Ancestral acquisitions, gene flow and multiple evolutionary trajectories of the type three secretion system and effectors in *Xanthomonas* plant pathogens

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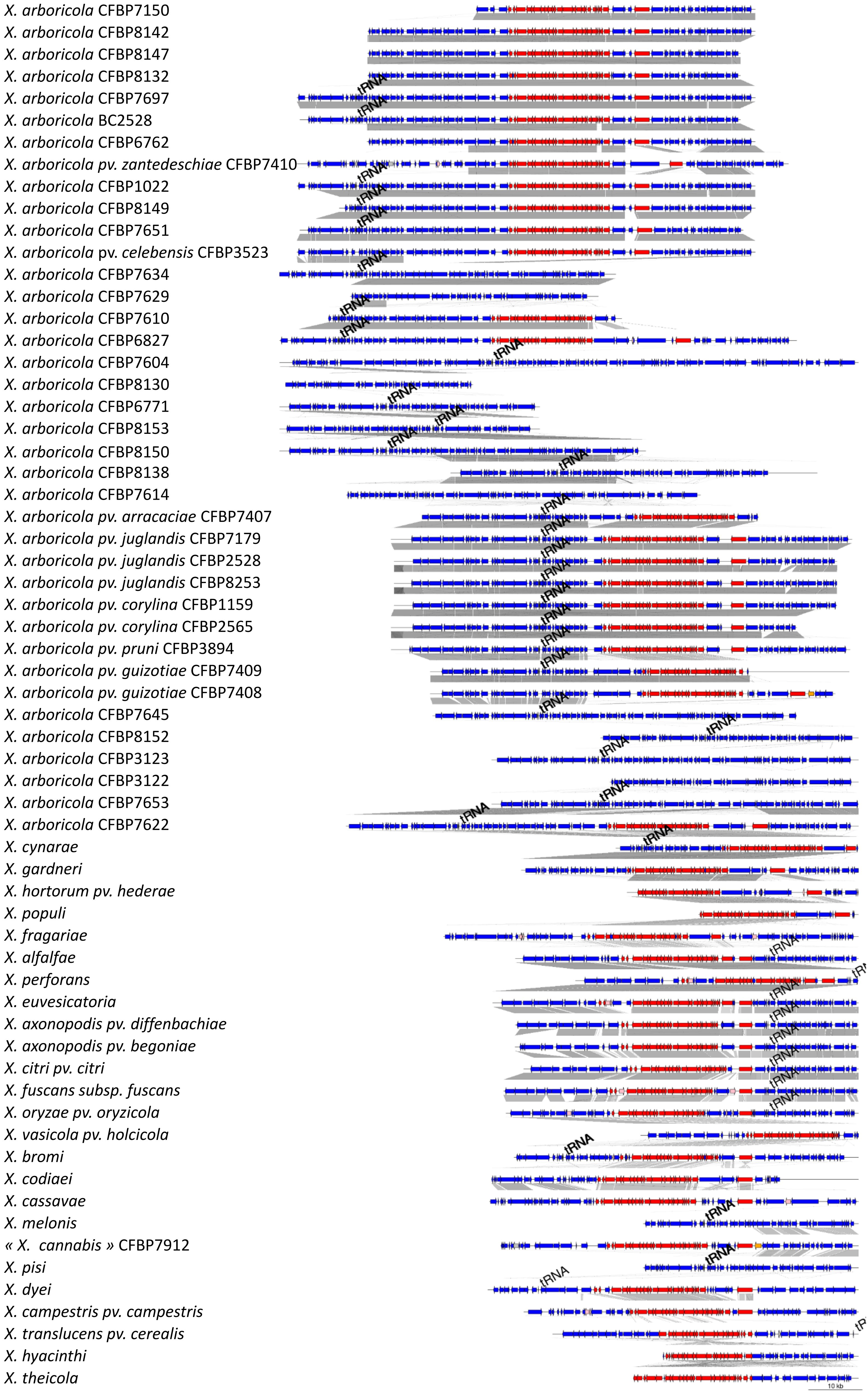
Supplementary Figure S1

FIGURE S1 Comparisons of genomic environments of T3SS clusters in different *Xanthomonas* species. Genomic environments (20 kb on each side) were compared using the R package GenoplotR. The genes of the T3SS cluster are represented by red arrows. Pink arrows represent genes encoding transposases, orange arrows represent integrons, grey arrows represent phages. Other genes are represented by blue arrows. DNA fragments showing BLASTn similarities are connected with grey shading. (A) Comparison of strains representing all the phylogenetic clusters identified in the *Xanthomonas* genus. Strains are ordered according to their phylogenetic relationships. (B) to (F) : examples of comparisons showing similarities and differences between genomic environments of T3SS cluster. Similar genomic environments of the T3SS cluster were highlighted by bars of the same color placed above the schematic representation of the sequences. (B) Comparison between the strain CFBP 7179 of *X. arboricola* used as reference (representing the genomic environment retrieved in *X. arboricola* strains), *X. bromi* (CFBP 1976) and *X. oryzae* (BAI3). This comparison reveals that the genomic environment of *X. bromi* T3SS cluster shares similarities with the one of *X. arboricola* but not with the one of *X. oryzae* (this latter is shared by other clade B species). (C). Comparisons showing the diversity of genomic environments of T3SS cluster in *X. codiaei*, "*X. cannabis*", *X. cassavae*, and *X. dyei* and absence of similarities (except for *X. dyei*) with the genomic environment of clade B represented in green. (D). Comparisons showing the mosaic structure of the genomic environment of T3SS cluster in *X. arboricola* pv. *guizotiae* and in *X. dyei*. (E). Comparison showing that the genomic environment of T3SS cluster in the group 1 species *X. translucens* shares no similarity with the one retrieved in group 2 species from clades A and B. (F). Comparison showing similarities between the genomic environments of T3SS cluster in the three group 1 species *X. translucens*, *X. hyacinthi* and *X. theicola*.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X. gardneri
X. hortorum pv. hederae
X. populi
X. fragariae
X. alfalfae
X. perforans
X. euvesicatoria
X. axonopodis pv. diffenbachiae
X. axonopodis pv. begoniae
X. citri pv. citri
X. fuscans subsp. fuscans
X. oryzae pv. oryzicola
X. vasicola pv. holcicola
X. bromi
X. codiaei
X. cassavae
X. melonis
« X. cannabis » CFBP7912
X. pisi
X. dyei
X. campestris pv. campestris
X. translucens pv. cerealis
X. hyacinthi
X. theicola
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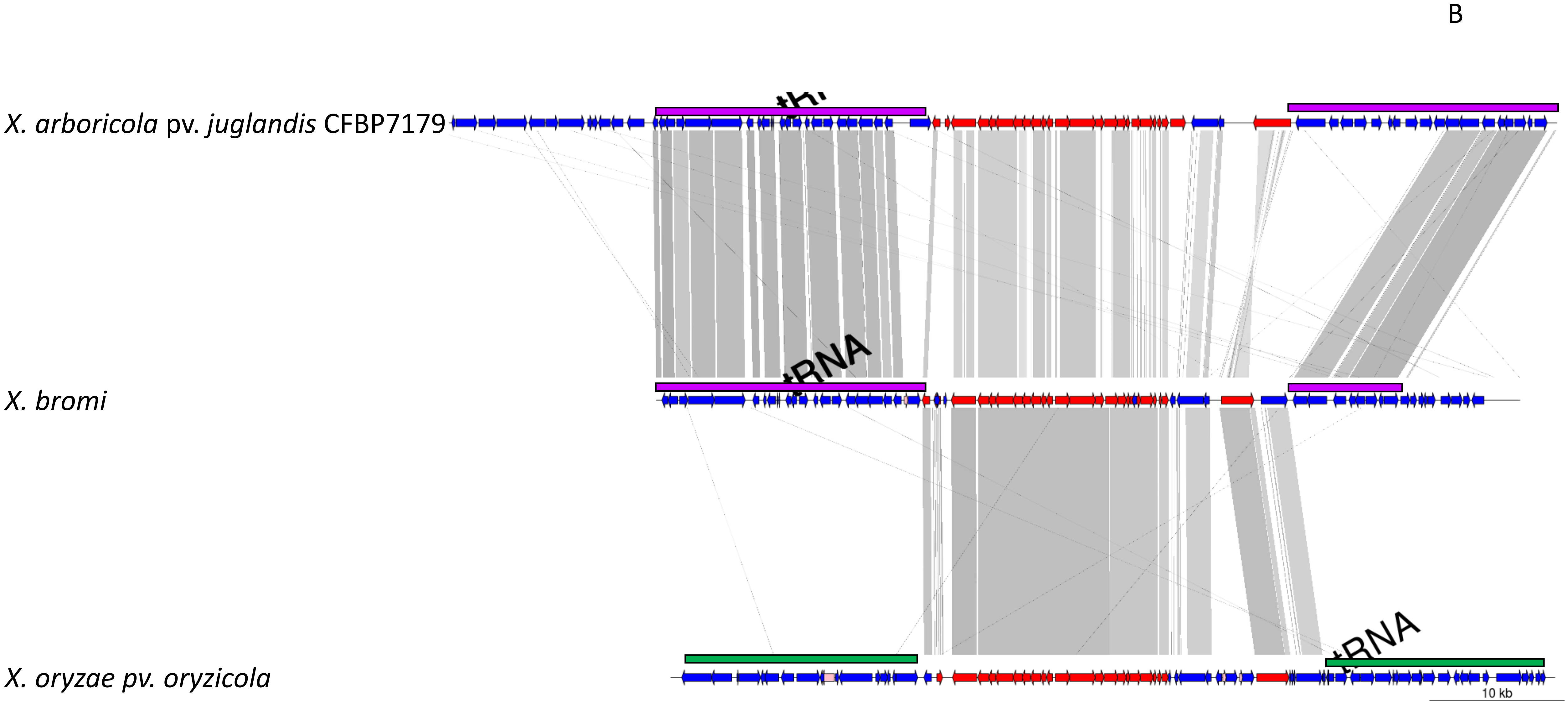
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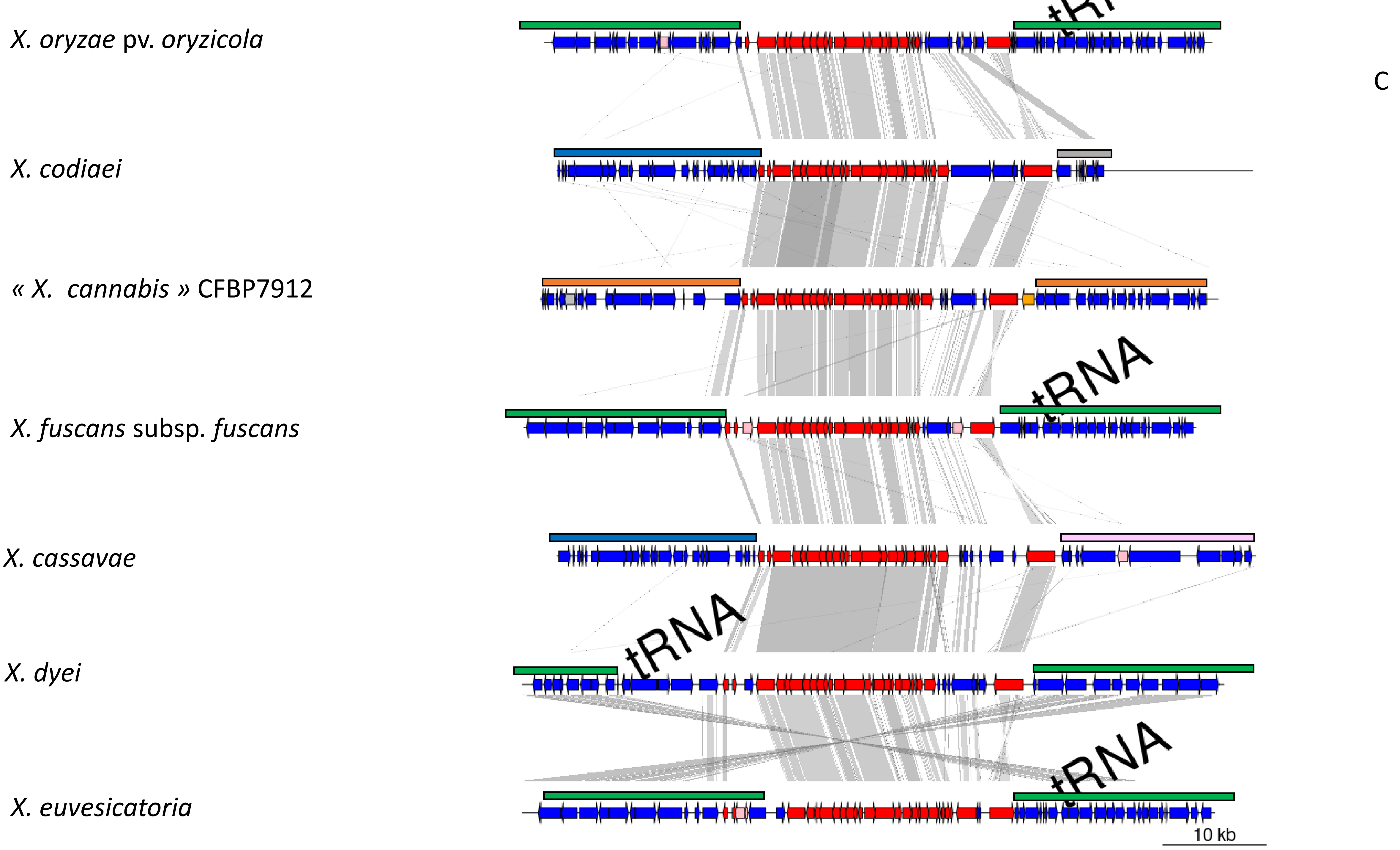
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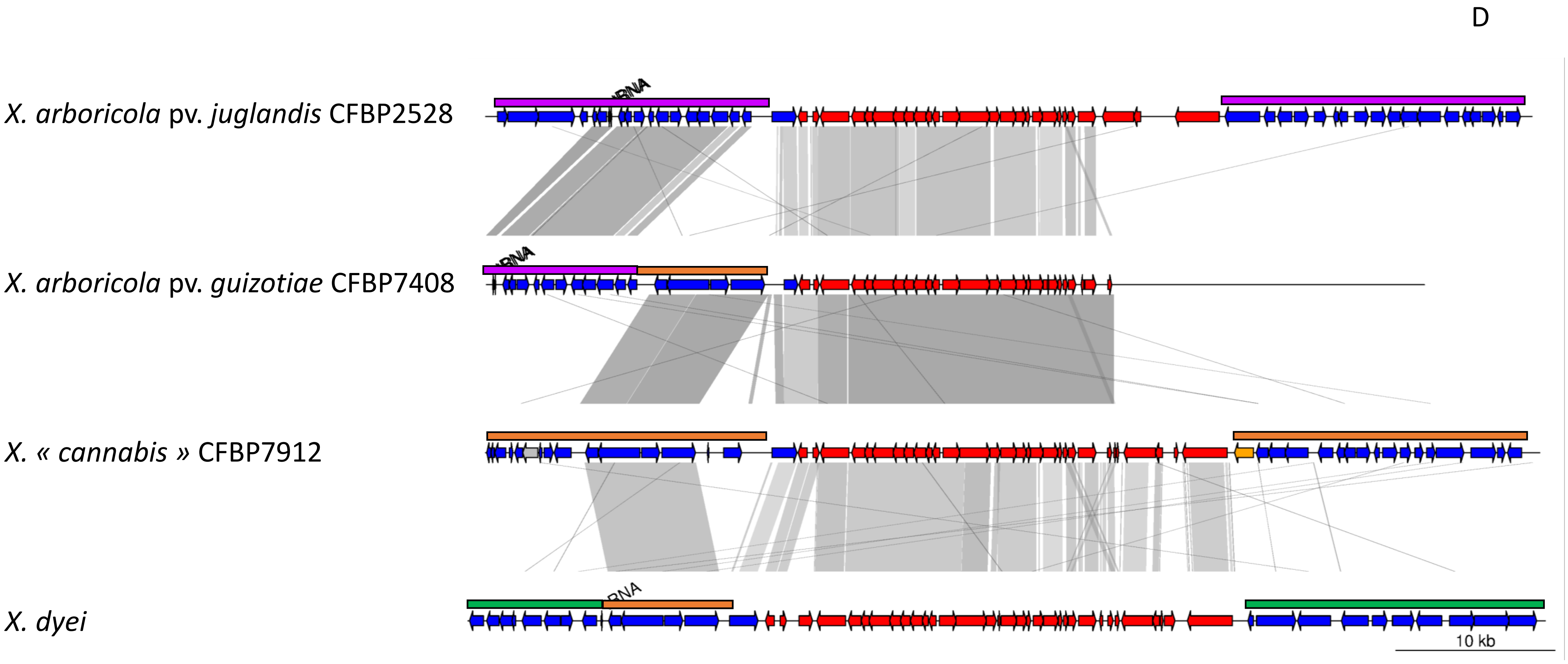
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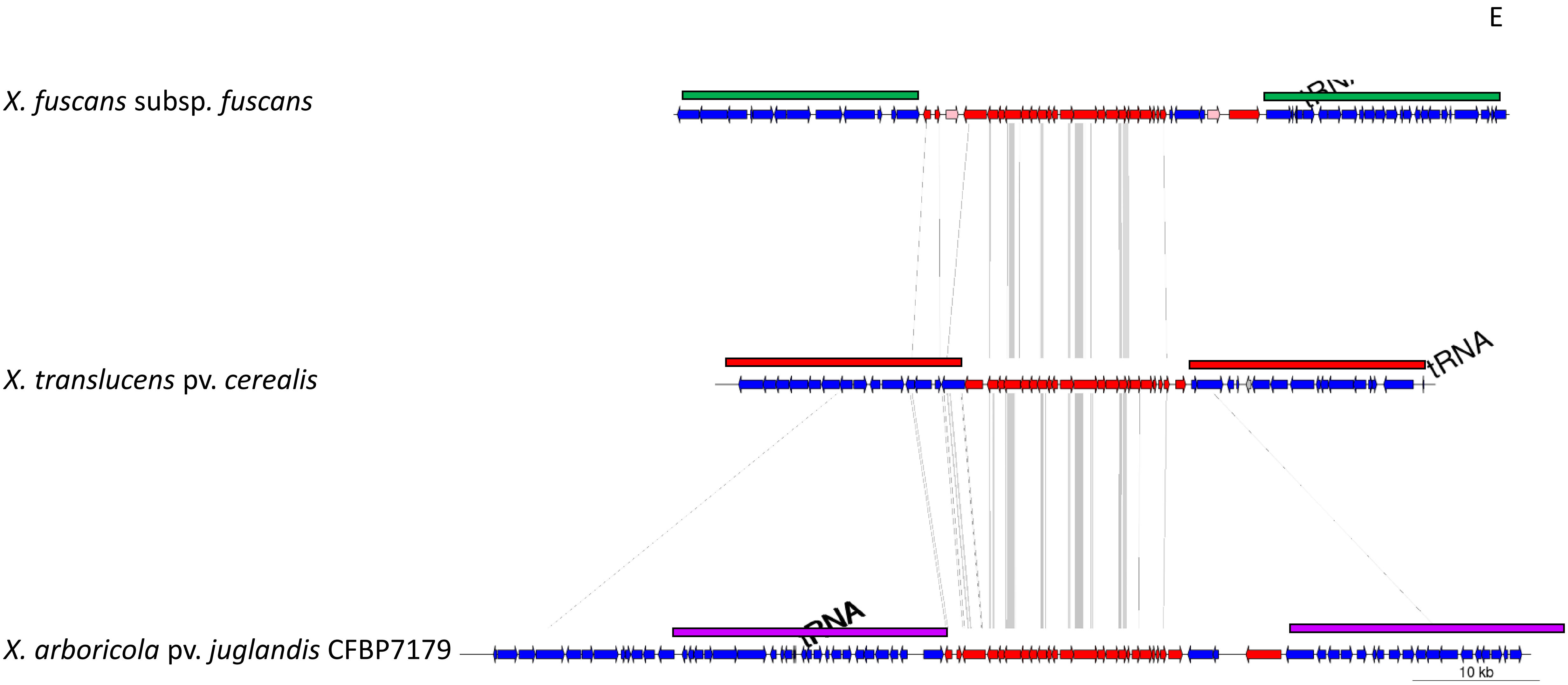
X. dyei
tRNA
10 kb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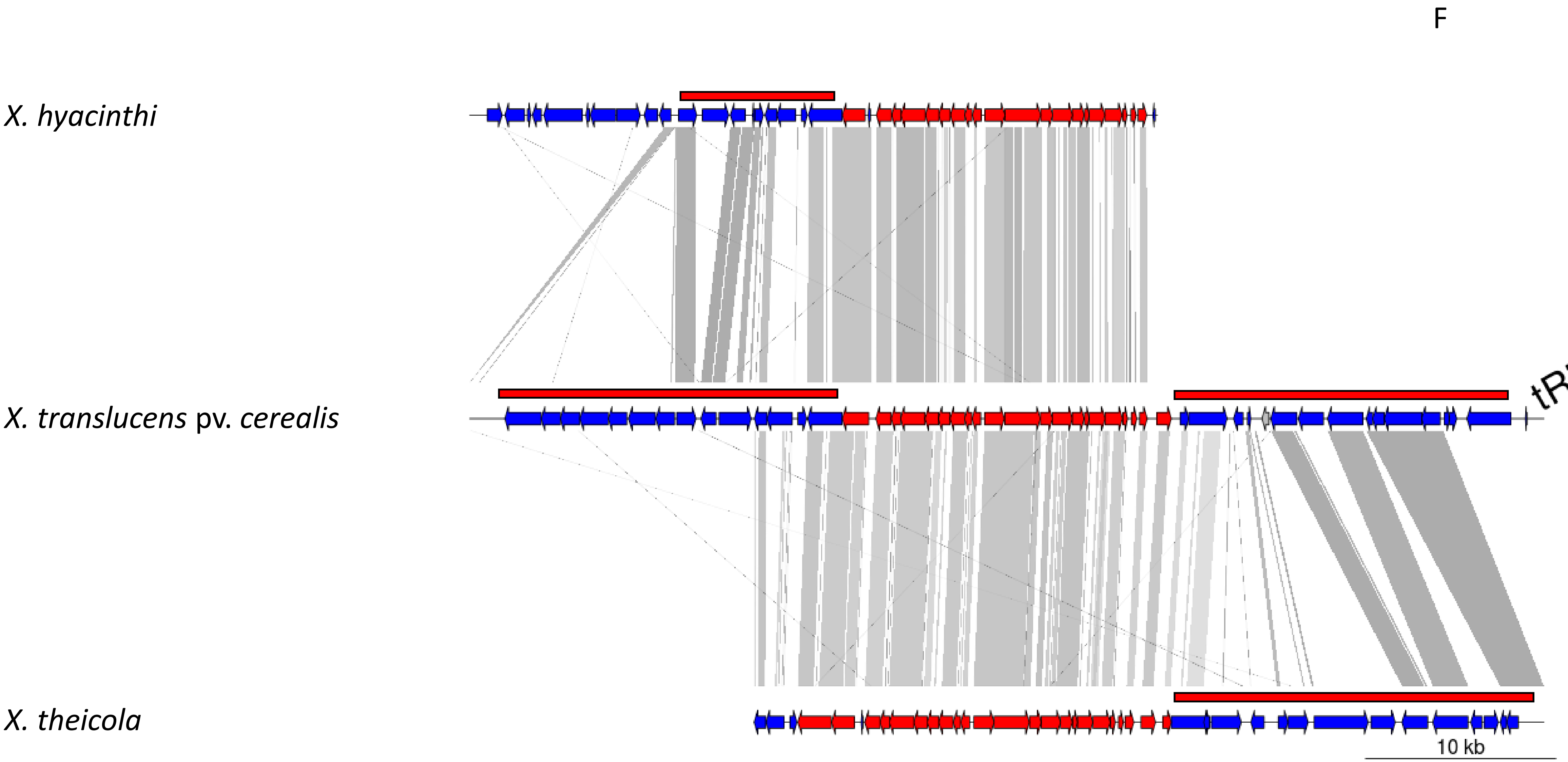
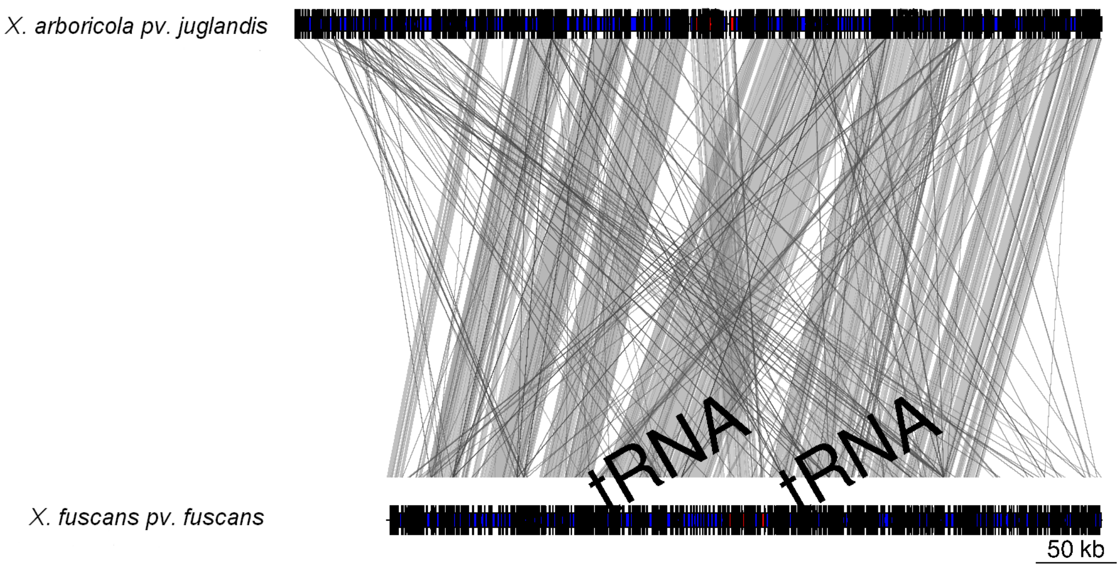

