



JOURNAL OF  
SYNCHROTRON  
RADIATION

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2 **Volume 27 (2020)**

3 **Supporting information for article:**

4 **Quantitative three-dimensional nondestructive imaging of whole  
5 anaerobic ammonium-oxidizing bacteria**

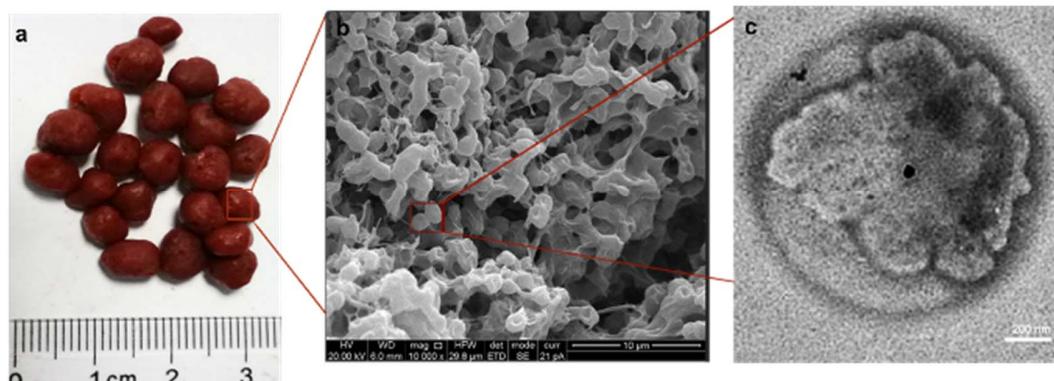
6 **Meng-Wen Peng, Yong Guan, Jian-Hong Liu, Liang Chen, Han Wang, Zheng-Zhe  
7 Xie, Hai-Yan Li, You-Peng Chen, Peng Liu, Peng Yan, Jin-Song Guo, Gang Liu, Yu  
8 Shen and Fang Fang**

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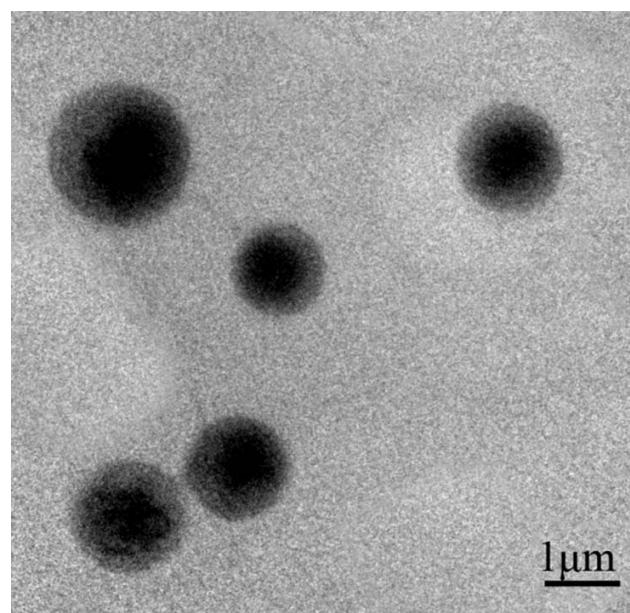


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14 **Figure S1** Anammox bacteria sampled from an EGSB reactor. (a) Anammox granules sampled from an  
15 EGSB reactor. (b) Scanning electron microscope (SEM) image of single anammox granule. (c)  
16 Transmission electron microscope (TEM) image of anammox bacteria isolated from anammox granule.

