

Supplemental materials

Table S1 Sample information, sequence abundance, and bacterial diversity of the reproductive organs and bacteriomes of both sexes, and the salivary glands and digestive organs of *Pycna repanda* males

Tissues	Samples	Effective Tags	OTUs	Richness indices		Diversity indices	
				Chao 1	ACE	Shannon	Simpson
Male's salivary glands	SGM 1	93471	354	646.25	604.16	0.9181	0.2039
	SGM 2	83839	364	628.27	625.85	1.7753	0.4604
	SGM 3	74025	257	425.17	409.44	1.2432	0.3089
	SGM 4	74529	212	298.06	296.67	0.7759	0.2280
Male's filter chamber	FCM 1	84216	418	632.44	665.01	1.2377	0.2782
	FCM 2	85398	277	434.76	422.49	1.2993	0.3696
	FCM 3	86855	248	419.03	444.43	0.7493	0.1738
	FCM 4	80812	234	359.08	390.42	0.9358	0.2640
Male's conical segment	CSM 1	77644	235	385.92	412.30	0.9165	0.2093
	CSM 2	85919	245	366.87	381.28	0.8002	0.1798
	CSM 3	79668	294	432.60	410.36	1.5108	0.3703
	CSM 4	103034	243	409.79	404.94	0.4899	0.0984
Male's midgut	MM 1	81107	252	453.28	455.93	0.7797	0.1543
	MM 2	85007	328	524.50	552.10	1.6130	0.3740
	MM 3	72193	209	370	364.26	0.9831	0.2310
	MM 4	92078	196	292.87	315.62	0.3405	0.0563
Male's hindgut	HM 1	104818	349	503.41	511.58	1.4454	0.3798
	HM 2	79333	349	449.16	422.48	1.7084	0.5277
	HM 3	82658	257	447.65	472.41	0.8856	0.2645
	HM 4	65707	221	310.76	315.66	1.6597	0.5421
Testes	TM 1	84440	425	613.25	634.81	2.3263	0.5660
	TM 2	81792	376	638.86	624.18	1.6014	0.3780
	TM 3	88302	339	534.03	482.70	2.2142	0.6357
	TM 4	85489	285	382	394.27	1.7336	0.4865
Male's bacteriomes	BM 1	70280	263	388.82	370.08	1.9265	0.5455
	BM 2	92568	347	590.89	535.82	1.5497	0.3819
	BM 3	66345	155	276.71	274.67	0.7379	0.1789
	BM 4	91484	191	262.03	294.75	0.9585	0.2309
Ovaries	OF 1	72703	296	426	419.67	2.9963	0.7855
	OF 2	78427	220	333.75	367.70	3.1249	0.7673
	OF 3	81942	220	350.57	329.40	2.9159	0.7603
	OF 4	81753	199	295.28	324.86	2.6241	0.7252
Female's bacteriomes	BF 1	73467	158	282.25	261.45	0.7997	0.2078
	BF 2	86450	203	329.40	310.47	1.5191	0.4700
	BF 3	85002	195	312.34	324.78	1.5589	0.5473
	BF 4	82809	148	214.96	219.37	1.1042	0.3089

Abbreviations: SGM, salivary glands of males; FCM, filter chamber of males; CSM, conical segment of males; MM, midgut of males; HM, hindgut of males; TM, testes of males; BM, bacteriomes of males; OF, ovaries of females; BF, bacteriomes of females.

Table S2 The 16S rRNA gene sequences of dominant symbionts harbored in *Pycna repanda*

