Response to Reviewers

First we want to thank recommender Iker Irisarri and the three reviewers for spending time to provide valuable input and constructive feedback for this work, which has enabled us to substantially improve the manuscript. We now present a revised version of our manuscript, carefully addressing all comments raised.

Revision round #1

Decision for round #1 : *Revision needed*

Minor revision

Recommender Iker Irisarri

Dear authors,

Thank you very much for submitting your study to PCI Genomics. As you have seen, the three Reviewers are very supportive of your study and I can only agree with them.

A recurrent suggestion was to add some comparative analyses with close relatives. I think, for example, a synteny analysis could strengthen the credibility of the current assembly.

Re: We have conducted a chromosome-scale collinearity analysis between the caucasian dwarf goby *Knipowitschia* cf. *caucasica (kc)* and the yellowstripe goby *Mugilogobius chulae (mc),* both belonging to the family of Gobiidae, to test the quality of genome scaffolding. We described the analysis in material and methods (line 109-122), described the results in line 149-151 and we have added a new figure showing our synteny results (Figure 4).

It seems the genome annotation is not publicly available. Unless there are strong objections (e.g. use in upcoming studies), I would strongly recommend to also publish the genome annotation data.

Re: On the ENA page, everything is public except for the Data Analysis -> Sequence assembly section. We talked to the ENA helpdesk, and the ERZ accessions are always private for assembled and template sequences. They said, when a project becomes public, its assemblies are displayed in the browser as sequence accessions (GCA_xxxxxx, LRxxxxxx) and not as analysis accessions. The assembly itself can be downloaded. We have uploaded the assembly as a flat file (with annotations). The structural genome annotation can be downloaded as follows: If you download the assembly in EMBL format from the ENA user interface site, the structural annotation is included and the fasta can be extracted automatically from this.

Also, two minor comments.

In line 22 unpublished data is mentioned. It would be good to at least mention the source.

Re: We added Lisa Heermann and Jost Borcherding, who have conducted the fishing and identified *Knipowitschia* cf. *caucasica* in the catches.

Please check line 24 makrozoobenthos;

- Thanks, we changed it to "macrozoobenthos".

Line 154 Junniorprofessorship

- Thanks, we changed it to "junior professorship".

Thank you!

Iker

Reviewer Tereza Manousaki

The paper by Schoenle et al. present the first genome assembly and annotation of the Caucasian dwarf goby *Knipowitschia cf. caucasica*, an invasive species of the river Rhine. This resource is critical for studying the species biology and the invasion processes.

The genome reference is of high quality and at chromosome level. Thus, I would encourage the authors to change the title to something similar to "Chromosome level genome reference of the Caucasian dwarf goby Knipowitschia cf. caucasica, a new alien Gobiidae invading the River Rhine" to make sure that the title represents better the quality of the resource produced.

Re: Revised as suggested.

From all sections, M&Ms, results and discussion everything is mostly clear. My only concern is the way BUSCO score is presented at the genome level which confuses me as I would expect to see the total percentage of single and duplicated genes instead of the percentage of the single and duplicated from the identified BUSCOs.

Re: Revised as suggested.

Finally, the gene prediction seems to have returned moderate results compared to the genome. For a direct comparison it would be easier to have both genome and annotation BUSCOed with the same database (e.g. Actinopterygii).

Re: Revised as suggested.

For improving the gene prediction, maybe the authors could consider to add protein hints from closely related species.

Re: Revised as suggested. We have now added protein hints from three closely related species (*Mugilogobius chulae, Boleophthalmus pectinirostris, Periophthalmus magnuspinnatus)* to the OrthdoDB v11 Metazoa subclade partition and have re-run braker3 with the RNA-Seq data and the combined protein database. We have changed the results in the manuscript regarding the genome annotation.

With best regards,

Tereza Manousaki

The genome note is well-written and provides a very clear description of rational for sequencing its genome, the methods used, etc. The genome itself is a valuable resource for further studies. I have only a few minor comments to add.

Specific Comments:

Introduction

Lines 28-32: A transition/connection is needed to clarify why a reference genome is important for subsequent studies, and subsequent analyses on local adaptations and population genetics.

Re: We have changed the text to the following for clarification: *"A reference genome is crucial as it provides a comprehensive map of the genetic material, enabling detailed population genomic analyses. These analyses will also allow the reconstruction of the invasion history of the species, helping to identify invasion routes (Jaspers et al., 2021) and to understand processes of rapid adaptation to local conditions in the novel environment (Szűcs et al., 2017; Yin et al., 2021)."*

Results

1. Line 104: It would be great if the authors add a table comparing the three assemblies to detail their differences and the rationale for selecting the best assembly.

Re: We have added a table (Table 1) to show the differences between the three assemblies.

2. Line 106-107, 115-116: I think you should use the percentage of total BUSCO for single and duplicated BUSCOs in parentheses, or at least ensure consistency in how these figures are presented.

Re: Revised as suggested.

Review by anonymous reviewer

This study reports a new chromosome level genome assembly and transcriptome analysis of the dwarf goby Knipowitschia cf. caucasica. This species is a recent invader in the River Rhine coming from the Caucasian region, predicted to have the potential to seriously impact the local species community due to its ecological habits. The generation of a high-quality genome will be of great benefit for thorough analyses of adaptation, phylogeographic reconstructions, and coevolutionary processes with local prey or competitor species.

Overall, I find the study well done and well presented. I only miss a comparative genome analysis with an existing genome of a closely related species. In the discussion the authors compare some parameters with the round goby Neogobius melanostomus.

Re: We have conducted a chromosome-scale collinearity analysis between the caucasian dwarf goby *Knipowitschia* cf. *caucasica (kc)* and the yellowstripe goby *Mugilogobius chulae (mc),* both belonging to the family of Gobiidae, to test the quality of genome scaffolding. We described the analysis in material and methods (line 109-122), described the results in line 149-151 and we have added a new figure showing our synteny results (Figure 4).