004b, Iglesias-Prieto et al. 2004, Tchernov et al. 2004, Warner et al. 2006, Sampayo et al. 2007, 2008, Thornhill et al. 2008).

The multi-copy nature of the rDNA gene family and differences in rates of concerted evolution (Dover 1982) can produce symbiont individuals that share identical dominant sequences, but also possess distinctive co-dominant variants that increase the diversity and complexity of DGGE banding patterns associated with a single ITS-2 'type'. The high number of symbiont types closely related to C1 in the present study highlight the intragenomic variation and/or radiations present in the *Symbiodinium* of this region. While they are given distinct names that correspond to diagnostic DGGE profiles, the degree to which these intragenomic variants are distinct from one another at the species level is beyond the scope of the present study. Indeed, further verification with multiple markers as well as ecological observations would clarify the systematic implications of the variation detected here.

The *Symbiodinium* communities identified from Western Australian coral reefs corroborated previously observed clade-level biogeographic patterns, revealed 16 novel *Symbiodinium* ITS-2 types from clades B and C, and increased the total known diversity of coral-*Symbiodinium* associations. In addition, a significant correlation between *Symbiodinium* in clade D and relatively warm and/or recently bleached sites was detected for multiple coral taxa, indicating that environmental history can influence symbiont dominance in some coral species. Given the variable environments, remote location, and novel symbiont diversity characterizing Western Australian reefs, future studies of this region have the potential to provide insights into how the coral-algal symbioses respond to environmental variation, climate change, and isolation by distance.

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