Symbiont types	16S rRNA gene sequences
Clone PR-16 (<i>Sulcia</i>)	<p>AGAGTTTGATCATGGCTCAGGATAAACGCTAGCGGAGGGCATAACACATGCA AGTCGAGGGGCATCAAAAAAATATAAGTTTACTTATGTTTTTTGTTTGGCAAC CGGCGTACGGGTGAGTAATACATACGTAACCTTCCTTATGCTGAAGGATAGCC CGAGGAACTTGGATTAATACTTCATAATACCATTTTTTATTAAGAAATTTGGTT AAAGTTATTATGGCATAAGATAGCGGATGTCCGATTAGTTAGTTGGTAAGGT AATGGCTTACCAAGACAATGATCGGTAGGGGTCCTGAGAGGGCATAACCCCA CATTGGGACTGAGACACGGACCAAACCTTCTACGGAAGGCAGCAGTGAGGAAT ATTGGTCAATGGAGGCAACTCTGAACCAGCCACTCCGCGTGTAGGAGGAAAG CCATTTAGGTTGTAACTACTTTTGTATATGAATAAAAAATTCTATTTTTAGAAA AAATTGAATGTAATATACGAATAAGTATCGGCCAACTCTGTGCCAGCAGCCGC GGTAAACCGGAGGATACGAGCGCTATCCGGATTTATTGGGTTTAAAGGGTGC GTAGGTGGTTTTTAAAGTCAGTAGTGAATCTTAAAGCTTAACTTTAAAAGTGC TATTGATACTGATAAACTAGAGTGAGGTTGGAGTAACTGGAATGTGTGGTGGAA GCGGTGAAATGCATAGAGATCACACAGAACCCGATCGCGAAAGCAGGTTAC TAAACATAGACTGACGCTGAGGCACGAAAGCATGGGGAGCAAACAGGATTAG ATACCCTGGTAGTCCATGCCGTAAACGATGATCACTAACTATTGGAGAATAATT CAGTGGTCAAGCGAAAGTGATAAGTGATCCACCTGAGGAGTACGACCGCAAG GTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAATCGGTGGAGCATGTGG TTTAATTCGATGATACGCGAGGAACCTTACCAAACTTAAATGTATTACGAATA AATTGGAAACAATTTAGTCATTAGACGGAGTACAAGGTGCTGCATGGTTGTGCG TCAGCTCGTGCCGTGAGGTGTGAGGTTAAGTCCTTTAACGAGCGCAACCCTT ATTATTAGTTACCATCAAGTAATGTTGGGACTCTAATAAGACTGCCGGCGTA AGCCGAGAGGAAGGCGGGGACGACGTCAAATCATCACGGCCCTTATGTTTTG GGCCACACACGTGCTACAATGGTCGGTACAGAGGGGAGCAACTGGGTGACC AGAAGCAGATCTTGAAAGCCGATCTCAGTTTCGATTGGAGTCTGCAACTCGAC TCTATGAAGCTGGAATCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAAT ATGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGTTTGAAGTA CCTAAAATCGGTGACCTTGAGAGAAGGGAAGTCCGAAGGTAAGTCAATAA CTAGGGCTAAGTCGTAACAAGGTAACCCAT ACGAGCGCGGGCGGCAGGCCTAACACATGCAAGTCGAGCGAACGTCCTTCG AAGGACGTAAGCGGCGGACGGGTGAGTAACACGTAAGAATCTACCTCGCTCA ACGGCACAACGCAGGGAAGCCTGCGCTAATTCCGTATGAATCCTGAATCAGG TAAAGATCCTTCGGAGCGAGATGAGCTTGCCTAGATTAGCTTGTGGTGGG GTAAATGCCACCAAGGCAACGATCTATAGCTGGTCTGGGAGGACGGACAGC CACACTGGGACTGGAACAAGGCCAGACCCCGTGGGGGTCAGCAGTGAGG AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGAGTGATTA GAAGTCAAACCTTCTGAAGTTTTGTGAAAATTGAAAACCTTTTTGCCAAACGA AATGATGATGGTAGTTTGGACGAATAAGCTCCGGCCAACTTCGTGCCAGCAG CCGCGGTAATACGAGGGGAGCAAGCGTTGTTCCGTTTTATTGGGCGTAAAGC GCGCGTAGGCGGCCACGCAAGTCGTTTCAGAAGTCAAACGATGGAGCTCAAC</p>
Clone PR-9 (<i>Hodgkinia</i>)	<p>AGAGTTTGATCATGGCTCAGGATAAACGCTAGCGGAGGGCATAACACATGCA AGTCGAGGGGCATCAAAAAAATATAAGTTTACTTATGTTTTTTGTTTGGCAAC CGGCGTACGGGTGAGTAATACATACGTAACCTTCCTTATGCTGAAGGATAGCC CGAGGAACTTGGATTAATACTTCATAATACCATTTTTTATTAAGAAATTTGGTT AAAGTTATTATGGCATAAGATAGCGGATGTCCGATTAGTTAGTTGGTAAGGT AATGGCTTACCAAGACAATGATCGGTAGGGGTCCTGAGAGGGCATAACCCCA CATTGGGACTGAGACACGGACCAAACCTTCTACGGAAGGCAGCAGTGAGGAAT ATTGGTCAATGGAGGCAACTCTGAACCAGCCACTCCGCGTGTAGGAGGAAAG CCATTTAGGTTGTAACTACTTTTGTATATGAATAAAAAATTCTATTTTTAGAAA AAATTGAATGTAATATACGAATAAGTATCGGCCAACTCTGTGCCAGCAGCCGC GGTAAACCGGAGGATACGAGCGCTATCCGGATTTATTGGGTTTAAAGGGTGC GTAGGTGGTTTTTAAAGTCAGTAGTGAATCTTAAAGCTTAACTTTAAAAGTGC TATTGATACTGATAAACTAGAGTGAGGTTGGAGTAACTGGAATGTGTGGTGGAA GCGGTGAAATGCATAGAGATCACACAGAACCCGATCGCGAAAGCAGGTTAC TAAACATAGACTGACGCTGAGGCACGAAAGCATGGGGAGCAAACAGGATTAG ATACCCTGGTAGTCCATGCCGTAAACGATGATCACTAACTATTGGAGAATAATT CAGTGGTCAAGCGAAAGTGATAAGTGATCCACCTGAGGAGTACGACCGCAAG GTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAATCGGTGGAGCATGTGG TTTAATTCGATGATACGCGAGGAACCTTACCAAACTTAAATGTATTACGAATA AATTGGAAACAATTTAGTCATTAGACGGAGTACAAGGTGCTGCATGGTTGTGCG TCAGCTCGTGCCGTGAGGTGTGAGGTTAAGTCCTTTAACGAGCGCAACCCTT ATTATTAGTTACCATCAAGTAATGTTGGGACTCTAATAAGACTGCCGGCGTA AGCCGAGAGGAAGGCGGGGACGACGTCAAATCATCACGGCCCTTATGTTTTG GGCCACACACGTGCTACAATGGTCGGTACAGAGGGGAGCAACTGGGTGACC AGAAGCAGATCTTGAAAGCCGATCTCAGTTTCGATTGGAGTCTGCAACTCGAC TCTATGAAGCTGGAATCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAAT ATGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGTTTGAAGTA CCTAAAATCGGTGACCTTGAGAGAAGGGAAGTCCGAAGGTAAGTCAATAA CTAGGGCTAAGTCGTAACAAGGTAACCCAT ACGAGCGCGGGCGGCAGGCCTAACACATGCAAGTCGAGCGAACGTCCTTCG AAGGACGTAAGCGGCGGACGGGTGAGTAACACGTAAGAATCTACCTCGCTCA ACGGCACAACGCAGGGAAGCCTGCGCTAATTCCGTATGAATCCTGAATCAGG TAAAGATCCTTCGGAGCGAGATGAGCTTGCCTAGATTAGCTTGTGGTGGG GTAAATGCCACCAAGGCAACGATCTATAGCTGGTCTGGGAGGACGGACAGC CACACTGGGACTGGAACAAGGCCAGACCCCGTGGGGGTCAGCAGTGAGG AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGAGTGATTA GAAGTCAAACCTTCTGAAGTTTTGTGAAAATTGAAAACCTTTTTGCCAAACGA AATGATGATGGTAGTTTGGACGAATAAGCTCCGGCCAACTTCGTGCCAGCAG CCGCGGTAATACGAGGGGAGCAAGCGTTGTTCCGTTTTATTGGGCGTAAAGC GCGCGTAGGCGGCCACGCAAGTCGTTTCAGAAGTCAAACGATGGAGCTCAAC</p>

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CACCGCCCGTCACACCATGGGAGCGGGGTCCGTGCGAAGCGTGAGCGAAGC
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CGCCCGTCACACCATGGGAGTG

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(*Hodgkinia*)

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GTACGGTTAAGTCCTATAACGAGCGCAACCCTCGTCCTTAGTTGCTTCCAAGC
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CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCTCGGGTCTGTAC
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CTCCGTCAGGGCGGACTTAGCGACCGAGGTG

Rickettsia

CACTTTATGAAATAATGCTAATACCGTATATTCTTCCGAGGAAAGATTTATC
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CTTTACTCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAAC
CCAACATTTCAACAACAGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAG
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CGACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCA
CGTTTTCGCACATGAGCGTCAGTCTTTATCCAGTGGATCGCCTTCGCCACTGG
TATTCCTCCACATCTCTACGCATTTACCGCTACATGTGGAATTCTATCCACCT
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GATTTACATTCAACTTAATTAACCGCTGCGTGCCCTTTACGCCAGTAATTC
CGATTAACGCTCGCACCCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGC
CCGGTGCTTCTTCTGTTGCTAACGTCAATTGCTAAGAGTATTAATCTTAACACC
TTTCCTCACAACCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACATGG
CATAGCTGCAGTAT

