GENOME NOTE

REVISED The complete and annotated mitochondrial genome of *Hemileia vastatrix* Race I, causal agent of coffee leaf rust

[version 2; peer review: 2 approved]

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Abstract

Hemileia vastatrix is the fungal pathogen responsible for coffee leaf rust (CLR), the most economically important disease of *Coffea arabica* worldwide. Recently, the nuclear genome of this fungus was completely deciphered. However, the mitochondrial genome of *H. vastatrix* has remained undercharacterized. Here, we present the complete, circularized mitochondrial genome of *H. vastatrix* Race I (isolate HvRI), assembled using a hybrid approach combining PacBio HiFi long reads and BGIseq short reads. The genome is 173,525 bp in length with a GC content of 33.1% and encodes 41 functional genes, including 15 protein-coding genes, 2 rRNAs, and 24 tRNAs. The assembly reveals significant structural complexity, driven by intron expansion in the *cox1* and *cob* genes. Notably, the *atp8* gene contains a group II intron, rare for this locus, whose internal open reading frame displays evidence of pseudogenization via internal stop codons.. We also characterized a putative replication initiation zone (~1.2 kb) defined by a poly-G homopolymer and conserved regulatory motifs. The mitogenome of the HvRI isolate does not contain *cob* mutations that lead to amino acid substitutions G143A and F129L associated with the quinone outside inhibitor (QoI) fungicide resistance. This high-quality mitogenome is an important resource for comparative mitogenomics, population diversity studies, and the molecular surveillance of QoI fungicide resistance.

Keywords

Hemileia vastatrix, Mitochondrial genome, Coffee leaf rust, PacBio HiFi, Fungicide resistance, Cytochrome b, atp8, Genome assembly

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 15 Jul 2026	✓ view	
version 1 19 May 2026	✓ view	✓ view

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2. **Martin Coetzee**, University of Pretoria, Pretoria, South Africa

Any reports and responses or comments on the article can be found at the end of the article.

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This article is included in the Plant Science gateway.

This article is included in the Pathogens gateway.

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Author roles: Marín-Ramírez GA: Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Maldonado CE**: Conceptualization, Data Curation, Formal Analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Padilla Hurtado BE**: Data Curation, Formal Analysis, Writing – Original Draft Preparation; **Brommonschenkel SH**: Funding Acquisition, Resources, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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REVISED **Amendments from Version 1**

In this revised version, we clarified the statement on fungal mitochondrial evolutionary rates to avoid overgeneralization. We have updated the Methods section to clarify how the paired-end data was handled in two stages related to trimming a quality filters. Finally, we added conventions for the colors used in the mitochondrial figure. Together, these changes improve the accuracy and clarity of the manuscript.

Any further responses from the reviewers can be found at the end of the article

Introduction

Hemileia vastatrix Berk. and Br., causal agent of coffee leaf rust (CLR), is the most devastating pathogen in global coffee production.[1] Although recent long-read sequencing efforts have resolved its large and repeat-rich nuclear genome (~748 Mb),[2] the mitochondrial genome of *H. vastatrix* remains undercharacterized.

Mitochondria are double-membrane organelles, present in high copy numbers per cell. They are essential for fungal metabolism and the site for oxidative phosphorylation.[3] In sexual eukaryotes, mitochondrial inheritance is predominantly uniparental; however, in fungi, both uniparental and biparental inheritance modes have been reported.[4] Another distinction across the tree of life is the mutation rate: while mitochondrial genomes evolve faster than nuclear genomes in animals, the opposite has been reported in specific fungal species.[5] Predominantly asexual species of the division Basidiomycota present SNP frequencies up to 7.69% in nuclear genomes but only up to 4.41% in their mitochondrial counterparts.[5] This slower mutation rate makes mitochondrial DNA markers useful for resolving deep evolutionary relationships between species.[6–8] Despite the slow mutation rate in Basidiomycota, high variability in genome size, gene order, and intron content has been found while maintaining a conserved core set of protein-coding genes, this genomic plasticity is valuable not only for phylogenetics of distant species but also for population genomics within a species.[9]

