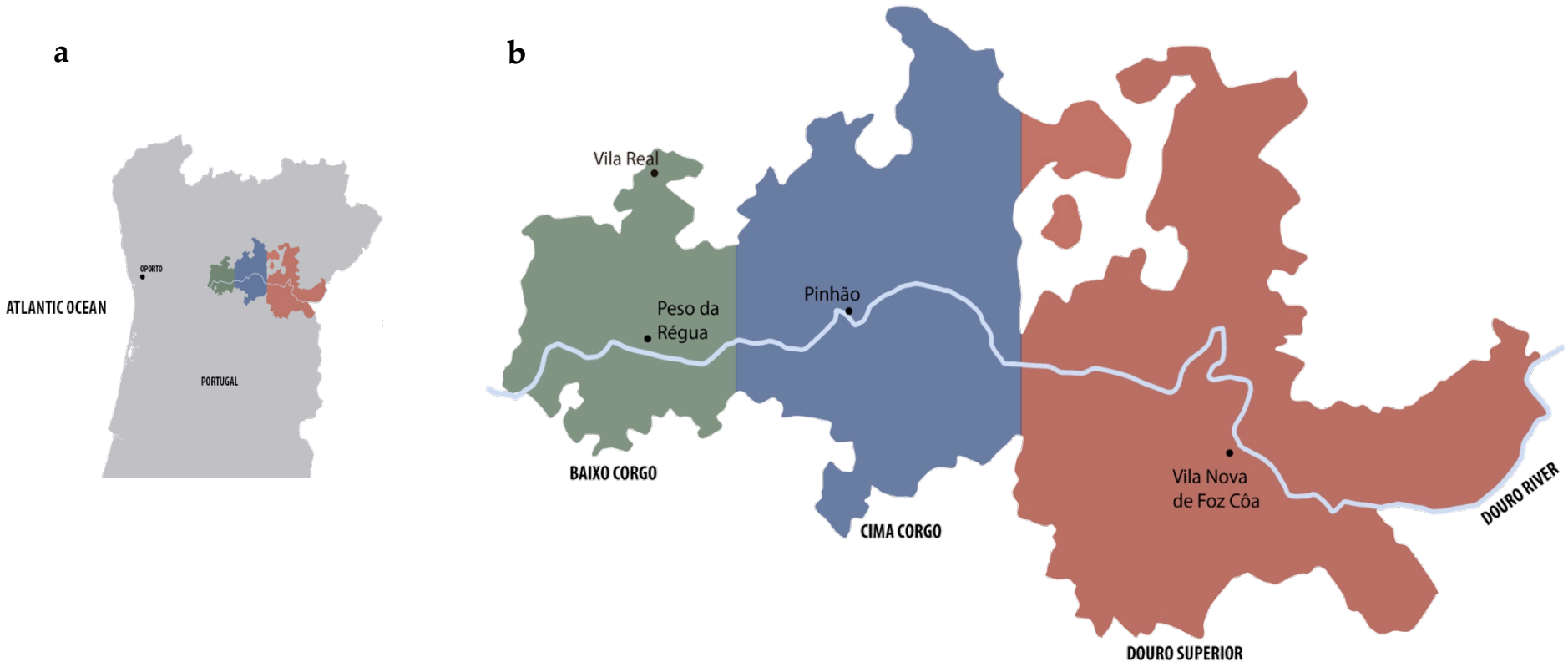


Figure S1. Schematic representation of the Douro demarcated region from where the indigenous *Hanseniaspora* strains had been recovered.

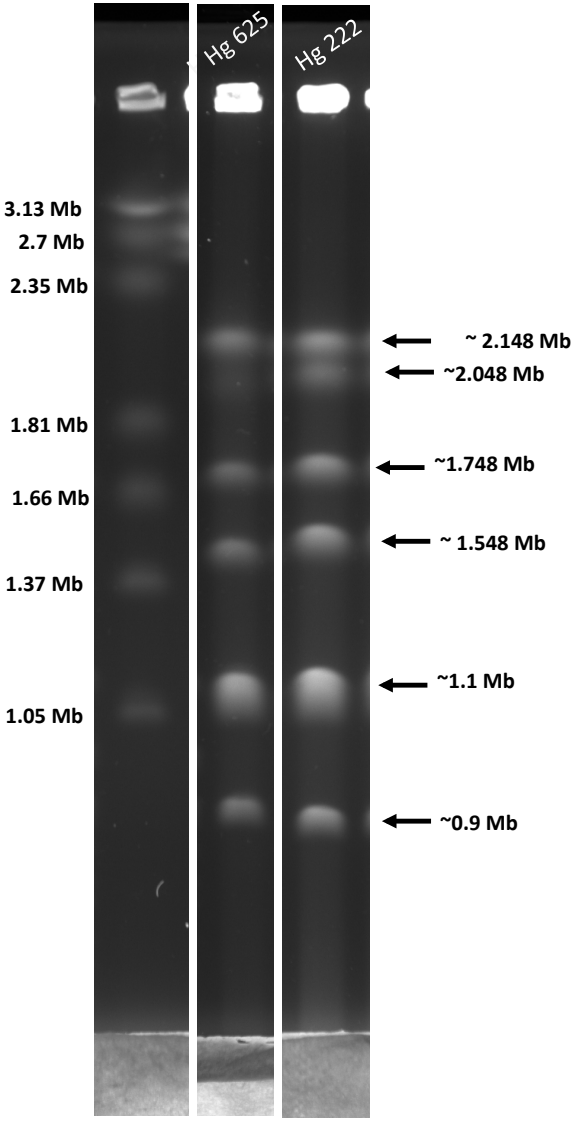


Figure S2. Karyotyping of *Hanseniaspora guilliermondii* UTAD222 and CBS625, based on PFGE, rendering clear five distinct chromosomal bands, one of them (at a molecular weight of about 1.1 Mb) being considered duplicated based on the higher intensity of the signal and also on a similar trait reported in other *H. guilliermondii* strains (Cadez et al., 2002). As a ladder the chromosomes of *Hansenula wingei* were used

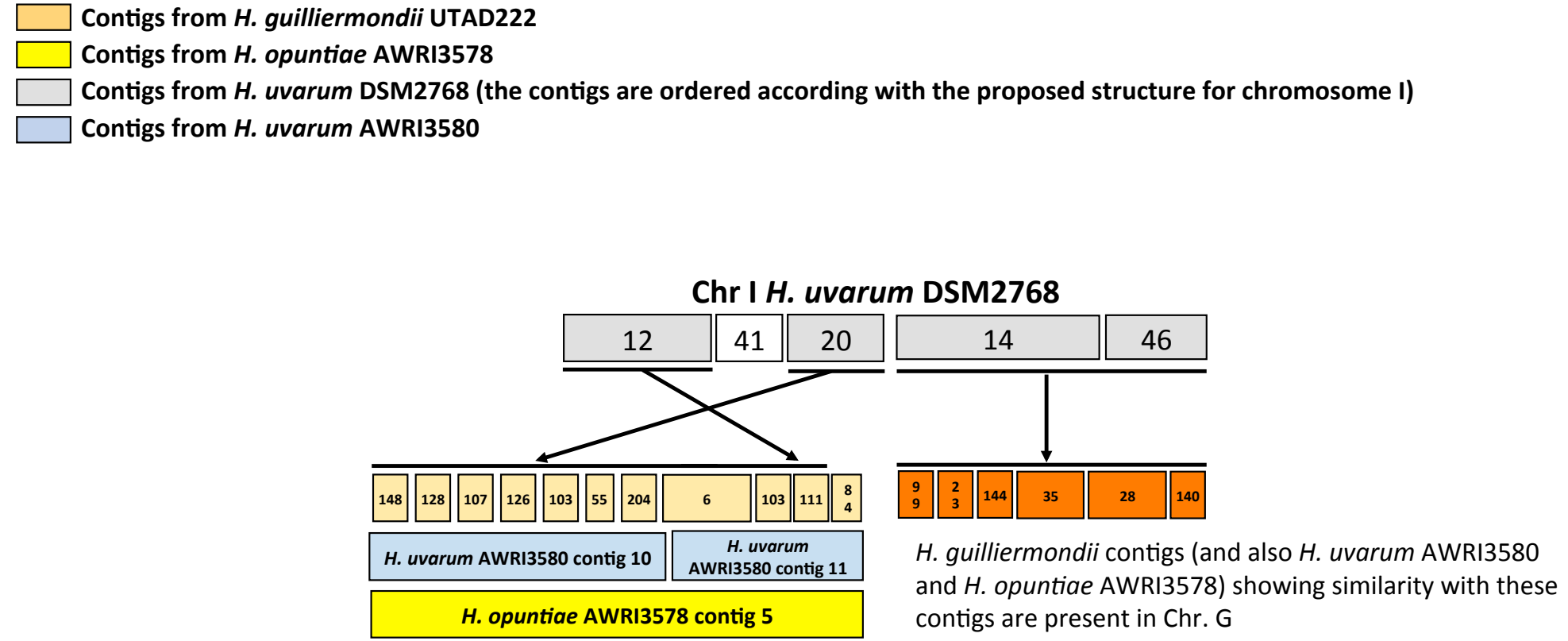


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome A. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains.

