

For research article

Response to Reviewer 2 Comments

1. Summary

Thank you very much for your valuable comments and suggestions on our manuscript. We appreciate the time and effort you have taken to review our work. These comments are invaluable and provide essential guidance for the revision and improvement of our paper, as well as for our research. We have carefully reviewed the comments and have made the necessary corrections, which we hope will be satisfactory. Additionally, we have submitted a revised manuscript through the system, with all changes prominently highlighted. The detailed revisions are as follows:

2. Questions for General Evaluation	Reviewer's Evaluation	Response and Revisions
Does the introduction provide sufficient background and include all relevant references?	Yes	
Are all the cited references relevant to the research?	Yes	
Is the research design appropriate?	Yes	
Are the methods adequately described?	Can be improved	
Are the results clearly presented?	Can be improved	
Are the conclusions supported by the results?	Can be improved	

3. Point-by-point response to Comments and Suggestions for Authors

Comments 1: *The mitochondrial genome (PQ766611) is not accessible, making it challenging to validate the results presented in the study.*

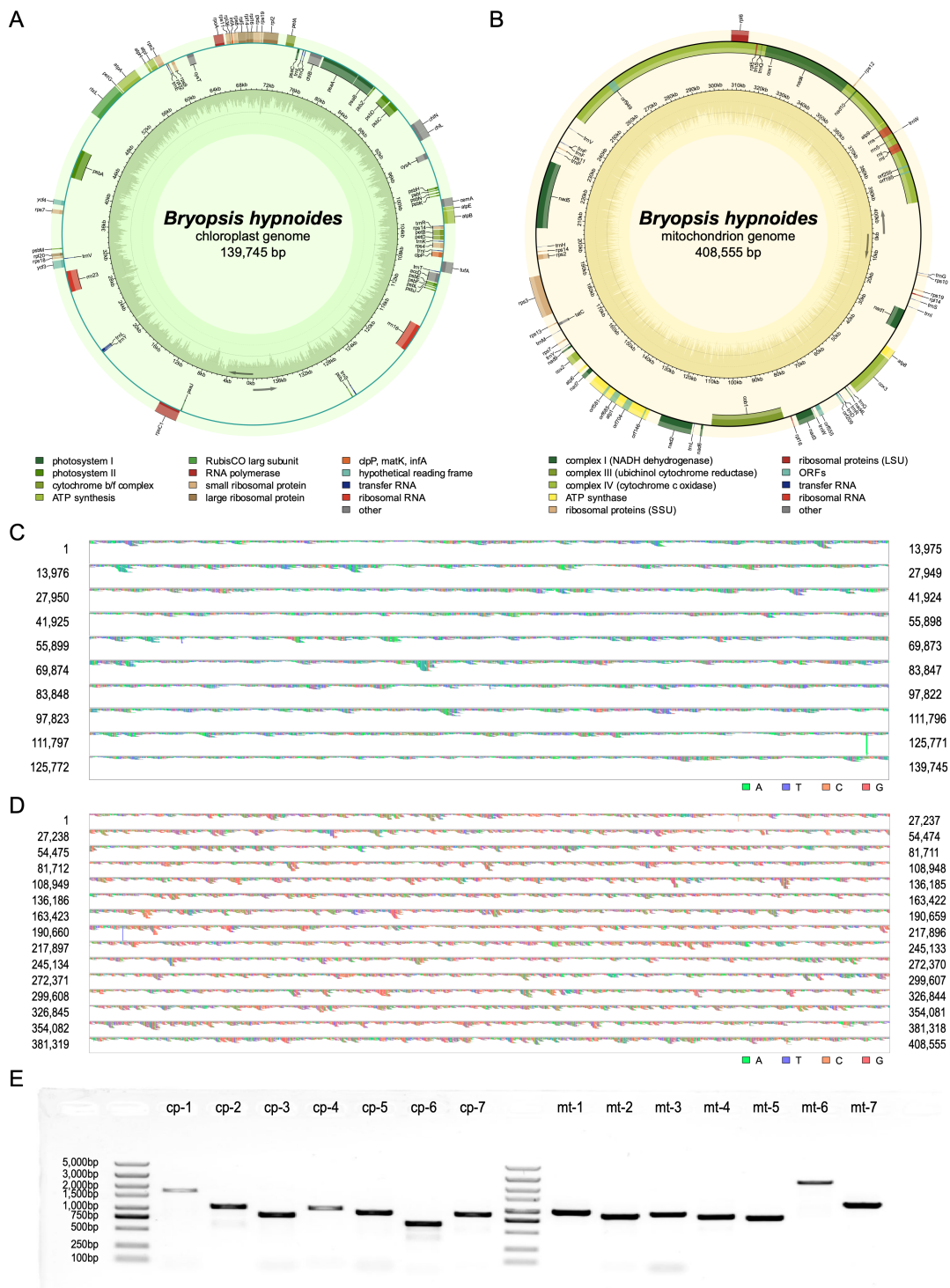
Response 1: Thank you for pointing this out. Regarding the mitochondrial genome sequence data, we submitted the data to GenBank on December 8, 2024, and subsequently received the GenBank accession number PQ766611 on December 17, 2024. Upon receiving your comment, we promptly reached out to GenBank via email to inquire about the possibility of an earlier release of the sequence information. In response, we were informed that "the scheduled release date for your submission is: Jan 20, 2025" (**Please see the Non-published Material for a copy of the email exchange**). To facilitate your review process and accommodate your needs, we have also uploaded the GenBank file of the mitochondrial genome to the Non-published Material section. This will allow you to access and review the sequence data

