

Supplementary Materials for

**Co-isolation and characterization of two
pandoraviruses and a mimivirus from a riverbank
in Japan**

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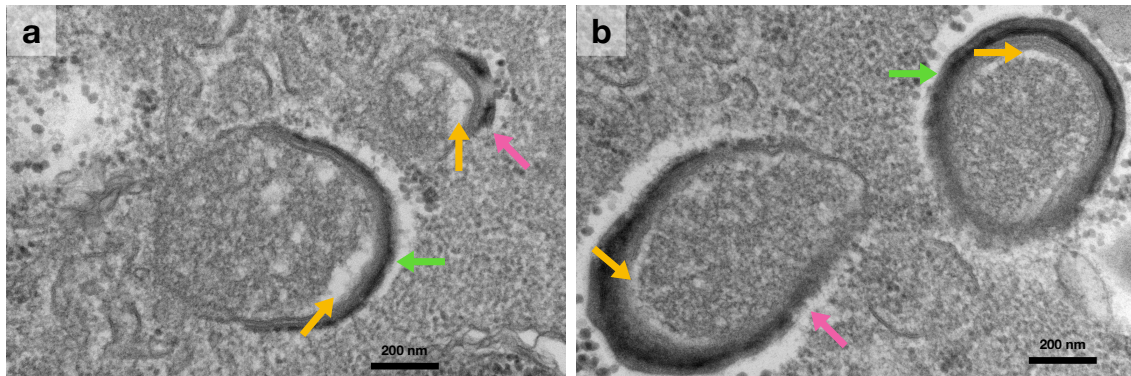


FIG S1 Pandoravirus morphogenesis started at the end of the viral particle that is opposite the ostiole-like apex. **(a)** *Pandoravirus hades*; **(b)** *P. persephone*. Scales are shown in all figures. Pink arrows: morphogenesis started at the ostiole-like apex. Green arrows: morphogenesis started at the opposite end of the ostiole-like apex. Yellow arrow: vacant space between the partially formed capsid and nucleocapsid.

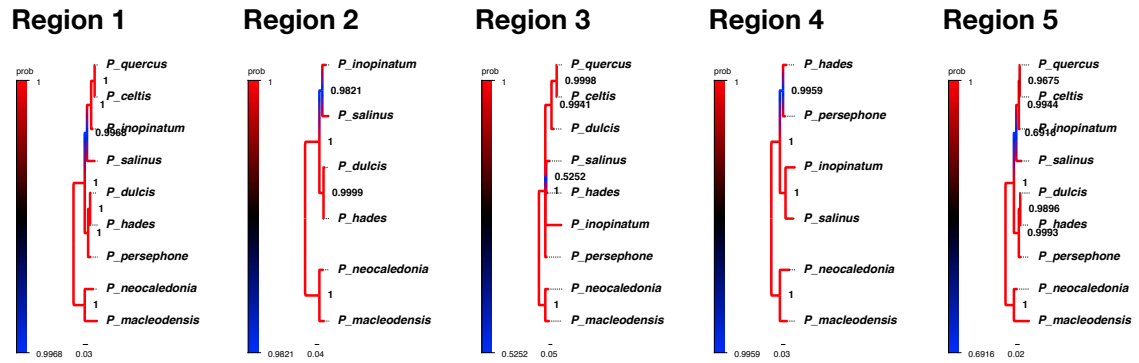


FIG S2 Bayesian phylogenetic trees of the specific regions of *polB*. Regions correspond to the *polB* alignment regions shown in Fig. 3. Color scale and node number: posterior probability. Scale bar: number of substitutions per site.

