

Table S1 The primers used in this study.

Group	Name	Primer (5'-3')	Size (bp)
Universal, 16S rRNA, PCR Pacbio	27F 1492R	AGRGTTYGATYMTGGCTCAG RGYTACCTTGTTACGACTT	1 500
Universal, 16S rRNA, qPCR	Com1 769R	CAGCAGCCGCGGTAATAC ATCCTGTTTGMTMCCCVCRC	270
Universal ,16S rRNA, PCR Illumina	338F 806R	ACTCCTACGGGAGGCAGCAG GGACTACHVGGGTWTCTAAT	468
un-Rhi (<i>Bartonella A</i>) 16S rRNA, PCR	Bar1-F Bar1-R	GGGGGAAAGATTTATCGGATT TGAAGAAATCTATCTCTAAATCT CA	800
un-Rhi (<i>Bartonella A</i>) 16S rRNA, qPCR	Bar2-F Bar2-R	GAAGCTAGCCGTTGGAAGTT TGAAGAAATCTATCTCTAAATCT CA	181
<i>Kocuria</i> 16S rRNA, PCR	Koc1-F Koc1-R	CCACACTGGGACTGAGACAC AGCCCCGAAAGGAAACAACA	709
<i>Kocuria</i> 16S rRNA, qPCR	Koc2-F Koc2-R	CTTATCCCAGAGTCCAAGGT ATGGCTCAGGACGAACGCTG	134

Table S5 Summary of Sequence and Base Counts for 16S rRNA Amplicon Sequencing

Sample	unfiltered reads number	Base number	Rarefied reads number
E1	48045	19624292	43749
E2	54282	22234827	43749
E3	54981	22343440	43749
AF1 (EA1)	60111	24500236	43749
AF2 (EA2)	54307	22133958	43749
AF3 (EA3)	61513	25094155	43749
AM1	71847	30347532	43749
AM2	64554	26483455	43749
AM3	60891	24866034	43749
SA1	63457	26416098	43749
SA2	58470	24100993	43749
SA3	61975	25355855	43749
C1	58542	23722967	43749
C2	58819	24001891	43749
C3	57769	23562187	43749
T1	53273	22101656	43749
T2	46312	19057626	43749
T3	49126	20174737	43749

Table S6 Hemolytic activity of isolated bacteria from *D. gallinae*

Bacterial genus	The width of the lysis zones (mm)	Hemolysis type
<i>Bacillus</i> sp.	3.74±0.26	β
<i>Clostridium</i> sp.	4.05±0.16	β
<i>Pseudomonas</i> sp.	1.69±0.12	α
<i>Enterococcus</i> sp.	1.06±0.08	α
<i>Proteus</i> sp.	7.54±0.36	β

Table S7 Recovery of reproductive capacity of adult female mites after antibiotic treatment

Reproduction parameters	First blood feeding (With antibiotics)	Second blood feeding (No antibiotics)	Third blood feeding (No antibiotics)	Fourth blood feeding (No antibiotics)
Oviposition rate (%)	64.93±20.08 ^a	92.62±0.86 ^{ab}	96.66±1.52 ^b	97.22±1.46 ^b
Fecundity	1.05±0.4 ^a	2.23±0.12 ^b	3.86±0.17 ^c	4.28±0.24 ^{cd}
Hatching rate (%)	22.54±13.19 ^a	69.91±3.45 ^b	96.28±1.96 ^c	95.72±2.00 ^{cd}

The data are expressed as the mean ± SD

a, b, c, d Values within the same row followed by different letters are significantly different

