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HORIZONS

Molecular and morphological methods for identifying plankton: what makes a successful marriage?

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Precise identification of species is critical for the study of biogeography of plankton and for applying laboratory culture results to the same organism in situ. Traditionally, identification has been based on knowledge of morphological traits transmitted from generation to generation of planktologists in monographs or at the bench. Despite recent rapid growth of molecular methods, taxonomists have been slow to incorporate molecular information in a formal way into species descriptions. Likewise, molecular biologists have often been less than thorough about making precise identifications of the species they sequence, as the large number of sequences in the public databases that are linked to mis- or unidentified species will attest. Although some have advocated for a new taxonomy built solely on a scaffold of DNA, for the present it seems wise to use a "total evidence" approach in identifying plankton, relying on both molecular and morphological information whenever possible. There is a large body of information on morphology, phenotypic variation, distribution and ecology of many species that is recorded in their formal descriptions, and this would be lost in a DNA-only approach. Without a successful marriage of molecular and morphological methods, it will be more difficult to solve the mystery of cryptic species. For now, we recommend that molecular approaches to identification be developed and extended where possible, that serious effort be committed to ensuring correct identification of species when DNA sequences are published and that new species of plankton should not be named based on morphology alone without supporting molecular information, especially for protists.

INTRODUCTION

Studies on the distribution and abundance of plankton in lakes and oceans, which have been going on for more than a century, have been hampered by the varying degree of precision in identification of individual species. Ecologically oriented biologists are usually the ones required to make the identifications, but they often do not have the taxonomic expertise to do so with the accuracy or consistency required to assign species. DNA-based methods are making it possible for rapid, inexpensive and precise identification of field-collected planktonic organisms, though the relationship of DNA sequences to morphological or biological species remains unclear. Moreover, the promise of DNA-based species

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identification raises questions regarding the process for linking extensive published taxonomic species descriptions, traditionally based on morphology, to molecular markers.

Our purpose is to discuss the "marriage" of morphological and molecular methods for the identification of planktonic organisms. The usefulness of molecular tools in plankton biodiversity studies seems inarguable and needs no review. A comprehensive survey of the literature in this vast field is also not possible in the space allocated here and we apologize in advance for omissions of some of the many papers that have contributed to rapid advances in recent years. Rather, we focus on challenges that have arisen when traditional and molecular methods give conflicting information. We will consider first the needs of planktologists with regard to identification, and briefly discuss traditional morphological and recent molecular methods as applied to plankton. Then, we present examples of successful marriages of the two approaches, highlight several emerging challenges, and discuss some implications of these challenges, especially the continual discovery of cryptic and rare genetic species. It should be pointed out at the start that "cryptic species" as we are using the term refers to genetic diversity hidden beneath apparent morphological homogeneity in one or a group of species. The term "cryptic diversity" is sometimes used to refer to the presence of unseen species (rare or inactive forms) in a natural assemblage. These may be morphologically diverse, just not observed (Fenchel *et al.*, 1997).

There is a considerable variation among researchers in both the specific needs for identification, and the level of precision required. In some cases, precise identification means answering a question such as "Is this the same species Lohmann described in 1908?" In others, the question might be "Is this the same organism I isolated last summer, whatever its formal name is?" The goal of identification may be extensive, for example to characterize diversity among all members of a community or guild (Doherty *et al.*, 2007); or it may be intensive, for example to describe the distribution and abundance over time of one or a few species (Costas *et al.*, 2007). The need for precise identification may not be the same for these alternatives. For example, one can describe the DNA diversity of a sample without naming the species within it, but if a description of the biogeographic distributions of individual species is the goal of the planktologist, precise identification is clearly essential. Protists provide special problems here, as discussed below, but it is obvious that misidentification of any species could lead to broad problems such as the descriptions of endemic species as widespread and cosmopolitan ones as endemic.

Traditional methods for identifying metazoan plankton involve examination of formalin-preserved collections. Extensive monographs are available for comparing a sample to named species, and this is especially effective for Crustacea, which often dominate the net-collected plankton. However, while precise identification is often possible for adult stages, some larval forms, for example many copepod nauplii, cannot be identified to species with light microscopy and may not ever have been definitively described.