In fungal phytopathogens, mitochondria are involved in virulence through the regulation of growth and resistance to antimicrobial compounds.[6,9] Strobilurin fungicides, also known as Quinone outside Inhibitors (QoI), suppress mitochondrial respiration by binding at the Qo site of cytochrome b (cob) within the cytochrome bc_1 complex (cyt bc_1), located in the inner mitochondrial membrane. This binding blocks electron transfer between cob and cytochrome c_1, which disrupts ATP production. In fungi, the cyt bc_1 comprises 10 or 11 subunits with a combined molecular mass of approximately 240 kD; all subunits are nuclear-encoded except *cob*, which is encoded by the mitochondrial genome.[10,11] Single-point mutations within the *cob* gene confer strobilurin resistance. The most significant mutation leads to the amino acid substitution of glycine (G) to alanine (A) at position 143 (G143A) of cob and confers a high level of resistance.[10,11] Additional mutations, such as F129L and G137R, also reduce the efficacy of chemical control based on QoI fungicides.[12,13]

Although a mitochondrial sequence was reported in a contig-level genome assembly of the *H. vastatrix* isolate Hv178a,[14] no complete, annotated, and curated mitochondrial genome has been made publicly available. In the *H. vastatrix* Race I genome,[2] the mitogenome was not assembled into a single sequence, requiring additional analysis. Here, we present a complete and circularized mitochondrial genome of *H. vastatrix* Race I, expanding the genomic resources for this fungus and providing a reference for comparative genomics and applied CLR management.

Methods

Biological material. A single isolate of *H. vastatrix*, identified as Race I (HvRI), was obtained from symptomatic leaves in a *Coffea arabica* var. Caturra plantation in the rural area of Pereira (Risaralda, Colombia) at an altitude of approximately 1,800 m a.s.l. (4°44′46.25″N, 75°36′14.59″W). The pathogen was multiplied on susceptible coffee plants (var. Caturra) maintained at 23 °C and 80% relative humidity to promote sporulation. Fresh urediniospores were harvested, vacuum-dried, and stored at −80 °C. Further details on the sample are presented in Angel *et al.* (2023)[2] and in the NCBI BioSample record SAMN32232808.

DNA isolation and sequencing. High molecular weight genomic DNA isolation, library construction, and sequencing were performed by the Arizona Genomics Institute (AGI, University of Arizona, USA). DNA was isolated from 3 g of urediniospores using a modified CTAB protocol.[15] A PacBio SMRTbell library was prepared for HiFi sequencing and run on a Sequel II system using two Single-Molecule Real-Time (SMRT) cells, targeting an expected 70X coverage of the nuclear genome. Short-read sequencing was also performed for HvRI. DNA was isolated using the DNeasy Plant Kit (Qiagen), and 50 million 150 bp paired-end (PE) reads were generated using the BGIseq/DNBseq platform by Complete Genomics (California, USA).

Mitogenome assembly. The mitochondrial genome was assembled using a hybrid approach. Initially, raw BGIseq reads (SRA SRR22911637) were adapter-trimmed using Trimmomatic v0.40.[16] A *de novo* assembly was then performed using NOVOPlasty v4.3.516,[17] which utilizes internal read quality filtering, using the *cob* gene sequence of *Puccinia striiformis* f. sp. *tritici* isolate CYR32 (GenBank MH891489.1) as a seed. The resulting contig was annotated with MFannot v2.017[18] using Genetic Code 4 (Mold, Protozoan, and Coelenterate Mitochondrial). The NADH dehydrogenase subunit 4 (*nad4*) gene from this preliminary assembly was subsequently used as a seed for a second round of NOVOPlasty. Finally, prior to error correction, the raw reads underwent quality trimming with Trimmomatic (leading/trailing $Q < 20$; 4-bp sliding window average $Q \geq 30$; minimum length 100 bp). These high-quality reads were mapped to the draft assembly using BWA-MEM v0.7.17,[19] and the assembly was polished with Pilon v1.24.[20]

