The authors would like to thank the reviewers for this second round of reviewing. You will find here our responses/actions to the first reviewer, the only one of the two to have requested additional information.

Material and & methods

(1) Did the authors create a museum voucher for the two samples they sequenced? I understand that the samples have been destroyed, but it is common practice to deposit a sample in a museum before sequencing a new reference genome, so it can serve as a reference for future taxonomic (re)identification. If this was done, please provide the voucher number and the name of the museum.

Answer: We agree and would have appreciated the opportunity to deposit the samples in a museum. However, *Lgh* and *Lpm* are invasive species. To work with these species, we sought authorization from the French government authorities (regional prefecture) to transport and store them for the purpose of our experiments. This authorization resulted in the drafting of a decree, published in the French Journal Official (arrêté number 2018/SEE/2423 see **Article 3** below), which clearly stipulates that the samples must be destroyed once the experiments are completed. We adhered to this requirement in full compliance with the decree, and consequently, we destroyed the samples as requested.

Article 3 – Conditions de prélèvement et de transport

Les plantes sont prélevées par le bénéficiaire sur les différents sites , mentionné à l'article 2.
Le bénéficiaire prend toutes les mesures nécessaires pour confiner les prélèvements. Il s'assure en fin d'expérimentation de la dévitalisation des plantes et de la gestion des déchets générés, et ce afin d'éviter toute dissémination dans le milieu.

(2) How were the short reads filtered? If the authors used the default parameters of fastp (not explicitly mentioned, but assumed based on the use of default settings for other software), the minimum quality threshold was 15, which seems rather low to me.

Answer : Indeed, we did not think to explicitly mention the parameters since the comparison of the quality thresholds Q15, Q20, and Q30 had no effect on the final result (66% of SR sequences retained).

Action : We have added a sentence to the text (lines 203-204)

Results

Plastome short read assembly.

(3) Please provide the total number of raw reads obtained and the mean quality before filtering. This information will help determine whether the number of chloroplast reads extracted is reasonable.

Answer: We regret to say that we find this question difficult to address, as we do not have sufficient information to determine what would constitute a 'reasonable number' of chloroplastic reads in plants, particularly in highly polyploid species like *Ludwigia*. Indeed, we do not know the ratio

between the nuclear genome, plastome, and mitogenome, nor whether this ratio varies depending on tissues or cell types. However, the fact that we obtained approximately 1900X coverage with SR chloroplast reads (line 289) suggests that the number of chloroplast reads is more than reasonable. Wouldn't you agree?

(4) In the Methods section, the authors mentioned producing two preliminary draft assemblies of Lgh, one with GetOrganelle and one with NOVOPlasty. Here, they state that they used the GetOrganelle draft to extract chloroplast short reads. What about the NOVOPlasty draft?

Answer : Not exactly. In the materials and methods section, we indicate that we conducted a draft assembly using both tools (lines 204-206). We opted to use the two haplotypes from GetOrganelle to recruit the chloroplast short reads, as NOVOPlasty did not offer any additional benefits.

Action : We add this precision lines 276-277

Plastome long read assembly.

(5) Please provide some metrics (particularly the N50) to assess the quality of the sequencing.

Answer : We have already provided the NGA50 values (similar to N50 but calculated using alignment block length and reference genome length instead of contig length and total contig size) for the short reads in Supp. Figure 1. The NGA50 values for Flye and Canu exceed 110 Kb, with zero misassemblies.

Action : We added NGA50 values for LR lines 296 and 297

Annotation

(6) The authors performed a new annotation of the Lo plastome. What differences were observed between the two annotations? Was the new annotation submitted to GenBank?

Answer : The reannotations include renaming genes, adding previously omitted introns, and syntactic reannotation of certain genes. Unfortunately, it is not possible to update the annotations of a genome on GenBank unless we are the original submitters of the genome sequence.

Action : A .gff file containing these annotations has been attached as additional file

(7) For Figure 3, please include the GenBank accession number directly on each graph.

Action : Done

Discussion

(8) Lines 547-519. Please cite some papers where this has been demonstrated previously. As currently written, the sentence implies that you discovered that hybrid assembly is superior to SR or LR assemblies alone, but others have already published similar findings before. If you prefer not to cite previous work, you should at least consider rephrasing the sentence to: « After conducting our research, we discovered that hybrid assembly, which incorporates both long and short read sequences, resulted in the most superior complete assemblies of Lgh plastome. »

Action : We added 'for *Lgh* plastomes' to the sentence in question (line 521-522). Moreover, different references had already been added to the previous version to indicate that this was not 'a discovery' (lines 522-524)."

(9) Line 526-528. I disagree with the statement that it was impossible to identify both haplotypes because only LR were available. In fact, LR should be the most suitable sequences for identifying both haplotypes, as they are the only ones capable of covering the SSC and its boundaries. When I used ptGAUL with LR alone, I successfully identified both haplotypes, provided I had sufficient raw reads. Please note that I am not the author of this software and have no relations with its developers.

Answer: Yes, this is generally true; however, it largely depends on the size of the long reads and the capability to obtain large fragments during DNA preparation. In our case, we did not find any long reads sufficiently large to cover the SSC/IR region. The sentence has been revised in the text to reflect this.

Action : see lines 531-534

In your LR assembly results for Lgh, you obtained haplotype 2 with CANU and haplotype 1 with FLYE. The correction with SR did not change the haplotype. It appears that, in your case, the haplotype identified may depend on the software used or on reconstructing one haplotype or the other by chance, rather than on whether SR correction is applied. Therefore, unless you can demonstrate that SR are absolutely necessary to observe both haplotypes, I suggest revising this statement.

Answer : We appreciate your feedback; however, without specific line references, we are unsure what this comment pertains to. We did not identify any section of the manuscript where we claim that the SR contributes to the discovery of haplotypes

Conclusion

(10) Line 643. Be careful, you did not observe two haplotypes for Lpm.

Action : The sentence was changed (lines 649-651).

Figures and Tables

(11) Table 1. Please replace ";" by "," for bp and ";" by "." for %.

Action : Done

(12) Table 2-3 + all the text. Gene names should be italicized, except for capital letters. Eg. psaB → *psa*B (*psa* = italic; B = non italic). Please ensure that this formatting is correct for every occurrence of a gene name throughout the text.

Action : Done

Data availability

(13) I understand that a whole genome analysis is underway, but this should not prevent the publication of chloroplast data alone (for example, the files obtained after filtering chloroplast reads with the draft assembly).

Answer : We have opted to provide access to the data upon request (see line 661), following an email exchange regarding its intended use. This approach is based on our expectation that some of this data may be reused for the construction of mitochondrial and nuclear genomes, given the frequent insertions of chloroplast DNA into these genomes. Once the complete genome is assembled, we will upload the data to the SRA along with the references to the relevant articles. We would also like to highlight that, while we agree with your perspective, many recent papers on plastomes do not make the sequence data available, either upon request or on public platforms. In comparison, providing access to the data upon request seems to us, for the moment, to be the best compromise for both us and the community.