

## Supplemental materials

**Table S1.** Cicada samples used for diagnostic PCR amplification in this study

Species	Symbiont	Numbers of positive individuals/total numbers of individuals tested								
		SG	FC	CS	MG	HG	TE	OV	FB	BA
<i>Karenia</i>	<i>Sulcia</i>	0/12	0/12	0/12	0/12	0/12	0/6	6/6	0/12	12/12
<i>caelatata</i>	YLS	0/12	0/12	0/12	0/12	0/12	0/6	6/6	12/12	0/12
<i>Tanna</i> sp.	<i>Sulcia</i>	0/10	0/10	0/10	0/10	0/10	0/5	5/5	0/10	10/10
	YLS	0/10	0/10	0/10	0/10	0/10	0/5	5/5	10/10	0/10

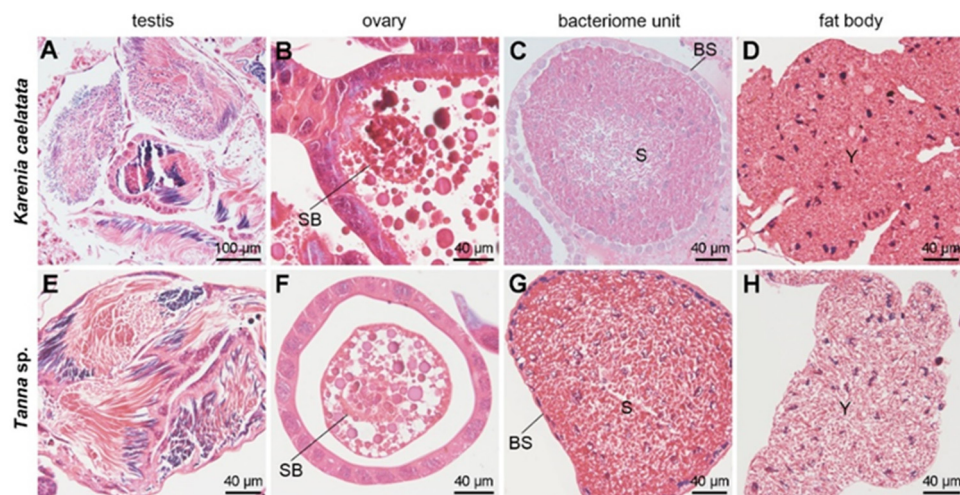
Abbreviations: SG, salivary glands; FC, filter chamber; CS, conical segment; MG, midgut; HG, hindgut; TE, testes; OV, ovaries; FB, fat bodies; BA, bacteriomes.

**Table S2.** NCBI blast results for the *Sulcia* and yeast-like fungal symbiont obtained from *Karenia caelatata* and *Tanna* sp.

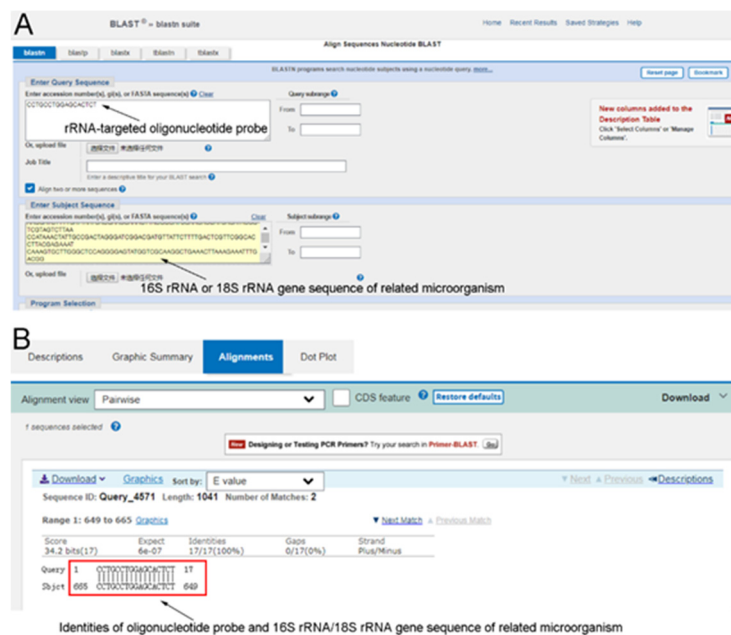
Symbionts	Query coverage	Representative sequences in GenBank	Percent identity (%)
<i>Sulcia</i> of <i>Tanna</i> sp.	100%	<i>Sulcia</i> of <i>Tanna japonensis</i> (LC370573.1)	99.40%
	100%	<i>Sulcia</i> of <i>Terpnosia vacua</i> (LC370693.1)	98.96%
	100%	<i>Sulcia</i> of <i>Te. nigricosta</i> (LC370570.1)	98.81%
<i>Sulcia</i> of <i>Karenia caelatata</i>	100%	<i>Sulcia</i> of <i>Meimunna iwasakii</i> (LC370740.1)	99.93%
	100%	<i>Sulcia</i> of <i>Me. kuroiwa</i> (LC370734.1)	99.93%
	100%	<i>Sulcia</i> of <i>Me. oshimensis</i> (LC370730.1)	99.85%
YLS of <i>Tanna</i> sp.	99%	YLS of <i>Mogannia minuta</i> (LC370999.1)	95.38%
	99%	YLS of <i>Euterpnosia chibensis</i> (LC370887.1)	95.11%
	99%	YLS of <i>Ta. japonensis</i> (LC370811.1)	93.49%
YLS of <i>Karenia caelatata</i>	94%	YLS of <i>Me. oshimensis</i> (LC370947.1)	92.95%
	93%	YLS of <i>Me. opalifera</i> (LC371018.1)	90.78%
	94%	YLS of <i>Ta. japonensis</i> (LC370901.1)	92.77%