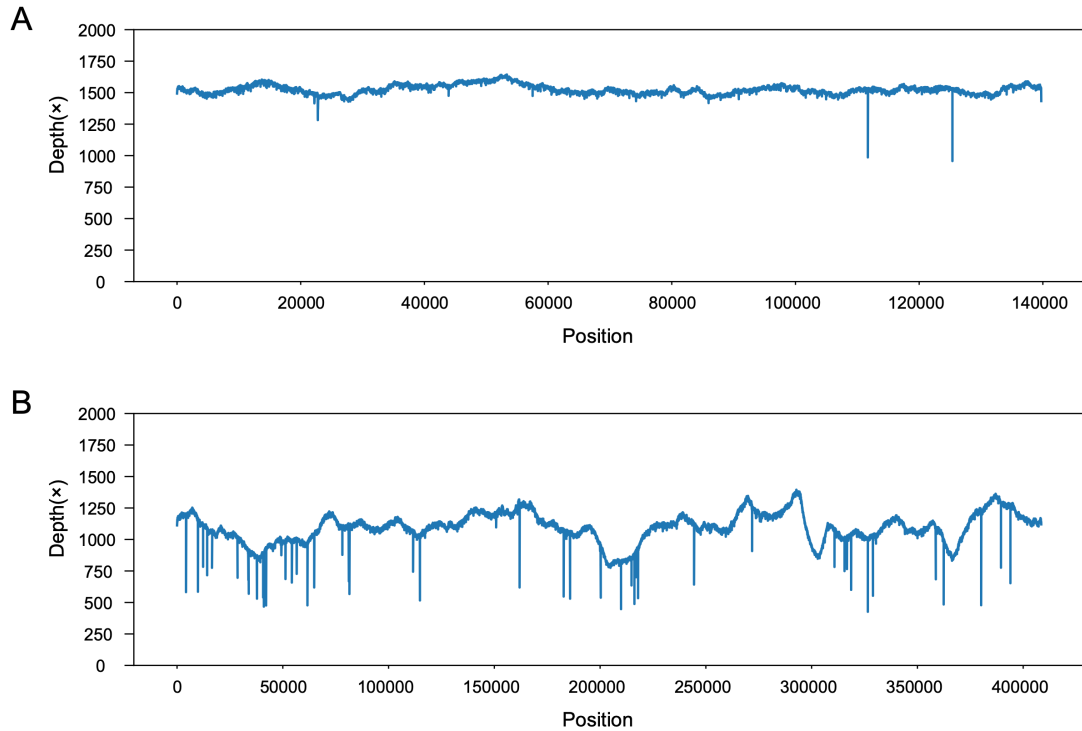
ahead of its official release on GenBank. We understand the importance of timely access to the data and assure you that we are doing everything within our power to expedite the release process. We sincerely apologize for any inconvenience this may have caused and appreciate your patience and understanding.

