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MORPHOLOGICAL COMPLEXITY, PLASTICITY, AND SPECIES DIAGNOSABILITY IN THE APPLICATION OF OLD SPECIES NAMES IN DNA-BASED TAXONOMIES

A paper by Belton et al. (2013) published in this issue of the *Journal of Phycology* addresses species boundaries in the *Caulerpa racemosa–peltata* complex. *Caulerpa* is a member of the siphonous green algae (order Bryopsidales), which consist of a single giant cell that forms a simple tube or one that branches to form a range of morphologies, from very simple branched tubes to much more complex architectures consisting of a medulla and cortex that can display elaborate macromorphological features (Hillis-Colinvaux 1984, Verbruggen et al. 2009a).

In *Caulerpa*, species display a complex morphology consisting of a stolon bearing root-like rhizoids and upright stalks (rachis) with lateral branchlets (ramuli; Fig. 1H). In "paradigm" *C. racemosa* the branchlets are spherical (Fig. 1E), whereas in *C. peltata* they are umbrella-like (Fig. 1A), although in reality one finds all sorts of intermediates between these morphologies (Fig. 1, A–E) as well as some other morphologies (Fig. 1, F–G). Furthermore, culture studies have provided evidence for habitat-induced phenotypic plasticity of the branchlets and the overall thallus appearance (Calvert 1976, Ohba and Enomoto 1987, Ohba et al. 1992). It is therefore no surprise that the *C. racemosa–peltata* complex has long troubled algal taxonomists.

Two centuries of taxonomic work on the complex have resulted in a Gordian knot of more than 50 formally described species and intraspecific taxa that have been merged back into *racemosa* and *peltata*, with several additional aberrant morphological variations on the same theme being described as separate taxonomic entities. Some workers have recognized the plasticity induced by microhabitat and chosen a system with few species and some ecomorphs (ecads) within them.

Over the last few decades, molecular work showed that the *C. racemosa–peltata* complex consists of multiple clusters that are likely to correspond to species (e.g., Famà et al. 2002, de Senerpont-Domis et al. 2003, Sauvage et al. 2013). The new work by Belton et al. (2013) applies objective methods to detect species boundaries in DNA data. Put simply, the method they use starts from a large haplotype tree and detects the transition between the type of branching one would expect to see above the species level (i.e., a Yule model) and the type of branching one would expect to see within species (i.e., a coalescent model). This transition should thus correspond to the species boundary and can be used to define species-level clusters (Pons et al. 2006, Fujita et al. 2012, Carstens et al. 2013, Payo et al. 2013). This method, used in combination with a second approach based on branch support, implied that the *C. racemosa–peltata* complex consists of 11 species.

But, accurate as it may be, the resulting DNA-based taxonomy does not resolve the taxonomic conundrum; it is only the first step. The toughest job is to choose appropriate names for the 11 species that are recovered with the DNA work. With several dozen existing species and variety names to choose from, and knowing that the species exhibit morphological plasticity, this is clearly a very difficult task. In fact, the discrepancy between the characters we use currently to discover species (mostly DNA) and the fact that we need to give new species names that take into account all the existing names which were based on a different set of features (predominantly morphological), has created much uncertainty and decision paralysis (De Clerck et al. 2013). Some have used DNA sequencing of type specimens as a solution (Hughey et al. 2002, Hayden et al. 2003, Gabrielson et al. 2011), although others have identified serious problems with this approach (Saunders and McDevit 2012). The poor preservation of many type specimens and the limited accessibility of types for destructive DNA work mean that this approach will not be feasible across the board, and we will more than likely continue to rely on morphological information to resolve the remaining problems. So the question of how likely we are to be able to assign old names to new taxa based on morphological comparison is a very relevant one to ask.