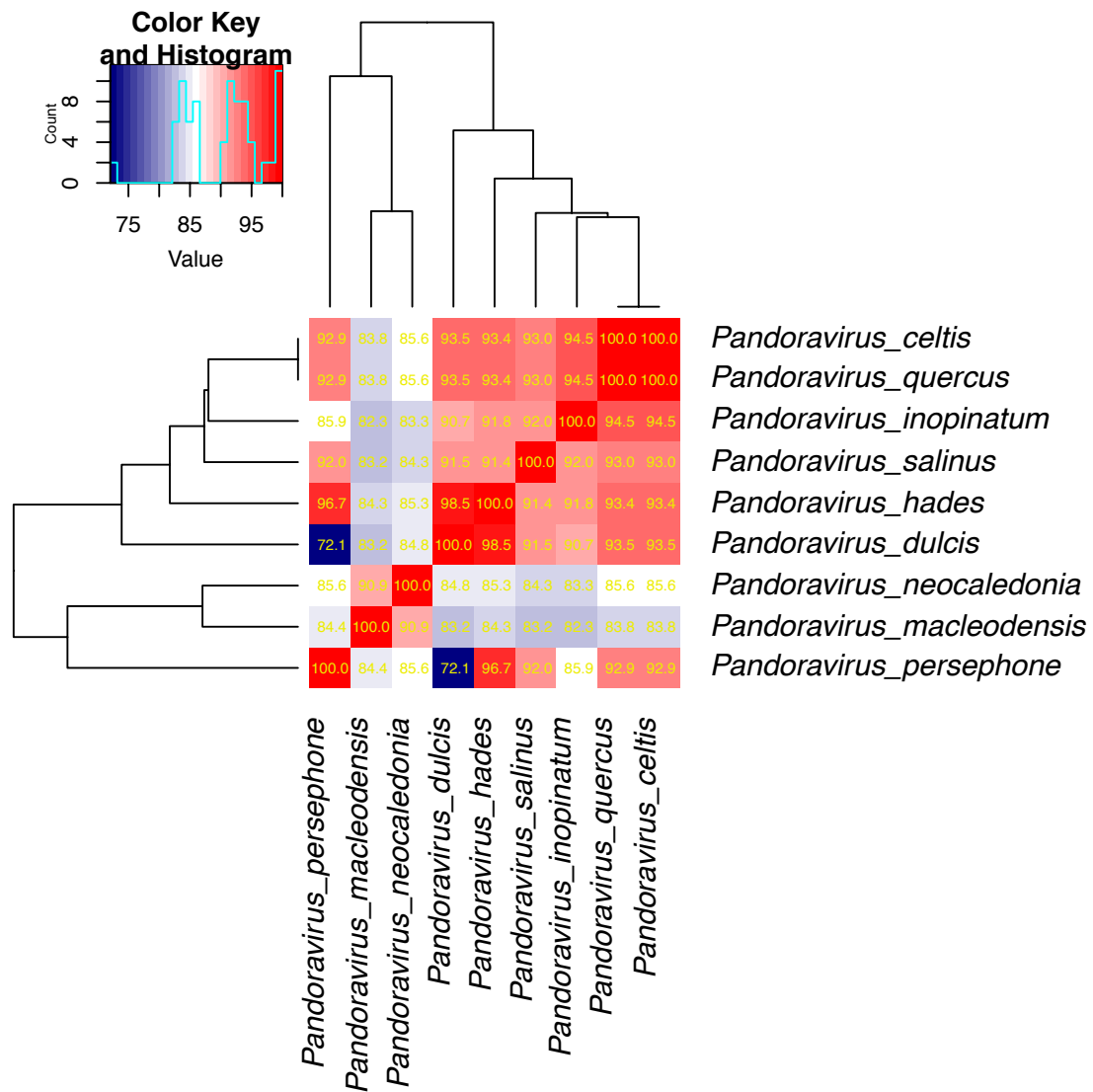


FIG S3 Heatmap of pairwise identities (%) among the *polB* sequences of pandoraviruses. Heatmap colors correspond to those shown in the key at the top left, in which a histogram of values is also shown. The pairwise identity values are given in yellow in each square.