Arsenophonus



Table S3 NCBI blast results for the 16S rRNA gene sequences of the representative clones obtained from the bacteriomes of *Pycna repanda* males

No. of representative clones of different RFLP profiles	The percentage in clone library	Closest match in GenBank	Identity to closest match (%)
Clone PR-16 (<i>Sulcia</i>)	63%	<i>Sulcia</i> of <i>Platypleura kaempferi</i> (LC370623.1)	99%
		<i>Sulcia</i> of <i>Pl. yaeyamana</i> (LC370627.1)	99%
		<i>Sulcia</i> of <i>Pl. kuroiwae</i> (LC370624.1)	99%
Clone PR-9 (<i>Hodgkinia</i>)	1%	<i>Hodgkinia</i> of <i>Pl. kuroiwae</i> (LC370476.1)	92.54%
		<i>Hodgkinia</i> of <i>Pl. yaeyamana</i> (LC370501.1)	91.76%
		<i>Hodgkinia</i> of <i>Pl. kaempferi</i> (LC370463.1)	91.72%
Clone PR-96 (<i>Hodgkinia</i>)	30%	<i>Hodgkinia</i> of <i>Pl. kuroiwae</i> (LC370476.1)	92.43%
		<i>Hodgkinia</i> of <i>Pl. yaeyamana</i> (LC370501.1)	91.71%
		<i>Hodgkinia</i> of <i>Pl. kaempferi</i> (LC370463.1)	90.78%
Clone PR-131 (<i>Hodgkinia</i>)	1%	<i>Hodgkinia</i> of <i>Pl. kuroiwae</i> (LC370476.1)	93.52%
		<i>Hodgkinia</i> of <i>Pl. yaeyamana</i> (LC370501.1)	92.65%
		<i>Hodgkinia</i> of <i>Pl. kaempferi</i> (LC370463.1)	91.49%
Clone PR-187 (<i>Hodgkinia</i>)	0.5%	<i>Hodgkinia</i> of <i>Pl. kuroiwae</i> (LC370476.1)	91.55%
		<i>Hodgkinia</i> of <i>Pl. yaeyamana</i> (LC370501.1)	91.03%
		<i>Hodgkinia</i> of <i>Pl. kaempferi</i> (LC370463.1)	90.52%
Clone PR-215 (<i>Hodgkinia</i>)	4.5%	<i>Hodgkinia</i> of <i>Pl. kuroiwae</i> (LC370476.1)	90.95%
		<i>Hodgkinia</i> of <i>Pl. yaeyamana</i> (LC370501.1)	90.44%
		<i>Hodgkinia</i> of <i>Pl. kaempferi</i> (LC370463.1)	90.52%

Hodgkinia of *Pl. kaempferi*
(LC370463.1)

92.45%

Table S4 Prevalence of dominant symbionts in different tissues of *Pycna repanda* using diagnostic PCR amplifications ^a

Symbiont	Numbers of positive individuals/total numbers of individuals tested ^b								
	SG	FC	CS	MG	HG	MT	TE	OV	BA
<i>Sulcia</i>	0/55	0/55	0/55	0/55	0/55	0/55	0/30	25/25	55/55
<i>Hodgkinia</i>	0/55	0/55	0/55	0/55	0/55	0/55	0/30	25/25	55/55
<i>Arsenophonus</i>	0/55	0/55	0/55	0/55	0/55	0/55	0/30	23/25	50/55 ^c
<i>Rickettsia</i>	55/55	55/55	55/55	55/55	55/55	55/55	30/30	0/25	0/55

^a A total of 55 individuals were used in this study, 30 males and 25 females.

^b Abbreviations: SG, salivary glands; FC, filter chamber; CS, conical segment; MG, midgut; HG, hindgut; MT, Malpighian tubules; TE, testes; OV, ovaries; BA, bacteriomes.

^c 50 individuals were positive detection, 27 males and 23 females.

Table S5 Ratio of *Hodgkinia* of dark cells to light cells in the bacteriomes ^a

	Average numbers of <i>Hodgkinia</i> cells counted in every individual ^a		Ratio of DC: LC
	Dark cells	Light cells	
Male (n=15)	~205	~1150	1: 5.598
Female (n=15)	~211	~1187	1: 5.619

^a *Hodgkinia* cells of different colors were found in all observed individuals. We randomly selected multiple sections from 15 males and 15 females for statistical analysis, aiming to estimate the ratio of *Hodgkinia* of dark cells to light cells. Abbreviations: DC, *Hodgkinia* of darker cells; LC, *Hodgkinia* of light cells.

Table S6 Individuals, tissues and observations for the study of transovarial transmission of bacteriome-associated symbionts.

Transmission process	Numbers of individuals examined ^a		Tissues	Numbers of bacteriocytes/ovarioles observed
	TEM	FISH		
<i>Sulcia</i> and <i>Arsenophonus</i> leaving the peripheral bacteriocytes	18	25	bacteriomes	~110
<i>Hodgkinia</i> emigrating from the central bacteriocytes	18	25	bacteriomes	~190
Symbionts migrating through the follicular cells into the perivitelline space	18	25	ovaries	~270
Symbionts forming a "symbiont ball" in each egg	18	25	ovaries	~230

^a The transmission process was similar in every individual examined. Mature females were used in the present study. Fourteen females were dissected for FISH and TEM. Eleven females were dissected for FISH. Four females were dissected for TEM.

Table S7 Statistical analysis showing *Hodgkinia* cells of darker color emigrating from central bacteriocytes through the multi-nuclear compartment to the hemolymph.

	Numbers of <i>Hodgkinia</i> cells counted	
	Dark cells	Light cells
FM 1	437	0
FM 2	393	0
FM 3	411	0
FM 4	221	0
FM 5	491	0
FM 6	401	0
FM 7	354	0
FM 8	284	0
FM 9	189	0
FM 10	253	0
FM 11	282	0
FM 12	167	0
FM 13	225	0
FM 14	309	0
FM 15	442	0
FM 16	228	0
FM 17	248	0
FM 18	459	0

Abbreviations: The numbers (i.e., 1, 2, 3, 4) after FM represented the biological replications.

Table S8 Statistical analysis revealing numbers of symbiont cells changing their shape during the transovarial transmission process.

Shape change	Symbiont species	Numbers of cells counted ^a
Irregular to roughly spherical	<i>Sulcia</i>	106
	<i>Hodgkinia</i>	892
Roughly spherical to irregular	<i>Sulcia</i>	168
	<i>Hodgkinia</i>	1262
Not obvious	<i>Arsenophonus</i>	1823

^a The shape change of related symbionts were observed in every examined individual and we randomly selected multiple sections from different individuals for the cell counts.