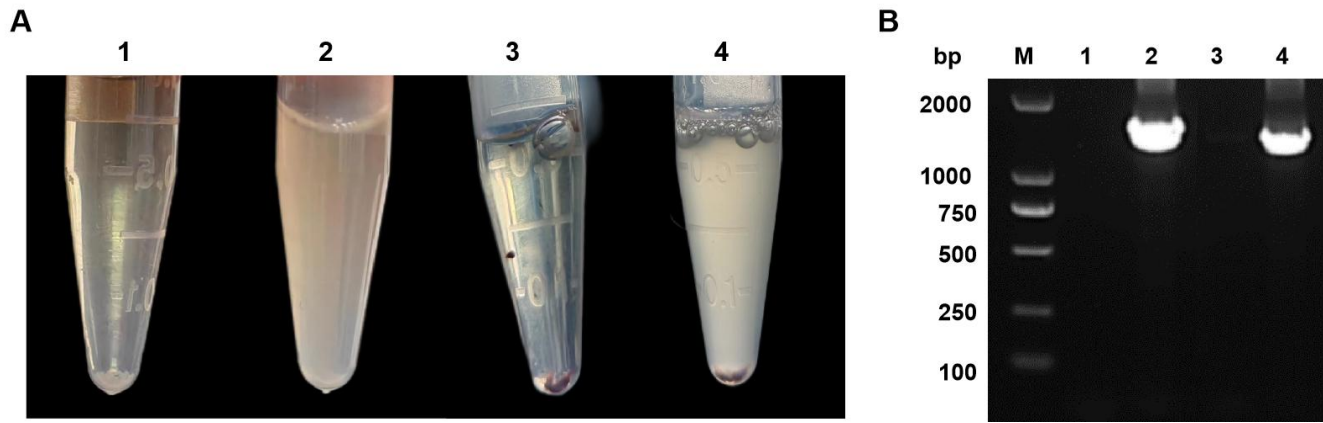


Fig. S1 Evaluation of surface sterilization efficiency *D. gallinae* mites and their eggs. **A** Photo images of LB media after external washing mites or eggs and subsequent thermal culturing. 1: surface-cleaned eggs; 2: no treatment eggs; 3: surface-cleaned mites; 4: no treatment mites. **B** PCR amplification of the bacterial 16S rRNA genes from the corresponding media in Fig A.

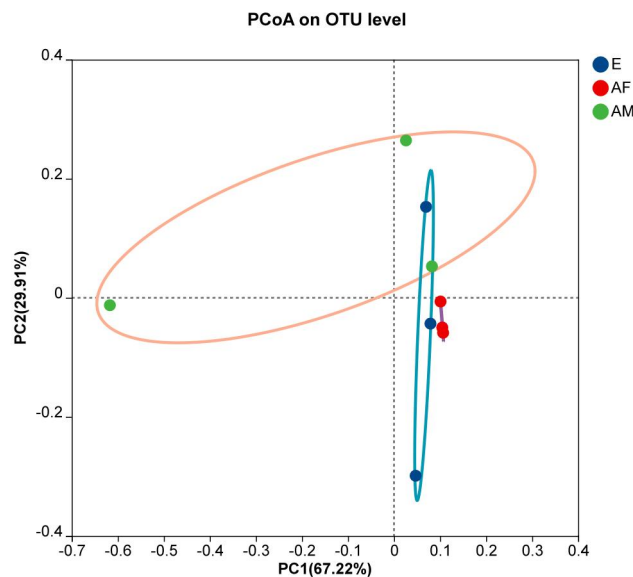


Fig. S2 The PCoA analysis for bacterial community composition in based on Bray-Curtis. Distances between points on the ordination plot reflect relative dissimilarities in microbiome structures. E, AF, and AM indicated the egg, fed adult female, and adult male, respectively.

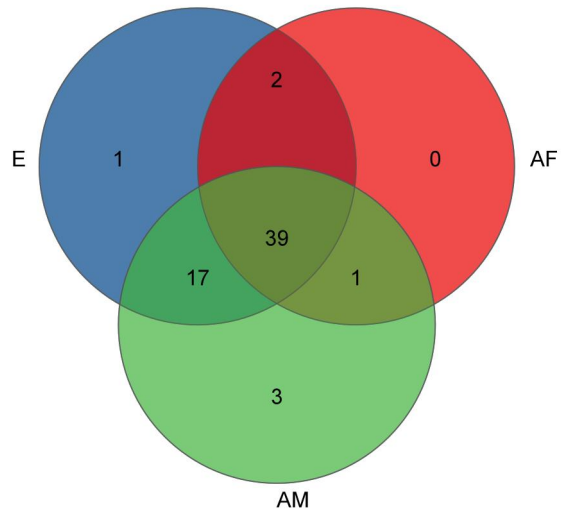


Fig. S3 Venn diagram representing the distribution of the OTUs across different groups of *D. gallinae*. Shared bacteria OTUs are shown in core. E, AF, and AM indicated the eggs, fed adult females, and adult males, respectively.

