Our answers are inline in green.

Dear Dr Tørresen,

Thank you for the prompt submission of a revised version of this manuscript. I have shared the manuscript again with one of the reviewers and the feedback was very positive. I will be happy to recommend this manuscript, which I'm sure will become a reference for other genomic studies thanks to the detailed and careful pipeline that you present.

However, I need to ask you to fix the paragraph in lines 384-389 before we move on to the recommendation, as it shows two versions of the same sentence. Besides, I have a few small suggestions and caught some typos. They are very minor things and I normally wouldn't have said anything, but since you are editing the text again, it is a good opportunity to go through them:

We noticed the duplication of text right after we had re-submitted to you, and then it was a bit late to change anything. Thank you for noticing. We have removed the extra sentence now.

- There is a double space in lines 33 ("the L. planeri"), 83 (". A central"), 136 ("steps: incubation"), 186 ("with -5SPM") and 260 (before and after the link to the GenomeEvaluation pipeline).

Thank you for noticing this. We searched for all the locations where we had double space and removed them. However, a couple of the suggestions here (line 260 for instance) looks like double space due to the text being justified.

- There is a space missing in lines 319 ("Omni-Creads") and 339 ("L. fluviatilis,the").

Thank you. We have added spaces in these locations.

- I would also write the full name of Petromyzon marinus in line 102, as this is the first time this species is named in the main text.

Thank you. We have done this.

- Change "comparative" to "comparable" in line 472.

Thank you for the suggestion, we have changed this.

- In lines 494 – 495, the sentence “If L. fluviatilis and L. planeri were two clearly differentiated species, we would expect more differences between the species than in a species.” is a bit awkward to read as is. I would rephrase it to say: “If L. fluviatilis and L. planeri were two clearly differentiated species, we would expect more differences between them than between the two L. fluviatilis specimens.” Or something along those lines.

We have changed the wording here to what you suggest.

- There are several paragraphs that read overly repetitive (e.g. 339-349, 504-513, among others). It would be easy to spot them with a careful read, but I leave the decision of rewriting these instances to you. This is a styling decision.

We see in retrospect that some of the text is quite repetitive. Some of this would require quite a bit of rewriting, and also change the style. As we mentioned in the previous round of review, we would like the text to be read independent of the figures and tables, and when reporting statistics for four different assemblies in the same paragraph, this can unfortunately end up repetitive.

Other than these minor fixes, the manuscript is great, and I will be happy to recommend it as soon as the new version of the text is available.

Thank you!

Sincerely,

Samuel Abalde

*by **Samuel Abalde**, 22 Apr 2025 08:42*
Manuscript: **https://doi.org/10.1101/2024.12.06.627158**
version: 4

Review by Quentin Rougemont, 17 Apr 2025 13:25

Dear Editors and authors,

I have now read the revised version of the manuscript “Comparison of whole-genome assemblies of European river lamprey (Lampetra fluviatilis) and brook lamprey (Lampetra planeri)“ by Tørresen et al.

I am happy to see that the authors have adequately addressed all of the comments from the previous round. I feel that my (minor) concerns were nicely clarified in the reply. The text also reads better and the quality of the manuscript is improved. This will be a nice workflow for new genome assemblies/annotation and look forward to using it.

Regarding the discussion on hifiasm options that influenced the assembly length, I found that increased values (i.e. above 0.7) of the -s parameter, that controls the similarity threshold for purging duplicate haplotigs, were increasing the length of the assembly. Same was true for –hom-cov. Other parameters seemed to have minor effects and across most of my assemblies default values are usually fine.

Thank you for this information.