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Supplementary information

An ancient ecospecies of *Helicobacter pylori*

Supplementary Figures

Supplementary Figure 1

Phylogenetic trees of the sequences of differentiated genes that returned at least one hit when blasted against the *H. cetorum* genome. The trees can be separated into four main scenarios: (A) The two ecospecies diverged after the divergence between *H. cetorum* and *H. pylori*, but before the hpAfrica2 branch split (scenario 1). In this scenario, *H. cetorum* is the deepest branch in the tree with the next deepest branch separating Hardy and Ubiquitous strains. The position of the HpAfrica2 branch is variable, likely due to ongoing genetic exchange with other *H. pylori* populations which cooccur in the same human populations in Southern Africa [33]. (B) The divergence takes place after the split with hpAfrica2 (scenario 2). This scenario is similar to (A) except that *H. acinonychis* is the second deepest branch after *H. cetorum*, meaning that human and primate strains cluster together. (C) Presence of gene flow (scenario 3). In this scenario, there are distinct Hardy and Ubiquitous clusters, but some Hardy or Ubiquitous strains have the other type. (D) Variant of scenario 1 but two copies are present. (E) The polymorphism is older than the divergence with *H. cetorum* (scenario 4). In this case, the deepest branch of the tree is between Hardy and Ubiquitous, with *H. cetorum* clustering with one or other.

SupplementaryFigure 2

ML phylogenetic tree of *vacA*, based on nucleotide sequences from complete *H. pylori* genomes and *H. cetorum* (AFI05407.1). The branches are colored based on their population and the Hardy strains are represented with a dot. Contrary to the *vacA* tree that is shown in **Extended Data Fig. 10D**, this tree was built using only complete genomes (in most of the non-complete genomes, *vacA* appears to be highly fragmented due to assembly issues). Only *one* copy of vacA from *H. cetorum* was included as they all clustered on the same branch. The leaves are labeled with strain name, plus the number of the copy of the gene in that strain (i.e., .1, and if there are more than one copy, .2 and .3). Note that, for the Hardy strains, the copies that are closer to the root (generally one copy per Hardy strain) are more *H. pylori*-like than the copies farther away.

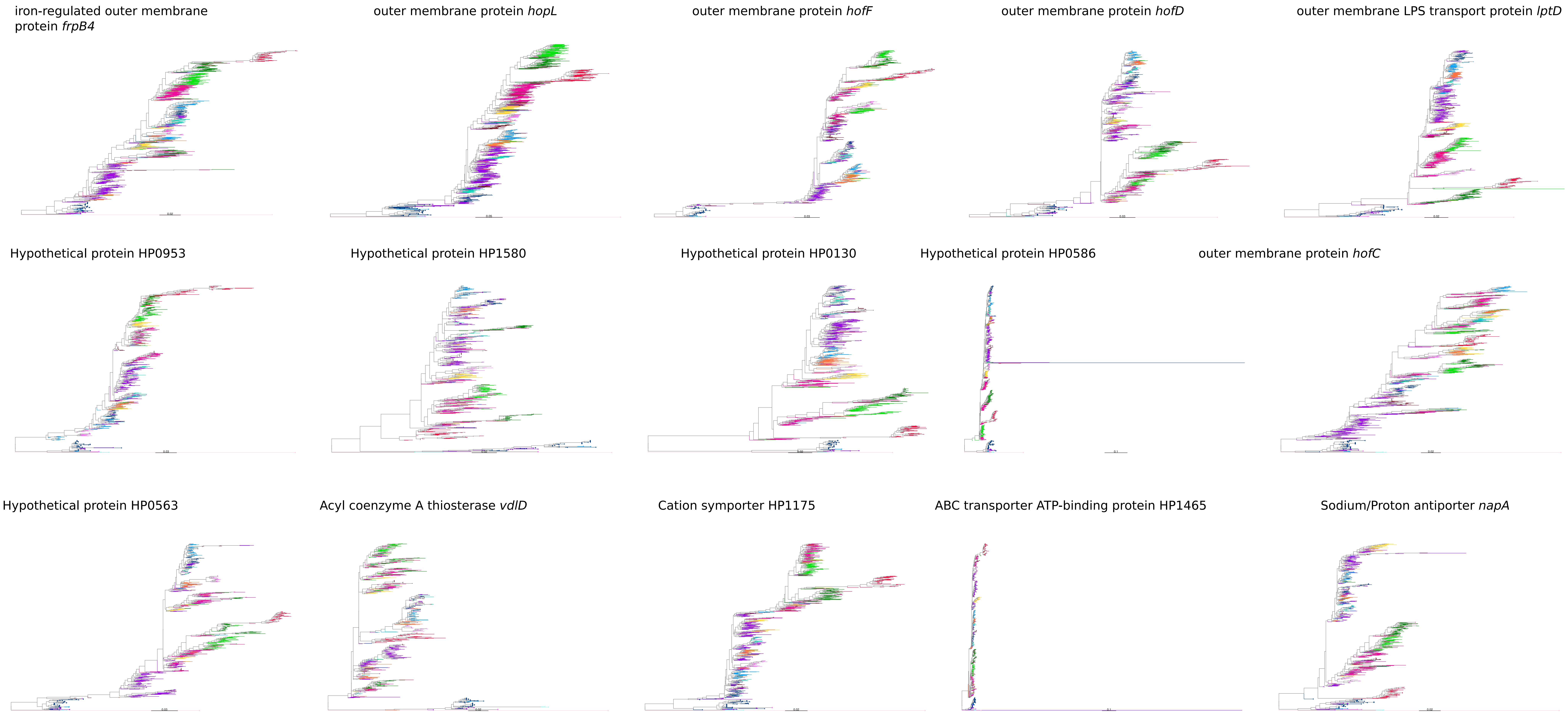
Supplementary Figure 3

Phylogenetic tree of *ureA* sequences for a sample of genomes. (A) For all *ureA* copies, (B) for the first copy, which is conserved in all *H. pylori* strains and (C) for the second copy that is only present in the Hardy strains and in some of the Ubiquitous strains from the same populations as the Hardy ones. The strains are colored based on their population, a dot at the end of the branch indicates a Hardy strain. *H. cetorum* sequences are also included.

Supplementary Figure 4

Phylogenetic tree of *ureB* sequences for a sample of genomes. (A) For all *ureB* copies, (B) for the first copy that is present in all strains and (C) for the second copy that is only present in the Hardy strains and in some of the Ubiquitous strains from the same populations as the Hardy ones. The strains are colored based on their population, a dot at the end of the branch indicates a Hardy strain. *H. cetorum* sequences are included.

