

CHAPTER 4

BIOGEOGRAPHY AND THERMAL NICHE EVOLUTION OF WARM-TEMPERATE MARINE MACROALGAE IN THE WESTERN INDO-PACIFIC OCEAN

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ABSTRACT

Here, an empirical approach is used to gain understanding in the biogeography and evolution of warm-temperate marine floras. The focus lies on the marine green algal *Codium decortdatum* species complex. We use climatic niche modeling and divergence time estimation to explore the historical biogeography and evolution of thermal variables within the complex. The climatic models were projected to the Last Glacial Maximum and the Pliocene to examine whether the Indian Ocean served as a thermal barrier for dispersal. Our results suggest that during the late Miocene – early Pliocene species within the *C. decortdatum* complex dispersed via episodic dispersal events. After geographic isolation, species have adapted to local conditions and their thermal niches have diverged from their closely related species.

INTRODUCTION

Knowledge of how species' climatic niches evolve can help us understand how they are distributed. Analyzing distributions of closely related taxa between geographically separated regions can yield important information about evolutionary lability of these climatic relationships or, alternatively, about their conservatism (Qian & Ricklefs, 2004). Both the tendency for related taxa to retain (e.g. Kozak & Wiens, 2006) or shift (e.g. Graham *et al.*, 2004) their ancestral ecological niche has been confirmed in the last decade and both mechanisms are assumed to promote diversification in different ways. The allopatric speciation model has dominated theories of speciation for decades. Also in the marine environment diversification as a result of long-term geographic isolation is regarded as the most prevalent mode of speciation, even though barriers to gene flow are often less visible (Palumbi, 1994). The emphasis, however, has always resided with the purely geographical setting while relatively little attention has been given to the possible influence of environmental and climatic factors that favor isolation between populations of marine organisms.

Among marine organisms, macroalgae are good models for evolutionary, macroecological and biogeographical research because of their broad distribution, variability in ecological dimensions and their sessile nature making them easier to collect (Bolton *et al.*, 2004; Verbruggen *et al.*, 2009b). One of the most mesmerizing patterns of macroalgal biodiversity is the apparent affinity of the marine floras of distant subtropical warm temperate regions in the Western Indo-Pacific, namely the Arabian Sea, SE Africa and SW Australia (Norris & Aken, 1985; Joosten & Van Den Hoek, 1986; Lüning, 1990). These regions harbor a rich species diversity with many endemic species (Phillips, 2001; Bolton *et al.*, 2004; Schils & Wilson, 2006) and similarities between them have often been depicted (Wynne, 2000; Schils & Coppejans, 2003; Verbruggen *et al.*, 2007; Bolton, 2010) and genetically confirmed (Pillmann *et al.*, 1997; Hommersand & Fredericq, 2003; Verbruggen *et al.*, 2007; De Clerck *et al.*, 2008). The marine floras of these regions also show similarities with the warm temperate flora of Japan (Børgesen, 1934; Hommersand, 1986; Wynne, 2000). Aside from the overall similarity of these regions' algal floras in terms of species diversity, the distinct regions feature morphologically similar congeners that are absent from the tropical seas separating them (Verbruggen *et al.*, 2007).

Several plausible explanations for the affinities between these distant subtropical algal floras have been proposed. To elucidate such affinities between S Africa and Australia Norris and Aken (1985) suggested that common taxa represent vicariant relics of a continuous distribution along the Cretaceous coast of Gondwanaland, distributions that were eventually fragmented by the northward migration of Africa, Australia and the Indian subcontinent. Hommersand (1986; 2007) proposed that descendents of lineages that originally evolved in warm-temperate SW Australia migrated through the Indian Ocean via the North Equatorial current as far as the east coast of S Africa and, occasionally, beyond the southern tip of Africa into the Atlantic Ocean. He postulated that such migrations took place during Miocene-Pliocene periods during which global sea surface temperatures decreased markedly. An alternative to this dispersal based scenario invokes a common ancestor that was distributed at low latitudes throughout the Indian Ocean during cool periods, with subsequent isolation in relatively small geographic areas taking place during warmer climatic periods (Verbruggen *et al.*, 2005).

Despite the existence of various biogeographic hypotheses, few studies have addressed the association between algal lineage diversification and its environmental setting in an explicit timeframe (but see Verbruggen *et al.*, 2009b). Phylogenetic analyses with relaxed molecular clock methods provide a means of tracing the evolution of a lineage in a temporal framework. Furthermore, biogeographical research and studies of speciation have rarely quantified

environmental factors that might have caused sister species to be allopatric (Kozak *et al.*, 2008). Yet several tools are available to estimate ecological niches of species and predict their responses to environmental change, so called ecological niche modeling (ENM). Integration of niche modeling techniques with time-calibrated phylogenies can yield valuable information about niche shifts and the evolution of environmental preferences (Yesson & Culham, 2006b; Yesson & Culham, 2006a). These advances could provide new understanding of biogeographical patterns and niche dynamics.

Here, we present an empirical approach to gain understanding in the biogeography and macroecological evolution of warm-temperate marine floras. More specifically, we focus on the green algal genus *Codium*, a widely distributed group that has its largest species diversity in warm-temperate regions. Our main goal is to understand the biogeography and niche evolution of a *Codium* species complex distributed in warm-temperate regions and to interpret observed patterns in consideration of the biogeographical hypotheses mentioned above. To achieve our goals, we combine phylogenetic, distributional and environmental data. First, we define a temporal framework for lineages within this complex. Second, we determine whether the thermal niches of these closely related entities have diverged or if they show a high degree of overlap. Third, we check if ecological niche models can be used to inform us about dispersal barriers and biogeographical patterns in geological time.

MATERIALS AND METHODS

STUDY SPECIES

We focus on the *Codium decortatum* complex, a clade containing five morphologically similar species present in different warm-temperate regions (Verbruggen *et al.*, 2007). *Codium cylindricum* (Holmes, 1896) is a Pacific species that occurs along the coasts of Japan and on both sides of the Baja California peninsula, Mexico. In the latter region, the species is known as *Codium amplivesiculatum* (Setchell & Gardner, 1924; Norris, 2010) but our genetic data show that it is conspecific with *Codium cylindricum*. *Codium decortatum* (Howe, 1911) has a very broad geographical area, ranging from the North Atlantic (Bermuda, Azores and North Africa) to the Caribbean and along the south coast of South Africa and into the Indian Ocean (Madagascar). The remaining three species have not been formally described and are here referred to as *C. duthieae* 1, 2 and 3 (Verbruggen *et al.*, 2007). The geographical area of *Codium duthieae* 1 is restricted to the Eastern coast of South Africa ranging just to the South of Mozambique. *Codium duthieae* 2 solely occurs on the southern Australian and Tasmanian coast. *Codium duthieae* 3 occurs in the Arabian Sea around the coasts of Oman, Yemen, India and Pakistan and was also recently collected in Madagascar.

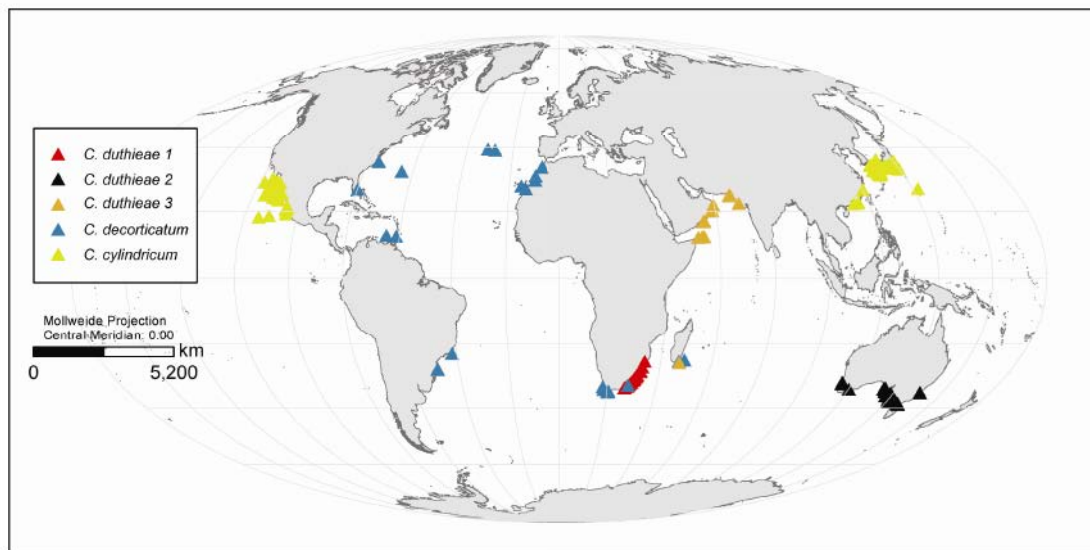


Figure 1: Species records of all *Codium decortatum* complex species.

