

Figure S1. Localization of Sulcia and Nasuia in the epithelial plug in the ovary of female N. cincticeps. $(a, b)$ Distribution of Sulcia and Nasuia close to the epithelial plug. Scale bars in $a: 10 \mu \mathrm{~m} ; b: 2 \mu \mathrm{~m}$. (c-e) Distribution of Sulcia and Nasuia within the epithelial plug. Scale bars in $c: 10 \mu \mathrm{~m} ; d$ and $e: 2 \mu \mathrm{~m} .(f, g)$ Distribution of Sulcia and Nasuia within the oocyte. Scale bars in $f: 10 \mu \mathrm{~m} ; g: 2 \mu \mathrm{~m}$. Panels $b, d, e$ and $g$ are enlargements of boxed areas in panels $a, c, d$ and $f$, respectively. Ep, epithelial plug; Fc, follicular cell; N, Nasuia; O, oocyte; Pd, pedicel; S, Sulcia. All micrographs are representative of at least three repetitions.


Figure S2. Copy number dynamics of 16S rRNA from (a) Nasuia and (b) Sulcia in the nymph and 1-7 days after emergence of $N$. cincticeps as detected by RT-qPCR assay.


Figure S3. Mean ( $\pm$ SE) proportion of RDV virions within the periplasmic spaces or on the outer membranes of Nasuia. The distribution of RDV virions was based on 43 Nasuia samples. Data from three independent experiments were tested with an independent-sample $t$-test at 0.05 level.


Figure S4. Phylogenetic relationships of porin orthologs of Nasuia from N. cincticeps with counterparts. The available sequences were aligned using Clustal W, and phylogenetic trees were reconstructed by neighbor-joining analysis with $P$-distance using MEGA 5.1. Reliability of the phylogenetic trees was estimated by calculating bootstrap confidence limits based on 1000 replicates.

(b)

(c)


Figure S5. Levels of Nasuia porin extracted from different tissues of N. cincticeps. (a) Specificity of antibody against Nasuia porin. Samples were separated by SDS-PAGE and detected with porin-specific antibody. Lanes: M, protein marker; 1 and 2, noninducible expression of porin in E. coli; 3, inducible expression of porin in E. coli; 4, purified porin (100 mm washing buffer); 5, purified porin (150 mm washing buffer); 6, purified porin (200 mm washing buffer). (b) Porin proteins extracted from female adult bacteriomes (lane 1), abdomen (segments 1, 2) (lane 2), abdomen (segments 35) (lane 3), and thorax and head (lane 4). (c) Immunofluorescence detection of the colocalization of RDV and Nasuia. Ovaries of viruliferous $N$. cincticeps were immunolabeled with RDV-FITC (green) for RDV and Porin-rhodamine (red) for Nasuia. Ep, epithelial plug; Fc, follicular cell; O, oocyte; Pd, pedicel. All images are representative of at least three replications.

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Table S1 Primers used in this study

| Oligonucleotide | Assay | Sequence ( $5^{\prime}-3{ }^{\prime}$ ) |
| :---: | :---: | :---: |
| EF1_F | qPCR | CAGTGAGAGCCGTTTTGAG |
| EF1_R | qPCR | AGGGCATCTTGTCAGAGGGC |
| Nasuia_F | qPCR | GGGGAAAACCTCGCGTTATA |
| Nasuia_R | qPCR | CCACTGCTGCCTCTCGTAAG |
| Sulcia_F | qPCR | GGGGACTCTAATAAGACTGC |
| Sulcia_R | qPCR | CTGAGATCGGCTTTCTGGAT |
| RDV-P8_F | qPCR | GCTTCGAGCTTGTGGACAGT |
| RDV_P8_R | qPCR | CGCACCAGCAGATTCCTTAT |
| RDV_P8_F | Y2H | GGCCATGGAGGCCATGTCACGCCAGATGTGGTTAG |
| RDV_P8_R | Y2H | $\begin{aligned} & \text { GGCCTCCATGGCCATCTAATTTGGTCTATAGTATCT } \\ & \text { TCCA } \end{aligned}$ |
| RDV_P2_F | Y2H | AGTGAATTCCACATGGCTTATCCTAACGACGTC |
| RDV_P2_R | Y2H | ATGGATCCCGTACTAAAAAACATCAGCGTGCTCTA |
| Nasuia_porin_F | Y2H | GGCCATGGAGGCCATGTCAAATTCAATTTATTT TTTTTTTATAG |
| Nasuia_porin_R | Y2H | $\frac{\text { GGCCTCCATGGCCTTAATCTAATAATTTTAAAAATT }}{\text { TTTTATAATAAG }}$ |
| Sulcia_OMP_F | Y2H | AGTGAATTCCACATGTATGGAGATAATCAAAAAAT |
| Sulcia_OMP_R | Y2H | ATGGATCCCGTACTAATATGCAATATCACATCCTAA AAAT |
| Nasuia_porin_F | Antibody generation | $\frac{\text { CGAGGGATCCGAATGTCAAATTCAATTTATTT }}{\text { TTTTTTTATAG }}$ |
| Nasuia_porin_R | Antibody generation | TTGAATTCGGATTTAATCTAATAATTTTAAAAATTT TTTATAATAAG |
| RDV_P8_F | Pull down | $\begin{aligned} & \text { GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGT } \\ & \text { CACGCCAGATGTGGTTAG } \end{aligned}$ |
| RDV_P8_R | Pull down | $\frac{\text { GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAT }}{\text { TTGGTCTATAGTATCTTCCA }}$ |
| Nasuia_porin_F | Pull down | CGTGGATCCCCGATGTCAAATTCAATTTATTT TTTTTTTATAG |
| Nasuia_porin_R | Pull down | $\frac{\text { GATGAATTCCGGTTAATCTAATAATTTTAAAAATTT }}{\text { TTTATAATAAG }}$ |