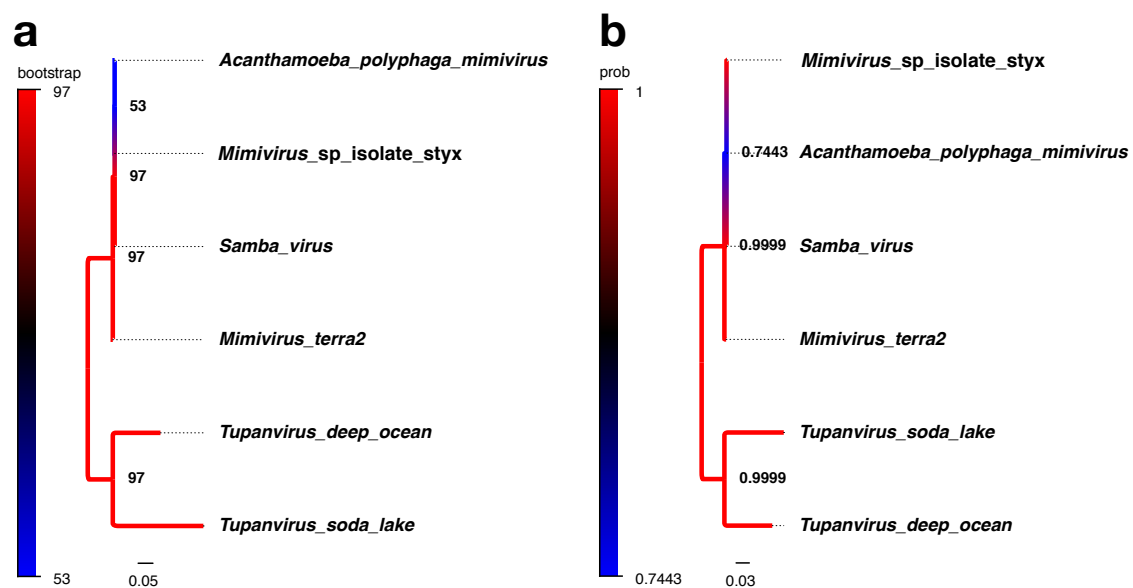


FIG S4 Phylogenetic analysis of the *polB* gene of members of the *Mimiviridae* family.

(a) Maximum likelihood phylogenetic tree. Color scale and node number: bootstrap value.

(b) Bayesian phylogenetic tree. Color scale and node number: posterior probability. Scale

bar: number of substitutions per site.

