

Evolutionarily recent dual obligatory symbiosis among adelgids indicates a transition between fungus- and insect-associated lifestyles

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Supplementary Information

'*Candidatus Mycetohabitans vallotii*' sp. nov.

Based on the well supported phylogenetic positioning of '*Candidatus Vallotia tarda*' within the clade formed by both currently recognized *Mycetohabitans* species, we propose the transfer of '*Candidatus Vallotia tarda*' (NCBI taxonomy ID 1177213) to the *Mycetohabitans* genus. To keep the naming consistent, we propose the specific name '*Candidatus Mycetohabitans vallotii*' (va.lo.tii) in honor of the researcher Vallot, who described *A. laricis* in 1836. '*Candidatus Mycetohabitans vallotii*' strains have a rod-shaped cell and co-inhabit the cytoplasm of bacteriocytes of *A. laricis/tardus* along with '*Candidatus Profftia tarda*' [1]. We propose the old species-specific name be used as a strain name, as '*Candidatus Mycetohabitans vallotii*' strain *tarda*. Given their monophyletic origin, the transfer of other species of the '*Candidatus Vallotia*' genus to the *Mycetohabitans* genus is reasonable [1, 2], however as multilocus sequence data are not available for those endosymbionts yet, we leave their species level re-designation open for future studies.

B vitamin synthesis by *Vallotia*

Vallotia likely produces six B vitamins (Figures S8, S11). For the synthesis of riboflavin, neither *yigB* nor *ybjI* [3] were found, however, the promiscuity of phosphatases has been documented [4]. Thus another phosphatase of *Vallotia*, among those belonging to the Haloacid dehalogenase-like hydrolase superfamily, like YigB, might perform this reaction. As is the case for other endosymbionts, *panD* is missing for the *de novo* synthesis of pantothenate, thus this would occur from L-valine and β -alanine in *Vallotia*. Regarding pyridoxine, *Vallotia* lacks *pdxB* and *serC*, but alternatives such as the ‘serendipitous pathways’, *thiG* and an unspecific transaminase might bypass these steps [5, 6]. *BioH* and *bioF* in biotin synthesis are missing from both symbionts, nonetheless, these are also notably absent in several symbiotic systems of aphids [7, 8]. *Vallotia* might still produce biotin, if either 8-amino-7-oxononanoate (KAPA) is imported or if these steps are taken over by the host. Finally, given their lack of *nadB* and *nadC* genes, both endosymbionts could synthesize NAD⁺ and NADP⁺ from the import of nicotinate.



Figure S1. (A) A gall of *Adelges laricis/tardus* collected with a spruce branch. (B) Adelgids in an opened gall.

