Reviewer Report

Title: Nanopore sequencing of long ribosomal DNA amplicons enables portable and simple biodiversity assessments with high phylogenetic resolution across broad taxonomic scale

Version: Revision 1 **Date:** 11/26/2018

Reviewer name: Shanlin Liu

Reviewer Comments to Author:

Most of the issues have been well addressed except for the ones regrading to mutation saturations of COI gene and the biased description of ITS regions.
The authors stated that "The phylogenetic resolution offered by short barcodes is very limited, as they contain only a restricted number of informative sites. This problem is exacerbated by the fast evolutionary rate of mitochondrial DNA, which leads to a quick saturation with mutations, increasing the probability of homoplasy." For DNA sequences, homoplasy can hardly be avoided due to its four-state nature. However, as I mentioned before, COI gene has > 600 sites, it is going to be extremely rare or, I would say, impossible for the entire gene getting saturated. The authors may want to provide citations here to illustrate how mutation saturation affect phylogenetic resolution?
For the ITS, as another reviewer also mentioned, ITS2 region is widely utilized to serve as barcode sequences for fungi and plants and less variable in length than other ITS regions. The authors may want to add an unbiased description of ITS regions in their main text.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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Are the conclusions adequately supported by the data shown? Choose an item.

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