A Scenario 1: The ecospecies diverged after *H. cetorum* but before the hpAfrica2 branch split



iron-regulated outer membrane protein *frpB4*

outer membrane protein *hopL*

outer membrane protein *hofF*

outer membrane protein *hofD*

outer membrane LPS transport protein *lptD*

Hypothetical protein HP0953

Hypothetical protein HP1580

Hypothetical protein HP0130

Hypothetical protein HP0586

outer membrane protein *hofC*

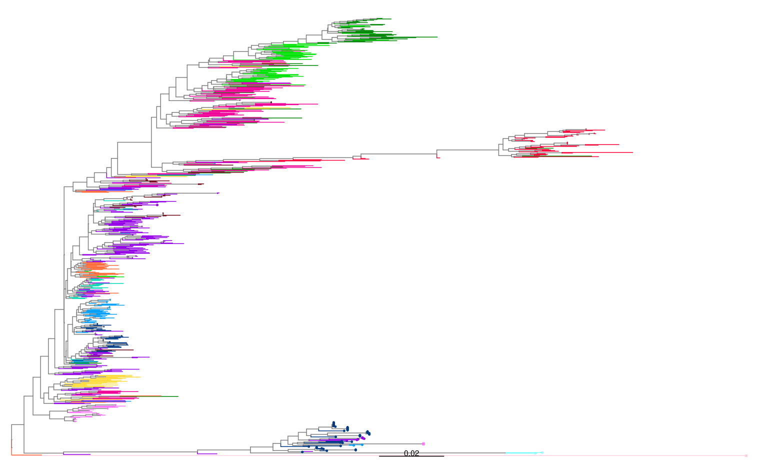
Hypothetical protein HP0563

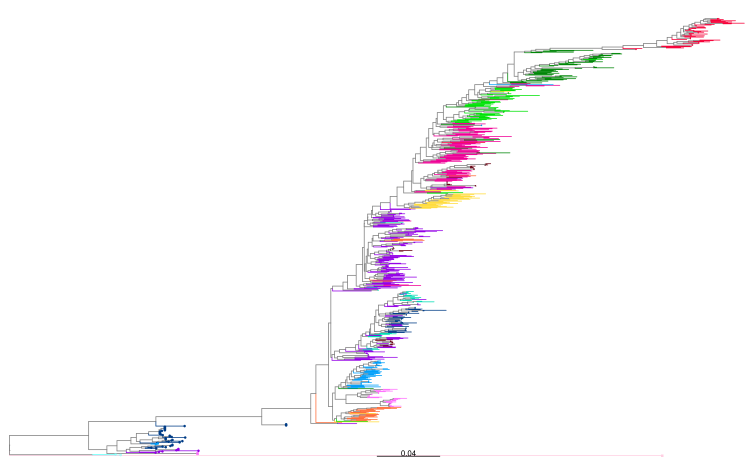
Acyl coenzyme A thiosterase *vdlD*

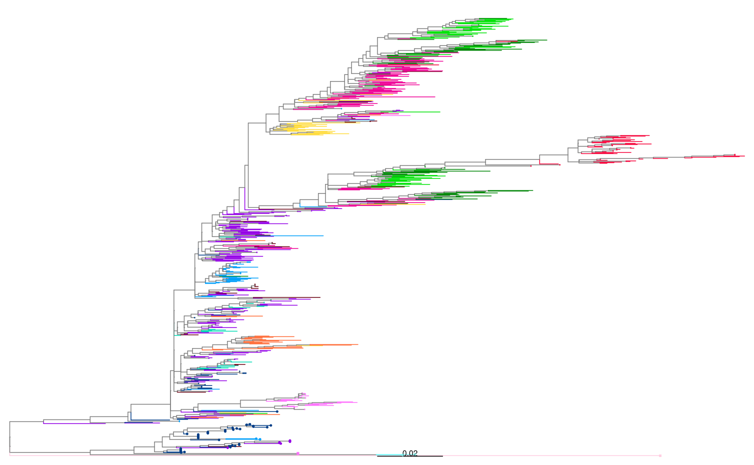
Cation symporter HP1175

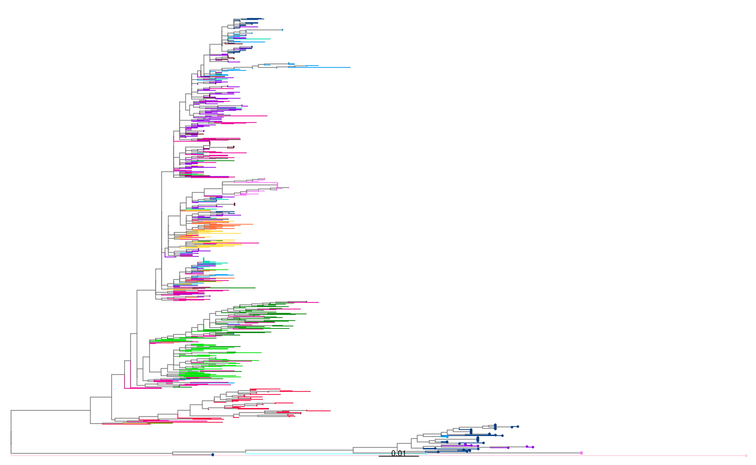
ABC transporter ATP-binding protein HP1465

