nature portfolio

Peer Review File

Genomic and evolutionary basis of parthenogenesis in a disease-vector tick species

Corresponding Author: Professor Weifeng Shi

Version 0:

Decision Letter:

22nd January 2026

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Professor Shi,

Your Article, "Genomic and evolutionary basis of parthenogenesis in *Haemaphysalis longicornis* ticks" has now been seen by 2 reviewers. You will see from their comments copied below that while they find your work of considerable potential interest, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be very interested in considering a revised version that addresses these serious concerns.

In particular, Reviewer 1 has raised concerns about the phylogenetic analysis and suggested that additional data will be needed for population genetics analysis in order to support your conclusions. Reviewer 1 has also mentioned potential issues with the genome assembly and pointed out that some NCBI links were unavailable during review. Additionally, Reviewer 2 has requested that you discuss the potential impact of polyploidy on genome assembly, discuss alternative explanations for results of the population genetics analysis, and tone down statements about structural variations and functions of candidate genes.

These points will need to be satisfactorily addressed before we can consider the manuscript further. If you choose to revise your manuscript taking into account all reviewer and editor comments, please highlight all changes in the manuscript text file and ensure that corresponding line numbers are included in the "Response to reviewers" file. Additionally, please ensure that all relevant data are accessible to the reviewers.

We hope you will find the reviewers' comments useful as you decide how to proceed. If you wish to submit a substantially revised manuscript, please bear in mind that we will be reluctant to approach the reviewers again in the absence of major revisions.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

If revising your manuscript:

* Include a “Response to reviewers” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already we suggest that you begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/natecolevol/info/final-submission. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

Please use the link below to submit a revised paper:

Link Redacted

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Ecology & Evolution or published elsewhere.

Nature Ecology & Evolution is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

Thank you for the opportunity to review your work.

Yours sincerely,

[REDACTED]
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[REDACTED]

Reviewer expertise:

Reviewer #1: phylogenomics, bioinformatics

Reviewer #2: parthenogenesis, population genetics

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This paper describes the genome sequencing and assembly of two strains of ticks from China, one of which is parthenogenetic. These genomes are of particular importance as the tick has medical relevance, and is in the process of invading and spreading across continents. In addition, implications for the genomic evolution of parthenogenesis and the effects it has on structuring populations are considered. Genome assembly was comprehensive and appears to have achieved the highest standard of results: haplotype resolved, chromosome scale, 90% complete (via BUSCO), and fully annotated. Key gene family expansions are reported, and an important signature of selection is detected on chromosome seven. Differential gene expression is analyzed for genes in that region, as well as an RNAi analysis that was performed on a detected fecundity related gene for a single targeted assay, showing that the region under selection is likely involved in reproduction (egg production) and mitosis. Population genomic analyses are used to characterize diversity over space and lineage, with surprising and confounding results suggesting greater diversity within the parthenogenetic strain than between strains, and that this diversity appears unrelated to geography. The paper shows considerable strengths in analysis and explanation, and is very nearly comprehensive in its approach. There are some aspects of interpretation, visualization, and discussion that may be improved, and especially as these impact the major conclusions of the manuscript.

Major revisions:

[1] I would suggest that the phylogenetic analyses throughout the paper require a bit more consideration.

1a. Can the authors clarify how they came to the conclusion of a nearly 30 million year divergence time between parthogenetic and bisexual strains? Examining figure S6, it appears that the molecular branch lengths between these are nearly 0, as is the same for the Varoa mites. But in the Figure 2a, the calibrated branch length is 2-30 my separated (for Varoa it remains ~0). Fossils do not seem to have been involved in calibrating this node specifically, as these were fossils much deeper in the tree.

1b. The phylogenetic tree of arachnids is perhaps a bit strange in this paper, as it has been a difficult area to resolve phylogenetically and is unlikely to be resolved using the data shown here. I believe that the gene expansions and contractions are the most important insight and the reason for the analysis, but I find these a bit hard to interpret across distances from Drosophila to spiders to mites. Can the authors clarify if these 12 species are the sum total of available high quality genomes, or why these were selected? Is there a way to frame this as a more targeted analysis, around the ticks in

question?

1c. Please clarify why figure 1c is rooted where it is? Why are the human IPA genes not inferred as the outgroup to the rest? If instead this is meant to represent an unrooted tree, another visualization should be selected.

1d. Please also clarify why they have rooted this tree as they have? They should also indicate lineage 2.2 here, and explain how they infer that PHael is the outgroup?

1e. A small point, but I couldn't see any nodes that have bootstrap 70-90%, as indicated by gray in the legend, is that right? Maybe instead color could indicate 90-95 and 95-100?

[2] In general, the genome assembly approach seems to me to be thorough and very clearly reported. The authors should be commended on assembling a triploid, relatively large and repeat rich, genome to haplotype chromosome scale resolution from a very small animal.

2a. I understood that genomes were assembled from DNA extracted from multiple individuals from sib-pair lines. Was there any evidence that assemblies were impacted by potential within-line variation? How was this assessed?

2b. Since these individuals are feeding on host blood, how was potential host DNA contamination accounted for? What about other contaminants? Were any screens or filters performed?

2c. I appreciate that the authors have provided temporary links for data accessibility, and confirm I could access 2 of the 4 of them, but the other two were unavailable, as was the github. I am inclined to believe this is an error on the NCBI side for the 2 links, but I wanted to ask for confirmation that these data will be released.

2d. The authors report a difference in CDS length between strains, but I can't quite see the difference in figure 2f. Is there another way to visualize these differences? More importantly, how can we determine if these differences are significant, and not influenced by technical artifacts of assembling a triploid genome?

[3] The population genomic results are perhaps the most surprising and confusing findings in the study! In particular, they suggest that the greatest division between gene exchange is within the parthenogenetic group, and not between it. But I am left confused as to how to interpret these findings.

3a. The authors state that their analyses suggest group 2.2 arose from within the PHaeL group, and not directly from BHaeL. I don't see how this can be concluded from the unrooted (?) phylogenetic tree, and the ADMIXTURE and Fst analyses seems to suggest a different story. Can the authors clarify how they came to this conclusion, and provide more data to support it.

3b. It should be stated somewhere in the manuscript if the reference PHaeL genome come from individuals that were detected as 2.2, 2.1, a mix of both, or can this be evaluated? I would ask to consider how this cryptic population structure might impact their genomic analyses.

3c. Please clarify how individuals in a parthenogenetic lineage might have mixed ancestry with a bisexual lineage, biologically (lines 505-508)?

[4] The differential expression and RNA interference analyses certainly elevate the level of this genomic research, though they stop short of a mechanistic understanding of how evolution acts on the genome to achieve triploid parthenogenesis.

4a. Please clarify why expression analyses were performed between stages 1 v 2 and 1 v 3, instead of 1 v 2 and 2 v 3?

4b. I might be misunderstanding, but it seems like the expression of the genes mentioned at line 229-230 goes down in midgut from stage 2 to 3, but the authors report that expression levels significantly go up with feeding time (l 232), is that correct?

4c. Please indicate how the genes for individual expression analyses were selected, and what pointed them to target the BIRC family genes for interference. Were other genes considered, targeted, or analyzed? Are there negative results that they can also describe in this study?

[5] The same colors are used to mean different things across figures, and in many cases across panels within a figure. Particularly confusing cases are found in Figure 3, where red vs blue is used to indicate strain in panel a, then the same colors for region in panel b, and then back to strain in panel c and d. I would ask to please consider using a standard color set for strain throughout the manuscript, and to choose distinct visualization approaches for other pieces of data (region, expression, etc).

Minor revisions:

- I believe there is a missing word at 797 "freshly", should "freshly dissected"

- Several figures could be reconsidered or moved to the supplement, to improve interpretation and increase the impact of the

paper. Examples include Figure 1c, perhaps the chromosomes could be aligned, and the circle simplified? Figure 2d, the network is difficult to read and interpret, especially at low resolution.

[redacted]

Reviewer #2 (Remarks to the Author):

This manuscript presents a comprehensive investigation into the genomic and evolutionary basis of polyploid parthenogenesis in H. longicornis. The generation of haplotype-resolved, chromosome-level genome assemblies for the triploid parthenogenetic strain alongside high-quality genomes for diploid bisexual strains, provide an important genomic resource for both evolutionary biology and vector research. The integration of comparative genomics, population resequencing, transcriptomic profiling, and functional RNAi experiments makes this study comprehensive and positions it as an important contribution to the field.

The genome assemblies themselves are high quality. However, the manuscript would benefit from a clearer explanation of the observed discrepancy between the k-mer–based genome size estimates and the final assembled genome sizes, particularly given the challenges of genome size estimation in triploid organisms. Explicit discussion of how polyploidy affects assembly inflation would help avoid confusion.

Comparative genomic and phylogenetic analyses are well executed and reveal lineage-specific gene family expansions and contractions that are biologically plausible. The expansion of cell cycle– and apoptosis-related gene families in the parthenogenetic lineage is a compelling finding. At the same time, the large number of contracted genes, many associated with male reproduction, fits well with theoretical expectations for obligate parthenogenesis. But, claims describing “structural uniformity” among haplotypes should be tempered to distinguish between conserved large-scale synteny and the presence of numerous smaller-scale structural variants.

The population genomic analyses is of strong value, however, while the interpretation that chromosome 7 harbors loci important for parthenogenetic reproduction is well supported, the discussion would benefit from a more explicit consideration of alternative explanations that could also generate chromosome-wide signals (e.g., reduced recombination, background selection, etc).

The identification of candidate genes on chromosome 7, including members of the ApoB family as well as Skp2 and Orc1, is biologically plausible and well supported by combined evidence from selection scans and ovarian expression profiles. However, consider referring to these genes as candidates rather than being definitively associated with parthenogenesis, as functional validation beyond correlative evidence is still lacking.

In the Discussion, statements regarding the evolutionary origins of the parthenogenetic lineage and the precise roles of candidate genes would benefit from clearer language to distinguish hypothesis from demonstrated mechanism.

In summary, a very interesting study that addresses a knowledge gap regarding polyploid parthenogenesis in animals.

Minor comments:
Ln 43 – Please give an example of major gene families that have contracted. Were any of significant value.
Ln 53 – Please given a one sentence potential explanation for this with citation. Increase in agriculture-urban boundaries?
Ln 65 – “differentiation” should be “differentiation”. Also the same error in the title of Fig. 4. Also, line 498.
Ln 78 – What other mechanisms are involved if not allo- or autopolyploidy?
Ln 79 – “enchance” should be “enhance”
Ln 83 – Ecological niche? How so?
Fig 3. Presenting the ADMIXTURE plot on a map also, would provide a better visual representation of patterns of genetic structure.
Ln 390 – “engorgement” should be “engorgement”
Ln 462 – spelling of ‘between’
Ln 491 – Can you confirm if these are facultative or obligate parthenogens, as the mechanisms governing these are quite different. For example, recent work by Sperling et al. 2023 has identified three genes linked to facultative parthenogenesis in Drosophila. It would be interesting to see how these genes act in H. longicornis.
Ln 494 – Spacing required “PHaeL<>and BHael”

Version 1:

Decision Letter:

8th May 2026

Dear Dr. Shi,

Thank you for submitting your revised manuscript "Genomic and evolutionary basis of parthenogenesis in Haemaphysalis longicornis ticks" (NATECOLEVOL-25103780A). It has now been seen again by the original reviewers and their comments

are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Ecology & Evolution, pending minor revisions to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

If you have not done so already, please ensure that you also email us a completed copy of the Reporting summary :

Reporting summary: https://www.nature.com/documents/nr-reporting-summary.pdf

We will now perform detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Ecology & Evolution. Please do not hesitate to contact me if you have any questions.

Sincerely,

[REDACTED]
--
[REDACTED]

Reviewer #1 (Remarks to the Author):

I thank the authors for their detailed comments to my original questions.

Reviewer #2 (Remarks to the Author):

Thank you for addressing my comments to the initial version. I feel that they revised version is stronger and add greatly to the literature of obligate parthenogenesis.