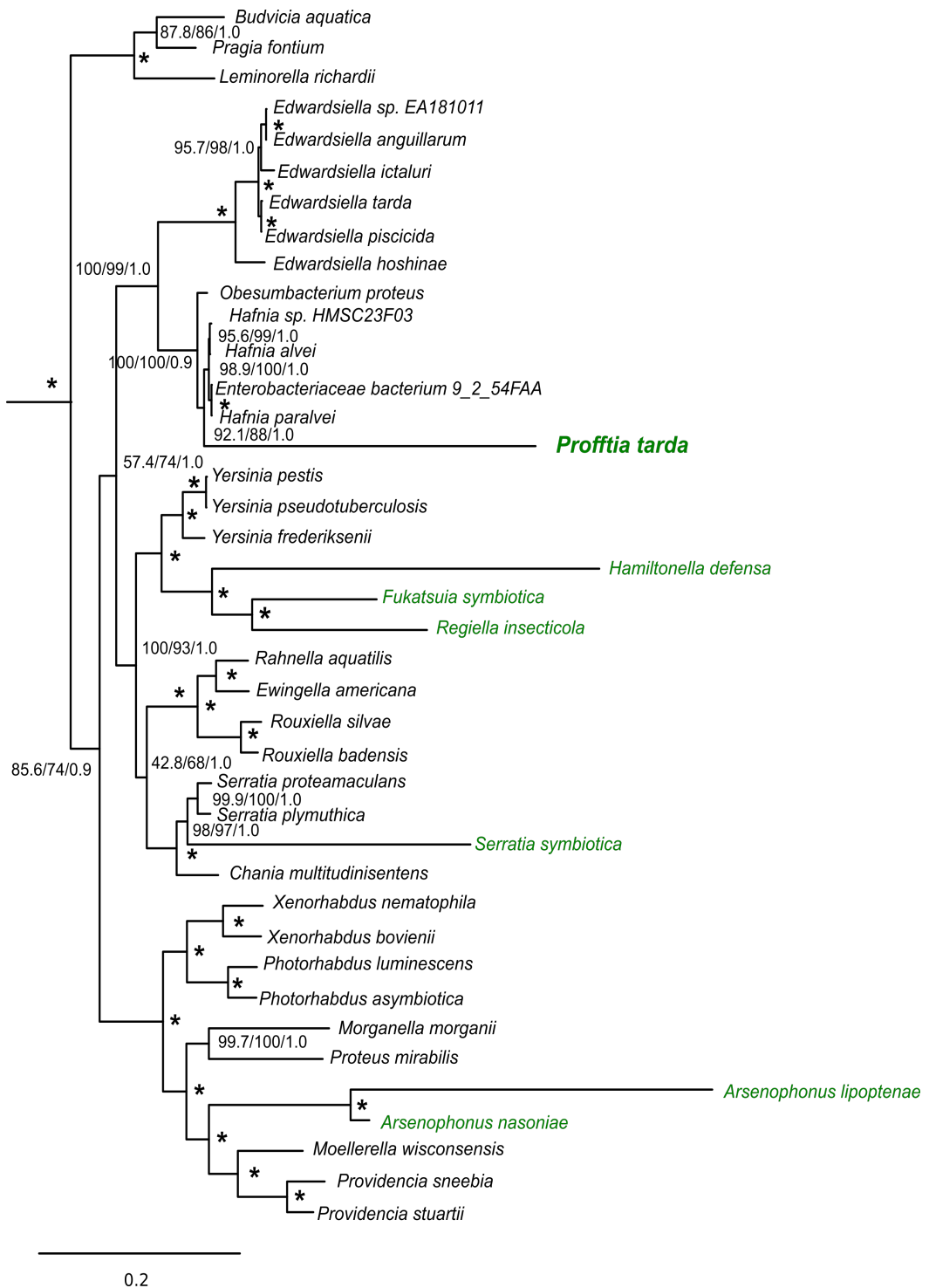


Figure S2. Phylogenomic analysis showing the affiliation of ‘*Candidatus Profftia tarda*’ within the Enterobacteriales. Insect symbionts are highlighted in green. *Xanthomonas campestris* [AE008922], *Stenotrophomonas maltophilia* [AM743169] and *Pseudomonas aeruginosa* [AE004091]) were used as outgroups. Maximum likelihood (IQTREE) and Bayesian trees (MrBayes) were based on a concatenated set of 45 proteins. Maximum likelihood tree is shown. SH-aLRT support (%) and ultrafast bootstrap support (%) values based on 1000 replicates, and Bayesian posterior probabilities are indicated on the internal nodes. Asterisks stand for a maximal support in each analysis (100% / 1). The branch length leading to *Profftia* indicated accelerated evolutionary rates and was similar to those of other obligate and facultative insect symbionts included in the analysis.