Comments 2: *Although the authors mention sequencing coverage depths (1933× for the chloroplast and 1551× for the mitochondrial genome), they do not explain how these thresholds were determined to ensure high confidence in the assembly. Was a specific cutoff used to define sufficient coverage? Additionally, was coverage uniform across the genome, or were there regions with lower coverage? The study asserts that no gaps were observed in the assemblies, but the exact criteria or tools used for this validation are not described in detail. For example, did the authors assess read mapping efficiency, and if so, what were the specific thresholds?*

Response 2: Thank you for your valuable suggestion. We have revised the corresponding part of the article according to your request. Regarding the concern about the specific cutoff for coverage depths during the assembly of the organellar genomes, we would like to clarify that we did not set a specific cutoff for this parameter. According to the user manual of Flye v2.9.2, "typically, a coverage depth of 40x is enough" for achieving reliable assemblies. In our study, the coverage depths for all samples far exceeded this threshold, ensuring a high confidence in our assembly results. **We have added a description of the coverage depth criteria in section 2.3 of the Materials and Methods (Page 3, Line 101-102) and details of assembly in section 2.3 of the Materials and Methods (Page 3, Line 98-99).**

We have updated the supplementary materials, Figure S2 depicts the coverage across the genome. As shown in the figure, the minimum coverage depths were 957x (for chloroplast) and 426x (for mitochondrial), respectively, which are significantly higher than the recommended 40x. We validated the absence of gaps in the assembled organellar genomes through two methods. Firstly, we manually inspected every site across the entire genome in Tablet to verify the presence of reads overlap at each site. Secondly, we visualized the depth at each genomic locus and examined for areas with exceptionally low depth to confirm the absence of gaps. The results of these analyses confirm that there are overlapping reads at every position (Figures 1C, D) and no regions of extremely low depth in either of the two organellar genomes (Figure S2). **We have added a description of this in section 2.4 of the Materials and Methods (Page 3, Line 111-118) and section 3.1 of the Results (Page 8, Line 209-219).** Figure 1 and Figure S2 are as follows:





Additionally, in response to your suggestion, we calculated the mapping efficiency using samtools. The results showed mapping efficiencies of 81.07% for the chloroplast and 74.78% for the mitochondrial. We acknowledge that these values are slightly lower than what might be expected in other organisms. However, this is a common phenomenon in algae sequencing due to potential contamination from microbial species. Despite this, we believe that our assembly and analysis pipeline have produced robust and reliable results.

Thank you once again for your valuable feedback. We hope that our responses and revisions address your concerns and enhance the quality of our manuscript.

Comments 3: *The discussion section provides valuable insights into the comparative genomics and evolutionary dynamics of *Bryopsis hypnoides*. However, it could be strengthened by connecting these findings to broader ecological or evolutionary contexts.*

Response 3: Thank you for your constructive comments on our discussion section regarding the comparative genomics and evolutionary dynamics of *B. hypnoides*. To further strengthen our discussion, we acknowledge that while we attempted to explore the evolutionary role of *Bryopsis* through phylogenetic analysis, our results were unfortunately limited by the scarcity of available organellar genome data. Despite these constraints, we remain confident that with the anticipated release of more organellar genome data, the significant evolutionary contributions of *Bryopsis hypnoides* will eventually come to light. We also hope these findings will contribute invaluable organellar genome data to the knowledge base of the Bryopsidales order and establish a foundational reference for future genetic evolution and phylogenetic investigations. **We refined the discussion section of the manuscript.** “The taxon Chlorophyta, which comprises the bulk of green algae, exhibits an extraordinarily wide variety of body structures[54]. Species in *Bryopsis* can develop thalli that reach over 30 centimeters in height, featuring characteristic side branches. Intriguingly, these thalli lack

cell walls to separate their numerous nuclei, resulting in a 'coenocytic' structure[2]. This unique feature has led to a lot of research in evolutionary biology[1, 2]. Despite the widespread lack of organellar genome data for green algae in public databases, we have observed correlations between phenotypes and evolution from the limited but available genome sequences of existing species. Our findings indicate that species with closer phylogenetic relationships exhibit more similar phenotypes. For instance, the coenocytic genus *Bryopsis* and the mononucleate genus *Ulva* can be distinguished based on mitochondrial phylogenetic trees. It is our belief that with the release of more organellar genome data and ecological data in the future, the significant evolutionary role under the adaption for *Bryopsis* and other green algae will ultimately be unveiled.”(Page 16-17, Line 470-482)

Comments 4: *Visualization quality could be improved to enhance clarity and accessibility for a general scientific audience.*

Response 4: Thank you for your advice. We agree with this comment. Following your advice, we have enhanced the clarity of all figures (Figures 1-5) in the manuscript. Please see in Page 6 Figure 1, Page 9 Figure 2, Page 10 Figure 3, Page 13 Figure 4, and Page 15 Figure 5.

4. Response to Comments on the Quality of English Language

5. Additional clarifications

Thank you once again for your constructive feedback. We have carefully considered all your comments and made the necessary revisions to the manuscript. We believe that these changes have significantly improved the quality and clarity of our work.