Evolutionary dynamics of algal traits and diversity

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With 4 figures

Abstract: Algae have a particularly rich evolutionary history that has not yet been comprehensively explored. We review statistical techniques to infer patterns of trait evolution and species diversification from phylogenies. We illustrate these methods using the evolution of algal thermal niches and its interaction with species diversification as a case study. We offer some perspectives for the application of these methods in other fields of phycology and the integration of micro- and macroevolutionary approaches.

Keywords: algae, comparative phylogenetic methods, diversification, macroevolution, niche evolution

Introduction

Algae exhibit an astonishing diversity of form and function. The various groups of algae derive from the merger of previously heterotrophic eukaryotic cells with autotrophic cells through the process of endosymbiosis (Keeling, 2013). These mergers have resulted in new organisms that can use a convenient and abundant energy source (sunlight, through photosynthesis) and accumulate genomic features and functions from both of the merged cells. Because of this, and their ancient origins, algae have diversified in almost all imaginable directions. Some have evolved multicellularity, some have colonized land, they feature an immense diversity of life cycles, have developed the ability to live in very high and very low light environments, range from very hot to icy habitats, and so on.

The study of algal evolution is a treasure trove of interesting discoveries, with many still to be made. We know quite a bit about the diversity of algal features, but their evolutionary history is yet to be characterized in detail. How frequently have algae evolved multicellularity and which molecular innovations have facilitated this? How quickly do algal environmental tolerances evolve, and consequently, how commonly do they invade tropical or cold-water environments? Has the emergence of a particular type of life cycle resulted in higher rates of speciation of the lineages having that life cycle? Many such questions, especially those dealing with speciation-extinction dynamics and the evolution of morphological traits, are typically explored using the fossil record in many groups of organisms. Regrettably, with a few notable exceptions, algae do not preserve well as fossils, so this approach is not realistic in most cases.

Luckily, an alternative is available. Phylogenetic trees also retain information about the evolutionary events that led to extant diversity, and statistical methods can be used to derive evolutionary insights from phylogenetic trees and information about contemporary species. These methods have been gradually developed over the last few decades, but the past five years (or so) have seen a surge in the interest in and development of these methods. In this paper, we will briefly review a selection of phylogenetic methods available for statistical inference of trait evolution and species diversification. We will look at some applications of these methods for studying the evolutionary dynamics of algal niches, and offer some perspectives of their future applications in algal evolution.

Evolutionary inference using phylogenies

A family of statistical techniques, sometimes referred to as comparative phylogenetic methods, is used to make evolutionary inferences from species phylogenies. We will illustrate the general concepts using the evolution of a continuous trait (e.g. body size) as the first example, with additional examples following. A more complete review of methods is given by O'Meara (2012).