F
X. hyacinthi
X. translucens pv. cerealis
tR
X. theicola
10 kb

Supplementary Figure S2

Supplementary Figure S2: Comparisons of large genomic environments of the T3SS clusters in different *Xanthomonas* species using a window of 200 kb upstream and downstream of the cluster. Genomic environments were compared using the R package GenoplotR. The genes of the T3SS cluster are represented in red; other genes are represented in blue. DNA fragments showing similarities are connected with grey shading.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tRNA
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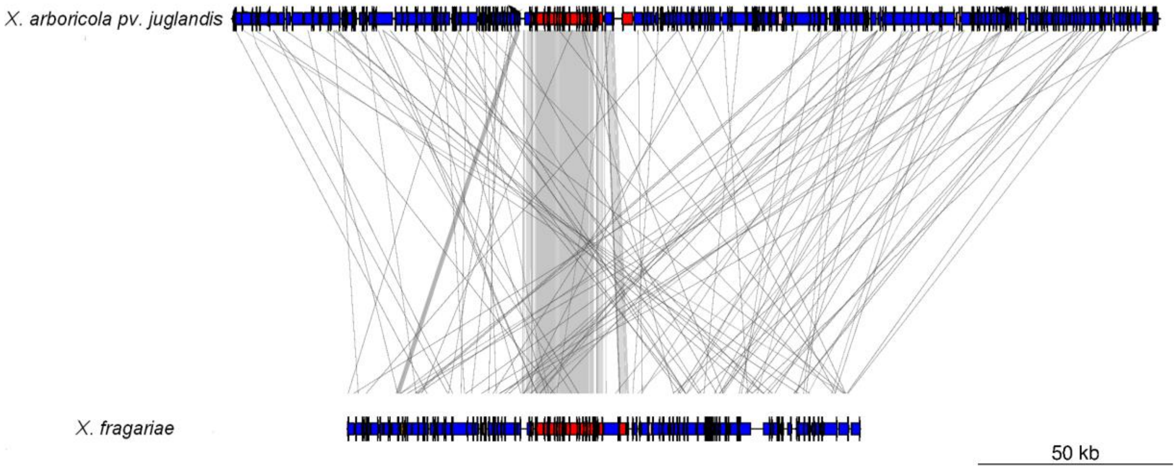
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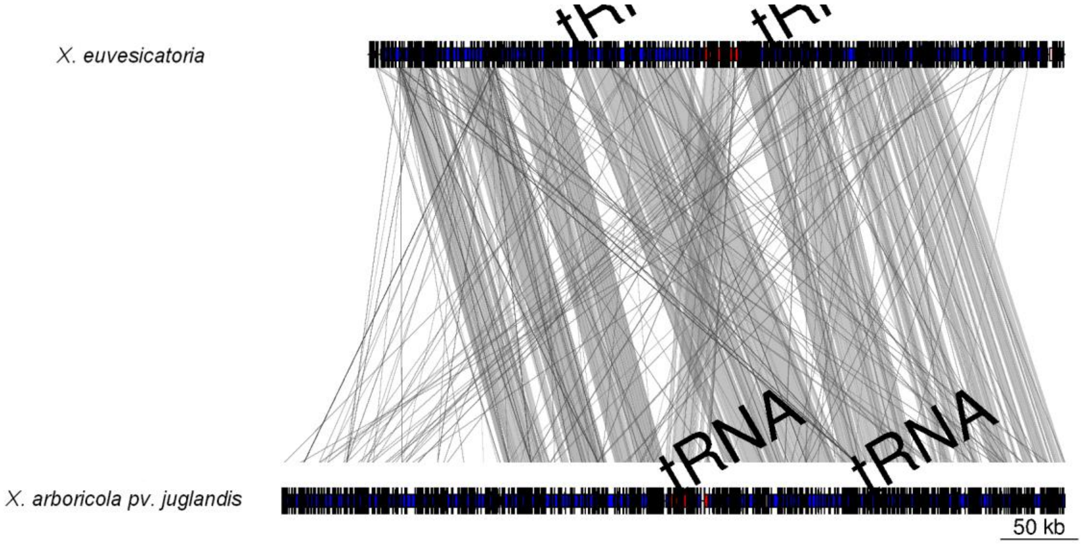
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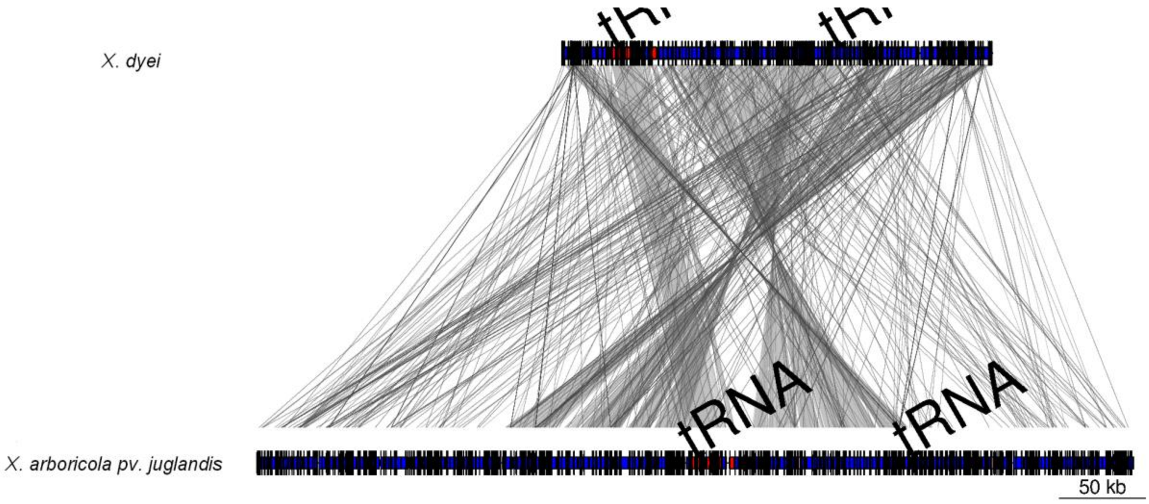
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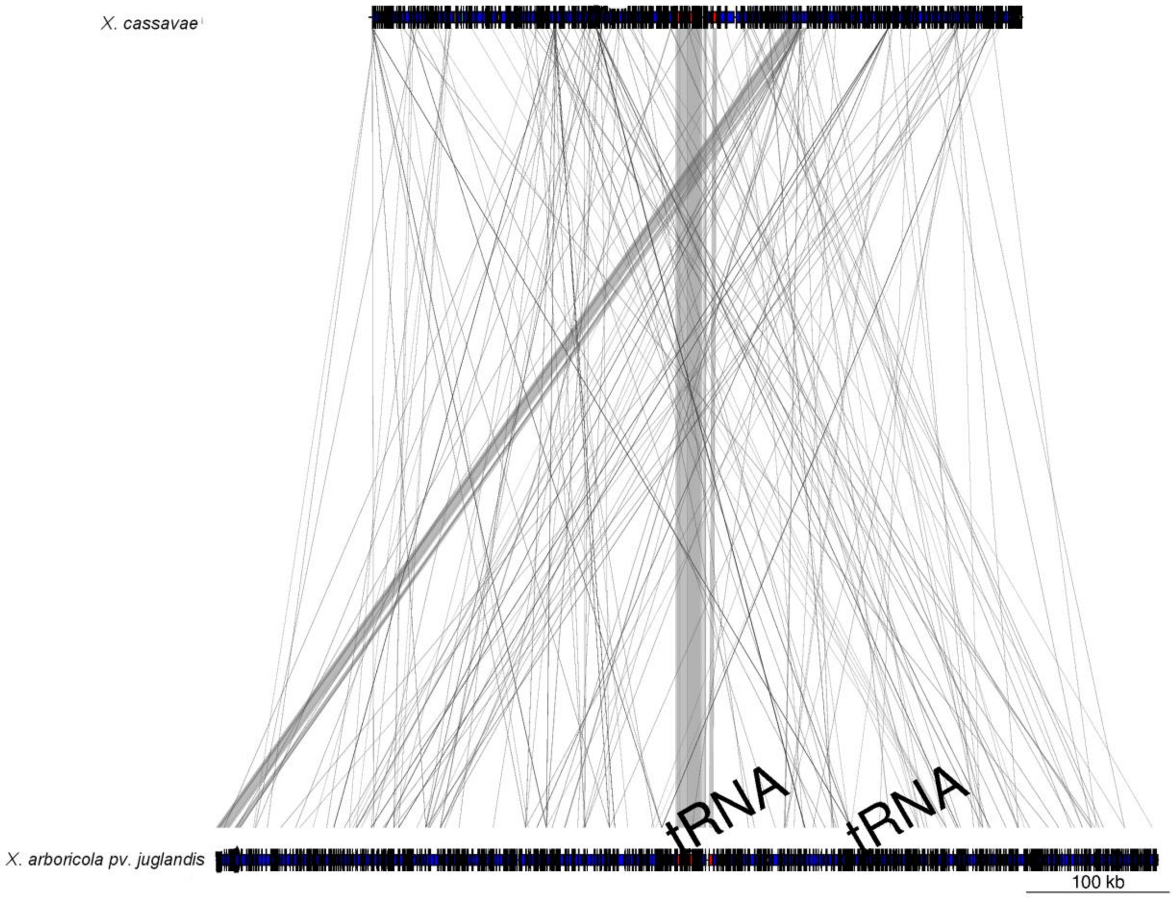
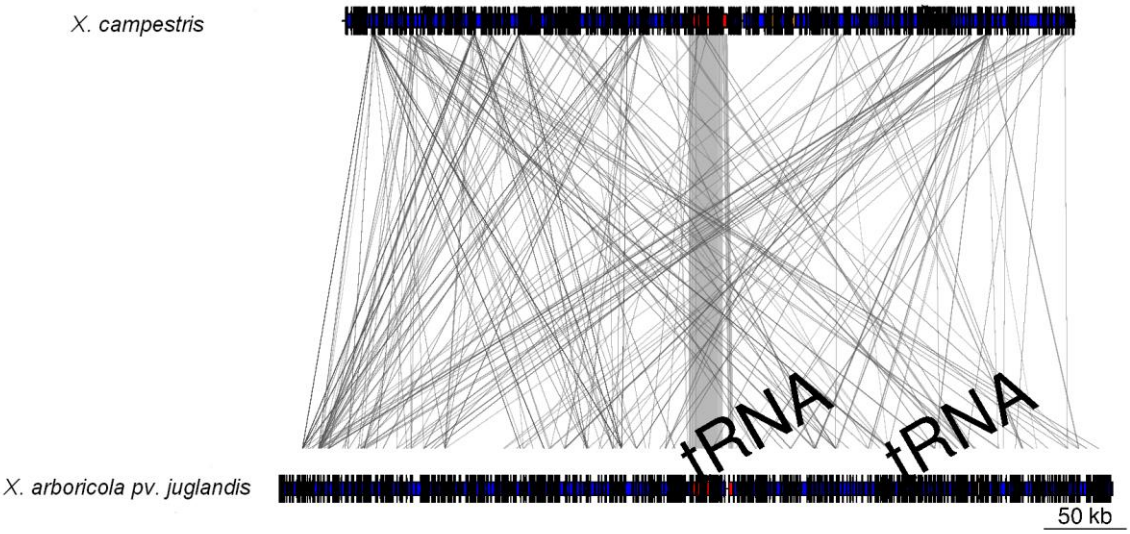
tRNA
tRNA
X. arboricola pv. juglandis
50 kb