Reviewer #1 (Remarks to the Author):

This paper describes the genome sequencing and assembly of two strains of ticks from China, one of which is parthenogenetic. These genomes are of particular importance as the tick has medical relevance, and is in the process of invading and spreading across continents. In addition, implications for the genomic evolution of parthenogenesis and the effects it has on structuring populations are considered. Genome assembly was comprehensive and appears to have achieved the highest standard of results: haplotype resolved, chromosome scale, 90% complete (via BUSCO), and fully annotated. Key gene family expansions are reported, and an important signature of selection is detected on chromosome seven. Differential gene expression is analyzed for genes in that region, as well as an RNAi analysis that was performed on a detected fecundity related gene for a single targeted assay, showing that the region under selection is likely involved in reproduction (egg production) and mitosis. Population genomic analyses are used to characterize diversity over space and lineage, with surprising and confounding results suggesting greater diversity within the parthenogenetic strain than between strains, and that this diversity appears unrelated to geography. The paper shows considerable strengths in analysis and explanation, and is very nearly comprehensive in its approach. There are some aspects of interpretation, visualization, and discussion that may be improved, and especially as these impact the major conclusions of the manuscript.

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[1] I would suggest that the phylogenetic analyses throughout the paper require a bit more consideration.

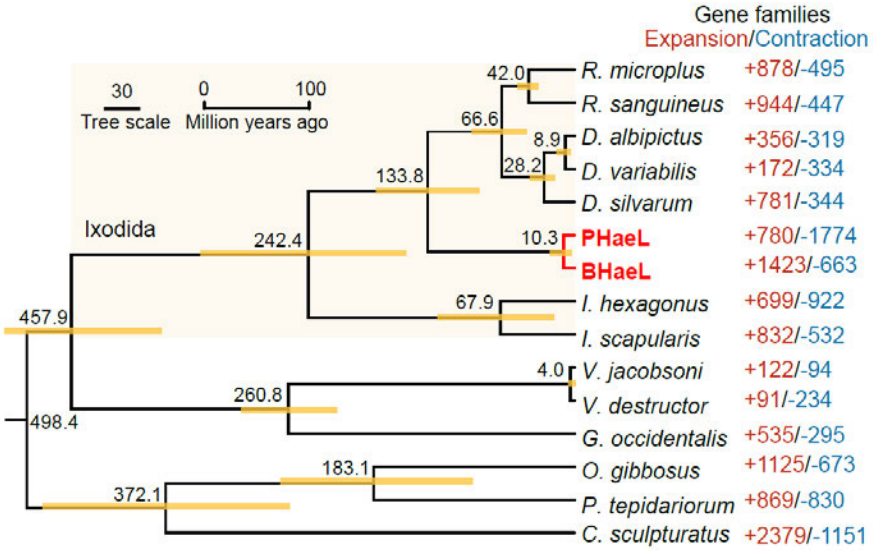
1a. Can the authors clarify how they came to the conclusion of a nearly 30 million year divergence time between parthogenetic and bisexual strains? Examining figure S6, it appears that the molecular branch lengths between these are nearly 0, as is the same for the Varoa mites. But in the Figure 2a, the calibrated branch length is 2-30 my separated (for Varoa it remains ~0). Fossils do not seem to have been involved in calibrating this node specifically, as these were fossils much deeper in the tree.

Response: We apologize for the confusion caused by the presentation of original Figure 2a and Figure S6 (now Supplementary Figure 7 in the revised manuscript). To estimate the divergence times we first constructed a phylogenetic tree using FastTree implemented in OrthoFinder. We then used this topology to estimate divergence times with MCMCTree. Following standard molecular clock analysis procedure, we removed the original branch lengths (representing

genetic distance) from the FastTree topology and applied fossil calibration points to infer the time-calibrated phylogeny shown in original Figure 2a.

The phylogenetic tree shown in original Figure S6 was a non-clock estimated by FastTree using the reference haplotype genomes of PHaeL, the BFHaeL genome, and reference species. In this tree, branch lengths represent the genetic distance between lineages, such that the extremely short branch lengths (near zero) reflect the close genetic distance of the conserved ORFs between all three PHaeL haplotypes and BHaeL. However, in the MCMCTree, branch lengths represent inferred divergence times, estimated under a relaxed molecular clock that accounts for heterogeneous evolutionary rates across lineages. Therefore, the apparent "discrepancy" of branch lengths in the two phylogenetic trees simply reflects the differing interpretations of branch length in the two phylogenetic methods.

We agree that the fossil calibration points originally used may not be optimal for estimating the relatively recent divergence time. For example, the divergence between *O. gibbosus* and *D. melanogaster* (536.6-600.0 MYA) are far deeper in the phylogenetic tree. To address this, we have re-estimated the divergence time using more suitable calibration points. Divergence times for *R. microplus-R. sanguineus* (37.6-52.5 MYA), *V. jacobsoni-V. destructor* (20.9-168.8 MYA), and *G. occidentalis*-(*V. jacobsoni*, *V. destructor*) (236.3-377.5 MYA) were retrieved from the TimeTree database and used as calibrations for the revised estimation (**Response Figure 1** and **revised Figure 2b**).



Response Figure 1. Phylogenetic analysis of *H. longicornis* and 13 Arachnida species, along with the evolution of gene families. The divergence times were calibrated using three fossil time constraints from the TimeTree database: *R. microplus-*

R. sanguineus (37.6-52.5 MYA), *V. jacobsoni-V. destructor* (20.9-168.8 MYA), and *G. occidentalis-(V. jacobsoni, V. destructor)* (236.3-377.5 MYA). Numerical values next to each node represent the estimated median divergence time, and yellow bars indicate the 95% highest posterior density (HPD) range. Red and blue numbers in the right panel indicate the numbers of expanded and contracted gene families with each branch, respectively.

Detailed results from the re-analysis are presented in our response to Comment 1b, and we have revised the corresponding methods section accordingly.

In the Methods section (lines 690-694):
"Divergence times for each node on the phylogenetic tree were estimated using the MCMCTREE program in PAML (v4.10.5)[91], employing three fossil-based calibration times taken from the TimeTree dababase: R. microplus-R. sanguineus (37.6-52.5 MYA), V. jacobsoni-V. destructor (20.9-168.8 MYA), and G. occidentalis-(V. jacobsoni, V. destructor) (236.3-377.5 MYA)".

1b. The phylogenetic tree of arachnids is perhaps a bit strange in this paper, as it has been a difficult area to resolve phylogenetically and is unlikely to be resolved using the data shown here. I believe that the gene expansions and contractions are the most important insight and the reason for the analysis, but I find these a bit hard to interpret across distances from Drosophila to spiders to mites. Can the authors clarify if these 12 species are the sum total of available high quality genomes, or why these were selected? Is there a way to frame this as a more targeted analysis, around the ticks in question?

Response: In the original manuscript, we first retrieved all available *Arachnida* genomes with scaffold- or chromosome-level assemblies, and their corresponding annotation files from NCBI (up to January 2024). Only 12 species with BUSCO completeness >90% and duplication <10% at the protein level were retained for phylogenetic analysis, with *D. melanogaster* designated as the outgroup. We agree that a more targeted analysis focusing on ticks would be better for this study. To achieve this and enhance the accuracy of our estimations, we re-analyzed the data using an updated set of reference species. We re-screened all tick genome assemblies at scaffold- and chromosome-levels from NCBI, and selected those with protein annotation with BUSCO completeness >90% and duplication <10%. Fortunately, this incorporated four additional tick species (*Rhipicephalus microplus*, *Dermacentor albipictus*, *Dermacentor variabilis*, and *Ixodes hexagonus*). As suggested, we removed the three *Acariformes* mites (*D. farinae*, *D. pteronyssinus*, and *O. nitens*) and *D. melanogaster* previously used to allow a more

targeted analysis. The updated reference species employed in the analysis have been summarized in **Response Table 1** (detailed information of the reference species has been provided in the revised Supplementary Table 9, and BUSCO assessment results in the revised Supplementary Figure 6).

Response Table 1. Information of the 13 reference genomes used in phylogenetic analysis

Order	Species	Genome level	BUSCO
Ixodida	*R. microplus*	Chromosome	C:97.7%[S:89.5%,D:8.2%]
Ixodida	*R. sanguineus*	Chromosome	C:96.2%[S:88.9%,D:7.3%]
Ixodida	*D. albipictus*	Chromosome	C:98.8%[S:95.7%,D:3.1%]
Ixodida	*D. variabilis*	Chromosome	C:98.2%[S:97.1%,D:1.1%]
Ixodida	*D. silvarum*	Chromosome	C:97.8%[S:91.9%,D:5.9%]
Ixodida	*I. hexagonus*	Chromosome	C:91.2%[S:87.8%,D:3.4%]
Ixodida	*I. scapularis*	Scaffold	C:90.2%[S:86.0%,D:4.2%]
Mesostigmata	*V. jacobsoni*	Scaffold	C:92.4%[S:91.1%,D:1.3%]
Mesostigmata	*V. destructor*	Scaffold	C:94.1%[S:92.6%,D:1.5%]
Mesostigmata	*G. occidentalis*	Scaffold	C:96.0%[S:90.0%,D:6.0%]
Araneae	*O. gibbosus*	Chromosome	C:95.7%[S:91.7%,D:4.0%]
Araneae	*P. tepidariorum*	Scaffold	C:96.0%[S:92.0%,D:4.0%]
Scorpiones	*C. sculpturatus*	Scaffold	C:92.6%[S:87.6%,D:5.0%]

Based on the updated data set and our two newly assembled genomes, we identified 840 single-copy orthogroups and reconstructed the phylogenetic tree using OrthoFinder. Divergence times for *R. microplus*-*R. sanguineus* (37.6-52.5 MYA), *V. jacobsoni*-*V. destructor* (20.9-168.8 MYA), and *G. occidentalis*-(*V. jacobsoni*, *V. destructor*) (236.3-377.5 MYA) were obtained from the TimeTree database and used as calibrations to estimate the divergence time between PHaeL and BHaeL (see above). We then estimated the species divergence times using MCMCTree. The revised analysis indicated that PHaeL and BHaeL diverged between 3.1-19.9 million years ago (MYA), with a median estimate of 10.3 MYA (**Response Figure 1** and **revised Figure 2b**). This constitutes a narrower interval than the original estimate of 2.8-35.2 MYA. The corresponding results and discussion have been made in the revised manuscript, along with revisions to Figure 2b. The Methods section has also been modified accordingly.

In the Results section (lines 191-194):

"Divergence time estimation based on fossil calibrations showed that PHaeL and BHaeL likely diverged between 3.1-19. 9 million years ago (MYA; 95% HPD), with a median estimate of 10.3 MYA (Fig. 2b)".

In the Discuss section (lines 486-487):

"Phylogenetic analysis revealed that PHaeL and BHaeL likely diverged > 3 million years ago, followed by subsequent pronounced lineage-specific evolution."

In the Methods section (lines 678-682):

"A phylogenetic tree was estimated using the genomes of thirteen Arachnida species (D. albipictus, D. variabilis, D. silvarum, R. microplus, R. sanguineus, I. hexagonus, I. scapularis, V. destructor, V. jacobsoni, G. occidentalis, P. tepidariorum, O. gibbosus, and C. sculpturatus), which were downloaded from NCBI (Supplementary Table 9), along with the genomes of PHaeL and BFHaeL."

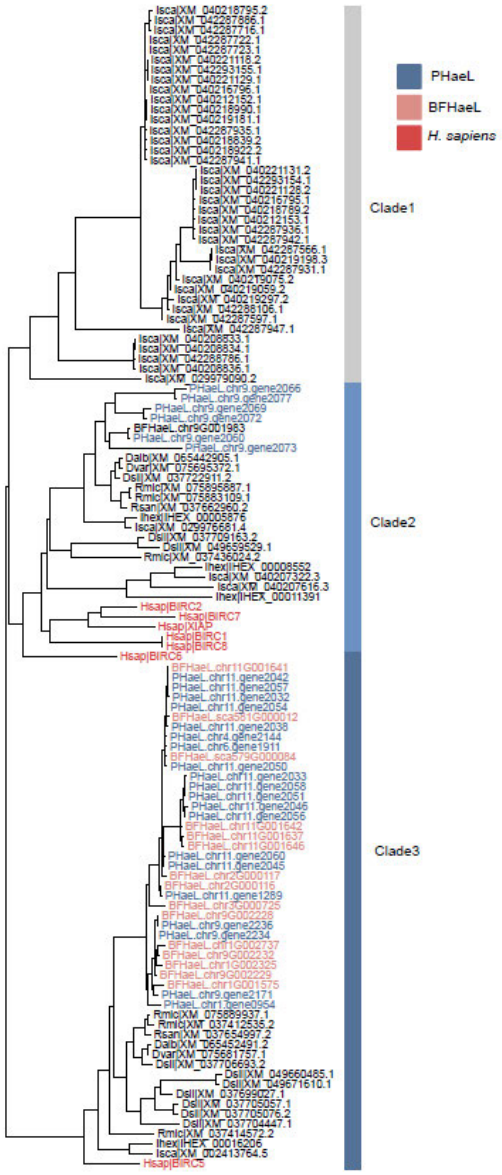
Additionally, as the reference species for divergence time estimation have been updated, we also re-performed the gene family expansion and contraction analyses accordingly. Gene family expansion and contraction enrichment analyses were also re-performed, and the updated results are provided in Supplementary Figure 8 and Supplementary Tables 10 and 11. The updated results are presented in the revised manuscript (lines 193-205), with corresponding revisions also made to Figure 2b. Importantly, these adjustments didn't change the overall conclusions of this study.