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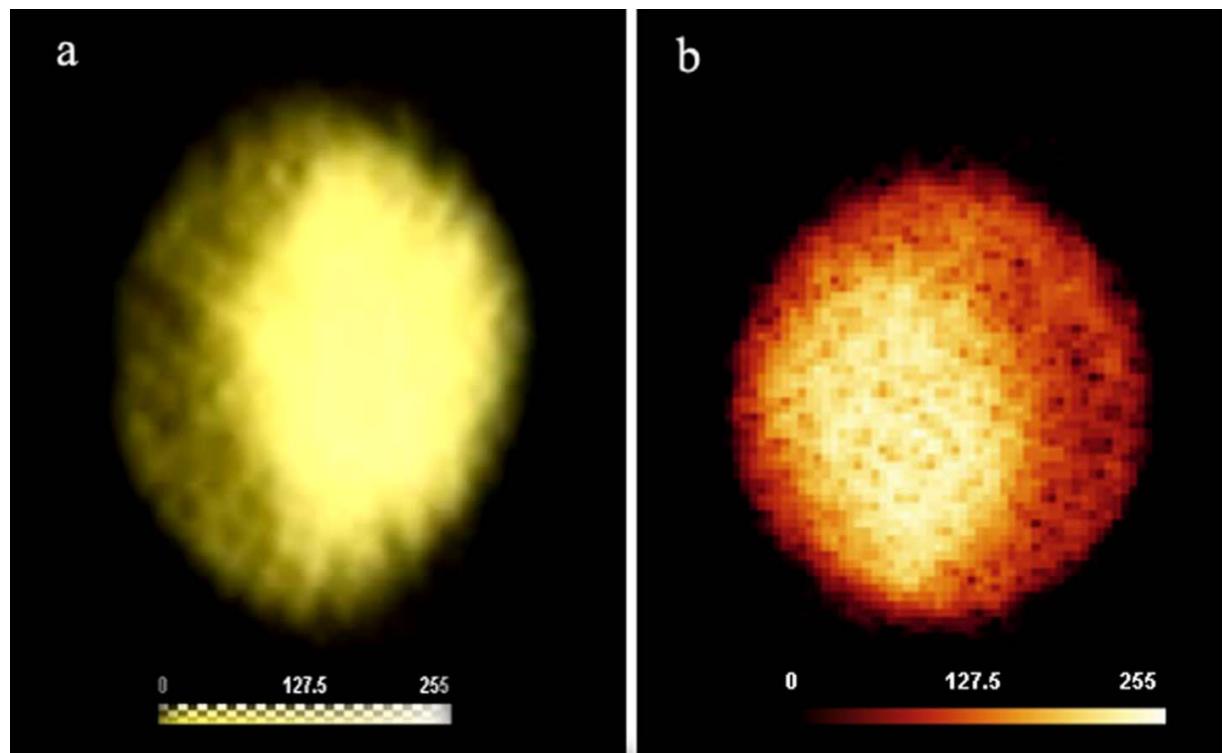


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20 **Figure S2** Two-dimensional synchrotron soft X-ray projection image of the anammox cells. Dark areas  
21 are anammoxosomes.