The techniques center around models of evolutionary change of the trait along the branches of a species tree. For a continuous trait, this could be a simple diffusion model (cf. drift) or a more complex model that pulls the trait towards a
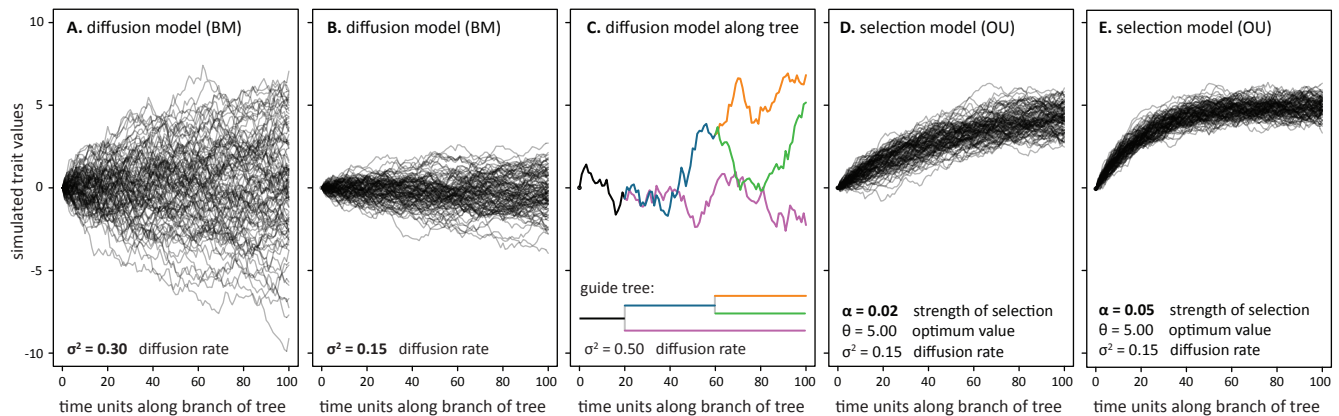
Fig. 1. Simulations of the evolution of continuous traits, illustrating trends in the expected distribution of trait values across 100 time units for different models. (A, B) Trait evolving according to a diffusion model (Brownian motion) results in a normal distribution of expected outcomes, with the spread being larger for higher values of σ^2. (C) Simulation of a trait along a three-taxon phylogeny, illustrating the inheritance of the parental trait in both sibling lineages at nodes and independent evolution along all the branches. (D, E) Trait evolving according to a model with selection (Ornstein-Uhlenbeck), the pull being towards an optimum value of 5.00 and the strength parameter α determining how quickly the trait evolves towards the optimum. All simulations start at a root value for the trait of 0.00.

certain optimum value over time (cf. selection), or any other model by which the trait may conceivably evolve (see below).

Once a model has been defined, it needs to be confronted with the data, which consist of observations (values) of the trait of interest for a set of species. With these data and a phylogenetic tree describing the relationships between the species, the model parameters are estimated using maximum likelihood optimization or Bayesian inference. Different competing evolutionary hypotheses can now be defined as alternative models, and the relative fit of those models to the data can be compared, yielding insight into which of the hypotheses most appropriately explains the observed variation in the trait.

Trait evolution

A very simple way of modelling the evolution of a continuous trait is by assuming a diffusion process (Brownian motion). This process is time dependent, with the expected change of the trait value during a time step having a normal probability distribution around the value before the time step. More specifically, the expected change can be written as

$$dX(t) = \sigma \, dB(t),$$

where $dX(t)$ is the change in the trait over the time step dt, σ is the rate of evolution of the trait, and $dB(t)$ is a normally distributed random variable with mean 0 and variance dt (notation following Butler & King 2004). When referring to time, in fact we mean units of branch length in the corresponding phylogenetic tree. For an ultrametric time-calibrated tree this corresponds to time in the conventional sense; otherwise it would typically represent the estimated amount of change in the molecular data, and

either approach can be useful (Litsios & Salamin 2012). It follows from the equation that the expected amount of change (diffusion) of the trait is thus proportional to its rate (Fig. 1A vs. B). At nodes in the tree, both sibling lineages start from the trait value prior to the split, but then continue on an independent evolutionary path (Fig. 1C). The diffusion model has two parameters, the rate of diffusion σ and the value of the trait at the root of the tree.

This model of evolution by diffusion is the basis from which more complex models of trait evolution are derived. For example, one could hypothesize that the evolution of a trait is faster in one part of the tree than in another. To test this, a model can be defined with different diffusion rates for different parts of the tree ($\sigma_1 \neq \sigma_2$), and the fit of this model can then be compared to that of the single rate model ($\sigma_1 = \sigma_2$) (O'Meara et al. 2006). Similarly, there are models with which you can evaluate the "early burst" type of evolution where most change in the trait occurs early on in the phylogeny (Harmon et al. 2010), and models that can evaluate the gradual vs. punctuational evolutionary behavior of the trait (Pagel 1999).

