

Document S5. Analyses of RvhB8 proteins across Rickettsiales species.

Assessment of divergence for RvhB8 paralogs across Rickettsiales species

RvhB8-I and RvhB8-II proteins were aligned for 15 Rickettsiales species: *Rickettsia bellii* str. RML369-C, *Rickettsia typhi* str. Wilmington, *Orientia tsutsugamushi* str. Boryong, *Orientia tsutsugamushi* str. Ikeda, Rickettsiaceae bacterium str. Os18, “*Candidatus* Midichloria mitochondrii” str. IricVA, *Neorickettsia sennetsu* str. Miyayama, *Neorickettsia risticii* str. Illinois, “*Candidatus* Xenolissoclinum pacificensis” str. L6, *Wolbachia* endosymbiont str. TRS (*Brugia malayi*), *Wolbachia* endosymbiont (*Drosophila melanogaster*), *Ehrlichia canis* str. Jake, *Ehrlichia chaffeensis* str. Arkansas, *Anaplasma phagocytophilum* str. HZ, *Anaplasma marginale* str. St. Maries. Complete proteins were aligned using MUSCLE (default parameters) (Edgar 2004), with percent identity matrices used to provide estimates of divergence (fig 1). (A) Pairwise divergence between RvhB8-I proteins. (B) Pairwise divergence between RvhB8-II proteins. NOTE: pairwise divergences between RvhB8-I and RvhB8-II proteins are shown in fig. 6A of the manuscript. Species within the same genus are colored similarly. NCBI GenBank accession numbers for all proteins are provided in Document S1.

Phylogeny estimations of RvhB8 proteins

Phylogenies were estimated on the global RvhB8 alignment, which included one outgroup VirB8 protein from *Yersinia frederiksenii* (WP_042562314) (fig 2). The alignment was trimmed of less conserved regions using Gblocks (Talavera and Castresana 2007). Phylogenies of the unmasked (295 aa) and masked (87 aa) alignments were estimated under maximum likelihood (ML) using RAxML v.7.2.8 (Stamatakis 2014), implementing a gamma model of rate heterogeneity and estimation of the proportion of invariable sites. Two separate analyses for each alignment employed the WAG or LG amino acid substitution models, resulting in four total ML-based phylogeny estimations. Branch support was assessed with 1000 bootstrap pseudoreplications. (A) WAG (unmasked): this tree is shown in fig. 6C of the manuscript. (B) LG (unmasked). (C) WAG (masked). (D) LG (masked). NCBI GenBank accession numbers for all proteins are provided in Document S1.

Relative conservation of RvhB8 paralogs across Rickettsiales species

The global RvhB8 alignment, which includes RvhB8-I and RvhB8-II proteins from 15 Rickettsiales species (see above). The assignment of predicted (top) and solved (bottom) structures are shown for *R. typhi* RvhB8-I and RvhB8-II, respectively. Predicted

transmembrane-spanning regions (Krogh et al. 2001) are colored blue. Five residues conserved across all RvhB8 proteins are colored black, with residues conserved only in RvhB8-I ($n = 15$) or RvhB8-II ($n = 1$) highlighted yellow.

REFERENCES

- Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32:1792–1797.
- Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* [Internet] 305:567–580. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11152613>
- Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.
- Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* [Internet] 56:564–577. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17654362>