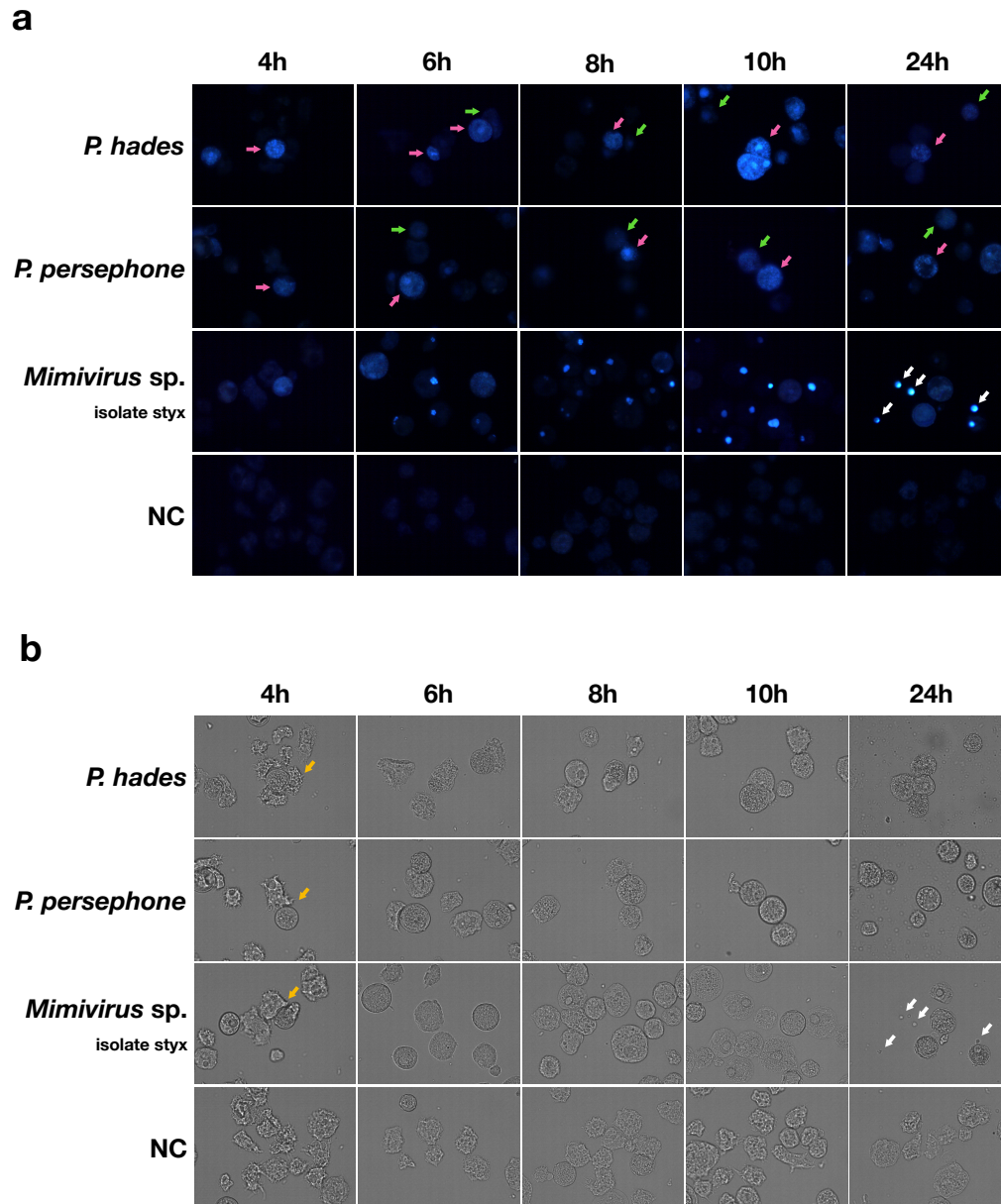


FIG S5 DAPI staining analysis of *P. hades*, *P. persephone*, and *Mimivirus* sp. isolate styx (Unmerged). **(a)** DAPI (4'6-diamidino-2-phenylindole). **(b)** Light. Green arrows: examples of partially stained cells. Pink arrows: examples of well-stained cells. White arrows: free virus factories. Yellow arrows: amoeba adhering to cells that exhibit cytopathic effects. NC: Uninfected amoeba cells as a negative control. Objective lens: x40.

Typing/sequencing analysis	Primer name	forward/reverse	sequence (5'-3')
<i>Pandoravirus</i>	P1	for	GTGAGCGTCAAGGGCATG
	P2*	for	CCAAACCTCGCACCACCTTTG
	P3	for	CGAGGGTCTATGTGATGCAGTG
	P4	for	ACCCATCCACAGAGAACTGG
	P5	for	CTACGATGCCATGTTTGGCG
	P6	for	CGATCGAGGTGTGGACCGAG
	P7	for	GCAACGACCAGACCTACGTGC
	P8	rev	CATCCACTGGGTGATCGGCGCC
	P8	rev	GGCGCCGATCACCCAGTGGATG
	P9	rev	CATCCACTGGGTGATCGGCGCC
	P10	rev	TCGAGGAGGCCACTCACATA
	P11	rev	GTGCATAAATGCGGTGATTG
	P12*	rev	TCGAGCGTGTACGATTTCGAG
<i>P. hades</i>	Phad_1	for	CAAAGCGTCCGTGTTGTAGC
	Phad_2	for	CCTGCCGCTCATGCTCATCAG
	Phad_3	rev	GTCTTTGTGCAGCACGTGCAG
	Phad_4	rev	AGGTTGCGTCAGACCATGATACATG
<i>P. persephone</i>	Ppers_1	for	GAGGAGATCTTTGAGCACTATGCGTC
	Ppers_2	rev	ACCCTCTTGCGCTGGTCTTTGAG
<i>Mimivirus</i>	M1*	for	GAGACGGATCATGTGGTTCCT
	M2*	rev	GAAGTGTCAAAGGGCTGCCA
RAPD analysis	Primer name	sequence (5'-3')	
	RAPD_1	CAATCGCCGT	
	RAPD_2	AGGGGTCTTG	
	RAPD_3	GAAACGGGTG	
	RAPD_4	AGGTGACCGT	
	RAPD_5	CAAACGTCCG	
	RAPD_6	GTTGCGATCC	

TABLE S1 PCR primers used in this study. Forward (for)/reverse (rev): primer directions based on *polB* gene coding direction. Primer names with asterisk: primers used for initial screening.

VIDEO S1 Lysis of *Acanthamoeba castellanii* cells infected with *Mimivirus shirakomae* and virus factory (VF) evacuation. (8 sec~): The small sphere (probable VF) appears immediately after lysis of the *A. castellanii* cell that exhibits cytopathic effects.