1c. Please clarify why figure 1c is rooted where it is? Why are the human IPA genes not inferred as the outgroup to the rest? If instead this is meant to represent an unrooted tree, another visualization should be selected.

Response: We apologize for the confusion caused by the rooting and presentation of original Figure 2b (not Figure 1c). In the original Figure 2b, an unrooted phylogenetic tree was constructed using the IAP sequences from five tick species, *D. melanogaster*, and *H. sapiens*. This unrooted phylogenetic tree was to illustrate the evolutionary relationships and clustering patterns among IAP homologs, rather than to infer a rooted phylogeny with a defined outgroup. Therefore, human IAPs were not set as the outgroup. Among these, Clade1 only comprised *I. scapularis*-specific proteins; Clade2 contained *D. melanogaster* Diap1/Diap2 and human

BIRC1-BIRC3, BIRC6-BIRC8, and XIAP; Clade3 included the Deterin genes from different ticks, *D. melanogaster*, and its human homolog (BIRC5). Therefore, the IAP sequences from humans are highly diverse and do not cluster together. However, based on the presence of human BIRC5 in Clade 3, we inferred that tick IAPs within clade3 might possess functions similar to those of BIRC5 from human.

As suggested, we have re-analyzed the IAPs family alongside updated reference species used in our gene family expansion and contraction analysis and improved the visualization. We re-identified the IAPs family members across all tick species and classified them into subfamilies using human IAPs as references. To avoid potential confusion caused by the phylogenetic tree in the original Figure 2b, we have revised the original Figure 2b and moved it to the supplementary materials as new Supplementary Figure 9 to display the phylogenetic tree of IAPs family members (**Response Figure 2** and revised **Supplementary Figure 9)**. Consistent with the original Figure 2b, tick IAPs were still classified into three clades in the revised phylogenetic tree. The revised phylogenetic tree reveals that BIRC5-type IAPs have been expanded in PHaeL (20 genes), compared to 15 genes in BHaeL and only 1-7 genes in other tick species.



Response Figure 2. Phylogenetic tree of IAPs family members. A phylogenetic tree was estimated using IAPs family members from nine tick species, including *I. scapularis* (Isca), *I. hexagonus* (Ihex), *D. albipictus* (Dalb), *D. variabilis* (Dvar), *D. silvarum* (Dsil), *R. microplus* (Rmic), *R. sanguineus* (Rsan), PHaeL, and BHaeL, together with the reference species *H. sapiens* (Hsap).

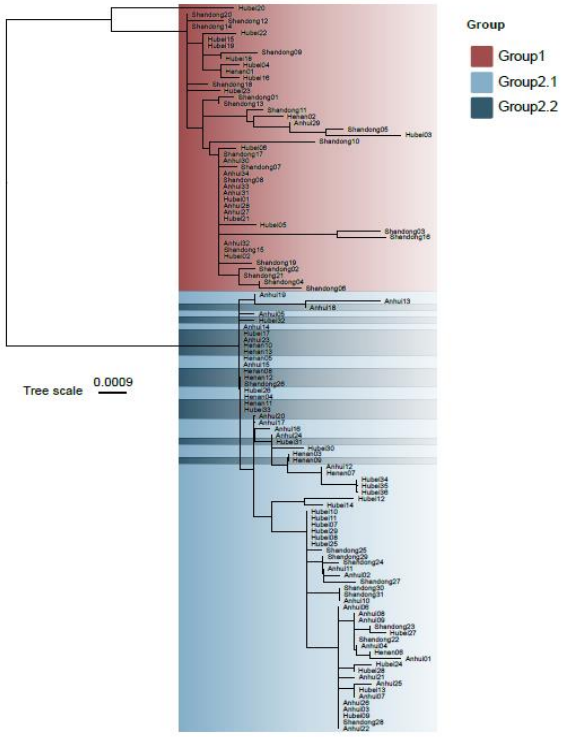
Accordingly, we have clarified this in the revised manuscript (lines 209-215):

"We identified all the IAP family members in nine tick genomes that could be classified into three clades (Supplementary Fig. 9). Twenty IAP family genes in clade 3 belonged to PHaeL, and higher than those in the other tick species: 15 for BHaeL, and only 1-7 genes in other tick species (Supplementary Fig. 9). In clade 3, the BIRC5 gene from Homo sapiens was reported to encode components of the chromosomal passenger complex, which could inhibit apoptosis

and promote cell proliferation (Supplementary Fig. 9)[33,34].”

1d. Please also clarify why they have rooted this tree as they have? They should also indicate lineage 2.2 here, and explain how they infer that PHael is the outgroup?

Response: We apologize for the confusion caused by the presentation of the phylogenetic tree in Figure 3b. This is an maximun likelihoox tree (FastTree) based on a 2100-bp mitochondrial gene fragment spanning a SNP marker mentioned a previous study. It was generated to distinguish the PHaeL and BFHaeL individuals collected from field, as they are morphologically indistinguishable. The tree is unrooted and was not intended for phylogenetic inference. Based on the phylogenetic structure inferred from the sequence data, PHaeL and BFHaeL clearly formed two distinct monophyletic groups, which allowed us to accurately distinguish the two lineages before re-sequencing. However, Lineage 2.2 (Group 2.2) samples did not form an independent clade in this phylogenetic tree (**Response Figure 3**), as this short mitochondrial gene segment harbors limited genetic variation and is therefore unable to resolve fine-scale population structure. Therefore, we have not indicated Lineage 2.2 (Group 2.2) in Figure 3b to avoid potential confusion.



Response Figure 3. Maximum likelihood tree inferred using a gene fragment (n = 2100 bp) covering the position 8,497 in the mitochondrial genome of PHaeL and BFHaeL. Different colors represent different groups.

1e. A small point, but I couldn't see any nodes that have bootstrap 70-90%, as indicated by gray in the legend, is that right? Maybe instead color could indicate 90-95 and 95-100?

Response: In the species divergence phylogenetic tree, every node exhibits a bootstrap support value of 100%, so the gray identifier in the original legend does not correspond to any observed data. To simplify the presentation and avoid misleading visual cues, we have removed all bootstrap support indicators from the revised Figure 2a, as shown in **Response Figure 1**.

[2] In general, the genome assembly approach seems to me to be thorough and very clearly reported. The authors should be commended on assembling a triploid, relatively large and repeat rich, genome to haplotype chromosome scale resolution from a very small animal.

2a. I understood that genomes were assembled from DNA extracted from multiple individuals from sib-pair lines. Was there any evidence that assemblies were impacted by potential within-line variation? How was this assessed?

Response: The genomes were assembled from DNA extracted from multiple individuals derived from sib-pair lines. These lines were established through successive generations of inbreeding, with stable genetic lines and highly homogeneous genetic background. In theory, the lines exhibit extremely low heterozygosity after inbreeding and are generally considered standardized materials for genomic research. Nevertheless, the assemblies were inevitably affected by potential within-line variation, as evidenced by the initial Hifiasm assemblies, particularly during genome assembly of the bisexual lineage.

The primary assembly contig graphs (p_ctg.gfa) for all three sample types were approximately 6 Gb in size, substantially larger than the expected haploid genome length, and the BUSCO assessments revealed duplication rates ranging from 47% to 69% (**Response Table 2**). These initial assemblies represent graph-based assemblies that retain allelic variation among haplotypes. To obtain final reference genomes, we processed these primary assemblies using purge_dups and related tools to remove redundant haplotigs. Compared to the parthenogenetic lineage, assembly of the bisexual lineage required significantly more computational resources (memory and time) and faced severe difficulties in redundancy removal.

Response Table 2. Characteristics of primary genome assemblies (p_ctg.gfa)

p_ctg.fa	Genome size(Mb)	Contig Num	Contig N50(Kb)	BUSCO (v5, arthropoda_odb10,n=1013)

PHaeL	5,915.39	3,083	9,136.13	C:95.2%[S:26.2%,D:69.0%],F:3.3%,M:1.5%
BFHaeL	5,932.91	27,007	765.11	C:94.3%[S:52.4%,D:41.9%],F:3.4%,M:2.3%
BMHaeL	6,158.14	21,365	991.92	C:94.0%[S:46.7%,D:47.3%],F:3.8%,M:2.2%

To address this issue, we implemented a series of stringent measures during sample selection, sequencing, and assembly validation to minimize the potential impact of within-line variation. First, during the sample preparation stage, we used the minimum number of individual ticks required for successful library preparation: genomic DNA pooled from 10 female (yielding 9.16 µg DNA) and 15 male ticks (yielding 7.68 µg DNA) was sufficient for a single HiFi library preparation. A more detailed description has been provided in the revised Methods section (lines 592-595). Second, to address the artifactual redundancy caused by within-line variation, particularly in the highly duplicated or collapsed repetitive regions, we applied several deduplication approaches to the initial contig assemblies, including HaploMerger2, HapSolo, Purge_dups, and purge_haplotigs. Among these, the best-performing deduplication approach varied across samples: Purge_dups was the best for PHaeL, Purge_haplotigs for BFHaeL, and a combination of Purge_dups and Purge_haplotigs for BMHaeL. Detailed procedures have been provided in the Methods section (line 612, lines 632-633, and line 636-638). Following deduplication, BUSCO assessments of the contig-level assemblies from the three ticks consistently showed low duplication rates of <10%, after which chromosome-scale scaffolding was performed (**Response Table 3**).

Response Table 3. Characteristics of he redundancy-removed genome assemblies

	Genome size(Mb)	Contig Num	Contig N50(Kb)	BUSCO (v5, arthropoda_odb10,n=1013)
PHaeL	2,678.54	803	10,000.0	C:92.0%[S:86.3%,D:5.7%],F:3.8%,M:4.2%
BFHaeL	3,095.14	6,203	2,942.5	C:90.4%[S:81.4%,D:9.0%],F:4.5%,M:5.1%
BMHaeL	3,080.15	5,608	3,120.6	C:90.0%[S:80.3%,D:9.7%],F:4.5%,M:5.5%

Finally, during chromosome scaffolding, we used HiCPro to filter Hi-C reads and obtained valid interaction pairs for contig anchoring and ordering, followed by manual curation in JuiceBox to remove misassembled or duplicated contigs. Our comprehensive quality evaluations confirmed the high assembly quality. These evaluations including Illumina read mapping, BUSCO completeness (>90%), and duplication metrics (<10%), indicated that within-line variation did not appreciably compromise the final assemblies. Detailed assessment statistics are provided in Table 1 of the revised manuscript.

2b. Since these individuals are feeding on host blood, how was potential host DNA contamination accounted for? What about other contaminants? Were any screens or filters performed?