PHYLOGENETIC INFERENCE AND DIVERGENCE TIME ESTIMATION

Three plastid markers (*rbcl* exon1, *tufA* and UCP6) were used to reconstruct phylogenetic relationships for 21 *Codium* species. For the *Codium decortatum* complex and five other closely related species four extra markers were added (*rbcl* exon2, *atpA*, *atpA* intron and UCP7) (Table S1). Using the Bayesian information criterion (BIC), a suitable partition strategy and suitable models of sequence evolution were selected. The guide tree used during the entire procedure was obtained

by a maximum-likelihood (ML) analysis of the unpartitioned concatenated alignment using a JC + Γ 8 model (Guindon & Gascuel, 2003). All likelihood optimizations and BIC calculations were carried out with Treefinder (Jobb *et al.*, 2004). The preferential model of sequence evolution and partitioning strategy (lowest BIC) was a GTR + G4 for which all genes were partitioned in three codon positions. This model was applied in all subsequent phylogenetic analyses.

ML analyses were performed using RAxML v. 7.2.8 (Stamatakis, 2006). Statistical branch support was assessed via bootstrapping with 100 replicates. Bayesian phylogenetic inference (BI) was carried out with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Two parallel runs, each consisting of four incrementally heated chains were run for 2 million generations, sampling every 2,000th generation. Convergence of log-likelihoods and parameter values was assessed in Tracer v1.5 (Rambaut & Drummond, 2007). An appropriate burn-in sample of trees (5%) was removed before constructing the majority rule consensus tree.

To obtain an ultrametric tree, a Bayesian phylogenetic analysis was conducted in BEAST v1.7.2 (Drummond *et al.*, 2012) under a GTR+G4 model with an uncorrelated lognormal (UCLN) relaxed molecular clock model and using a Yule tree prior. Three Markov Chain Monte Carlo (MCMC) analyses were run for 10 million generations, sampling every 10,000th generation. The output was diagnosed for convergence using Tracer v.1.5, and summary statistics and trees were generated using the last 9 million generations with TreeAnnotator v1.5.3 (Rambaut & Drummond, 2007). The tree was calibrated by assuming an age of 153 (SD 35) million years (Myr) for the crown node of the genus (Verbruggen *et al.*, 2009a).

NICHE MODELING

We used a correlative ecological niche modeling approach to estimate the climatic niche of species within the *Codium decorticatum* complex using species occurrences and climatic data. We also projected the inferred niche onto past climatic circumstances (Last Glacial Maximum and Pliocene) in order to visualize potential climatic barriers.

Occurrence data

Species occurrence records are primarily obtained from recently collected specimens for which the identification has been confirmed with DNA barcodes. These records are complemented with herbarium specimens and verified literature data that were carefully re-examined. For specimens that were not geotagged in the field, georeferencing was conducted using Google Earth, only including records that could be unambiguously assigned to a precise locality. ArcGIS (ESRI, Redlands, California, USA) was used to map the coordinates for visual verification. Our final dataset of unique georeferenced records ranged between 24 (*C. duthieae* 1) and 119 (*C. cylindricum*) unique localities per species (Table S2).

Climate data

Climate data were derived for three different time periods. For present-day conditions, we used Bio-ORACLE, a marine environmental dataset for global-scale applications, to derive sea surface temperature (SST) data (minimum, maximum and mean) (Tyberghein *et al.*, 2012). Climate data for the Last Glacial Maximum (LGM) were obtained from model-interpolated GLAMAP data (Paul & Schäfer-Neth, 2003). The global monthly LGM SST data originally had a spatial resolution of one

degree and were interpolated (bilinear) to match the 9km resolution of Bio-ORACLE. SST data for the Pliocene was derived from the Pliocene Research, Interpretation and Synoptic Mapping Project (PRISM3) (Dowsett *et al.*, 2009; Dowsett *et al.*, 2010) at a spatial resolution of 2 degrees (left at default spatial resolution). This dataset documents the global climate in the mid-Pliocene warm period using various proxies (e.g., foraminifera, diatoms, ostracods, pollen and plant macrofossil data). To be consistent with the Bio-ORACLE data set, we built the same types of grids (minimum, maximum, mean and SST range) based on the monthly data for both past periods (LGM & Pliocene).

Because the 4 temperature-related layers we use are correlated, principal component analysis (PCA) was conducted in ArcGIS to reduce data dimensionality and generate a set of new independent variables from the original unstandardized Bio-ORACLE data. The first two PCA axes accounted for more than 99% of the underlying environmental variation. The factor loadings from this analysis were used to construct PC axes for both the LGM and Pliocene time period. These PC axes were used for subsequent niche modeling.

Modeling technique

We reconstructed niche models using MaxEnt3.3.3k (Phillips *et al.*, 2006; Phillips & Dudik, 2008). This machine learning algorithm yields largely better results than other presence-only niche modeling methods (Elith *et al.*, 2006) and performs well even when the number of occurrence records available for modeling is small (e.g. Wisz *et al.*, 2008). MaxEnt allows for model performance evaluation by calculating the area under the ROC (receiver operating characteristic) curve (AUC), based on training and test data, which represent the ability of the model to distinguish presence data from background data (Phillips *et al.*, 2006). All analyses were replicated 10 times using a subsampling approach with 30% of localities for model testing. Background points were randomly chosen based on an equal area map in order not to bias towards the higher latitudes. All other settings were left at their defaults.

MaxEnt was also used to predict areas of suitable thermal conditions during the LGM and Pliocene by creating the models on the species' present thermal distribution range and projecting them onto past sea surface temperature grids.

NICHE COMPARISONS

We compared the thermal niches of all five members of the *Codium decortatum* complex using several methods. First, we examined the level of divergence in environmental space. We conducted a PCA on all species within the complex to assess the overall level of environmental divergence but also quantified how pairs of sister taxa overlapped in environmental space. Second, we calculated overlap of the inferred ecological niche models, which are a representation of the species' environmental affinities in geographic space.

A PCA was conducted with all five species of the *Codium decortatum* complex. Data for all occurrence localities were extracted from the original Bio-ORACLE temperature layers. We also assessed niche overlap for each species pair from separate PCAs. Statistical significance of the possible niche shifts between species was evaluated using multivariate analyses of variance (MANOVAs) of the first and second principal component, a method fully detailed by Graham *et al.* (2004). Statistical analyses were conducted using R (R Development Core Team, 2008).

As a complement to the PCA analyses, we also used computed overlap statistics using niche models. The predicted thermal niche of all five species was compared using indices for niche overlap D and I, as proposed by Warren et al. (2008) implemented in the package ENMTools 1.3 (Warren *et al.*, 2010). The significance of both indices, ranging from 0 (no overlap) to 1 (identical niche models), was evaluated with two randomization tests (detailed in Warren *et al.*, 2008). First, we tested for 'niche equivalency' by comparing the predicted niches to a null distribution (100 pseudoreplicates) that was generated by randomly resampling the pooled sample of occurrence points for both species with the condition that sample sizes for both species were kept constant. This procedure tests whether the observed niche similarity differs significantly from that expected under the null hypothesis that the two species are ecologically equivalent.

ANCESTRAL NICHE RECONSTRUCTIONS

To examine thermal niche evolution in the *Codium decorticum* complex, we reconstructed ancestral states for all SST variables. We used Bayesian MCMC to construct the posterior distribution for the states at internal tree nodes as implemented in the Phytools R package (Revell, 2011). We used extracted temperature data from sampling localities and add the five closest *Codium* taxa in order to get a more comprehensive picture of the ancestral niche of the *C. decorticum* clade.

In addition, we applied an approach that allowed taking intraspecific variability of thermal preferences into account (Evans *et al.*, 2009). Niche models created by MaxEnt are combined with individual SST layers to produce a probability distribution for each variable (Predicted Niche Occupancy (PNO)). This distribution of thermal tolerances gets sampled for data to reconstruct. We also took phylogenetic uncertainty into account by repeating this analysis over 1000 samples from the posterior distribution of chronograms. The estimation of ancestral climatic tolerances sensu Evans et al. (2009) was conducted in the R package Phyloclim (<http://www.christophheibl.de/Rpackages>).

RESULTS

PHYLOGENY AND DIVERGENCE TIME ESTIMATION

The resulting phylogenetic trees were generally congruent with previous hypotheses for this group (Verbruggen *et al.*, 2007). Thirteen of the 19 nodes in the tree were recovered with Bayesian posterior probabilities greater than 0.95 (Figure S1). Phylogenetic relationships remained poorly resolved among the three *Codium duthieae* species despite the addition of four extra plastid markers.

The root age used in this study was based on previous molecular dating of siphonous green algae (Verbruggen *et al.*, 2009a). Using this root age, the estimated crown group age for the *Codium decorticatum* complex is about 13.4 Myr (24.5-4.1 Myr 95% highest posterior density [HPD]). *Codium decorticatum* diverged during the Late Miocene at about 7.5 Myr ago while the three *Codium duthieae* lineages diverged in the Early Pliocene (~ 5.3-3.6 Myr ago).

ECOLOGICAL NICHE ANALYSES

The ecological niche models of all species within the *Codium decorticatum* complex had mean test AUC values ranging from 0.833 to 0.974 (Table 1, Figure 2) indicating reliable model performances (Phillips *et al.*, 2006). Overall, maps of predicted species distribution showed broad suitability outside their realized ranges. These areas show similar temperature conditions indicating the potential distribution of the species. One exception is the niche model for *Codium cylindricum*, which shows a less extensive extrapolation towards other areas.