For microbial eukaryotes (protists), precise identification using microscopy can be more problematic. Diatoms and other large forms that have distinctive hard parts can usually be identified to morphospecies, but small flagellates often cannot. In ciliates, special methods (e.g. silver staining or electron microscopy) are required for precise identification to the morphospecies level. These methods in themselves are too time consuming and expensive to be applied routinely for the most ecological studies, and they also sometimes obscure information that is ecologically significant such as the observation of functional chloroplasts in mixotrophic ciliates (Stoecker and Silver, 1987; Stoecker, 1999). Probably the greatest single problem for precise identification of microbial eukaryotes is that species are often inadequately described in the older literature. A thumbnail sketch accompanied by a one-paragraph description is sometimes all the modern planktologist has to go on when trying to identify a collected species (e.g. Kahl (Kahl, 1932) for ciliates), and it is impossible to reconcile that kind of description with the emerging picture of low morphological diversity underlain by high genetic diversity, as discussed below.

Although size and shape may be important factors in prokaryote evolution (Young, 2006), bacterioplankton have almost no morphology that is discernible by transmitted light or fluorescence microscopy. For several decades now, microbial ecologists have used clade-specific fluorescent probes or other molecular methods for identifying taxa in a sample (e.g. Amann *et al.*, 1995; Fuhrman and Ouverney, 1998). They have thus happily (for them) bypassed the molecules versus morphology problem and hence identification of bacteria (and archaea) will not be discussed further.

DNA-based methods

DNA barcoding has probably been the most widely applied molecular method for identifying plankton (e.g. Bucklin *et al.*, 2007; Lin *et al.*, 2008; Webb *et al.*, 2006; for zooplankton, see www.cmarz.org). The goal of barcoding is to use short sequences of one or a few genes (so far mostly from the mitochondrion) to identify known species and to aid in the discovery of new ones. This technique is relatively simple, is applicable to all

life stages of a given species, can be performed on parts of an organism, is culture-independent and is objective. Because specialized training is not needed (beyond knowledge of the polymerase chain reaction), it has been said that barcoding "democratizes" access to systematics (Stoeckle *et al.*, 2004). Ironically, the exactly opposite argument can also be made, based on the significant contributions to morphotaxonomy that have been made by scientists in the developing world using universally available microscopy (e.g. Björnberg, 1963, 1972). Proponents of barcoding have suggested that the future technology may make it possible to create a hand-held device that would enable instantaneous precise identification of a specimen. Criticisms of the barcoding approach, some of which have already led to changes in the way it is done, include the fact that there probably is not a single gene that is appropriate for barcoding all organisms, and that rates of change in a locus may be heterogeneous within or between clades (Moritz and Cicero, 2004).

Ironically, one reason used to justify the use of barcodes for species identification is that the number of adequately trained taxonomists is insufficient and continues to shrink (Hebert *et al.*, 2003), yet some have claimed that barcoding will inevitably lead to a DNA-only approach to systematics and a subsequent complete loss of human taxonomic expertise (Will and Rubinoff, 2004; DeSalle *et al.*, 2005). It has also been suggested that advances in sequencing technologies may overtake the single-gene barcode approach by enabling rapid genomics of species, even during routine sampling, making the current mitochondrial-based barcoding seem not ambitious enough. Some critics have also pointed out that the barcode metaphor is unfortunate because it implies that species are static and that boundaries between them are never imprecise, and also that proponents of barcoding have made the assumption that morphological taxonomy, as the gold standard to which barcodes are set, is a permanent and static body of information (Tautz *et al.*, 2003).