A

RvhB8-I

	<i>R. bellii</i>	<i>R. typhi</i>	<i>Orientia (Boryong)</i>	<i>Orientia (Ikeda)</i>	Os18	<i>M. mitochondrii</i>	<i>N. sennetsu</i>	<i>N. risticii</i>	<i>X. pacificiensis</i>	<i>Wolbachia (Bmal)</i>	<i>Wolbachia (Dmel)</i>	<i>E. canis Jake</i>	<i>E. chaffeensis</i>	<i>A. phagocytophilum</i>	<i>A. marginale</i>
<i>R. bellii</i>	-----	76.9	42.5	42.5	46.5	28.5	28.8	25.3	31.2	32.6	32.7	28.1	28.2	29.4	31.0
<i>R. typhi</i>	76.9	-----	41.6	41.6	47.8	29.7	28.8	25.8	31.2	34.4	35.0	29.4	29.2	29.9	31.0
<i>Orientia (Boryong)</i>	42.5	41.6	-----	100	64.6	29.8	30.0	29.2	29.2	34.7	33.5	30.7	30.7	31.9	30.2
<i>Orientia (Ikeda)</i>	42.5	41.6	100	-----	64.6	29.8	30.0	29.2	29.2	34.7	33.5	30.7	30.7	31.9	30.2
Os18	46.5	47.8	64.6	64.6	-----	28.6	28.8	27.6	31.2	35.3	34.1	32.6	30.4	32.9	30.0
<i>M. mitochondrii</i>	28.5	29.7	29.8	29.8	28.6	-----	34.5	33.1	35.1	35.1	35.7	35.1	34.5	36.9	38.9
<i>N. sennetsu</i>	28.8	28.8	30.0	30.0	28.8	34.5	-----	91.6	35.3	43.2	41.6	38.0	37.5	39.1	37.3
<i>N. risticii</i>	25.3	25.8	29.2	29.2	27.6	33.1	91.6	-----	33.9	40.5	38.9	36.8	36.2	35.2	34.9
<i>X. pacificiensis</i>	31.2	31.2	29.2	29.2	31.2	35.1	35.3	33.9	-----	40.2	41.3	40.4	39.9	38.0	35.2
<i>Wolbachia (Bmal)</i>	32.6	34.4	34.7	34.7	35.3	35.1	43.2	40.5	40.2	-----	81.4	52.2	54.0	50.7	51.8
<i>Wolbachia (Dmel)</i>	32.7	35.0	33.5	33.5	34.1	35.7	41.6	38.9	41.3	81.4	-----	48.9	48.9	49.1	50.2
<i>E. canis Jake</i>	28.1	29.4	30.7	30.7	32.6	35.1	38.0	36.8	40.4	52.2	48.9	-----	93.6	62.4	69.2
<i>E. chaffeensis</i>	28.2	29.2	30.7	30.7	30.4	34.5	37.5	36.2	39.9	54.0	48.9	93.6	-----	62.5	67.5
<i>A. phagocytophilum</i>	29.4	29.9	31.9	31.9	32.9	36.9	39.1	35.2	38.0	50.7	49.1	62.4	62.5	-----	83.4
<i>A. marginale</i>	31.0	31.0	30.2	30.2	30.0	38.9	37.3	34.9	35.2	51.8	50.2	69.2	67.5	83.4	-----

