Dear editor and reviewer,

We thank you for your comments. Please find below our answers.

Line 81: This sentence does not really reflect the findings of the Ihrmarks paper and contradicts the findings in the preprint which shows a high divergence rate for all pipelines.

Response: Thank you for this remark. We have rephrased the sentence to meet the Ihrmarks paper results.

"In addition, using the entire ITS region barcode leads to additional bias resulting in lower representation of species with longer amplicons in the datasets (Ihrmark et al., 2012)."

We have also completed the discussion to better discuss the Irhmark's results in comparison with our results:

" Indeed, some ITS barcoding primers may have mismatches with the sequence of fungal species of interest, such as *Yarrowia* species (Ihrmarks et al. 2012, Tedersoo and Lindahl, 2016). Finally, although ITS1 and ITS2 seem to be the best barcodes for distinguishing between species and, according to our results, their variation in size does not appear to introduce a large bias, their difference in size may hinder sequence alignment and therefore beta diversity estimates that take phylogenetic distances into account.

L114: I guess "downside" not "downfall" is meant here.

Response: Thank you, it has been corrected.

L124: Building correct biological sequences is beside the point of traditional de novo clustering.

Response: we completely agree, thank you, the word "correct" has been replaced by "representative".

Ad Methods:

L330: I thank the authors for their answer but apparently no changes were made in this respect in the preprints methods. To be more explicit: A difference that can make a lot of difference, especially when talking about perfect matches to reference sequences, is what is compared with that reference sequence - is it an OTU represented by a centroid, a Swarm seed or a denoised sequence variant? This is not implicit for every pipeline and "following authors guidelines" is too unspecific for USEARCH and Qiime. I guess for USEARCH the authors refer to "recommended procedures" at https://drive5.com/usearch/manual/. There both, OTU clustering and denoising, are given which makes this reference ambiguous on how things have been done in the preprint. Similar is true for Qiime. It should not be

necessary for the reader to screen the code in the supplementary just to get the information if the respective pipeline was using ZOTUs, ASVs or OTUs.

Response: We agree with your comment, which we misinterpreted last time. We have therefore added information about the tools/subcommands used for each bioinformatics approach presented in our paper in the "Benchmark of metabarcoding approaches" section.