When a trait is assumed to be under selection, this can also be facilitated in the models (Butler & King 2004). For this, a second term is added to the change in the character:

$$dX(t) = \sigma \, dB(t) + \alpha \, [\theta - X(t)] \, dt$$

The first term represents diffusion as above, while the second adds a directional pull of strength α towards an optimal trait value θ. The pull towards the optimum is proportional to the difference between the optimum and the current value of the trait $[\theta - X(t)]$, which means that the further the trait value is situated from the optimum, the stronger the pull towards the optimum will be. The strength parameter also determines how strong the pull is, with higher values lead-

ing to quicker evolution of the trait towards its optimum value (Fig. 1D vs. E). Qpeg"ci ckp."xctkcvkqpu"qp"vj ku"vj go g ecp"dg"o cf g."uq"vj cv"f khhgtgpv"r ctu"qh"vj g"vtgg"ecp"j cxg"f khhgtgpv"ugpgevkxg"tgi ko gu.""g0 0f khhgtgpv"tctk/"qr ko c" $_3$." $_4$"qt"f khhgtgpv"uvtgpi vj u"qh"ugngevkqp"" $_3$." $_4$*Dgcwkgw"gv"cn0"4234+0"Qt vj g"qr vko wo "xcnwg"kuugnh"eqwnf "gxqnxg"cnqpi "vj g"vtgg"*J cp/ugp"gv"cn0"422; +0

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Models of trait evolution can also be used to estimate ancestral trait values for the interior nodes of a phylogeny (Paradis 2012). Ancestral trait estimation is sensitive to model misspecification and becomes harder for faster-evolving traits because they retain less signal about ancestral states, and results should be interpreted cautiously (Martins 1999).

Diversification

Phylogeny-based modelling techniques can also contribute to our understanding of diversification dynamics, i.e. how speciation and extinction have generated the biodiversity of the group under study (Mooers et al. 2007). Each node in the tree represents a past lineage splitting (speciation) event, and branch lengths can be taken to represent the interval between speciation events (assuming that the phylogenetic tree is a chronogram and that no extinctions have taken place). If two sister lineages have produced widely different numbers of extant species, then one could propose the hypothesis that they diversify at different rates. These types of patterns can be analyzed with models of lineage diversification.

The simplest diversification model is the speciation-only model (Yule model), which has a single parameter (the speciation rate λ) that is constant throughout the tree (Fig. 2A). One step up in terms of complexity is the birth-death model, which includes both the rate of speciation λ and the rate of extinction μ, which are both assumed constant. Additional models have been developed to permit testing for differences in λ and μ in different lineages of the phylogeny (Rabosky et al. 2007) or to evaluate the hypoth-

esis that diversification changes over time (Figs 2B,C) (Rabosky 2006, Morlon et al. 2011, Stadler 2011). One example of the latter is the density-dependent model in which diversification slows down over time because, as the number of species in the lineage grows, niches and geographic areas are filled and opportunity for speciation is reduced (Fig. 2B). Another set of models offers the interesting possibility of investigating the relationship between diversification and species traits by treating λ and/or μ as a function of the trait value (Fig. 2D) (Paradis 2005, FitzJohn 2010, Maddison et al. 2007).

While these models offer great possibilities for investigating how diversification might proceed through the evolution of a lineage, it is important to realize that different processes can sometimes lead to similar results and that sampling schemes can bias results, both of which can make it difficult to identify the most suitable model for certain datasets (e.g. Rabosky 2009, Morlon et al. 2011, Höhna et al. 2011).

Comparison between alternative models, both for trait evolution and diversification dynamics, is typically done using information criteria in a maximum likelihood framework (e.g., Butler & King 2004) or with reversible jump MCMC or Bayes factors in a Bayesian inference framework (Pagel & Meade 2006, Eastman et al. 2011). It was recently shown that common information criteria tend to choose overly complex models, and that it is therefore useful to assess the power of the test in the context of the type of dataset and shape of the phylogeny being studied (Boettiger et al. 2012).