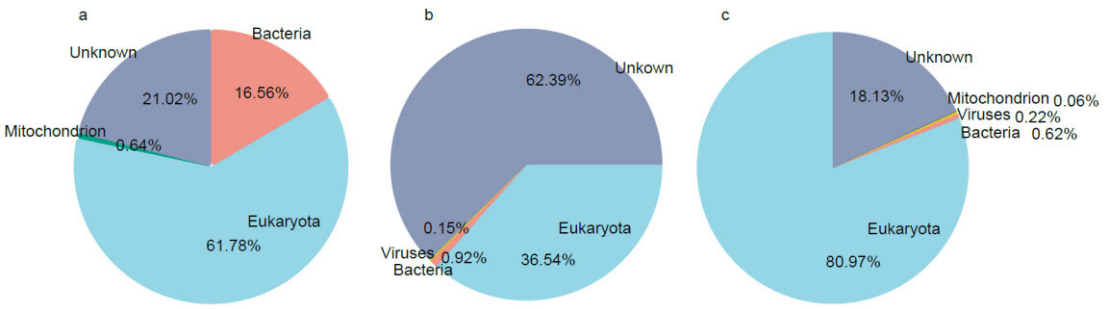
Response: In our view, host DNA and other exogenous contaminants are unlikely to have substantially affected our assembly results, as we implemented multiple quality control procedures during sample preparation, sequencing, and bioinformatic analyses. First, contamination was minimized through careful sample selection: (1) the larvae of PHaeL were newly hatched and they haven't feed on host blood; (2) adult bisexual *H. longicornis* (post-molting nymphs) were starved for four weeks before sequencing, thus minimizing host DNA contamination. A more detailed description has been included in the revised Methods section (lines 590-594). Second, we assessed the HiFi data for exogenous contamination prior to genome assembly. We randomly selected a subset of sequences from the HiFi data sets of parthenogenetic and bisexual female/male *H. longicornis* ticks, and aligned them against the nucleotide database from NCBI to evaluate the presence of exogenous contamination. As shown in **Response Table 4**, only a limited proportion of reads (0.57%-1.75%) were identified as potential contaminants (e.g., HiFi reads of Mammal or Microorganism origin). These low-abundance contaminant reads typically assemble into small, identifiable fragments and are subsequently excluded during chromosome scaffolding and manual curation due to their inconsistency with Hi-C interaction signals. Therefore, the impact of these low-level contaminants on the final assembly quality is negligible.

Response Table 4. Summary of nucleotide alignment results for randomly selected HiFi reads from PHaeL, BFHaeL, and BMHaeL ticks.

	PHaeL	BFHaeL	BMHaeL
HiFi reads	Number/Percentage	Number/Percentage	Number/Percentage
Unkown	3,175/90.71%	1,427/71.35%	1,446/72.3%
Arthropods	282/8.06%	503/25.15%	480/24.00%
Mammal	8/0.23%	11/0.55%	20/1.00%
Microorganism	12/0.34%	20/1.00%	15/0.75%
Others	23/0.66%	39/1.95%	39/1.95%
Total	3,500/100%	2,000/100%	2,000/100%

However, as suggested, we have filtered the genome assemblies by removing unplaced contigs that may originate from contaminants. Accordingly, we extracted all unanchored

contigs shorter than 10 Mb and aligned them against the nt database, including 157 contigs for PHaeL, 654 for BFHaeL, and 1,787 for BMHaeL, respectively. We used BioPerl (v1.7.8) to split each query sequence into 10 kb non-overlapping windows. Each subset was then aligned against the NCBI nt database (2023 release) using blastn (BLAST+, v2.16.0+) with the parameter e-value of 1e-10. Contigs with top hits to bacteria or viruses were identified as potential contaminants. For PHaeL, 16.56% (26/157) of the contigs, with a total length of 4.90 Mb, had top hits to bacteria (**Response Figure 4a**). For BFHaeL, 0.92% (6/654) and 0.15% (1/654) of the contigs (total length of 0.87 Mb) had top hits to bacteria and viruses, respectively (**Response Figure 4b**). For BMHaeL, 0.62% (11/1,787) and 0.22% (4/1,787) of the contigs (total length of 1.06 Mb) had top hits to bacteria and viruses, respectively (**Response Figure 4c**).



Response Figure 4. Proportions of contigs with top hits to potential contaminant sources based on nt database alignment. (a) PHaeL, (b) BFHaeL, (c) BMHaeL.

After filtering of putative contaminant sequences we deposited the updated genome assemblies in the public database under version V2. These modifications have further improved the accuracy of the chromosome-scale assemblies without affecting the overall conclusions of the study.

2c. I appreciate that the authors have provided temporary links for data accessibility, and confirm I could access 2 of the 4 of them, but the other two were unavailable, as was the github. I am inclined to believe this is an error on the NCBI side for the 2 links, but I wanted to ask for confirmation that these data will be released.

Response: We apologize for any confusion caused regarding the data repository. All genome assemblies and related data generated in this study have been deposited in the China National Center for Bioinformation (CNCB). We have re-examined all provided links and confirm that

all the four datasets and the GitHub repository are now fully accessible.

The links are as follows:

[CRA030983]: https://ngdc.cncb.ac.cn/gsa/s/OC9Rz0Qk

[CRA030867]: https://ngdc.cncb.ac.cn/gsa/s/8QhE48s6

[CRA030902]: https://ngdc.cncb.ac.cn/gsa/s/VT3yI7A7

[Genome of PHaeL]:

https://ngdc.cncb.ac.cn/gwh/Assembly/reviewersPage/lRNvbRacKMQjtnWaouvNNZhgKSTtboiniKhkSYgQJzlwJGQJuvprZhzlGESevtVS

[Genome of BFHaeL and BMHaeL]:

https://ngdc.cncb.ac.cn/gwh/Assembly/reviewersPage/JRFyByUvNjiUvWGBnglKdrSJgzZnlpAvRENDimneGdFtARpxnRDYEgiyEFWSaIic

GitHub link: https://github.com/lvqiang0120/protocols-for-parthenogenesis-in-Haemaphysalis-longicornis

All data will be made publicly available upon acceptance of the manuscript.

2d. The authors report a difference in CDS length between strains, but I can't quite see the difference in figure 2f. Is there another way to visualize these differences? More importantly, how can we determine if these differences are significant, and not influenced by technical artifacts of assembling a triploid genome?

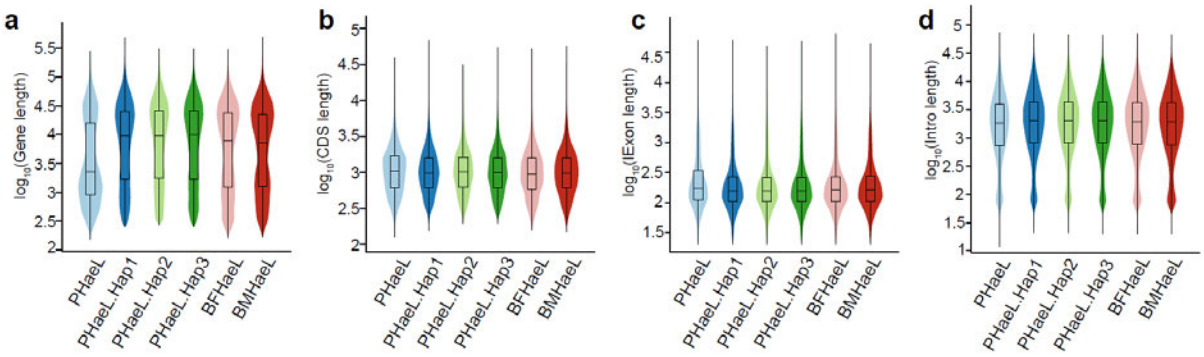
Response: We agree that the original visualization in Figure 1f (not figure 2f) did not clearly illustrate the differences between lineages. To address this, we have now compared the mean CDS lengths of our assemblies with those of published *H. longicornis* genomes, including one parthenogenetic genome (GCA_048455015.1) and two bisexual genomes (GCA_013339765.2 and GCA_022414705.1) (**Response Table 5**).

Response Table 5. Summary of gene structure statistics for *H. longicornis* genomes

Genome	Strain	Level	Protein number	Average protein length	Average exon length(bp)	Average cds length(bp)
PHaeL (this study)	Parthenogenetic	Chromosome	32,947	445.4	397.7	1339.1
PHaeL.Hap1 (this study)	Parthenogenetic	Chromosome	26,859	429.2	329.3	1290.6
PHaeL.Hap2 (this study)	Parthenogenetic	Chromosome	25,861	436.5	327.3	1312.5
PHaeL.Hap3 (this study)	Parthenogenetic	Chromosome	25,801	428.9	325.2	1289.6
GCA_048455015.1	Parthenogenetic	Chromosome	39,615	545.0	339.9	1,637.9
BFHaeL (this study)	Bisexual	Chromosome	32,123	418.4	317.2	1258.1
BMHaeL (this study)	Bisexual	Chromosome	33,583	431.2	326.3	1296.5
GCA_022414705.1	Bisexual	Scaffold	24,189	NA	NA	1297.2
GCA_013339765.2	Bisexual	Chromosome	25,651	294.2	296.0 0	885.5

In our assemblies, the mean CDS lengths are 1339.1 bp for PHaeL, 1258.1 bp for BFHaeL, and 1296.5 bp for BMHaeL (**Response Table 5**). The mean CDS length for the parthenogenetic genome (GCA_048455015.1) was 1637.9 bp, and that for the bisexual genome (GCA_022414705.1) was 1297.2 bp (**Response Table 5**). The variation between our new PHaeL and BHaeL assemblies fell within the normal range of genomic variation, reflecting a close evolutionary relationship between the parthenogenetic haplotypes and the bisexual lineage.

We apologize for the imprecise description of gene structure characteristics. This has now been corrected in the revised manuscript (lines 149-156). Additionally, we have revised the original Figure 1e-h by removing the gene structure features of closely related tick species (*D. silvarum*, *R. sanguineus*, and *I. scapularis*). We have also presented the structural characteristics of our newly assembled genomes using violin plots in **Response Figure 5** (revised Supplementary Figure 4), which reveals highly consistent distribution patterns of coding sequence (CDS) lengths across different genomes **(Response Figure 5b)**.



Response Figure 5. Distribution map of the gene elements in the six newly assembled genomes, including gene length, CDS length, exon length, and intron length. Different tick species and parthenogenetic haplotypes are represented by different colors.

The sentence has been rewritten as (lines 149-156):

"The average length of the coding sequence (cds) of PHaeL was 1,339.1 bp, compared to 1,258.1 bp in BFHaeL and 1,296.5 bp in BMHaeL, respectively (Supplementary Table 4). The three haplotypes of PHaeL (PHaeL.Hap1-3) contained 25,801-26,859 genes, with mean protein lengths ranging from 428.9 to 436.5 aa, mean exon lengths from 325.2 to 329.3 bp, mean CDS lengths from 1,289.6 to 1,312.5 bp, and mean intron lengths from 3,453.9 to 3,514.5 bp (Supplementary Table 4). These values were highly consistent across the three haplotypes and comparable to those observed in the BHaeL assemblies (Supplementary Fig. 4 and Supplementary Table 4)."

[3] The population genomic results are perhaps the most surprising and confusing findings in the study! In particular, they suggest that the greatest division between gene exchange is within the parthenogenetic group, and not between it. But I am left confused as to how to interpret these findings.

3a. The authors state that their analyses suggest group 2.2 arose from within the PHaeL group, and not directly from BHaeL. I don't see how this can be concluded from the unrooted (?) phylogenetic tree, and the ADMIXTURE and Fst analyses seems to suggest a different story. Can the authors clarify how they came to this conclusion, and provide more data to support it.

Response: We agree that relying solely on the phylogenetic tree, which was midpoint-rooted for visualization purposes, is insufficient to infer the evolutionary relationship among groups. Therefore, we combined the phylogenetic tree with ADMIXTURE and F_{ST} analyses to jointly interpret the genetic relationships among group 1, group 2.1, and group 2.2.

The ADMIXTURE analysis revealed the shared ancestral genetic components among populations. Across K values from 3 to 5, the ADMIXTURE analysis indicated that group 1, group 2.1, and group 2.2 forms three distinct genetic groups (Figure 3e). However, when K=2, the model-based clustering analysis grouped Group 1 and Group 2.1 as sharing the same ancestral origin. Moreover, a clear and gradual tendency of introgression (the proportion of the blue ancestry component) was observed from Group 2.2 to Group 2.1 and even to Group 1, indicating frequent hybridization among them. Consistent with this observation, F_{ST} between Group 1 and Group 2.1 was much lower than that of Group 2.2 versus Group 1 or Group 2.2 versus Group 2.1. These findings suggest a scenario in which Group 2.1 arose from hybridization between bisexual Group 1 and parthenogenetic Group 2.2. Therefore, the genetic background of parthenogenetic Group 2.1 is closer to Group 1 than Group 2.2. As discussed in response to comment 3c, laboratory studies have shown that triploid zebrafish and parthenogenetic *Drosophila* can mate with diploid males and produce viable offspring (Ou, Y. et al. 2024; Sperling, A.L. et al. 2023), supporting the possibility that ancestral group 2.2 individuals may have hybridized with group 1, resulting in the formation of parthenogenetic group 2.1 descendants.