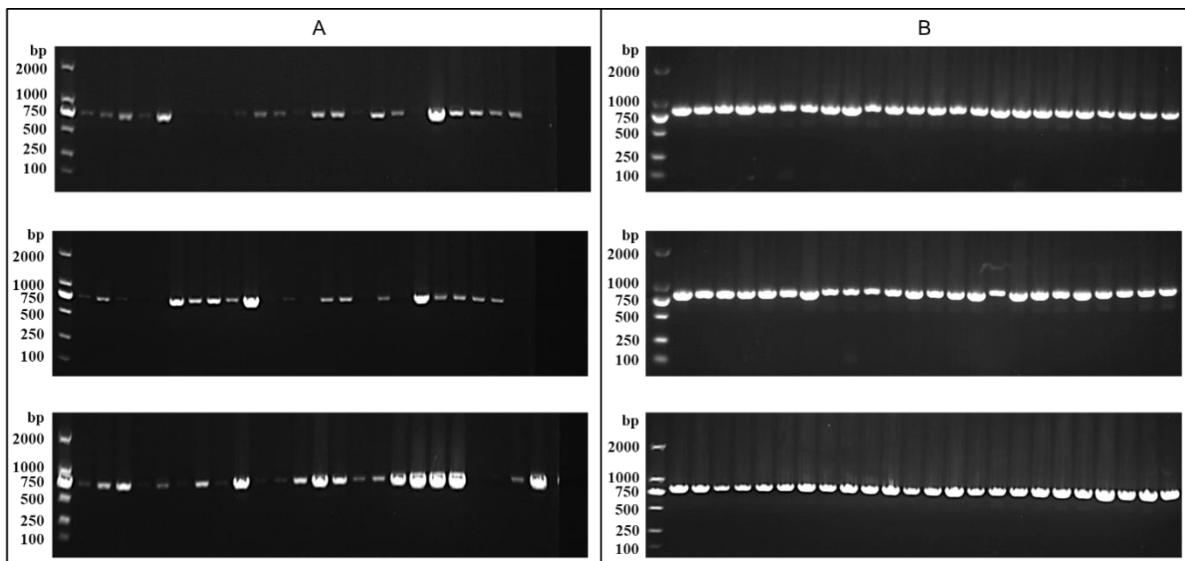


Fig. S4 Detection of infection rates of core bacteria in individual adult female mites by specific PCR amplification. A *Kocuria*; B *Bartonella A*.