The resulting short-read assembly served as a reference to recruit PacBio HiFi reads (SRA SRR22911636) using minimap2.[21,22] Reads with a mapping quality of at least Q30 were extracted by Samtools v1.21[23] and *de novo* assembled using NextDenovo v2.5.5[24] and NOVOLoci v0.5.[17] Although NOVOLoci is optimized for Oxford Nanopore (ONT) data, its seed-and-extend algorithm was experimentally applied here to the PacBio HiFi reads.

Contigs obtained from NextDenovo and NOVOLoci were aligned to the NOVOPlasty assembly to verify mitochondrial identity. The selected sequences were self-aligned using the nucmer module of MUMmer v3.23,[25] and coordinates extracted with the show-coordinates utility were used to trim overlaps and define the circular genome. The three independent assemblies were aligned using MAFFT v7.525[26] and manually inspected using Unipro UGENE[27] to resolve discrepancies. The final consensus sequence, derived primarily from the NextDenovo assembly, was selected for annotation.

Functional annotation. The mitochondrial genome was annotated using MFannot v2.0 (Genetic Code 4)[18] and MITOS v2.1.10 (Genetic Code 4; RefSeq 89 Fungi).[28,29] Predicted features from both pipelines, including protein-coding genes (PCGs), transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs), were merged and manually curated. Coding sequences (CDSs) were inspected for start and stop codon integrity. To verify gene boundaries, sequences were aligned using BLASTX[30] against the NCBI nr database. In cases of potential truncations or missing regions, particularly regarding fungal orthologs in the order Pucciniales, open reading frames (ORFs) were manually inspected in all three relevant reading frames, and intron-exon boundaries were refined through comparative analysis.

Results

Mitochondrial genome assembly and validation. The *de novo* assembly using BGIseq short reads and NOVOPlasty produced a single circular contig of 173,497 bp with an average coverage of 2,411X (2,769,734 aligned reads). A second assembly using a conspecific seed (*nad4* derived from the first round) yielded an identical length (173,497 bp) and consistent coverage depth (2,392X), confirming the stability of the short-read assembly graph.

Polishing of the BGIseq assembly with Pilon identified and corrected three single nucleotide polymorphisms (SNPs), validating 99.87% (173,266/173,497 bases) of the initial sequence as error-free. To further verify structural integrity

Table 1. Genome assembly metrics and annotation features of the Hemileia vastatrix Race I mitochondrial genome.

Property	Value
Organism	*Hemileia vastatrix Berk. and Br.*
Isolate	Race I (HvRI)
BioSample Accession	SAMN32232808
Genome Size	173,525 bp
Topology	Circular
GC Content	33.10%
Gene Content	41 functional genes (15 PCGs, 2 rRNAs, 24 tRNAs)
Sequencing Technology	PacBio Sequel II (HiFi); BGIseq/DNBseq (PE 150 bp)
Assembly Tools	NextDenovo v2.5.5; NOVOPlasty v4.3.5; NOVOLoci v0.5
Coverage Depth	1,240X (PacBio HiFi); 2,411X (BGIseq)
Raw Data Accession (SRA)	SRR22911636 (PacBio); SRR22911637 (BGIseq)
GenBank Accession	PX759424