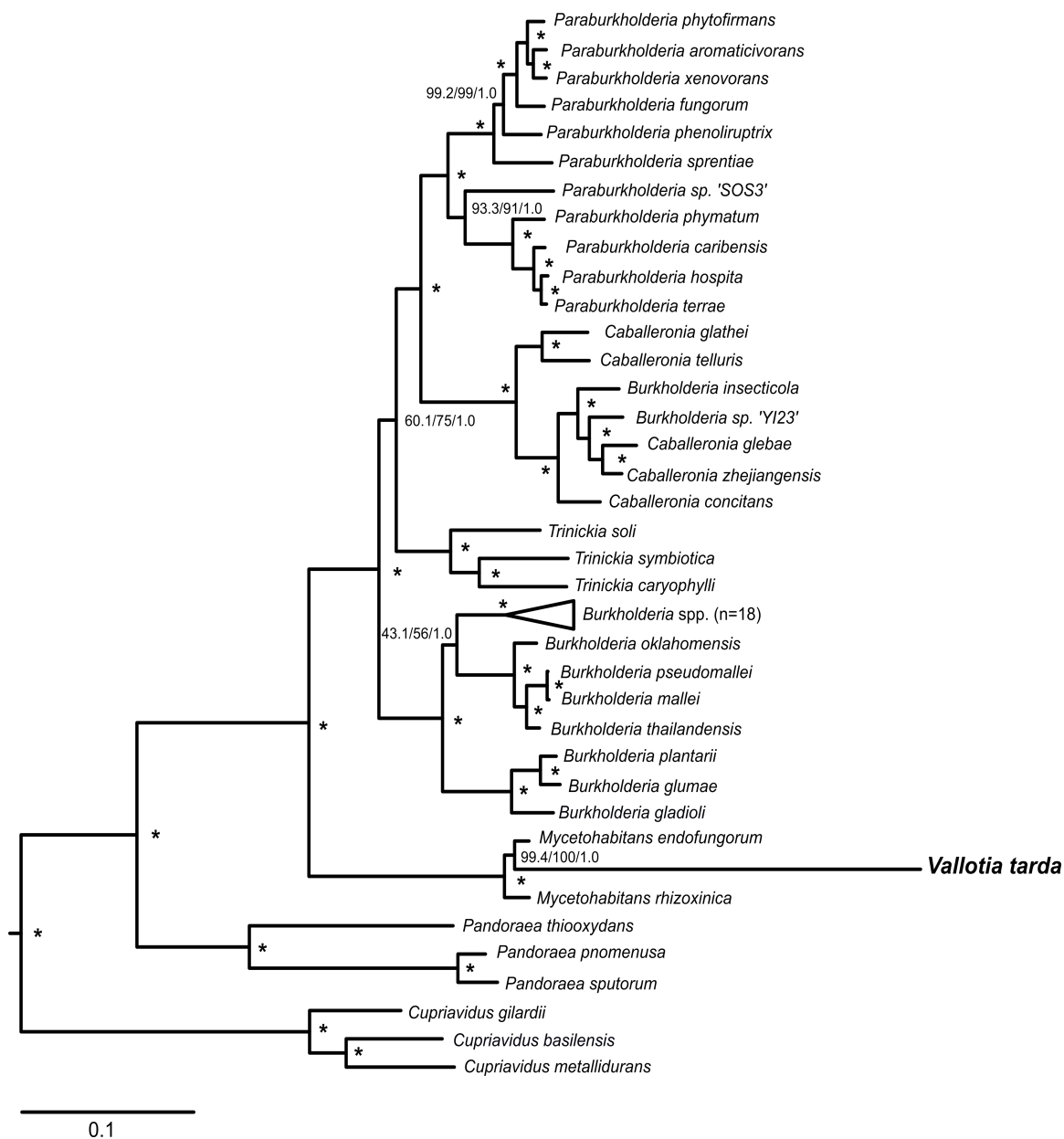


Figure S3. Phylogenomic analysis showing the affiliation of ‘*Candidatus Vallotia tarda*’ within the *Burkholderiaceae*. Selected members of *Oxalobacteraceae* (*Janthinobacterium agaricidamnorum* [HG322949], *Collimonas pratensis* [CP013234] and *Herbaspirillum seropedicae* [CP011930]) were used as outgroups. Maximum likelihood (IQTREE) and Bayesian analyses (MrBayes) were performed based on a concatenated set of 108 proteins. Maximum likelihood tree is shown. SH-aLRT support (%) and ultrafast bootstrap support (%) values based on 1000 replicates, and Bayesian posterior probabilities are indicated on the internal nodes. Asterisks stand for a maximal support in each analysis (100% / 1).

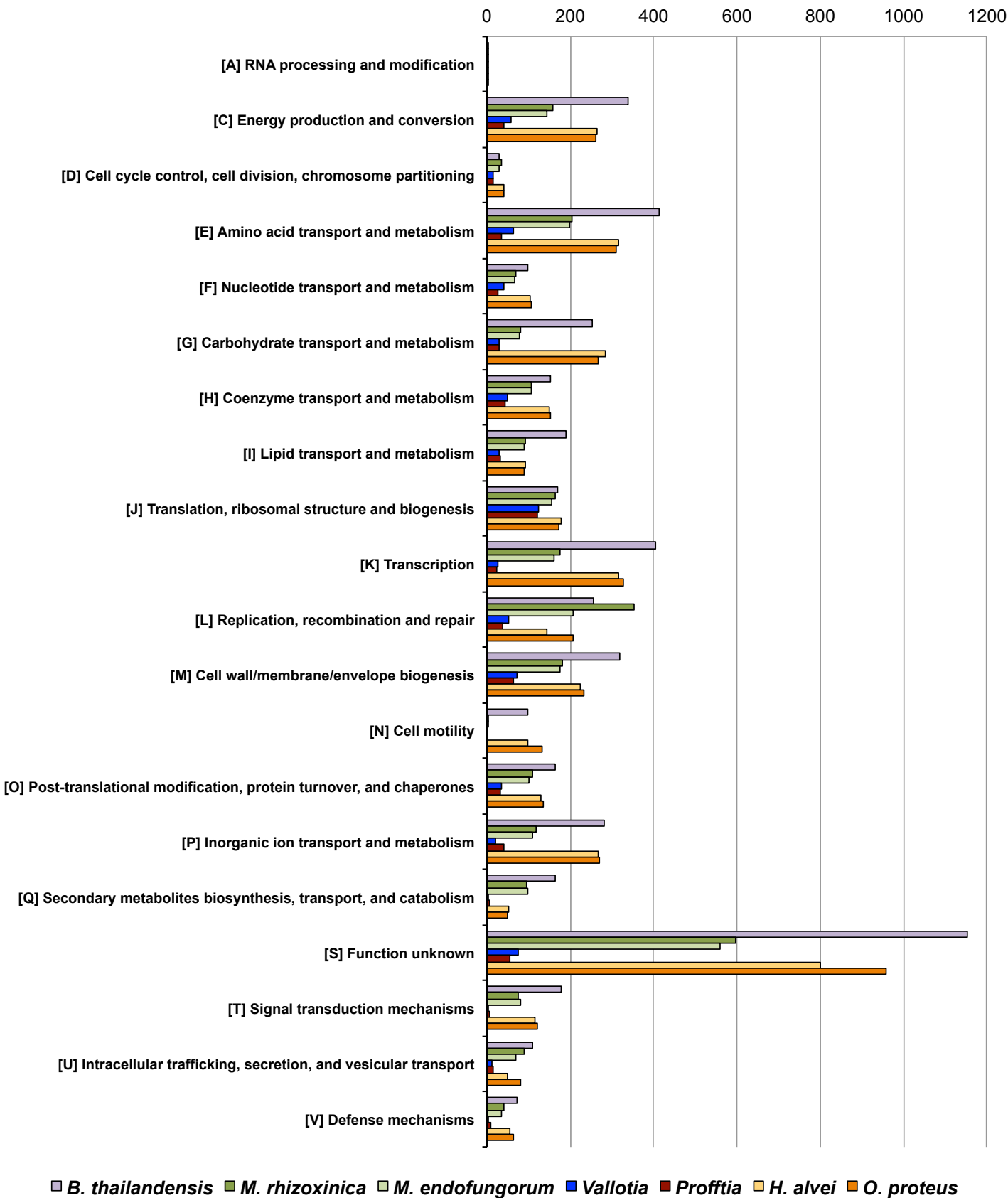


Figure S4. Functional reduction in *Vallotia* and *Profftia*, as shown by the number of genes among main functional categories according to the EggNOG classification in the genomes of *Profftia*, *Vallotia*, and closely related bacteria. The fungus-associated endosymbionts, *Mycetohabitans rhizoxinica* [FR687359.1, FR687360.1, FR687361.1], and *M. endofungorum* [GCA_002927045.1] are the closest relatives of *Vallotia*. Free-living bacteria involve the *Vallotia*-related *Burkholderia thailandensis* [CP008785.1], and *Profftia*-related *Hafnia alvei* [CP036514.1], and *Obesumbacterium proteus* [CP014608.1].