X. cassavae
tRNA
tRNA
X. arboricola pv. juglandis
100 kb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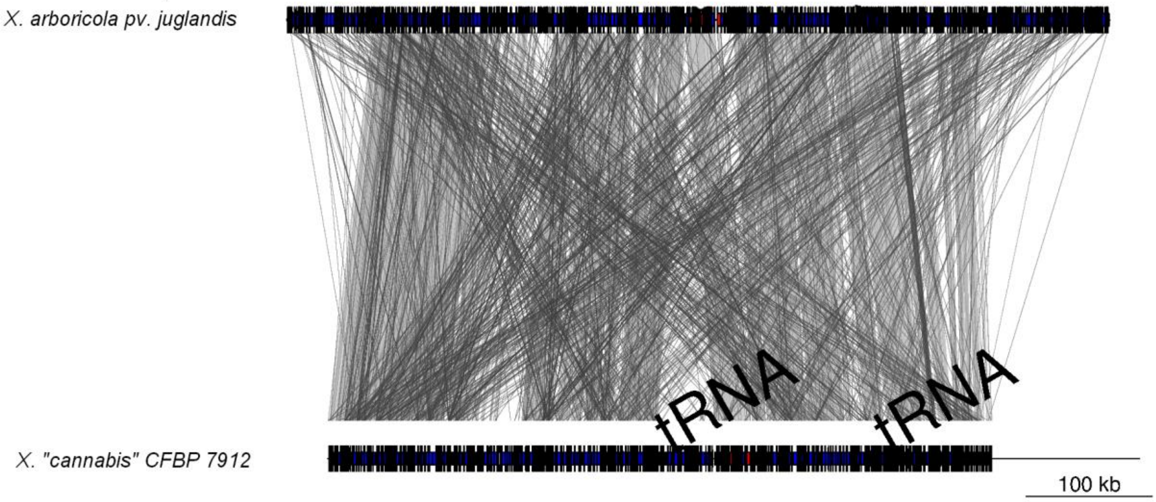
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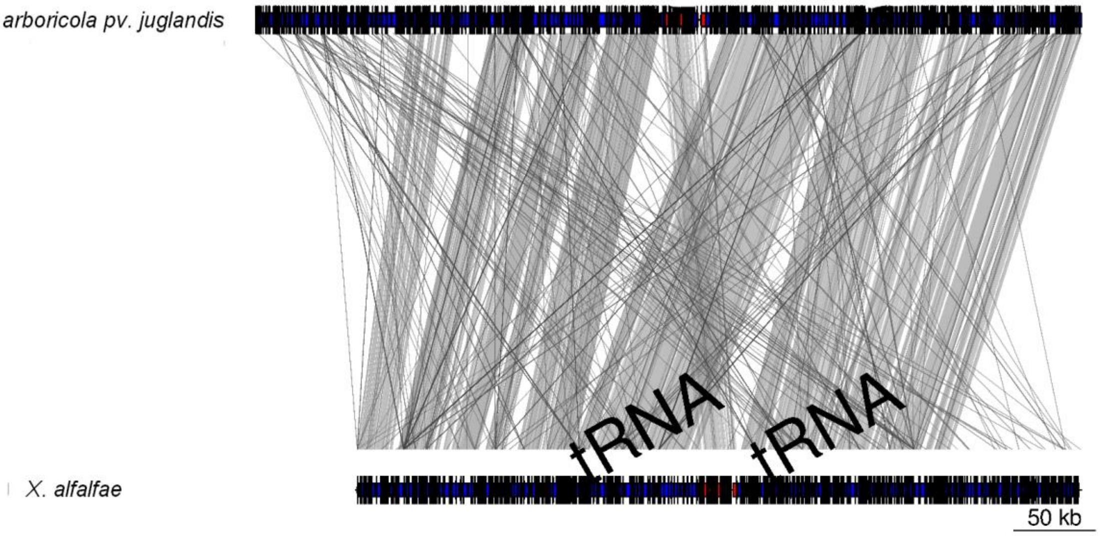
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tRNA
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50 kb

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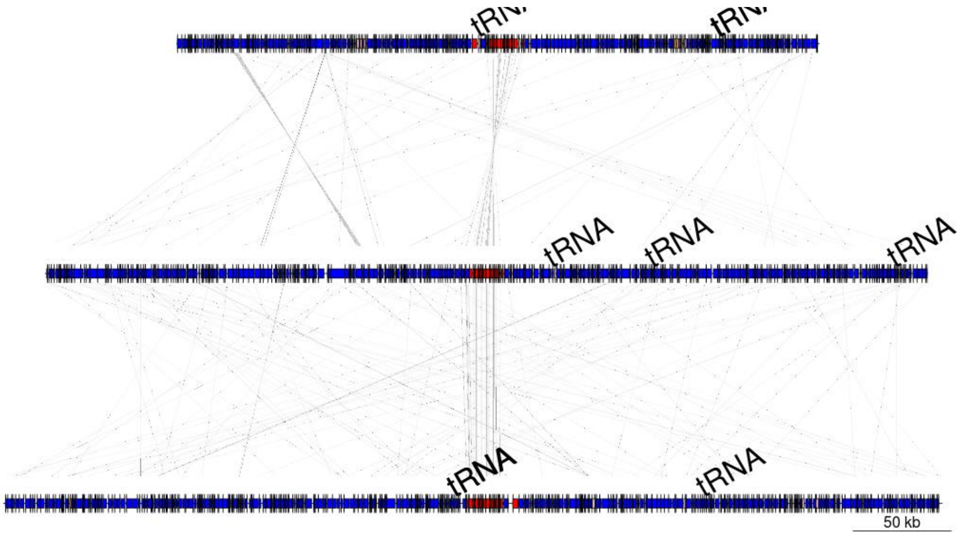
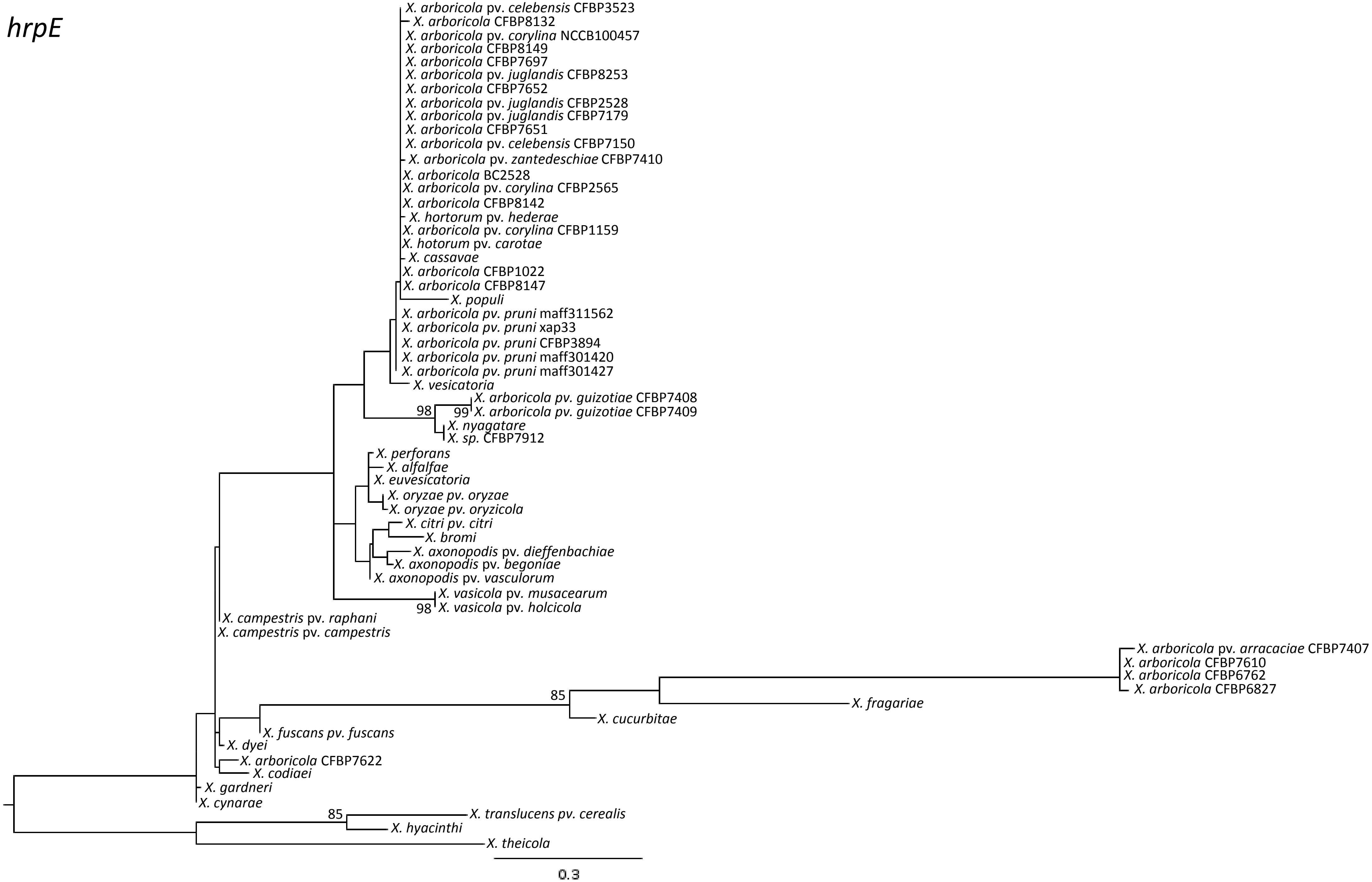


Supplementary Figure S3

Supplementary Figure S3: individual maximum likelihood phylogenies built for each *hrc/hrp* gene coding for the T3SS. Bootstrap scores (1000 bootstraps) higher than 85% are displayed at each node.