Table S9 Ratio of three different symbionts in the “symbiont balls” at the posterior pole of the oocytes

	Numbers of different symbionts counted ^a			Ratio of S: H: A
	<i>Sulcia</i>	<i>Hodgkinia</i>	<i>Arsenophonus</i>	
FM 1	185	1661	686	1: 8.978: 3.708
FM 2	193	1718	702	1: 8.902: 3.637
FM 3	189	1690	693	1: 8.942: 3.667
FM 4	179	1638	679	1: 9.151: 3.793
FM 5	194	1756	705	1: 9.052: 3.634
FM 6	185	1672	685	1: 9.038: 3.703
FM 7	183	1651	678	1: 9.022: 3.705
FM 8	195	1753	720	1: 8.990: 3.692
FM 9	188	1684	691	1: 8.957: 3.676
FM 10	185	1659	684	1: 8.968: 3.697
FM 11	196	1773	722	1: 9.046: 3.684
FM 12	183	1640	679	1: 8.962: 3.710
FM 13	192	1730	707	1: 9.010: 3.682
FM 14	187	1748	697	1: 9.250: 3.727
FM 15	194	1742	724	1: 8.979: 3.732
FM 16	190	1697	701	1: 8.932: 3.689
FM 17	197	1765	728	1: 8.959: 3.695
FM 18	187	1669	685	1: 8.925: 3.667

^a We randomly selected multiple sections from every individual to estimate the ratio of the three different symbionts. Abbreviations: A, *Arsenophonus*; S, *Sulcia*; H, *Hodgkinia*; FM, females. The numbers (i.e., 1, 2, 3, 4) after FM represented the biological replications.

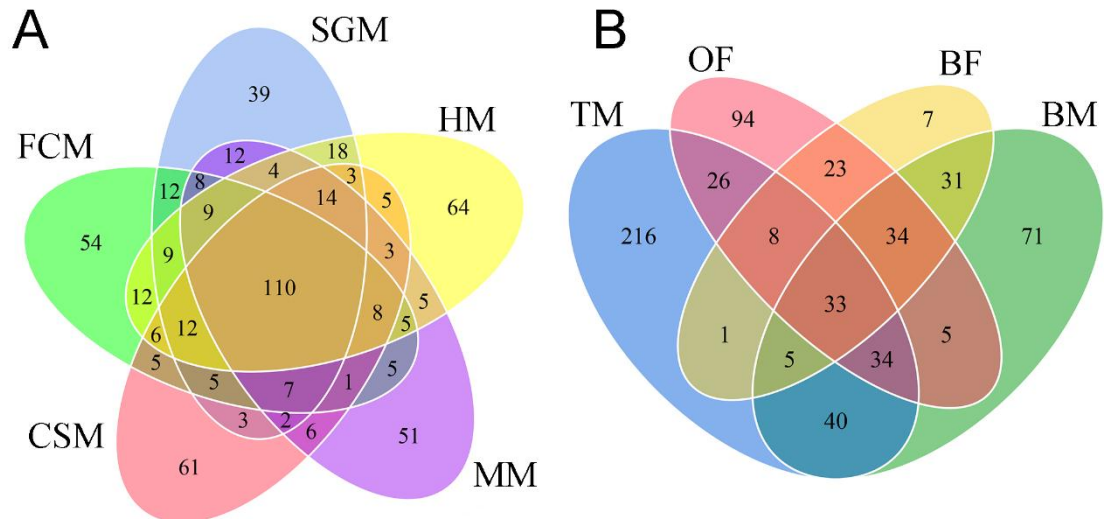


Fig S1 Venn diagrams showing numbers of the OTUs shared among different tissues of *Pycna repanda*. (A) Numbers of the OTUs are shared among the salivary glands, filter chamber, conical segment, midgut and hindgut of *Py. repanda* males. (B) Numbers of the OTUs shared among the bacteriomes and reproductive organs in both sexes. Abbreviations: SGM, salivary glands of males; FCM, filter chamber of males; CSM, conical segment of males; MM, midgut of males; HM, hindgut of males; TM, testes of males; BM, bacteriomes of males; OF, ovaries of females; BF, bacteriomes of females.

