LETTER

Reply to Kiss: Internal transcribed spacer (ITS) remains the best candidate as a universal DNA barcode marker for *Fungi* despite imperfections

The goals of our study (1) can be summarized as follows in response to the work by Kiss (2):

i) To produce enough data across the subphyla defined by the Fungal Tree of Life projects in order to allow a comprehensive statistical comparison of multiple markers as possible barcodes for fungi.

ii) To provide convincing arguments for the selection of a different marker than the default DNA barcode *CO1* for *Fungi*.

The internal transcribed spacer (ITS) locus was always favored in the mycological community as barcode marker, but no comparative study had yet been attempted using a common set of taxa for multiple candidate markers. We are keenly aware of several shortcomings of the ITS as a barcode marker for fungi, and we feel that we discussed these shortcomings adequately in our paper (1). This includes one problem raised in the work by Kiss (2), of heterogeneous copies in single individuals, by referring to a sampling of diverse studies where this finding is pointed out in detail. We also included data from *Glomeromycota* in the supplemental analyses to indicate the use of this region, even for multinucleate fungi with high numbers of ribosomal variants that may require cloning before sequencing (1). Finally, we discussed the importance of defining secondary barcode-like markers in specific lineages where ITS does not separate closely related species. International collaborations are already underway to document such secondary markers (1). Despite all these pitfalls, we found that ITS performed remarkably well in the diverse dataset that we assembled (1).

The basic details of data standards for the ITS barcoding of fungi are being debated elsewhere and were outside the scope of this paper. These discussions remain open to input and are available on the BarcodeConnect site (http://connect.barcodeoflife.net/group/fungi). The results of these deliberations will be presented for consideration by the Consortium for the Barcode of Life committee, which has the authority to formally designate the ITS as the fungal barcode and recommend criteria required to assign the barcode flag to GenBank or BOLD submissions. Specific recommendations based on the data that we generated in our paper will be part of the final decision-making process (1). These recommendations will include suggestions on the use of cloning or direct amplification.

We anticipate the debate on appropriate barcode markers to persist as technology continues to make an impact. However, the urgent need to accelerate sampling and accurate documentation of fungal biodiversity requires that we proceed with the best knowledge available now. A single DNA barcode marker provides a tool to lower the percentage of unclassified sequences that emerges with ambitious environmental sequencing projects. This should continue despite the danger that some percentage of ITS variation is still unaccounted for. We need to have the best possible names for DNA sequences—names that are attached to biological information. In the final analysis, we still believe that ITS as a DNA barcode provides the best possible path to achieve this goal in *Fungi*.

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1. Schoch CL, et al. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proc Natl Acad Sci USA* 109: 6241–6246.
2. Kiss L (2012) Limits of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as species barcodes for *Fungi*. *Proc Natl Acad Sci USA* 109: E1811.

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The authors declare no conflict of interest.

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