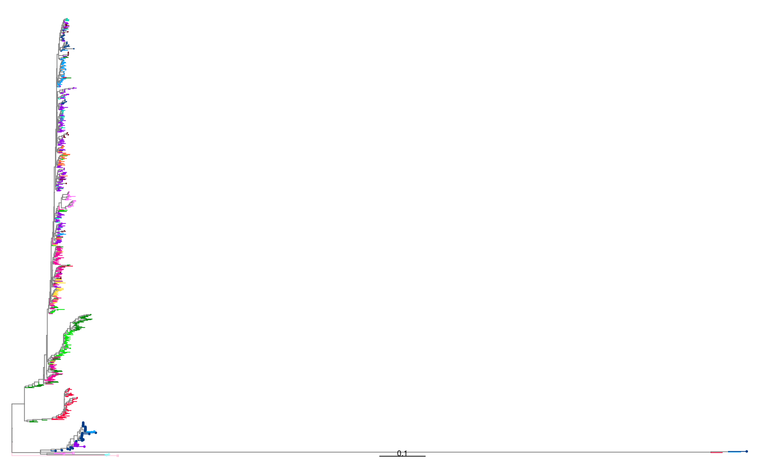
Sodium/Proton antiporter *napA*

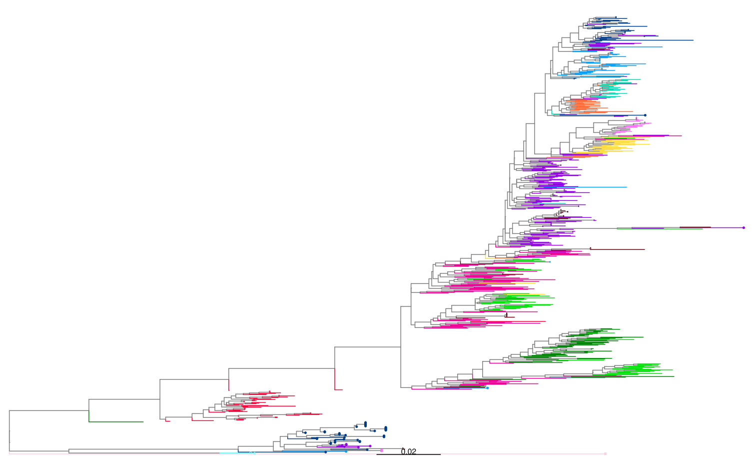

Gamma-glutamyltransferase *ggt*

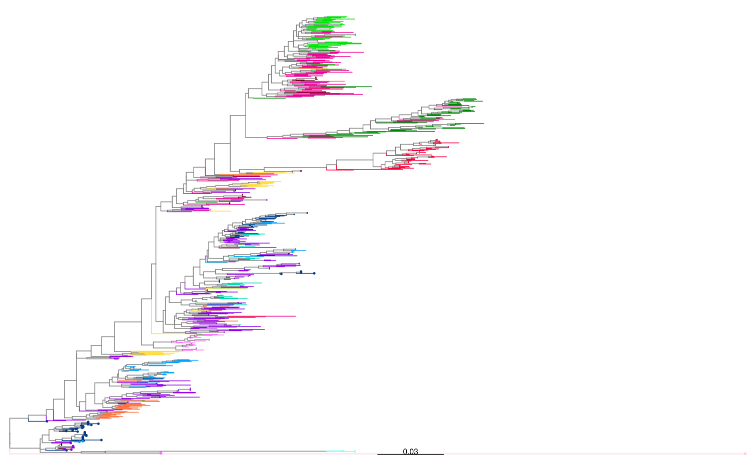

Perisplasmic competence protein *comH*

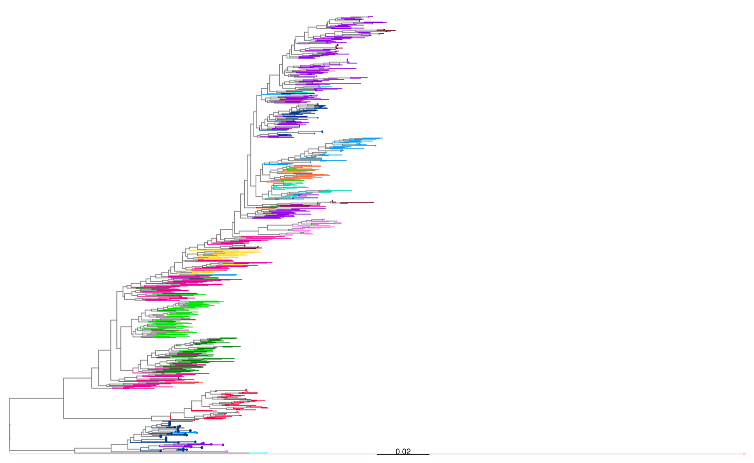

Magnesium and cobalt transport protein *corA*

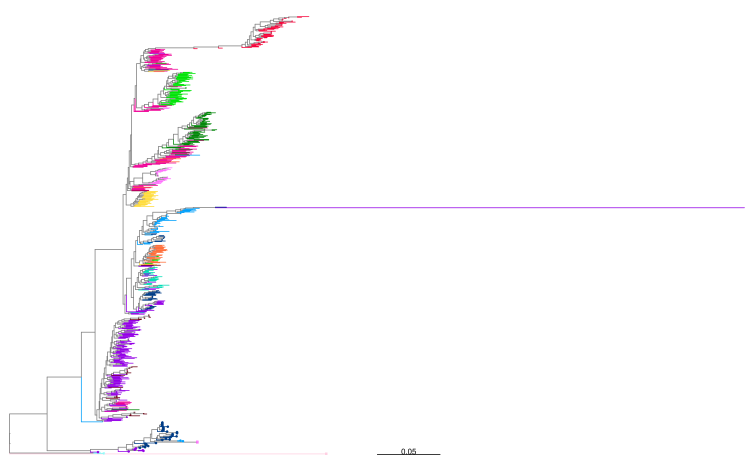

Response regulator *arsR*

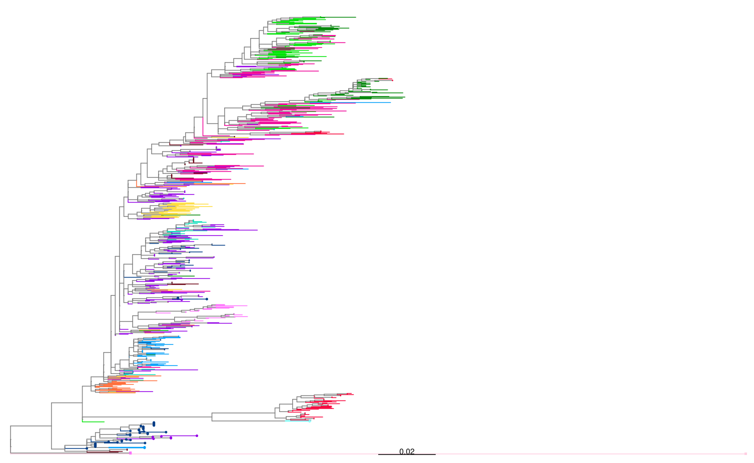

Cysteine-rich protein E %2C beta-lactamase *hcpE*