Table 1: Mean test AUC values (area under the receiver operation characteristics curve) of niche models per species, Schoener's index D and Hellinger's index I of niche overlap, and assessment of niche equivalency via randomization tests.

Species X	Species Y	AUC (\pm SD) X	AUC (\pm SD) Y	D	I
<i>C. duthieae 1</i>	<i>C. cylindricum</i>	0.974 (0.008)	0.968 (0.011)	0.198***	0.445***
<i>C. duthieae 1</i>	<i>C. duthieae 2</i>	0.974 (0.008)	0.944 (0.006)	0.340***	0.632***
<i>C. duthieae 1</i>	<i>C. duthieae 3</i>	0.974 (0.008)	0.950 (0.026)	0.282***	0.574***
<i>C. duthieae 1</i>	<i>C. decorticatum</i>	0.974 (0.008)	0.833 (0.029)	0.528***	0.806***
<i>C. duthieae 2</i>	<i>C. cylindricum</i>	0.944 (0.006)	0.968 (0.011)	0.207***	0.467***
<i>C. duthieae 2</i>	<i>C. duthieae 3</i>	0.944 (0.006)	0.950 (0.026)	0.056***	0.190***
<i>C. duthieae 2</i>	<i>C. decorticatum</i>	0.944 (0.006)	0.833 (0.029)	0.569***	0.840***
<i>C. duthieae 3</i>	<i>C. cylindricum</i>	0.950 (0.026)	0.968 (0.011)	0.318***	0.593***
<i>C. duthieae 3</i>	<i>C. decorticatum</i>	0.950 (0.026)	0.833 (0.029)	0.342***	0.615***
<i>C. decorticatum</i>	<i>C. cylindricum</i>	0.833 (0.029)	0.968 (0.011)	0.401***	0.724***

Significance values (95% level) of niche overlap are indicated by asterisks; ***P < 0.001

Paleo-ENMs for the Pliocene show similar predictions as for present day models (Figure 2). The projection for *C. duthieae 3* predicts remarkably less suitable habitat in the Pliocene. For *C. duthieae 1*, *C. duthieae 2* and *C. decorticatum*, predictions onto LGM circumstances show similar geographical patterns, however it is clearly visible that predictions narrow towards the tropics. Projections for *C. duthieae 3* and *C. cylindricum* yielded less predicted suitable area.

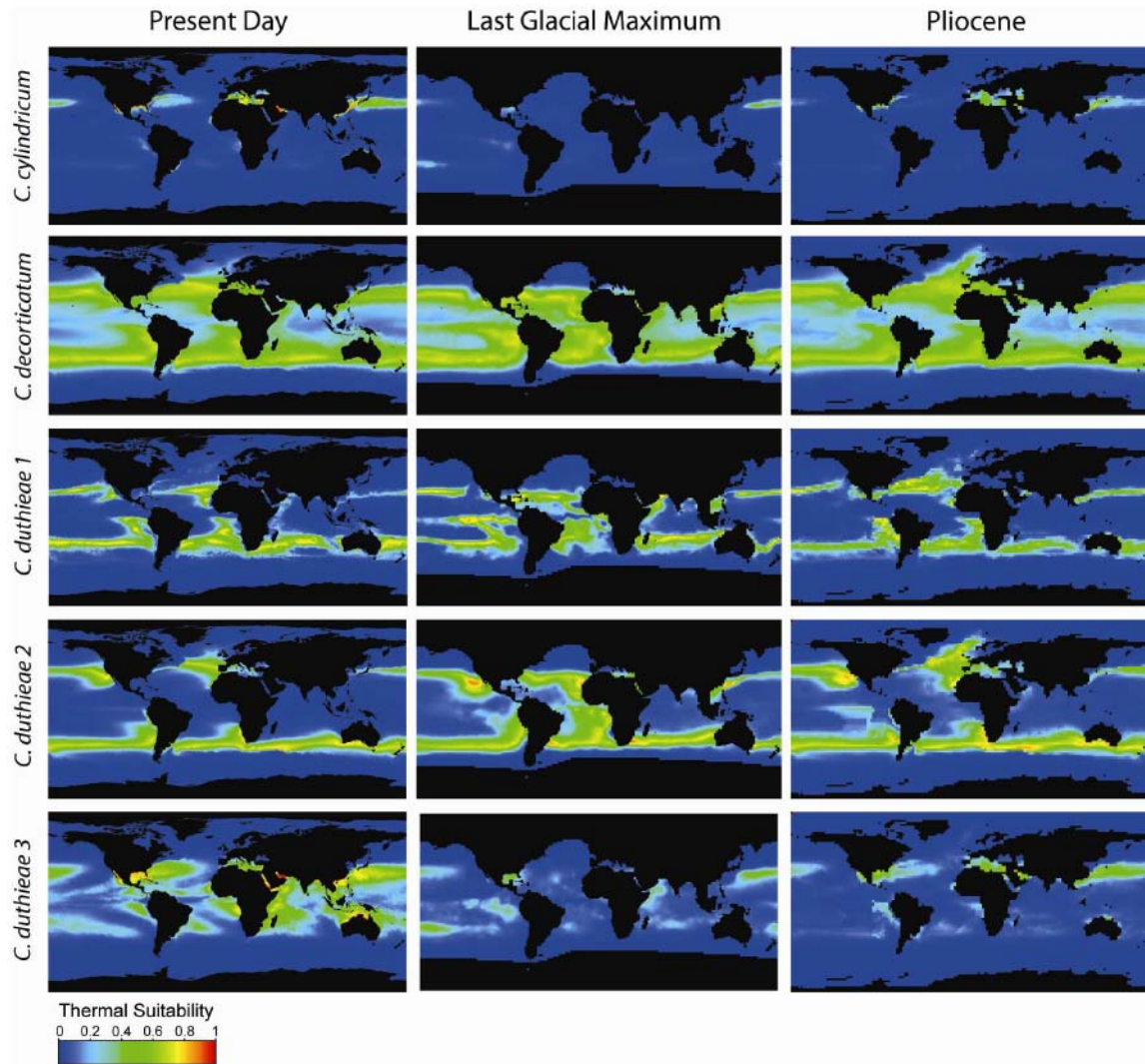


Figure 2: Predicted thermal niche models of *Codium* species inferred from temperature layers and occurrence points. The left column depicts present day habitat suitability. The second and third column represent projections onto past temperature circumstances, respectively the Last Glacial Maximum (~18,000 kyr) and the Pliocene (Mid Piacenzian 3.29 – 2.97 myr). Predicted habitat suitability is indicated with colors along a gradient; warmer colors indicating areas with better predicted conditions.

Thermal profiles show that all five *Codium* species primarily occur in warm temperate environments (Table 2). One species, *C. duthieae* 2 experiences somewhat colder temperatures in S Australia, whereas the Arabian *C. duthieae* 3 mainly occurs in warmer waters. Environments of the Pacific species *C. cylindricum* that occur both along the coasts of Japan and Baja California, are characterized by temperatures ranging from 20.7-32.2 °C in the warmest month to 17.4-25.1 °C in the coldest month. Predicted niche occupancy (PNO) plots (Figure 3) indicate a broad range of suitable temperatures for *Codium decorticaum*, a species occurring in both tropical and temperate areas. The PNO profile for *Codium cylindricum* clearly shows the broad temperature range of this species.

Table 2: Thermal data for the *Codium decorticaum* clade. Sea Surface temperature data in degrees Celsius, standard deviation between parentheses.

Species	maximum sst	mean sst	minimum sst	sst range
<i>Codium cylindricum</i>	28.27 (2.25)	22.31 (2.52)	17.21 (3.15)	11.05 (2.81)
<i>Codium decorticaum</i>	24.99 (3.37)	21.86 (3.37)	19.17 (3.55)	5.82 (1.80)
<i>Codium duthieae</i> 1	25.62 (1.52)	22.86 (1.38)	20.49 (1.31)	5.13 (0.38)
<i>Codium duthieae</i> 2	20.92 (2.02)	17.86 (2.03)	15.01 (2.25)	5.91 (1.67)
<i>Codium duthieae</i> 3	28.89 (0.77)	26.21 (0.51)	22.65 (0.97)	6.24 (1.17)

Table 3: Results of MANOVA. This analysis, using the PC scores as dependent variables and species as categorical variables, shows differences in the thermal space of the five species using the first two principal component scores. The multivariate analysis represents an overall statistic to determine whether separation in environmental space was significant while the univariate analysis is a test to determine which PCA axis accounts for significance in the overall test.

Species comparison	multivariate		univariate	
	F stat		F stat PC1	F stat PC2
<i>C. duthieae</i> 1 - <i>C. cylindricum</i>	146***		15.724***	83.952***
<i>C. duthieae</i> 1 - <i>C. duthieae</i> 2	68***		108.15***	0.2383 (Ns)
<i>C. duthieae</i> 1 - <i>C. duthieae</i> 3	50***		104.58***	0.0282 (Ns)
<i>C. duthieae</i> 1 - <i>C. decorticaum</i>	50**		9.1588**	2.3048 (Ns)
<i>C. duthieae</i> 2 - <i>C. cylindricum</i>	165***		156.39***	48.832***
<i>C. duthieae</i> 2 - <i>C. duthieae</i> 3	69***		330.03***	0.3282 (Ns)
<i>C. duthieae</i> 2 - <i>C. decorticaum</i>	69***		24.652***	1.3546 (Ns)
<i>C. duthieae</i> 3 - <i>C. cylindricum</i>	147***		68.991***	10.005**
<i>C. duthieae</i> 3 - <i>C. decorticaum</i>	51***		62.509***	0.1032 (Ns)
<i>C. decorticaum</i> - <i>C. cylindricum</i>	147***		0.8887 (Ns)	98.325***

Significance values are indicated by asterisks; ***P < 0.001, **P<0.01, *P < 0.05, Ns: not significant.