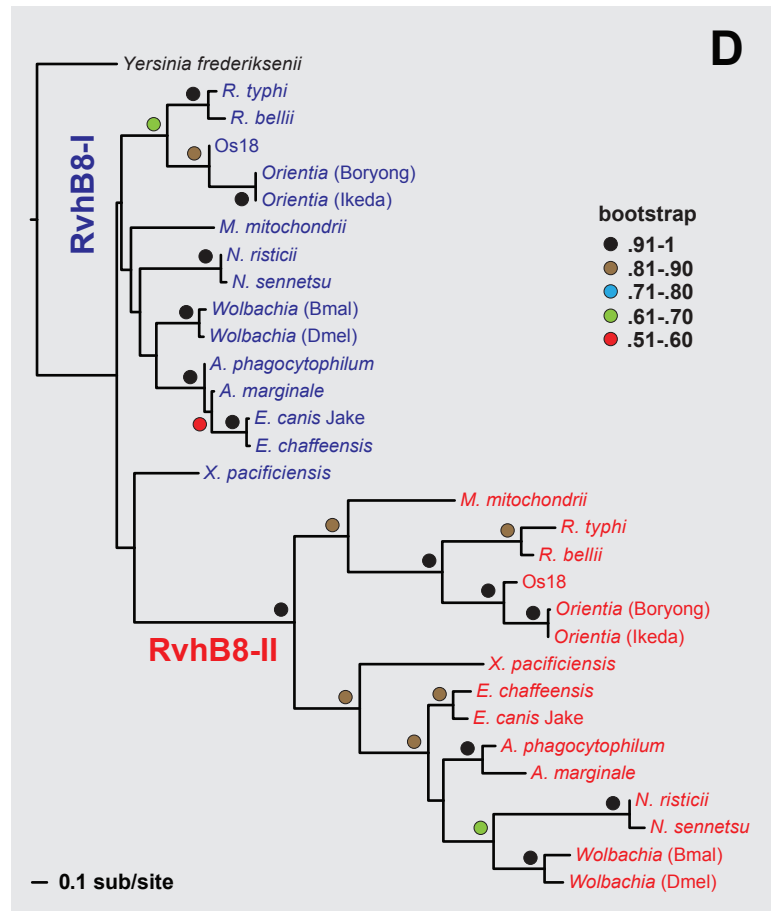
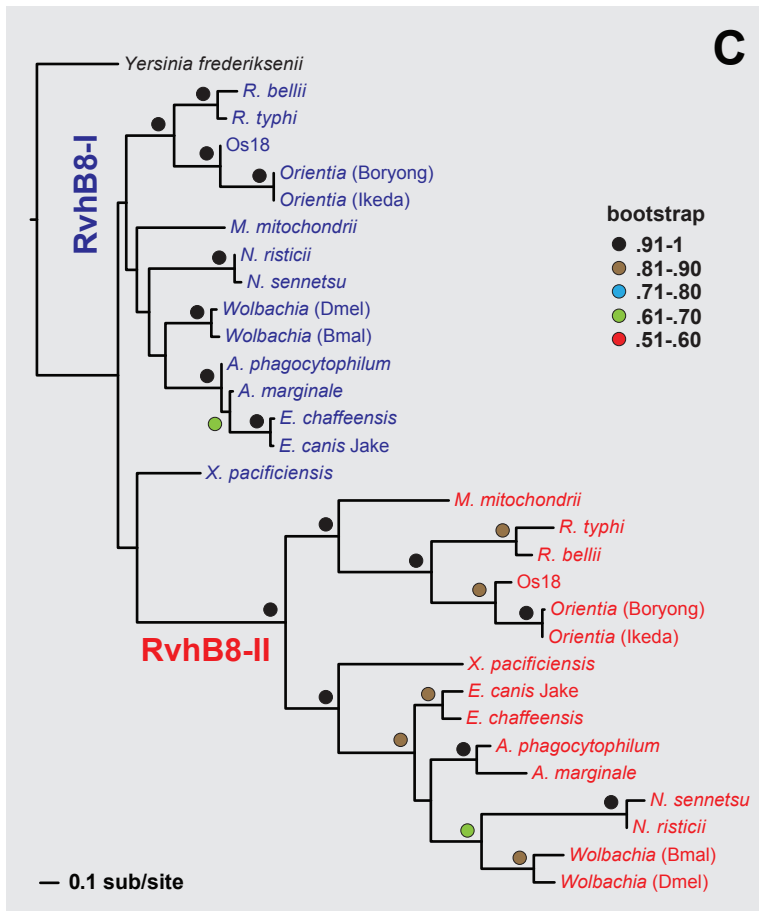