In this article, I aim to quantify how the morphological complexity of a taxon affects the diagnosability of its species (i.e., identification success at the

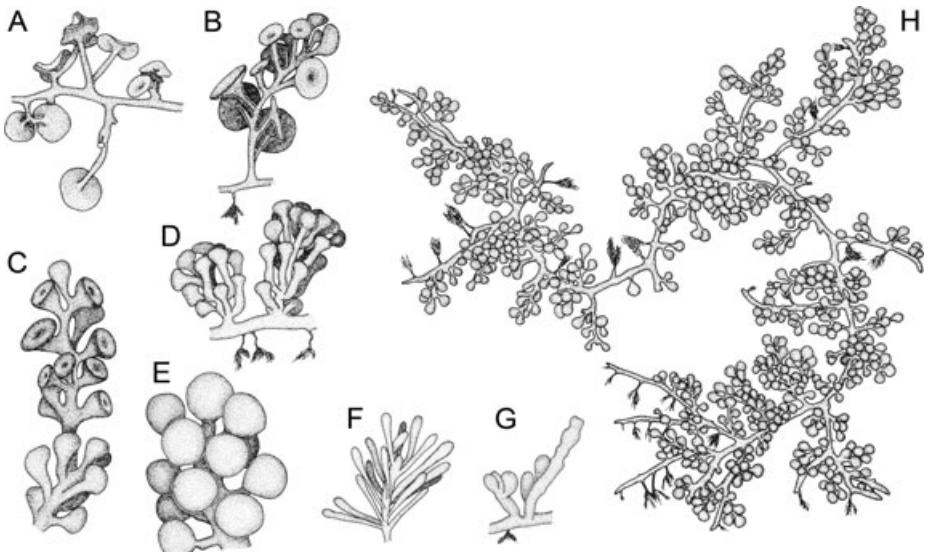

FIG. 1. Illustration of the morphological variability observed in the *Caulerpa racemosa–peltata* complex. (A–E) The morphological continuum between the umbrella-like branchlets characteristic of *C. peltata* (A) and the round branchlets typical of *C. racemosa* (E). (F and G) Two additional morphologies that used to be recognized as varieties under *C. racemosa* prior to the study by Belton et al. (2013). (H) A typical thallus of a *Caulerpa* with vesiculate ramuli, the stolons bearing root-like rhizoids and upright assimilators bearing laterals. The drawings are reproduced from Coppejans and Beeckman (1989), with permission from Schweizerbart publishing (www.schweizerbart.de).

species level), and how morphological plasticity in response to habitats influences this relationship. Based on these results, I will discuss the uncertainty inherent in reconciling old species names with DNA-based taxonomies.

As Madeleine van Oppen and coworkers pointed out nearly two decades ago, many groups of algae suffer from a "low-morphology problem" leading to the presence of cryptic species (i.e., morphologically indistinguishable species; van Oppen et al. 1996). In continuation of this idea, I calculated that, if the morphology of a taxon can be scored as a set of X binary characters, and morphological species boundaries are defined by a minimum of a one-character difference, the theoretical number of morphologically diagnosable species (N) increases exponentially with the number of characters available ($N = 2^X$). Consequently, chances of encountering multiple species with identical morphologies increase quickly in taxa of lower morphological complexity (Verbruggen et al. 2009b).

While such reasoning is useful conceptually, it is unlikely that all theoretically possible morphologies will be produced in the course of the evolution of a lineage. To obtain a more realistic image of morphospace occupancy, I have now simulated character data using sensible models of character evolution. In summary, this approach consists of generating phylogenetic trees containing a number of species (between 10 and 400), and subsequently letting a set of traits (i.e., morphological characters) evolve along this phylogeny at a rate that corresponds to those measured for a real algal morphometric data set. The result of this exercise is a set of values for each trait for each species in the phylogeny. Those can then be compared with each other to evaluate how many of the species can be reliably distinguished from one another. For a more detailed description of the simulations, see the author's blog at http://phycoweb.wordpress.com/.

As could be expected, only a small subset of all possible character combinations were produced during the evolution of the simulated lineages. For example, when lineages of 400 species were simulated, only ~50 distinct morphologies were produced in lineages with 20 characters, and only ~20 morphologies in lineages with 10 characters (Fig. 2A). It is evident that more complex lineages (i.e., with more characters) reach higher actual numbers of diagnosable species than simpler lineages. Consequently, complex lineages have a higher fraction of species pairs that are distinguishable (Fig. 2B), and these fractions are not influenced by the number of species in the lineages.

The same pattern returns if continuous rather than binary characters are used: 54.2% of species pairs could be distinguished for lineages with 10 characters, whereas this number increased to 72.5% for organisms with 20 characters (Fig. 3, 1st vs. 4th boxplot). In conclusion, it appears to be a general rule that species of more complex lineages (i.e., those having more characters) are more easily distinguishable from one another than species of simpler lineages.