ATP-dependent nuclease *addB*

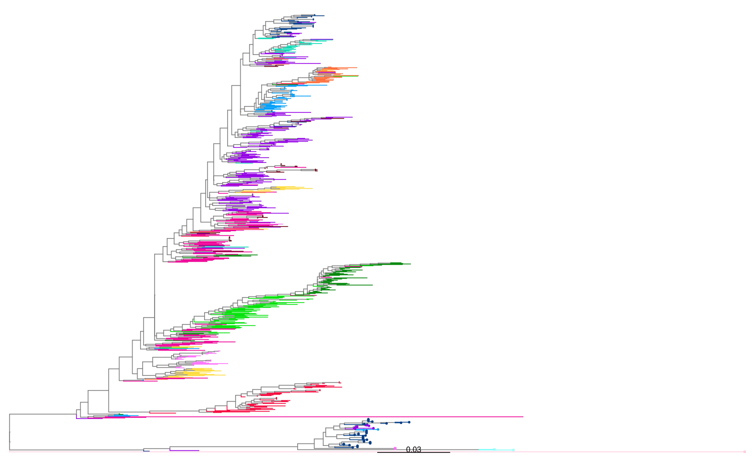

Chaperone *surA*

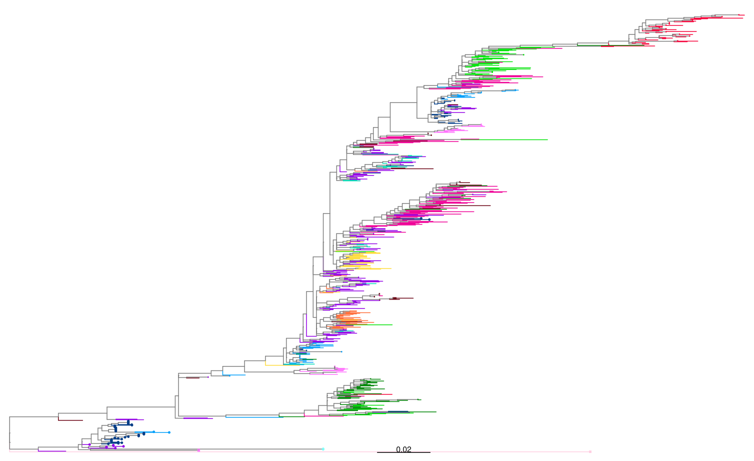

Serine protease *htrA*

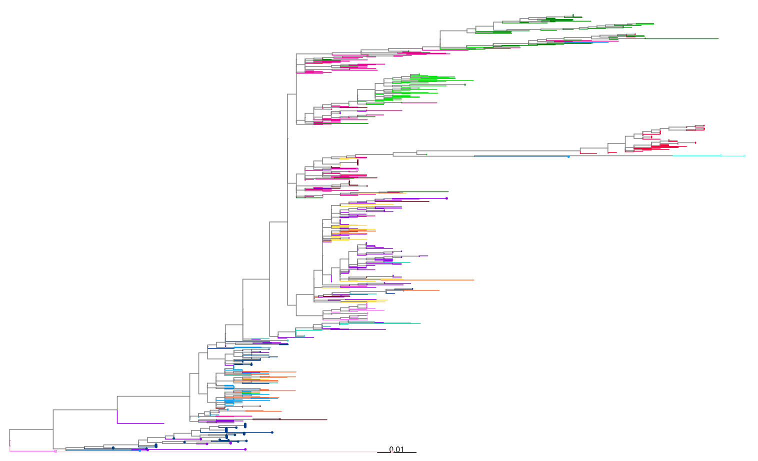

Protective surface antigen *D15*

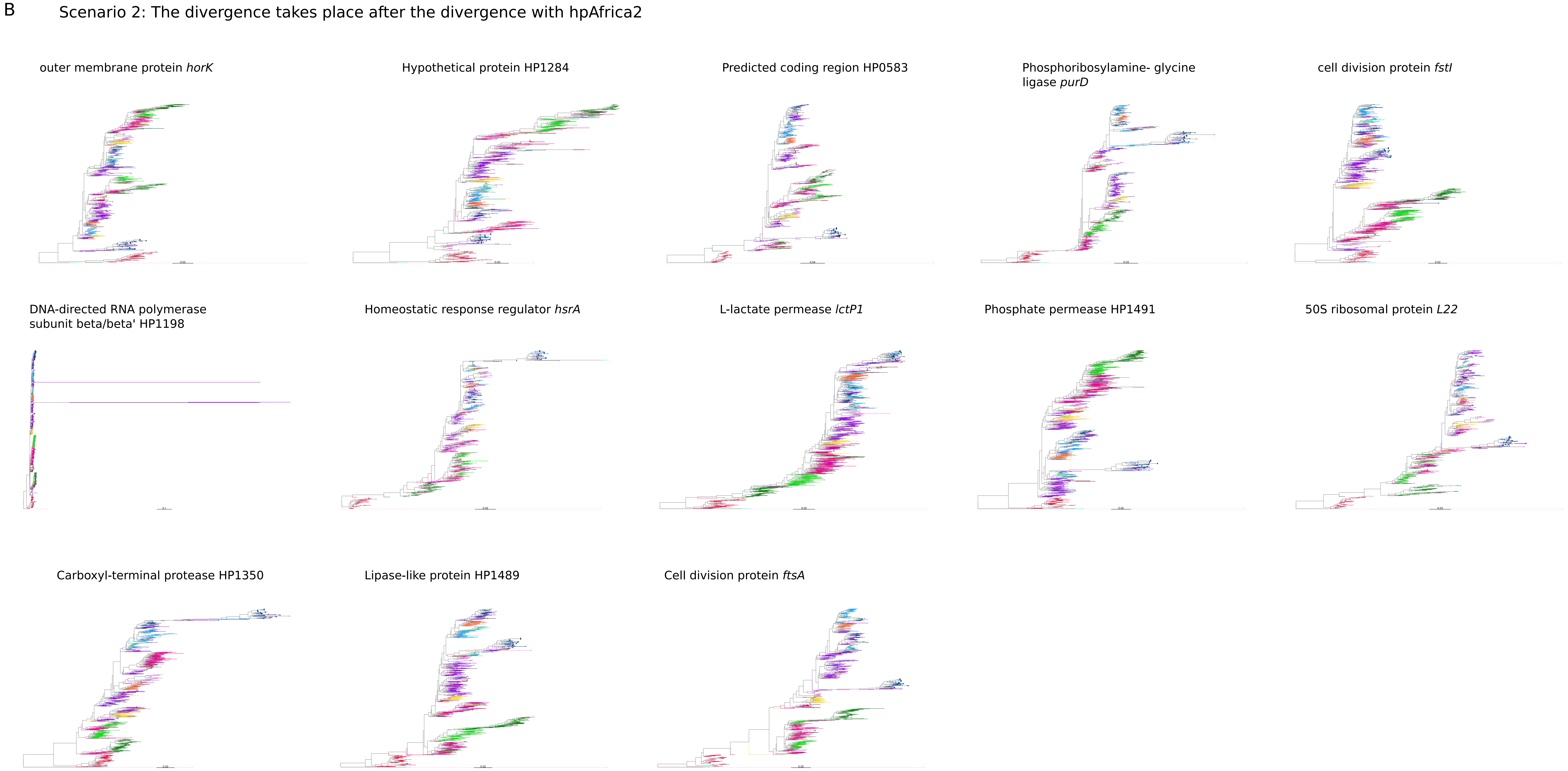

Putative glycerol-3-phosphate acyltransferase *plsY*


Glucose/galactose transporter *gluP*


ABC transporter permease HP1466


Disulfide isomerase HP0231


Ferric uptake regulation protein *fur*