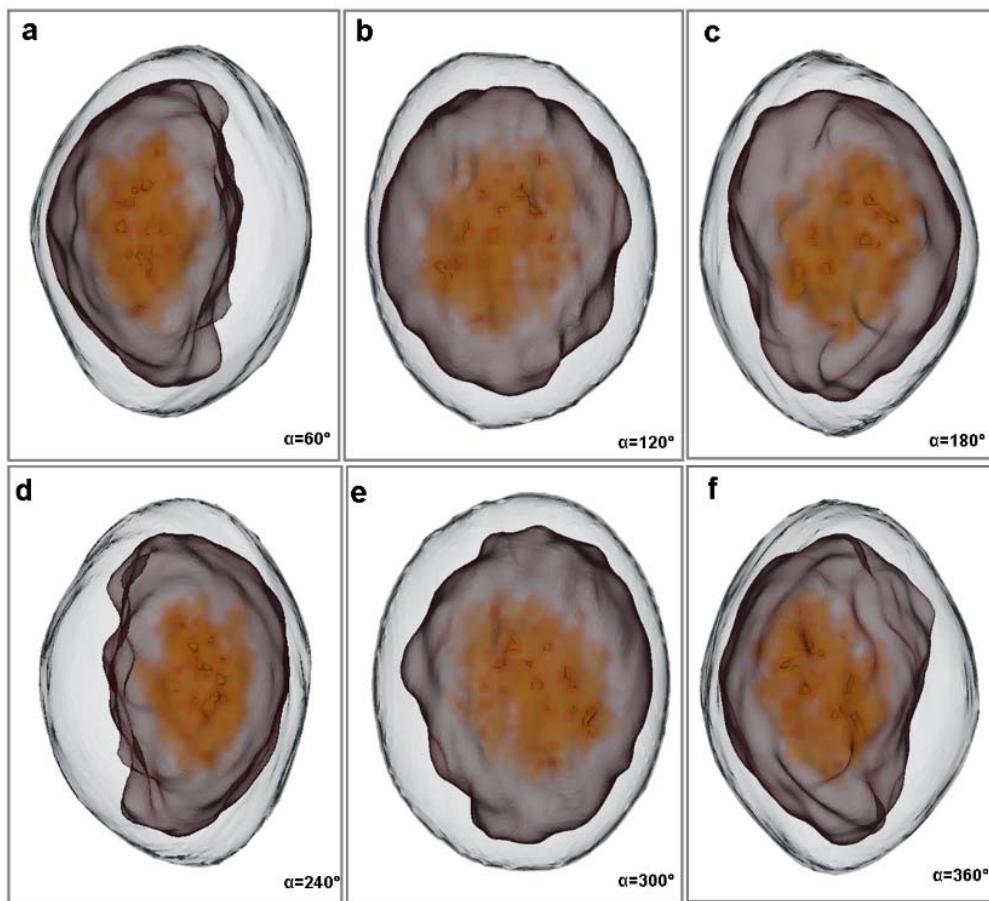
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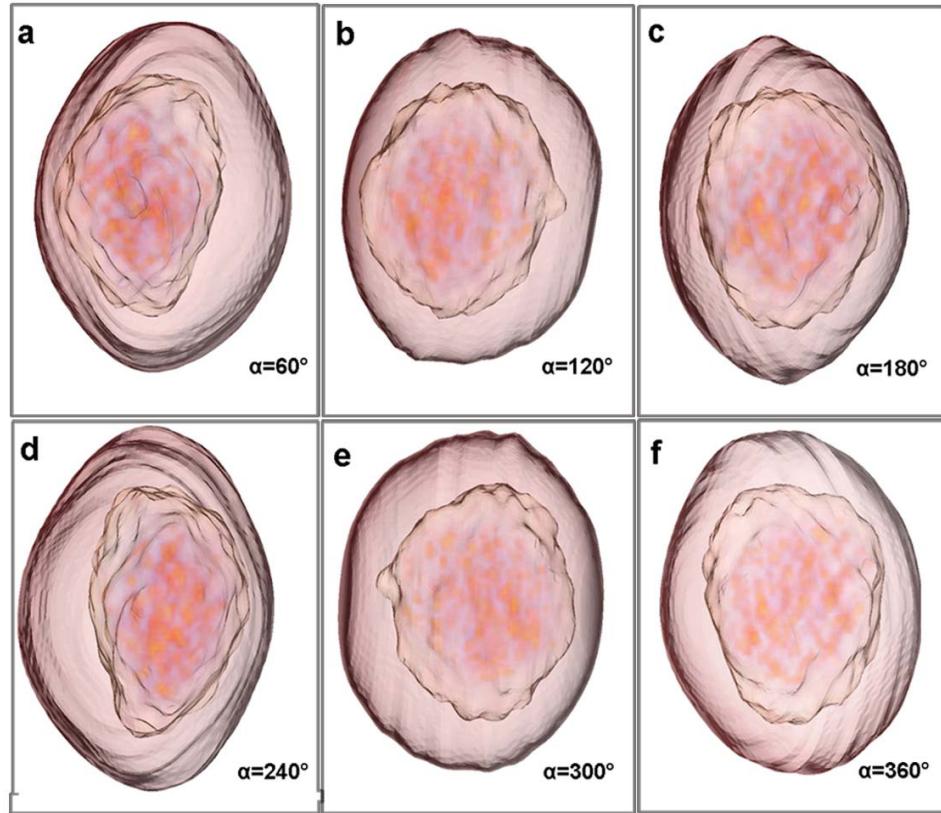
25 **Figure S3** Synchrotron soft X-ray imaging of anammox bacteria with the FBP algorithm. (a) Projection  
26 image of the intact anammox bacteria cell. (b) Slice image of a layer in the anammox bacteria cell.



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28 **Figure S4** The segmentation images of anammox cell at different rotation angles. (a), (b), (c), (d), (e),  
29 and (f) correspond to 60°, 120°, 180°, 240°, 300°, and 360°, respectively. ( $\alpha$  represents the rotation  
30 angle).