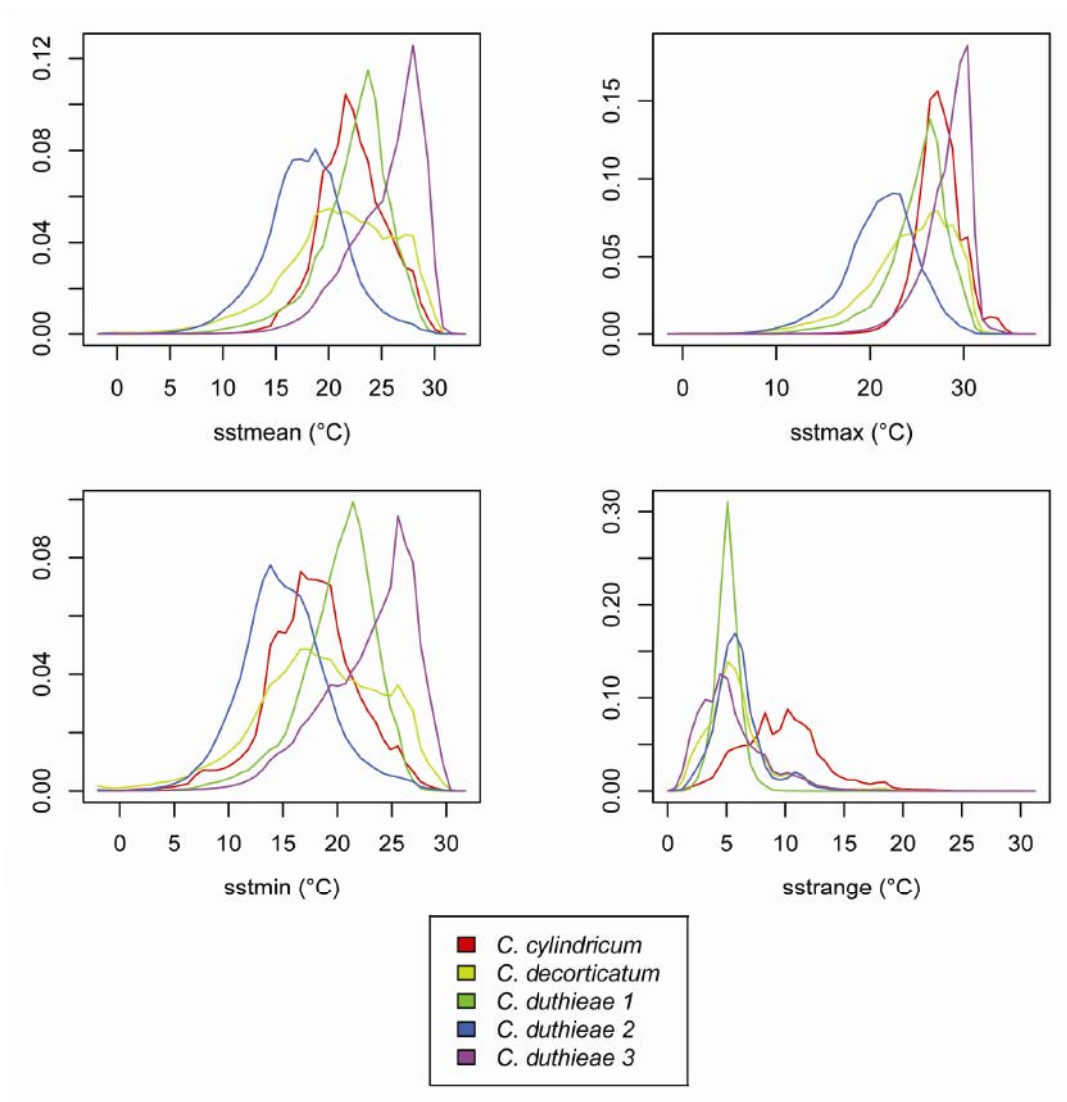


Figure 3: Predicted niche occupancy of the 5 taxa in the *Codium decorticans* complex. These plots represent unit area histograms of suitability, illustrating a species' (predicted) occupancy of each sst variable. Pictures were developed using the R package Phyloclim (<http://www.christophheibl.de/Rpackages>).

or the overall PCA on all five species within the *Codium decorticans* clade, the first and second principal components explained nearly all variation (PC1 = 61.5% and PC2 = 38.2%) among the extracted data from the four Bio-ORACLE layers (Figure 4, Table S4). Mean, maximum and minimum sea surface temperature loaded on the first axis, whereas SST range was responsible for the factor loading on the second axis. MANOVAs were calculated on both PC1 and PC2 on all species pairs within the *C. decorticans* clade (see Table S4 for detailed results of PCAs). Overall separation in environmental space was statistically significant for all species pairs (Table 3). Post-hoc analyses indicated that PC1 was responsible for this separation in all cases except for *C. decorticans* / *C. cylindricum*. Divergence along PC2 was only statistically significant in species comparisons with *C. cylindricum*. This result is expected and corroborates the observation that *C. cylindricum* grows in areas with substantial seasonal temperature differences.

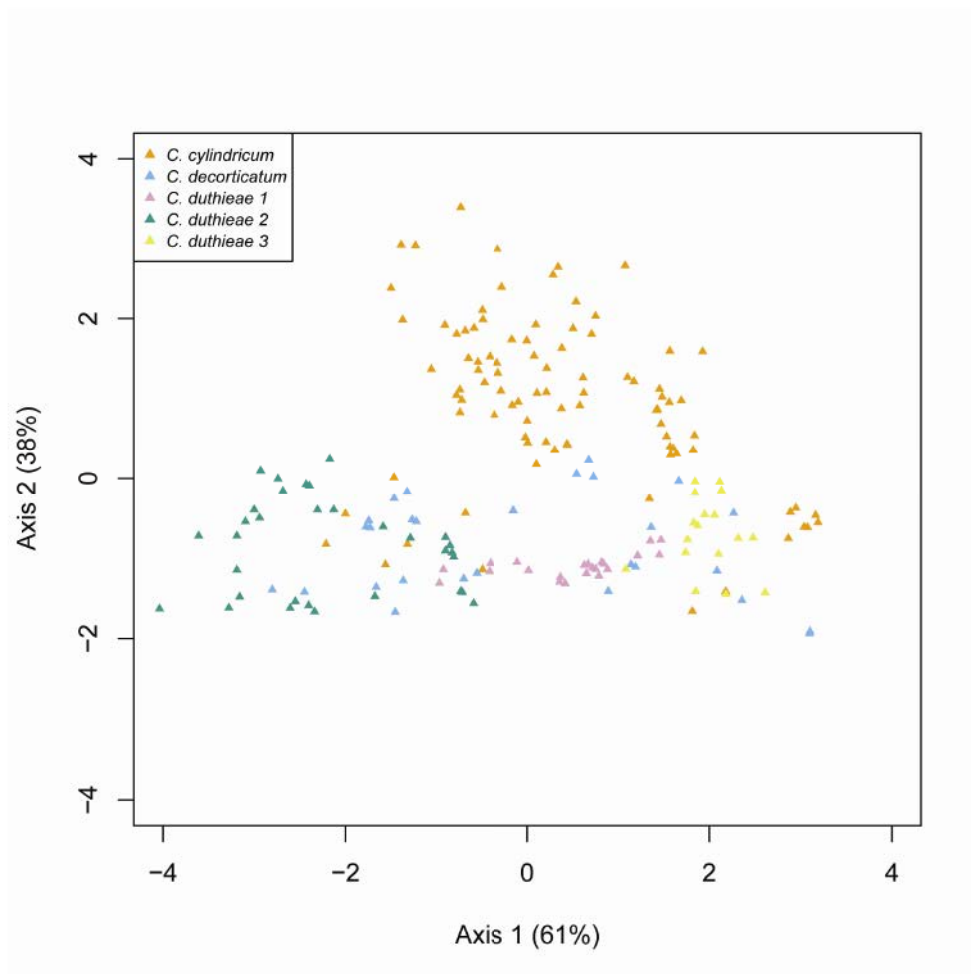


Figure 4: Principal components analysis depicting the position of the 5 focal *Codium* species in thermal niche space, with each data point representing a unique species locality.

Overlap of ecological niche models resulted in D values ranging from 0.06 to 0.57 and I values ranging from 0.19 to 0.84 (Table 1). Values of 0 symbolize no overlap whereas values of 1 indicate identical niche models. We find the highest niche overlap between *C. decorticans* and *C. duthieae 2* and between *C. decorticans* and *C. duthieae 1*. Niche overlap between *C. duthieae 2* and *C. duthieae 3* is noticeably very low.