B Scenario 2: The divergence takes place after the divergence with hpAfrica2

outer membrane protein *horK*

Hypothetical protein HP1284

Predicted coding region HP0583

Phosphoribosylamine- glycine ligase *purD*

cell division protein *fstI*

DNA-directed RNA polymerase subunit beta/beta' HP1198

Homeostatic response regulator *hsrA*

L-lactate permease *lctP1*

Phosphate permease HP1491

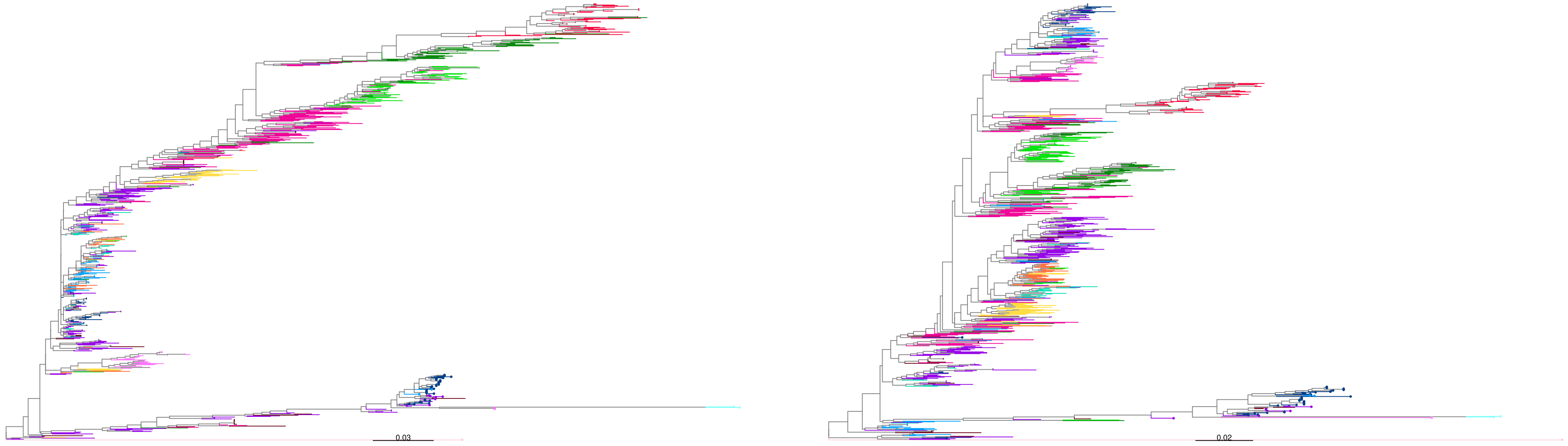
50S ribosomal protein *L22*