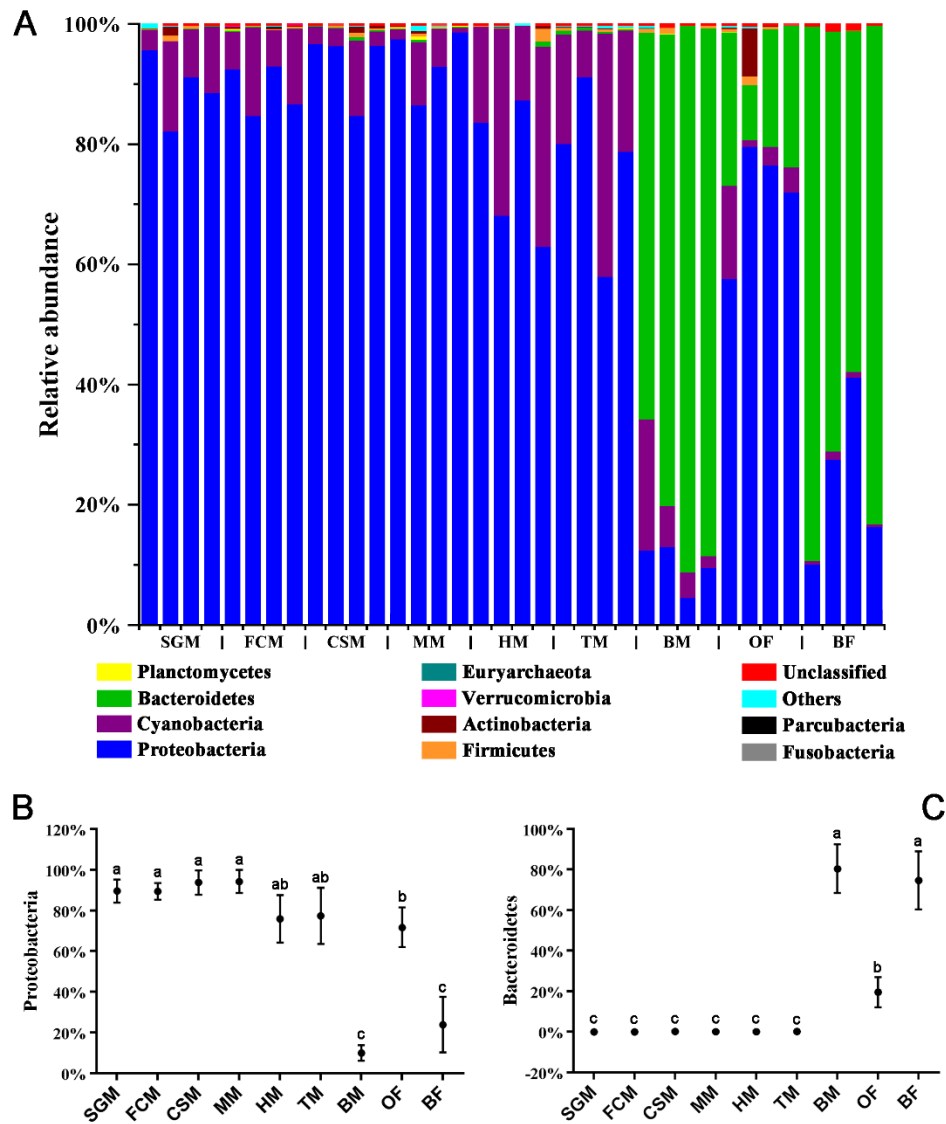


Fig S2 Bacterial communities of the salivary glands, bacteriomes, reproductive and digestive organs of *Pycna repanda* at the phylum level and comparative analysis of the differences of dominant phyla among different tissues. (A) Bacterial composition of the salivary glands, bacteriomes, reproductive and digestive organs. (B) Comparative analysis of the differences of Proteobacteria among different tissues. (C) Comparative analysis of the differences of Bacteroidetes among different tissues. Different letters represent significant difference at the 0.05 level. Abbreviations: SGM, salivary glands of males; FCM,

filter chamber of males; CSM, conical segment of males; MM, midgut of males; HM, hindgut of males; TM, testes of males; BM, bacteriomes of males; OF, ovaries of females; BF, bacteriomes of females.

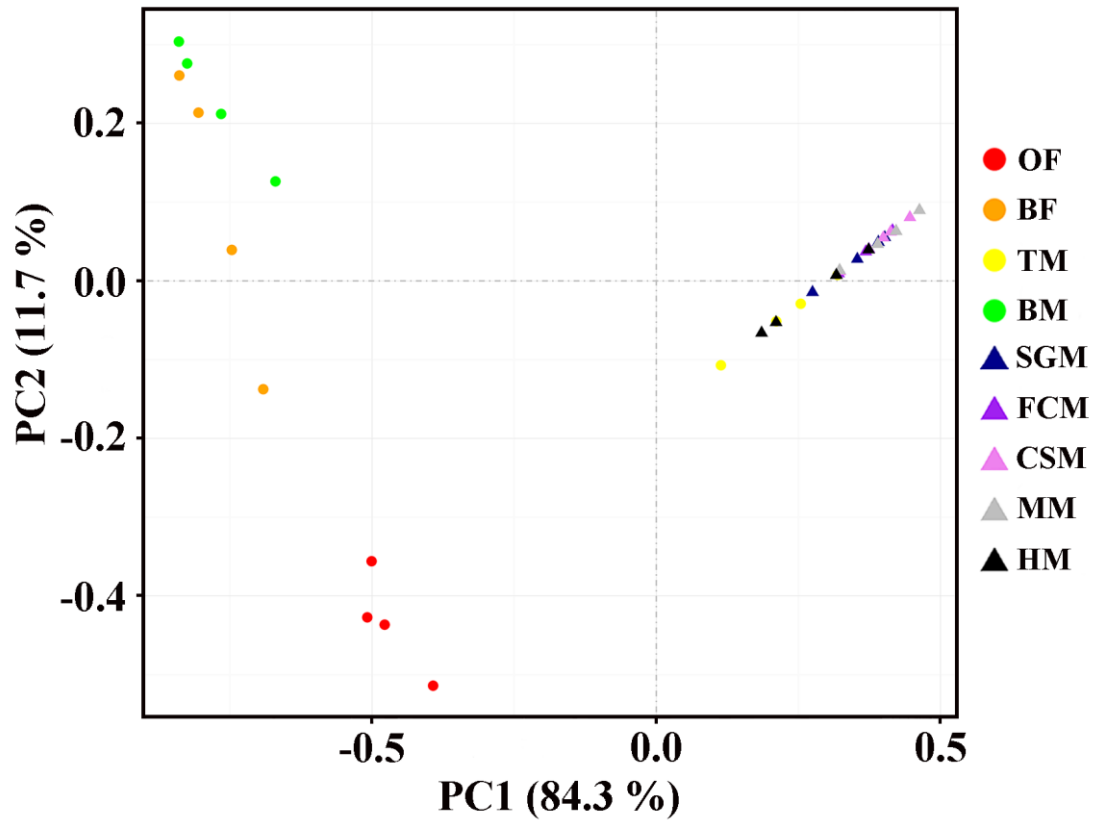


Fig S3 Principal component analysis revealing the similarity of bacterial communities among different tissues. Abbreviations: SGM, salivary glands of males; FCM, filter chamber of males; CSM, conical segment of males; MM, midgut of males; HM, hindgut of males; TM, testes of males; BM, bacteriomes of males; OF, ovaries of females; BF, bacteriomes of females.

