

Supplementary Materials

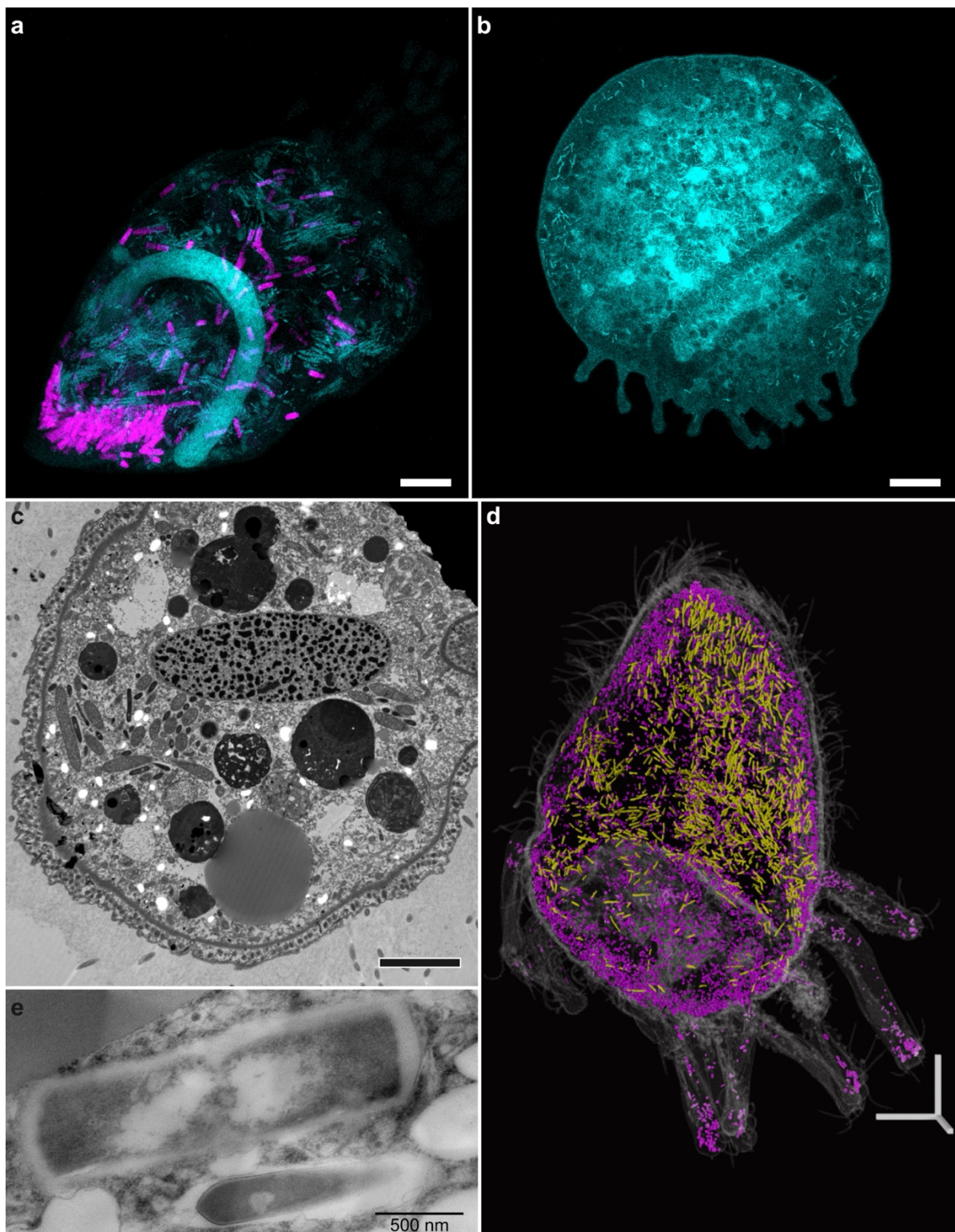


Fig. S1. Methanogenic symbionts of *Legendrea loyzeae*. (a) FISH signal of *Ca. Methanotherix pelomyxae* (archaeal probe with DAPI staining). (b) Autofluorescence of clustered *Methanobacterium* sp. endosymbiont. (c) TEM showing spatial distribution of symbionts in the cytoplasm. (d) 3D FIB-SEM reconstruction showing the distribution of the new type of MROs (purple) and *Methanobacterium* sp. endosymbiont (yellow). (e) TEM

showing the ultrastructure of *Ca. Methanotherix pelomyxae* (above) and *Methanobacterium* sp. (below) symbionts. Scale bars: A, B, D 10 μm , C 5 μm .