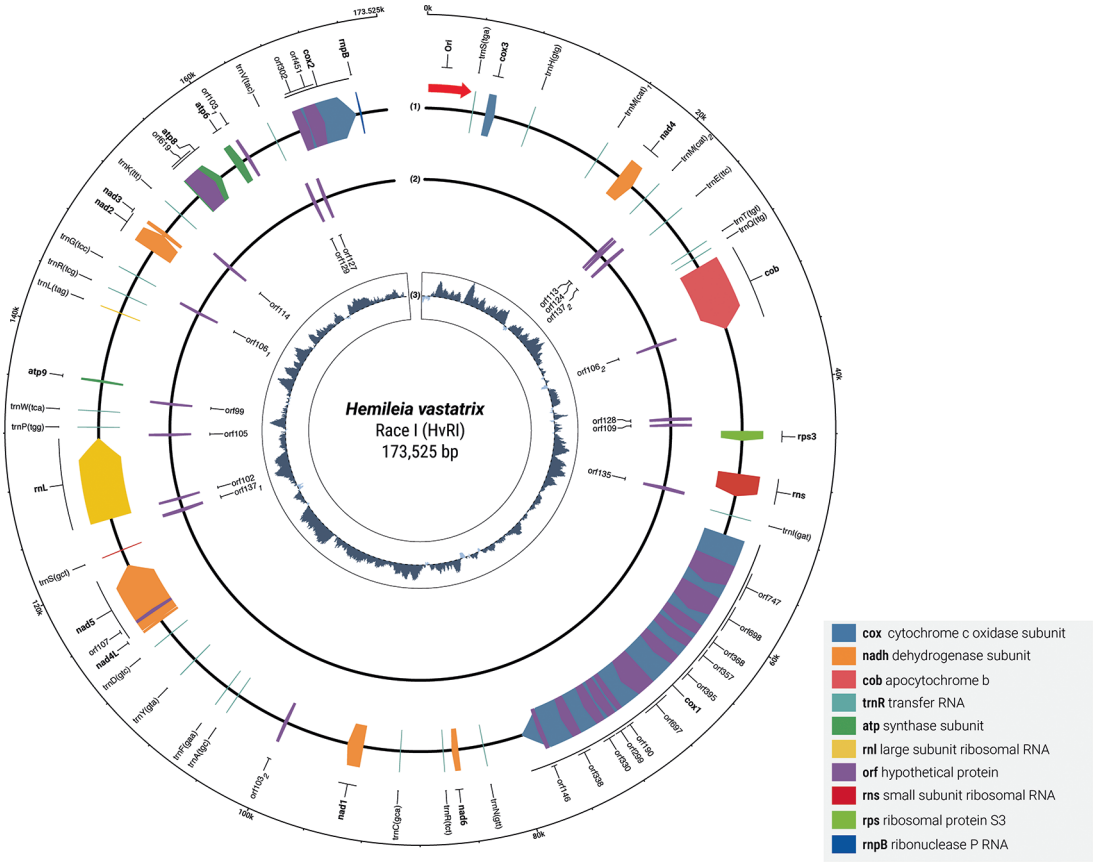

Figure 1. Circular map of the *Hemileia vastatrix* Race I mitochondrial genome (173,525 bp). Tracks are arranged from outermost to innermost: (1) Genes encoded on the forward (+) strand; (2) Genes encoded on the reverse (−) strand; and (3) GC skew calculated using a 400 bp sliding window (dark gray: positive values; light gray: negative values). Gene blocks are color-coded by functional category. The *rnpB* gene is located near the 170.6 kb mark on the outer track. Radial tick marks indicate genomic coordinates in kilobases (kb). The map is oriented such that the putative replication initiation zone (approx. 62 kb) is positioned at the top.

and sequence accuracy, an independent assembly was generated using PacBio HiFi reads. The HiFi-based assembly, generated by NextDenovo and NOVOLoci, produced a circularized sequence of 173,525 bp with 1,240X coverage.

Comparative alignment between the polished short-read assembly and the HiFi assembly revealed a high degree of concordance. The minor length discrepancy (28 bp difference) was attributed to small indels (1–2 bp) located exclusively in homopolymeric regions, which are known artifacts in short-read assemblies. Crucially, the three SNPs corrected by Pilon in the short-read assembly were confirmed by the HiFi data. Due to the superior handling of repetitive regions by long-read sequencing, the HiFi consensus (173,525 bp) was selected as the final reference sequence.