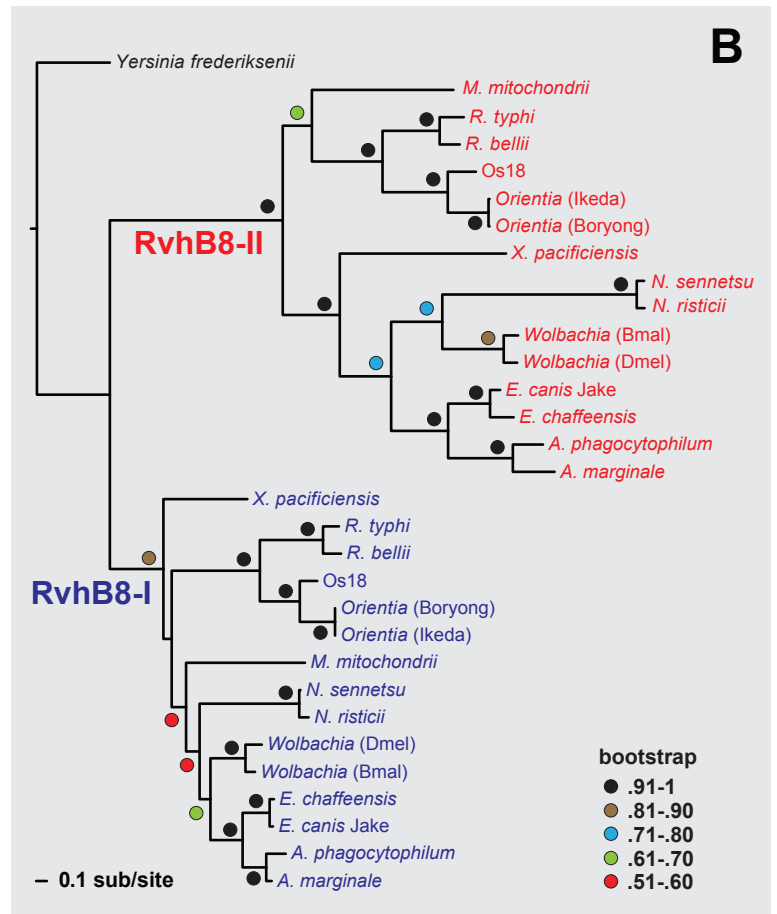
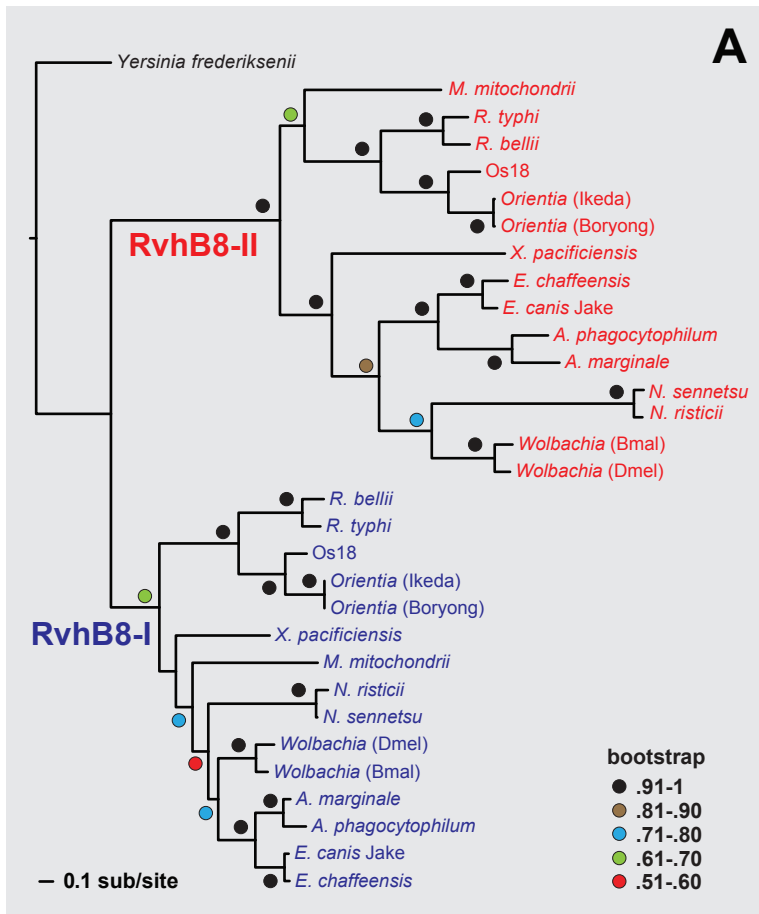
B

