

The manuscript entitled “Sequencing, de novo assembly of *Ludwigia* plastomes, and comparative analysis within the Onagraceae family” presents the first sequencing and assembly of complete plastomes for *Ludwigia peploides* (*Lpm*) and *Ludwigia grandiflora* (*Lgh*) using a hybrid assembly approach. It identifies two plastome haplotypes in *Lgh* and suggests the likely presence of two haplotypes in *Lpm*, expanding our understanding of plastome diversity within the *Ludwigia* genus.

It's an interesting manuscript that's well written and organized. However, there are a few things that, in my opinion, can be improved as mentioned below:

Evaluation of the various components of the article

- **Title and abstract**

- Does the title clearly reflect the content of the article? [] Yes, [] No (please explain), [] I don't know
- Does the abstract present the main findings of the study? [] Yes, [] No (please explain), [] I don't know

- **Introduction**

- Are the research questions/hypotheses/predictions clearly presented? [] Yes, [] No (please explain), [] I don't know
- Does the introduction build on relevant research in the field? [] Yes, [] No (please explain), [] I don't know

- **Materials and methods**

- Are the methods and analyses sufficiently detailed to allow replication by other researchers? [] Yes, [] No (please explain), [] I don't know
- Are the methods and statistical analyses appropriate and well described? [] Yes, [] No (please explain), [] I don't know

Some methods sections lack clear and detailed methods, such as phylogenetic tree building.

The phylogenetic tree analyses need to be described in more detail.

- **Results**

- In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [] Yes, [] No (please explain), [] I don't know

No “negative” results.

- Are the results described and interpreted correctly? [] Yes, [] No (please explain), [] I don't know

- **Discussion**

- Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [] No (please explain), [] I don't know
- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [] No (please explain), [] I don't know

List of comments, questions and suggestions

Abstract

The abstract provides a concise overview of the study and key findings. However, it might be further improved by clarifying the importance of the study: Explain why these *Ludwigia* species are important. Provide which methods or specific sequencing technologies were used to generate the plastid genomes. Provide numerical information on the plastid genome size of three *Ludwigia* species.

Line 12-13: I suggest split this sentence in two: "The Onagraceae family, which belongs to the order Myrtales, consists of approximately 657 species and 17 genera, including the genus *Ludwigia* L., which is comprised of 82 species."

Introduction

What is the importance of *Ludwigia* species? I suggest including 1-2 sentences in the introduction to highlight the importance of these species. Additionally, provide a more detailed rationale for the importance of studying plastid genomes in *Ludwigia* species.

Line 44, 107: *Ludwigia* needs to be italicized. Please check throughout the manuscript carefully.

Line 63: Add Reference.

Line 67: Add Reference.

Line 95-97: The sentence seems not complete "Recent long-read sequencing (> 1000 bp) provides strong evidence that terrestrial plant plastomes have two structural haplotypes, present in equal proportions and differing in IR orientation of the [22]."

Materials and methods

Why did authors not perform short-read Illumina sequencing and long-read Oxford Nanopore sequencing on both *Ludwigia peploides* (*Lpm*) and *Ludwigia grandiflora* (*Lgh*)?

The phylogenetic methods should be described in more detail, including information on the selected species, outgroup species, and the data used in phylogenetic analyses.

Which DNA extraction kit was used?

Line 179, 190, 217: Please add a reference to all tools you used.

Line 191-192: Why does the number of ONT reads have a high difference between *Lgh* and *Lpm*?

Line 230: *Ludwigia* needs to be italicized. Species and genus names are sometimes given in italics, sometimes not. Please check throughout the manuscript carefully.

Results

Provide details about the quality of Illumina and Oxford Nanopore Sequencing reads, including information on the quantity of data obtained, how much data was used for the assembly, and its coverage.

Line 319: There is a mention to Table 2, but I think the authors meant Table 1.

Line 315–319: In general variations results, it is better if also provide numerical information. In general, providing numerical information would enhance the clarity of the variations observed.

Line 346-347: In the results, the authors mentioned comparing *Ludwigia* sp. junctions with those of other Onagraceae plastomes (Figure 5). The authors should provide details about the analysis and the species used in the methods section.

In the phylogenetic tree, the authors only utilized matK sequences for constructing the tree. Why did the authors not include additional gene or protein sequences to enhance the phylogenetic analysis? Additionally, the authors should specify the source of the matK sequences.

In the content, the authors mention "Add. Figure," but below the figure, the author used "Supp. Figure."

Discussion

It would be better if the authors discussed and expanded on how the identification of two plastome haplotypes in *Ludwigia grandiflora* contributes to advancing phylogenetic and evolutionary studies.