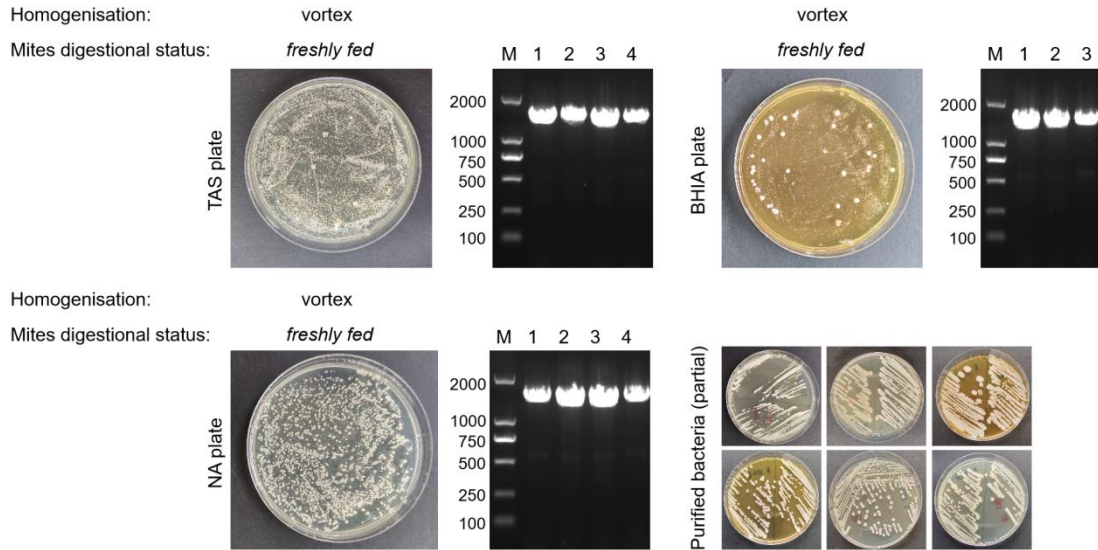


Fig. S5. Agar cultivation and 16S rRNA gene amplification of bacteria isolated from dissected midguts of *D. gallinae* mites. A homogenisation procedure was applied on midguts of freshly fed *D. gallinae* adult females. Samples from different plates were taken for DNA isolation and 16S rRNA amplification with subsequent Sanger sequencing. Individual sequences are shown below as Supplementary Data S1.

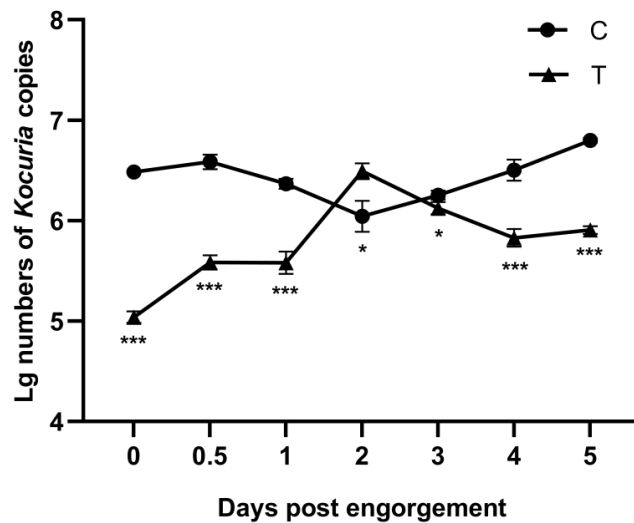


Fig. S6 The effect of antibiotics treatment on *Kocuria* abundance in *D. gallinae*. The quantification of numbers of copies of *Kocuria* 16S rRNA gene in control and treatment groups. The data are Log10 transformed. Asterisks indicate the statistical significance: *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001 in the Student's t test.