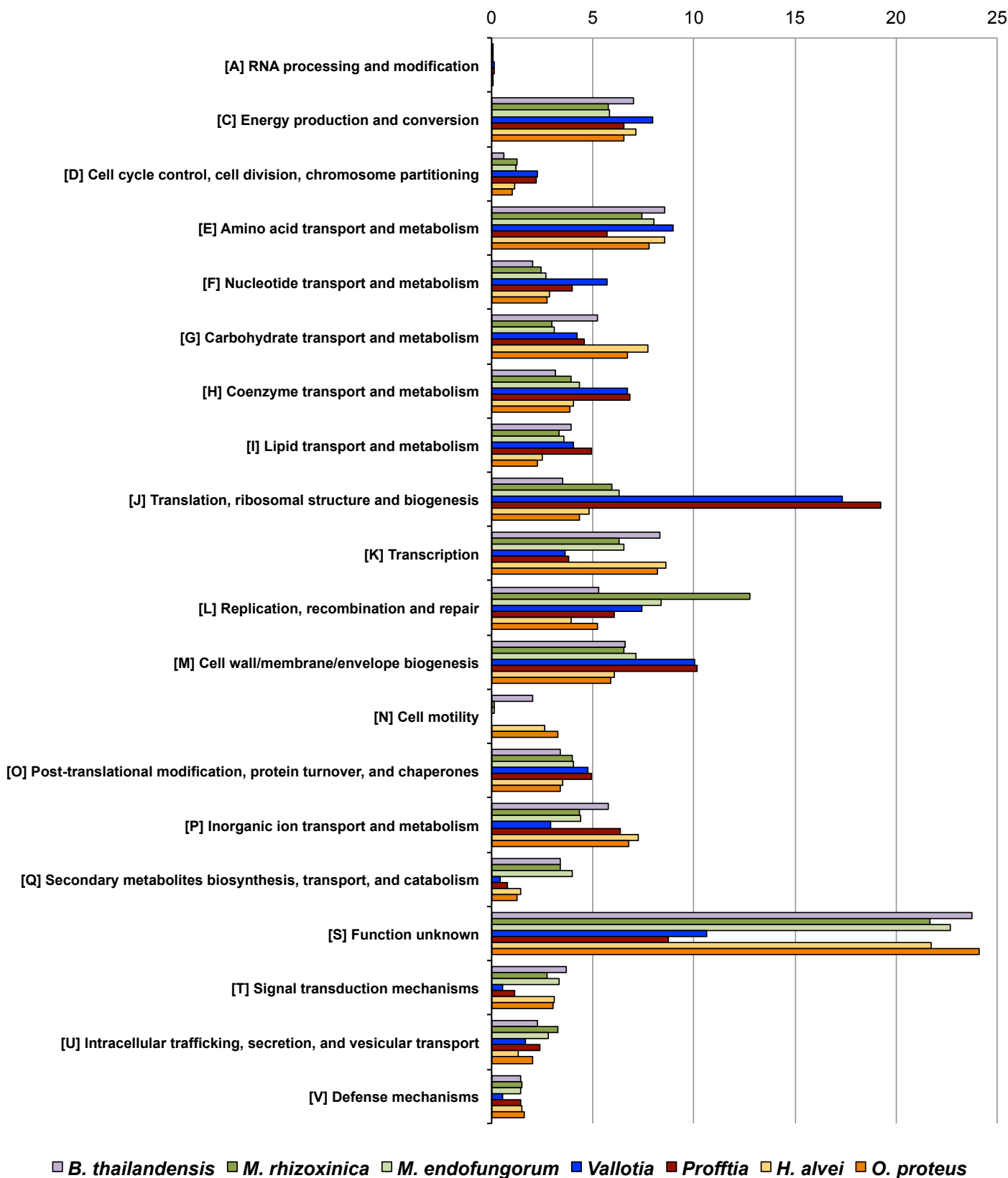


Figure S5. Proportion (%) of genes among main functional categories according to the EggNOG classification in the genomes of *Profftia*, *Vallotia*, and closely related bacteria. The fungus-associated endosymbionts, *Mycetohabitans rhizoxinica* [FR687359.1, FR687360.1, FR687361.1], and *M. endofungorum* [GCA_002927045.1] are the closest relatives of *Vallotia*. Free-living bacteria involve the *Vallotia*-related *Burkholderia thailandensis* [CP008785.1], and *Profftia*-related *Hafnia alvei* [CP036514.1], and *Obesumbacterium proteus* [CP014608.1].