RvhB8-II

	<i>R. bellii</i>	<i>R. typhi</i>	<i>Orientia (Boryong)</i>	<i>Orientia (Ikeda)</i>	Os18	<i>M. mitochondrii</i>	<i>N. sennetsu</i>	<i>N. risticii</i>	<i>X. pacificiensis</i>	<i>Wolbachia (Bmal)</i>	<i>Wolbachia (Dmel)</i>	<i>E. canis Jake</i>	<i>E. chaffeensis</i>	<i>A. phagocytophilum</i>	<i>A. marginale</i>
<i>R. bellii</i>	-----	71.1	37.5	37.9	39.0	26.3	17.0	16.5	21.3	24.1	23.2	19.1	21.3	15.7	18.1
<i>R. typhi</i>	71.1	-----	38.5	39.0	40.4	25.1	17.6	17.1	21.8	25.7	24.8	22.4	25.1	18.1	19.2
<i>Orientia (Boryong)</i>	37.5	38.5	-----	98.3	60.8	26.3	17.1	16.1	22.2	23.7	21.0	21.0	25.9	21.6	21.3
<i>Orientia (Ikeda)</i>	37.9	39.0	98.3	-----	60.8	26.3	16.6	15.6	21.8	23.7	21.0	21.0	25.5	21.2	21.3
Os18	39.0	40.4	60.8	60.8	-----	26.8	18.5	18.0	21.9	22.4	19.2	21.0	23.8	20.3	20.5
<i>M. mitochondrii</i>	26.3	25.1	26.3	26.3	26.8	-----	15.1	15.1	23.9	23.3	22.2	25.1	27.2	21.8	20.1
<i>N. sennetsu</i>	17.0	17.6	17.1	16.6	18.5	15.1	-----	87.9	23.3	26.2	27.1	21.8	22.3	22.9	19.9
<i>N. risticii</i>	16.5	17.1	16.1	15.6	18.0	15.1	87.9	-----	22.4	26.2	27.5	21.8	22.3	22.4	20.4
<i>X. pacificiensis</i>	21.3	21.8	22.2	21.8	21.9	23.9	23.3	22.4	-----	23.5	22.7	23.5	25.2	25.0	21.2
<i>Wolbachia (Bmal)</i>	24.1	25.7	23.7	23.7	22.4	23.3	26.2	26.2	23.5	-----	76.9	35.2	34.3	33.5	29.2
<i>Wolbachia (Dmel)</i>	23.2	24.8	21.0	21.0	19.2	22.2	27.1	27.5	22.7	76.9	-----	33.2	33.5	32.3	28.2
<i>E. canis Jake</i>	19.1	22.4	21.0	21.0	21.0	25.1	21.8	21.8	23.5	35.2	33.2	-----	78.2	45.5	44.1
<i>E. chaffeensis</i>	21.3	25.1	25.9	25.5	23.8	27.2	22.3	22.3	25.2	34.3	33.5	78.2	-----	43.2	43.2
<i>A. phagocytophilum</i>	15.7	18.1	21.6	21.2	20.3	21.8	22.9	22.4	25.0	33.5	32.3	45.5	43.2	-----	60.7
<i>A. marginale</i>	18.1	19.2	21.3	21.3	20.5	20.1	19.9	20.4	21.2	29.2	28.2	44.1	43.2	60.7	-----

Fig. 1 (Document S5)

Fig. 2 (Document S5)