Chr A *H. guilliermondii* UTAD222

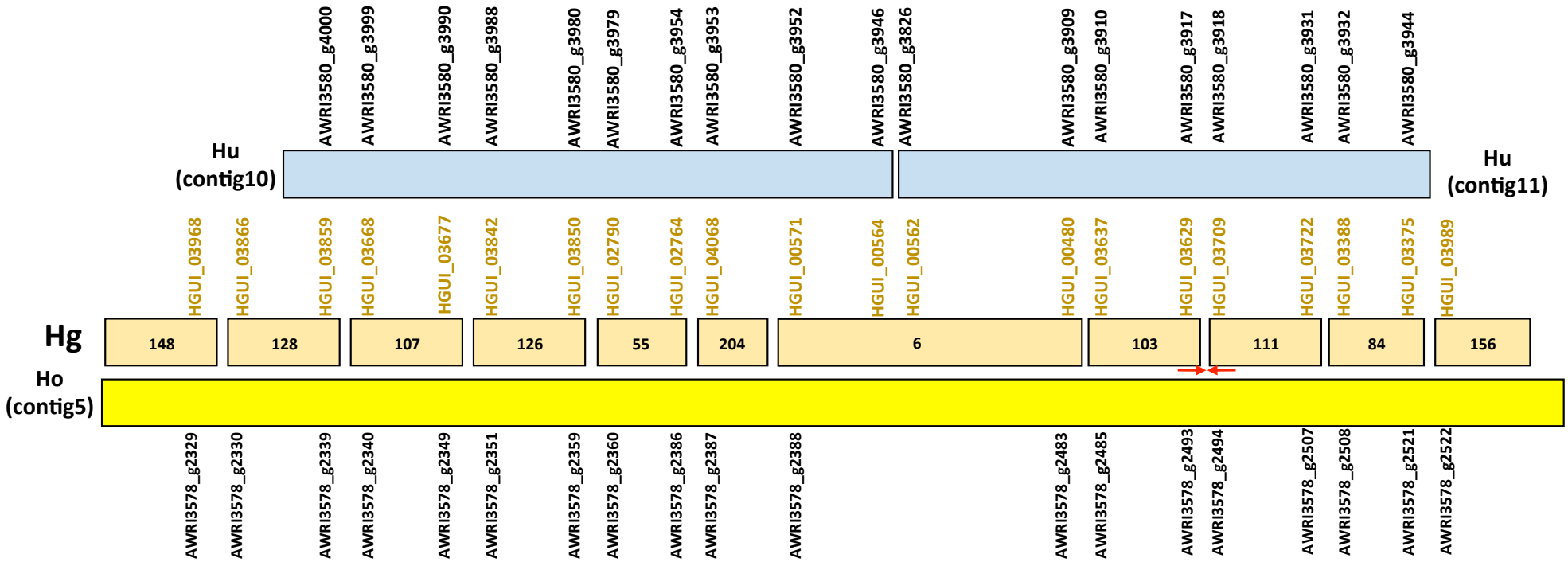


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome A of Hg. The results obtained shows a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs in this genomic showing a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae*. The junction between contig 103 and 111 in *H. guilliermondii* UTAD222 was experimentally confirmed by PCR (results not shown).

Chr B *H. guilliermondii* UTAD222

- Contigs from *H. guilliermondii* UTAD222
- Contigs from *H. opuntiae* AWRI3578
- Contigs from *H. uvarum* DSM2768 (the contigs are ordered according with the proposed structure for chromosome II)
- Contigs from *H. uvarum* AWRI3580
- Contigs from *H. uvarum* DSM2768 (these are contigs allocated to chromosomes other than chromosome II)

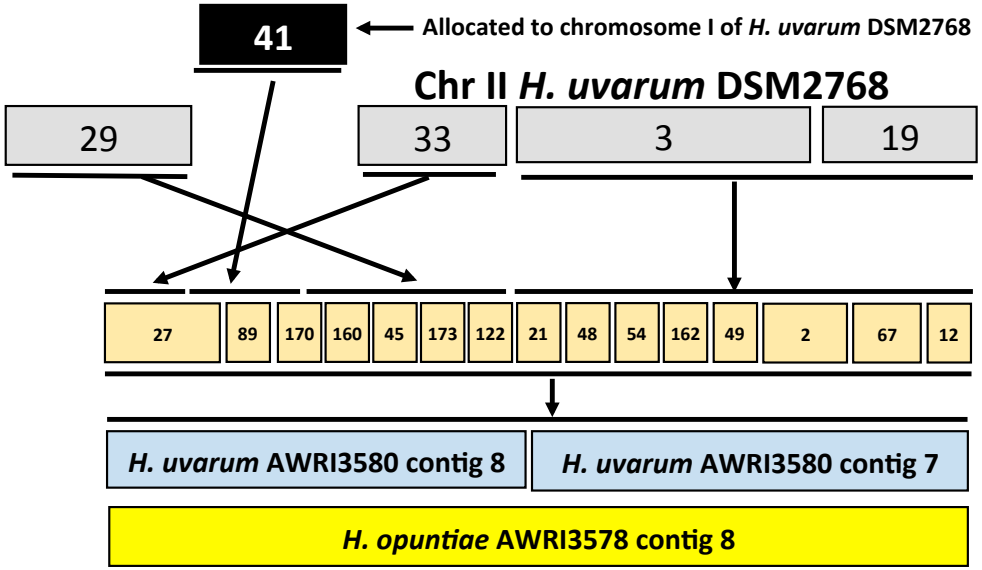


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome B. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains.

Chr B *H. guilliermondii* UTAD222

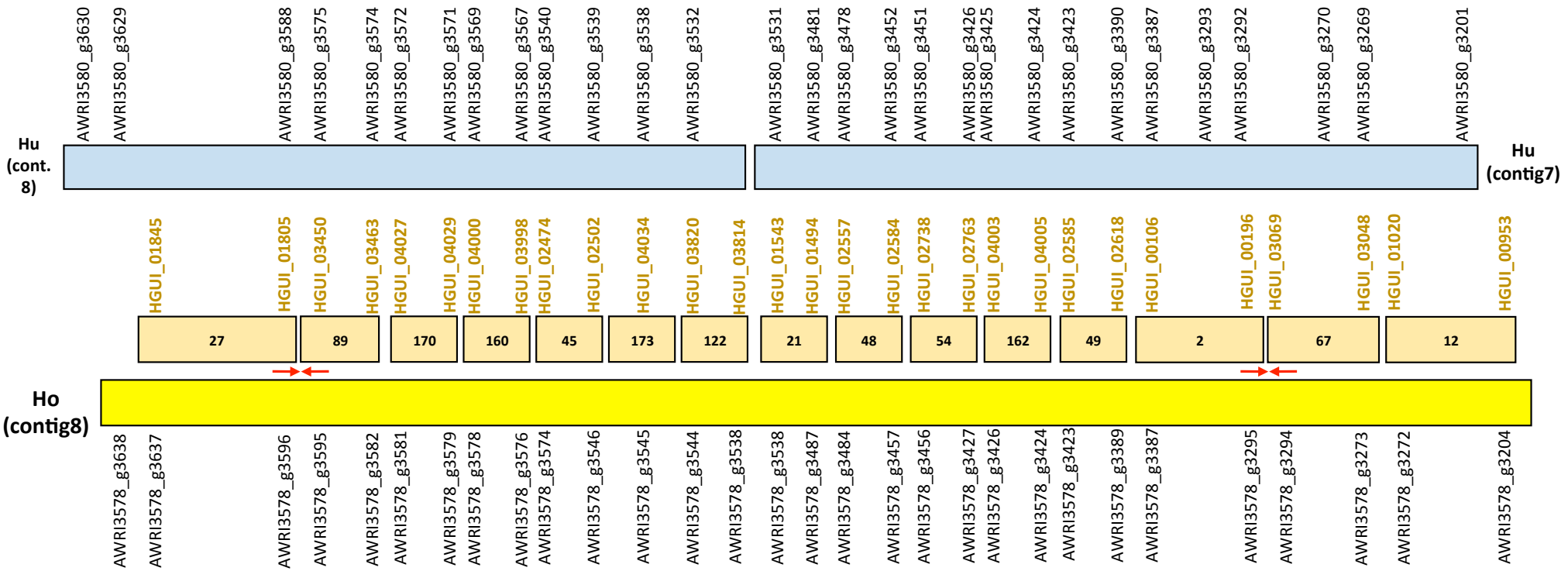


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome B of Hg. The results obtained shows a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs suggesting a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae* strains. The junction between contigs 89/27 and 2/67 in *H. guilliermondii* UTAD222 were experimentally confirmed by PCR (results not shown).

Chr C *H. guilliermondii* UTAD222

Contigs from *H. guilliermondii* UTAD222
Contigs from *H. opuntiae* AWRI3578
Contigs from *H. uvarum* DSM2768 (the contigs are ordered according with the proposed structure for chromosome III)
Contigs from *H. uvarum* AWRI3580