The creation of clone libraries of environmental DNA sequences (most often using all or part of the small subunit or other ribosomal gene) is another molecular technique that has been widely used for the identification of plankton (e.g. Lopez-Garcia *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Stoeck and Epstein, 2003; Bass and Cavalier-Smith, 2004; reviewed in Epstein and López-García, 2008). This method purports to answer the question "How many different kinds of things are out there, whether or not we can identify them morphologically?" Although this method has generated an enormous amount of data and led to the consensus that global diversity of plankton is much greater than had been thought, its application to identification of plankton is dependent entirely upon the number of sequences in DNA databases that have been properly linked to named species. For most groups this number is small, and this has led to some confusion. For example, a deep-branching SSU clade from the Southern Ocean was initially described as a "new eukaryotic lineage", but later turned out to be the cosmopolitan and apparently rapidly evolving ciliate *Mesodinium rubrum*, which had not been sequenced before (see discussion in Fenchel, 2005). Another issue is that accessions to public genetic databases are not currently peer-reviewed. Thus, the databases potentially contain many highly precise DNA sequences measured on incorrectly identified organisms. Given the current rate of new accessions (as of Feb 2008, GenBank contained more than 82 million sequence records), peer-review is not possible. A "wiki" model (community policing of accuracy) may be possible, but this has not been implemented in the large genetic databases to date.

DGGE and T-RFLP are the two other methods for analyzing the fingerprints of natural communities using DNA (see review in Caron *et al.*, 2004). Both promise more rapid sample processing than clone libraries, and are usually done in conjunction with some clone library construction to identify unique sequences more precisely. In one recent study, DGGE was compared with microscopic examination of plankton samples from the Bay of Fundy. Both methods showed high levels of diversity, but many abundant diatoms and other large phytoplankton that were seen in the samples did not appear in the DGGE, in which heterotrophs and unknown lineages were more common (Savin *et al.*, 2004). While barcoding seeks to use DNA sequences of organisms that have already been precisely identified by taxonomists (and to identify situations where DNA sequences suggest that there are new species to be described), the clone library and other environmental sequence approaches generally put the DNA at the front of the cart and challenge taxonomists to catch up with the high molecular diversity they are revealing.

One direct marriage of molecular and morphological methods is the application of fluorescence *in situ* hybridization (FISH) in microscopic observations, principally of protists. With this method, morphological identification under the microscope can be confirmed using oligonucleotide probes designed to hybridize with only a single species. In the case of the ciliate genus *Euplotes*, in which a great number of morphospecies have been described, FISH is able to discriminate multiple species from within a mixture (Petroni *et al.*, 2003). This method is only limited by the ability to find true species-specific sequences and to make slide

preparations suitable for examining both hybridization and important morphological features.

HARMONY AND DISCORD IN THE MARRIAGE

There are many cases where morphological and molecular methods produce concordant results in classifying groups of organisms and many cases where they disagree. The latter include cases where molecular methods find more species than the morphologists can see and, more rarely, where morphology divides species that are essentially genetically identical. Here we review a few instructive examples, mostly from studies of metazoan holozooplankton.

When molecules and morphology agree

Pseudocalanus is a genus of marine copepods that is of ecological importance, especially in boreal regions. Identification and taxonomy have been difficult due to both morphological similarity among species and great variation in body size, which is correlated with intraspecific and interspecific variations in genome size (Corkett and McLaren, 1978; McLaren *et al.*, 1989). Based on careful morphological analysis of samples collected throughout the range of the genus, Frost (Frost, 1989) was able to discriminate among seven species, and this work was subsequently confirmed by allozyme analyses (Sevigny *et al.*, 1989). Given the ecological importance of this species as food for the larvae of commercially important fish, a DNA-based method was developed to discriminate among co-occurring congeners. This method confirmed that two of the species, *Pseudocalanus moultoni* and *P. newmani*, co-occur on Georges Bank, but with different distributions, suggesting some level of niche separation (Bucklin *et al.*, 2001; McGillicuddy and Bucklin, 2002).

A similar felicitous correspondence of molecular and morphological results was found by Ueda and Bucklin (Ueda and Bucklin, 2006), who studied two populations of *Acartia pacifica*, an estuarine/coastal copepod with an apparent ability to live at a very wide range of salinities (>20). Upon closer examination, morphological traits were found that could reliably separate this single species into two. When sequences of the mitochondrial cytochrome oxidase (mtCOI) and 16S ribosomal genes confirmed species-level degrees of divergence, the authors erected a new species, *A. ohtsukai*, for the brackish water form. In this case, ecological information led to morphological studies that were confirmed via DNA.