Niche evolution as a case study

Niche evolution refers to changes of ecological traits over time. It is a very timely topic to study, as it will help us understand species' responses to climate change as well as spread of invasives, parapatric speciation, patterns of biodiversity, etc. Niche evolution can be studied at different timescales, ranging from the geological to the ecological. Near the geological timescale end of the spectrum, this involves multi-species analyses of ecological traits in a phylogenetic context as described above. Studies at ecological timescales more typically focus on micro-evolutionary processes within a single species.

The evolution of niches on geological timescales can be investigated through the relationships between phylogenies and niche traits (Fig. 3). Since temperature plays a major role in algal survival and distributions (Breeman 1988, Lüning 1990, Eggert 2012), this is an obvious niche trait to study. We will illustrate the process of analyzing the evolution of species' sea surface temperature (SST) affinities with our work on the green algal genus *Halimeda* (Verbruggen et al. 2009, Marcelino 2012). The workflow is illustrated in Fig 3. Based on a large number of georeferenced collections and good insights in species boundaries based on DNA barcodes and morphometric analyses (Verbruggen et al. 2005), it is straightforward to approximate the SST affinities of species by extracting average annual SST values from a GIS database (Bio-ORACLE: Tyberghein et al. 2012).
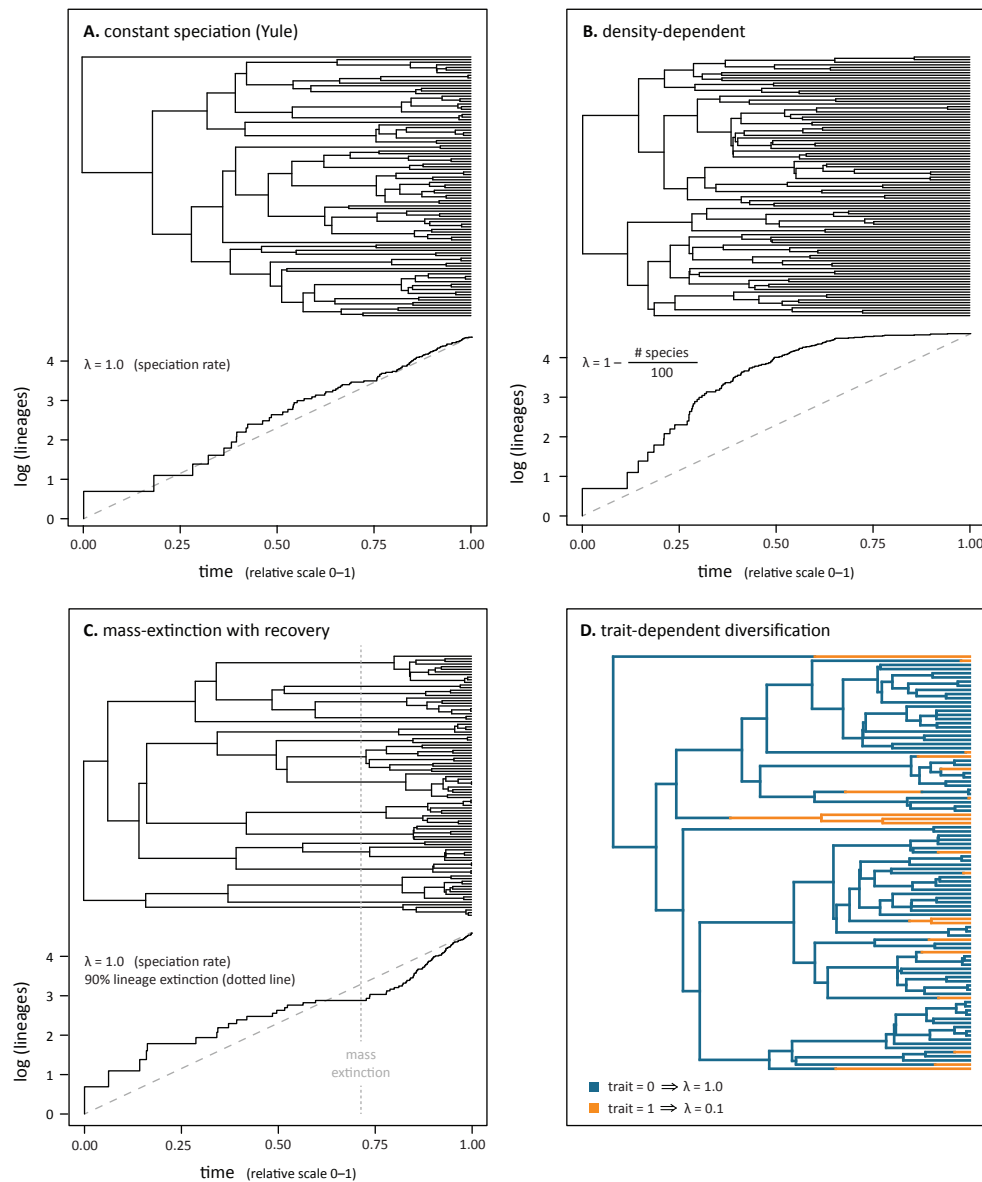
Fig. 2. Simulations of diversification dynamics, illustrating the different tree shapes they result in. (A) Speciation at a constant rate results in an exponential increase of the number of lineages through time, i.e. a linear lineages through time (LTT) plot. (B) The density dependent model has high rates of speciation in the beginning, with longer branches (i.e. less speciation) towards more recent times. (C) Simulation using a model with a mass extinction results in long internal branches and denser branching near the tips of the phylogeny. (D) For a model in which speciation rates are dependent on the state of a trait, one gets longer branches and less diversity for lineages with one state of the trait (orange) and shorter branches with more diversity for lineages with the other state of the trait (blue). All models illustrated have background extinction rates of zero; the only form of extinction used is a mass extinction in panel C. Note that lineage through time plots only serve to visualize the simulated patterns of diversification. It is not advised to derive conclusions about diversification dynamics from LTT plots alone, because a variety of mechanisms can result in virtually identical LTT plots.