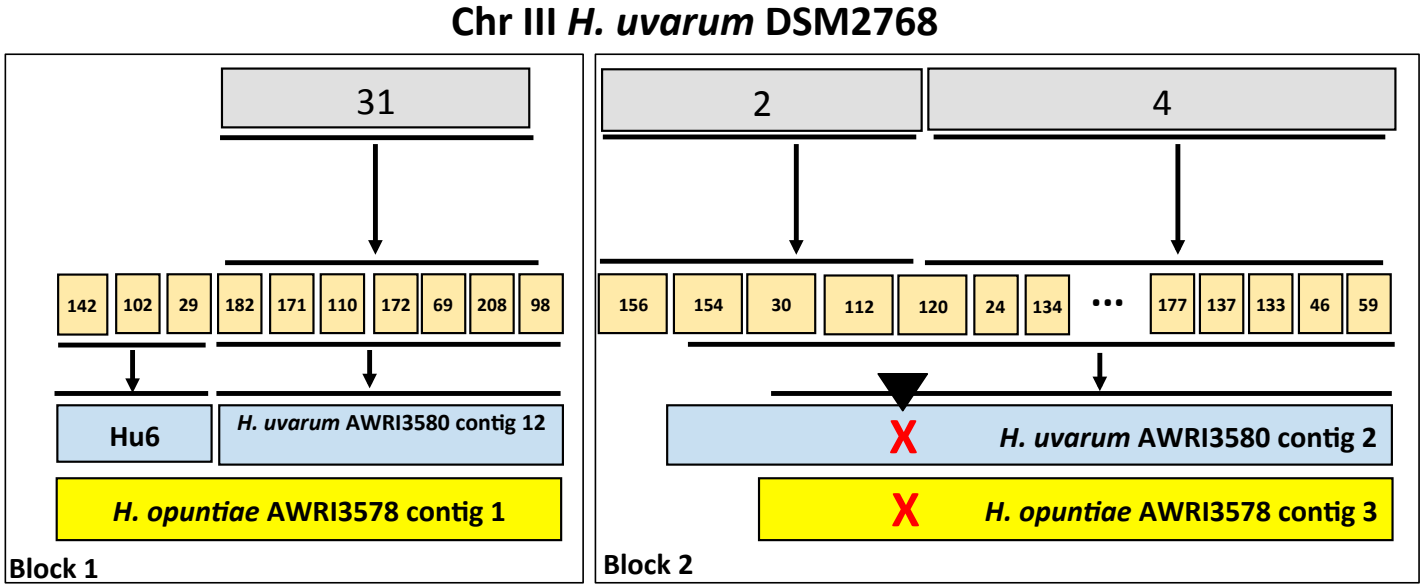
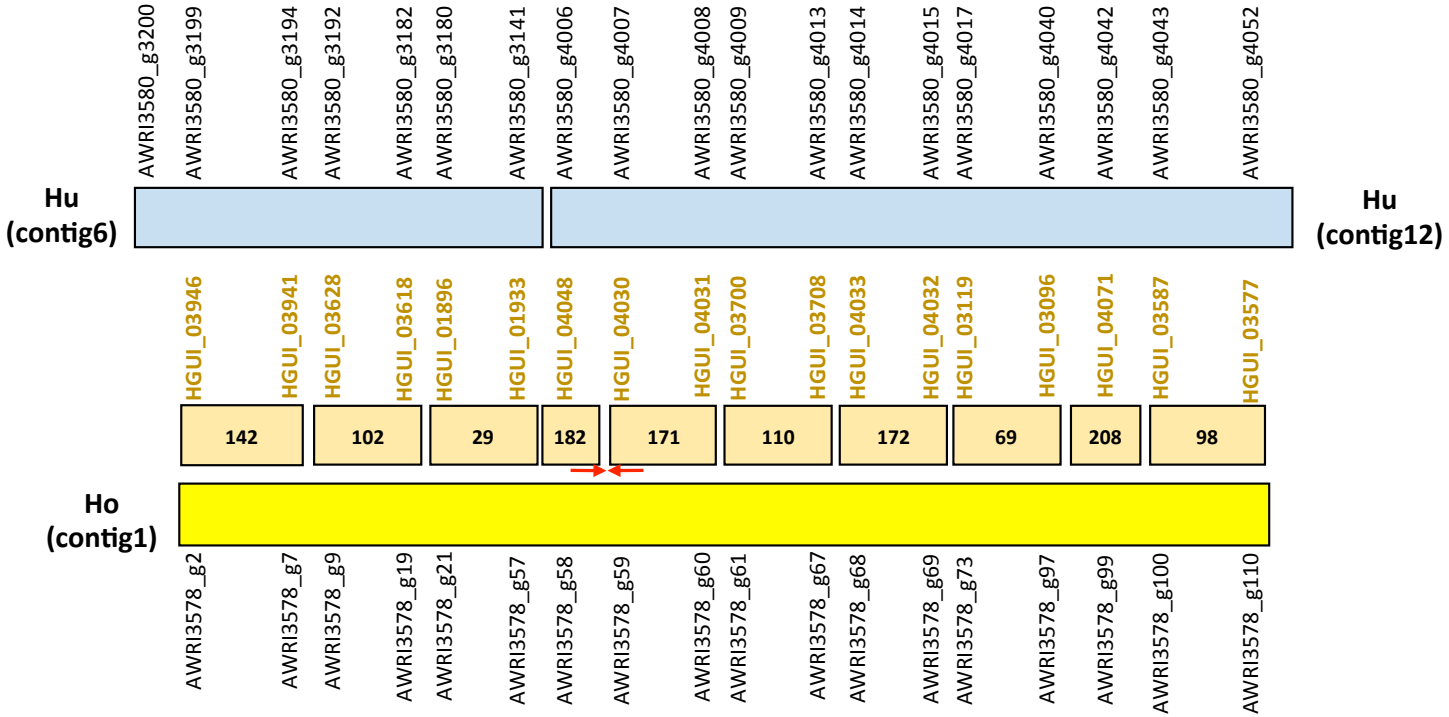


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome C. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains. In this case it is evident a significant genomic alteration in the second block (indicated by the black triangle) since in Hg it is not detected a genomic portion present in Hu/Ho, this absent region being found in in chromosome F, as confirmed by PCR.

Chr C *H. guilliermondii* UTAD222 (Block1)



Chr C *H. guilliermondii* UTAD222 (Block 2)

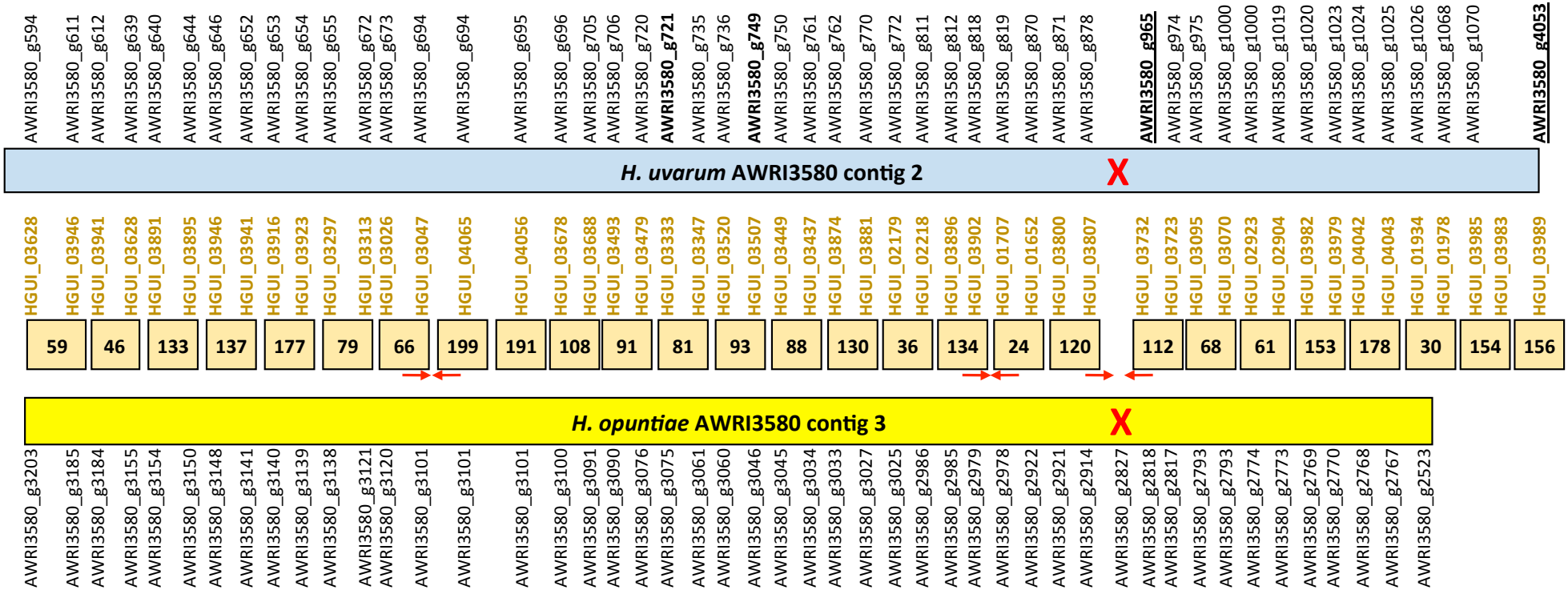


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome C of Hg. The results obtained shows a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs suggesting a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae* strains. A break in co-linearity in the region that was found to be absent in Hg (indicated in the figure with a red X).The junction between contigs 66/99; 134/24 and 120/112 in *H. guilliermondii* UTAD222 were experimentally confirmed by PCR (results not shown).