However, phylogenetic tree topology showed that group 2.2 clustered within group 2.1, rather than forming a sister lineage to group 2.1. The apparent conflict between the population structure inferred from ADMIXTURE/F_{ST} and the phylogenetic tree topology suggests that the

phylogenetic tree topology may be disproportionately influenced by loci that retain ancestral or introgressed variants (e.g., organellar genomes or regions under selection). Such discordance between genome-wide ancestry and locus-specific phylogenies is frequently observed in hybrid lineages and highlights the mosaic nature of hybrid genomes (Kunishima, T. et al, 2025; Gebreselase, H.B, 2024).

Despite this, we acknowledge the theoretical possibility of independent origins of Group 2.1 from Group 1 through genetic mutations, rather than through the hybridization scenario. Under this scenario, Group 2.2 may arise through independent mutations from either Group 1 or other historical bisexual *H. longicornis* populations. However, although it cannot be entirely excluded, a single origin of parthenogenetic species is a rare event in nature, making multiple independent origins even less likely.

As suggested, we have revisited the genetic relationships among Group 1, Group 2.1, and Group 2.2, and substantially revised the corresponding sections in the Discussion. The revised text is presented below:

"ADMIXTURE analysis showed that BHaeL constituted a single genetic cluster (Group 1), whereas PHaeL comprised two distinct subgroups (Group 2.1 and Group 2.2) at K values from 3 to 5. However, at K=2, Group 1 and Group 2.1 clustered into a single shared ancestral lineage. In addition, a clear and gradual tendency for introgression (i.e., the proportion of the blue ancestry component) was observed from Group 2.2 to Group 2.1, and even to Group 1, indicative of frequent hybridization among them. Consistent with this observation, the F_{ST} between Group 1 and Group 2.1 is much lower than that of Group 2.2 versus Group 1 or Group 2.2 versus Group 2.1. Collectively, these findings suggest that Group 2.1 likely originated through hybridization between bisexual Group 1 and parthenogenetic Group 2.2. Laboratory studies have shown that triploid zebrafish and parthenogenetic Drosophila can mate with diploid males and produce viable offspring[42,45], supporting the possibility that ancestral Group 2.2 individuals may have hybridized with Group 1, resulting in the formation of parthenogenetic Group 2.1 descendants. Despite this, we cannot fully exclude that Groups 2.1 and 2.2 of PHaeL had different origins, although this should be considered highly unlikely. Notably, our phylogenetic analysis also revealed that Group 2.2 clustered within Group 2.1 rather than forming a sister lineage. This inconsistency between population structure (ADMIXTURE, F_{ST}) and phylogenetic tree topology may reflect the disproportionate influence

of loci retaining ancestral or introgressed variation (i.e., organellar genomes or selected regions), a phenomenon commonly observed in hybrid lineages and which reflects the mosaic nature of hybrid genomes[46,47].” (lines 505-524)

References:

Ou, Y. et al. Formation of different polyploids through disrupting meiotic crossover frequencies based on cntd1 knockout in Zebrafish. *Mol Biol Evol* **41** (2024).

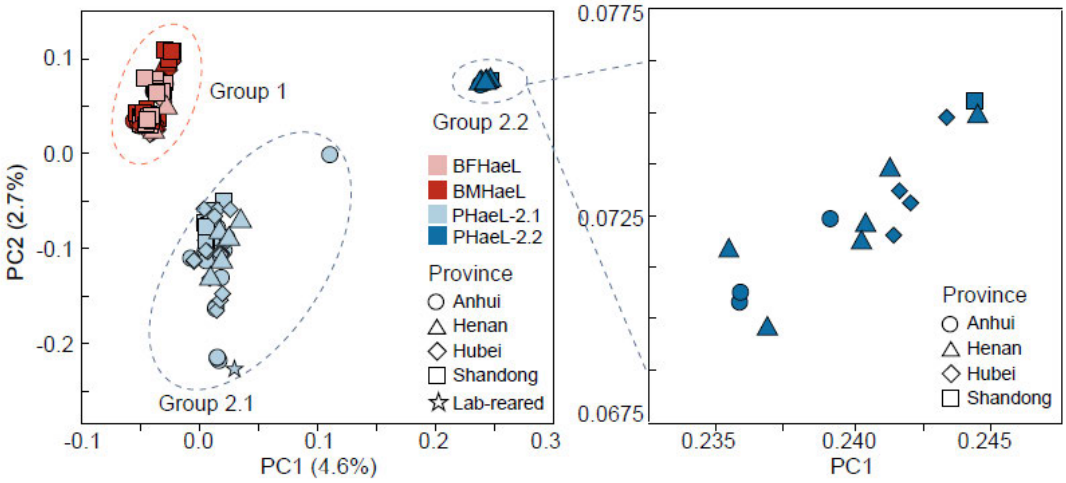
Sperling, A.L., Fabian, D.K., Garrison, E. & Glover, D.M. A genetic basis for facultative parthenogenesis in Drosophila. *Curr Biol* **33**, 3545-3560.e13 (2023).

Kunishima, T. et al. Genetic population structure of Japanese freshwater crab, Geothelphusa dehaani species complex using genome wide SNPs. Scientific Reports **15**, 23781 (2025).

Gebreselase, H.B., Nigussie, H., Wang, C. & Luo, C. Genetic Diversity, Population Structure and Selection Signature in Begait Goats Revealed by Whole-Genome Sequencing. Animals (Basel) **14**(2024).

3b. It should be stated somewhere in the manuscript if the reference PHaeL genome come from individuals that were detected as 2.2, 2.1, a mix of both, or can this be evaluated? I would ask to consider how this cryptic population structure might impact their genomic analyses.

Response: To address this, we randomly selected approximately 10× depth of Illumina reads from the PHaeL assembly individual and re-performed population structure analysis together with the field-collected population samples. The analyses of PCA, phylogenetic tree, and ADMIXTURE all placed the lab-reared PHaeL individual within group 2.1, not a mix of 2.2 and 2.1 (**Response Figure 6** and **revised Figure 3c**).

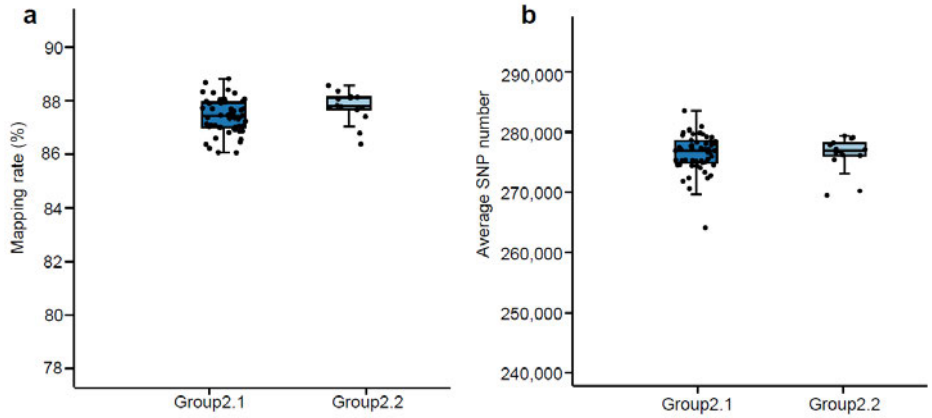


Response Figure 6. PCA of 69 PHaeL and 110 BHaeL individuals (depth >10×) based on 284,509 SNPs at fourfold degenerate sites (4d-SNPs), together with one lab-reared PHaeL individual (marked with a blue star). Different shapes represent different

geographic origins.

Accordingly, we have clarified this in the revised manuscript:
"*For the PHaeL populations, individuals were further grouped into two distinct groups without any geographic pattern, with the lab-reared PHaeL individual clustering within group 2.1 (Fig. 3c).*" (lines 258-260).

In addition, regarding the potential impact of this cryptic population structure on genomic analyses, we indirectly assessed its impact by comparing alignment rates and SNPs at fourfold degenerate sites (4d-SNPs) counts between subgroups (**Response Figure 7**).



Response Figure 7. Assessment of reference genome bias between PHaeL subgroups. (a) Comparison of mapping rates between group 2.1 and group 2.2. (b) Comparison of the average number of 4d-SNP sites per individual between the two subgroups.

First, we compared the mapping rates of resequencing data from different subgroups: we did ±0.21%) (t-test, $P = 0.062$), indicating that our reference genome didn't introduce alignment bias between the two subgroups (**Response Figure 7a**). Second, we used bcftools to calculate the average number of 4d-SNPs per individual in each group. This showed that group 2.1 and

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3c. Please clarify how individuals in a parthenogenetic lineage might have mixed ancestry with a bisexual lineage, biologically (lines 505-508)?

Response: Based on our ADMIXTURE and F_{ST} analyses we inferred that the parthenogenetic lineage exhibits a mixed ancestry component associated with the bisexual lineage. Biologically, we propose that the parthenogenetic lineage originated from a chromosome doubling event in bisexual lineages, and some of them may have initially retained the capacity to mate with bisexual individuals during their early emergence. Moreover, a degree of gene flow was maintained between parthenogenetic and bisexual lineages, particularly involving PHaeL Group 2.1. Although it is difficult to reconstruct this historical process, our inference is biologically reasonable supported by numerous laboratory studies that have successfully induced polyploidy or parthenogenesis in other species. For example, after artificially inducing triploidy from diploid zebrafish, triploid males were highly sterile, whereas triploid females retained the ability to produce eggs that could be fertilized normally, though with extremely low embryonic survival rates (only 5%) (Ou, Y. et al. 2024). Tetraploid females crossed with males and produced viable triploid offspring (Ou, Y. et al. 2024). In addition, studies on parthenogenesis induction in *D. melanogaster* showed that parthenogenetic females also retained the ability to mate with males and exhibited partial fertility (Sperling, A.L. et al. 2023).

We have addressed these points in our response to Comment 3a, and have clarified the likely biological processes in the revised Discussion (lines 513-517). The revised content and references are already detailed in the response to Reviewer Comment 3a.

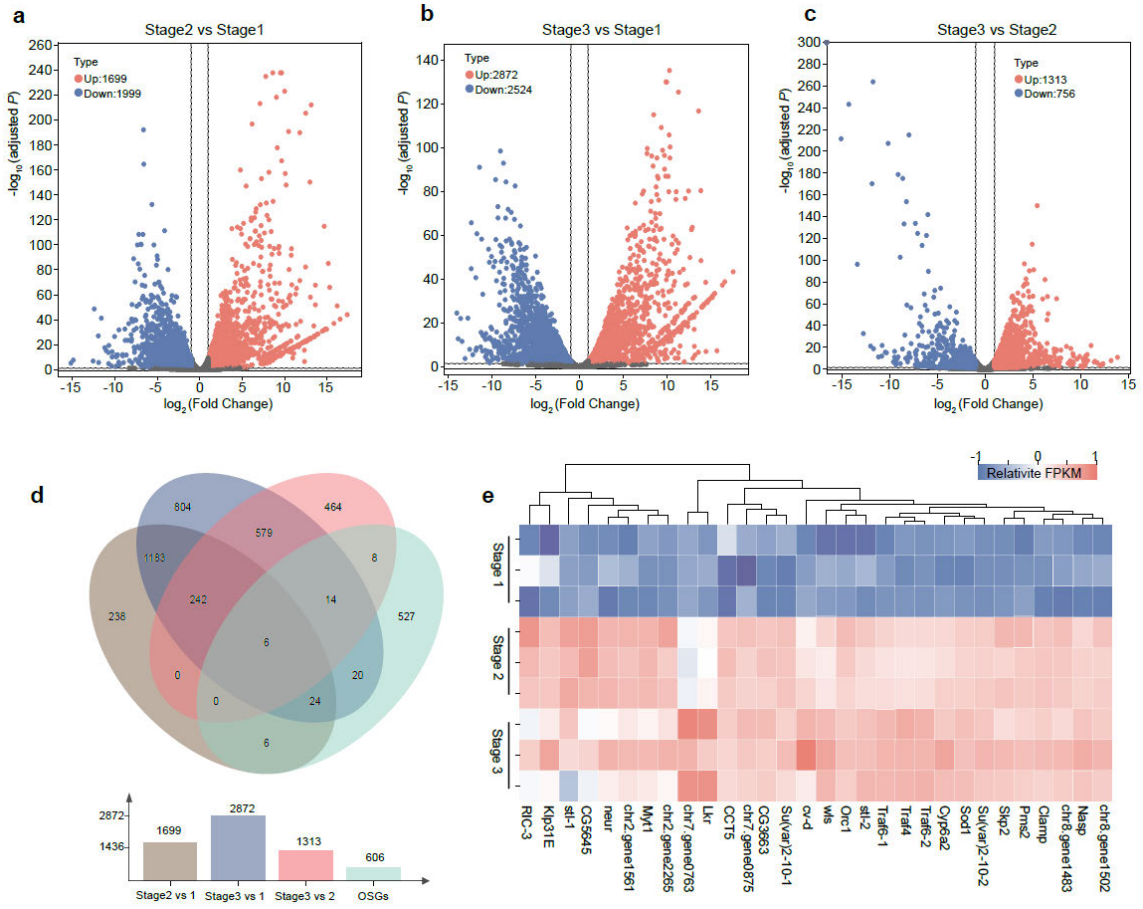
[4] The differential expression and RNA interference analyses certainly elevate the level of this genomic research, though they stop short of a mechanistic understanding of how evolution acts on the genome to achieve triploid parthenogenesis.