Carboxyl-terminal protease HP1350

Lipase-like protein HP1489

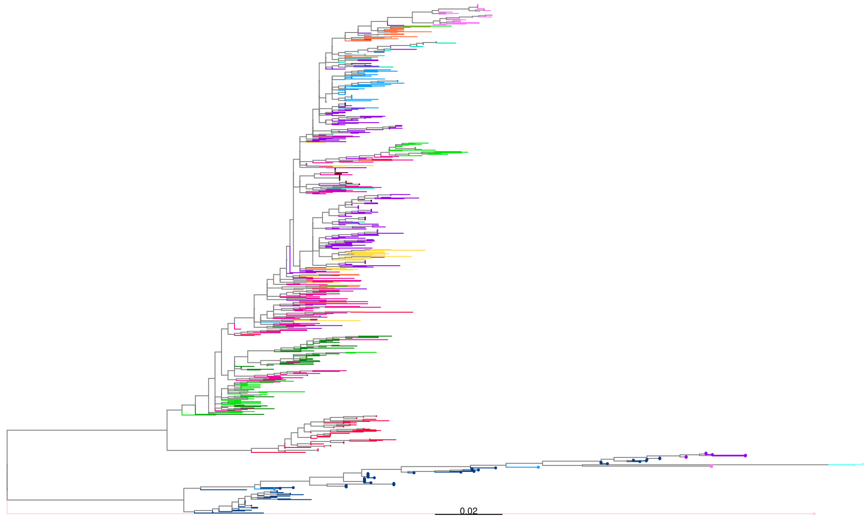
Cell division protein *ftsA*

C Scenario 3: Presence of gene flow,in at least part of the gene

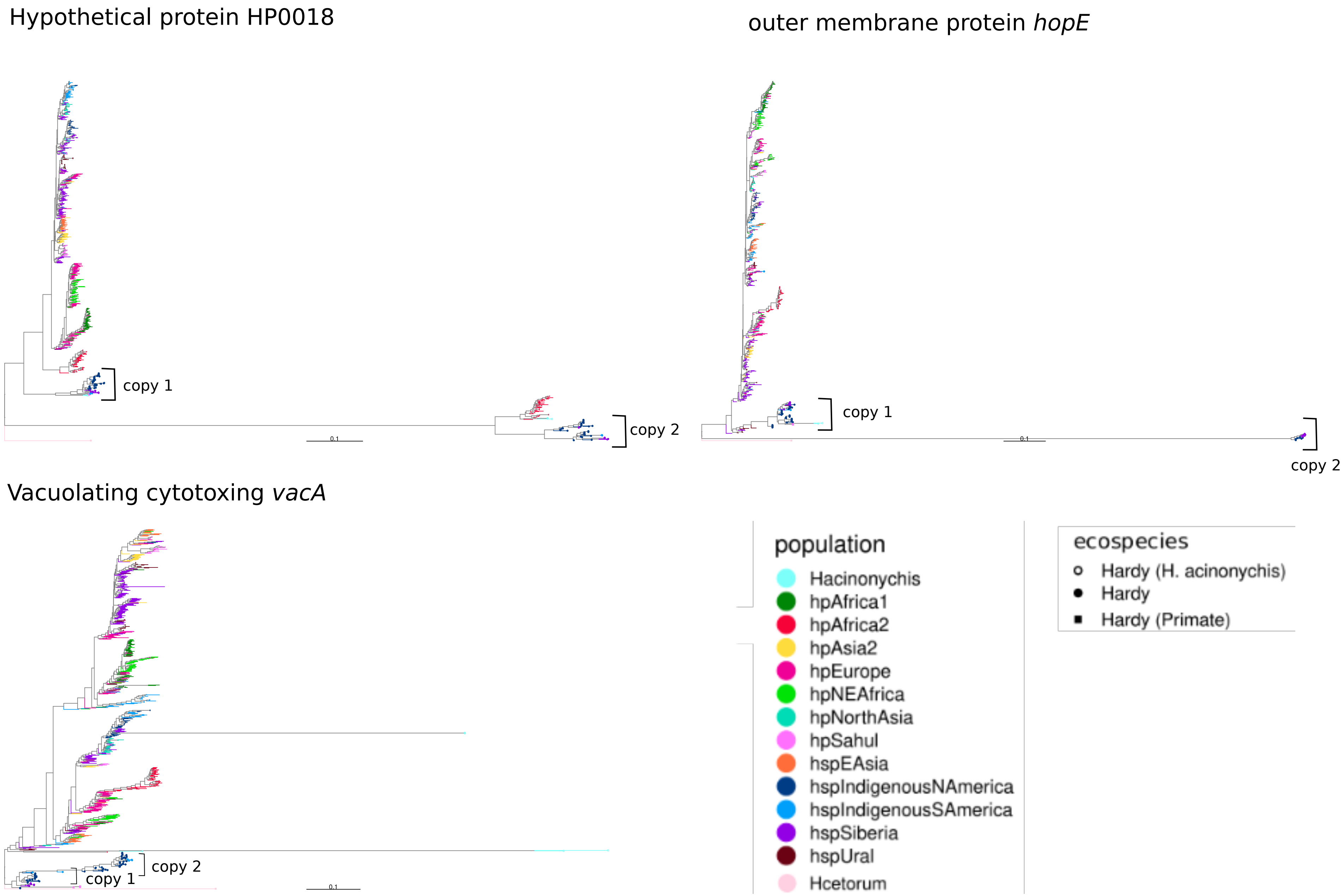

outer membrane protein *horL*

Methyl-accepting chemotaxis protein HP0599

lpp20 lipoprotein



D Scenario 1bis: The ecospecies diverged after *H.cetorum* but before the hpAfrica2 branch split. Two copies are present