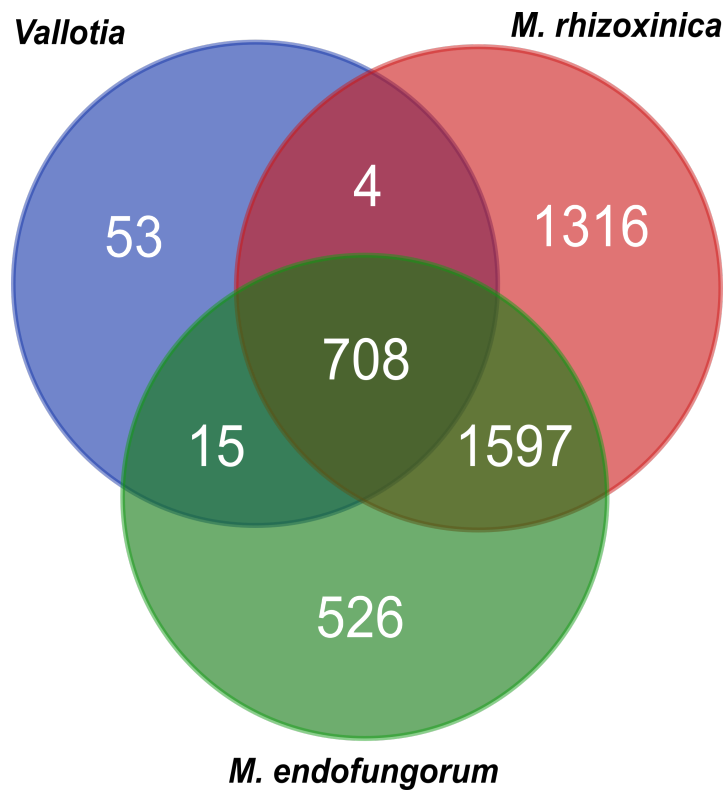


Figure S6. Venn diagram showing the pan-genome of the insect endosymbiont, *Vallotia*, and related fungus-associated endosymbionts, *M. rhizoxinica* [FR687359.1, FR687360.1, FR687361.1] and *M. endofungorum* [GCA_002927045.1].

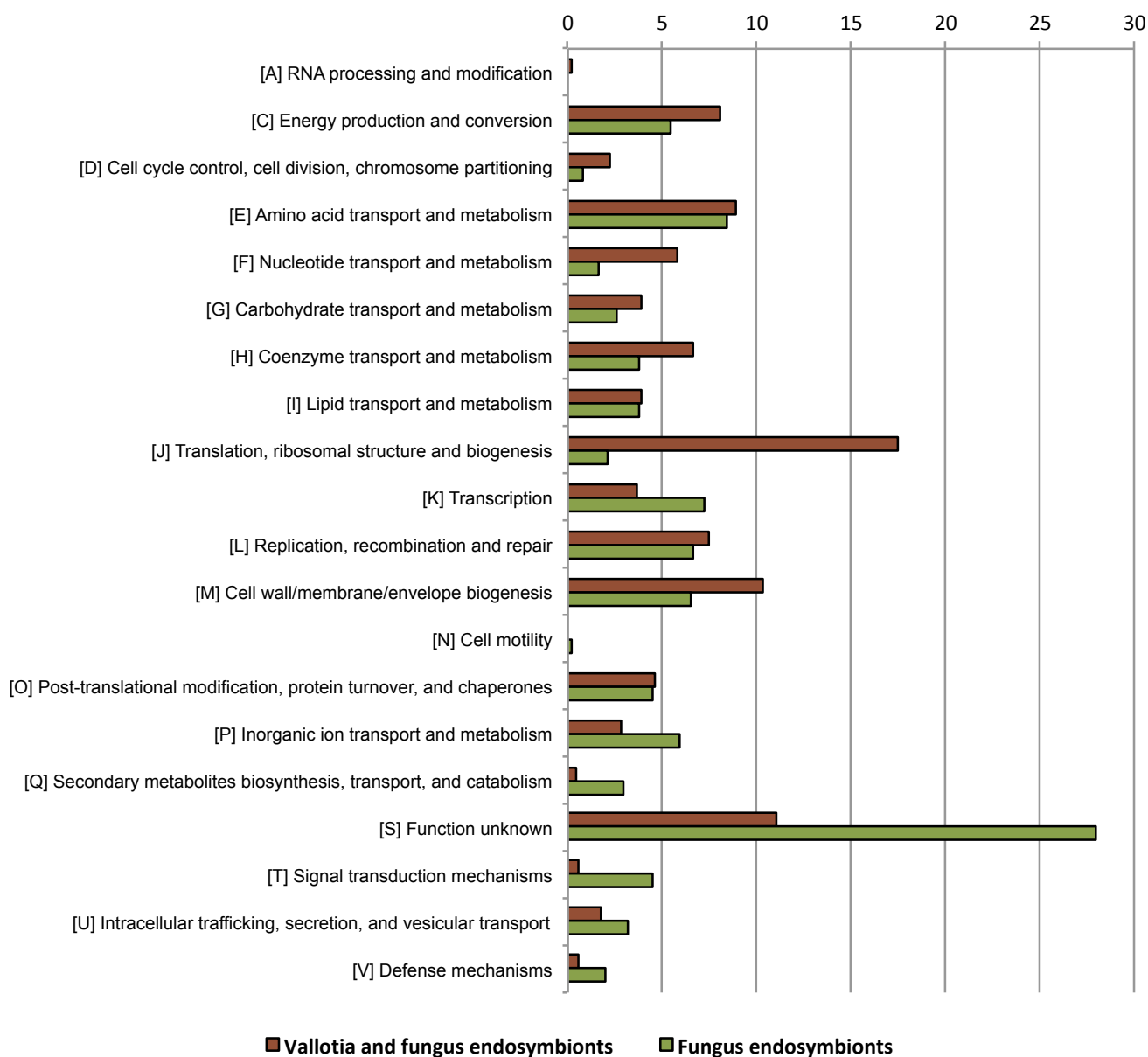


Figure S7. Proportion (%) of genes shared by *Vallotia* and fungus-associated endosymbionts (shown in brown) compared to those shared only by the fungus-associated endosymbionts (shown in green) – *M. rhizoxinica* [FR687359.1, FR687360.1, FR687361.1] and *M. endofungorum* [GCA_002927045.1] – among the main functional categories according to the EggNOG classification.