4a. Please clarify why expression analyses were performed between stages 1 v 2 and 1 v 3, instead of 1 v 2 and **2 v 3**?

Response: We performed expression analyses between stages 1 v 2 and 1 v 3 to identify genes potentially associated with parthenogenesis based on their expression profiles during ovarian development in adult females following blood feeding. In *H. longicornis*, ovarian and embryonic development are markedly activated during the partially-feeding (Stage 2, 96 h post-feeding) and engorgement (Stage 3, detached freely) stages compared to the unfed stage (Stage 1). We hypothesized that candidate genes related to parthenogenesis would be significantly up-

regulated at one or both post-blood feeding stages (Stage 2 and/or Stage 3) relative to Stage 1.

We acknowledge that genes consistently up-regulated across both comparisons (Stage 1 v 2 and Stage 2 v 3) may play a particularly important role. However, as suggested, differentially expressed genes (DEGs) between Stage 3 and Stage 2 were identified using DESeq2 after filtering out lowly expressed genes (mean FPKM < 1) (**Response Figure 8a** and **Supplementary Figure 20a**). Significant DEGs were then selected based on a threshold of | $\log_2$FoldChange > 1 | and adjusted *P*-value < 0.05. We re-analyzed differential expression for Stage 2 vs Stage 1 and Stage 3 vs Stage 1, after filtering out low-expression genes (**Response Figure 8b** and **8c; Supplementary Figure 20b** and **20c**).



Response Figure 8. Screening potential parthenogenesis-regulating genes by integrating the analysis of signatures of natural selection and transcriptome dynamics during ovarian development. (a-c) Volcano plots of differentially expressed genes (DEGs) in ovarian tissue: (a) Stage 2 vs Stage 1; (b) Stage 3 vs Stage 1; (c) Stage 3 vs Stage 2. (d) Venn diagram showing the identification of stage-specific up-regulated genes from the intersection of 606 overlapping selected genes (OSGs; identified by F_{ST} and XP-CLR) and stage-specific DEGs. (e) Expression heatmap of the resulting 30 overlapping candidate genes.

Our re-analysis identified 1,699 genes significantly up-regulated in Stage 2 (vs Stage 1), 2,872 genes in Stage 3 (vs Stage 1), and 1,313 genes in Stage 3 (vs Stage 2) (**Response Figure 8a-c; Supplementary Figure 20a-c**). A total of 30 overlapping genes were identified as stage-specific up-regulated genes from the intersection between the 606 overlapping selected genes (OSGs) screened by F_{ST} and XP-CLR analyses. Notably, six genes (one *Apob*, one *Lkr*, and four *Traf* family members) exhibited a consistent up-regulation pattern, showing significant elevation in Stage 2 and sustained through Stage 3 (**Response Figure 8e; Supplementary Figure 20e**). The characteristics of the *Apob* family were detailed in the original manuscript. *Lkr* encodes a seven-transmembrane GPCR with the product of LK as a ligand, signals via intracellular calcium, and regulates Malpighian tubule ion balance and feeding (Radford et al., 2002). Traf encodes a TNF-receptor-associated factor adaptor mediating downstream signaling through receptor interactions, promoting Toll-related pro-inflammatory effects, and inducing apoptosis via egr-activated JNK (Shen et al., 2001; Ma et al., 2014). Functional annotation suggests that Traf is a promising candidate for future study. These re-analyses have been integrated throughout the revised manuscript, specifically in the Results, Discussion, Supplementary Figure 20, and Methods.

Results (lines 347-353):

"To identify putative parthenogenesis-related genes, we jointly analysed the 606 overlapping selected genes (OSGs) previously screened by F_{ST} and XP-CLR analyses with stage-specific up-regulated genes (ranging from 1,313 to 2,872 across differentially developmental groups); this yielded 30 overlapping differentially expressed genes (Supplementary Figure 20). Notably, six genes (one Apob, one Lkr, and four Traf family members) exhibited a consistent pattern of up-regulation, with significant elevation in Stage 2 that was sustained through Stage 3."

Discussion (lines 549-552):

"In addition, Traf encodes a TNF-receptor-associated factor adaptor that functions as a key component of Toll signaling and regulates embryonic development[53,54]. Four Traf family genes were found within the genomic region under selection and exhibited consistent ovarian up-regulation at stage 2 and stage 3, warranting further study."

Methods (lines 723-727):

"Differential expression analysis was then performed using DESeq2 (v1.18) after filtering out lowly expressed genes (mean FPKM < 1) to compare distinct developmental stages of the ovary

(Stage 2 vs. Stage 1, Stage 3 vs. Stage 1, and Stage 3 vs. Stage 2). Significant DEGs were then selected based on a threshold of | log_2FoldChange > 1 | and adjusted P-value < 0.05."

Supplementary Figure 20: *The original Supplementary Figure 19 has been replaced by a new Supplementary Figure 20 in the revised manuscript. The replacement contained new volcano plots, Venn diagrams, and heatmap reflecting these specific comparisons (also shown in Response Figure 8 above)."*

References:

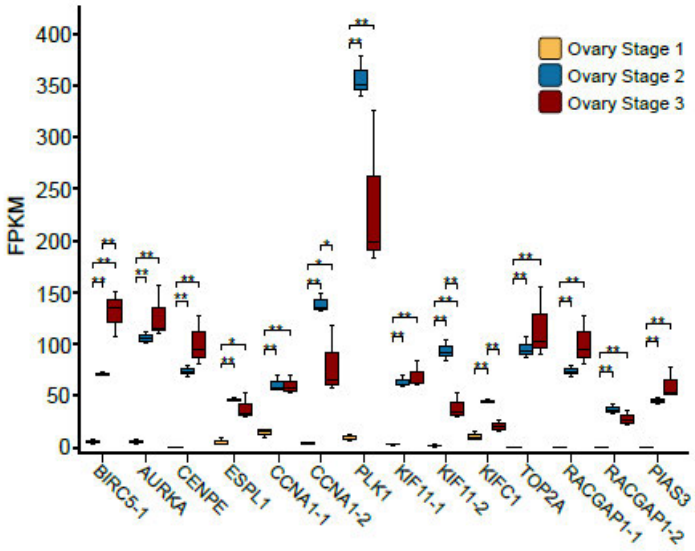
Radford, J.C., Davies, S.A., Dow, J.A.T. (2002). Systematic G-protein-coupled receptor analysis in Drosophila melanogaster identifies a leucokinin receptor with novel roles. J. Biol. Chem. 277(41): 38810--38817.

Shen, B., Liu, H., Skolnik, E.Y., Manley, J.L. (2001). Physical and functional interactions between Drosophila TRAF2 and Pelle kinase contribute to Dorsal activation. Proc. Natl. Acad. Sci. U.S.A. 98(15): 8596--8601.

Ma, X., Li, W., Yu, H., Yang, Y., Li, M., Xue, L., Xu, T. (2014). Bendless modulates JNK-mediated cell death and migration in Drosophila. Cell Death Differ. 21(3): 407--415.

4b. I might be misunderstanding, but it seems like the expression of the genes mentioned at line 229-230 goes down in midgut from stage 2 to 3, but the authors report that expression levels significantly go up with feeding time (l 232), is that correct?

Response: We must clarify that the corresponding description in the originally manuscript lines 229-230 refers to the ovary-specific high expression of BIRC5 and its interacting proteins, not the midgut. We have carefully checked the transcriptome analysis and the FPKM values of the genes, and confirmed that the expression values were calculated correctly. In addition, we have redrawn the original Supplementary Figure 9 and presented it as the revised Figure 2f (**Response Figure 9**), which shows the expression profiles of proteins directly interacting with BIRC5 in the ovary.



Response Figure 9. **Expression of BIRC5 and its direct interacting proteins in ovaries across different blood-feeding stages**. The y axis represents the FPKM value, * and ** indicate independent t-test *P* value below 0.05 and 0.01 , respectively.

As shown in the **Response Figure 9**, the expression levels of CCNA1-2 (PHaeL.chr5.gene2296), KIF11-2 (PHaeL.chr9.gene0593), and KIFC1 (PHaeL.chr9.gene1067) significantly decreased from Stage 2 to Stage 3 in the ovary. However, CCNA1-2 and KIF11-2 were significantly upregulated at Stage2 and Stage3 compared with Stage 1, and KIFC1 was significantly upregulated at Stage 2 compared with Stage1. Thus, all three genes were significantly highly expressed post blood feeding (either Stage2 or Stage3).

To make this clearer, we have revised this sentence as follows:
"Interestingly, most of the genes that interact directly with BIRC5, including AURKA, CENPE, ESPL1, CCNA1, PLK1, KIF11, KIFC1, TOP2A, RACGAP1, and PIAS3, were specifically expressed in the ovary of PHaeL after blood-sucking, with significant upregulation at either Stage 2 or Stage 3 (Fig. 2e and 2f)." (lines 227-230)

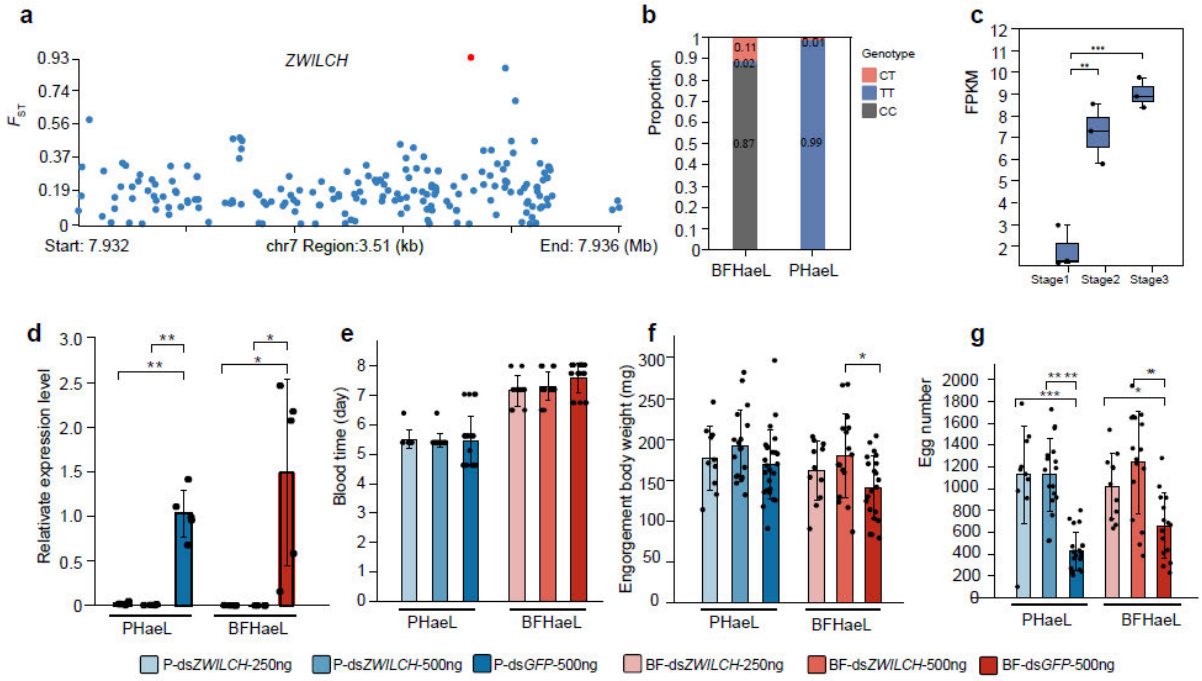
4c. Please indicate how the genes for individual expression analyses were selected, and what pointed them to target the BIRC family genes for interference. Were other genes considered, targeted, or analyzed? Are there negative results that they can also describe in this study?
Response: The genes selected for individual expression analyses were identified based on a combination of weighted gene correlation network analysis (WGCNA) and protein-protein interaction (PPI) analysis, which have been mentioned in the Methods section (lines 715-722) and visualized in the corresponding results (Fig. 2c and Fig. 2d). First, a total of 5,931

tissue-specific genes were identified and divided into 19 modules by WGCNA. Among these modules, six modules (M7, M11, M13, M14, M17, and M18) were significantly associated with the 14 BIRC5-type IAPs family genes that showed specific expression in the ovary (Fig. 2c, Supplementary Figure 10 and Supplementary table 13). We then performed PPI analysis within these six modules to screen genes that directly interact with BIRC5. The candidate genes chosen for individual expression analysis included AURKA, CENPE, ESPL1, CCNA1, PLK1, KIF11, KIFC1, TOP2A, RACGAP1, and PIAS3, because they exhibited direct interactions with BIRC5 and showed ovary-specific and blood-sucking-induced expression patterns. We focused on the BIRC5 (IAPs family) for functional interference because:

1. The BIRC5 gene families were related to cell cycle regulation in PHaeL and have undergone significant expansion
2. The BIRC5-type IAPs family genes displayed ovary-specific expression
3. BIRC5 was located at a key regulatory position in the PPI network
4. Its interacting genes were upregulated following blood feeding, suggesting their involvement in ovarian or embryonic development.