But how about selection? We know that habitat has a major influence on the morphology of organisms. For example, macroalgae from very different phylogenetic backgrounds (Rhodophyta, Ulvophyceae, and

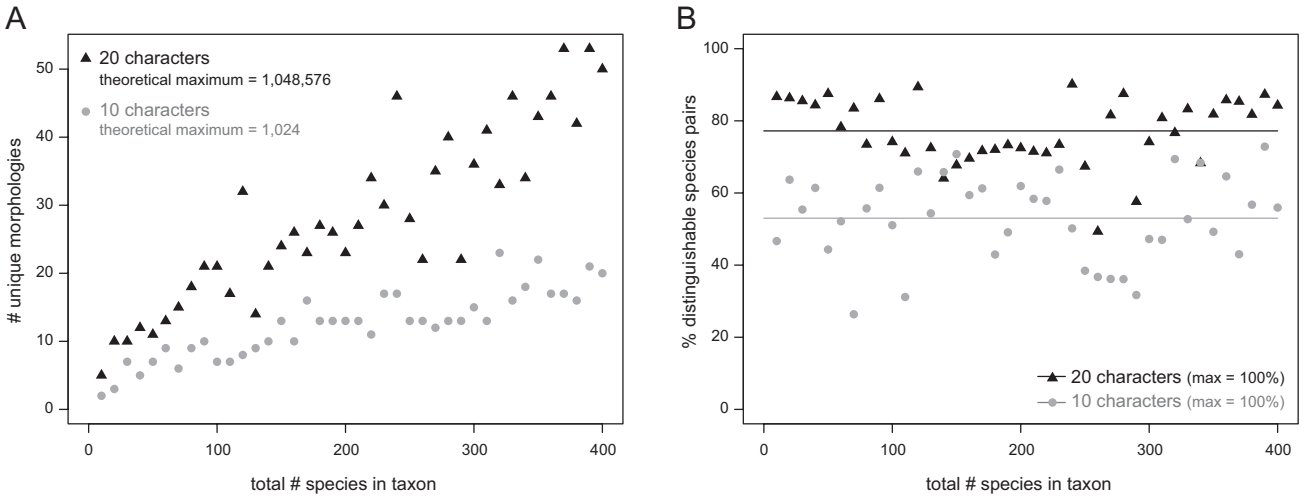

FIG. 2. Results for evolutionary simulations of binary morphological traits. (A) The number of unique morphologies increases with the total number of species in the taxon, but that there are always much fewer unique morphologies than there are actual species. It also shows that feature-rich organisms (black triangles) have more unique morphologies than feature-poor organisms (gray dots). (B) The simulated species' morphologies have been compared one by one to calculate the percentage of species that can be distinguished morphologically.

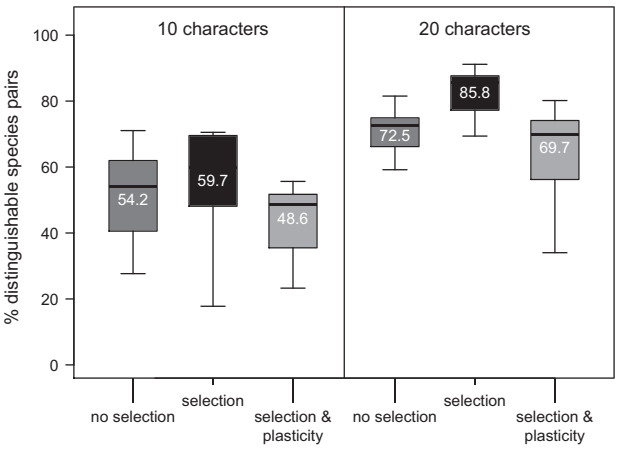

FIG. 3. Results for the evolutionary simulation of continuous traits. The boxplots depict the percentage of distinguishable species pairs (a measure of species diagnosability) in simulations under the conditions listed along the *x*-axis and above the box plots. Whereas simulations assuming habitat selection (boxplots 2 and 4) led to increased species diagnosability, assuming morphological plasticity in response to the environment had a detrimental effect on diagnosability (boxplots 3 & 6). The differences between conditions were more pronounced for more complex organisms (right side of graph) and these organisms also had higher overall species diagnosability.