Using a Brownian motion model of trait evolution (workflow ① in Fig. 3B), we have estimated ancestral SST affinities by likelihood maximization. This indicated that *Halimeda* has tropical origins and that while 4 out of 5 sections show strong niche conservatism for warm tropical waters, the other section (*Halimeda*) has been able to colonize colder waters at least three and possibly five times independently (Fig. 4). Using a model that permits measuring the relative contribution of punctuational vs.

gradual change, it was shown that SST evolve predominantly gradually rather than in speciation-associated bursts (Verbruggen et al. 2009).

Further investigations on the genus' thermal niche have shown that section *Halimeda* is under a different evolutionary regime than the other clades. The thermal niche in section *Halimeda* appears to evolve according to a Brownian motion expectation. In contrast, the evolutionary pattern of the other clades is better explained by the
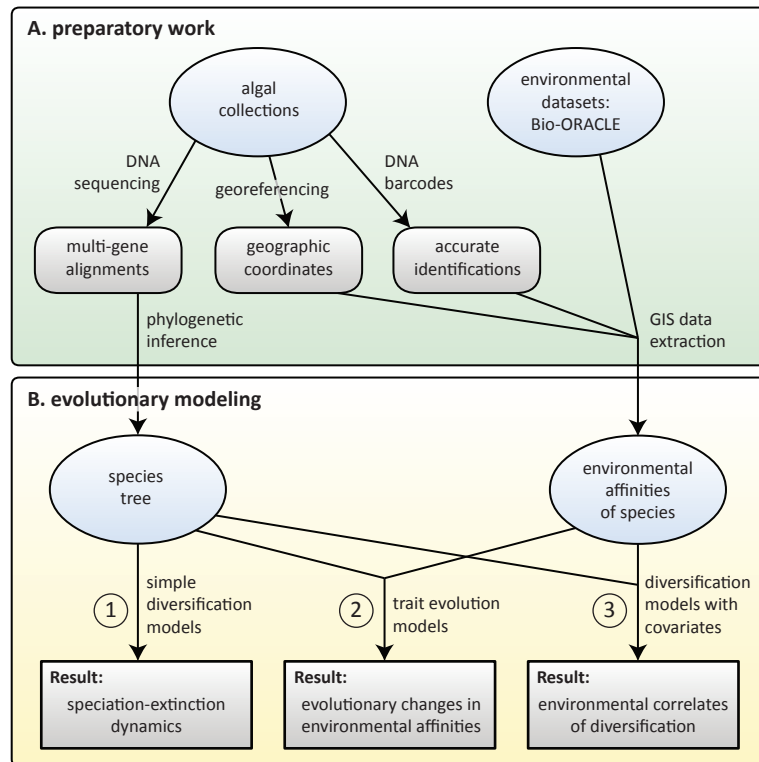
Fig. 3. Generalized workflow for studying evolutionary dynamics of niches and their interactions with diversification. Initial screening of many algal collections with DNA barcodes provides insight into species boundaries and allows for accurate identification of specimens. Subsequently, a species tree is generated, generally by sequencing multiple genes for one specimen of each species and phylogenetic analysis of the resulting alignment. The DNA barcodes provide information about species membership and by querying an environmental GIS dataset with the geographic coordinates of the collections, a dataset characterizing the environmental affinities of the species can be obtained. Using the modelling techniques described in the text, one can subsequently infer diversification dynamics, evolutionary patterns in environmental affinities, and environmental correlates of diversification for the group of interest.

Ornstein-Uhlenbeck model (Fig. 1D-E). The model indicates that these sections have an optimum SST preference of $\theta = 28°C$ and are retained close to this temperature by a strong selective force ($\alpha = 0.22$).

Of course, SST affinities do not evolve independently from other species features, and it is relevant to ask which other species traits may facilitate evolution along the temperature axis. In the tropics, many *Halimeda* species live on sheltered reef slopes or in lagoons, but several other species inhabit reef crests, where they are exposed to higher variations in temperature and other physical disturbances. One could hypothesize that adaptations to such environments enhance survival in colder water and facilitate evolution along the SST axis. Using models designed to test this hypothesis, we found that species living in exposed habitats have thermal preferences evolving 3X faster than species inhabiting sheltered areas (Marcelino 2012).

All the work mentioned above focuses on the evolution of species traits (workflow ① in Fig 3B). We have also studied the relationship between the rate of diversification and niche evolution (workflow ② in Fig. 3B). Here, we used the green algal genus *Codium*, a genus with ca. 3X more species than *Halimeda*. Species-rich taxa make better models to study diversification because large numbers of species are needed to accurately infer model parameters. Our work on *Codium* showed that a model in which the rate of diversification of a lineage is a function of the SST affinity of that lineage provides a much better fit to the data than a simple model of constant diversification throughout the tree (Verbruggen et al., unpublished). More specifically, we found that lineages with higher SST showed higher rates of diversification. While the observed correlation between SST and diversification is statistically sound, it should not necessarily be seen as a causal relationship because other factors co-varying with SST may impact on diversification.