Chr D *H. guilliermondii* UTAD222

Contigs from *H. guilliermondii* UTAD222
Contigs from *H. opuntiae* AWRI3578
Contigs from *H. uvarum* DSM2768 (the contigs are ordered according with the proposed structure for chromosome IV)
Contigs from *H. uvarum* AWRI3580

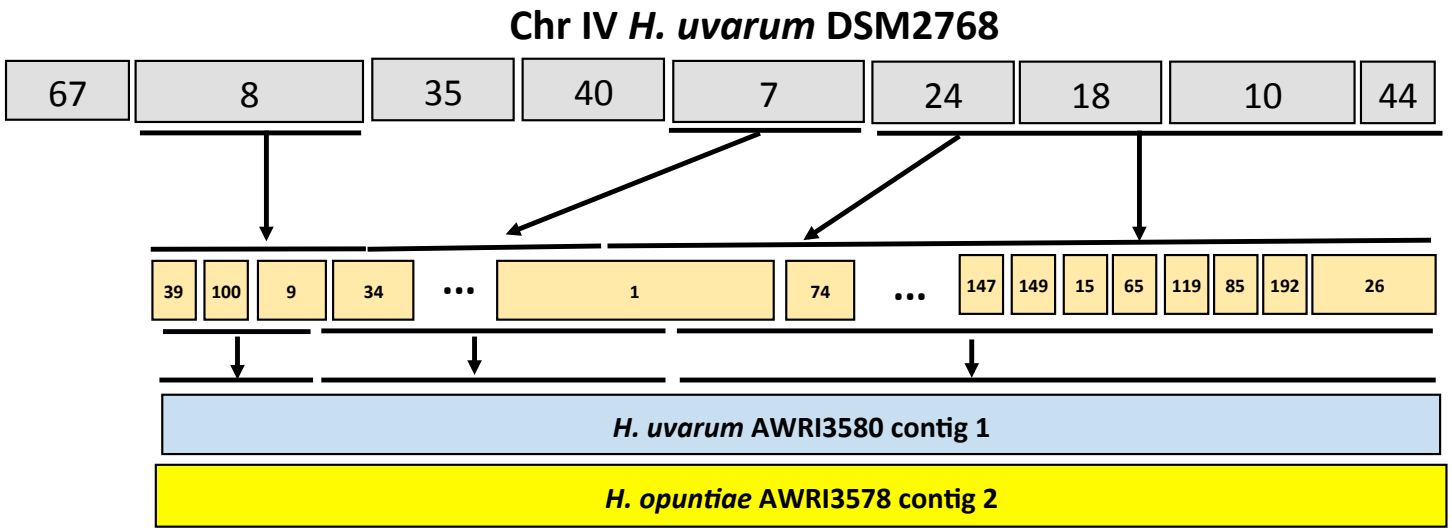


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome D. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains.

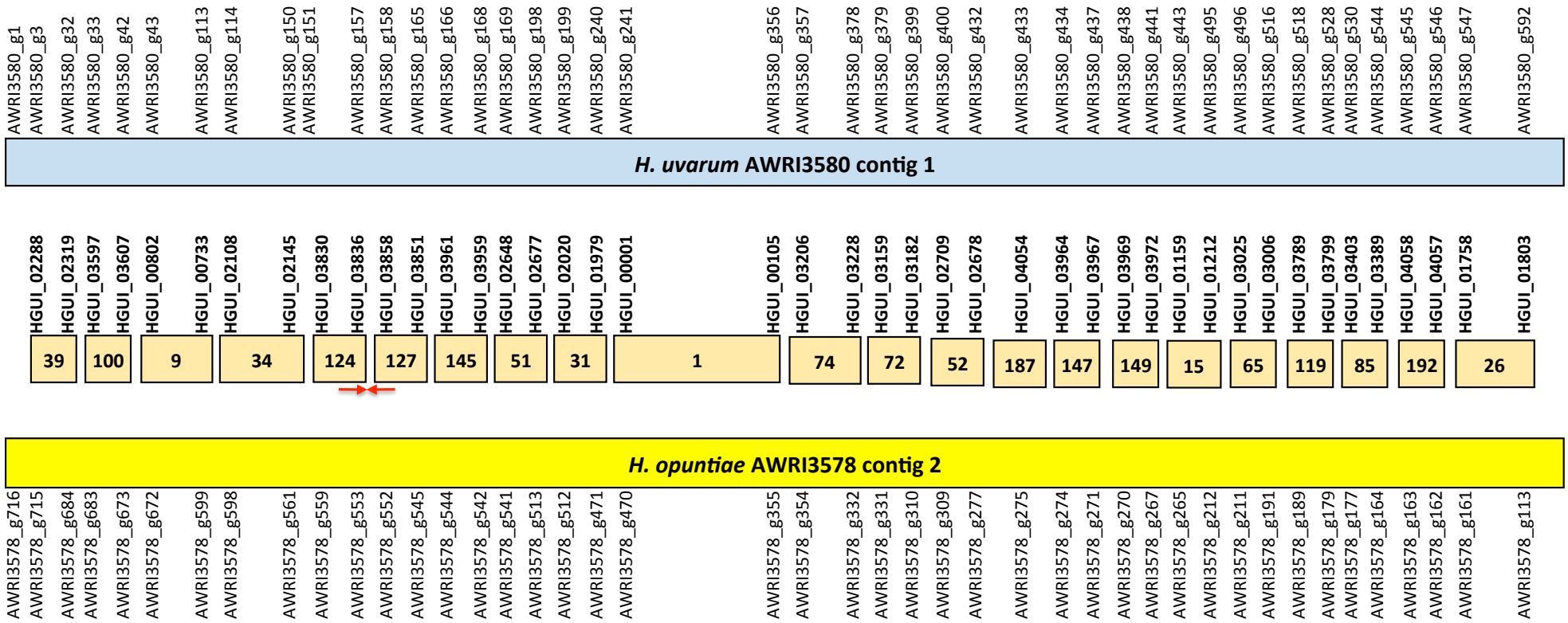


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome D of Hg. The results obtained shows a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs suggesting a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae* strains. The junction between contigs 124/127 in *H. guilliermondii* UTAD222 was experimentally confirmed by PCR (results not shown).

Chr E *H. guilliermondii* UTAD222

- Contigs from *H. guilliermondii* UTAD222
- Contigs from *H. opuntiae* AWRI3578
- Contigs from *H. uvarum* DSM2768 (the contigs are ordered according with the proposed structure for chromosome V)
- Contigs from *H. uvarum* AWRI3580

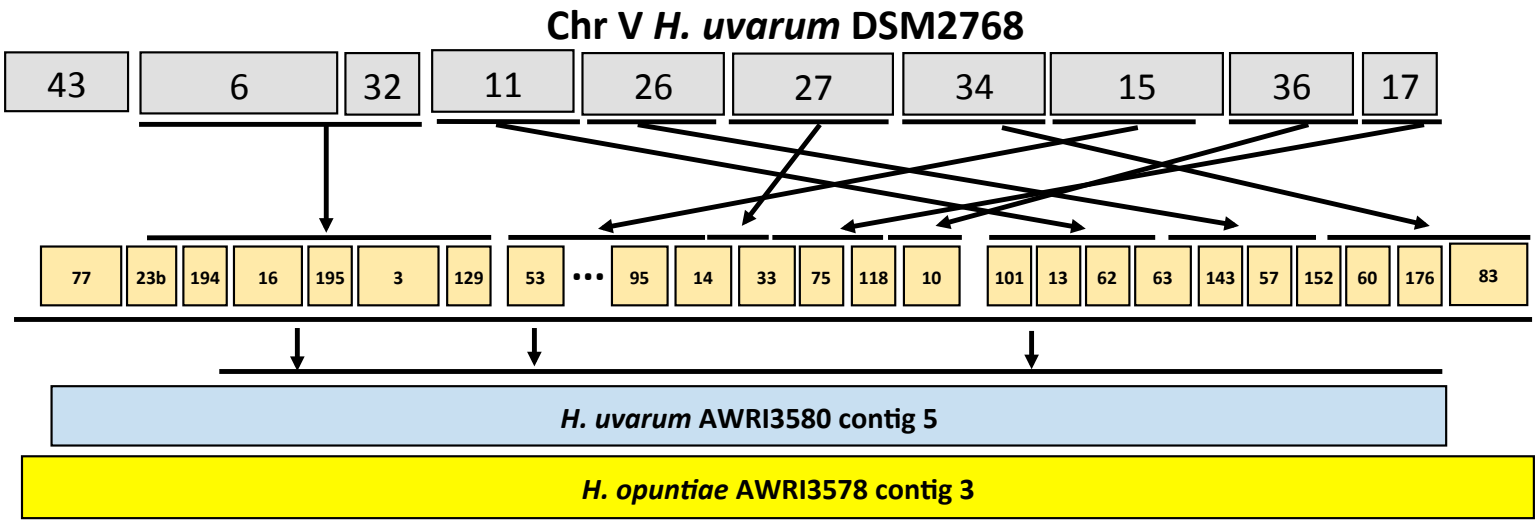


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome E. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains.

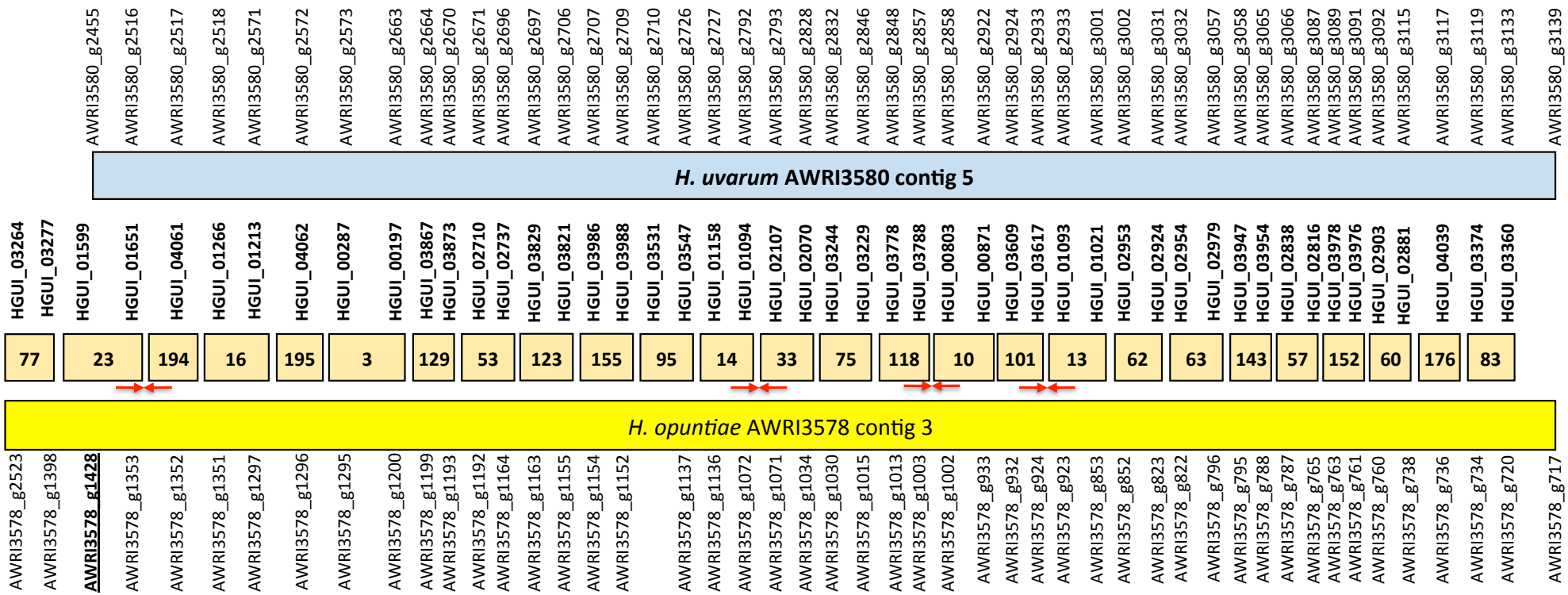


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome E of Hg. The results obtained shows a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs suggesting a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae* strains. Despite this, it was possible to identify a lack of co-linearity in part of contig 23, this being identified as a red X. The junction between contigs 23/294, 14/33, 118/10, 101/13 in *H. guilliermondii* UTAD222 was experimentally confirmed by PCR (results not shown).