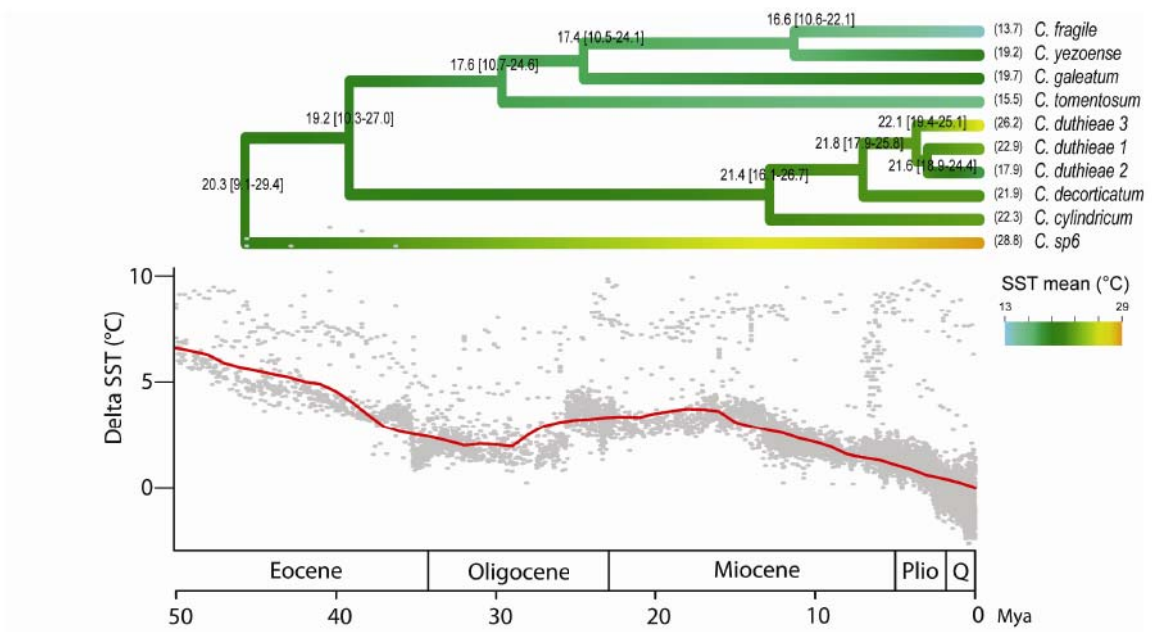


Figure 5: Calibrated phylogenetic tree of 10 *Codium* species including the *C. decortatum* complex. Ancestral state estimations for mean sea surface temperature are plotted on the tree. The mean ancestral state of 1,000,000 Bayesian generations is given in the branch nodes and indicated by a color gradient (95% confidence interval between square brackets). At the bottom, the ocean temperature curve (in red) is based on a high-resolution deep-sea oxygen isotope record (grey dots; $\delta^{18}O$; Veizer *et al.*, 1999). Delta SST represents the temperature difference between past and current temperatures. A 10-Ma geological time-scale indicates the timeframe, spanning epochs since 50 Ma (Plio, Pliocene; Q, Quaternary).

A mean sea surface temperature affinity of 21.4°C (95% HPD: 16.1-26.7) was inferred for the basal split of the *Codium decortatum* complex (Figure 5). *Codium duthieae* 3 evolves towards warmer waters, while the opposite is true for *Codium duthieae* 2. Similar results were obtained in the analysis of the history of niche occupancy for separate temperature variables (Figure 6).

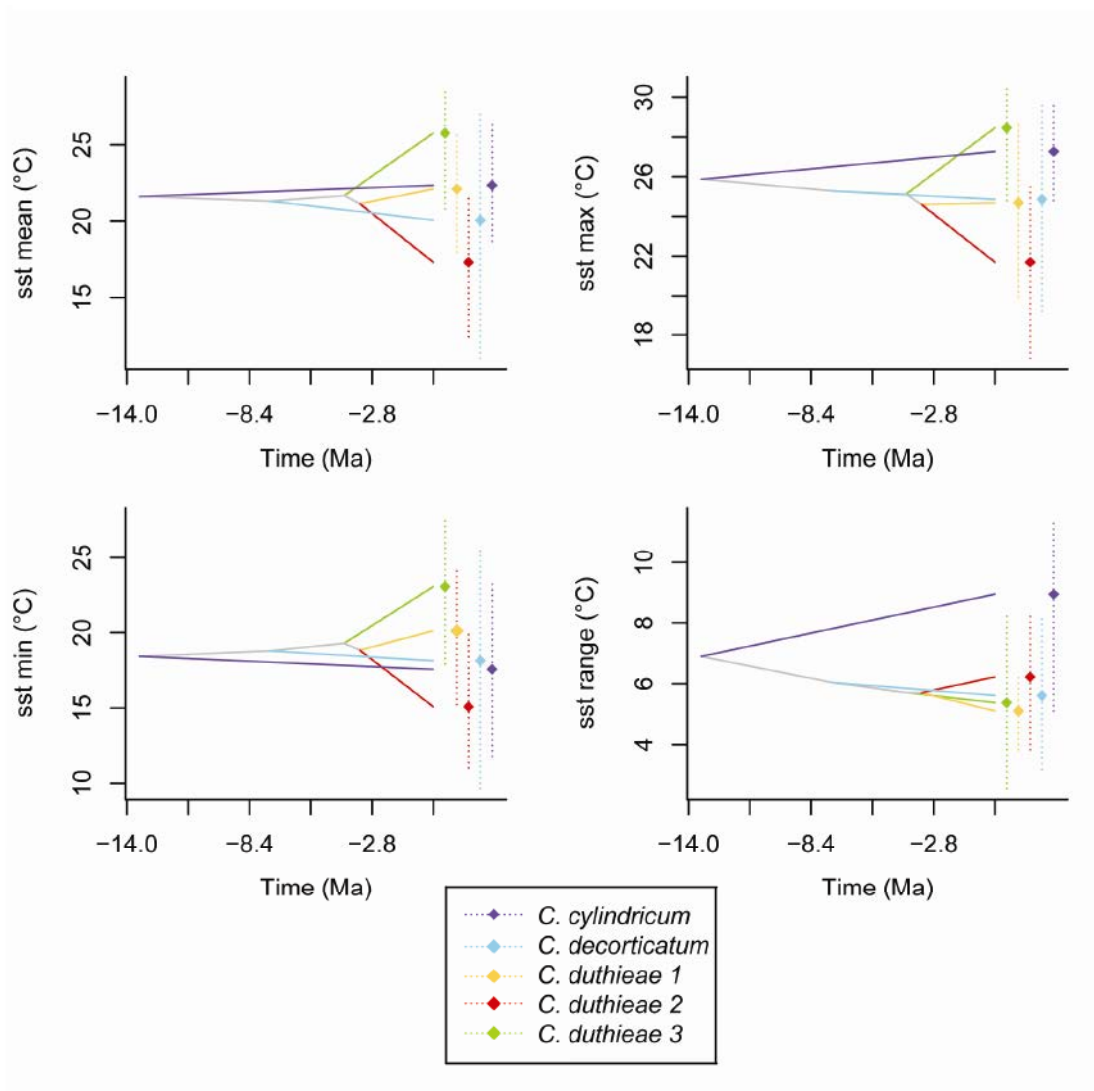


Figure 6: Ancestral reconstructions of thermal niches. The x-axis represents time. The 80% central density of thermal tolerance for each extant species is indicated by a vertical dashed line, the mean by a diamond symbol. The graphs were developed using the R package Phyloclim (<http://www.christopheibl.de/Rpackages>).

DISCUSSION

The approach presented here explores the ability to study macroecological and biogeographical patterns using a combination of phylogenetic, geographic and environmental data. The temporal framework of diversification in combination with the results from thermal niche studies allow us to discuss observed patterns of subtropical to temperate algal floral affinities in light of previously proposed hypotheses.

BIOGEOGRAPHY

According to our phylogenetic results, the *Codium decorticatum* complex diversified from the Mid-Miocene (13.4 mya) onwards with the most recent inferred split dated in the Pliocene (3.4 mya). Considering the distributions of *Codium* species in this study, it is unlikely that tectonic events affected the diversification of the complex. It was hypothesized that disjunct warm temperate species ranges in the Western Indo-Pacific could represent vicariant relics of a previously continuous distribution along the Cretaceous coast of Gondwanaland at a time when the connection between the coastlines of Africa and Australia were more or less continuous (Norris & Aken, 1985; Hommersand, 1986). Our results refute this hypothesis for the *C. decorticatum* complex, as species divergence times are much too recent to account for a Cretaceous common ancestor.