More generally speaking, testing causal hypotheses is beyond the reach of the evolutionary modeling approach. Experimental procedures can offer complementary insights into causal mechanisms (Weber & Agrawal 2012, Reusch 2014). Besides examining the phenotype and assessing the potential for thermal adaptation in breeding experiments (e.g. Clark et al. 2013), it is useful to perform genome scans of populations to identify genes under selection along temporal and/or geographical thermal gradients (e.g. Jueterbock 2013).
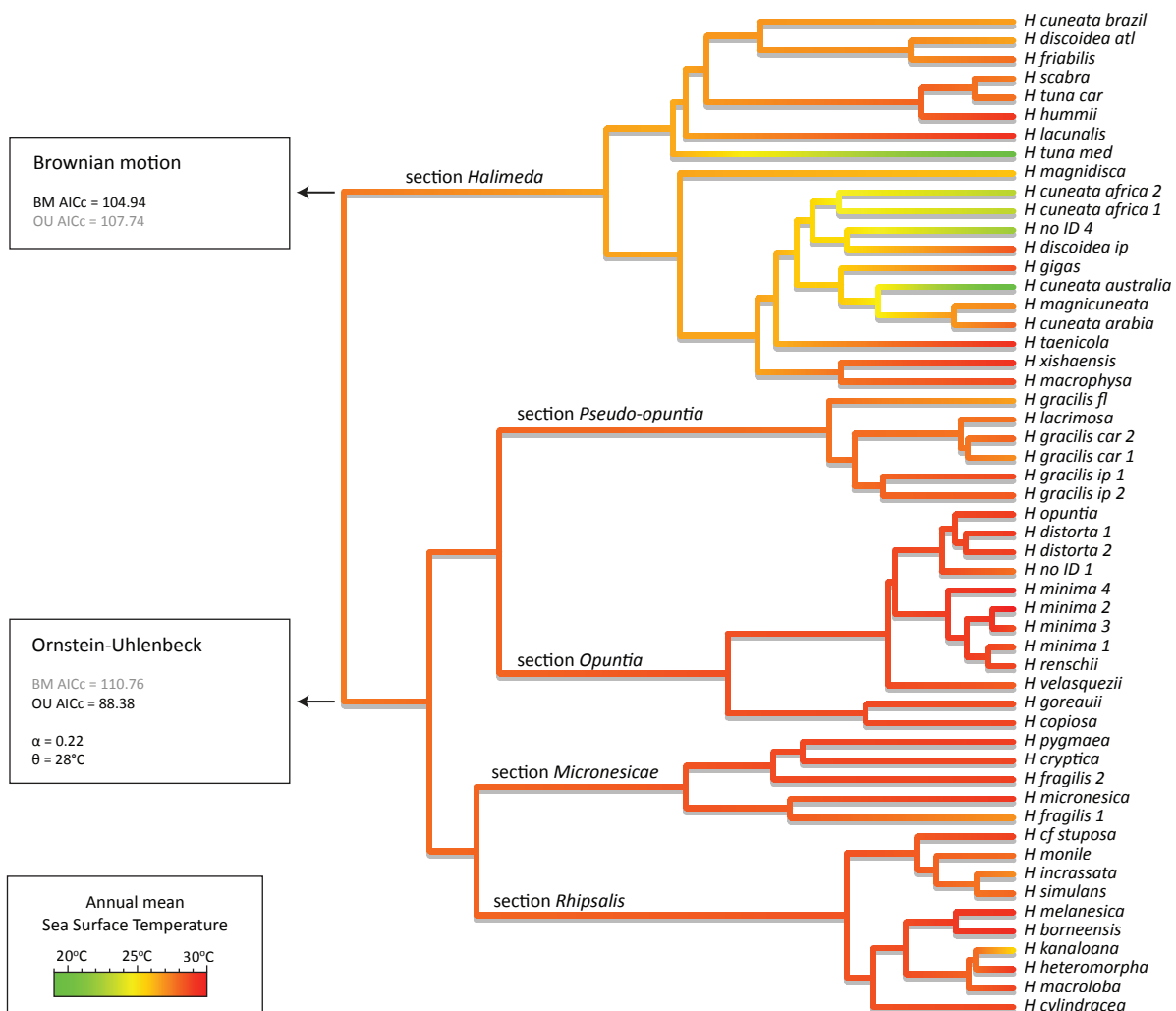
Fig. 4. Phylogeny of the seaweed genus *Halimeda* and the estimated ancestral character states of its thermal niche. The mean sea surface temperature for each species was based on occurrence records and an environmental dataset, and ancestral states were estimated using a maximum likelihood optimization of a Brownian motion model. We analyzed the evolution of thermal niches individually in section *Halimeda*, which has several cold water species, and the remaining four sections of the genus that are restricted to warm waters. The model of trait evolution was chosen according to the Akaike Information Criterion corrected for sample sizes (AICc). In section *Halimeda* the simple diffusion model was favoured over a model incorporating selection. The Ornstein-Uhlenbeck model, which incorporates a selection term (see text), provides a better fit than the BM model for the other clades of the genus. The parameter optimization would suggest that these lineages show strong conservatism, with selection towards an optimum temperature of ca. 28°C.