Chr F *H. guilliermondii* UTAD222

Contigs from *H. guilliermondii* UTAD222
Contigs from *H. opuntiae* AWRI3578
Contigs from *H. uvarum* DSM2768 (the contigs are ordered according with the proposed structure for chromosome VI)
Contigs from *H. uvarum* DSM2768 (these are contigs allocated to chromosomes other than chromosome VI)
Contigs from *H. uvarum* AWRI3580

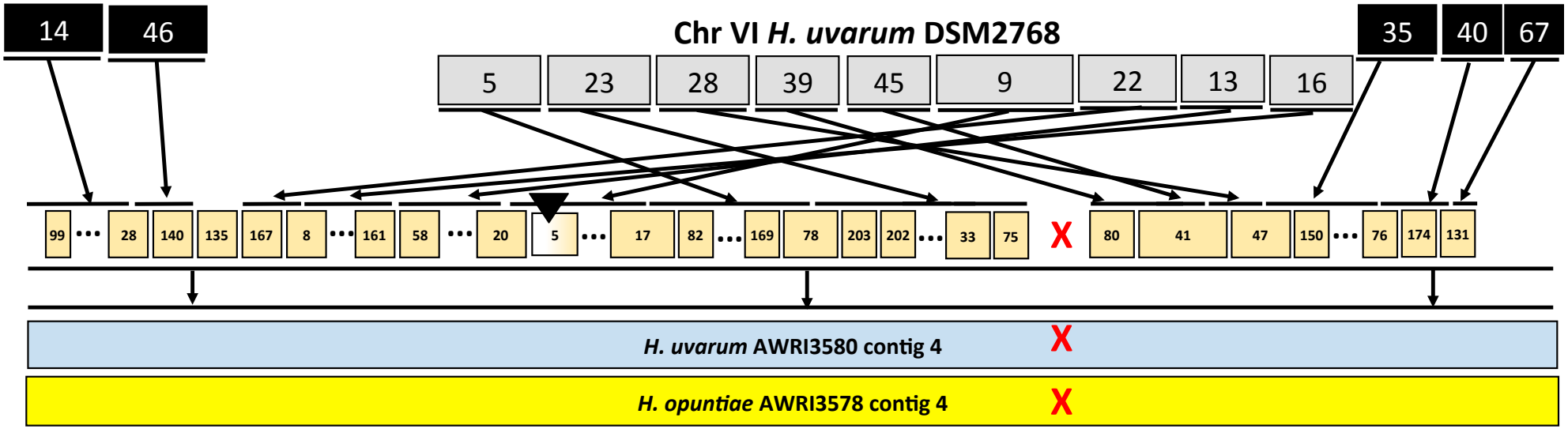
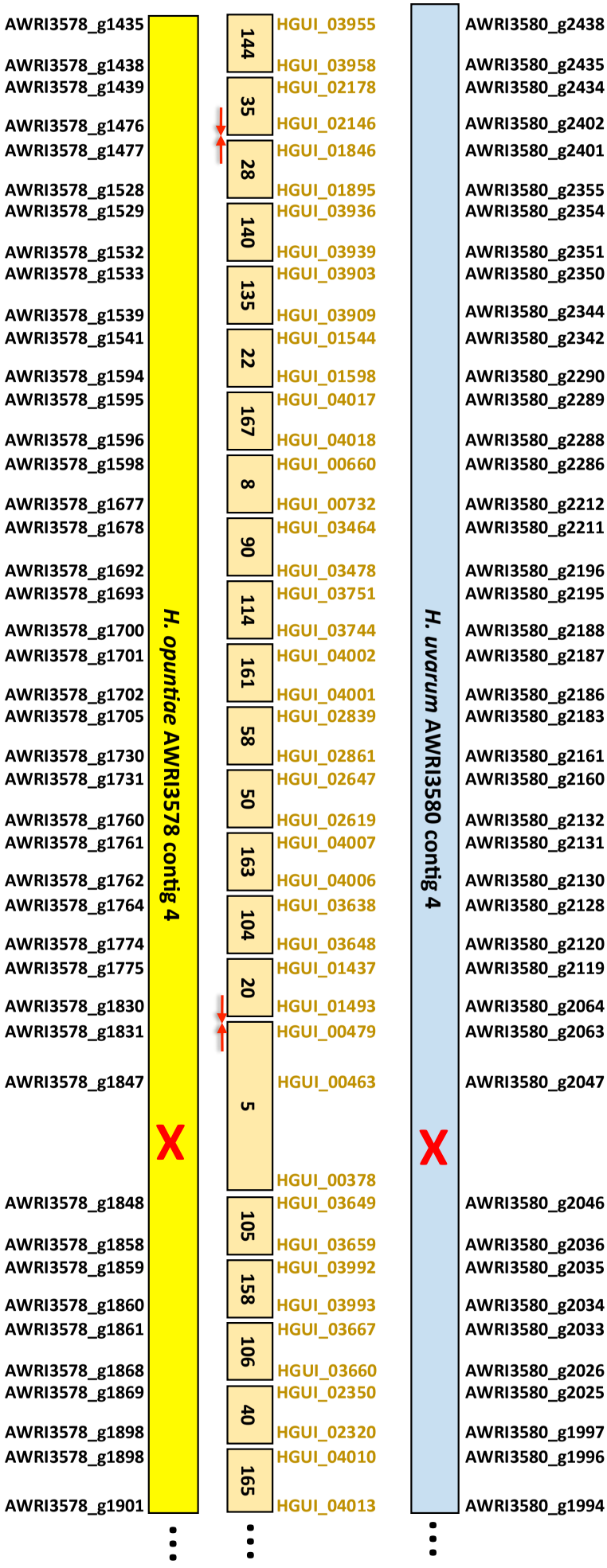


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome F. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains. In this chromosome were observed two significant genomic alteration comparing with the H. uvarum AWRI3580 and H. opuntiae AWRI3580 counter-partners since in the Hg chromosome it is detected a genomic portion present in Hg (in contig 5) that has homology with other Hu/Ho chromosomes (2 and 7, respectively). This apparent insertion is indicated with a black triangle. There is also a portion of Hu chr. 4 and Ho chr. 4 (between Hg contigs 75 and 80) that is absent in Hg. This part absent in Hg is indicated with a red X.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AWRI3578_g1860
AWRI3578_g1861
AWRI3578_g1868
AWRI3578_g1869
AWRI3578_g1898
AWRI3578_g1898
AWRI3578_g1901
H. opuntiae AWRI3578 contig 4
144
35
28
140
135
22
167
8
90
114
161
58
50
163
104
20
5
105
158
106
40
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HGUI_03660
HGUI_02350
HGUI_02320
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HGUI_04013
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AWRI3580_g1996
AWRI3580_g1994
H. uvarum AWRI3580 contig 4