Instead, a dispersal-based hypothesis, originally proposed by Hommersand (1986; 2007), is far more likely to account for the current distributions of all species within the *C. decorticatum* complex. Hommersand suggested that species which originally evolved in warm-temperate environments of SW Australia have descendants that migrated through the Indian Ocean via the North Equatorial current as far as the east coast of S Africa and, exceptionally beyond, into the Atlantic Ocean. Likewise, species originated in warm-temperate climates in East Asia could migrate through the tropics via the Arabian Sea into S Africa. *Codium duthieae* 2 (SW Australia), *C. duthieae* 3 (Arabian Sea), *C. duthieae* 1 (SE Africa), *C. decorticatum* (Atlantic Ocean) and *C. cylindricum* (Japan) seem to support this hypothesis of biogeographic affinities. Species intolerant of tropical temperatures may have dispersed across equatorial waters at shallow depths during periods of climatic cooling (Darwin, 1859; Hubbs, 1952; Lindberg, 1991). It was assumed that such migrations took place during Miocene-Pliocene periods in which the temperature decreased markedly. The divergence times inferred in the present study corroborate this time setting.

During the Pliocene (~5-2 Myr ago), when the *C. duthieae* species diverged, global sea surface temperatures were approximately 3°C warmer than at present (Ravelo *et al.*, 2004). Our model approach permitted to predict areas of suitable thermal conditions during this period. We realize that this projection represents only a snapshot (3.29 – 2.97 myr ago) in geological time; however, it clearly indicates that the Indian Ocean was too warm to allow for a continuous range of the common ancestor of the *C. duthieae* clade. Model projections onto the LGM (~18 kyr ago), the period in the Earth's Cenozoic history when ice sheets reached their maximum extent, show that even during these cool periods the low latitudes of the Indian Ocean were not predicted as suitable. Hence, the current distribution pattern among *C. duthieae* species can only have arisen by means of long-distance dispersal. Only for *Codium decorticatum*, models indicate a broad range across low latitudes during glacial maxima, which is still reflected in the species broad geographic distribution which spans tropical to temperate regions in the Atlantic Ocean.

Dispersal among marine organisms is considered to be a function of larval behavior, life history strategies, drifting capabilities, oceanographic features, habitat availability and environmental limits (Bellwood *et al.*, 1998; Cowen *et al.*, 2000; Gillespie *et al.*, 2012). In *Codium*, mechanisms for dispersal include motile flagellated reproductive cells (Ramus, 1972) and thallus fragmentation (Fralick & Mathieson, 1972). Occasional long-distance dispersal is possible as detached thalli can float due to internal oxygen entrapment (Dromgoole, 1982). This, in combination with the ability to withstand temporary desiccation (Schaffelke & Deane, 2005), makes successful long distance dispersal possible. The observed distribution pattern, whereby 3 entities are nearly exclusively confined to warm temperate pockets in the Indian Ocean despite their divergence approximately 3.4 mya, serves as evidence for the exceptional nature of such events. Likewise, the presence of *C. duthieae* 3 in southern Madagascar provides evidence, be it circumstantial, that *Codium* species are capable of occasionally dispersing over long distances and bridging large areas of unsuitable environmental conditions. At such low frequency, dispersal events are so rare that they can allow for genetic differentiation (bottlenecks and Founder effects) and eventually even allopatric speciation (Thiel & Haye, 2006).

As to the directionality of dispersal events, our phylogeny remains largely inconclusive. The short branch lengths separating the *C. duthieae* entities and lack of support keep us from putting forth a specific hypothesis. Furthermore, hypotheses about directionality can be seriously confounded by extinction events (Crisp *et al.*, 2011), the importance of which is difficult to estimate based on a phylogeny of extant taxa (e.g. Rabosky, 2009; Rabosky, 2010; Morlon *et al.*, 2011; Stadler, 2011). Hommersand (1986) favored dispersal from Australia through the northern part of the Indian Ocean to South Africa by way of the islands of Indonesia and the North Equatorial Current. We should also consider there was also a possibility for dispersal from South Africa to Australia, for example via the South Indian Current (southern part of the Indian Ocean gyre). The fact that most of the warm, salty waters of the Agulhas current, the largest ocean jet current in the Southern Hemisphere, feeds back into the subtropical gyre (Zahn, 2009), lends credibility to this theory. Alternatively, migration by means of the Antarctic circumpolar current (west wind drift) could also be possible. A phylogeographic study on a widespread coastal sea star (*Patiriella exigua*) revealed colonization of Australia from southern Africa via the west wind drift (Waters & Roy, 2004). Such dispersal pattern is not unique among marine organisms and has since gained more evidence (Waters, 2008; Fraser *et al.*, 2009; Zemlak *et al.*, 2009; Macaya, 2010; Nikula *et al.*, 2010). It seems that most taxa for which evidence of long distance dispersal by means of the Antarctic Circumpolar Current has been presented have affinities for cold temperate rather than warm temperate waters. It is questionable whether a warm-temperate *Codium* species could reach the Antarctic Circumpolar Current and as such be dispersed via this route. Furthermore, species would have to endure suboptimal temperatures for a very long time in order to reach the coast of Australia.

NICHE EVOLUTION

The climatic analyses and ENM results for the *C. decortdatum* clade yield some insights in the evolution of the thermal niche. All five species are associated with temperate to tropical conditions, but there is considerable variation in their thermal characteristics. *Codium cylindricum* experiences temperatures ranging from 17 to 28°C, nearly encompassing the tolerances of the other four species. All species occupy significantly different thermal niches based on multiple lines of evidence. We observed a common trend of environmental divergence among sister species. This was reflected in the MANOVA and PCA results that showed clear separation of sister species in ecological space. For all species comparisons, except for those with *C. cylindricum*, the first principal axis (temperature) was responsible for this separation. PCAs involving *C. cylindricum* showed that species were very similar along this first axis, while there were more differences along the second

axis (seasonality). This species occurs in both Mexico and Japan, temperate regions experiencing high temperature ranges. Here, one could argue that *C. cylindricum* is biased towards a certain season. Large differences in seasonal temperatures cause some organisms to be limited by winter cold and other by summer heat (Adey & Steneck, 2001). However, by comparing sampling dates and monthly temperature values, we confirm the presence of this species on the Japanese and northern Mexican coasts all year around and thus the species appears to be able to persist across a wide temperature range. This effect is also reflected in the thermal niche model, which is dominated by seasonality (the second PCA axis) and hence shows only limited amounts of suitable habitat at a global scale compared to other species in the complex (Figure 2, present-day). Although this model represents the correct distribution of the species, we cannot rule out that *C. cylindricum* has the potential to survive in areas with a smaller seasonal temperature range.

By incorporating ecological niche models, climatic and geographic distributions could be estimated beyond the sample locations. Overlap tests based on these models showed that not a single species' niche was found to be equivalent to another, regardless of the measure of similarity used (Table 1). The highest values of niche equivalency were presented by comparing *C. decorticans* to the other species. These high values are due to the fact that *C. decorticans* is characterized by a broad environmental niche that overlaps with all other species. The lowest niche overlap was observed between *C. duthieae* 2 from Australia and *C. duthieae* 3 from the Arabian Sea. These values are corroborated by the results of the PCA (Figure 4), which separates both species completely on the first axis. The potential niches of both species are also virtually mutually exclusive in geographical space (Figure 2). When comparing equivalency of niches, however, it is important to take scale effects into account (Pearman *et al.*, 2008). Here, we must consider scale affect along a thermal axis. One can imagine species being close to one another with respect to the entire range of temperatures observed on earth, but different from each other within the "temperate" subset of temperatures. Thus, despite the significant differences observed, we can consider that all species have retained their affinities for warm-temperate environmental conditions.

Thermal niche models for *Codium duthieae* 1, *C. duthieae* 2 and *C. decorticans* predict good suitability along the South American coast. Interestingly, Setchell (1937) described "*Codium fernandezianum*" from the Juan Fernandez Islands, an archipelago off the coast of Chile, stating 'It may be questioned whether the Juan Fernandez plants should all be placed under the same species and it may also be questioned, in case it may be decided that they are all only variants of one species, whether to segregate them from *Codium decorticans*.' Setchell's uncertainty as to exactly how to call the Chilean entity is not surprising given the morphological similarity between species in the *C. decorticans* complex, but it does indicate that a species belonging to the complex may be present in the Juan Fernandez Islands. Whether it is a proper species (Setchell decided to call it *C. fernandezianum*) or that it consists of a population of *Codium duthieae* 2 that migrated along the Antarctic circumpolar current, or whether yet another explanation is needed, remains to be determined.

CONCLUSION

Using a combination of a calibrated phylogeny, ecological niche models and projections of those models into the past, we have shed light on the biogeography and macroevolutionary pattern of a warm-temperate complex of seaweed species. Diversification of the *Codium decorticatum* complex could be placed in the late Miocene – early Pliocene. This timeframe in combination with the thermal and geographic distribution of the complex favor a dispersal hypothesis. Because niche projections give evidence for a tropical barrier impeding regular connection between current geographic ranges, we hypothesize that rare transoceanic dispersal events are likely to be responsible for this pattern. Low frequency dispersal would allow for genetic differentiation and founder speciation as species adapted to their local thermal environment.

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AUTHOR CONTRIBUTIONS

LT and HV conceived and designed the study. LT, HV and ODC constructed the phylogenetic alignments. HV carried out the phylogenetic analyses. LT prepared the species and environmental data sets, and performed all niche related analyses. LT wrote the manuscript and HV and ODC gave comments.