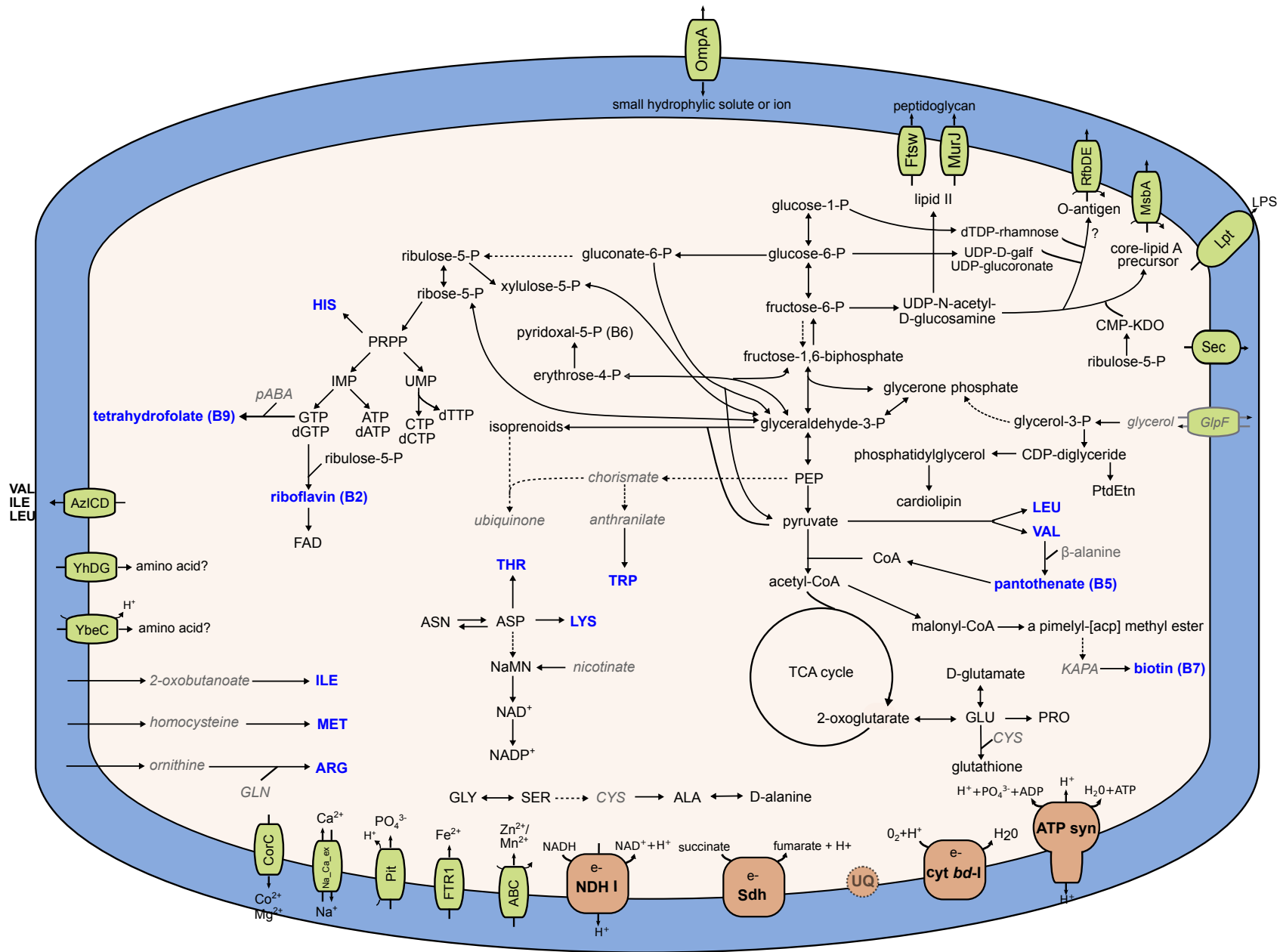


Figure S8. Metabolic pathway reconstruction of *Vallotia*. Dashed lines indicate missing or incomplete pathways. Compounds of which biosynthesis or transport could not be confirmed based on the genomic data are in grey italics. Essential amino acids and B vitamins produced by *Vallotia* are shown in blue.