When molecules and morphology do not agree

While the two examples illustrated above indicate harmonious marriages of morphological and molecular approaches, there are a growing number of cases where morphological and molecular methods disagree. These principally involve cases where molecular methods find more species than the morphologists can distinguish, revealing the presence of "cryptic" species in the plankton (Ciros-Perez *et al.*, 2001; Dawson, 2003; Goetze, 2003; Ortells *et al.*, 2003; Katz *et al.*, 2005; Slapeta *et al.*, 2006; Chen and Hare, 2008, to name just a few examples).

Estuaries and other coastal environments are places where strong temporal and spatial gradients in temperature, salinity and other environmental factors may structure plankton populations, and several studies have shown genetic variation within morphologically uniform estuarine populations (Lee, 2000; Lee and Frost, 2002; Rynearson *et al.*, 2006). *Acartia* spp., for example, are ecologically important copepods that are numerically dominant in estuaries worldwide. Species are difficult to tell apart morphologically (Enrique Carrillo *et al.*, 1974; Ueda 1986; McKinnon *et al.*, 1992), and historic misidentifications and accidental introductions via shipping have caused biogeographic distributions to be unclear. Caudill and Bucklin (Caudill and Bucklin, 2004) identified multiple deeply divergent clades within the single morphospecies *Acartia tonsa*. Sampling populations along the Atlantic and Gulf coasts of the USA, they found distinct geographic distributions of haplotype frequencies in the mitochondrial 16S ribosomal gene, except in closely adjacent systems. This suggests that dispersal is limited and genetic exchange is very small. Phylogenetic analysis revealed four deeply divergent clades, having within-clade haplotype differences of <2% and between-clade differences (10–14%) almost as high as those found for separate species in other calanoids (19–28%). Similarly, Chen and Hare (Chen and Hare, 2008) examined two deeply divergent clades of *A. tonsa* from Chesapeake Bay and correlated their distributions within the Bay to salinity variations, suggesting that there were two separate species adapted to different salinity regimes. To date, efforts to discriminate these molecularly defined clades by morphology have not been successful.

When genetically highly similar species appear to be morphologically distinct

Although a theme of the morphological/molecular marriage has been the frequent discovery of

DNA-based cryptic species, there have been instances where morphological distinctions have not been supported by DNA evidence. The copepods *Calanus euxinus* and *C. helgolandicus* were separated into distinct species based on morphological differences (mainly size) and apparent mating incompatability *in vitro* (Fleminger and Hulsemann, 1987; Hulsemann, 1991). Genetic studies, however, indicate that recent gene exchange has occurred between populations of the two species, suggesting that they are not genetically isolated (Papadopoulos *et al*., 2005). This has brought the status of *C. euxinus* as a valid species into question and a call for more research on disjunct populations from the Northeast Atlantic and the Black Sea (Unal *et al*., 2006).

When morphology feeds back to molecular findings

The Scyphozoan genus *Aurelia* includes examples of cryptic species being uncovered by molecular methods and verified by a closer look at the morphology (Dawson and Jacobs, 2001; Dawson, 2003, 2005). For example, Gershwin (Gershwin, 2001) verified DNA-based observations of cryptic diversity and showed from morphological observations that various eastern Pacific populations of *Aurelia* were not the supposedly cosmopolitan *Aurelia aurita*, but a separate species, *A. labiata*, which may itself be comprised of a number of species or varieties. She resurrected the name *A. labiata* from descriptions by earlier morphological taxonomists. Ironically, in this case it was the gradual lumping of forms into a single species (*A. aurita*) by observers who ignored earlier taxonomic work that led to the false impression of cosmopolitanism, rather than the inability of morphologically oriented taxonomists to recognize differences.