Genome annotation and features. The HvRI mitochondrial genome has a GC content of 33.1% typical of the order Pucciniales. Assembly metrics and genomic features are summarized in Table 1. Annotation identified a total of 41 functional genes, comprising 15 protein-coding genes (PCGs), two rRNAs (*rnl* and *rns*), and 24 tRNAs (Figure 1).

The genome is characterized by significant structural complexity, particularly in the cytochrome *c* oxidase subunit I (*cox1*) and *cob* genes, which contain multiple introns harboring LAGLIDADG and GIY-YIG homing endonucleases. The expansion of these intronic regions and intergenic spacers contributes to the large genome size observed in HvRI mitochondrial genome compared to those of other rust fungi, which ranges from 31,825 bp in *Phakopsora pachyrhizi*[31] to 102,521 bp of *Puccinia striiformis* f. sp. *tritici*.[32]

Of particular interest is the ATP synthase subunit 8 (*atp8*) gene. While introns are rare in fungal *atp8* genes, this locus contains an intron. BLASTX analysis of this region revealed sequence similarity to intron-encoded proteins, providing

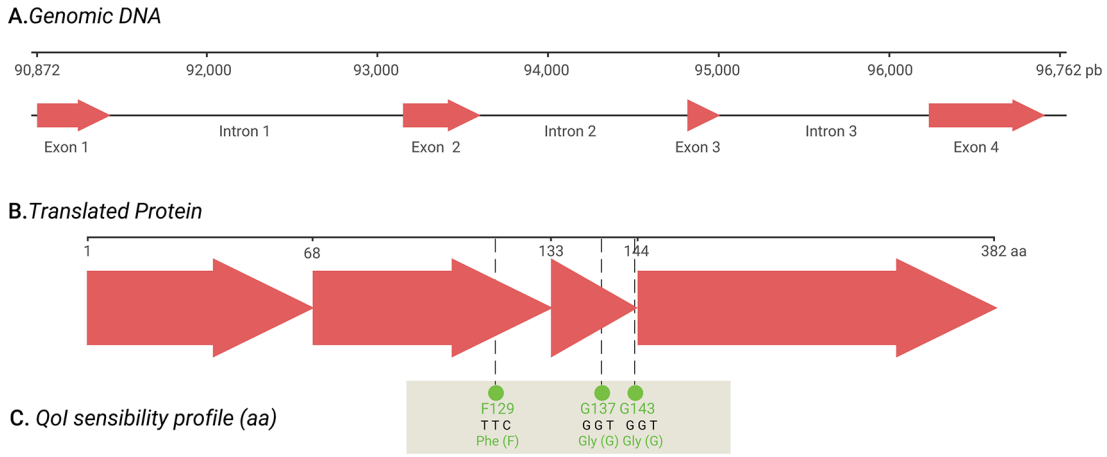

Figure 2. The *cob* gene in *Hemileia vastatrix* Race I. The schematic illustrates the transition from the genomic DNA scale (bp) to the functional protein scale (aa). A. The genomic track shows four exons separated by extensive introns, spanning coordinates 90,872 to 96,762 bp. B. In silico splicing yields a 382-residue protein. C. The quinone outside inhibitor (QoI) susceptibility profile identifies three critical wild-type markers: F129 (TTC), G137 (GGT), and G143 (GGT). No QoI resistance-associated substitutions were detected, establishing this assembly as a susceptible reference for coffee leaf rust.

evidence of intron acquisition through group II intron retrohoming, likely mediated by a reverse transcriptase/maturase. Furthermore, the intronic ORF appears to have accumulated nonsense mutations resulting in several internal stop codons, indicating that this intronic element may be undergoing pseudogenization or functional loss in this isolate.

The putative replication initiation zone was identified within an approximately 1,140-bp region, beginning at position 62,683. The 5′ boundary is defined by a poly-G homopolymer, which serves as a potential initiation site for RNA priming. Downstream of this signal, we identified two copies of the conserved regulatory motif AGACCCGACC. The regulatory zone ends with the second copy of the conserved motif at position 63,779, serving as a replication checkpoint. The 3′ boundary is defined by a distinct AT-rich melting peak (maximum AT at position 63,817), after which the sequence composition reverts to standard GC content.