Fig. S2. RAxML phylogenetic tree of CoA-transferases.

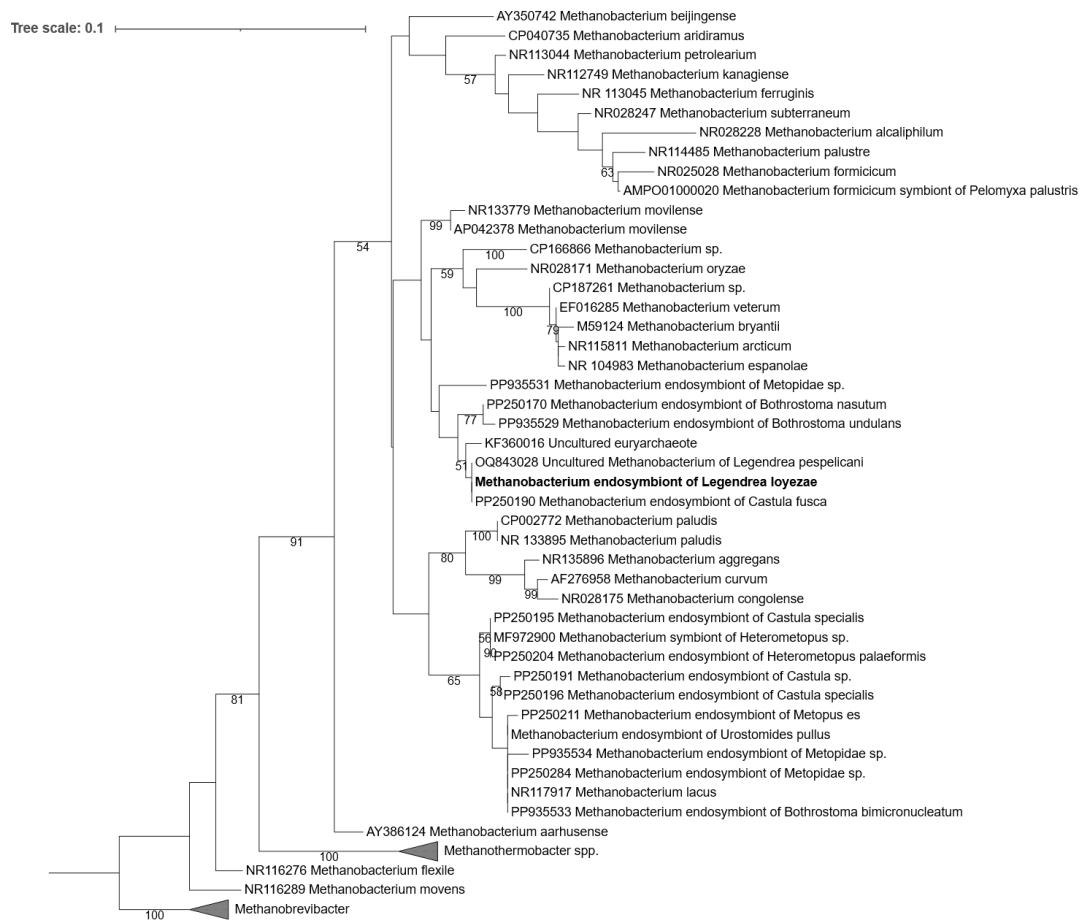


Fig. S3. RAxML phylogenetic tree based on 16S rRNA gene showing the position of *Methanobacterium* sp. symbiont from *Legendrea loyezae*.