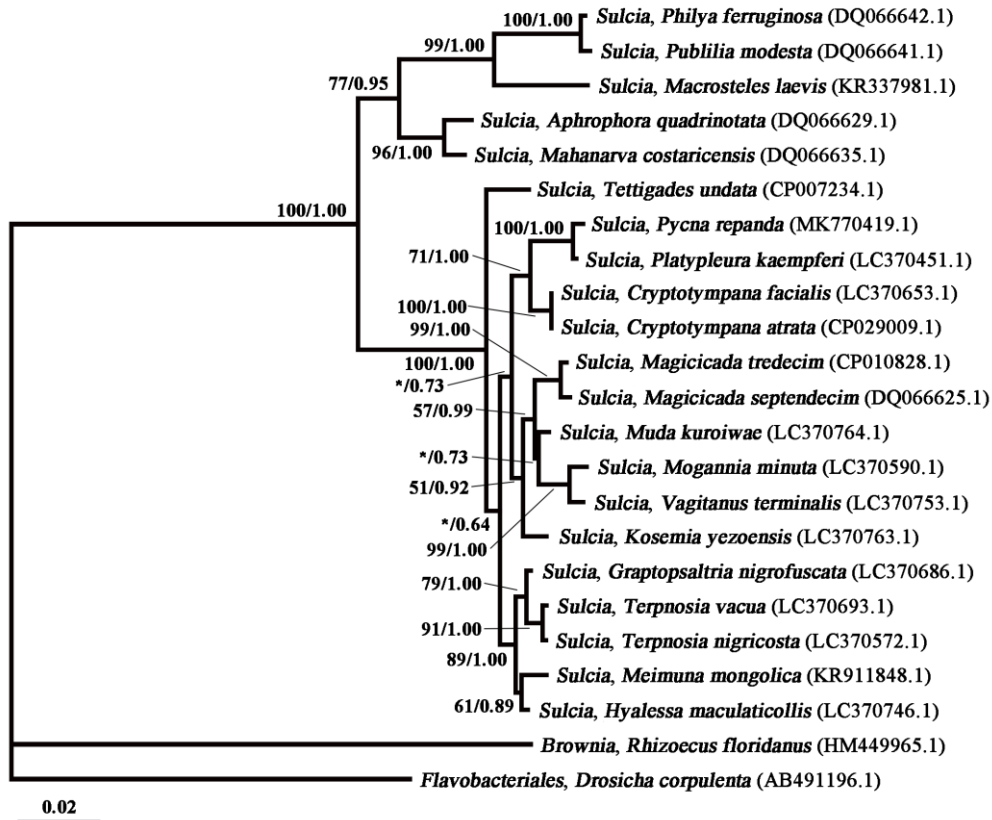


Fig S4 Phylogenetic relationship inferred from bacterial 16S rRNA gene sequences of *Sulcia* symbionts of cicadas and other representative hemipteran insects. Asterisks representing support values less than 50% and bootstrap support values more than 50% are shown on each node in the order of the maximum-likelihood/Bayesian inference. Bootstrap support values and posterior probabilities of the maximum-likelihood/Bayesian inference are shown near branches. The scale bar represents 0.02 substitutions per nucleotide site.

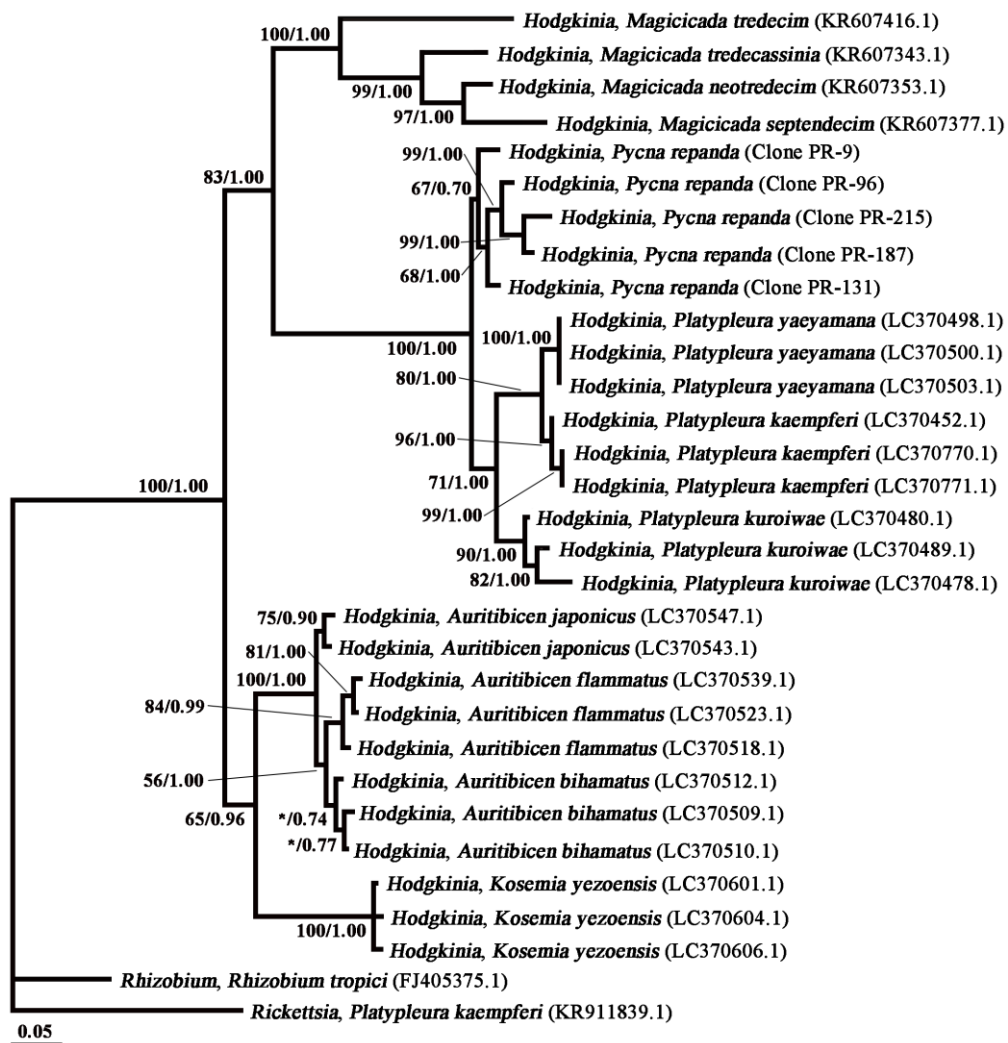


Fig S5 Phylogenetic relationship inferred from bacterial 16S rRNA gene sequences of *Hodgkinia* symbionts of cicadas. Asterisks representing support values less than 50% and bootstrap support values more than 50% are shown on each node in the order of the maximum-likelihood/Bayesian inference. Bootstrap support values and posterior probabilities of the maximum-likelihood/Bayesian inference are shown near branches. The scale bar represents 0.05 substitutions per nucleotide site.