hrpE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X. cassavae
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X. arboricola pv. guizotiae CFBP7408
98
99
X. arboricola pv. guizotiae CFBP7409
X. nyagatare
X. sp. CFBP7912
X. perforans
X. alfalfae
X. euvesicatoria
X. oryzae pv. oryzae
X. oryzae pv. oryzicola
X. citri pv. citri
X. bromi
X. axonopodis pv. dieffenbachiae
X. axonopodis pv. begoniae
X. axonopodis pv. vasculorum
X. vasicola pv. musacearum
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X. campestris pv. raphani
X. campestris pv. campestris
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X. arboricola CFBP7610
X. arboricola CFBP6762
X. arboricola CFBP6827
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X. fragariae
X. cucurbitae
X. fuscans pv. fuscans
X. dyei
X. arboricola CFBP7622
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X. theicola
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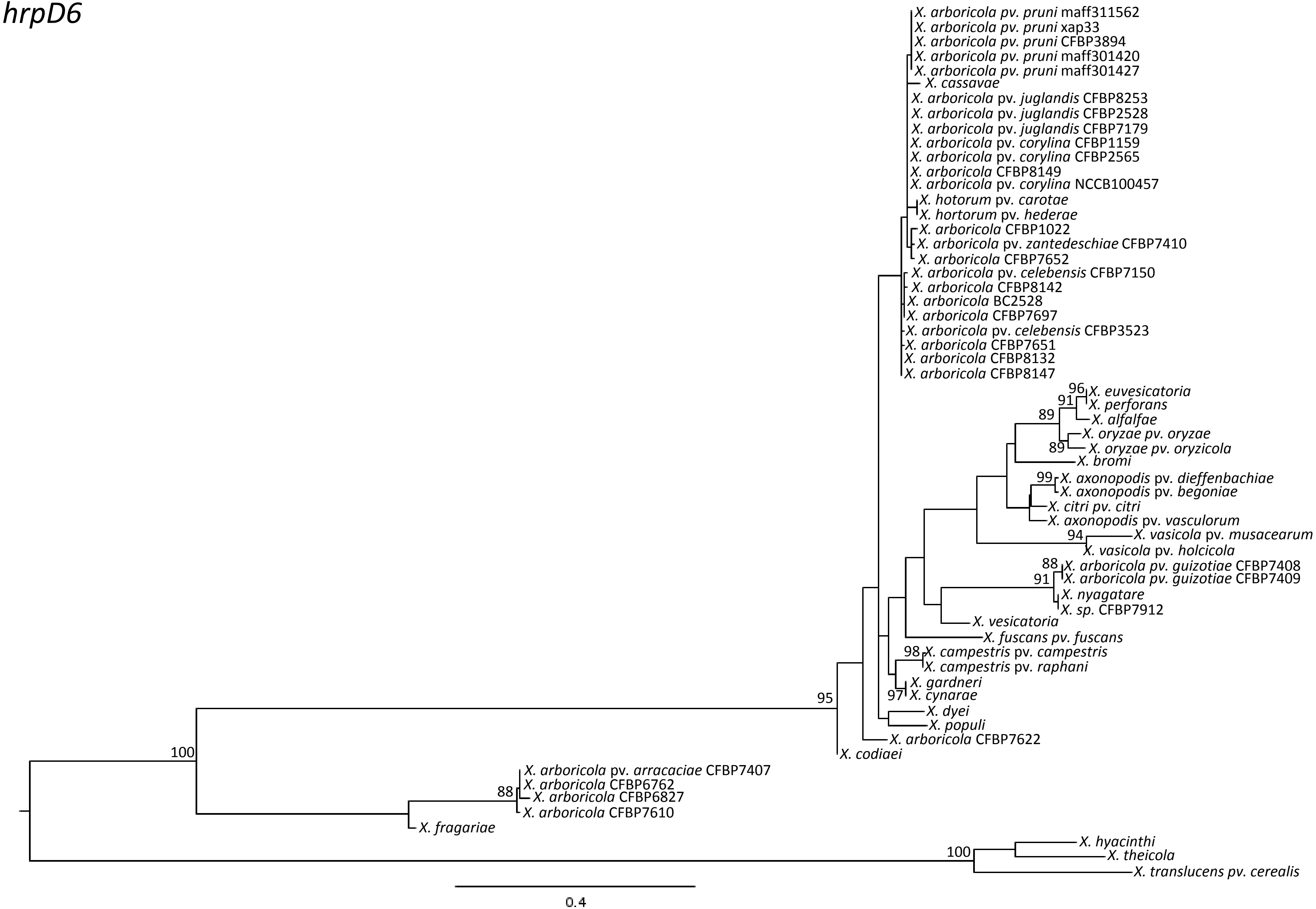
hrpD6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X. cynarae
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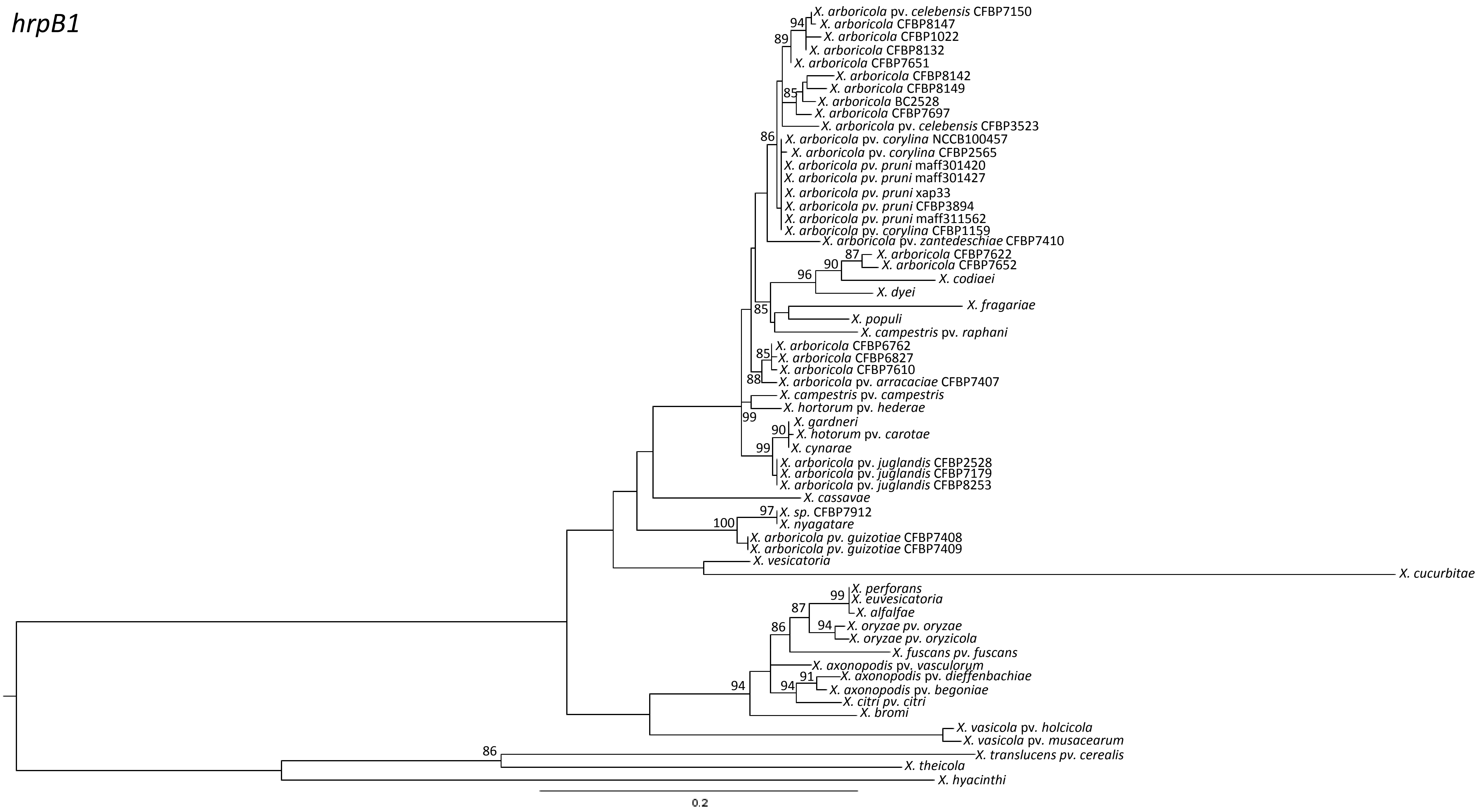
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X. dyei
X. fragariae
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X. bromi
X. vasicola pv. holcicola
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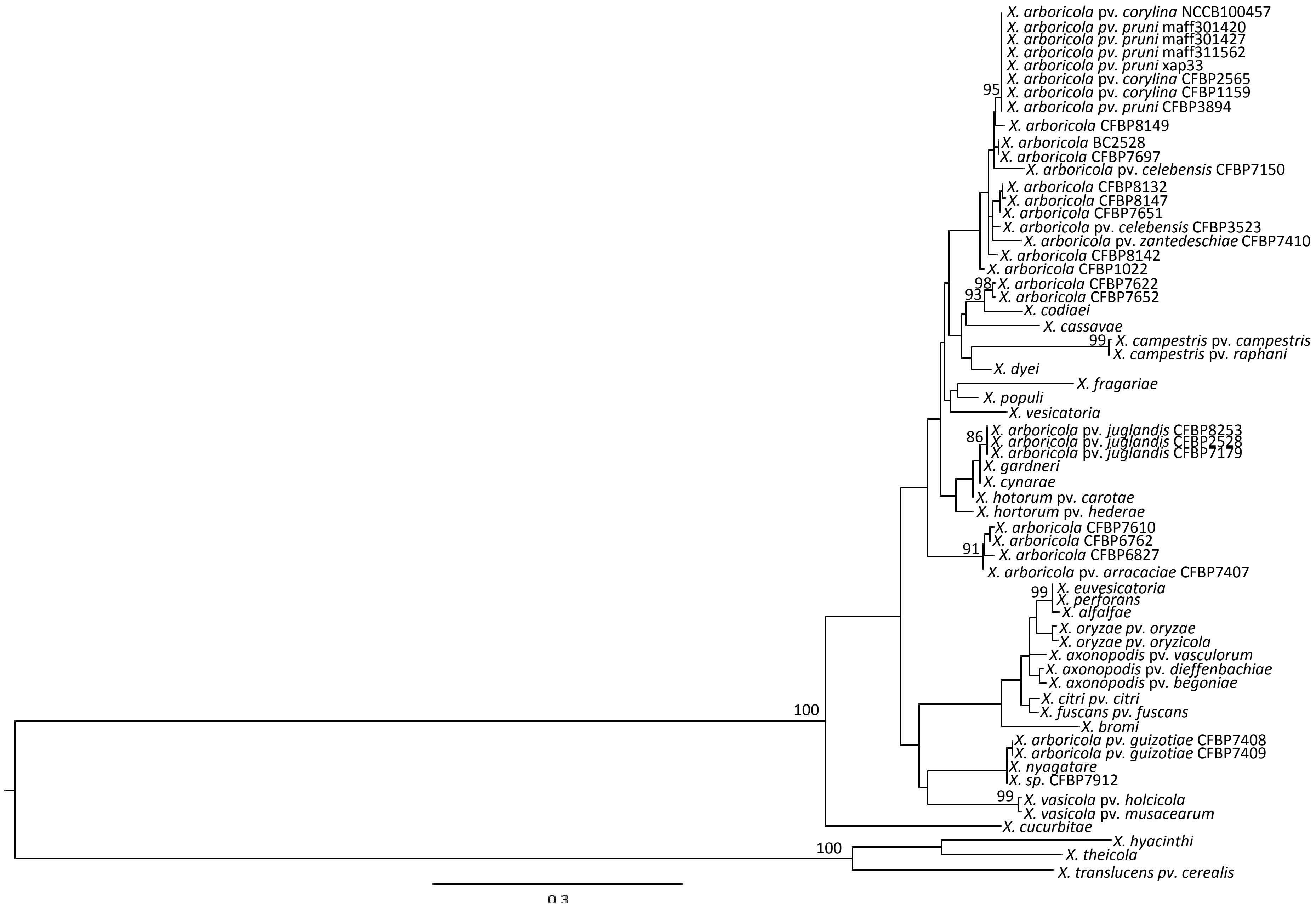
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X. axonopodis pv. begoniae
X. citri pv. citri
X. fuscans pv. fuscans
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X. bromi
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X. sp. CFBP7912
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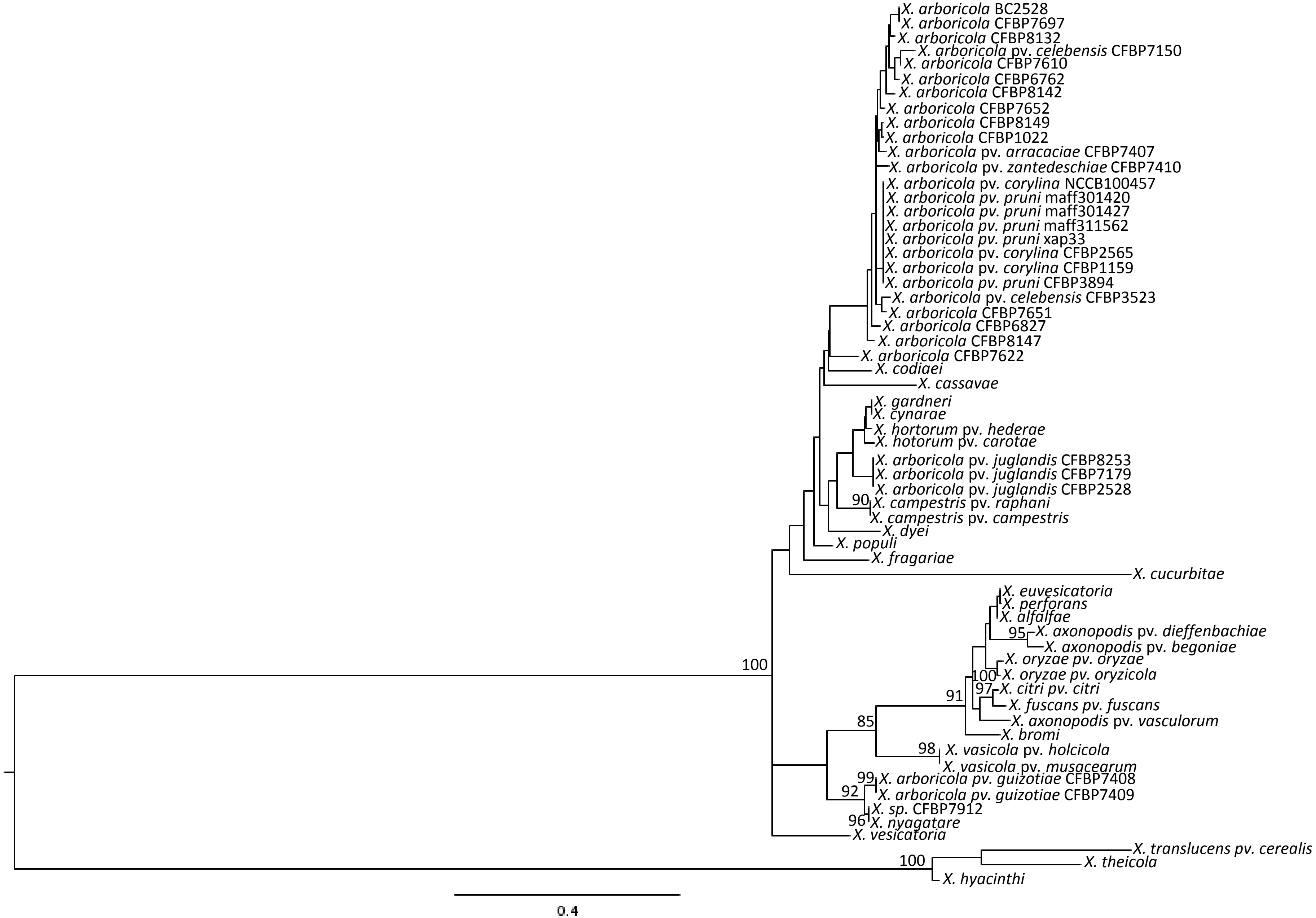
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X. gardneri
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X. hotorum pv. *carotae*
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X. axonopodis pv. *begoniae*
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91
97
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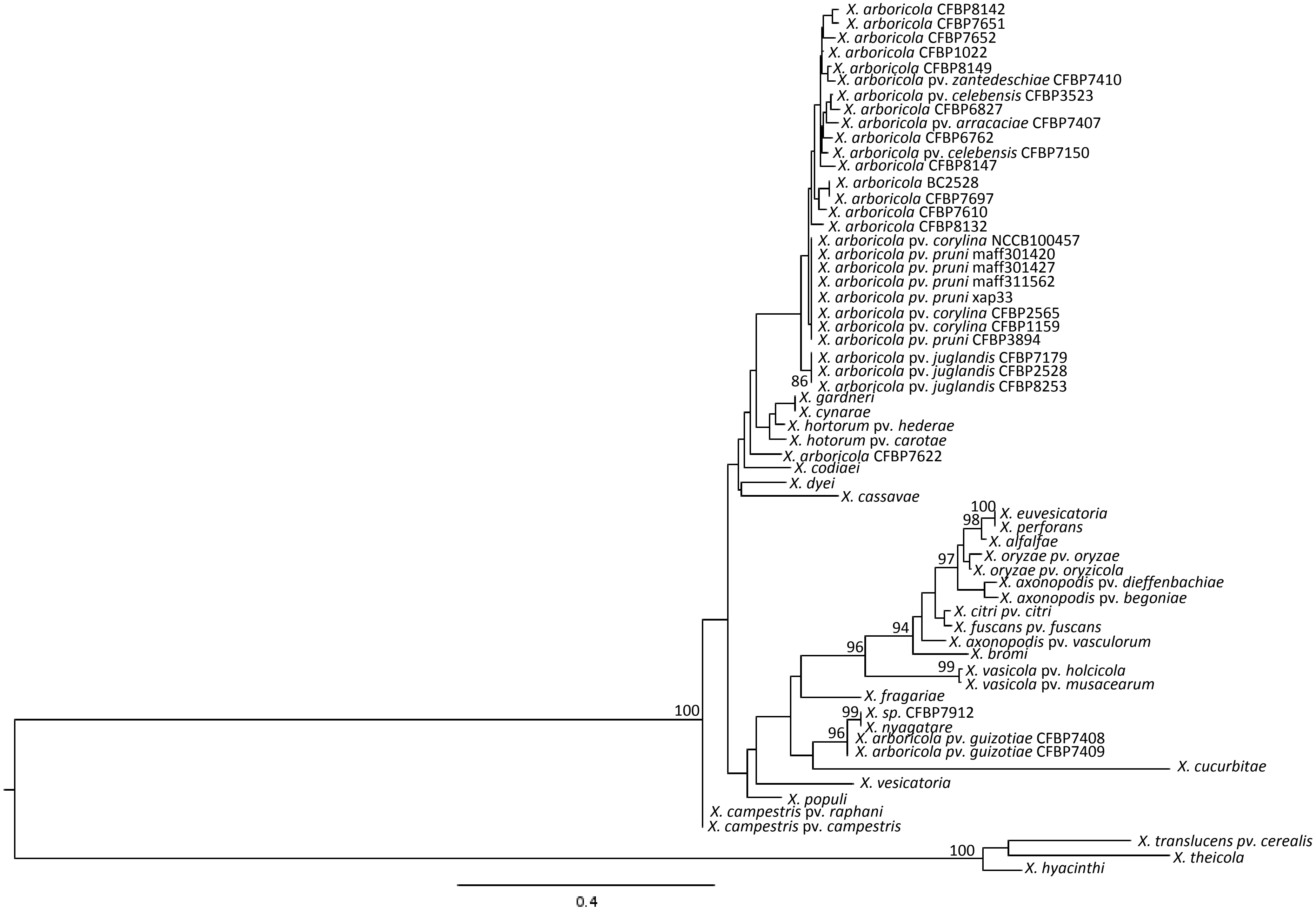
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X. cynarae
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X. euvesicatoria
98
X. perforans
X. alfalfae
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96
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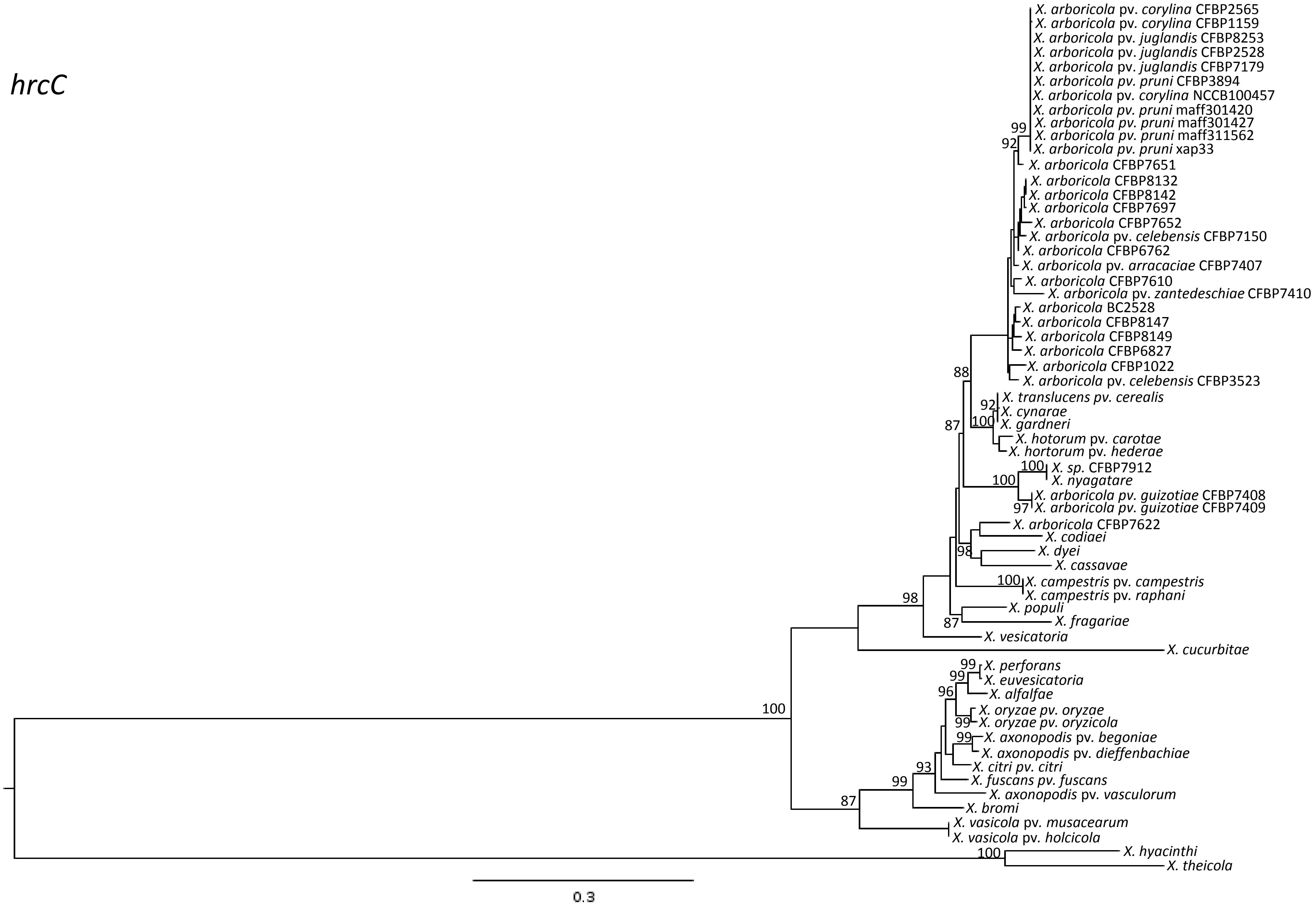
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100
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X. dyei
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100
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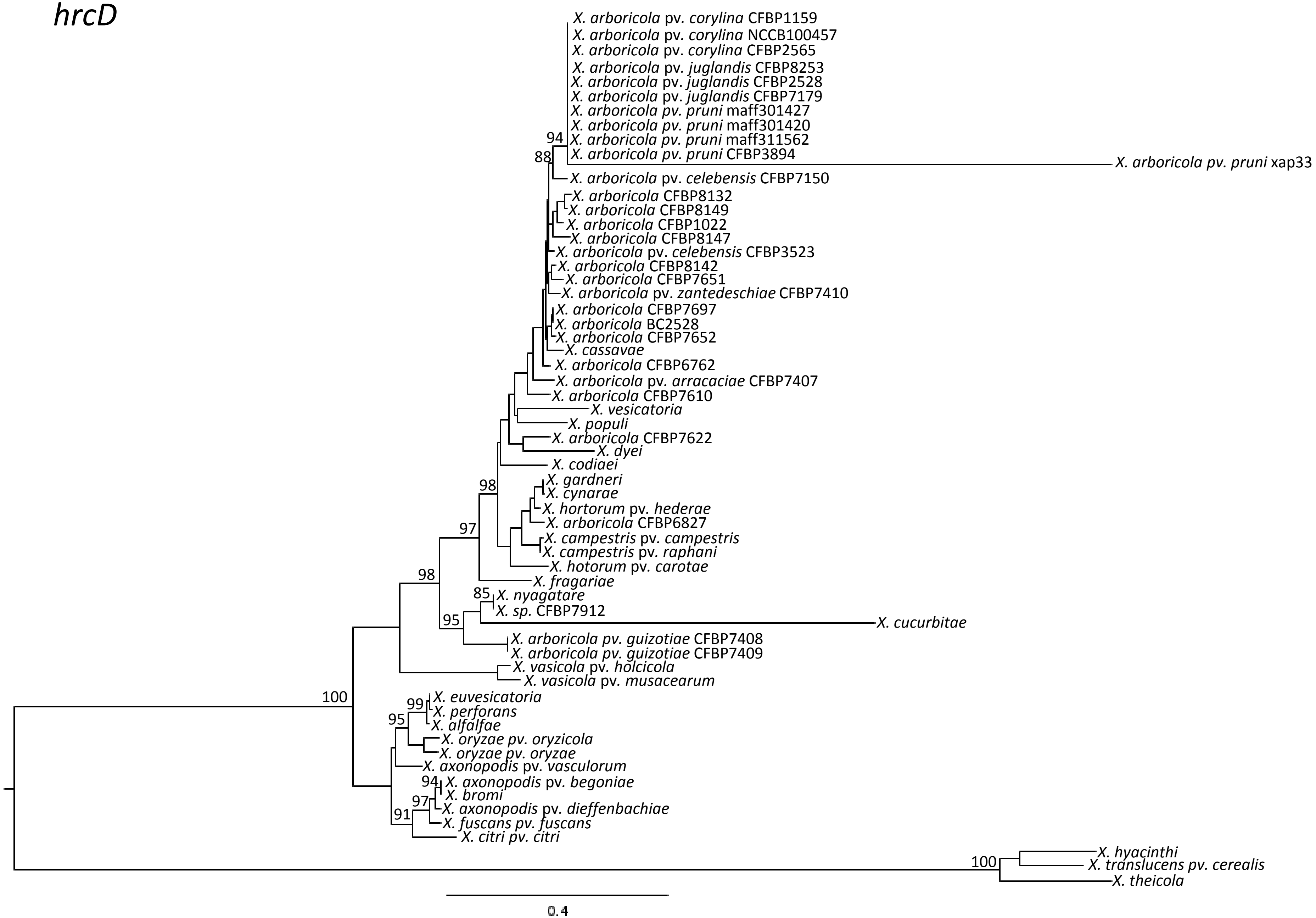