When only larvae are cryptic

There are many examples of good morphological species in the holozooplankton, especially among copepods, for which morphological identification of larvae is either very difficult or impossible. Considering that studies of life history processes and secondary production may depend on accurate identification of larval stages, this can be a critical problem (Peterson and Kimmerer, 1994). In some such cases, molecular tools can verify larval identity rapidly and inexpensively. For example, Kiesling *et al*. (Kiesling *et al*., 2002) designed species-specific primers that could be used in a rapid microtiter plate-based hybridization assay to discriminate the nauplii of 13 copepod species.

Problems with protists

The inability to culture many protists means that many species descriptions have been based on observation of field-collected specimens. While it is possible to make careful morphological observations on such material, it has been difficult to obtain morphological and molecular information from the same population. An exception in some cases can be provided by the ciliates, whose naturally highly amplified genome makes it possible to pick one or a few individuals from a natural population and obtain DNA sequences (Katz *et al*., 2005). In the particular case of tintinnid ciliates, whose taxonomy is based strictly on morphology and morphometrics of a secreted external shell, or lorica, it is possible to obtain microscopic images of a single individual for morphological classification and subsequently amplify one or more genes from that same individual. This would enable direct linking of the morphological and the molecular data, and presumably lead to resolution of the controversy over whether lorica morphology adequately separates true tintinnid species (Alder, 1999; Duff *et al*., 2008; Fig. 1).

There is an increasing number of cases where molecular information on protist morphospecies has revealed cryptic diversity (Medlin, 1997; Sáez *et al*., 2003; Foissner *et al*., 2008). For example, when Katz *et al*. (Katz *et al*., 2005) sequenced DNA from wild

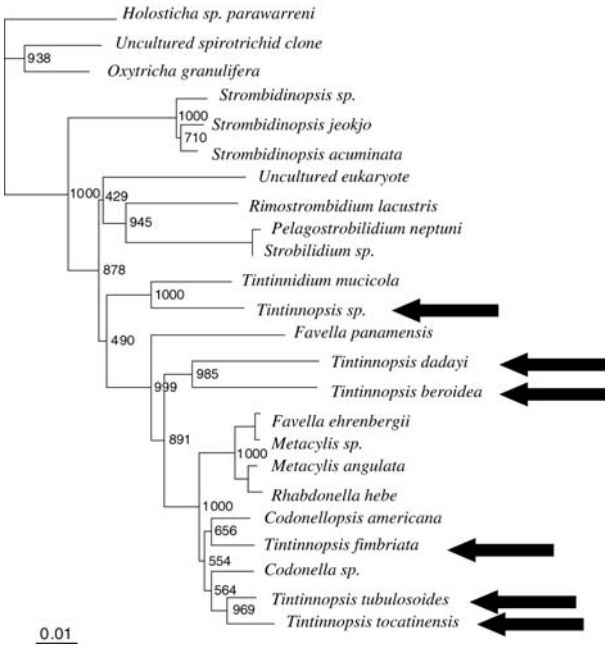

Fig. 1. Neighbor-joining tree from 18S sequences of tintinnids and some other members of the class Spirotrichea available on GenBank. Bootstrap values are based on 1000 iterations. Arrows indicate *Tintinnopsis* spp., whose positions indicate either a lack of monophyly in the genus or the tendency to misidentify it.