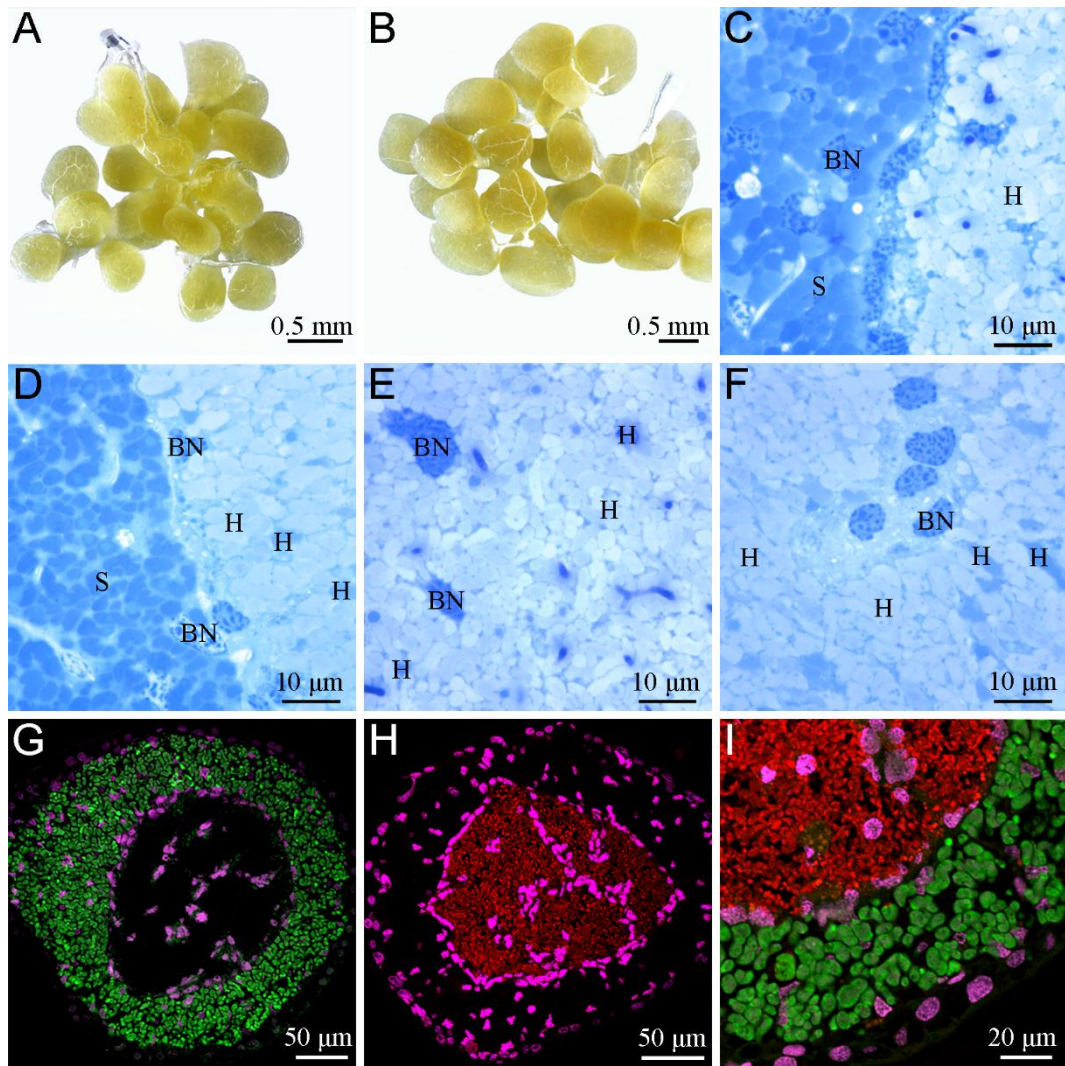


Fig S6 General morphology of the bacteriomes and localization of primary symbionts in the bacteriome units in *Pycna repanda*. (A) General morphology of the bacteriomes of male adult. (B) General morphology of the bacteriomes of female adult. (C) The distribution of symbionts in the bacteriocytes of males. (D) The distribution of symbionts in the bacteriocytes of females. (E) The central bacteriocytes harboring *Hodgkinia* of different colors in males. (F) The central bacteriocytes harboring *Hodgkinia* of different colors in females. (G) Negative control using only *Sulcia*-specific probe (green) showing *Sulcia* hosted in the peripheral bacteriocytes. (H) Negative control using only *Hodgkinia*-specific

probe (red) showing *Hodgkinia* harbored in the central bacteriocytes. (I) FISH analysis distinguishing *Hodgkinia* (red) (in the central bacteriocytes) from *Sulcia* (green) (in the peripheral bacteriocytes). Magenta, green and red represent bacteriocyte nucleus, *Sulcia* and *Hodgkinia*, respectively. Abbreviations: BN, bacteriocyte nucleus; H, *Hodgkinia*; S, *Sulcia*.

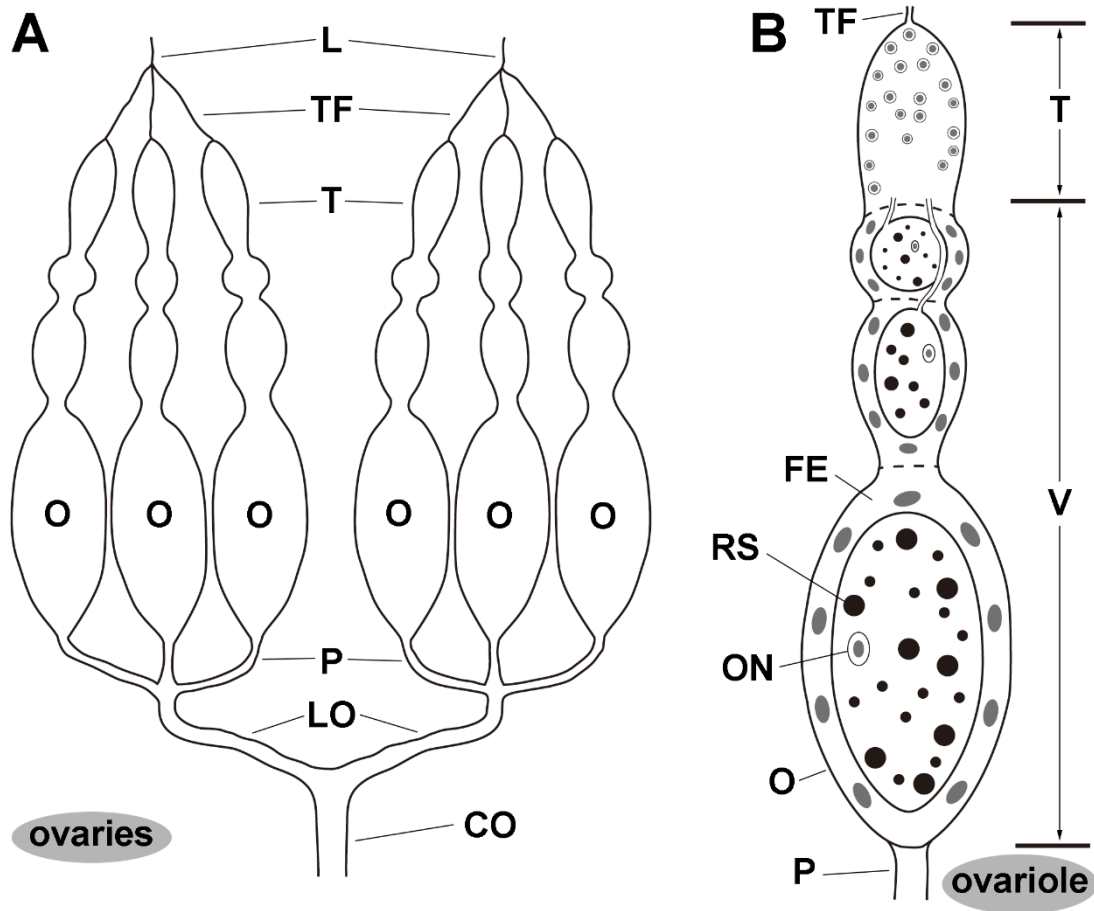


Fig S7 Schematic representation illustrating the general morphology of ovaries and ovariole of *Pycna repanda*. (A) The general morphology of the ovaries. (B) The morphology of the ovariole. Abbreviations: CO, common oviduct; FE, follicular epithelium; L, ligament; LO, lateral oviduct; O, oocyte; ON, oocyte nucleus; P, pedicel; RS, reserve substances; TF, terminal filament; T, tropharium; V, vitellarium.