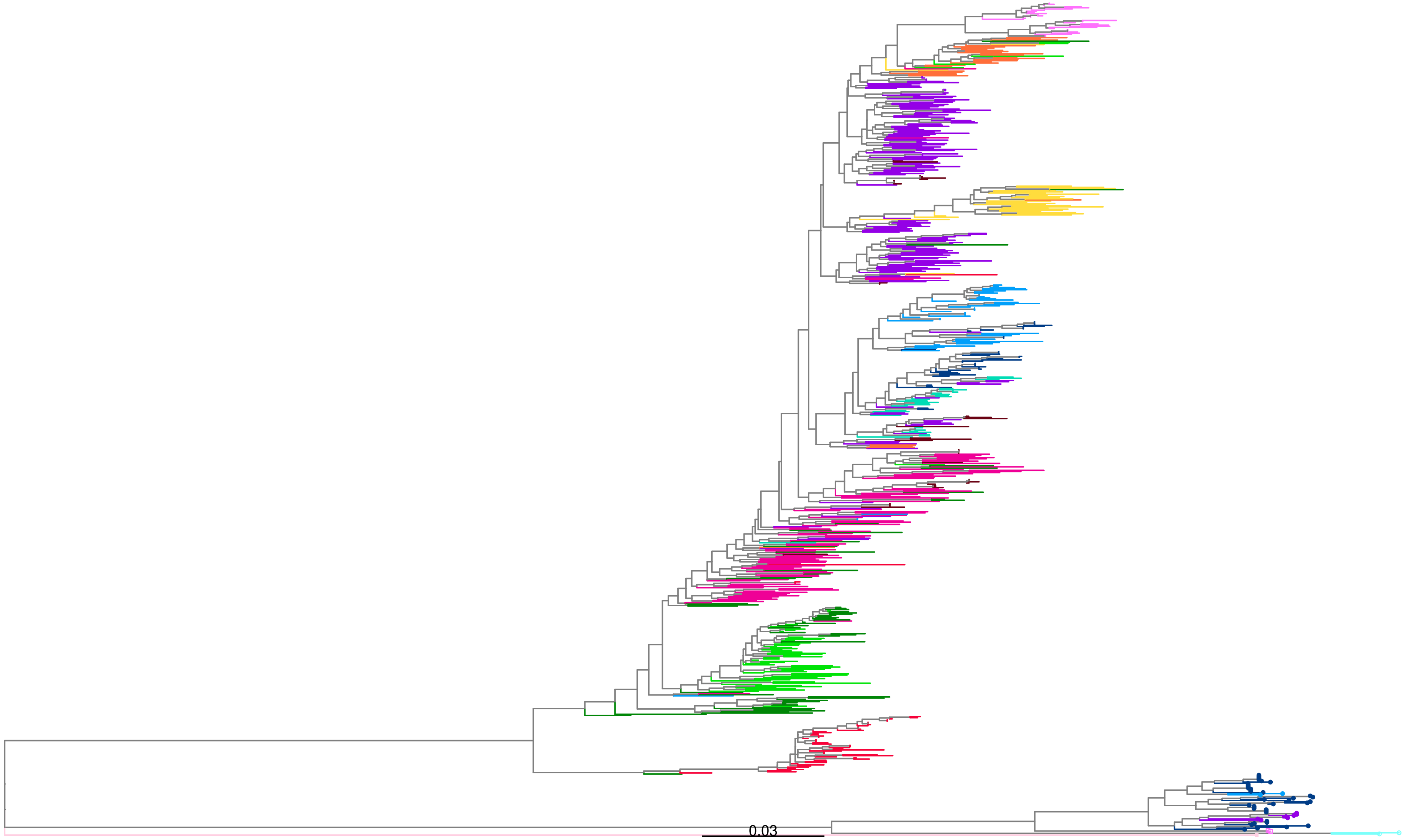
Hypothetical protein HP0018

outer membrane protein *hopE*

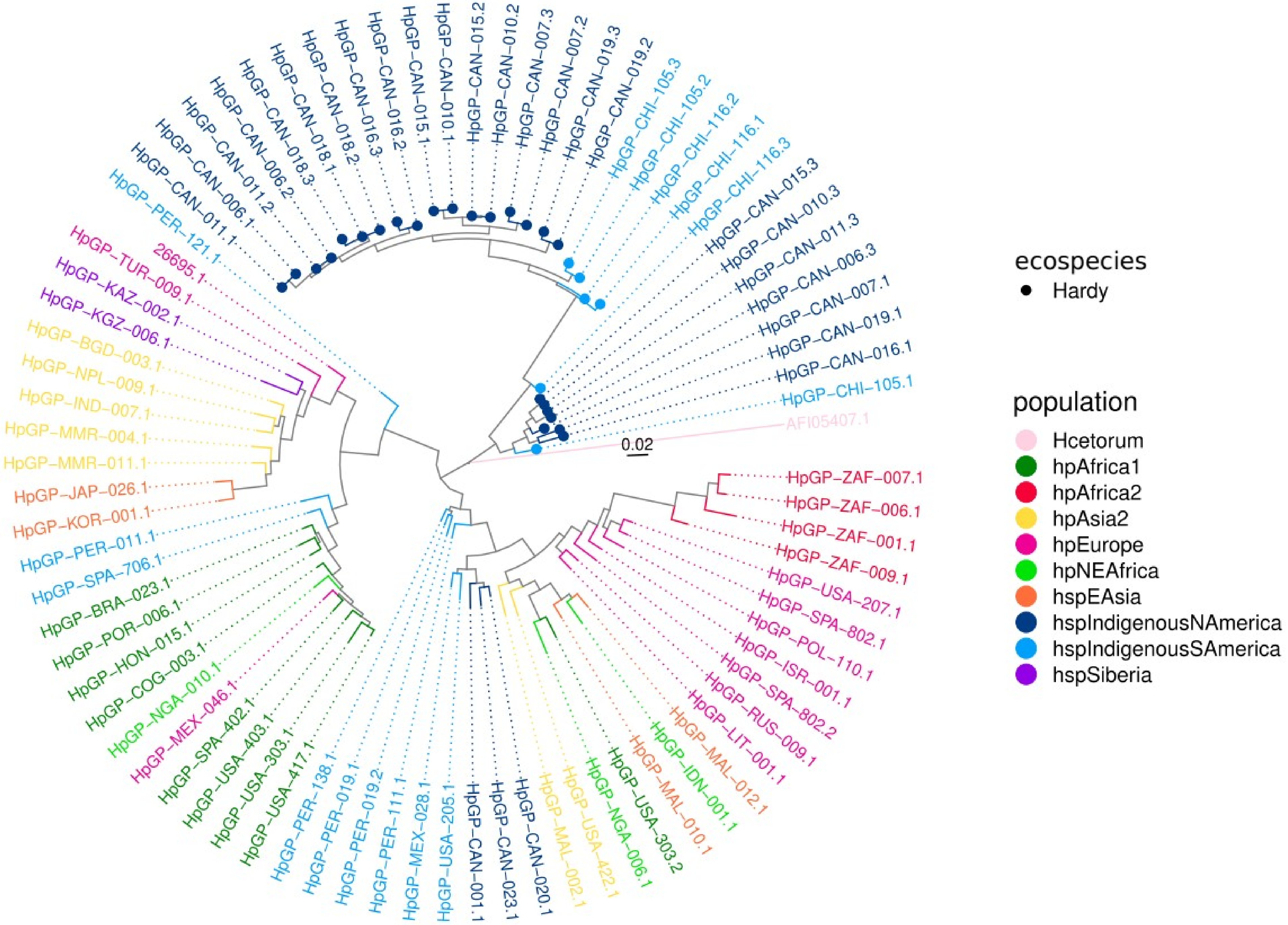
Vacuolating cytotoxing *vacA*

E Scenario 4: The polymorphism is older than *H. cetorum*

outer membrane protein *hopF*

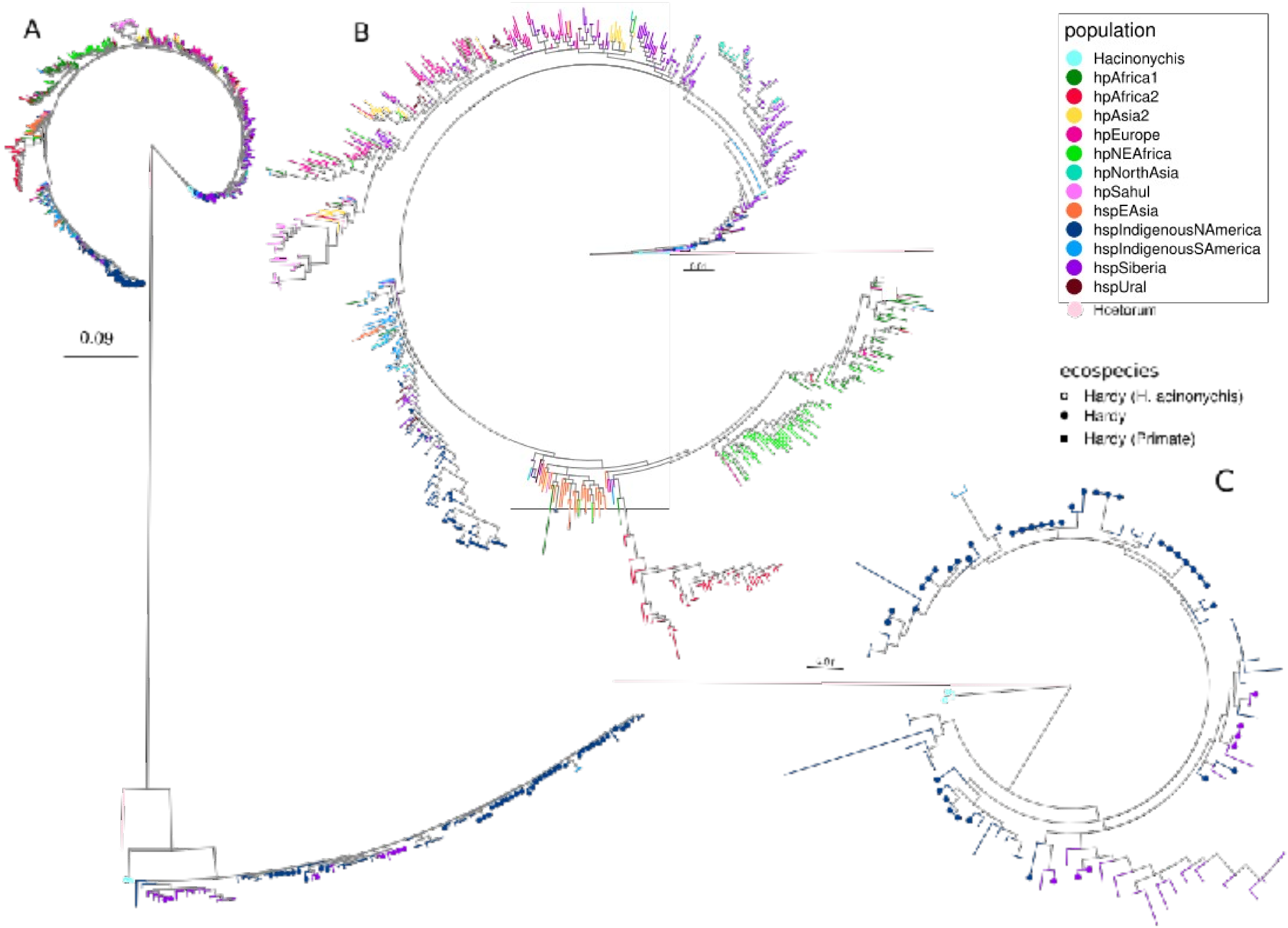


Extended Data Figure 10: **Phylogenetic trees of the sequences of differentiated genes that returned at least one hit when blasted against the *H. cetorum* genome.**