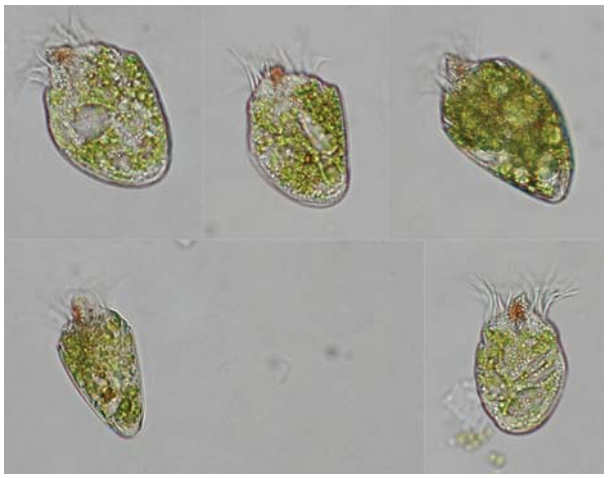

Fig. 2. These images show the morphological variation in ciliates collected from a single population in a tide pool in Dublin Bay, Ireland in 2002. All of the ciliates are grass-green and contain an orange–red eyespot. We initially identified them as *Strombidium oculatum*, a species that has been well studied by other researchers, but DNA sequencing of the ITS region of the ribosomal genes suggests that there are at least a dozen different forms that differ by as much as 16% (Katz *et al.*, 2005).

populations of the well-known tidepool oligotrich ciliate *Strombidium oculatum*, they found evidence for multiple species. Sequences of the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal genes indicated at least 10 distinct haplotypes differing by up to 15%, with four of them being common (Fig. 2). Cryptic species have also been found in molecular studies of Foraminifera (de Vargas *et al.*, 1999), and subsequent observations have verified morphological differences among phylotypes. On the other hand, for the smallest protists, morphology is almost non-existent. For example, the ubiquitous small prasinophyte *Micromonas pusilla* (ca. 2 μm) barely consists of nucleus, mitochondrion and chloroplast, with a single flagellum. When Slapeta *et al.* (Slapeta *et al.*, 2006) sequenced several genes from 17 different isolates, representing the Pacific, Atlantic, Indian and Mediterranean basins, they found this morphologically homogeneous collection to consist of five well-defined clades whose initial divergence appears to have taken place some 60 million years ago. At least three of the clades appear to be globally distributed. There is an accumulating mass of such data for other protist groups showing not only some species with cosmopolitan distributions, but also high levels of contemporaneous cryptic diversity in the same samples (Foissner *et al.*, 2008), as well as evidence for microbial endemism (Boenigk *et al.*, 2006). Given the increasing accumulation of examples of cryptic species in protists as well as problems with incorrectly identified species in the public DNA databases, it would seem wise to avoid naming new protist species based only on morphology of field-collected specimens without sequence information that would allow future workers to verify observations on similar or identical species.

DISCUSSION

Although the tug-of-war between molecular and morphological data on species identity has been sometimes cast in terms of either/or, for the time being both approaches are needed. There are no formal barriers in including molecular information in a species description (e.g. ICZN, 1999), and there are many examples of modern species descriptions that incorporate both kinds of information (e.g. Modeo *et al.*, 2003). Dawson (Dawson, 2005) and Jenner (Jenner *et al.*, 2004) have advocated a "total evidence" approach to species description and identification, including both molecules and morphology. The real problem is trying to decide what to do about cryptic species, when one of the partners in the marriage (e.g. morphology) cannot contribute.

Discordance between molecules and morphology reveals the dynamic nature of processes that generate biological diversity and contribute to the longstanding debate on the nature of species. In some cases, genetic, morphological and reproductive isolation aspects of the species concept have been shown to be concordant with molecular data (Ortells *et al.*, 2003; Amato *et al.*, 2007). In most cases, we do not have the requisite data to evaluate the degree to which cryptic species clusters are truly separate genetic or ecological entities. Cryptic species have been long known in biology, at least since the elucidation of *Paramecium* mating types by Sonneborn in the 1950s (Sonneborn, 1975). It has been suggested that marine habitats may somehow be more amenable to the development of cryptic species than terrestrial ones (Knowlton, 1993) and some early success stories where molecular methods demonstrated real diversity beneath apparent morphological homogeneity were based on marine examples (*Symbiodinium* spp.; Rowan and Powers, 1991).

Although large discrete jumps in phylogenetic trees constructed from DNA distance matrices are persuasive, caveats remain where molecular evidence for cryptic species has not been confirmed by closer morphological examination or experimental demonstration of reproductive isolation or unique ecology. For example, dinoflagellates and some other microbial eukaryotes often have large genomes that include many copies of individual genes, including paralogs and pseudogenes. Such intragenomic variation has been shown in the ribosomal loci for the toxic dinoflagellates *Alexandrium* spp.

