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DNA barcoding of pelagic cnidarians: current status and future prospects

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Abstract A review of the current status of DNA barcoding in pelagic cnidarians is given. Most current studies tend towards using the 16S mitochondrial ribosomal RNA gene for barcoding purposes in pelagic cnidarians, judged more appropriate for this group than the mitochondrial COI gene. Although further studies on mitochondrial genome structure and the prevalence of nuclear insertions of mitochondrial sequences (NUMTs) are advised, empirically it seems that the sequence fragment of the 16S gene that is currently being used is robust enough to apply DNA barcoding to a range of outstanding questions concerning the taxonomy, ecology and biology of pelagic cnidarians.

Key words: DNA barcoding, Cnidaria, COI, 16S

Introduction

Pelagic cnidarians are ubiquitous members of marine ecosystems. They occur from brackish waters in river estuaries (e.g. Nishikawa et al. 2009) to abyssal depths in the open ocean (e.g. Lindsay 2005); and from ice-covered Antarctic waters (Toda et al. 2014, Lindsay et al. 2014) to regions of hydrothermal venting in tropical, oligotrophic waters (Lindsay et al. 2015). Although often treated as "pest" species by some fisheries due to their propensity to "bloom" into vast aggregations that clog nets and clean the water of all available prey or larval fish, they are themselves the target of some very lucrative fisheries (Nishikawa et al. 2009, Nishikawa et al. 2014). Due to their gelatinous nature they are often damaged severely during collection and species identifications in such cases are extremely difficult—even by an accomplished taxonomist, of which very few exist. Several groups have an asexual polyp stage and a sexual medusa stage, which have very different morphologies, and these stages are sometimes known by different scientific names. One diverse group of pelagic cnidarians, the hydrozoan siphonophores, are, in effect, a swimming polyp colony with multiple zooid types—all with different morphologies. The medusoid, sexual stages can remain attached to the polygastric colony or can be released to live as free-living "eudoxids". Many of these eudoxids were also described as species in their own right and given their own scientific names. In some cases, such as with calycophoran siphonophores of the Genus *Chuniphyes*, the eudoxids of the known species are morphologically indistinguishable from each other. Furthermore, a single net sample invariably contains many species of siphonophores, and original species descriptions sometimes are amalgamations of zooids from multiple species. The most famous example of this is perhaps that of *Agalma elegans* (*pro parte* M. Sars, 1846), which contains parts of 2 different species: *A. elegans* and the distantly related *Nanomia cara* (Totton, 1954).

As such, pelagic cnidarians are a taxon where the application of DNA barcoding techniques would greatly benefit our understanding of their taxonomy, biology and ecology. The present paper introduces the current status and future prospects of DNA barcoding applied to pelagic cnidarians.

Current Status

The most commonly used locus for DNA barcoding in animals is the Folmer region of the cytochrome oxidase I (COI)