Phaeophyceae) have converged onto very similar body architectures in similar environments (e.g., Littler and Littler 1980, Steneck and Dethier 1994). The simulations I have presented so far have assumed that the habitat in which a lineage lives does not influence its morphology.

To evaluate the effect of habitat selection on the morphological distinctness of species, a second set of simulations was done. The lineages were first allowed to evolve into five different habitats. Subsequently, the evolution of morphological traits was simulated, with half of the traits evolving toward different optimal trait values dictated by the habitat in which the lineage lives and the other half evolving free of selection. As it turns out, this increases the overall morphological distinctness of species. For simulations of 10 characters, a nonsignificant rise was observed from 54.2% to 59.7% distinguishable species pairs, and for simulations of 20 characters a significant rise from 72.5% to 85.8% was observed (Fig. 3).

This is a somewhat counterintuitive result because one would expect selection to lead to morphological similarity of species living in the same habitat, hence reducing the percentage of distinguishable species. While this reasoning is true, it is incomplete because it ignores the 50% of characters that are not under habitat selection. Put simply, selection subdivides the morphologies into five habitat-specific categories, thereby subdividing the species distinguishability problem into five smaller subproblems (one for each habitat). These smaller subproblems are easier to solve with the remaining characters that are not under selection. As a concrete example one could think of *Ulva* and *Porphyra*. These have very similar leaf-like overall appearances that can be taken to be the result of evolution into the same environment. Yet it is easy to distinguish between them using a range of other characters that are not (or less) determined by their habitat. It is likely that increasing the percentage of characters under selection in the simulation will result in a decrease rather than an increase of species distinctness. Such further experiments are relevant because in genera like *Caulerpa* most measurable characters

are related to thallus structure and thus prone to habitat selection.

But clearly, selection is only part of the story. So far, I have assumed that every species lives in a single habitat. In most organisms, and this is certainly true for algae, one also has species that live in multiple environments and feature adaptive morphological plasticity in response to those environments (e.g., de Senerpont-Domis et al. 2003, Demes et al. 2009, Monro and Poore 2009). To accommodate this reality, a second layer of complexity was added to the simulations. First, a "plasticity trait" was simulated along the phylogeny. This trait can switch on and off, resulting in parts of the tree having morphological plasticity and other parts of the tree not having it. Subsequently, the lineages were allowed to evolve into five habitats as above, with the exception that lineages with plasticity occupied all five habitats rather than one. Finally, the evolution of morphological traits was simulated along the phylogenies, with 50% of the characters under habitat selection as above and 50% not under selection. In lineages with plasticity, there were five parallel paths of evolution, each toward the optimal morphology for one of the habitats.

For these simulations, species distinguishability was drastically less than for the previous simulations (48.6% for the simpler organisms, 69.7% for the more complex organisms; Fig. 3). In other words, plasticity has a strongly negative effect on the potential to recognize species based on their morphology. Any advantages brought about by habitat selection (i.e., subdivision of the species distinguishability problem into subproblems) are completely wiped out by the presence of species that have distinctive morphologies in the different habitats they inhabit.

There are three main messages we can take home from this simulation exercise. First, there are substantially fewer unique morphologies than there are species in lineages with simple structures. In other words, we can expect cryptic diversity to abound and indeed, this appears to be the case in algal taxonomy. As such, for any given algal taxon, we should expect to be unable to distinguish between at least some and possibly many of its species based on morphology alone. The second conclusion is that these problems are more pronounced in simple organisms than in more complex organisms, but even in complex organisms there are generally much fewer distinct morphologies than there are species. Third, habitat-induced plasticity drastically reduces the likelihood that one can distinguish between species based on morphology, even in complex organisms.

These simulation results, in combination with what we have learned from empirical studies over the last few decades, have clear consequences for how species-level taxonomy is best approached in algae. Most importantly, morphological features have lost credibility as the primary species delimitation criterion. They can still play an important role in the initial discovery of unknown entities in the field or in collections, but should not be trusted to be informative about species boundaries without verification by other methods (preferably DNA-based). Importantly, the results also suggest that one should not assume that because two individuals look alike, they are going to belong to the same species because a substantial proportion of species can be expected to be cryptic. This has consequences for how field-work is carried out. Rather than sampling one or a few individuals of each morphological type, we should move to a sampling design where many individuals of similar morphology are investigated in detail.