Perspectives

There are countless examples of how evolutionary modelling could further our understanding of algal biology and how applying them to algal datasets would improve our knowledge of evolutionary biology more broadly. Several important traits are particularly frequent or diverse in algae, and there is much we have yet to learn about their evolution. How did different life cycles evolve and does the origin of particular life cycle features impact on rates of speciation and extinction (Cock et al. 2014, John 1994)? When did multicellularity and the diversity of seaweed growth forms

arise and what has its effect on diversification been (LoDuca & Behringer 2009, Knoll 2011)? What are the evolutionary dynamics associated with transitions from a photosynthetic lifestyle to parasitism (Blouin & Lane 2012)?

Physiological traits can be analyzed in the same way if comparative data across multiple species are collected. And there are several interesting questions to address, including the origins of carbon concentrating mechanisms (Giordano et al. 2005, Edwards & Smith 2010) and the evolutionary trends observed in trace element utilization (Quigg et al. 2003, Quigg et al. 2011), just to name two. The same is true for genome traits such as genome content (Smith et

al. 2013), the diversification of gene families (Cock et al. 2010), molecular evolutionary dynamics of genes of interest (Cocquyt et al. 2009), selection on genes in association with environmental change, etc.

These sorts of hypotheses become testable through evolutionary modelling techniques. Different scenarios about the evolution of a trait (or of diversification) are expressed as alternative models, which are then confronted with the available data in statistical analyses, and allow weighing the evidence for each of them. By doing so, we will be able to judge the relative credibility of different hypotheses and measure uncertainty about those hypotheses.

Despite the promise of these techniques, it is important to realize that inferring past evolutionary dynamics from data of contemporary species is an innately difficult task, and in some cases the information available may not suffice to come to clear conclusions (Losos 2011). As with all modelling endeavours, having more informative data leads to more accurate estimation of model parameters and more power to distinguish between competing models (Boettiger et al. 2012). For evolutionary models, increasing the amount of data comes down to including more species, i.e. using bigger phylogenies and having trait data for each species in those phylogenies. So, in order to answer more complex questions with confidence, we will need to broaden up our studies to higher taxa and assemble big datasets of comparative data. And, as mentioned before, some questions may be impossible to answer, but at least the modeling approach offers the possibility to test whether it is reasonable to assume that they can be answered. A useful approach in this context is the one described by Boettiger et al. (2012), in which simulations under alternative models are used to assess the power of the model comparison, i.e. to provide an indication of whether the phylogenetic tree is large and structured enough to permit distinguishing between evolutionary hypotheses. Furthermore, as is always the case, it is important to interpret the results critically and consider biological realism along with statistical significance (e.g., Losos 2011).

Interactions between macroevolutionary work at the geological timescale and microevolutionary work looking into the benefits and costs of adaptations also offer an interesting avenue of future research. While the microevolutionary work on its own lacks generality and the macroevolutionary approach on its own falls short in addressing causal mechanisms, both methods generate hypotheses that can be tested using the other method, yielding more profound insights into the evolutionary dynamics of traits and diversification (Weber & Agrawal 2012).

There is no doubt that evolutionary modelling techniques are a useful addition to one's toolbox. Fortunately, many methods for evolutionary analysis have been implemented in R, an open-source statistical computing platform. Paradis (2012) offers an excellent introduction to using these tools, and an overview of implemented methods is available online at http://cran.r-project.org/web/views/Phylogenetics.html.

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