**Table S3.** The primers for amplification of dominant symbionts in this study

Target	Primer name	Primer sequence (5'–3')	References
<i>Sulcia</i>	10_CFB_FF	AGAGTTTGATCATGGCTCAGGATG	Moran et al., 2005
(16S rRNA)	1515_R	GTACGGCTACCTTGTTACGACTTAG	
YLS	Fng18S_82F	GAAACTGCGAATGGCT	Matsuura et al., 2018
(18S rRNA)	NS4	CTTCCGTCAATTCCTTTAAG	
YLS	Fng18S_82F	GAAACTGCGAATGGCT	Matsuura et al., 2018
(18S rRNA)	Fng18S_106 7R	TMTCGTAAGGTGCCGA	
YLS	Hyp325F	GTTTGGGTAGTGGCCAAAC	Matsuura et al., 2018
(18S rRNA)	Hyp760R	CCTGCCTGGAGCACTCT	
YLS	CFS2-CS	TTCAACGAGGAAYCCCTA	Matsuura et al., 2018
(18S rRNA)	LSU_CS-1R	GCTTCACTCGCCGTTAC	
YLS	ITS_CS-1F	CATCGAATCTTTGAACGC	Matsuura et al., 2018
(28S rRNA)	NLB1_CS	CTTTACCTCATAAAACTGAGNTCG	
YLS	RPB1-OhpF1	GTCTWCCACCCGGGCTT	Matsuura et al., 2018
(RPB1)	RPB1-OhpR1	GGCRATGTCGTTGTCCAT	
YLS	RPB2-OphF1	YAAGAAGCGYCTCGACCT	Matsuura et al., 2018
(RPB2)	RPB2-OphR2	GGCAGACTGGTACGTGTT	
YLS	Ef1α-OphF1	CCGGCCACCGTGACTTCAT	Matsuura et al., 2018
(Ef1α)	Ef1α-Oph221 8R	ATGACACCGACGGCRMCGGTYTG	



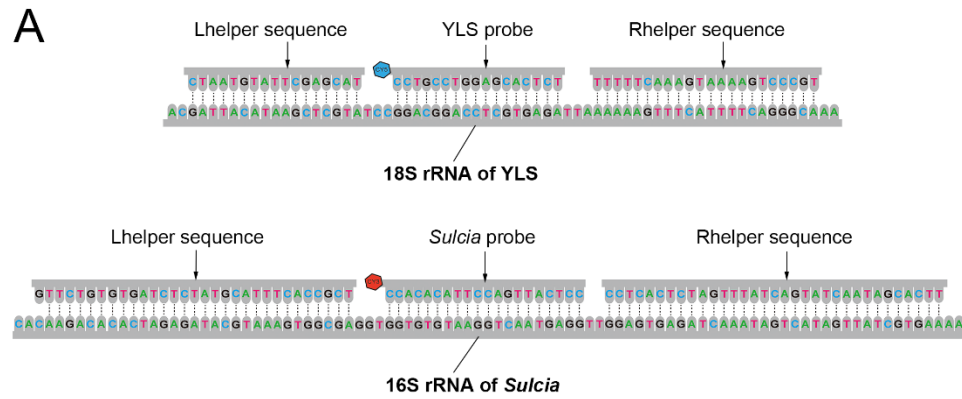
**Figure S1.** Histological microscopy showing the distribution of *Sulcia* and YLS in the testes, ovaries, bacteriomes and fat bodies of *Karenia caelata* and *Tanna* sp. Abbreviations: BS, bacteriome sheath; S, *Sulcia*; Y, yeast-like fungal symbiont.



**Figure S2.** The identities of rRNA-targeted oligonucleotide probe and 16S rRNA/18S rRNA gene sequence of targeted microorganisms.

<b>Solution</b>	<b>Time</b>	<b>Temperature</b>
Xylene	20 min	room temperature
↓		
Xylene	20 min	room temperature
↓		
100% ethanol	10 min	room temperature
↓		
100% ethanol	10 min	room temperature
↓		
90% ethanol	5 min	room temperature
↓		
80% ethanol	5 min	room temperature
↓		
70% ethanol	5 min	room temperature
↓		
Double-distilled water	5 min	room temperature
↓		
Hematoxylin	8 min	room temperature
↓		
Double-distilled water	30 s	room temperature
↓		
Eosin	10 min	room temperature
↓		
Double-distilled water	30 s	room temperature

**Figure S3.** The detailed protocol for the paraffin sections stained with hematoxylin and eosin.



**B**

<b>Solution</b>	<b>Time</b>	<b>Temperature</b>
Xylene	20 min	room temperature
↓		
Xylene	20 min	room temperature
↓		
100% ethanol	10 min	room temperature
↓		
100% ethanol	10 min	room temperature
↓		
90% ethanol	5 min	room temperature
↓		
80% ethanol	5 min	room temperature
↓		
70% ethanol	5 min	room temperature
↓		
Double-distilled water	5 min	room temperature
↓		
Hybridization buffer	8-12 h	37 °C
↓		
2xSSC	~1 h	37 °C
↓		
Double-distilled water	5 min	room temperature
↓		
DAPI solution	~3 min	room temperature
↓		
Double-distilled water	2 min	room temperature

**Figure S4.** Schematic diagram showing the target sites of probe sequences and adjacent unlabeled helper sequences and the detailed protocol using for the hybridization procedure.