So how should species be delimited? Neutral DNA markers are an obvious and easily obtainable alternative source of information. They are relevant about species boundaries, much less prone to convergence, plasticity and cryptic behavior than morphological features, and there are appropriate statistical methods that can be used to test alternative hypotheses about species limits based on them (Fujita et al. 2012). That said, with the currently used sequencing and analysis methods, DNA taxonomy is not a silver bullet. Recent speciation events may be hard to detect reliably with the most commonly used markers, and with single-marker approaches in general due to effects of lineage sorting and introgression (Mattio and Payri 2010, Neiva et al. 2010, Zardi et al. 2011). However, with the improvements in sequencing technologies that we are experiencing, one can soon expect a shift from single-marker to multimarker approaches, which will drastically improve our ability to distinguish between closely related species, hopefully even in the face of hybridization (e.g., Zardi et al. 2011). Leliaert et al. (2014) provide a review of DNA-based species delimitation in phycology.

Even after DNA sequencing takes central stage in species delimitation, morphological characterization of species will remain a critical task. Features like shape, size, and color will remain our first visual point of access to the algae we study and our first clue to their identity for the time to come. Algal morphology is of great ecological relevance, as is illustrated by the body of work on algal functional morphology as well as the various references to morphological plasticity made in this paper and elsewhere. It also serves as a key feature in research into functional genomics and physiology, and we need to characterize it to understand the evolutionary dynamics of algal shapes and their functionality.

In species-level taxonomy, morphological features are increasingly being used as secondary defining features, after more reliable features have been used to define species boundaries. Should DNA sequences suggest that there are multiple species in a set of samples, the logical next step is to search for morphological clues that support or contradict

this hypothesis. This approach, which is sometimes called "molecular-assisted alpha taxonomy" or "reverse taxonomy," has been widely adopted and is hugely successful in algal taxonomy. As part of this approach, morphological features will also continue to play a major role in nomenclature, more specifically in the process of fitting old names into modern, DNA-based taxonomies. As is evident from the simulations presented here, this process will often involve dealing with uncertainty because not all species will have unique morphologies. And rather than letting this uncertainty grind algal taxonomy to a standstill, we should be pragmatic in making educated decisions in the face of uncertainty to move algal taxonomy forward.

All of this relates back to the revision of the *C. racemosa–peltata* complex in a very direct way. Nomenclatural decisions need to be made in the face of uncertainty, in this case in a situation where relatively few morphological characters are available and where they are under strong influence of habitat-induced plasticity. Belton et al. (2013) do this very judiciously. They use appropriate molecular tools to delimit species. Following species delimitation, they proceed with an in-depth morphological examination of the clades they recovered and of many relevant historic synonyms. Based on all this, and despite the many remaining uncertainties, they make informed decisions to provide a much-needed taxonomic update, and in that respect I consider it to be an exemplary study. We finally have a proper species name for one of the most notorious invasive seaweeds of the world (*C. cylindracea*). We now know that one of the species, which is very abundant in tropical and subtropical habitats around the world, is extremely morphologically plastic, and it has a proper name (*C. chemnitzia*). And we now realize that besides this morphologically highly variable species, there is a whole array of other species with narrower morphological bounds, and the majority of these have now been given appropriate names.

Of course, no scientific study comes without caveats. Belton et al. have not sequenced any types, and they were unable to get new collections from several type localities. They also have not formally quantified morphological variation and used statistical tools to match the morphology of types with that of sequenced samples. Doing that extra work could have reduced uncertainty somewhat but would certainly not have eliminated it. Inevitably, this is not the final word about this troublesome species complex. It is possible that some of the applied names will need to be revised in the future or that multimarker work will suggest altering some of the species boundaries. But, as the Pareto principle states, 80% of the effects come from 20% of the causes, or translated to this situation, a majority of correct conclusions can be reached with limited information. That is, in my opinion, what Belton and co-workers have achieved through careful consideration of alternatives and pragmatic decision-making. Reducing the remaining uncertainties and making further taxonomic refinements will take substantial and continued effort from the algal systematics community.

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