(Scholin *et al.*, 1993, 1994; Kim *et al.*, 2004). Intragenomic variation could lead to overestimates of diversity or the incorrect finding of cryptic species, as suggested for the dinoflagellate cluster *Symbiodinium* spp. (Thornhill *et al.*, 2007).

A new "paradox of the plankton"?

The apparent coexistence of cryptic species clusters in the plankton brings us back to the famous "paradox of the plankton" (Hutchinson, 1961)—How can such a large number of species co-exist in a homogeneous environment without competitive exclusion? Hutchinson thought that one answer to this paradox, at least for lake phytoplankton, is that many species are temporarily plankton, having emigrated seasonally from non-homogeneous habitats in the littoral sediments, and hence were not coexisting on a time scale commensurate with competition to extinction. For the ocean, he realized that this explanation would not suffice and suggested that synergistic relationships among species, differential susceptibility to predation or failure to achieve equilibrium were likely factors that allowed competitors to co-exist (Hutchinson, 1961).

The isotropic nature of the pelagial and the ability of ocean currents to disperse plankton over great distances has underlain the long-held assumption that most marine species are cosmopolitan and has probably led to a good deal of taxonomic "lumping" in the past (see the example of *Aurelia* spp. discussed above). A version of this idea has recently formed the core of the revived "everything is everywhere" debate in microbial biogeography. It has been argued that the enormous absolute population sizes of microbes make both extinction and allopatric speciation extremely unlikely and hence species persist for many millennia in the plankton (Fenchel *et al.*, 1997; Finlay *et al.*, 1996, 1998; Finlay and Fenchel, 1999).

The idea that the massive population sizes of microbial plankton makes even local extinction extremely difficult was also put forward by Hutchinson, in this case to question the idea that most plankton are opportunistic species and that disturbance wipes the slate clean for a succession of species to coexist by overlapping in time. Hutchinson was thinking of lakes, but a marine example is illustrative: one common large oligotrich in temperate coastal waters is the Strombidiid *Laboea strobila*. This ciliate reaches a population size of about 10^6 individuals m^{-3} during summer in the coastal North Atlantic (McManus and Fuhrman, 1986). Even if we restrict its abundance to the upper 10 m, for a 1000 km coastline and a 10 km wide offshore range, the absolute population size of this organism in the western North Atlantic alone would be on the order of 10^{17} individuals. Given its distribution on at least both sides of the Atlantic (McManus and Fuhrman, 1986; Agatha *et al.*, 2004), this is a conservative estimate of its census population. It is difficult to imagine anything short of a global catastrophe eliminating 100% of such a large and rapidly dispersing population.

Given the large population sizes of at least some planktonic morphospecies, it is possible that members of a cryptic species cluster are indeed true biological species that occupy the same niche locally, but no one of them can ever be permanently eliminated locally because they will always have a global reservoir to provide new immigrants. This is consistent with recent observations that suggest random, or "neutral", assembly of protist communities (Dolan *et al.*, 2007). Under this model, the community assemblage at any site is primarily determined by abundance in and migration from surrounding environments.

The possible explanations for maintenance of genetically diverse cryptic species beg the question as to how they managed to diverge in the past. Perhaps, ancient patterns of ocean circulation, different from those of today, were more conducive to regional isolation of planktonic species. We know that both the thermohaline and surface circulation patterns have varied dramatically in the past and may have been more conducive to isolation (e.g. Li and Keller, 1999; Erbacher *et al.*, 2001, as argued in Slapeta *et al.*, 2006). Glacial/interglacial cycles and major global extinction events might also have played a role in the process of isolation.