Analysis of the *cob* gene architecture in the HvRI mitogenome revealed a conserved wild-type allelic configuration. Specifically, mutations that lead to non-synonymous substitutions associated with QoI fungicide resistance are absent in this isolate. The genomic coordinates for these markers were localized within Exon 2 (codon 129) and Exon 3 (codons 137 and 143), as illustrated in the structural mapping of the gene (Figure 2). It will be important to characterize the *cob* sequence of isolates collected in coffee fields with reported fungicide control failures.

Ethical approval

Ethical approval and consent were not required.

Large Language Models (Gemini Advanced/ChatGPT) were used exclusively for grammatical editing and language refinement of the author's original text. The authors reviewed and take full responsibility for the content.

Data availability

The mitochondrial genome sequence was deposited in GenBank under the accession number PX759424.1. The associated Bio-Sample and SRA IDs are SAMN32232808, SRR22911637, and SRR22911637 respectively.

NCBI GenBank: Hemileia vastatrix Race I mitochondrion, complete genome. Accession number PX759424. https://www.ncbi.nlm.nih.gov/nuccore/PX759424.1 (Marin-Ramirez; Maldonado; Padilla and Brommonschenkel, 2026).

NCBI BioSample: MIGS Eukaryotic sample from *Hemileia vastatrix.* Accession number SAMN32232808.

NCBI Sequence Read Archive (SRA): PacBio HiFi WGS of *Hemileia vastatrix* Race I: spores. Accession number SRR22911636. https://www.ncbi.nlm.nih.gov/sra/SRX18869071

NCBI Sequence Read Archive (SRA): BGIseq of *Hemileia vastatrix* Race I: spores. Accession number SRR22911637. https://www.ncbi.nlm.nih.gov/sra/SRX18869070

Acknowledgements

The authors wish to thank the Plant Pathology team at CENICAFE for the *in vivo* maintenance of the Race I isolate and spore production, as well as the team at the Laboratory of Genetics and Genomics of Plant-Pathogen Interaction (LGGIPP - Bioagro UFV) for their support.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 16 July 2026

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✔ **Theo Llewellyn**
[1] Comparative Fungal Biology, Jodrell Laboratory Royal Botanic Gardens Kew Richmond, England, UK
[2] Imperial College London Department of Life Sciences, London, England, UK

No further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Fungal genomics, secondary metabolism, evolutionary ecology, fungal phylogenetics, lichenology, symbiosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 07 July 2026

https://doi.org/10.5256/f1000research.195807.r494852

✔ **Martin Coetzee**
University of Pretoria, Pretoria, South Africa

The manuscript is well written, and the mitochondrial genome reported here will be a valuable

resource for future research on *Hemileia vastatrix* Berk. & Broome. In agreement with the other reviewer, I do not find any major flaws in the methods or results. I also have no major concerns about the manuscript and no substantial additions to the comments already provided by the previous reviewer. However, the authors should still address the remaining comments raised in the earlier review.

Are the rationale for sequencing the genome and the species significance clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbial genomics and evolution

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 08 Jul 2026

Gustavo Marin

Dear Prof. **Martin Coetzee**,

We sincerely thank the reviewer for the positive evaluation of our manuscript and for recognizing the value of the mitochondrial genome presented here for future research on *Hemileia vastatrix*. We are also grateful for the reviewer's agreement that there are no major concerns regarding the methods or results.

As suggested, we have carefully addressed all remaining comments raised by **Reviewer** (Theo Llewellyn) in the revised manuscript, including clarification of the wording regarding fungal mitochondrial evolutionary rates and the addition of the quality-filtering and polishing details in the Methods section. These revisions have improved the precision, reproducibility, and overall clarity of the manuscript

Sincerely,

On behalf of all authors

Competing Interests: No competing interests were disclosed.