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97
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86
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89
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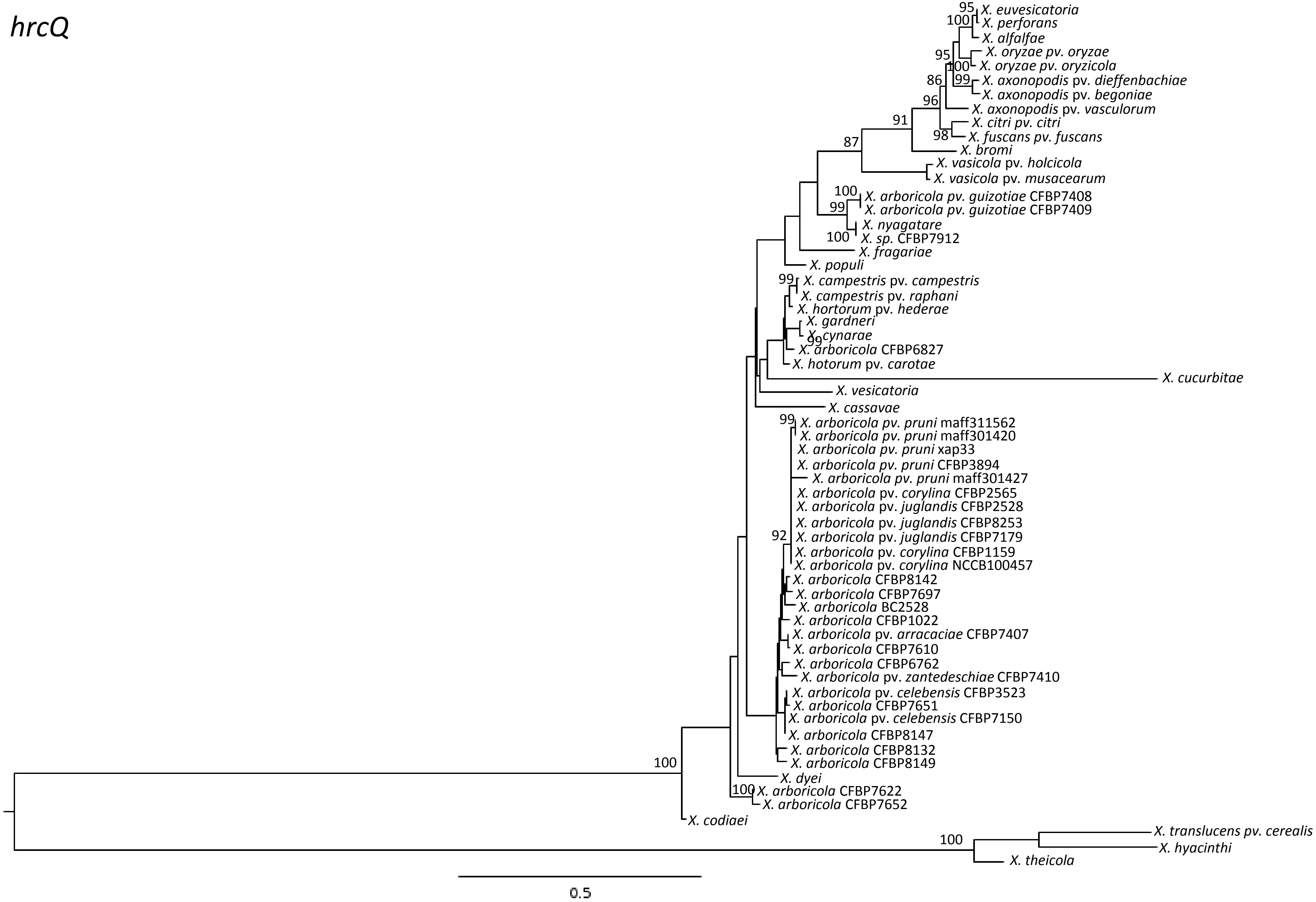
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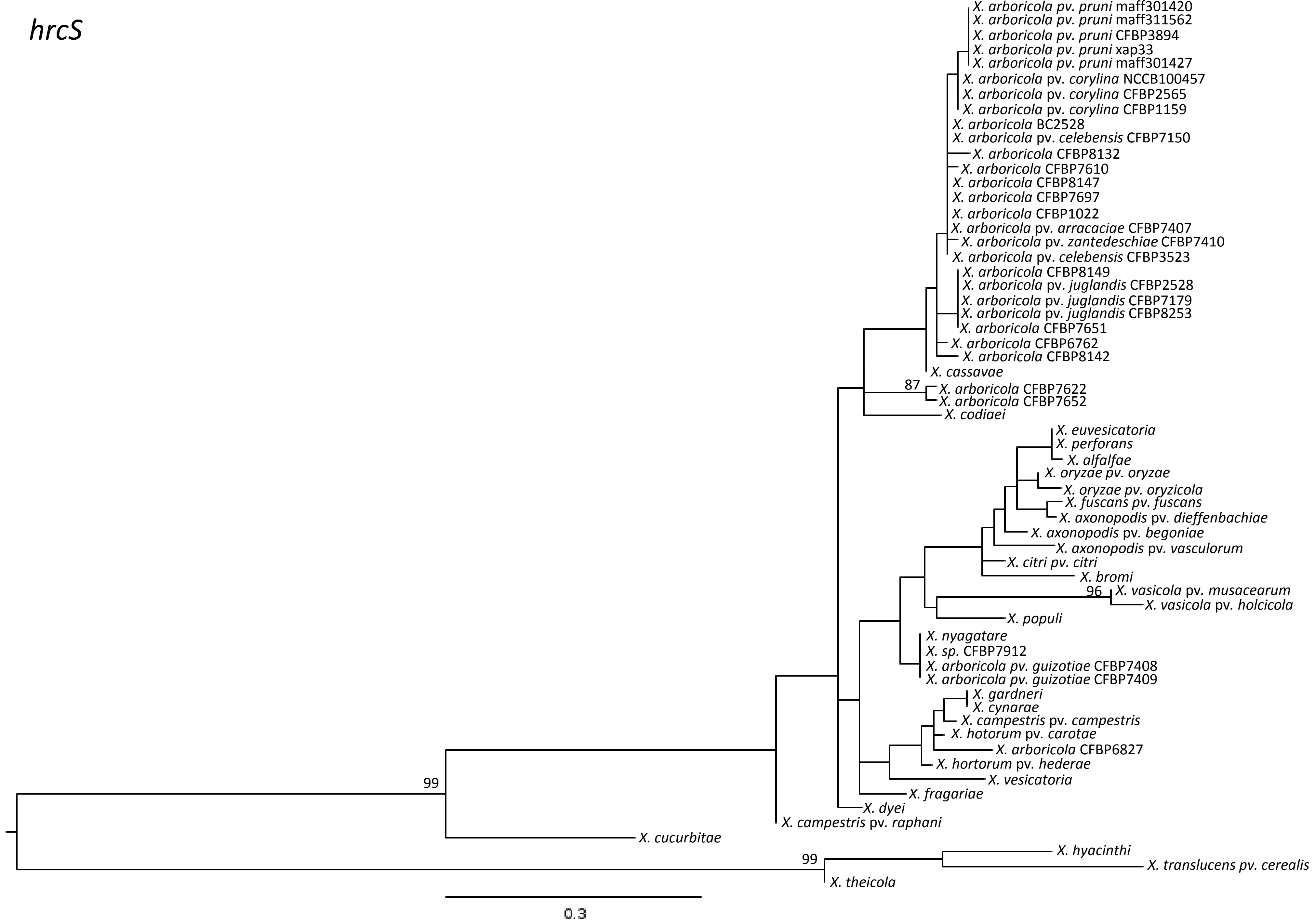
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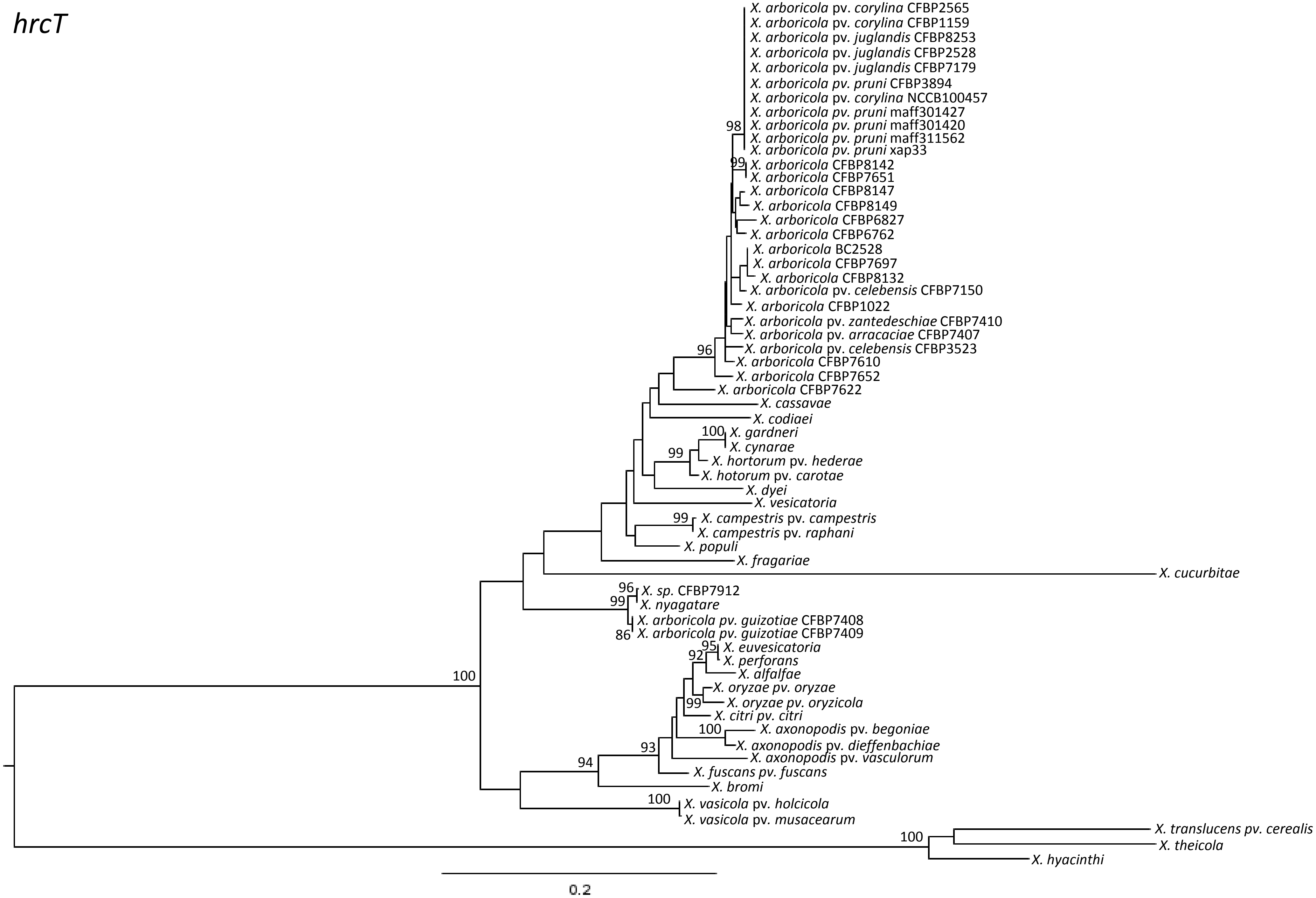
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99
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86
X. arboricola pv. guizotiae CFBP7409
95
X. euvesicatoria
92
X. perforans
X. alfalfae
X. oryzae pv. oryzae
99
X. oryzae pv. oryzicola
X. citri pv. citri
100
X. axonopodis pv. begoniae
X. axonopodis pv. dieffenbachiae
93
X. axonopodis pv. vasculorum
94
X. fuscans pv. fuscans
X. bromi
100
X. vasicola pv. holcicola
X. vasicola pv. musacearum
100
X. translucens pv. cerealis
X. theicola
X. hyacinthi
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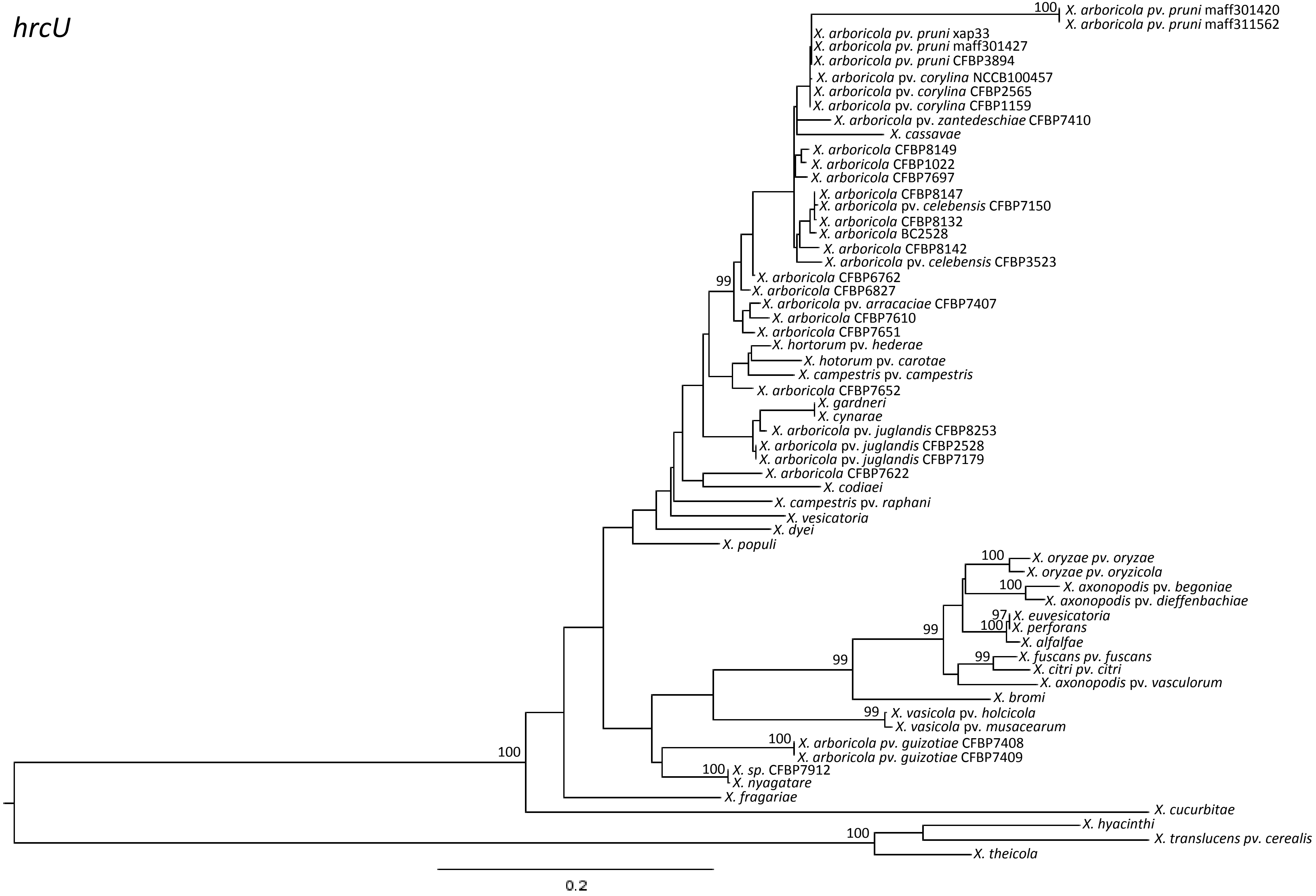
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X. arboricola pv. pruni CFBP3894
X. arboricola pv. corylina NCCB100457
X. arboricola pv. corylina CFBP2565
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X. arboricola CFBP8149
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X. arboricola CFBP8147
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X. arboricola CFBP8132
X. arboricola BC2528
X. arboricola CFBP8142
X. arboricola pv. celebensis CFBP3523
99
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X. arboricola CFBP6827
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X. arboricola CFBP7651
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X. hotorum pv. carotae
X. campestris pv. campestris
X. arboricola CFBP7652
X. gardneri
X. cynarae
X. arboricola pv. juglandis CFBP8253
X. arboricola pv. juglandis CFBP2528
X. arboricola pv. juglandis CFBP7179
X. arboricola CFBP7622
X. codiaei
X. campestris pv. raphani
X. vesicatoria
X. dyei
X. populi
100
X. oryzae pv. oryzae
X. oryzae pv. oryzicola
100
X. axonopodis pv. begoniae
X. axonopodis pv. dieffenbachiae
97
X. euvesicatoria
100
X. perforans
X. alfalfae
99
99
X. fuscans pv. fuscans
X. citri pv. citri
X. axonopodis pv. vasculorum
99
X. bromi
99
X. vasicola pv. holcicola
X. vasicola pv. musacearum
100
X. arboricola pv. guizotiae CFBP7408
X. arboricola pv. guizotiae CFBP7409
100
X. sp. CFBP7912
X. nyagatare
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X. fragariae
X. cucurbitae
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X. hyacinthi
X. translucens pv. cerealis
X. theicola
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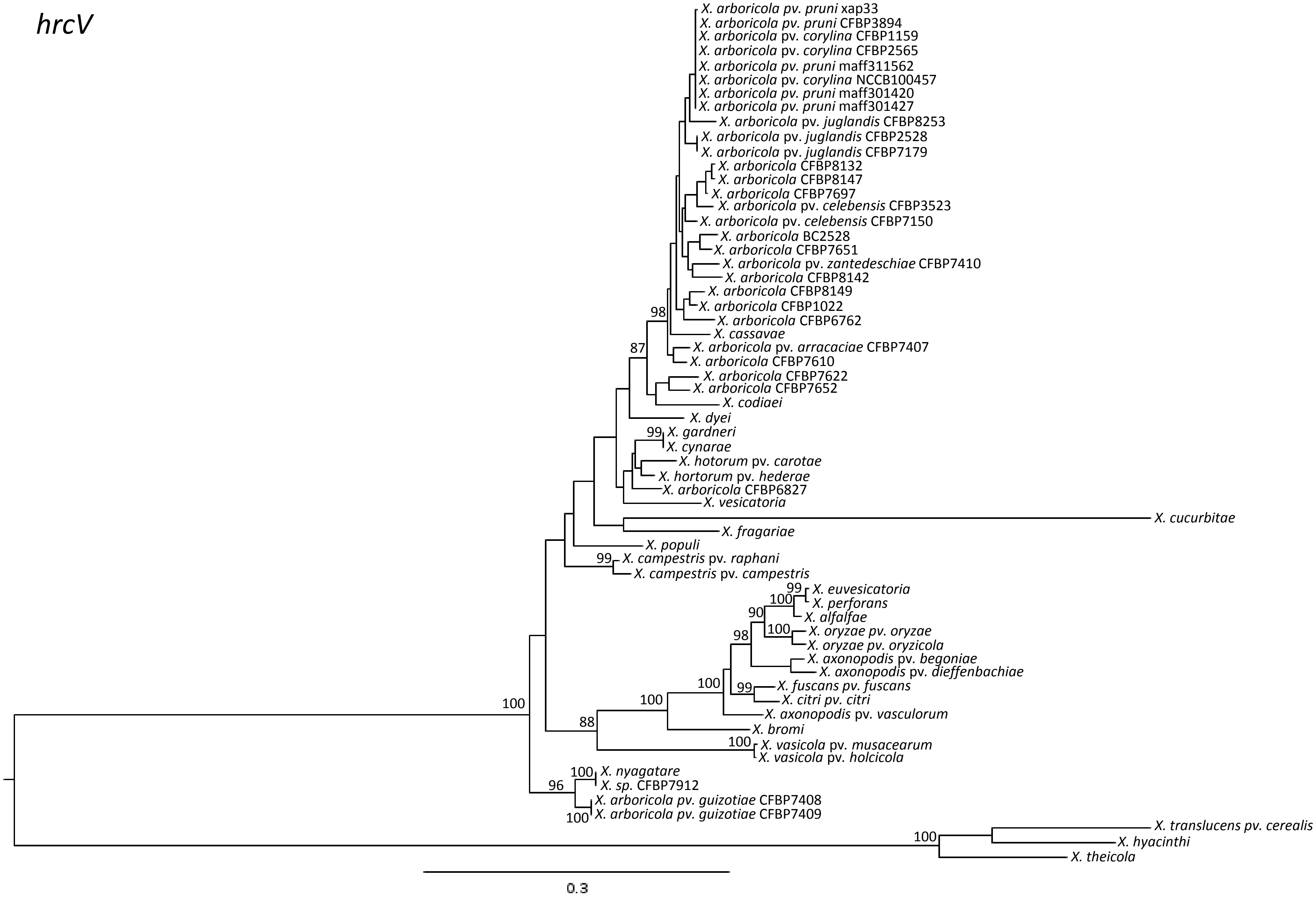
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X. arboricola pv. pruni maff301420
X. arboricola pv. pruni maff301427
X. arboricola pv. juglandis CFBP8253
X. arboricola pv. juglandis CFBP2528
X. arboricola pv. juglandis CFBP7179
X. arboricola CFBP8132
X. arboricola CFBP8147
X. arboricola CFBP7697
X. arboricola pv. celebensis CFBP3523
X. arboricola pv. celebensis CFBP7150
X. arboricola BC2528
X. arboricola CFBP7651
X. arboricola pv. zantedeschiae CFBP7410
X. arboricola CFBP8142
X. arboricola CFBP8149
X. arboricola CFBP1022
98
X. arboricola CFBP6762
X. cassavae
87
X. arboricola pv. arracaciae CFBP7407
X. arboricola CFBP7610
X. arboricola CFBP7622
X. arboricola CFBP7652
X. codiaei
X. dyei
99
X. gardneri
X. cynarae
X. hotorum pv. carotae
X. hortorum pv. hederae
X. arboricola CFBP6827
X. vesicatoria
X. cucurbitae
X. fragariae
X. populi
99
X. campestris pv. raphani
X. campestris pv. campestris
99
X. euvesicatoria
100
X. perforans
90
X. alfalfae
100
X. oryzae pv. oryzae
98
X. oryzae pv. oryzicola
X. axonopodis pv. begoniae
X. axonopodis pv. dieffenbachiae
100
99
X. fuscans pv. fuscans
100
X. citri pv. citri
100
X. axonopodis pv. vasculorum
88
X. bromi
100
X. vasicola pv. musacearum
X. vasicola pv. holcicola
100
X. nyagatare
96
X. sp. CFBP7912
X. arboricola pv. guizotiae CFBP7408
100
X. arboricola pv. guizotiae CFBP7409
100
X. translucens pv. cerealis
X. hyacinthi
X. theicola
0.3