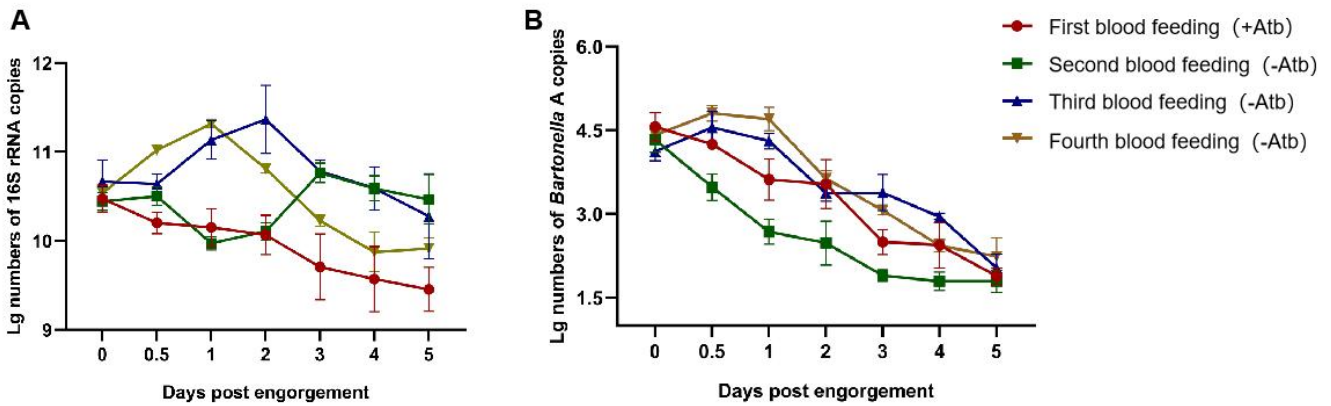


Fig. S7. Recovery of bacterial load of adult female mites after antibiotic treatment. The quantification of numbers of copies of 16S rRNA gene in different groups. The data are Log₁₀ transformed. **A** Numbers of copies obtained from adult females by universal primers. **B** Numbers of copies obtained from adult females by *Bartonella A* specific primers. +Atb: The mites fed on OTC-treated chicken. -Atb: The mites fed on non-treated chicken.

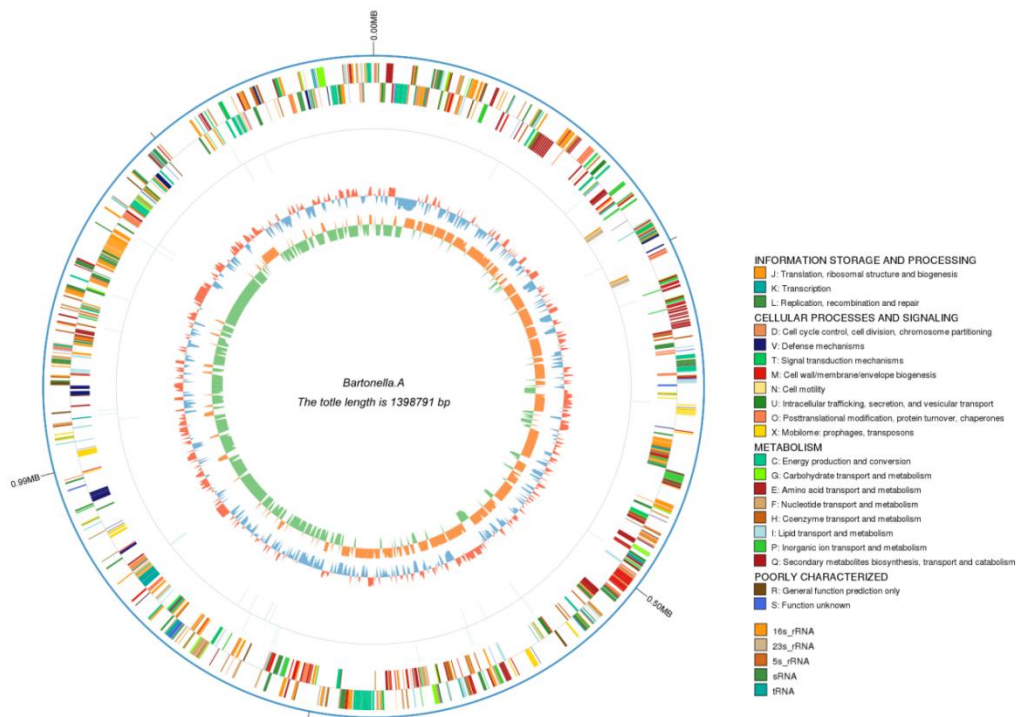


Fig. S8. Circular genome representation map of the *Bartonella A* symbiont. From outside to the centre: genome sequence of coordinates, genes on forward strand (colour by COG categories), genes on reverse strand (colour by COG categories), ncRNA, GC content, GC skew.