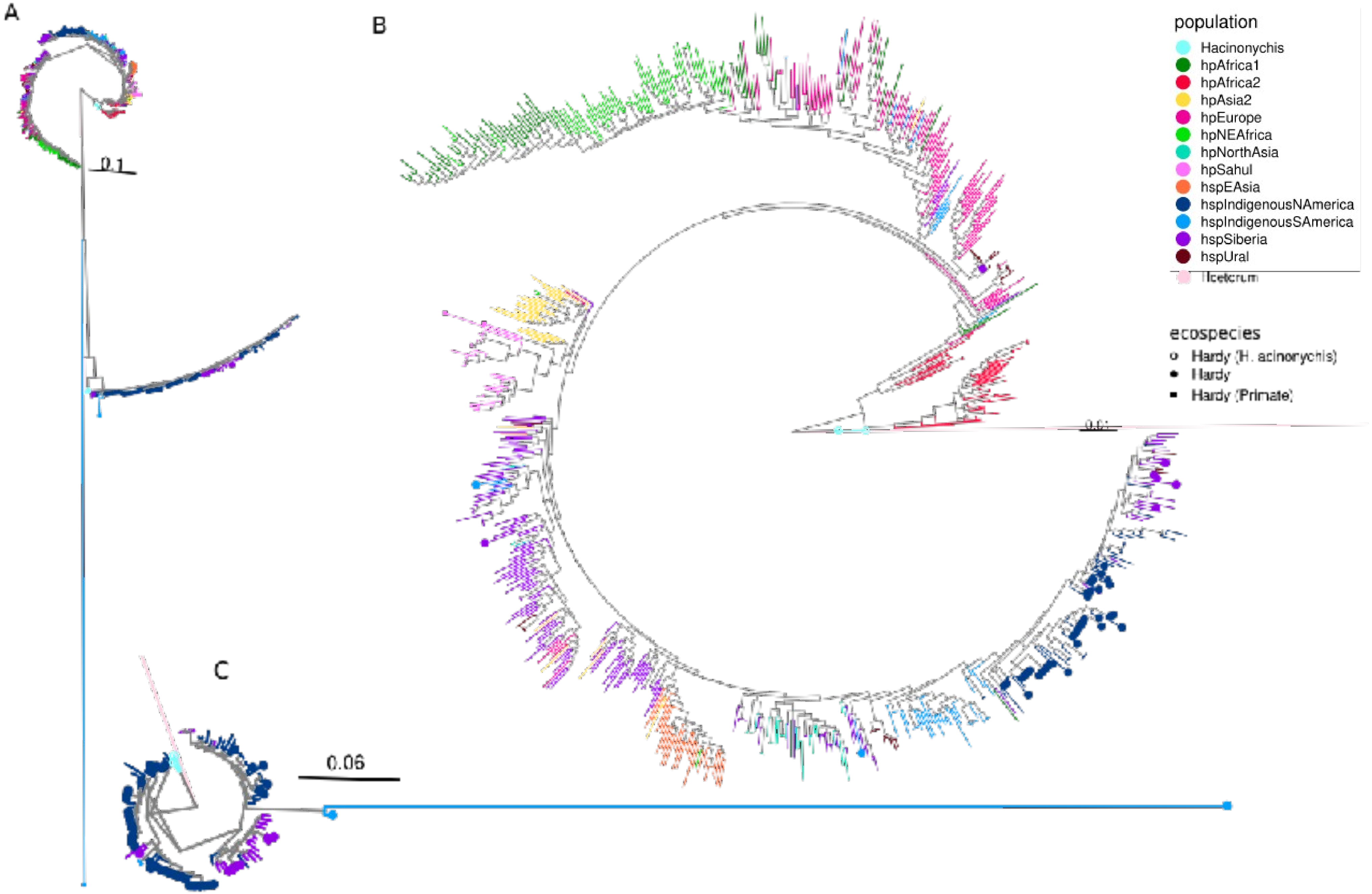


Extended Data Figure 11:

ML phylogenetic tree of *vacA*, based on nucleotide sequences from complete *H. pylori* genomes and *H. cetorum* (AFI05407.1).



Extended Data Figure 12: **Phylogenetic tree of *ureA* sequences for a sample of strains.**



Extended Data Figure 13: **Phylogenetic tree of *ureB* sequences for a sample of strains.**