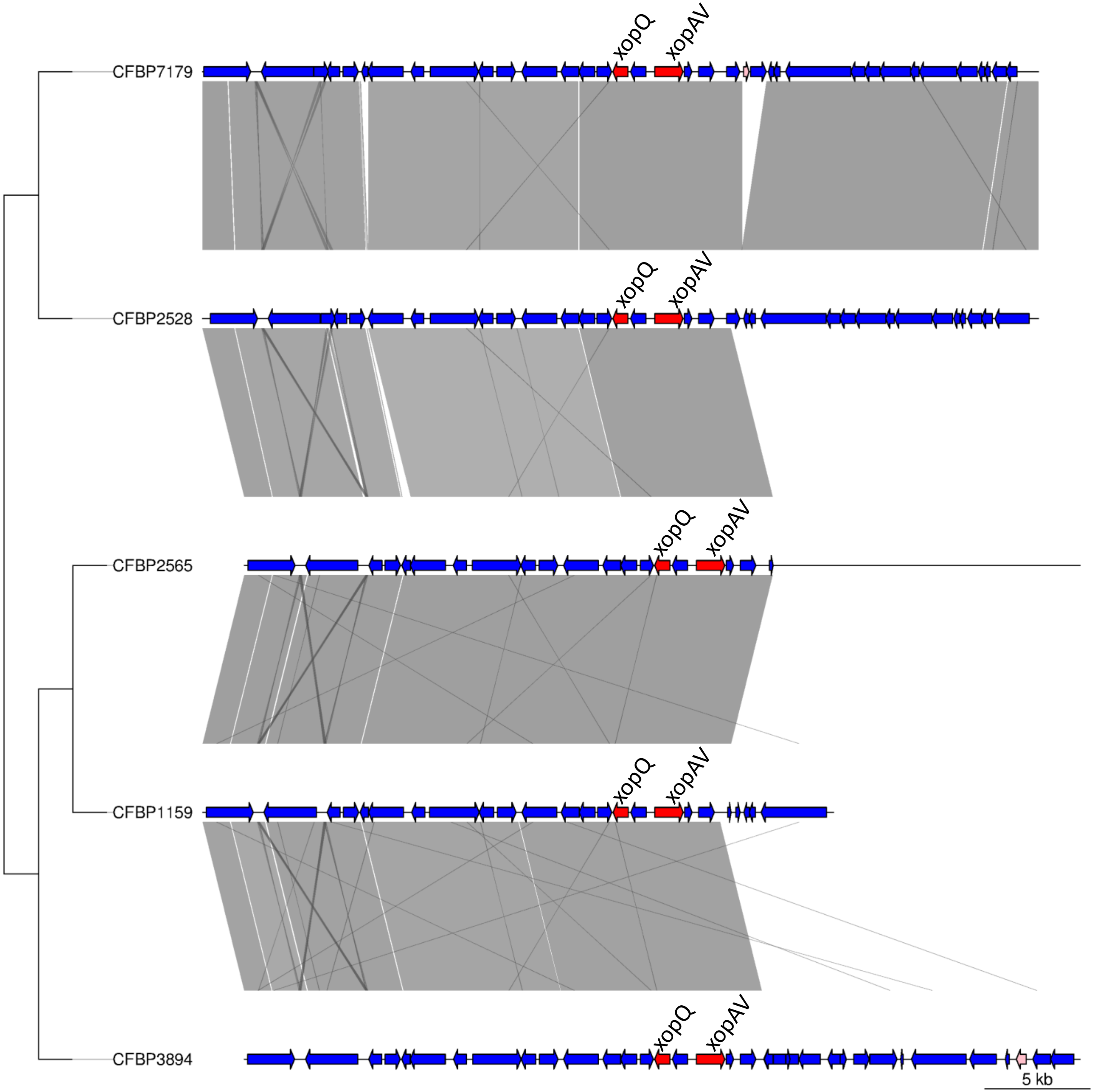
Supplementary Figure S4

Supplementary Figure S4: Genomic environments of *avrBs2* in *Xanthomonas arboricola* strains. On the left, the dendrogram corresponds to the phylogenetic relationship between strains inferred from the core genome. On the right, genomic environments of *avrBs2* insertion site, within a window of 20 kb upstream and downstream of *avrBs2*. Red arrows represent *avrBs2*. Pink arrows represent genes encoding transposases. Blue arrows represent other genes within genomic environments. DNA fragments sharing similarities are connected with gray shading.