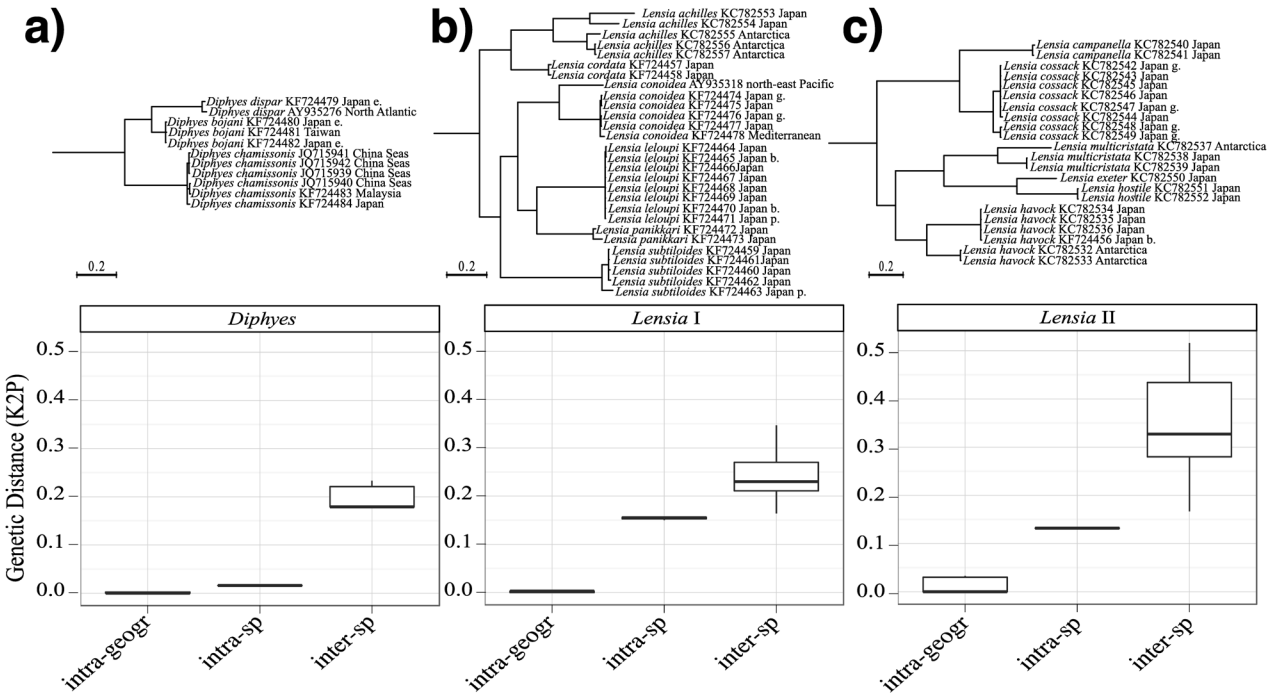

Fig. 1. Phylogenetic trees based on Bayesian analyses (see Grossmann et al. 2014 for details) and box-whisker plots of genetic distance for the 16S rDNA gene. (a) for *Diphyes* species, (b) for *Lensia* species from the 5-ridged clade (c) for *Lensia* species from the non-5-ridged clade. intra-geogr: between samples of the same species from the same location, intra-sp: between samples of the same species from far-removed locations, inter-sp: between species within the same genus and/or clade. In part redrawn after Grossmann et al. (2014, Fig. 2: 168).

gene, a region of just over 600 base pairs in length coded on the mitochondrial genome (Bucklin et al. 2011). The most speciose class in the phylum Cnidaria is Anthozoa, containing approximately 70% of the more than 10,000 accepted cnidarian species (WoRMS, 2014). Several studies have found very low levels of sequence variation in the Anthozoa and one study suggested that the mitochondrial (mt) genome of scleractinian corals evolves 50–100 times more slowly than the mt genome of other animals (Hellberg 2006). In both scleractinian and octocorallian anthozoans, the absence of a significant gap between intra- and interspecific genetic distance values imposes a limit on the use of COI for species identification, whereby sequence divergence measurements higher than a certain cutoff can differentiate species but it does not necessarily follow that measurements under that cutoff point signify that two individuals belong to the same species (McFadden et al. 2011), but see Stampar et al. (2014).

Mitochondrial genome structure in the Anthozoa is circular, while medusozoan mt genomes are contained in linear chromosomes (Kayal et al. 2012). Ancestrally, the medusozoan mt genome appears to have been coded on a single linear chromosome and to have secondarily split into eight linear chromosomes in the Cubozoa, and two linear chromosomes in a subset of the Hydridae, within the Hydrozoa (Kayal et al. 2012). Although most cnidarians with a pelagic life-history stage appear to have a single copy of the COI gene, a subset of the Hydrozoa, non-inclusive of the Trachylina, have two copies, or one full and one partial copy, of COI at the ends of their linear chromosome/s (Kayal et al. 2012). Although the existence of multiple copies of a barcoding gene would not seem to be the ideal situation, COI has been successfully used for species identification and delimitation in both single-copy medusozoans such as the Scyphozoa (e.g. Nishikawa et al. 2014, Piraino et al. 2014), and in the Hydrozoa (e.g. Ortman et al. 2010, Bucklin et al. 2010a, Bucklin et al. 2010b).

Recently Zheng et al. (2014) compared the utility of both the COI and the 16S mitochondrial genes for DNA barcoding in hydrozoans and concluded that, at least in the restricted area of coastal Chinese waters, either gene is suitable. However, they stressed that the 16S gene has three advantages over the COI gene: it is more easily amplified, it provides phylogenetic information as well as being useful for barcoding, and more sequences exist for it than COI in GenBank (Zheng et al. 2014). The very fact that more hydrozoan 16S than COI se-

quences exist in GenBank points to the already-reached consensus among taxonomists that 16S is the preferred gene of choice for this taxon（e.g. Grossmann et al. 2013, Grossmann et al. 2014）. Zheng et al.（2014）showed that 16S was a good barcoding gene for the hydrozoan Orders Anthoathecata and Leptothecata, within a restricted geographic area. The 16S gene's usefulness extends to the Order Siphonophorae and it remains a good barcoding gene even when samples are from areas as geographically-removed as the Mediterranean, the Antarctic, and off Japan（Fig. 1）. The phylogenetic information it encodes can both identify possible cryptic species（e.g. within *Lensia achilles* Totton, 1941 and *L. havock* Totton, 1941, see Fig. 1b & 1c）and indicate where new Genera need to be erected（e.g. Fig. 1b vs 1c, and Grossmann et al. 2014）.