RvhB8-I

RvhB8-II

- R. bellii*
- R. typhi*
- Orientia* (Boryong)
- Orientia* (Ikeda)
- Os18
- M. mitochondrii*
- N. sennetsu*
- N. risticii*
- X. pacificiensis*
- Wolbachia* (Bmal)
- Wolbachia* (Dmel)
- E. canis* Jake
- E. chaffeensis*
- A. phagocytophilum*
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- Wolbachia* (Dmel)
- E. canis* Jake
- E. chaffeensis*
- A. phagocytophilum*
- A. marginale*

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RvhB8-I

RvhB8-II

- R. bellii*
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- Orientia* (Boryong)
- Orientia* (Ikeda)
- Os18
- M. mitochondrii*
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- N. risticii*
- X. pacificiensis*
- Wolbachia* (Bmal)
- Wolbachia* (Dmel)
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- A. phagocytophilum*
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- E. canis* Jake
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Fig. 3 (Document S5)

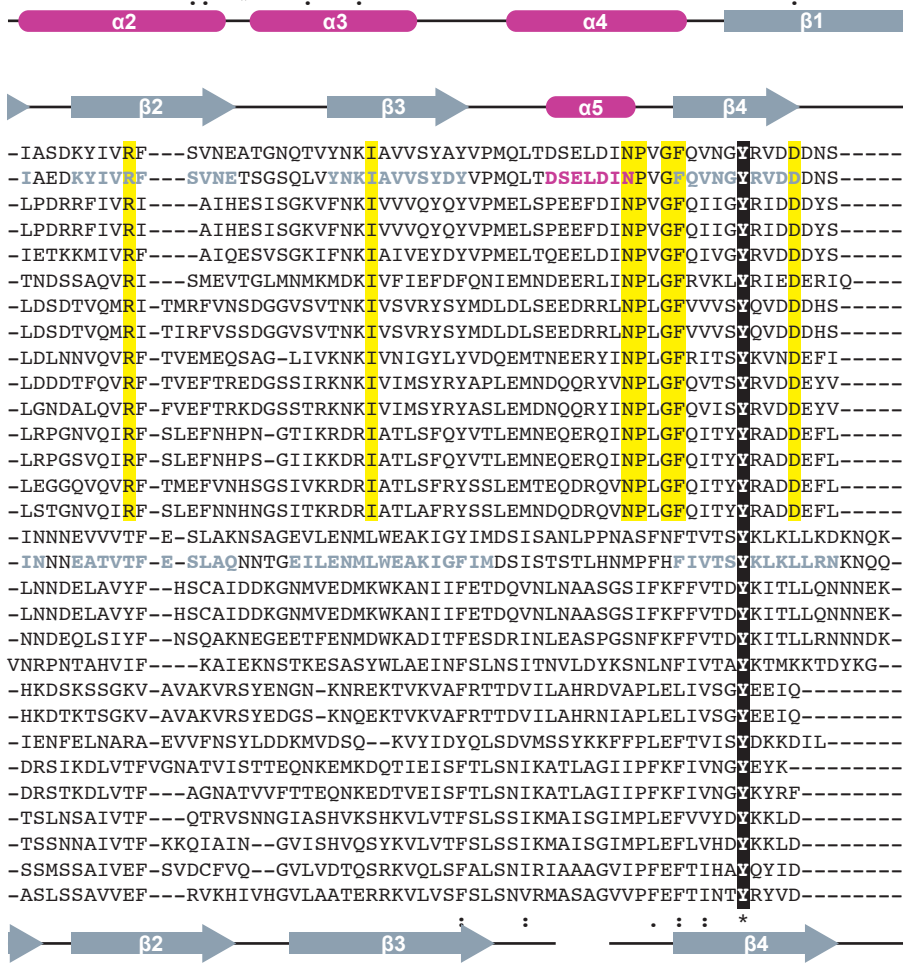
RvhB8-I

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RvhB8-I

RvhB8-II

Fig. 3 (Document S5)