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APPENDIX

Table S1: Data availability matrix. Graphical representation of the concatenated alignment, showing the availability of sequence data.

	atpA + intron	Rbcl exon1	Rbcl exon2	tufA	UCP6	UCP7
<i>C. adhaerens 1</i>		GenBank: XX strain: Dorset length: 760 nt		GenBank: XX strain: HEC15691 length: 905 nt	GenBank: EF107854 strain: SMG05.35 length: 357 nt	
<i>C. arabicum 1</i>		GenBank: EF107969 strain: JH9 length: 775 nt		GenBank: XX strain: SGAD0509370 length: 911 nt	GenBank: EF107856 strain: SD0509370 length: 357 nt	
<i>C. bursa</i>		GenBank: EF107970 strain: KRK001 length: 775 nt		GenBank: XX strain: G.113 length: 873 nt	GenBank: XX strain: G.113 length: 358 nt	
<i>C. cf.dimorphum</i>		GenBank: XX strain: HV1877 length: 722 nt		GenBank: XX strain: HV1867 length: 911 nt	GenBank: EF107875 strain: C29 length: 267 nt	
<i>C. coralloides</i>		GenBank: EF107977 strain: KRK003 length: 772 nt		GenBank: XX strain: HV03269 length: 750 nt	GenBank: EF107869 strain: KRK003 length: 356 nt	
<i>C. cylindricum</i>	GenBank: XX strain: HV1730 length: 872 nt	GenBank: XX strain: HV1730 length: 750 nt	GenBank: XX strain: HV1730 length: 515 nt	GenBank: new strain: HV1730 length: 890 nt	GenBank: EF107872 strain: C223 length: 268 nt	GenBank: XX strain: HV1730 length: 331 nt
<i>C. decorticatum</i>	GenBank: XX strain: H.0775 length: 925 nt	GenBank: XX strain: H.0775 length: 741 nt	GenBank: XX strain: H.0775 length: 516 nt	GenBank: XX strain: H.0775 length: 1026 nt	GenBank: EF107873 strain: CDNC07 length: 357 nt	GenBank: XX strain: H.0775 length: 561 nt
<i>C. dimorphum</i>		GenBank: EF107981 strain: H.0771 length: 760 nt		GenBank: XX strain: H.0771 length: 682 nt	GenBank: EF107874 strain: H.0771 length: 344 nt	
<i>C. duthieae 1</i>	GenBank: XX strain: G.300 length: 925 nt	GenBank: XX strain: G.300 length: 750 nt	GenBank: XX strain: G.300 length: 515 nt	GenBank: XX strain: HEC15348 length: 928 nt	GenBank: EF107877 strain: KZN2K4.1 length: 357 nt	GenBank: XX strain: HEC15348 length: 563 nt
<i>C. duthieae 2</i>	GenBank: XX strain: G.291 length: 925 nt	GenBank: XX strain: G.291 length: 750 nt	GenBank: XX strain: LT105 length: 515 nt	GenBank: XX strain: G.291 length: 920 nt	GenBank: EF107878 strain: H0691 length: 381 nt	GenBank: XX strain: G.291 length: 667 nt
<i>C. duthieae 3</i>	GenBank: XX strain: DHO.003 length: 881 nt	GenBank: EF107994 strain: DHO.006 length: 750 nt	GenBank: XX strain: DHO.003 length: 516 nt	GenBank: XX strain: DHO.006 length: 928 nt	GenBank: EF107879 strain: DHO2.002 length: 358 nt	GenBank: XX strain: DHO.006 length: 621 nt
<i>C. fragile</i>	GenBank: XX strain: JAP128 length: 932 nt	GenBank: XX strain: JAP128 length: 721 nt	GenBank: XX strain: JAP128 length: 515 nt	GenBank: XX strain: JAP128 length: 928 nt	GenBank: EF107889 strain: C41 length: 317 nt	GenBank: XX strain: JAP128 length: 560 nt
<i>C. galeatum</i>	GenBank: XX strain: G.111 length: 853 nt	GenBank: XX strain: G.111 length: 750 nt	GenBank: XX strain: G.111 length: 515 nt	GenBank: XX strain: G.111 length: 920 nt	GenBank: EF107892 strain: H0794 length: 380 nt	GenBank: XX strain: G.111 length: 495 nt
<i>C. isthmocladum 1</i>		GenBank: XX strain: PEUFR48498 length: 787 nt		GenBank: XX strain: HV907 length: 937 nt	GenBank: EF107905 strain: HV907 length: 357 nt	
<i>C. megalophysum</i>		GenBank: EF108058 strain: HEC15349 length: 763 nt		GenBank: XX strain: G.919 length: 801 nt	GenBank: EF107917 strain: KZN2K4.29 length: 358 nt	
<i>C. minus 1</i>		GenBank: AB102959 strain: C43 length: 676 nt		GenBank: XX strain: JAP077 length: 911 nt	GenBank: EF107918 strain: C43 length: 271 nt	
<i>C. saecotum</i>		GenBank: XX strain: H.0974 length: 741 nt		GenBank: XX strain: HA0096 length: 905 nt	GenBank: XX strain: H.0974 length: 358 nt	
<i>C. sp6</i>	GenBank: XX strain: LL0470 length: 892 nt	GenBank: XX strain: LL0470 length: 725 nt	GenBank: XX strain: LL0470 length: 515 nt	GenBank: XX strain: LL0470 length: 768 nt	GenBank: EF107951 strain: LL0470 length: 358 nt	GenBank: XX strain: LL0470 length: 562 nt
<i>C. spinescens</i>		GenBank: EF108075 strain: H.0693 length: 765 nt		GenBank: XX strain: G.112 length: 846 nt	GenBank: EF107931 strain: H.0693 length: 356 nt	
<i>C. tomentosum</i>	GenBank: XX strain: HV1344 length: 863 nt	GenBank: XX strain: HV1344 length: 750 nt	GenBank: XX strain: HV1344 length: 515 nt	GenBank: XX strain: HV1344 length: 982 nt	GenBank: XX strain: CT0V01 length: 268 nt	GenBank: XX strain: HV1344 length: 585 nt
<i>C. yezoense</i>		GenBank: XX strain: HV1855 length: 674 nt		GenBank: XX strain: HV1855 length: 905 nt	GenBank: EF107945 strain: C53 length: 308 nt	

Table S2: Species Occurrence Data: References

Species	Source	# Specimens	# Unique localities
<i>Codium cylindricum</i>	GENT Herbarium, SAP Herbarium, TNS Herbarium	133	119
<i>Codium decorticatum</i>	GENT Herbarium, PARIS Herbarium, Smithsonian Institution	45	27
<i>Codium duthieae 1</i>	GENT Herbarium; (Silva, 1959)	26	24
<i>Codium duthieae 2</i>	GENT Herbarium; Adelaide Herbarium; Herbarium Western Australia	69	40
<i>Codium duthieae 3</i>	GENT Herbarium; (Wynne, 2001); (Nizamuddin, 2001)	34	24

Table S3: Covariance matrix and principal component analysis on 4 Bio-ORACLE layers (sstmean, sstrange, sstmax, sstmin). Analyses conducted in ArcGIS 10 (ESRI).

Covariance matrix				
	range	sstmean	sstmin	sstmax
sstrange	1	0.01255	-0.10419	0.17146
sstmean	0.01255	1	0.99289	0.98699
sstmin	-0.10419	0.99289	1	0.96196
sstmax	0.17146	0.98699	0.96196	1

PCA				
	PC1	PC2	PC3	PC4
sstrange	0.00453	0.81532	-0.04316	-0.57738
sstmean	0.5814	-0.04661	-0.81228	-0.00054
sstmin	0.57304	-0.38893	0.43286	-0.57706
sstmax	0.57757	0.4264	0.38855	0.57761

Table S4: Principal component analyses on extracted *Codium* occurrence point data from Bio-ORACLE.

		PC1	PC2	PC3	PC4
All five species	sstmax	-0.524	0.459	0.435	0.571
	sstmean	-0.635	0.006	-0.772	0.000
	sstmin	-0.566	-0.371	0.462	-0.573
	sstrange	0.043	0.807	-0.029	-0.588
<i>C. duthieae 1</i> vs <i>C. cylindrium</i>	sstmax	-0.240	0.746	0.454	-0.424
	sstmean	-0.601	0.266	-0.754	0.000
	sstmin	-0.630	-0.131	0.456	0.615
	sstrange	0.430	0.596	-0.132	0.665
<i>C. duthieae 1</i> vs <i>C. duthieae 2</i>	sstmax	-0.540	0.351	0.431	0.632
	sstmean	-0.572	0.117	-0.812	0.000
	sstmin	-0.574	-0.079	0.393	-0.714
	sstrange	0.228	0.926	-0.027	-0.300