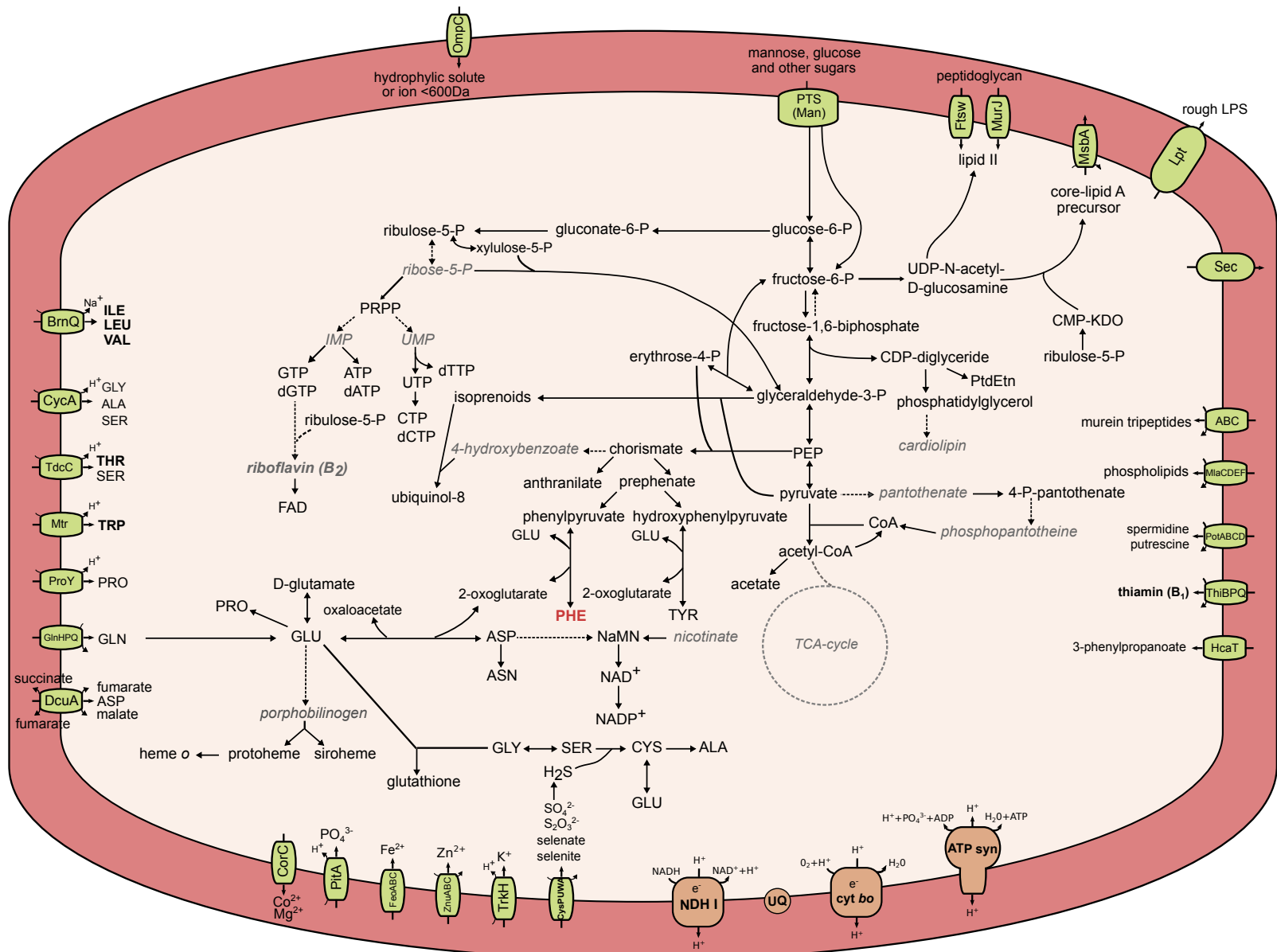


Figure S9. Metabolic pathway reconstruction of *Profftia*. Dashed lines indicate missing or incomplete pathways. Compounds of which biosynthesis or transport could not be confirmed based on the genomic data are in grey italics. Bolded texts indicate essential amino acids and B vitamins. Essential amino acids and B vitamins produced by *Profftia* are shown in red.