Reviewer Report 10 June 2026

https://doi.org/10.5256/f1000research.195807.r489837

Theo Llewellyn
[1] Comparative Fungal Biology, Jodrell Laboratory Royal Botanic Gardens Kew Richmond, England, UK
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The authors provide a concise and well-written article detailing a new high-quality mitochondrial genome sequence for the causal agent of coffee leaf rust *Hemileia vastatrix*. There are no major flaws I could identify and only a couple of minor points to address, which I have listed below.

Paragraph 2: 'the opposite is often observed in fungi' this sentence requires a citation. It also feels overly generalised as mitochondrial evolutionary rates vary across the kingdom. Whilst this may be true for some asexual *Basidiomycota*, I think 'often observed in fungi' does not accurately represent this.

Methods: which quality-filtering thresholds and tools were applied to reads that were then mapped back to contigs with pilon? I could not find this in the text

Figure 1: needs a colour legend for the functional categories

Are the rationale for sequencing the genome and the species significance clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?
Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Fungal genomics, secondary metabolism, evolutionary ecology, fungal phylogenetics, lichenology, symbiosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 08 Jul 2026

Gustavo Marin

Dear Prof. **Theo Llewellyn**,

We would like to sincerely thank you for the careful evaluation of our manuscript and for the constructive comments and suggestions. We greatly appreciate the time and effort invested in reviewing our work. Your feedback has helped us improve the clarity, precision, and methodological transparency of the manuscript. Below, we provide a point-by-point response to the comments.

Comment 1
"the opposite is often observed in fungi" this sentence requires a citation. It also feels overly generalised as mitochondrial evolutionary rates vary across the kingdom. Whilst this may be true for some asexual Basidiomycota, I think 'often observed in fungi' does not accurately represent this.

- **Response**: We agree that our original wording was too broad and could be interpreted as overgeneralizing mitochondrial evolutionary patterns across fungi. In response, we revised the sentence to be more precise and cautious, and we added appropriate citations supporting the observation that, in some fungal lineages, nuclear genomes may exhibit higher polymorphism than mitochondrial genomes, while acknowledging that these patterns are not universal across the kingdom. *We replaced the original statement with a more nuanced phrasing that avoids generalization and reflects the heterogeneity of mitochondrial evolutionary rates in fungi.*

Comment 2
Methods: which quality-filtering thresholds and tools were applied to reads that were then mapped back to contigs with pilon? I could not find this in the text

- **Response:** We have revised the Methods section to explicitly describe the preprocessing and polishing workflow. Specifically, the raw BGIseq reads were adapter-trimmed and converted to Phred+33 format using Trimmomatic v0.40. After the initial assembly with NOVOPlasty v4.3.5, the reads were further quality-trimmed prior to polishing using Trimmomatic with the following parameters: leading and trailing base quality < 20, a 4-bp sliding window requiring an average quality score of at least 30, and a minimum read length of 100 bp. The filtered reads were then aligned to the draft assembly with BWA-MEM v0.7.17 and the assembly was polished with Pilon v1.24. *To improve clarity, we also suggest incorporating the following text into the manuscript.*

We again thank the reviewer for the constructive feedback, which has significantly improved

the manuscript. We hope that the revisions adequately address the concerns raised and enhance the quality and reproducibility of our study.

Sincerely,

On behalf of all authors.

Competing Interests: No competing interests were disclosed.

Author Response 09 Jul 2026

Gustavo Marin

Dear **Reviewers**,

Paragraph 2: 'the opposite is often observed in fungi' this sentence requires a citation. It also feels overly generalised as mitochondrial evolutionary rates vary across the kingdom. Whilst this may be true for some asexual Basidiomycota, I think 'often observed in fungi' does not accurately represent this.