At present there are 1655 partial 16S sequences ostensibly belonging to the Hydrozoa deposited in GenBank, compared to 678 COI sequences. Unfortunately, the level of quality control in sequences found on GenBank is highly variable and blindly using such sequences as a basis for identifying species is wrought with danger. Even when taxonomists were involved in the work, mistakes occur. For example, of the COI sequences reported by Ortman et al.（2010）, one sequence labeled as the siphonophore *Nectopyramis* ［sic］ *diomedeae*（GenBank ID: GQ120030）is actually a sequence from an ostracod contaminant, while another labeled as the physonect siphonophore *Forskalia contorta*（GenBank ID: GQ119984）is in fact a protist contaminant（personal observation）. The 16S gene is not immune to this problem, with a point in case being the misidentification of an animal that should have been identified as *Vogtia serrata*（Moser, 1925）being reported as *V. pentacantha*（GenBank ID: AY937362）（personal observation from data in Dunn et al. 2005, unpublished sequences of *V. serrata* from Japan, and in situ images of the ROV-collected specimen from Monterey Bay）, and a sequence（GenBank ID: EU294001）that should be assigned to *Solmissus marshalli* Agassiz & Mayer, 1902 being currently assigned to *S. incisa*（Fewkes, 1886）, and vice versa（GenBank ID: EU294002）（personal observation from data in Collins et al. 2008 and unpublished sequences from Japan）.

Doubtless many more examples of such mis-assignments or mis-identifications will come to light in the future, but only if voucher specimens for each sequence are kept and made available for examination by taxonomists. It is obvious, however, that DNA barcoding in the pelagic Cnidaria is still in a too-immature state to have full confidence in species identifications based solely on DNA sequences harvested from GenBank by someone unfamiliar with the specimen-sequence dataset.

Another area that needs to be researched before the semi-automatic species identification holy grail of DNA barcoding is reached in the pelagic cnidarians is the spectre of nuclear insertions of mitochondrial sequences（NUMTs）. Song et al.（2013）recently reported that multiple full and partial copies of both COI and 16S mitochondrial genes occur within the nuclear genome of *Hydra magnipapillata* Itô, 1947［＝*Hydra vulgaris* Pallas, 1766］（Hydrozoa: Hydroidolina: Anthoathecata: Hydridae）with sequence similarities to the mitochondrial genes lying between 83–99.8％. Very few NUMTs were found in the nuclear genomes of two anthozoans, with slowly-evolving circular mitochondrial genomes（Song et al. 2013）, but it remains unclear in other hydrozoans with linear chromosomes as to the prevalence of COI and 16S pseudogenes that encompass the "barcoding regions" of these sequences.

Future Prospects

Although some concerns remain as to the extent that pseudogenes might affect the accuracy of DNA barcoding in pelagic cnidarians, in practice, 16S appears empirically to "work"（Grossmann et al. 2013, Grossmann et al. 2014, Zheng et al. 2014）. As further 16S sequences, vetted by taxonomists and based on extant voucher specimens, are added to GenBank and similar databases, anomalies due to contamination or mistaken species identifications should become apparent. In addition to the sequences in open-access databases, the present authors have around 270 extra, presently unpublished, gold-standard 16S barcoding sequences for which voucher specimens exist. In several cases these sequences have revealed discrepancies in the current taxonomy of various hydrozoan groups, such as in the Narcomedusae（Collins et al. 2008 & unpublished data）, where the families Cuninidae, Solmarisidae and Aeginidae all appear to be polyphyletic. It is also apparent that considerable cryptic species diversity exists but is currently masked by historical, rampant synonymization. The careful taxonomic revisions and determination of morphological character matrices, which appear to corroborate the DNA-based phylogenies, take time.

By concentrating on the utility of 16S as a barcoding gene and, for the time being, ignoring the phylogenetic information that it encodes, it is already possible to start investigating several important aspects of the ecology of pelagic cnidarians.

Although a lack of hard body parts makes partially-digested cnidarians almost impossible to identify in the stomach contents of their predators, our preliminary data from shrimp and fish stomachs and from seabird scats suggests that DNA barcoding will be extremely useful for dietary studies. Linking asexual and sexual generations that are commonly known by different scientific names is also possible at the present time（e.g. Grossmann et al. 2013, Grossmann et al. 2014）and advances in amplifying DNA sequences from formalin-preserved specimens（Zhang 2010 and references therein）may allow the validity of taxonomic synonymies to be tested on historical sample collections. Species identifications of processed or partially-processed jellyfishes destined for human consumption is another future application and DNA barcodes are already being determined for this purpose（Nishikawa et al. 2014）.

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