CFBP8147
avrBs2
CFBP8132
avrBs2
CFBP7150
avrBs2
CFBP8142
avrBs2
CFBP7697
avrBs2
BC2528
avrBs2
CFBP6762
avrBs2
CFBP7410
avrBs2
CFBP1022
avrBs2
CFBP3523
avrBs2
CFBP7651
avrBs2
CFBP8149
avrBs2
CFBP7634
avrBs2
CFBP7629
avrBs2
CFBP7652
avrBs2
CFBP7610
avrBs2
CFBP6827
avrBs2
CFBP7604
CFBP8150
CFBP6771
CFBP8153
CFBP8130
CFBP8138
CFBP7614
CFBP7407
avrBs2
CFBP7179
avrBs2
CFBP2528
avrBs2
X12710
avrBs2
CFBP1159
avrBs2
CFBP2565
avrBs2
CFBP3894
avrBs2
CFBP7409
tRNA
avrBs2
CFBP7408
tRNA
avrBs2
CFBP7645
CFBP8152
CFBP3123
CFBP3122
CFBP7653
CFBP7622
5 kb

Supplementary Figure S5

Supplementary Figure S5: Genomic environments of 10 predicted type three effector (T3E) genes specific to *Xanthomonas arboricola* group A strains. On the left, the dendrogram corresponds to the phylogenetic relationship between strains. On the right, genomic environments of predicted T3E genes, within a window of 20 kb upstream and downstream of the gene, are represented. Red arrows represent predicted T3E genes. Pink arrows represent genes encoding transposases. Green arrows represent phages. Blue arrows represent other genes within genomic environments. DNA fragments sharing similarities are connected with gray shading.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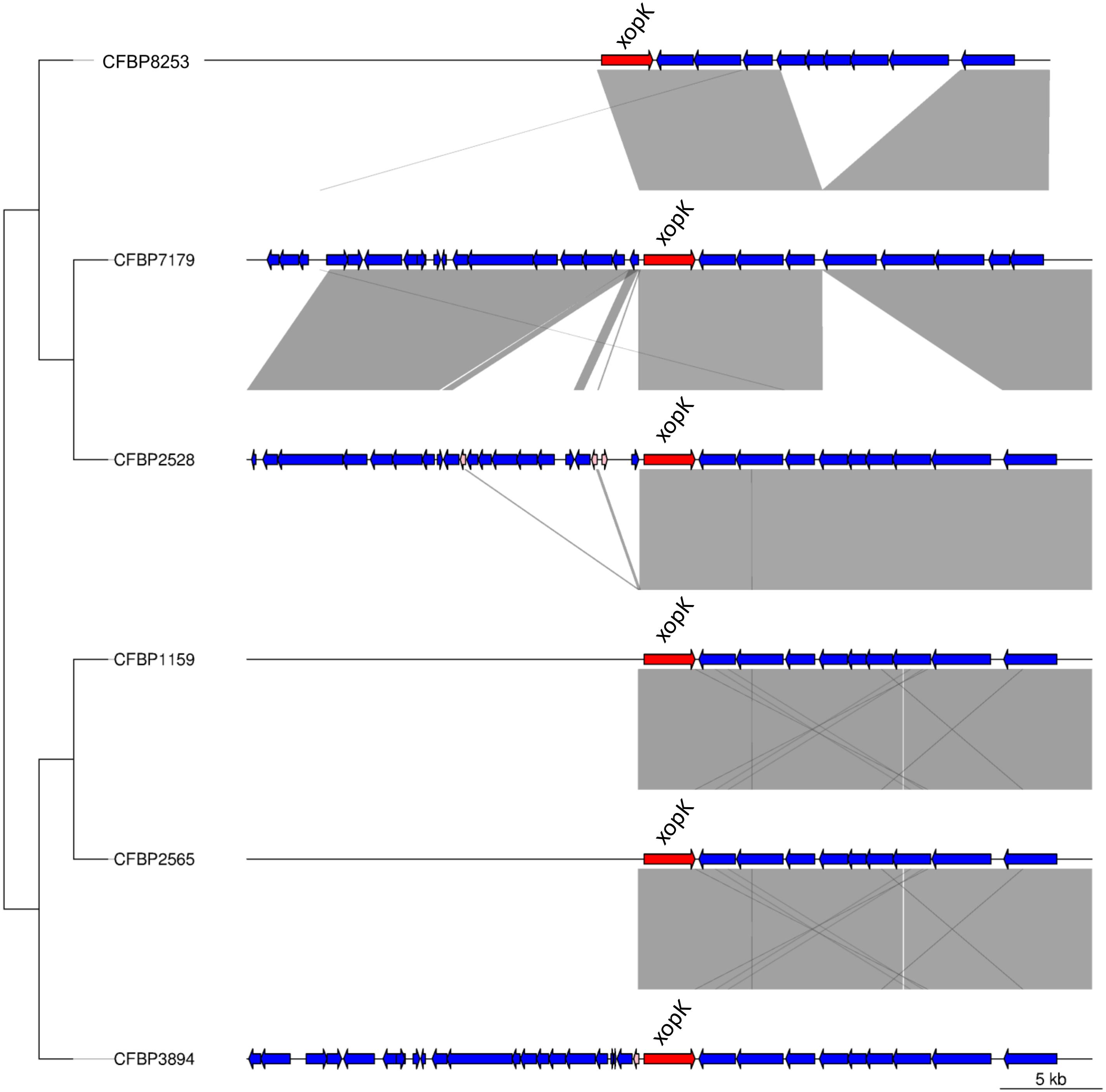
xopAV
CFBP7179
CFBP2528
CFBP2565
CFBP1159
CFBP3894
5 kb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CFBP8253
xopK
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CFBP2528
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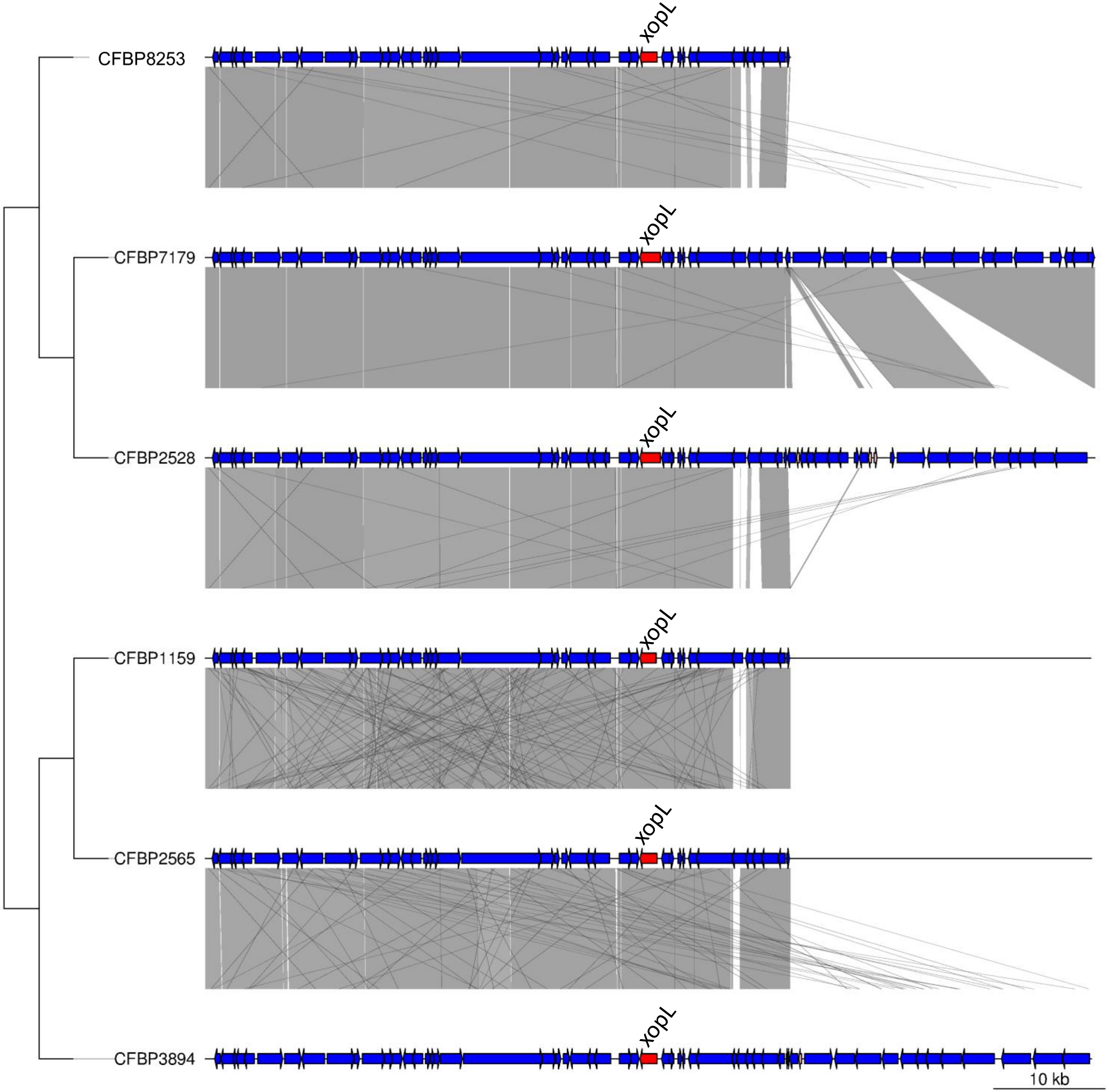
CFBP3894
5 kb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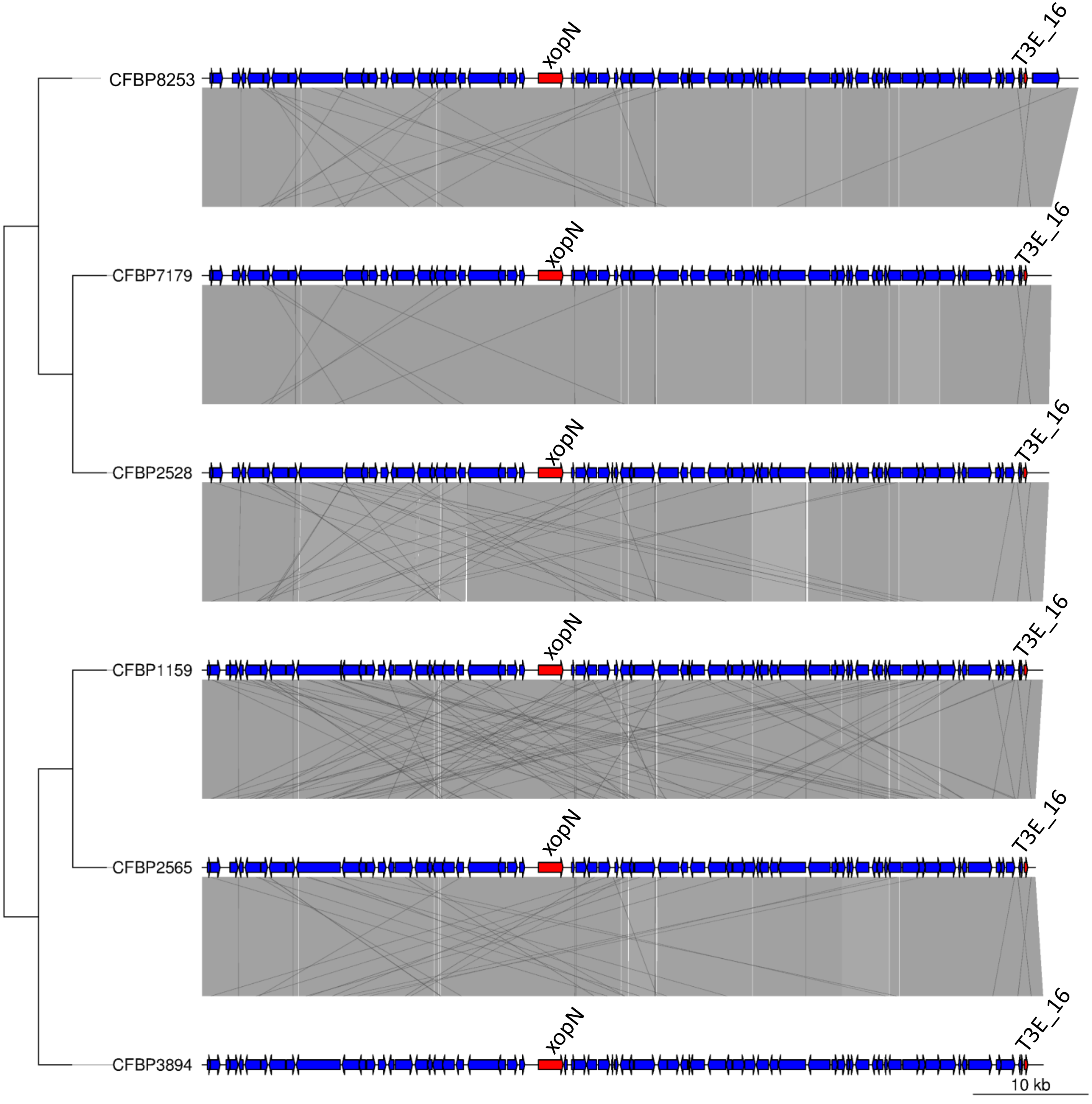
CFBP8253
xopL
CFBP7179
xopL
CFBP2528
xopL
CFBP1159
xopL
CFBP2565
xopL
CFBP3894
10 kb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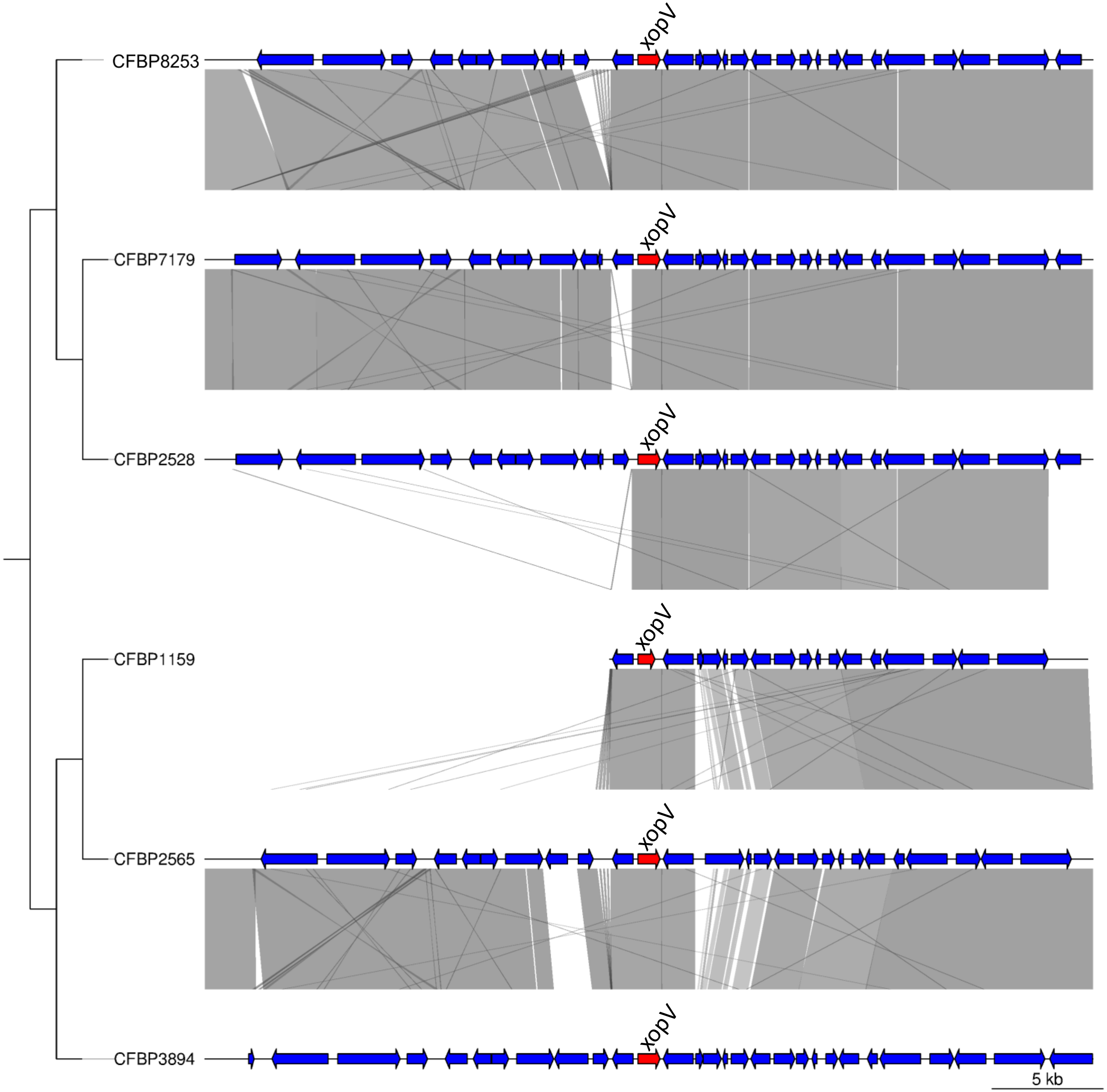
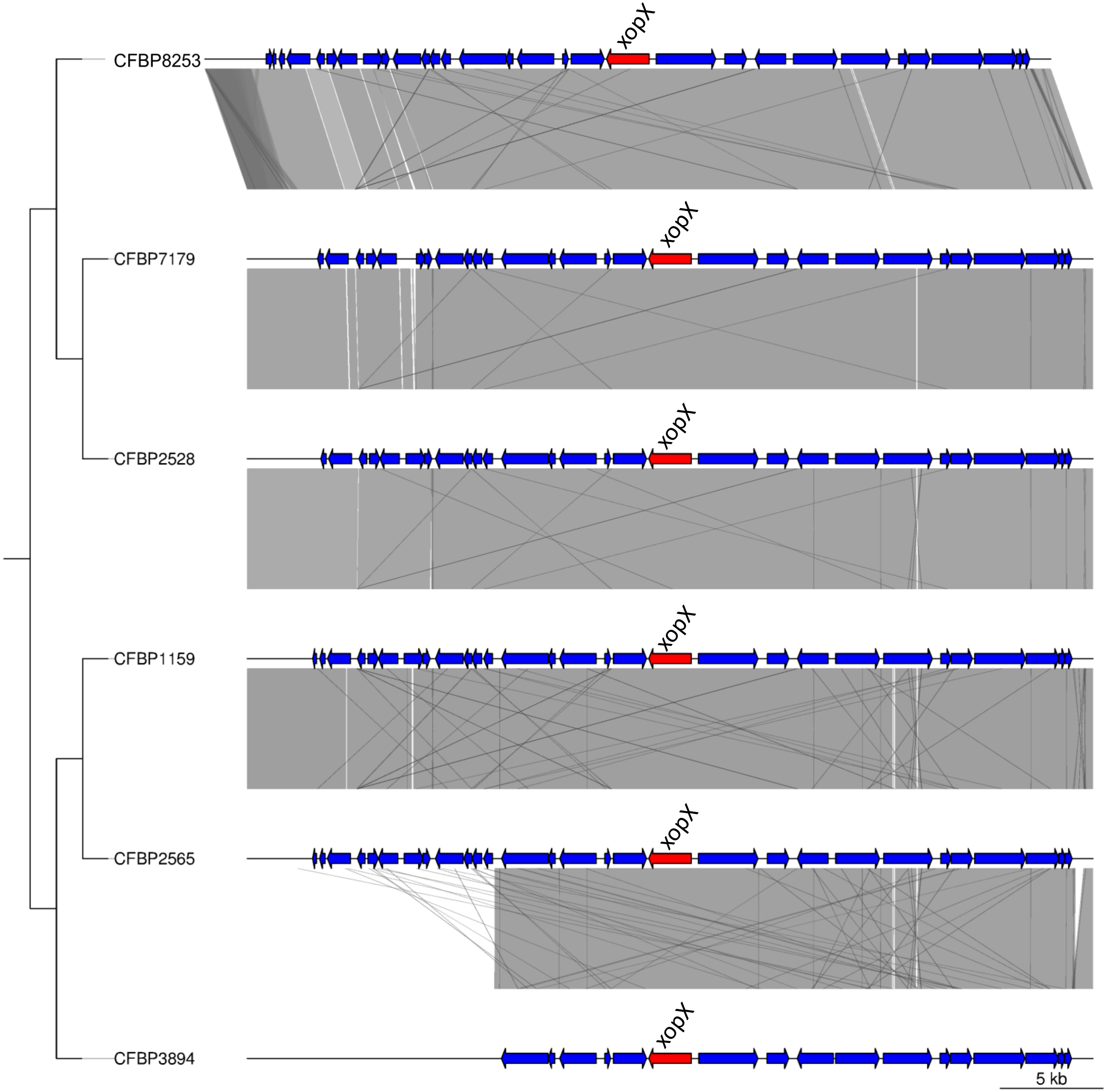
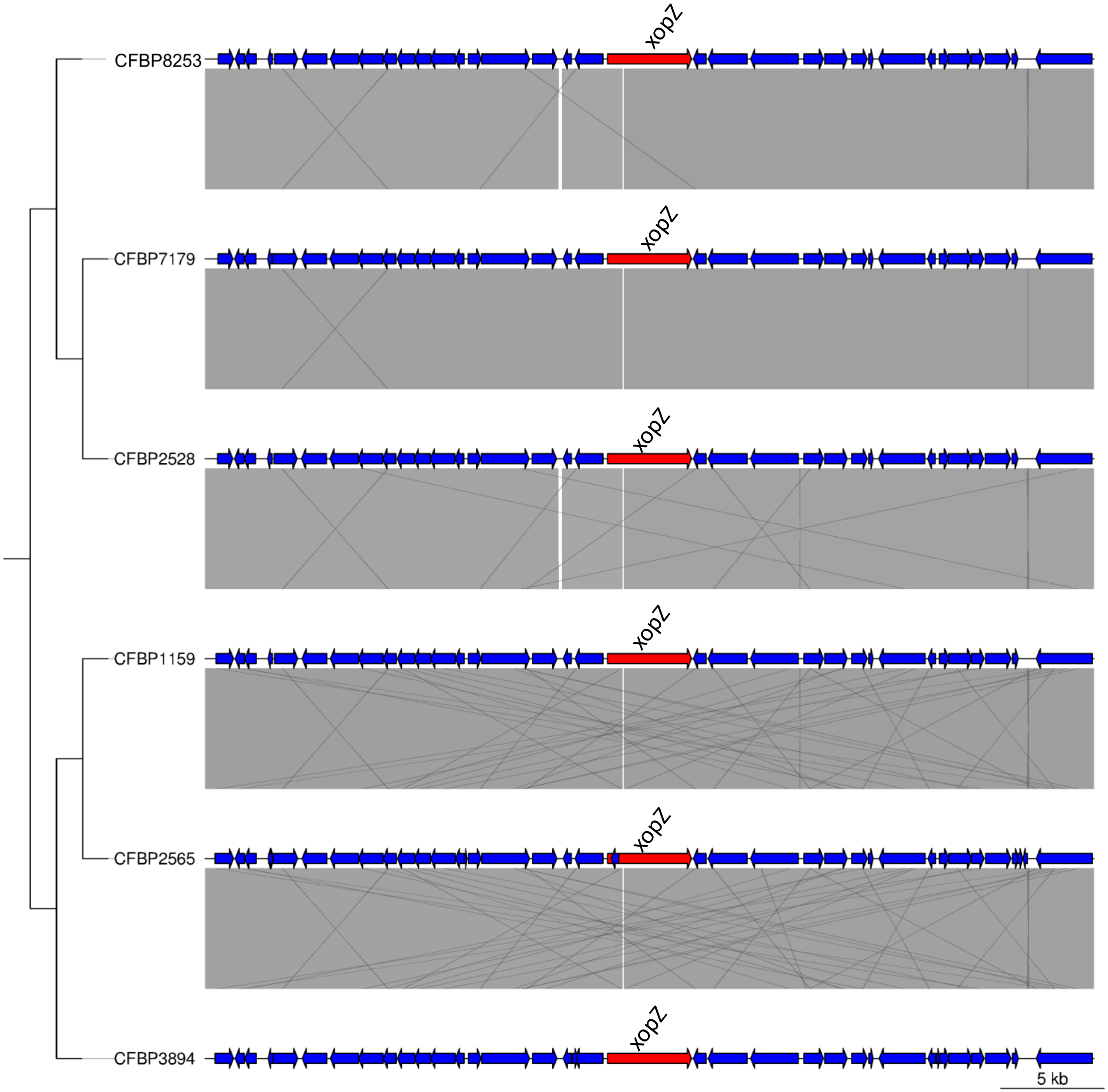
T3E_16
CFBP8253
CFBP7179
CFBP2528
CFBP1159
CFBP2565
CFBP3894
10 kb