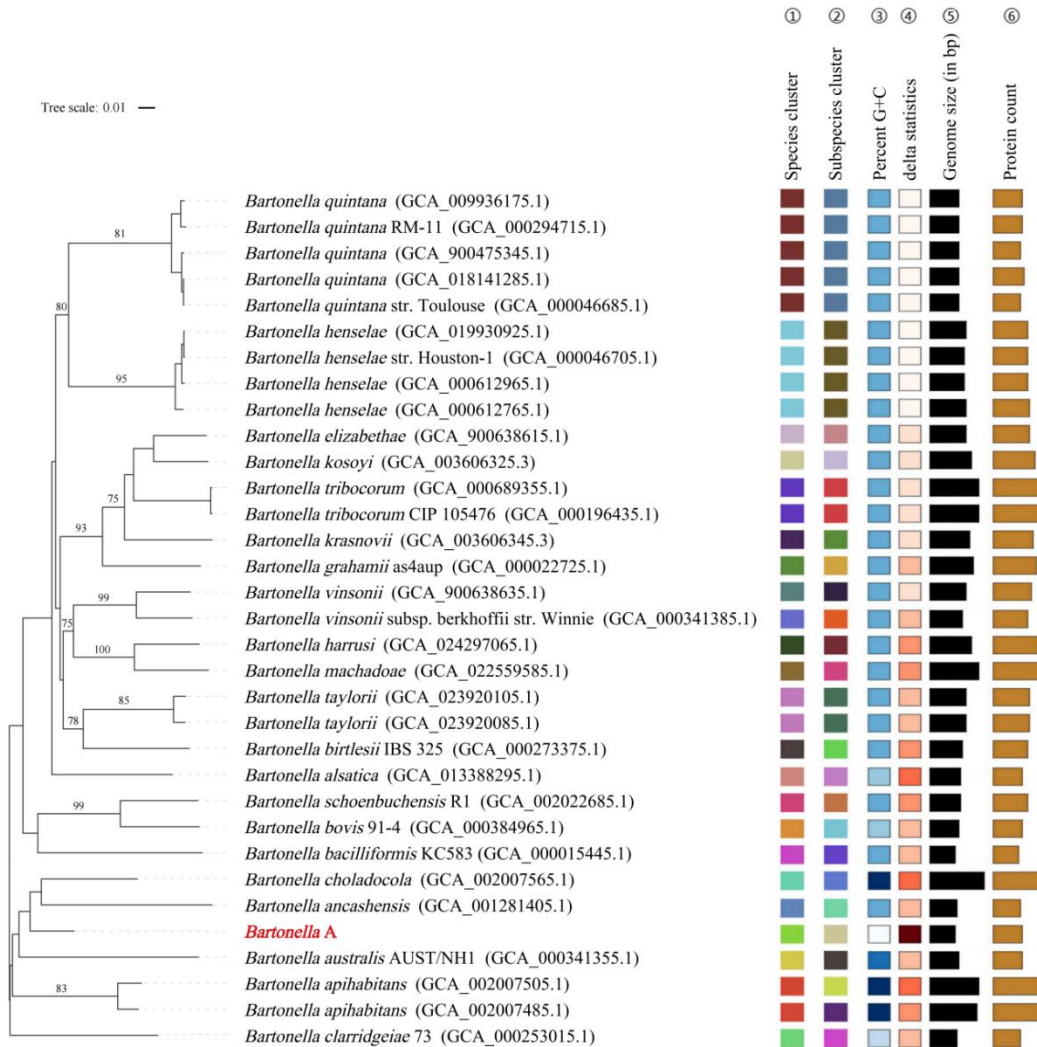


Fig. S9. Whole-genome-based phylogeny. The resulting intergenomic distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1. Branch support was inferred from 100 pseudo-bootstrap replicates each, and values > 75% are shown above branches. Leaf labels are annotated by affiliation to species ① and subspecies ② clusters, genomic G+C content ③, δ

values ④, overall genome sequence length ⑤, number of proteins ⑥. Numbers in brackets indicate NCBI accession numbers.

Supplementary Data S1. Nucleotide sequences of 16S rRNA amplicons acquired from agar plates culturing and electrophoretic separation above.

>TSA1 *Staphylococcus*

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>TSA2 *Staphylococcus*

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>TSA3 *Staphylococcus*

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>TSA4 *Koucria*

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>NA1 *Escherichia*

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>NA2 *Staphylococcus*

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>NA3 *Staphylococcus*

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>NA4 *Escherichia*

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>BHIA1 *Staphylococcus*

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