Reviewer Report

Title: Chromosome-level reference genome of tetraploid Isoetes sinensis provides insights into evolution and adaption of lycophytes

Version: Revision 1 **Date:** 8/17/2023

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Reviewer Comments to Author:

The authors have addressed most of my questions.Minor concerns remain:1.Page 6 Synteny between seed free and seed plant genomes: I do not think these two blocks could be considered as synteny blocks between I. sinensis and A. thaliana and and Z. mays (FigS4C). The genes in A. thaliana are completely out of order and three out of the four genes (evm.model.Chr1.1789, 1794, 1797) in I. sinensis are tandemly duplicated homologs to Zm00001d046136_T001 from maize.2.Table 1: Gap ratio means nothing, and should be removed. "Average" should be added to Gene/CDS/Exon/Intron length or number.3.Fig1B: Why the texts in the first box are not center aligned as the rest boxed ? "Genome polishing", "chromosome scaffolding by HiC".4.Fig1C: the legend of circos plot should be 'a/b/c/d/e' rather than "A/B/...". Track a has no unit (Mb). Track b and e show repeat density histogram and number of ncRNA, but I did not see the scale bars.5.Fig2: Copia and Gypsy should be italic.6.Data availability: None of accession numbers the author provided are accessible now and genome annotation should be also deposited. Before accepting this paper, these data should be public and available. Personally I hope the genome assembly, and annotation can be also submitted to NGDC.

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