- *Vallotia*
- *Profftia*
- Both
-→ None

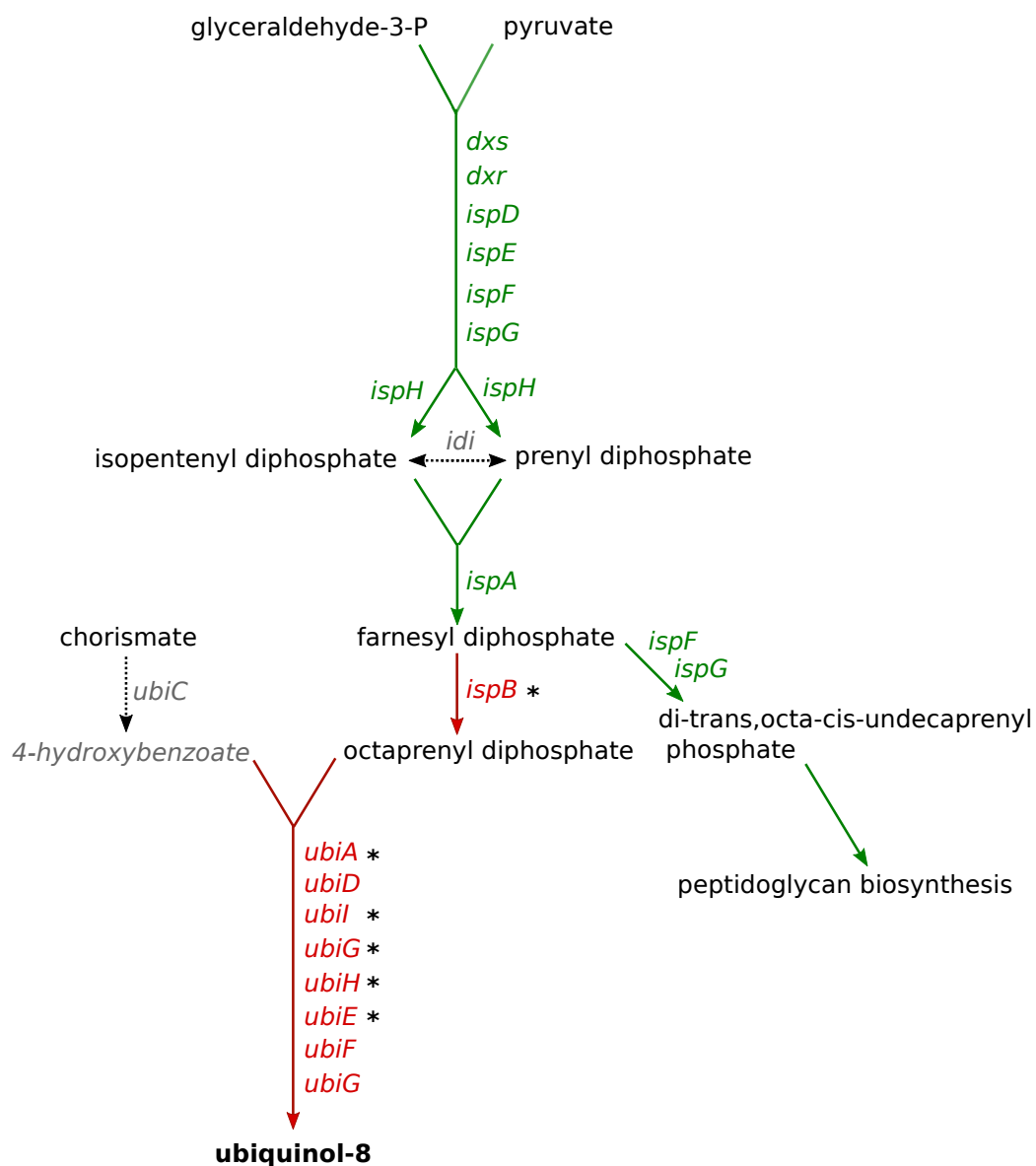


Figure S10. Ubiquinone biosynthesis in *Profftia* and *Vallotia*. *Profftia* has a complete gene set for the pathway. Asterisks indicate pseudogenized genes in *Vallotia*, which is not able to synthesize ubiquinone. Farnesyl diphosphate can be used for peptidoglycan synthesis by both symbionts.

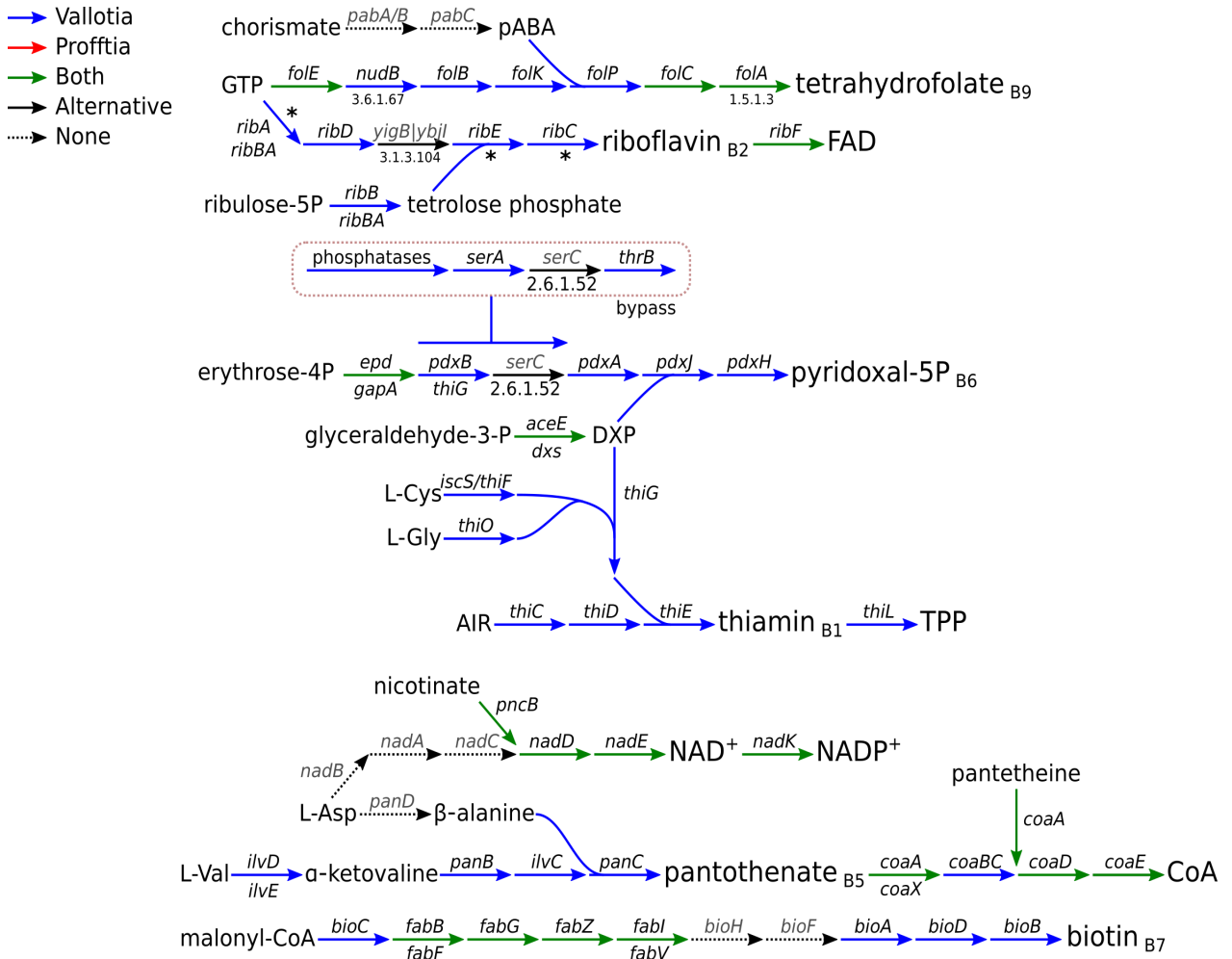


Figure S11. B vitamin synthesis as inferred based on the presence of genes in *Vallotia* and *Profftia*. Missing genes are shown in grey. Asterisks indicate pseudogenes of *Profftia*.

Supplementary references

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