Mitochondria are double-membrane organelles, present in high copy numbers per cell. They are essential for fungal metabolism and the site for oxidative phosphorylation.3 In sexual eukaryotes, mitochondrial inheritance is predominantly uniparental; however, in fungi, both uniparental and biparental inheritance modes have been reported.4 Another distinction across the tree of life is the mutation rate: while mitochondrial genomes evolve faster than nuclear genomes in animals, the opposite is often observed in fungi. Predominantly asexual species of the division Basidiomycota present SNP frequencies up to 7.69% in nuclear genomes but only up to 4.41% in their mitochondrial counterpar ts.5 This slower mutation rate makes mitochondrial DNA markers useful for resolving deep evolutionary relationships between species.6–8 Despite the slow mutation rate in Basidiomycota, high variability in genome size, gene order, and intron content has been found while maintaining a conserved core set of protein-coding genes, this genomic plasticity is valuable not only for phylogenetics of distant species but also for population genomics within a species.9

Response

We agree with the reviewer that our original statement was overly generalized, given the vast evolutionary diversity within the fungal kingdom. We have revised the text to clarify that slower mitochondrial evolution is not universal but has been documented in specific lineages, seamlessly leading into our cited example of Basidiomycota (Reference 5).

Reviewer comment:

Methods: which quality-filtering thresholds and tools were applied to reads that were then mapped back to contigs with pilon? I could not find this in the text

Original paragraph:

The mitochondrial genome was assembled using a hybrid approach integrating multiple tools. Initially, a de novo assembly was performed using NOVOPlasty v4.3.516 with BGIseq reads (SRA SRR22911637), using the cob gene sequence of Puccinia striiformis f. sp. tritici isolate CYR32 (GenBank MH891489.1) as a seed. The resulting contig was annotated with MFannot v2.017 using Genetic Code 4 (Mold, Protozoan, and Coelenterate Mitochondrial). The NADH dehydrogenase subunit 4 (nad4) gene from this preliminary assembly was then used as a seed for a second round of NOVOPlasty. Finally, the assembly was polished with Pilon v1.2418 by mapping quality-trimmed reads back to the draft assembly using BWA-MEM v0.7.17.19

Edited paragraph:

The mitochondrial genome was assembled using a hybrid approach. Initially, raw BGIseq reads (SRA SRR22911637) were adapter-trimmed using Trimmomatic v0.40 (Beier et al., 2026). A de novo assembly was then performed using NOVOPlasty v4.3.516, which utilizes internal read quality filtering, using the cob gene sequence of Puccinia striiformis f. sp. tritici isolate CYR32 (GenBank MH891489.1) as a seed. The resulting contig was annotated with MFannot v2.017 using Genetic Code 4 (Mold, Protozoan, and Coelenterate Mitochondrial). The NADH dehydrogenase subunit 4 (nad4) gene from this preliminary assembly was subsequently used as a seed for a second round of NOVOPlasty. Finally, prior to error correction, the raw reads underwent quality trimming with Trimmomatic (leading/trailing Q < 20; 4-bp sliding window average Q $\geq$ 30; minimum length 100 bp). These high-quality reads were mapped to the draft assembly using BWA-MEM v0.7.17, and the assembly was polished with Pilon v1.24.

Beier, S., Bolger, A.M., Bolger, M.E., Schwacke, R. and Usadel, B., 2026. Trimmomatic: a decade of feature-rich, high-performance NGS read preprocessing. Bioinformatics, 42(6), p.btag331.

Reviewer Comment:

"Methods: which quality-filtering thresholds and tools were applied to reads that were then mapped back to contigs with pilon? I could not find this in the text"

Response:

We thank the reviewer for pointing this out. This comment made us realize that the initial data preprocessing was not clearly explained. We have updated the Methods section to clarify how the paired-end data was handled in two stages. First, we removed adapters using Trimmomatic v0.40, allowing NOVOplasty to use its own internal read filtering for the assembly. Second, we clarified that a strict quality filtering step (leading/trailing Q < 20; 4-bp sliding window average Q $\geq$ 30; minimum length 100 bp) was applied using Trimmomatic prior to mapping the reads for Pilon polishing.

Competing Interests: No competing interests were disclosed.