These lines of evidence collectively indicated that BIRC5 and its network are functionally important and therefore selected for further functional investigation.

Regarding whether other genes were considered, targeted, or analyzed, the answer is yes. In addition to *BIRC5*, we also analyzed *ZWILCH* (PHaeL.chr7.gene0134), a gene involved in mitotic spindle assembly checkpoint signaling and kinetochore protein localization (Williams et al., 2003). For *ZWILCH*, PHaeL carries a nonsynonymous C-to-T mutation (exon 15: c.C1685T) relative to BFHaeL, located at position 7,934,560 on chromosome 7 of the BFHaeL genome within exon15 (**Response Figure 10a**). Population genotyping revealed that 99% of parthenogenetic individuals were homozygous for the T allele, and only 1% were heterozygous (CT), whereas 87% of bisexual female *H. longicornis* individuals were homozygous for the C allele (**Response Figure 10b**). Compared to Stage 1, *ZWILCH* exhibited significantly higher expression levels at Stage 2 and Stage 3 following blood feeding (**Response Figure 10c**). We therefore performed dsRNA knockdown targeting *ZWILCH* both in PHaeL and BFHaeL to evaluate its impact on blood feeding and egg production.



Response Figure 10. Genetic signatures, expression patterns, and functional characterization of the *ZWILCH* gene. a, Local Manhattan plot of a ~3.5 kb region near the *ZWILCH* gene locus G1685A:chr7:7,934,560; the Y-axis represents the fixation index (F_{ST}) values. The red dot represents the nonsynonymous single nucleotide polymorphism (SNP) (G1685A) at position 7,934,560 on chromosome 7, with an F_{ST} value of 0.93. **b**, Genotype frequencies of the G1685A SNP in *ZWILCH* among PHaeL and BFHaeL population samples. **c**, Box plot of *ZWILCH* gene expression levels (FPKM values) in the ovaries of PHaeL at different blood-sucking stages. **d**, Evaluation of the *ZWILCH* knockdown efficiency in PHaeL and BFHaeL. **e**, Effect of the *ZWILCH* gene knockdown on the blood-sucking time in PHaeL and BFHaeL. **f**, Effect of the *ZWILCH* gene knockdown on the engorgment body weight in PHaeL and BFHaeL. **g**, Effect of the *ZWILCH* gene knockdown on the egg number in PHaeL and BFHaeL.

Two doses of ds*ZWILCH* (250 ng/tick and 500 ng/tick) were used to knock down the *ZWILCH* expression in the ovaries of both populations, with the same dsGFP control (500 ng/tick) used in the ds*BIRC5* knockdown experiment. In PHaeL, the *ZWILCH* knockdown efficiencies were 98.02% (low-dose: ds*ZWILCH*-250 ng/tick) and 99.37% (high-dose: ds*ZWILCH*-500 ng/tick) at 4 days post-blood-sucking, respectively (**Response Figure 10d**). The silencing of *ZWILCH* (99.92% in the ds*ZWILCH*-250 ng group, and 99.99% in the ds*ZWILCH*-500 ng group) at 4 days post-blood-sucking was also successful in BFHaeL (**Response Figure 10d**). Both for PHaeL and BFHaeL, the knockdown of *ZWILCH* did not influence the blood-sucking time to engorgement (**Response Figure 10e**). In PHaeL, the engorged body weight was increased in both ds*ZWILCH*-treated groups relative to the ds*GFP* control (176.87 ± 39.94 mg in the 250

ng group and 191.77 ± 43.26 mg in the 500 ng group vs 168.60 ± 42.21 mg in the ds*GFP*-500 ng group; **Response Figure 10f**). Similarly, in BFHaeL, ds*ZWILCH* treatment also led to increased engorged body weight (161.47 ± 36.90 mg in the 250 ng group and 180.11 ± 51.68 mg in the 500 ng group vs 141.01 ± 38.77 mg in the dsGFP-500 ng group; **Response Figure 10f**). Although all ds*ZWILCH*-treated groups exhibited higher mean engorged body weights than their corresponding controls, the difference was only statistically significant in the ds*ZWILCH*-500 ng group of BFHaeL (t-test, $P = 0.013$). Oviposition was substantially increased in all ds*ZWILCH*-treated groups of PHaeL and BFHaeL relative to their corresponding ds*GFP* groups. In PHaeL, egg production reached 1129.5 ± 444.17 in the 250 ng group and 1127.39 ± 334.03 in the 500 ng group, compared to 428.6 ± 171.32 in the dsGFP control (**Response Figure 10g**). A similar trend was observed in BFHaeL, where oviposition reached 1022.55 ± 299.33 (250 ng group) and 1239.75 ± 474.03 (500 ng group) versus 659.07 ± 288.40 in the control (**Response Figure 10g**). Furthermore, the eggs laid by ds*ZWILCH*-treated ticks exhibited uniform morphology and size, which were similar to those produced under natural conditions.

Studies in *Drosophila* have shown that *ZWILCH* mutations lead to anaphase lagging and premature sister chromatid separation upon spindle checkpoint activation, resulting in 21% aneuploid larval brain cells and aberrant chromosome distribution in 20% of anaphases (Williams et al., 2003). In our study, silencing of *ZWILCH* in both PHaeL and BFHaeL did not reduce oviposition; on the contrary, egg production increased, and the eggs exhibited completely normal morphology. These results reveal that *ZWILCH* is related to reproduction of ticks, although they are weird and difficult to interpret. Therefore, given the lack of sufficient experimental evidence, we consider that the current *ZWILCH* results are not yet suitable for any publication.

Reference:

Williams, B.C., Li, Z., Liu, S., Williams, E.V., Leung, G., Yen, T.J., Goldberg, M.L. (2003). Zwilch, a new component of the ZW10/ROD complex required for kinetochore functions. Mol. Biol. Cell 14(4): 1379--1391.

[5] The same colors are used to mean different things across figures, and in many cases across panels within a figure. Particularly confusing cases are found in Figure 3, where red vs blue is used to indicate strain in panel a, then the same colors for region in panel b, and then back to strain in panel c and d. I would ask to please consider using a standard color set for strain

throughout the manuscript, and to choose distinct visualization approaches for other pieces of data (region, expression, etc).

Response: We have now standardized the color scheme throughout the manuscript. We have redrawn all panels of Fiure 2 and panels a, b, c, and e of Figure 3 to improve clarity and visual presentation.

Minor revisions:

- I believe there is a missing word at 797 "freshly", should "freshly dissected"

Response: We have made this change. (line 818)

- Several figures could be reconsidered or moved to the supplement, to improve interpretation and increase the impact of the paper. Examples include Figure 1c, perhaps the chromosomes could be aligned, and the circle simplified? Figure 2d, the network is difficult to read and interpret, especially at low resolution.

Response: We have revised Figure 1, Figure 2, Figure 3, and several supplementary figures. In response to comment 2d, we have removed Figures 1e-h and replaced them with a violin plot, which is presented as the new Supplementary Figure 4. In response to Comments 1a and 1b, we have redrawn the species phylogenetic tree as Figure 2b (previously Figure 2a) and Supplementary Figures 6-8 (originally Supplementary Figures 5-7). Furthermore, we have redrawn the synteny plot between the haplotype genome of PHaeL and the BFHaeL genome (originally Supplementary Figure 4b) and presented it as Figure 2a**.**

In response to Comment 1c, we have redrawn the original Figure 2b and moved it to the supplementary materials as new Supplementary Figure 9 to display the phylogenetic tree of IAPs family members. Figure 2d has been simplified. The revised Figure 2d presents the interaction confidence between BIRC5 and its direct binding partners in an interaction heatmap. In addition, we have reformatted Supplementary Figure 10, and redrawn Supplementary Figure 20 (previously Supplementary Figure 19) following updated reanalysis. At the same time, minor adjustments were made to several figures to improve their appearance.

Reviewer #2 (Remarks to the Author):

This manuscript presents a comprehensive investigation into the genomic and evolutionary basis of polyploid parthenogenesis in H. longicornis. The generation of haplotype-resolved, chromosome-level genome assemblies for the triploid parthenogenetic strain alongside high-quality genomes for diploid bisexual strains, provide an important genomic resource for both

evolutionary biology and vector research. The integration of comparative genomics, population resequencing, transcriptomic profiling, and functional RNAi experiments makes this study comprehensive and positions it as an important contribution to the field.

- The genome assemblies themselves are high quality. However, the manuscript would benefit from a clearer explanation of the observed discrepancy between the k-mer–based genome size estimates and the final assembled genome sizes, particularly given the challenges of genome size estimation in triploid organisms. Explicit discussion of how polyploidy affects assembly inflation would help avoid confusion.

Response: In triploid organisms the presence of three distinct haplotypes with high heterozygosity poses a significant challenge for assembly algorithms. Generally, highly divergent allelic regions are not collapsed into a single consensus sequence but are instead assembled as separate contigs (a phenomenon known as haplotype inflation or assembly duplication). This leads to an assembled genome size exceeding the true biological genome size estimated by k-mer analysis. This discrepancy is a common technical challenge in genome assembly and size estimation for polyploid non-model organisms. Nevertheless, the quality of our assemblies has been validated by subsequent assessments of continuity and completeness, confirming their suitability for downstream analyses.

As suggested, we have added a paragraph in the Discussion section to address this issue (lines 463-465).

"The resulting assembly for the triploid PHaeL lineage exceeds 2.6 Gb, which is larger than the initial k-mer-based estimates, likely due to the high proportion of repetitive sequences in the genome (nearly 60%). The quality of all new assemblies was validated through high BUSCO completeness (>90%), Illumina read mapping rates (>95%), and genome coverage (>99%), demonstrating both high continuity and completeness."

- Comparative genomic and phylogenetic analyses are well executed and reveal lineage-specific gene family expansions and contractions that are biologically plausible. The expansion of cell cycle– and apoptosis-related gene families in the parthenogenetic lineage is a compelling finding. At the same time, the large number of contracted genes, many associated with male reproduction, fits well with theoretical expectations for obligate parthenogenesis. But, claims describing "structural uniformity" among haplotypes should be tempered to distinguish between conserved large-scale synteny and the presence of numerous smaller-scale structural

variants.

Response: We agree that the description of "structural uniformity" should be tempered to distinguish between conserved large-scale synteny and the presence of numerous smaller-scale structural variants. As suggested, we have therefore revised the relevant description in the revised manuscript.

"Comparative genomic analysis revealed high collinearity between the parthenogenetic and bisexual genomes, with a stable chromosomal architecture maintained among the three haplotypes of parthenogenetic strain." (lines 38-41)

- The population genomic analyses is of strong value, however, while the interpretation that chromosome 7 harbors loci important for parthenogenetic reproduction is well supported, the discussion would benefit from a more explicit consideration of alternative explanations that could also generate chromosome-wide signals (e.g., reduced recombination, background selection, etc).

Response: We agree that the strong chromosome-wide signals on chr7 may also arise from evolutionary mechanisms other than direct selection on parthenogenesis-related loci. As suggested, we have therefore expanded the Discussion to explicitly consider these alternatives in the revised manuscript (lines 529-534).

