LETTER

Limits of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as species barcodes for *Fungi*

In PNAS, the work by Schoch et al. (1) proposed nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as the sole universal barcode for fungi. The work by Schoch et al. (1) stated that "the proposal will satisfy most fungal biologists but not all" (1).

I am among those biologists who are not so satisfied and not really surprised either, because ITS has been the most widely used marker for species identifications in mycology since the development of the first universal primers to amplify this DNA region in fungi. Its use as an unofficial fungal species barcode has also been criticized (2). It was also criticized because of its limits, including intraspecific variation. This aspect was discussed in the work by Schoch et al. (1) from different perspectives except one: the methodological dimension of revealing intraspecific variation in the ITS region.

All fungal and other genomes contain multiple copies of the ITS region. Divergent intragenomic ITS sequences were found in several fungal groups (3–5) and other organisms. Such intragenomic variation can be detected by both direct sequencing and sequencing after cloning the PCR-amplified ITS region; obviously, the latter method will reveal more variation, depending on the number of cloned PCR products sequenced (4, 5). Thus, it is essential to specify how to sequence the ITS when used as a fungal species barcode: directly or after cloning? This issue was not addressed in the work by Schoch et al. (1). Ideally, only ITS sequences determined by direct sequencing of PCR products amplified from voucher specimens should be considered as fungal species barcodes.

The use of the ITS as a species barcode is especially important when dealing with environmental samples (1). In these samples, sequencing after cloning is the most common way to determine the ITS sequences. However, a large part of the conclusions on the barcode value of the ITS was made in the work by Schoch et al. (1) and other works based on direct sequencing works, which may not have revealed all of the existing variation in many samples.

Many phylogenetically closely related and well-founded fungal species and especially, the sibling/cryptic species differ in only one or a few nucleotide positions at the ITS level (3, 5). This divergence is similar to the intragenomic variation revealed in several fungal species. Moreover, the number of variable nucleotide positions detected in ITS sequences depends on the way of sequencing. Therefore, ITS is not a powerful tool to distinguish many closely related fungal species, especially if determined after cloning. ITS is, however, a robust marker when dealing with phylogenetically more distant, albeit congeneric, fungal species. It should be recommended as a universal DNA barcode marker for *Fungi* with these limits in addition to the limits highlighted in the work by Schoch et al. (1).

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