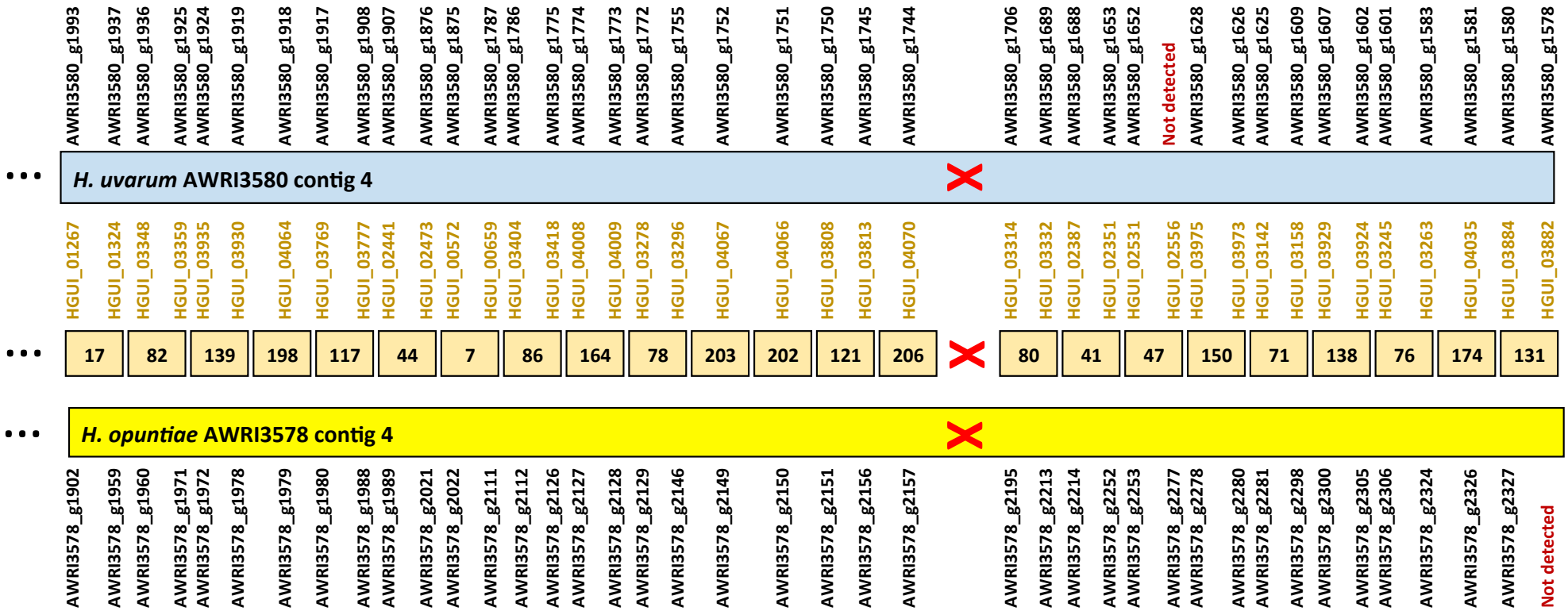


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome F of Hg. The results obtained shows a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs suggesting a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae* strains. Despite this, it was possible to identify a lack of co-linearity in part of contig 23, this being identified as a red X. The junction between contigs 23/294, 14/33, 118/10, 101/13 in *H. guilliermondii* UTAD222 was experimentally confirmed by PCR (results not shown).

Chr G *H. guilliermondii* UTAD222

Contigs from *H. guilliermondii* UTAD222
Contigs from *H. opuntiae* AWRI3578
Contigs from *H. uvarum* DSM2768 (the contigs are ordered according with the proposed structure for chromosome VII)
Contigs from *H. uvarum* DSM2768 (these are contigs allocated to chromosomes other than chromosome VII)
Contigs from *H. uvarum* AWRI3580

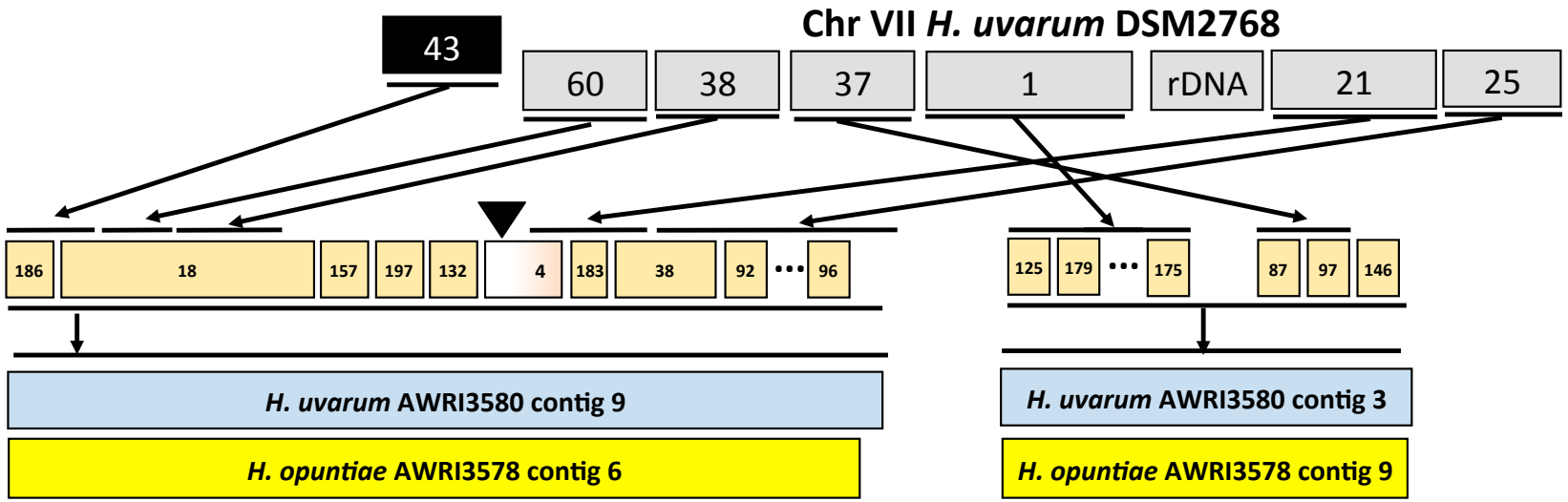
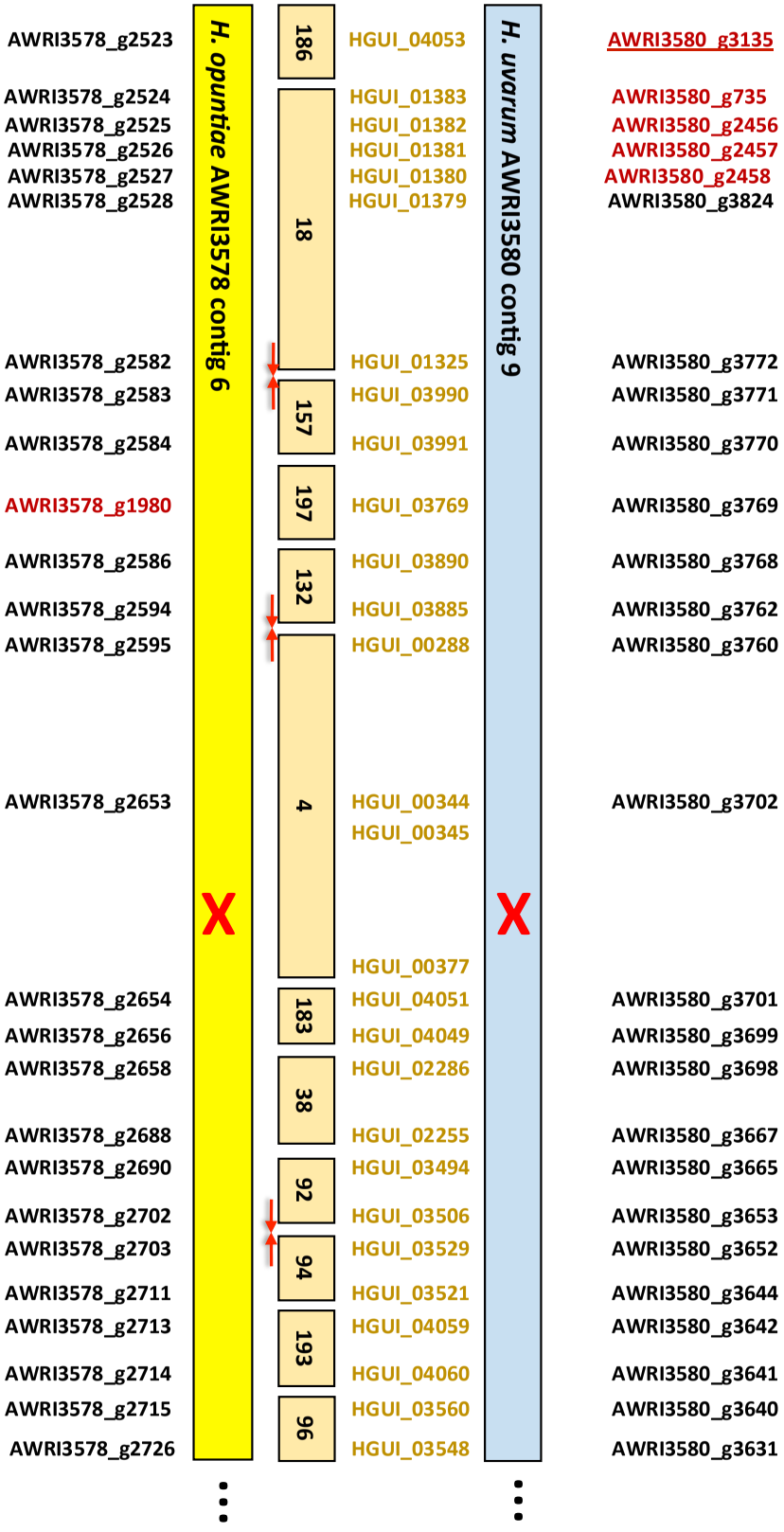


Figure S3 (A). Proposed structure of *H. guilliermondii* chromosome G. The structure of this chromosome was proposed using as a basis the one defined for *H. uvarum* DSM2768 subsequently fine-tuned using whole genome-alignment between contig sequences of *H. guilliermondii* UTAD222, *H. opuntiae* AWRI3578 and *H. uvarum* (DSM2768 and AWRI3580 strains). The back lines indicate similar regions between the different strains. In this Hg chromosome is observed an insertion (in contig 4) showing no similarity with Hu ctg 9 or Ho ctg 6 but identical to Hu ctg 4 and Ho ctg 4. This apparent insertion in the Hg chromosome is indicated with a black triangle. Junction between contig 132 and 4 in Hg UTAD222 was experimentally validated.