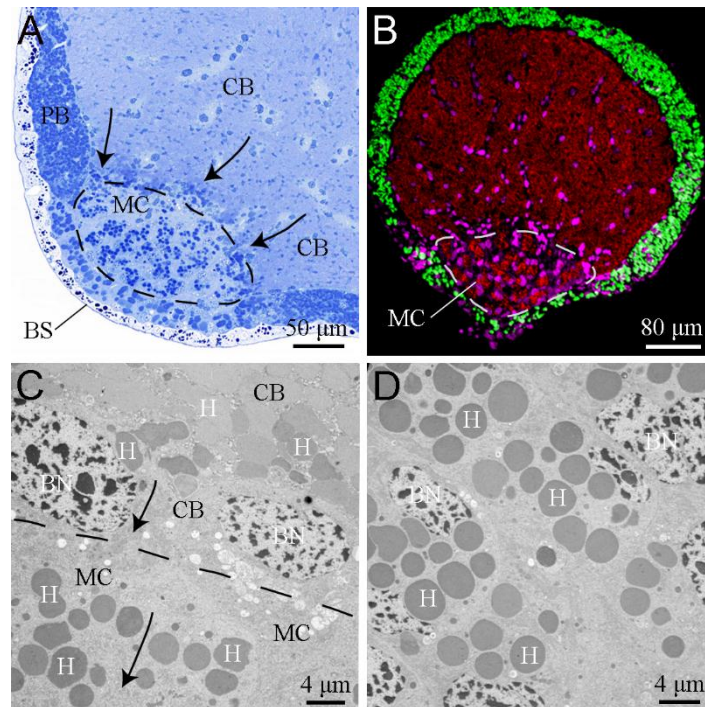


Fig S8 *Hodgkinia* emigrating through the multi-nuclear compartment from central bacteriocyte. (A) Histological observation showing the *Hodgkinia* cells emigrating through the multi-nuclear compartment from the central bacteriocytes of females. (B) Fluorescent microscopy showing the *Hodgkinia* cells emigrating through the multi-nuclear compartment from the bacteriocytes. Magenta, green and red represent bacteriocyte nucleus, *Sulcia* and *Hodgkinia*, respectively. (C–D) Ultrastructural observation showing *Hodgkinia* cells of darker colors emigrating through the multi-nuclear compartment from central bacteriocytes. Abbreviations: BN, bacteriocyte nucleus; CB, central bacteriocyte; H, *Hodgkinia*; MC, multi-nuclear compartment (encircled with black/white dotted line); PB, peripheral bacteriocyte; black arrows, *Hodgkinia* emigrating from central bacteriocyte through the multi-nuclear compartment.

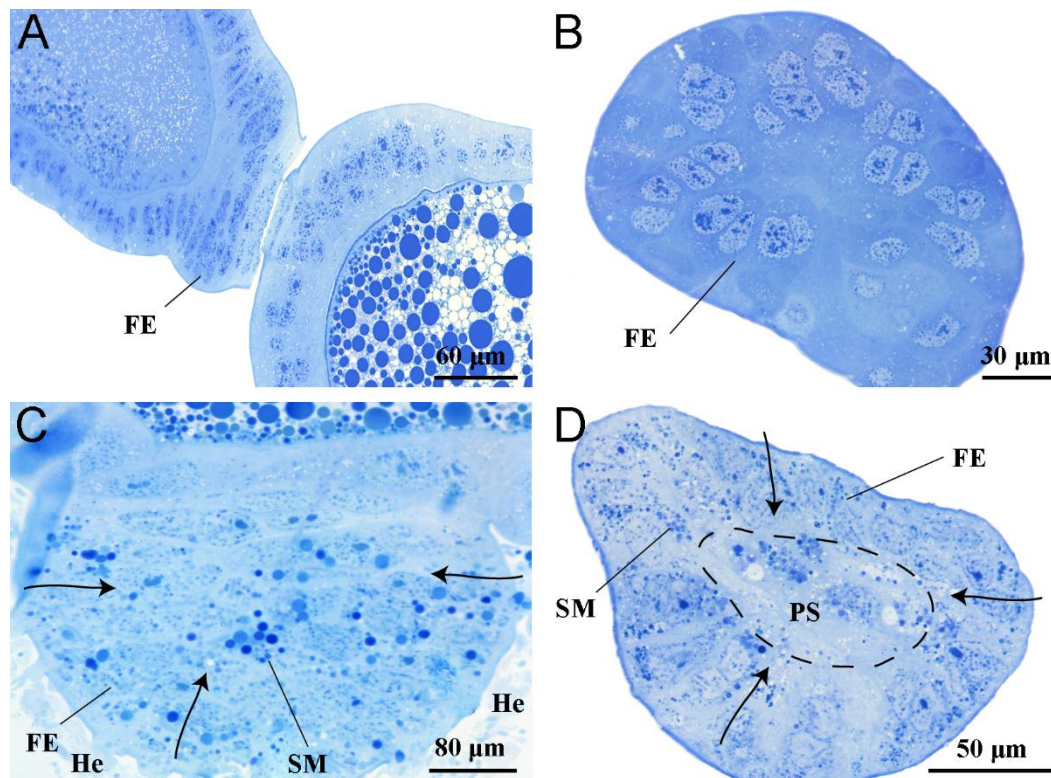


Fig S9 Symbionts invading the follicular epithelium before migrating into the perivitelline space of the ovariole. (A–B) Follicular epithelium remained its inherent shape before invasion of symbionts (A, longitudinal section; B, cross section). (C–D) Symbionts entered the cytoplasm of the follicular epithelium, resulting in the increased volume of follicular epithelium. Abbreviations: FE, follicular epithelium; He, hemolymph; PS, perivitelline space (encircled with black dotted line); SM, symbiotic microorganisms (C, longitudinal section; D, cross section); black arrows, symbiotic microorganisms emigrating through the follicular epithelia into the perivitelline space.