Tree scale: 0.1

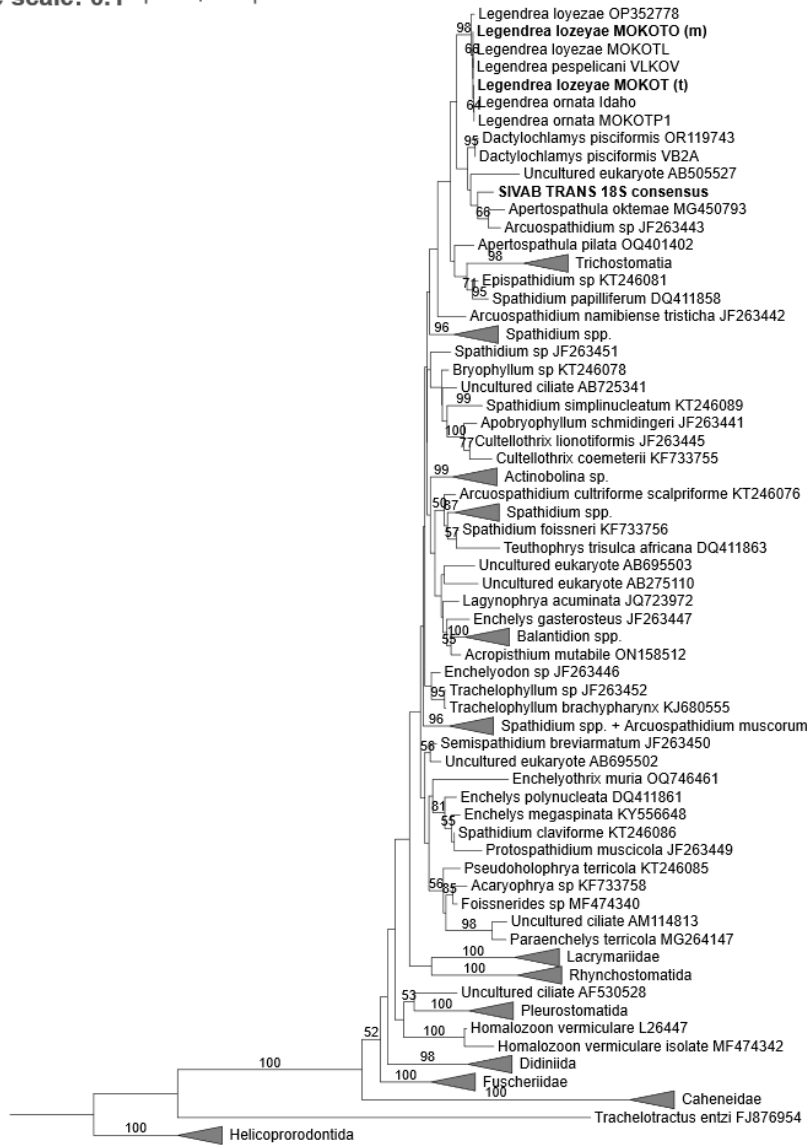


Fig. S4. RAxML phylogenetic tree based on 18S rRNA gene showing the relationship of *Legendrea loyzeae* and the SIVAB ciliate.

Legendrea loyezae

| Cells used (11) | Method | Strain |
|-----------------|------------------|--------------------|
| 3 | TRANSCRIPTOME | MOKOT |
| 2 | METAGENOME | MOKOT |
| 1 | TEM | MOKOT |
| 1 | FIB-SEM | MOKOT |
| 3 | FISH | MOKOT (2), 7TL (1) |
| 1 | Autofluorescence | MOKOT |

Spathidium sp.

| Cells used (3) | Method | Strain |
|----------------|---------------|--------|
| 3 | TRANSCRIPTOME | SIVAB |

| Strain | Habitat | Locality | Coordiantes |
|--------|------------------|---------------------------------------------------|--------------------------|
| MOKOT | hypoxic sediment | Small pond, Modřany, Prague, Czechia | 49.9901275N, 14.4012394E |
| 7TL | hypoxic sediment | Uni-Fleet-Süd, University campus, Bremen, Germany | 53.1072294N, 8.8463003E |
| SIVAB | hypoxic sediment | Travertine spring pool, Sivá Brada, Slovakia | 49.0064050N, 20.7229667E |

Table S5. Cells of *Legendrea loyezae* used in this study, strains, and localities.

In individual files:

Fig. S5. Mitochondrial-related organelle (MRO) single protein trees collection.

Table S1. Spatial analysis of pairwise distances between prokaryotic symbionts.

Table S2. Contaminants in metagenomic and transcriptomic data.

Table S3. Annotated putative *Methanobacterium* sp. proteins involved in methanogenesis

Table S4. *Legendrea loyezae* and SIVAB MRO-targeting prediction tables

Movie S1. FIB-SEM based 3D model of *Legendrea loyezae* and their prokaryotic symbionts. FIB-SEM 3D model of *Legendrea loyezae* and their prokaryotic symbionts. Ca. *Methanotherix pelomyxae* (red), Ca. *Syntrophilus obsequens* (blue), *Methanobacterium* sp. (yellow), Ca. *Xociella bifrons* (green), mitochondrion-related organelle (purple), macronucleus (light blue), and oral basket (black). Scale bar: 10 μ m.