H. opuntiae AWRI3578 contig 6
H. uvarum AWRI3580 contig 9
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AWRI3578_g2524
AWRI3578_g2525
AWRI3578_g2526
AWRI3578_g2527
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AWRI3578_g2713
AWRI3578_g2714
AWRI3578_g2715
AWRI3578_g2726
186
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157
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4
183
38
92
94
193
96
HGUI_04053
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HGUI_03521
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HGUI_03548
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AWRI3580_g3631

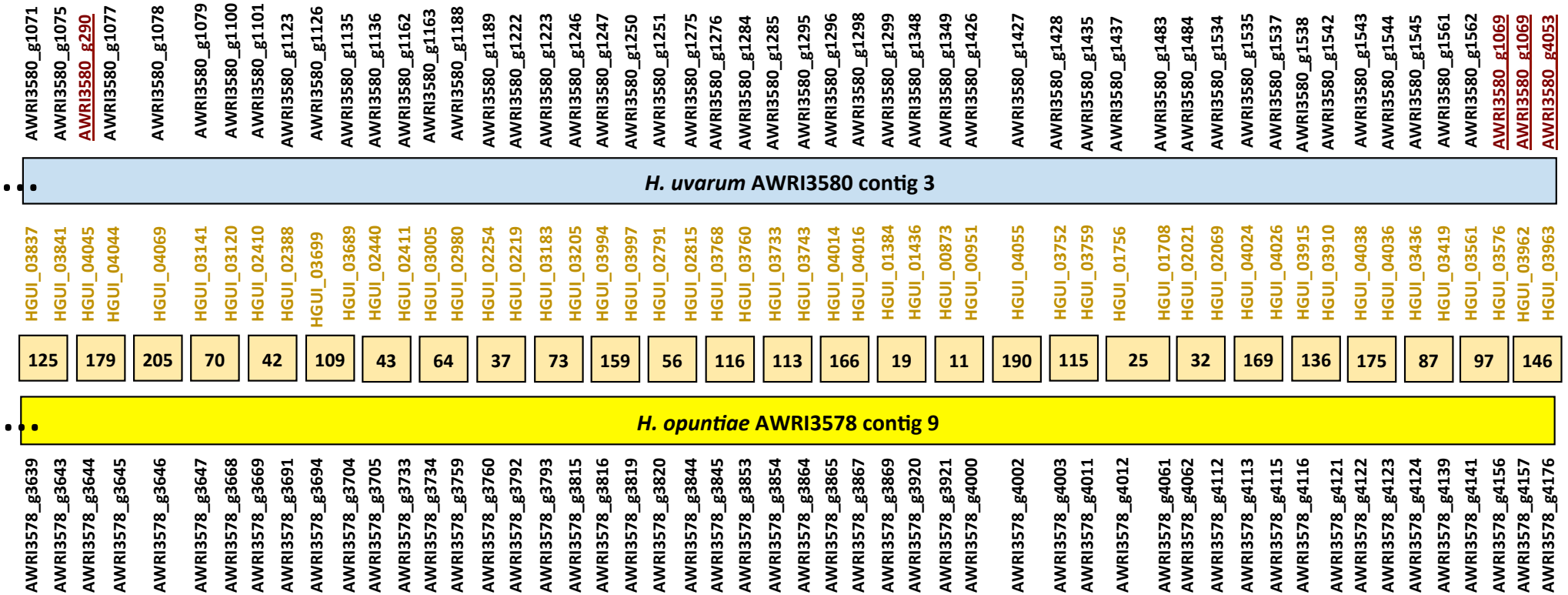


Figure S3 (B). Pairwise alignments were performed using all predicted H. guilliermondii, H. uvarum AWRI3580 and H. opuntiae AWRI3578 proteins. Afterwards, the homologous Hg/Ho and Hg/Hu protein pairs were mapped in the contig organization suggested for chromosome G of Hg. The results obtained shows (with the exception of two regions highlighted in red) a contiguous positioning of Ho/Hg and Hg/Hu homologous protein pairs suggesting a strong co-linearity in this chromosomal region of *H. guilliermondii*, *H. uvarum* and *H. opuntiae* strains. The junction between contigs 18/157, 132/4, 92/94 in *H. guilliermondii* UTAD222 was experimentally confirmed by PCR (results not shown).

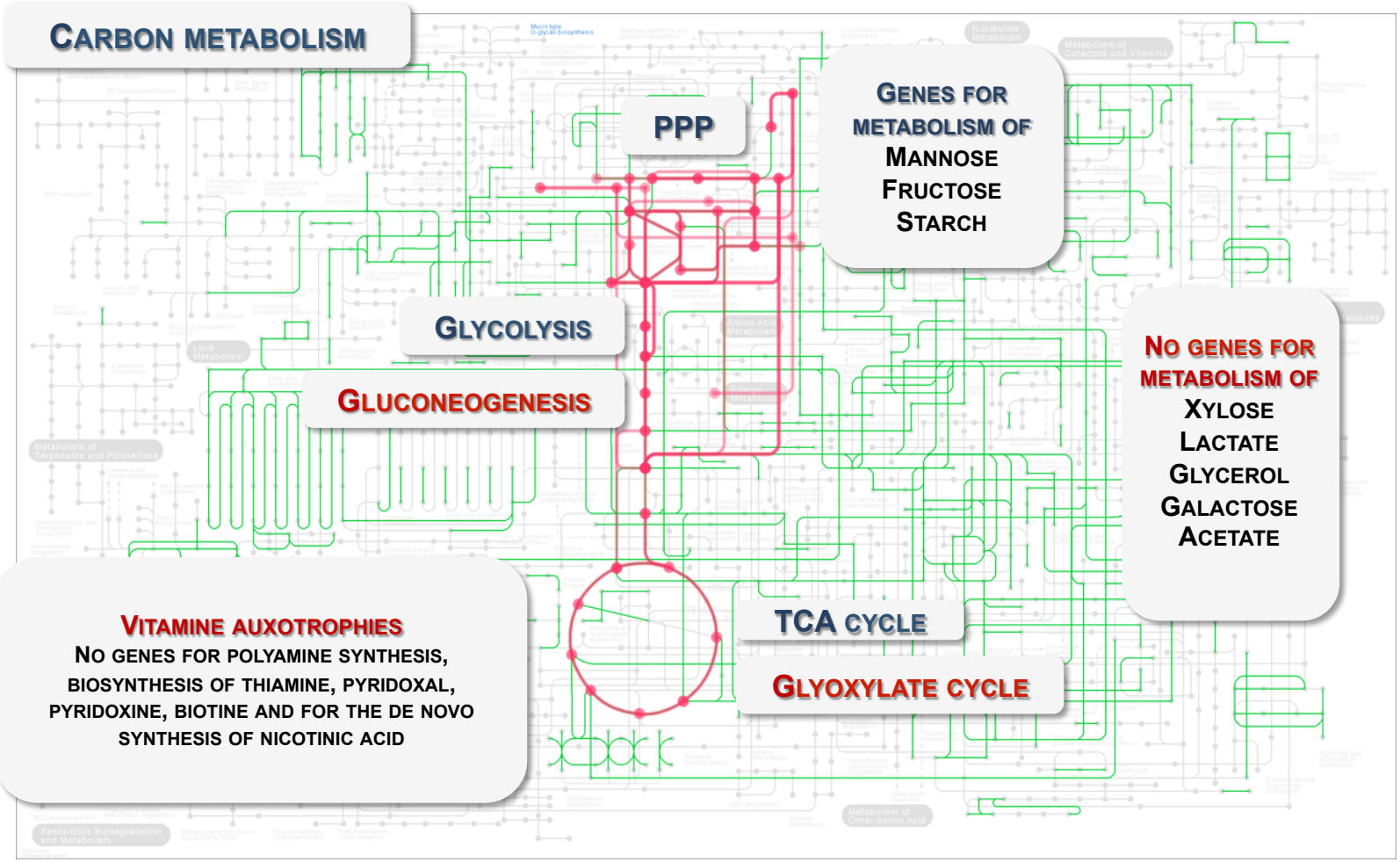


Figure S4. Metabolic reconstruction of *H. guilliermondii* focused on carbon metabolism, as unveiled by KEGG Koala metabolic reconstruction tool