<i>C. duthieae 1 vs C. duthieae 3</i>	sstmax	-0.564	0.029	0.380	0.733
	sstmean	-0.559	-0.100	-0.823	0.000
	sstmin	-0.493	-0.528	0.398	-0.565
	sstrange	-0.356	0.843	0.139	-0.379
<i>C. duthieae 1 vs C. decortdatum</i>	sstmax	0.538	0.406	0.440	0.594
	sstmean	0.601	0.067	-0.796	0.000
	sstmin	0.578	-0.257	0.414	-0.655
	sstrange	-0.126	0.875	-0.021	-0.468
<i>C. duthieae 2 vs C. cylindricum</i>	sstmax	0.583	0.307	0.418	0.625
	sstmean	0.620	-0.125	-0.774	0.000
	sstmin	0.487	-0.521	0.474	-0.516
	sstrange	0.193	0.786	0.028	-0.586
<i>C. duthieae 2 vs C. duthieae 3</i>	sstmax	-0.576	0.157	0.413	0.688
	sstmean	-0.583	-0.006	-0.812	0.000
	sstmin	-0.572	-0.196	0.412	-0.682
	sstrange	-0.026	0.968	0.012	-0.249
<i>C. duthieae 2 vs C. decortdatum</i>	sstmax	-0.557	0.340	0.428	0.625
	sstmean	-0.600	0.025	-0.800	0.000
	sstmin	-0.572	-0.276	0.421	-0.648
	sstrange	0.052	0.899	-0.011	-0.436
<i>C. duthieae 3 vs C. cylindricum</i>	sstmax	-0.331	0.764	0.411	0.370
	sstmean	-0.584	0.174	-0.793	0.000
	sstmin	-0.589	-0.140	0.403	-0.686
	sstrange	0.450	0.605	-0.198	-0.626
<i>C. duthieae 3 vs C. decortdatum</i>	sstmax	0.563	0.308	0.433	0.634
	sstmean	0.596	0.040	-0.802	0.000
	sstmin	0.571	-0.268	0.411	-0.658
	sstrange	-0.048	0.912	0.009	-0.407
<i>C. cylindricum vs C. decortdatum</i>	sstmax	-0.412	0.610	0.454	0.502
	sstmean	-0.632	0.139	-0.762	0.000
	sstmin	-0.608	-0.261	0.457	-0.595
	sstrange	0.246	0.735	-0.070	-0.628

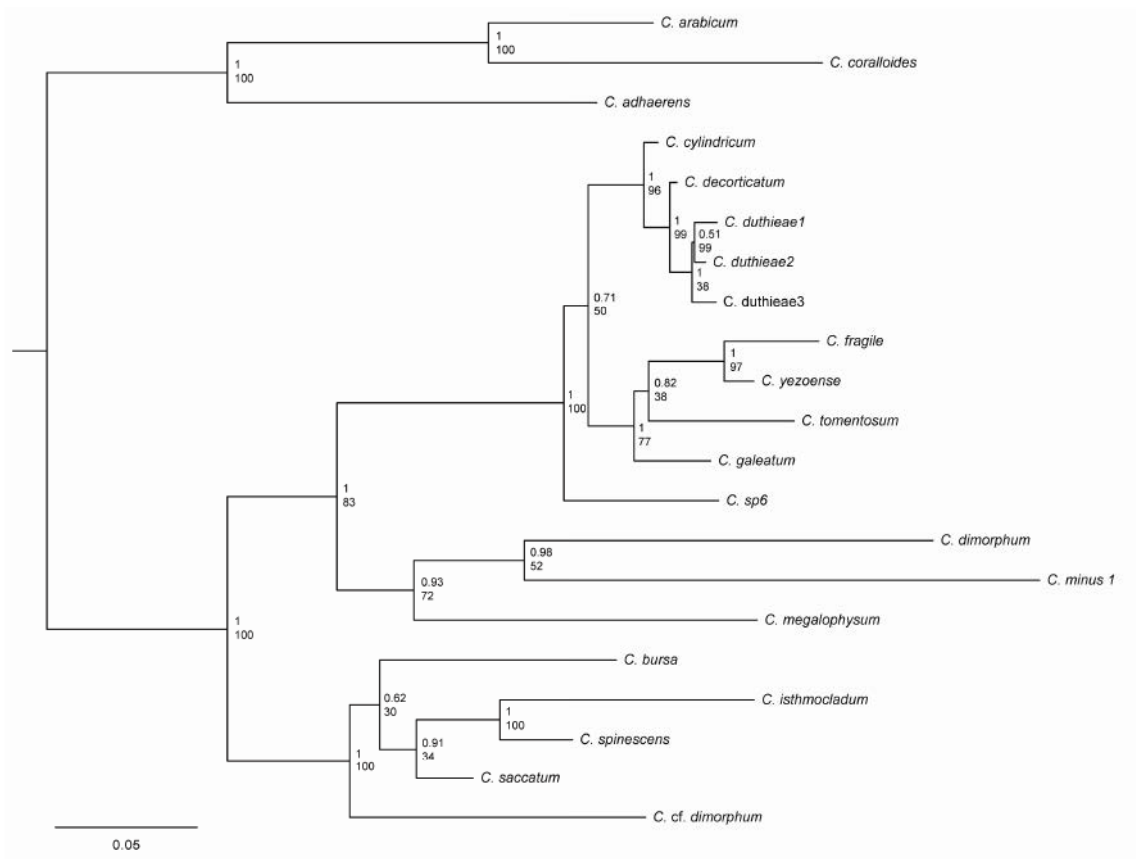


Figure S1: Bayesian phylogenetic tree of 21 *Codium* species. Numbers at nodes indicate posterior probabilities (top) and ML bootstrap values (bottom).

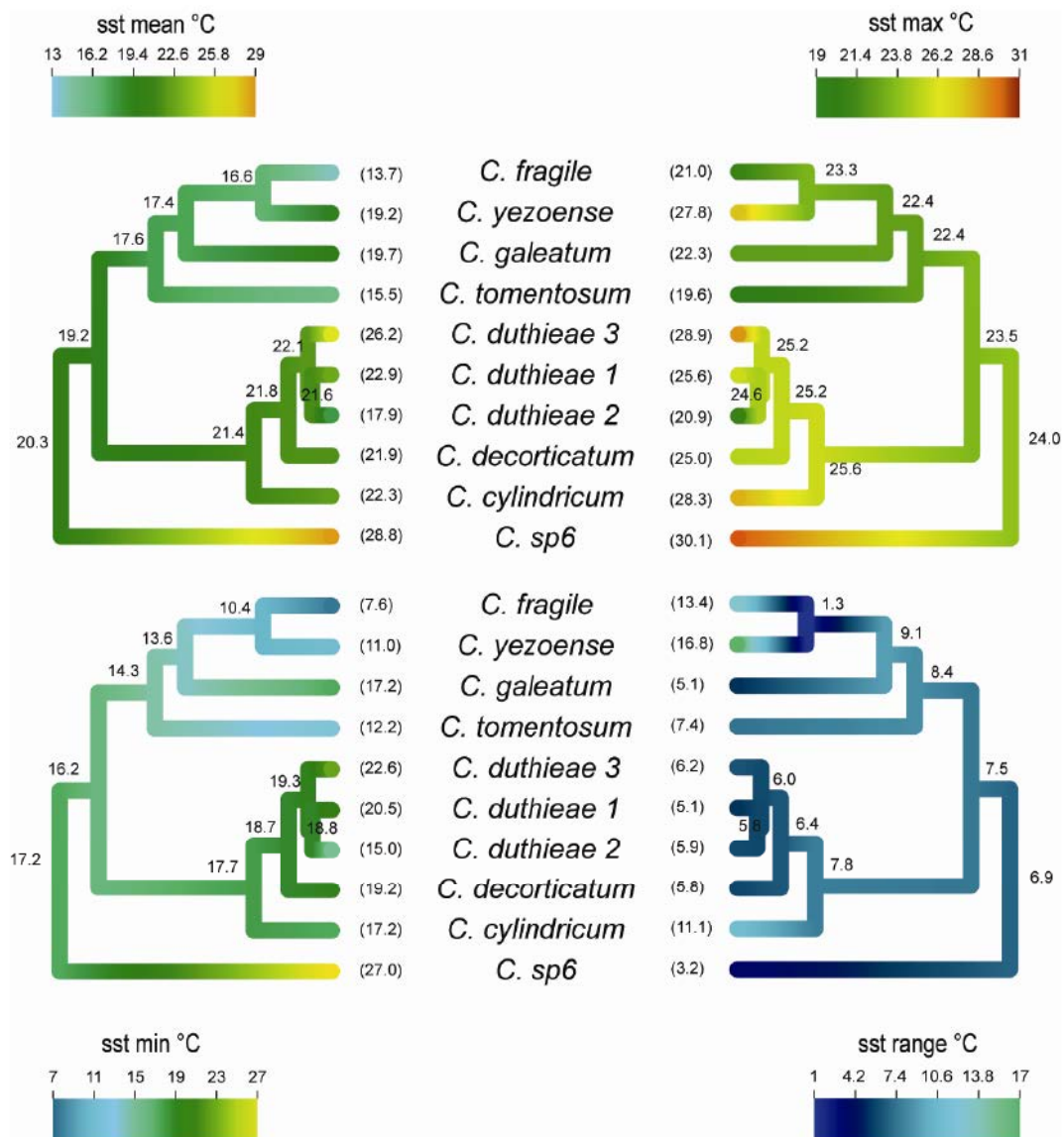


Figure S2: Inferred evolutionary history of niche features in 10 *Codium* species. Ancestral values for sea surface temperature (sstmean, sstmin, sstmax and sstrange) are plotted along the phylogeny. Numbers plotted at nodes indicate the inferred ancestral values. These values were obtained using a Bayesian MCMC approach (Revell, 2011). The color gradient indicates the temperatures.