xopV
CFBP8253
xopV
CFBP7179
xopV
CFBP2528
xopV
CFBP1159
xopV
CFBP2565
xopV
CFBP3894
5 kb



CFBP8253
xopX
CFBP7179
xopX
CFBP2528
xopX
CFBP1159
xopX
CFBP2565
xopX
CFBP3894
xopX
5 kb


xopZ
CFBP8253
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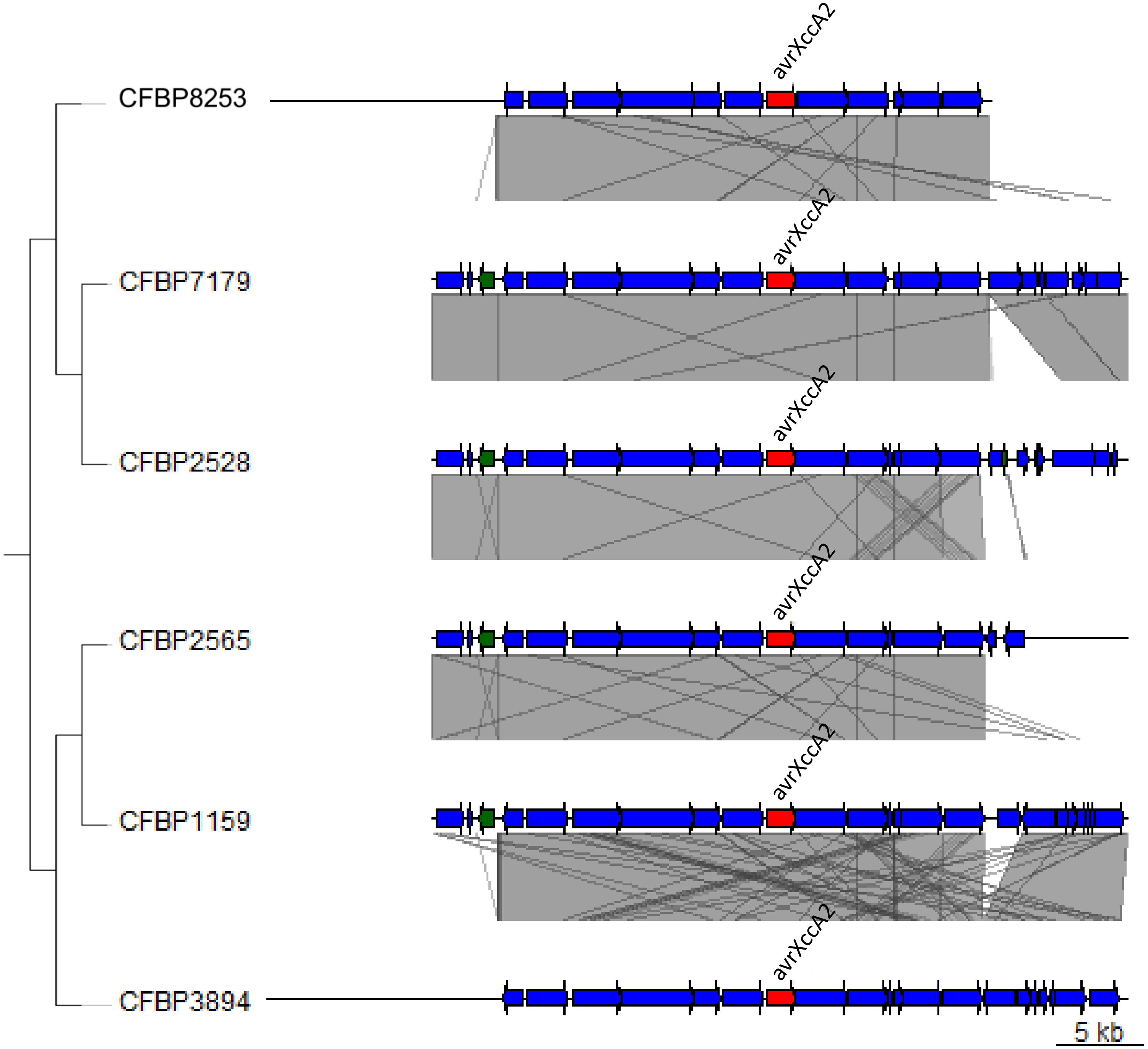
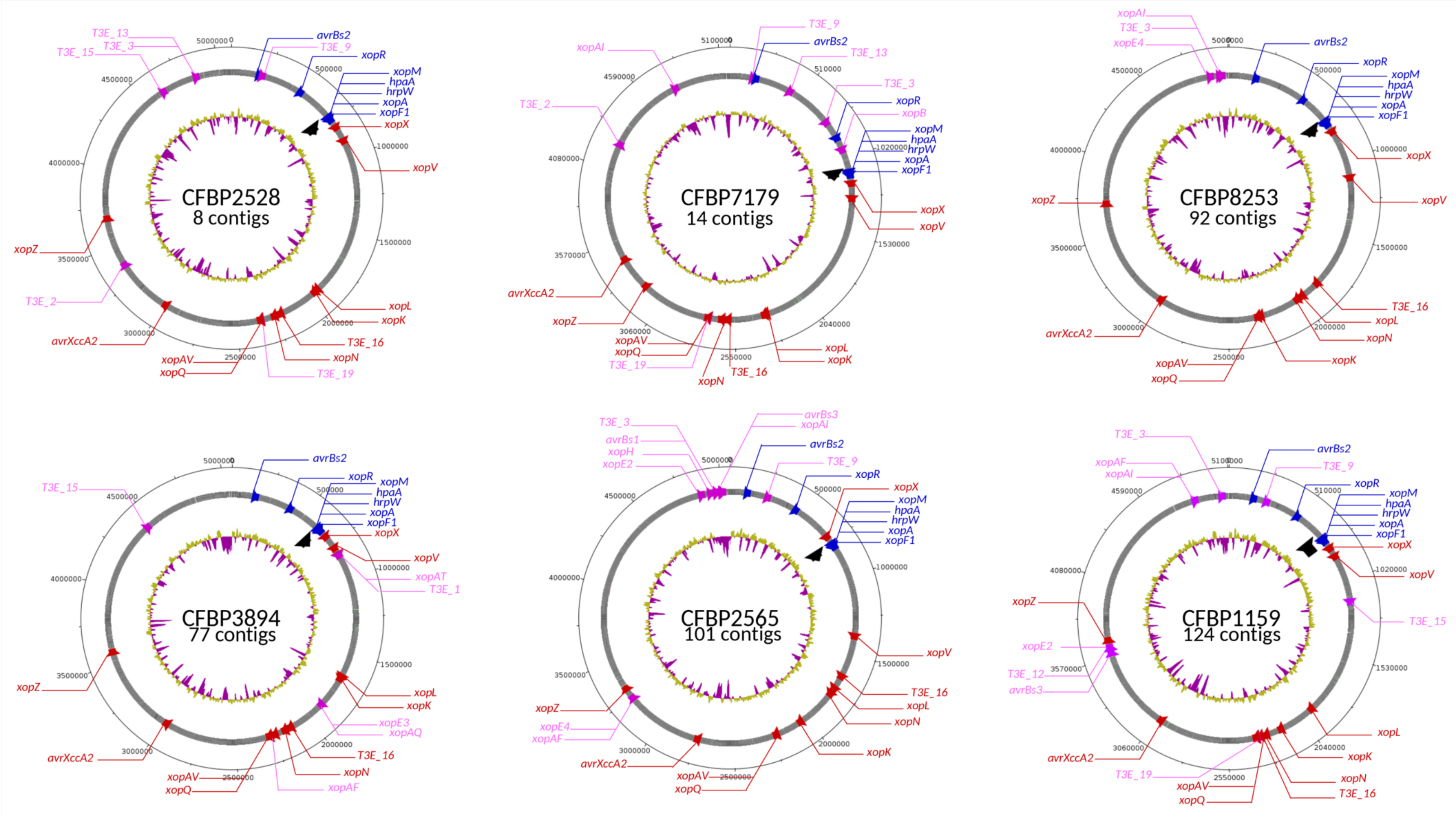
CFBP1159
CFBP2565
CFBP3894
5 kb


avrXccA2
CFBP8253
CFBP7179
CFBP2528
CFBP2565
CFBP1159
CFBP3894
5 kb



Supplementary Figure S6: Graphical circular representation of the draft genomes of *X. arboricola* strains belonging to the three successful pathovars : pv. *juglandis* (CFBP 2528, CFBP 7179, CFBP 8253), pv. *pruni* (CFBP 3894) and pv. *corylina* (CFBP 2565, CFBP 1159). The contigs were ordered by Mauve using the CFBP 2528 genome sequence as reference. The blue arrows represent the core T3E genes, the red arrows represent the 10 TE3 genes acquired by the common ancestor of these strains, the pink arrows represent the T3E genes independently acquired by the strains. The black arrows represent the T3SS coding genes.

Table S1 : Results of Shimodaira-Hasegawa tests comparing phylogenies of T3SS coding genes in *Xanthomonas*

		H1 : topologies alternatives															
		hrc_concat	*hrcC*	*hrcD*	*hrcJ*	*hrcN*	*hrcQ*	*hrcR*	*hrcS*	*hrcT*	*hrcU*	*hrcV*	*hrpE*	*hrpB1*	*hrpB2*	*hrpB4*	*hrpB7*
H0: alignement	hrc_concat	0,96034	0	0,88536	0,04023	0	0,00083	0,16132	0,00578	0,20897	0,0206	0,00684	0,02441	0,03873	0,11108	0,3825	0,01626
	hrcC	0,37406	0,91395	0,34218	0,00832	0,52414	0,72117	0,65727	0,00259	0,26576	0,0002	0,46417	0	0,94543	0,89079	0,82633	0,7081
	hrcD	0,00025	0	0,94864	0,43053	0	0,04018	0,00005	0	0,00233	0	0,12352	0	0	0,41333	0,3802	0,00036
	hrcJ	0,05185	0	0	0,92988	0,07593	0	0,02137	0,04262	0	0,29572	0,43796	0	0,29101	0,73003	0,46092	0,02644
	hrcN	0	0,00001	0,00012	0,05916	0,91767	0,23817	0,03422	0,33393	0,0009	0,00322	0,27644	0	0,53221	0,42616	0,16445	0,09918
	hrcQ	0	0	0,85718	0,94507	0,9462	0,95422	0,69799	0,1154	0	0,08249	0,8301	0	0,76662	0,75029	0,90246	0,78262
	hrcR	0	0,01155	0,19194	0,35578	0,66565	0,28707	0,90366	0,27447	0,73229	0,08124	0,21551	0	0,32863	0,97158	0,02542	0,55015
	hrcS	0	0	0,00005	0,00029	0,00041	0,05747	0,0254	0,76005	0,0018	0,11881	0,01921	0	0,00016	0,00014	0,00008	0,0001
	hrcT	0	0	0,03912	0,00017	0,00778	0,06186	0,0903	0,17226	0,90986	0,30794	0,51963	0,00227	0,66663	0,19137	0,80863	0,72965
	hrcU	0	0	0,00026	0,03987	0,00043	0	0,00025	0,92947	0,00792	0,8608	0,03076	0,92051	0,62439	0,20338	0,0045	0,00021
	hrcV	0	0	0,46891	0,13923	0,06054	0,03686	0,04388	0,49159	0,00934	0,00053	0,94393	0,05171	0,02328	0,00002	0,91582	0,46595
	hrpE	0	0,00187	0,01405	0	0,02687	0,00386	0	0,00001	0,00011	0,02048	0	0,8257	0,01079	0,08082	0	0,00646
	hrpB1	0	0,00572	0,03533	0,48392	0,2558	0,04064	0,30653	0,00634	0,00264	0,19341	0,01187	0	0,92934	0,11034	0,50696	0,68073
	hrpB2	0	0	0,06997	0,956	0,00082	0,02319	0,70404	0,00021	0,00054	0,92429	0,97765	0,03508	0,00551	0,96611	0,9265	0,62853
	hrpB4	0	0	0	0,95477	0,28745	0,00553	0,03089	0	0	0,02795	0,00873	0	0,44886	0,0748	0,86887	0,29673
	hrpB7	0	0	0,00349	0,00318	0,38469	0,6936	0,81701	0,90497	0,02205	0,56477	0,00167	0	0,67116	0,94118	0,01916	0,9435