"Independent of directional selection on specific parthenogenesis-related loci, the genome-wide patterns observed on chromosome 7 may be caused by reduced recombination, potentially driven by structural variants[40], transposable element accumulation[41], or expanded centromeric regions[42]. In addition, background selection acting on deleterious mutations can remove linked neutral variation and generate broad selective signatures through hitchhiking effects[43]."

References:

Akopyan M, Tigano A, Jacobs A, Wilder AP, Baumann H, Therkildsen NO. Comparative linkage mapping uncovers recombination suppression across massive chromosomal inversions associated with local adaptation in Atlantic silversides. (2022). Mol Ecol. 31(12):3323-3341.

Stanek TJ, Leung W, Shaffer CD. et al., Recombination Suppression Drives Expansion of the Drosophila Dot Chromosome. (2025). Mol Biol Evol.42(12):msaf304.

Slotman MA, Reimer LJ, Thiemann T. et al., Reduced recombination rate and genetic differentiation between the M and S forms of Anopheles gambiae s.s. (2006). Genetics. 174(4):2081-93.

Wang J, Street NR, Scofield DG, Ingvarsson PK. Natural Selection and Recombination Rate Variation Shape

Nucleotide Polymorphism Across the Genomes of Three Related Populus Species. (2016) Genetics. 202(3):1185-200.

- The identification of candidate genes on chromosome 7, including members of the ApoB family as well as Skp2 and Orc1, is biologically plausible and well supported by combined evidence from selection scans and ovarian expression profiles. However, consider referring to these genes as candidates rather than being definitively associated with parthenogenesis, as functional validation beyond correlative evidence is still lacking.

Response: We agree that these genes should be described as candidates rather than definitively associated with parthenogenesis in the absence of functional validation. Accordingly, we have revised the manuscript.

"Population resequencing of 179 individuals revealed their differentiation into distinct two subpopulations, with chromosome 7 harboring high genetic differentiation and several genes (including one ApoB family gene, as well as Skp2, and Orc1) ***likely candidates for parthenogenesis****."* (lines 43-46).

- In the Discussion, statements regarding the evolutionary origins of the parthenogenetic lineage and the precise roles of candidate genes would benefit from clearer language to distinguish hypothesis from demonstrated mechanism.

Response: As suggested we have revised the Discussion to more clearly distinguish between hypotheses and demonstrated mechanisms.

Regarding evolutionary origins (lines 472-476):

"This high degree of similarity suggests that PHaeL and BHaeL share a common ancestor and that the polyploidy in PHaeL ***likely*** *occurred without large-scale genomic rearrangements, such as chromosomal fusions or fragmentations. PHaeL may have* ***potentially*** *occurred through spontaneous polyploidization via production of unreduced gametes or polyspermy."*

Regarding candidate gene functions (lines 554-557):

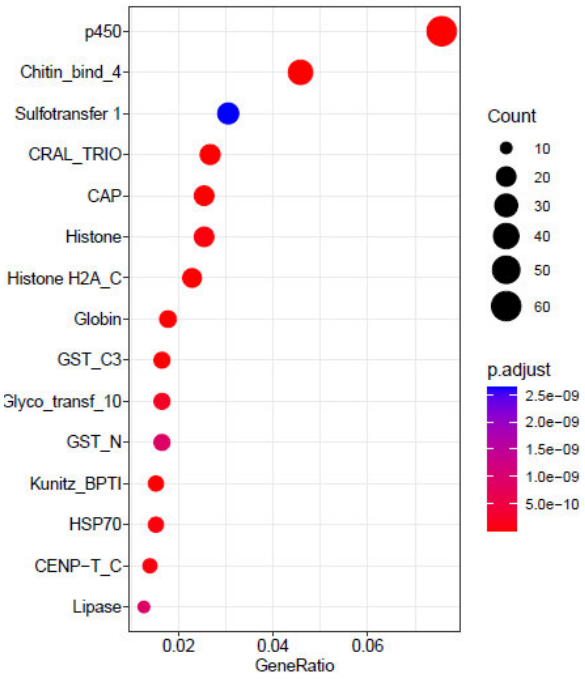
"Our findings therefore suggest that genes related to embryonic development and mitotic regulation are likely involved in the adaptive evolution of parthenogenesis and genetic differentiation between the PHaeL and BFHaeL populations, although needs to be confirmed in vivo."

- In summary, a very interesting study that addresses a knowledge gap regarding polyploid parthenogenesis in animals.

Minor comments:

Ln 43 – Please give an example of major gene families that have contracted. Were any of significant value.

Response: To further identify major gene families, we performed domain enrichment analysis of proteins encoded by contracted genes in PHaeL. Consistent with the GO term enrichment results, the most significantly enriched domains were associated with xenobiotic metabolism (P450, GST_N/GST_C_3, Sulfotransfer_1, Lipase), cuticle structure (Chitin_bind_4, Glyco_transf_10), and male reproduction (CAP, CRAL_TRIO, Kunitz_BPTI) (**Response Figure 11** and revised **Supplementary Figure 8c**). Notably, families with significant enrichment values included the CAP superfamily (Cysteine-Rich Secretory Proteins, Antigen 5, and Pathogenesis-related 1 proteins). CAP proteins exhibit multifaceted functions within the male reproductive system, playing pivotal roles during spermatogenesis, sperm maturation, and fertilization (Jamsai et al., 2009). Specifically, the member CRISP2 is localized to the acrosomal region and tail of round spermatids, serving as a core regulatory factor in spermatogenesis (Jamsai et al., 2009; Hu et al., 2018). Meanwhile, CRISP1 and CRISP4 act synergistically to promote sperm motility, capacitation, and fertilization capacity (Hu et al., 2018).



Response Figure 11. Protein domain enrichment analysis of contracted protein families in PHaeL**.**

We have added the significantly enriched/contracted gene families to the Results sections of the revised manuscript.

In the Results section:

"Notably, these contracted families were predominantly composed of proteins encoding CAP domains involved in spermatogenesis, as well as families related to xenobiotic metabolism (e.g., P450s, GSTs) and cuticle structure (e.g., chitin-binding proteins) (Supplementary Fig. 8c)[25-27]." (lines 202-205)

References:

Jamsai D, Rijal S, Bianco DM, O'Connor AE, Merriner DJ, Smith SJ, Gibbs GM, O'Bryan MK. A novel protein, sperm head and tail associated protein (SHTAP), interacts with cysteine-rich secretory protein 2 (CRISP2) during spermatogenesis in the mouse. (2009). Biol Cell. 102(2):93-106.

Hu J, Merriner DJ, O'Connor AE, Houston BJ, Furic L, Hedger MP, O'Bryan MK. Epididymal cysteine-rich secretory proteins are required for epididymal sperm maturation and optimal sperm function. (2018). Mol Hum Reprod. 24(3):111-122.

Ln 53 – Please given a one sentence potential explanation for this with citation. Increase in agriculture-urban boundaries?

Response: As suggested, we have added an explanation with a supporting citation (lines 53-55):

"The expanding geographic range and global spread of some tick species have led to a rise in tick-borne diseases in recent decades and now pose a major threat to public health[3,4]."

Reference:

Estrada-Pena, A. & de la Fuente, J. The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Res* **108**, 104-28 (2014).

Fang, L.Q. *et al.* Emerging tick-borne infections in mainland China: an increasing public health threat. *Lancet Infect Dis* **15**, 1467-1479 (2015).

Ln 65 – "differentiation" should be "differentiation". Also the same error in the title of Fig. 4. Also, line 498.

Response: Changed as suggested (line 332 and line 505).

Ln 78 – What other mechanisms are involved if not allo- or autopolyploidy?

Response: In addition to allo- or autopolyploidy, parthenogenesis can arise through other mechanisms. Endosymbiotic bacteria such as *Wolbachia* and *Rickettsia* are known to induce thelytokous parthenogenesis in various arthropods (Weeks et al. 2001, Giorgini et al. 2010). Gene mutations can also alter reproductive modes, as documented in insects like *Apis mellifera capensis* and the parasitoid wasp *Lysiphlebus fabarum* (Aumer et al. 2017, Sandrock et al. 2011). Furthermore, hybridization has been implicated in the origin of parthenogenesis in multiple lineages, including stick insects and vertebrates such as the flowerpot snake (Lv, Y. et al. 2025). We have added these alternative mechanisms in the revised manuscript.

"Parthenogenesis can originate through diverse mechanisms, including spontaneous gene mutations, interspecific hybridization, and manipulation by endosymbiotic bacteria such as Wolbachia and Rickettsia, as observed in various insects, rotifers, and vertebrates[16-18]*."* (lines 75-77)

References:

A. R. Weeks and J. A. Breeuwer. Wolbachia-induced parthenogenesis in a genus of phytophagous mites [J]. Proc Biol Sci, 2001, 268(1482): 2245-2251.

M. Giorgini, U. Bernardo, M. M. Monti, et al. Rickettsia symbionts cause parthenogenetic reproduction in the parasitoid wasp Pnigalio soemius (Hymenoptera: Eulophidae) [J]. Appl Environ Microbiol, 2010, 76(8): 2589-2599.

D. Aumer, M. H. Allsopp, H. M. G. Lattorff, et al. Thelytoky in Cape honeybees (Apis mellifera capensis) is controlled by a single recessive locus [J]. Apidologie, 2017, 48(3): 401-410.

C. Sandrock and C. Vorburger. Single-locus recessive inheritance of asexual reproduction in a parasitoid wasp [J]. Curr Biol, 2011, 21(5): 433-437.

Lv, Y. *et al.* Genomic insights into evolution of parthenogenesis and triploidy in the flowerpot snake. *Sci Adv* **11**, eadt6477 (2025).

Ln 79 – "enchance" should be "enhance"

Response: Changed as requested (line 83).

Ln 83 – Ecological niche? How so?

Response: The statement that the transition from bisexual reproduction to obligate parthenogenesis can impact a species' ecological niche is supported by the widely observed phenomenon of geographical parthenogenesis. This classic pattern describes how clonally

reproducing, all-female lineages of plants and animals are more frequently found at higher latitudes and altitudes, on islands, and in disturbed habitats compared to their sexual counterparts (Hörandl E. 2009; Burke et al. 2019). The relevant references have been added to the revised manuscript (line 86).

References:

Hörandl, E. (2009). Geographical parthenogenesis: general purpose genotypes and frozen niche variation. In *Lost Sex* (pp. 99-116). Springer.

Burke, N. W., & Bonduriansky, R. (2019). The geography of sex: sexual conflict, environmental gradients and local loss of sex in facultatively parthenogenetic animals. *Philosophical Transactions of the Royal Society B*, 374(1777), 20190022.

- Fig 3. Presenting the ADMIXTURE plot on a map also, would provide a better visual representation of patterns of genetic structure.

Response: We have now incorporated the ADMIXTURE results into a geographic map to better visualize the patterns of genetic structure. We have now incorporated the ADMIXTURE results into a geographic map to better visualize the patterns of genetic structure (**Response Figure 12** and **revised Figure 3a**).

Response Figure 12. Geographic distribution of the PHaeL and BHaeL individuals used in this study. Different colors represent different tick types.

In the revised Figure 3a, we provide a composite map integrating sampling locations with pie charts representing individual ancestry proportions inferred by ADMIXTURE. This clearly illustrates the spatial distribution of genetic clusters and their relationship with geographic distance.

Ln 390 – “engorgement” should be “engorgement”

Response: Changed as requested (line 392).

Ln 462 – spelling of ‘between’

Response: Changed as requested (line 471).

Ln 491 – Can you confirm if these are facultative or obligate parthenogens, as the mechanisms governing these are quite different. For example, recent work by Sperling et al. 2023 has identified three genes linked to facultative parthenogenesis in Drosophila. It would be interesting to see how these genes act in H. longicornis.

Response: The answer is yes. We confirm that the parthenogenetic *H. longicornis* strain used in this study is an obligate parthenogen. This is supported by a previous study conducted at Hebei Normal University, in which parthenogenetic and bisexual *H. longicornis* males were co-reared. During blood feeding, no mating behavior was observed between parthenogenetic females and bisexual males, and all offspring produced were all female ticks (Chen et al. 2012).

Reference:

Z. Chen, X. Yang, F. Bu, et al. Morphological, biological and molecular characteristics of bisexual and parthenogenetic Haemaphysalis longicornis [J]. Vet Parasitol, 2012, 189(2-4): 344-352.

Ln 494 – Spacing required “PHaeL<>and BHael”

Response: Changed as requested (line 500).