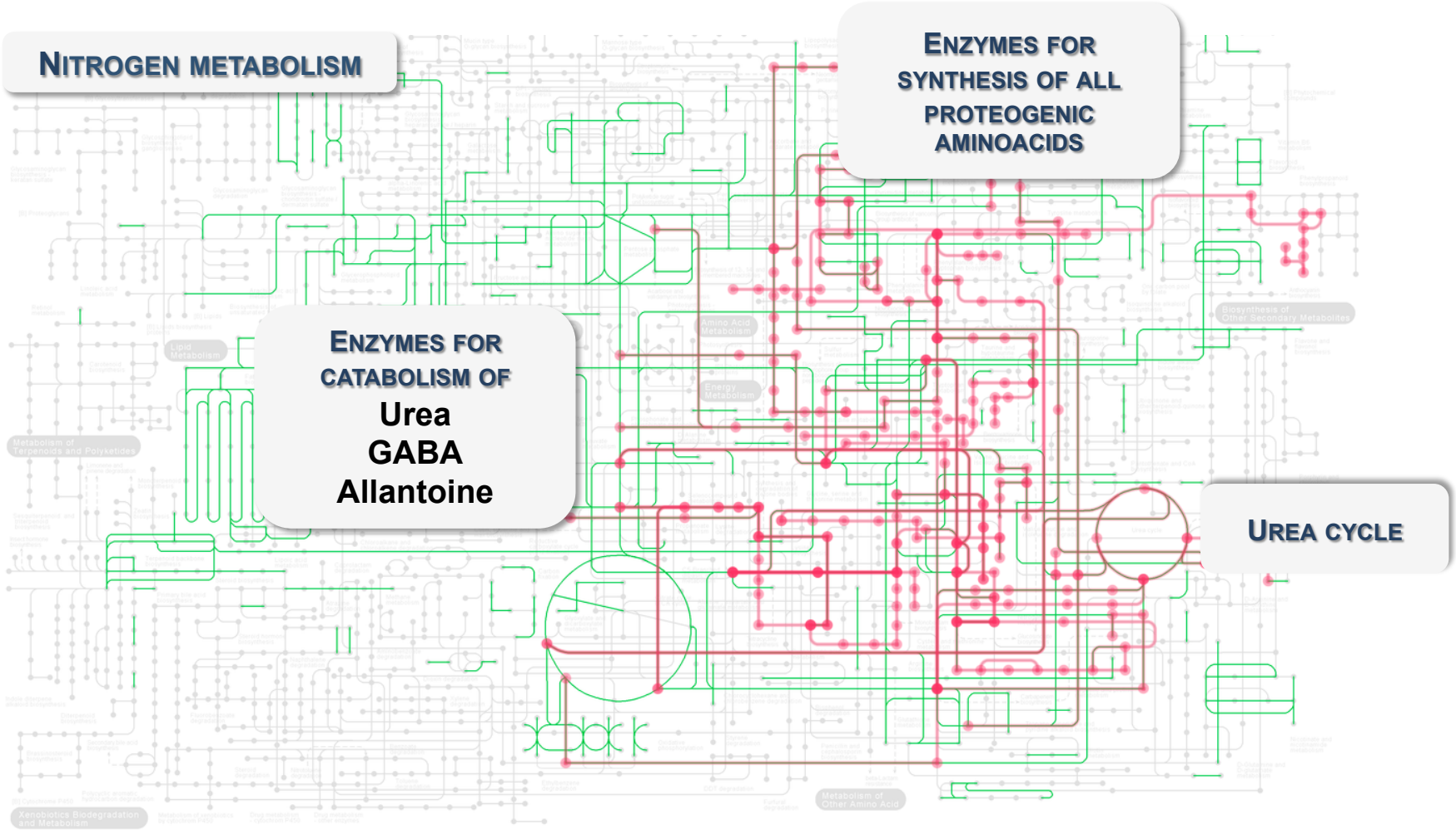
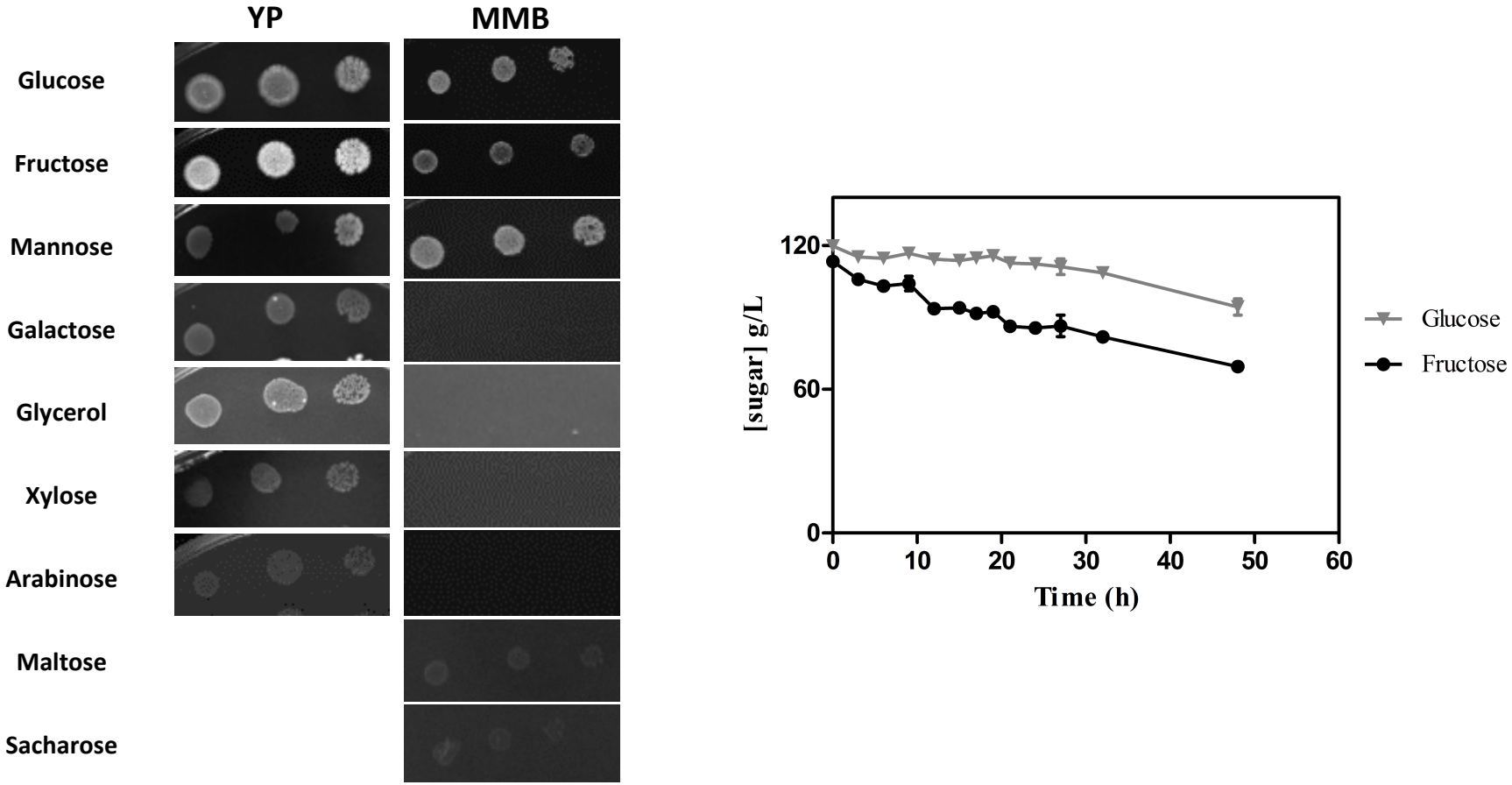
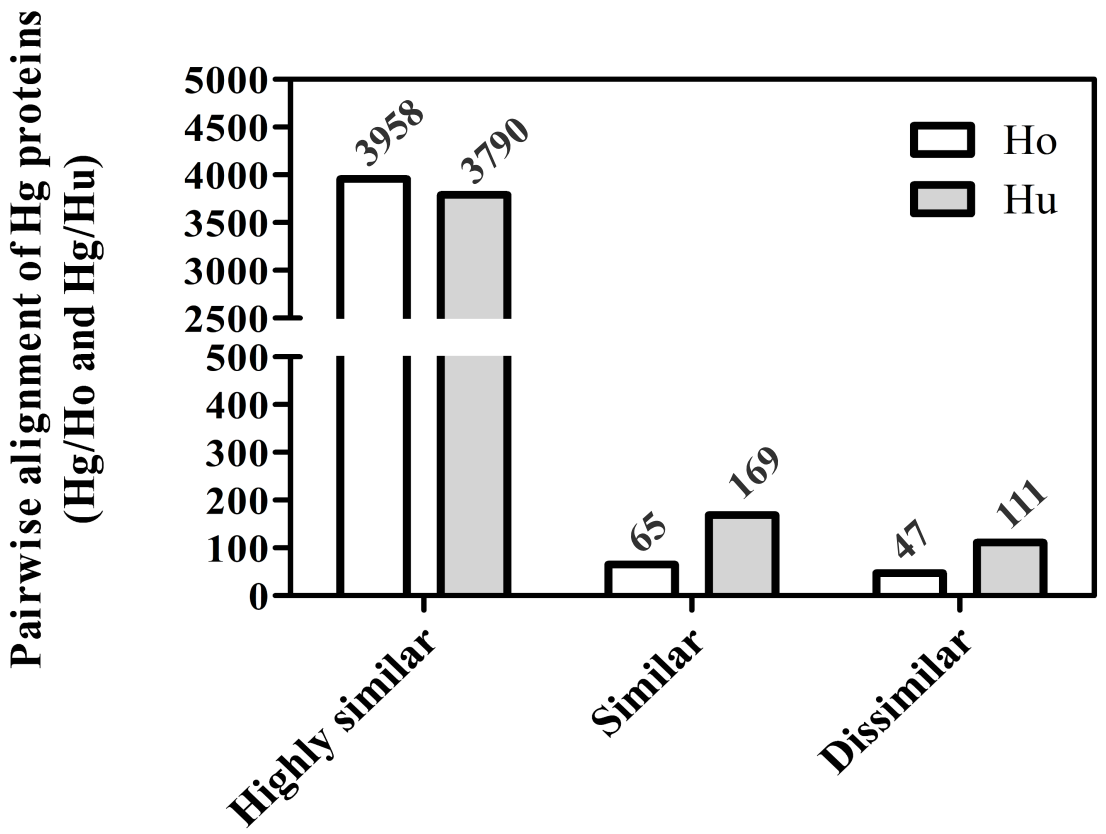


Figure S5. Metabolic reconstruction of *H. guilliermondii* focused on nitrogen and sulphur metabolism, as unveiled by KEGG Koala metabolic reconstruction tool



Supplementary figure S6. (left) Growth of *H. guilliermondii* UTAD222 in rich (YP) or in mineral (MMB) medium having different sugars as the sole sources of carbon and energy; (right) Growth of *H. guilliermondii* UTAD222, at 25ºC and 180 rpm, in rich YP medium having 10% glucose and 10% fructose as the sole carbon source



Supplementary figure S7. Comparison of the proteomes of *H. guilliermondii* UTAD222 with the one of *H. uvarum* AWRI3580 and *H. opuntiae* AWRI3578. The graph shows the number of proteins considered highly similar(associated e-value of the alignment lower than $1x10^{-50}$ and an identity above 50%), similar (e-value between $1x10^{-50}$ and $1x10^{-20}$ and/or an identity value below 50%) or dissimilar (e-value equal or above $1x10^{-20)}$. On top of the columns it is indicated the number of pairwise alignment (Hg vs other species) considered