The apparent widespread occurrence of cryptic species clusters in the plankton also reminds us that we do not really know much about the spatial distributions of plankton, especially microbes, on the scale at which they live. Hutchinson anticipated this to some degree as well, arguing that patchiness, or a "heterogeneously diverse" environment, could also result in coexistence of species occupying the same niche. As yet we do not know enough to infer that cryptic species are in fact occupying the same niche rather than partitioning the environment in ways we do not yet understand. For example, in the case of the cryptic tidepool cluster *Strombidium* spp. noted above, only one of the haplotypes has ever come up in culture. This haplotype has been isolated by us from both sides of the North Atlantic and from the eastern South Atlantic. None of the other haplotypes has ever come up in culture for us despite our best efforts. This suggests that there are true differences among the haplotypes in diet or other requirements, and hence that they are not ecologically equivalent and may not be competing for the same resources, which is a key assumption of the Hutchinson's paradox. In this

regard, the morphology challenged prokaryotes provide an excellent example of how small differences in ribosomal genes may be accompanied by significant divergence in ecological properties. Six "ecotypes" of the unicellular cyanobacterium *Prochlorococcus*, differing by $<3\%$ in the ribosomal genes, have been shown to have distinct temperature and light preferences and to be distributed along environmental gradients that are consistent with niche differentiation (Moore *et al.*, 1998; Rocap *et al.*, 2003; Johnson *et al.*, 2006). This suggests that we should not assume that small differences in the ribosomal genes of microbes reflect neutral divergence between otherwise identical organisms when we have scant information about distributions or other niche components in the field.

Where is this marriage headed?

Recognizing the perils of predicting impacts of emerging technologies far into the future (in 1900, US Postmaster General Charles Smith predicted "the extension of the pneumatic tube system to every house, thus ensuring immediate delivery of mail"; Schaer, 1999), a few cautious forecasts may be made. First, it seems likely that the speed and efficiency of sequencing will continue to go up, making possible the collection of enormous clone libraries of environmental sequences and correspondingly more genetic information to be gathered on individual identified morphospecies. Computational methods to deal with such a blizzard of data and to relate it to environmental information are being advanced as well (e.g. Lozupone *et al.*, 2006). This will probably solve the cryptic species problem in that reliance on just one or a few genes as markers of species identity will not be necessary. We currently quibble about 1 versus 2% cutoffs for "operational taxonomic units" for the SSU gene, for example, but with information on differences between hundreds or thousands of pairs of genes, we will surely be able to agree on whether two individuals are members of the same gene pool.

But what if the answer is that there are truly one or two orders of magnitude more species than we currently estimate, as suggested by observations of the rare biosphere (Sogin *et al.*, 2006: Doherty *et al.*, 2007)? Will there be enough alpha taxonomists in the next generation or two of biologists to catalogue all of these newly found organisms? Will the pace of formal description keep up with new genetic discoveries? Will we ever be able to name all species or will a new system be required for cataloging life's diversity purely by DNA sequences?

Currently, morphology based taxonomy remains in the ascendancy, but it seems inevitable that eventually the classification of life will be built on a scaffold of DNA. This could take a long time, depending on the true number of species in the biosphere, or it could take a shorter time, depending ironically on the rate of extinction of the morphotaxonomists. The problem for biologists in general is to manage the transition successfully. The problem for planktologists in particular is to maintain the continuity between the past and the future in plankton studies.

Even with the accelerating pace of extinctions, it does not seem possible that Earth's full biodiversity can adequately be catalogued in morphology based monographs, especially given the continuing loss of expertise. Indeed, some have argued that DNA should be the scaffold upon which systematics hangs, regardless of availability of taxonomists (Tautz *et al.*, 2003) and the "democratization" of taxonomy made possible by the proliferation of thermal cyclers and sequencing facilities may ultimately prove to be a boon for systematics. So what do we do about the morphological perspective, and its data? Perhaps current planktologists should consider ourselves to be living in something of a "golden age" of taxonomy, in which there is still enough human capital to enable the links to be made between the monographs of the past and the DNA databases of the future. Our challenge is to facilitate this linkage so that the rich compendia of knowledge about morphology, behavior, biogeography and ecology of planktonic organisms will not be obscured by the anticipated deluge of genomic information. As this information becomes available, we must ensure that we do not lose sight of the centrality of the individual organism, its population context and its niche. As always, the study of these elements will enable us to understand the living world and how it continues to evolve, especially under the influence of human activities.

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