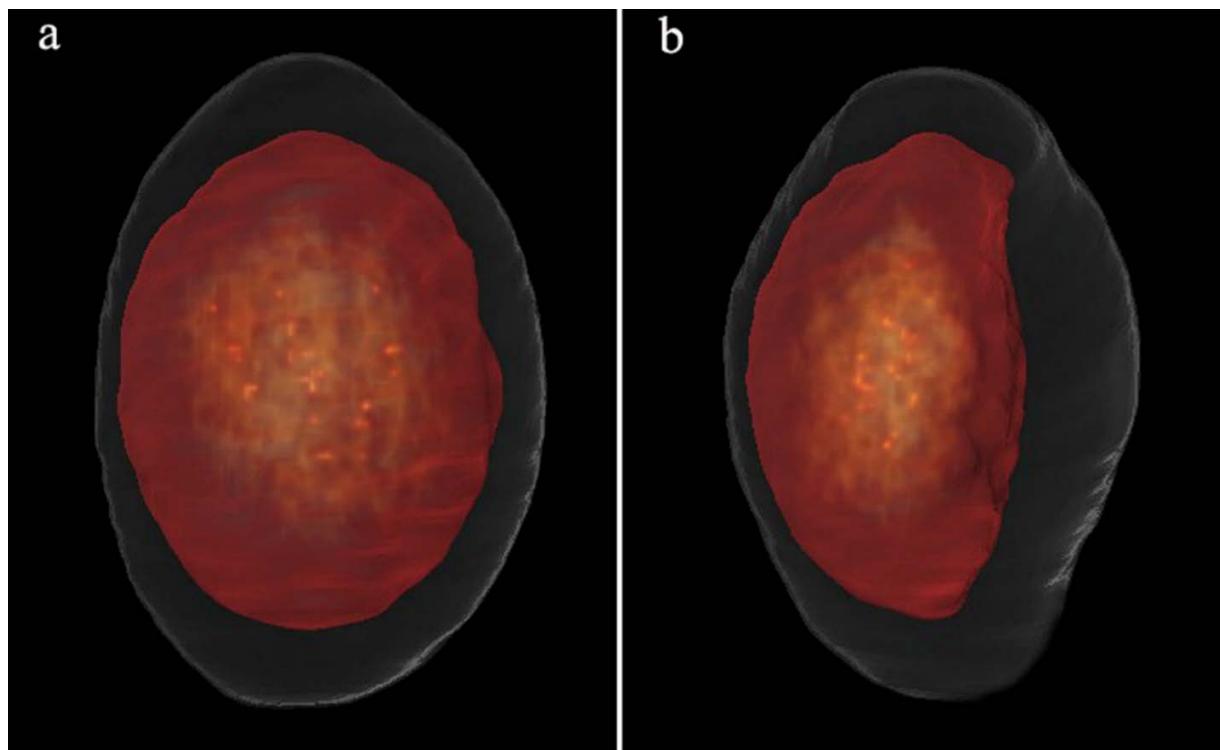
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33 **Figure S5** The segmentation images of anammox cell at different rotation angles. (a), (b), (c), (d), (e),  
34 and (f) correspond to  $60^\circ$ ,  $120^\circ$ ,  $180^\circ$ ,  $240^\circ$ ,  $300^\circ$ , and  $360^\circ$ , respectively. ( $\alpha$  represents the rotation  
35 angle).

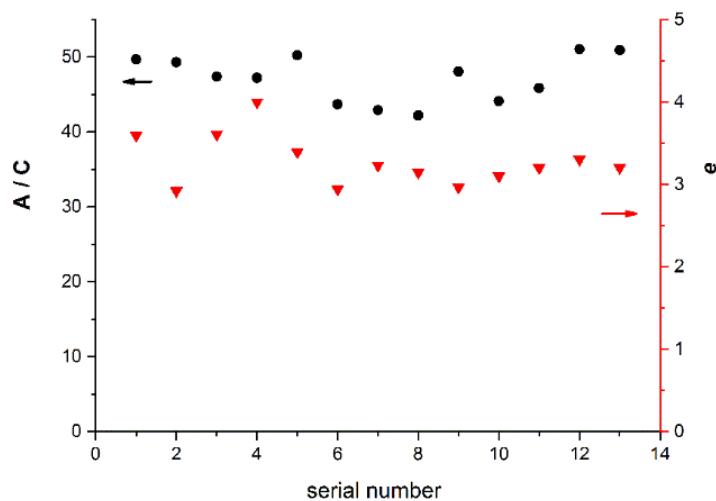
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38 **Figure S6** The segmentation image of the anammox cell. At minimum eccentricity (a) and maximum  
39 eccentricity (b).

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42 **Figure S7** Statistics of the anammox bacteria morphology. The red triangles represent the eccentricity  
43 of the anammoxosome organelle. The black circles represent the A/C volume ratio (A: anammoxosome  
44 C: anammox bacteria cell).

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#### 46 Multimedia Files

47 **Movies S1.** 3D reconstruction of anammox bacteria with the FBP algorithm.

48 **Movies S2.** Tomogram of the intact anammox bacteria reconstructed with the FBP algorithm.

49 **Movies S3.** 3D reconstruction of anammox bacteria with the TV-SART algorithm

50 **Movies S4.** Tomogram of anammox bacteria reconstructed with the TV-SART algorithm.

51 **Movies S5.** Segmentation of the anammox bacteria based on LAC values.

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**Table S1.** The identified proteins in anammoxosome organelles analyzed by the shotgun method.

| No | Accession  | Protein description            | Microbe origins                    | # Peptides | # Unique Peptides | Coverage | Molecular mass (kDa) | Isoelectric point | SAF (%) |
|----|------------|--------------------------------|------------------------------------|------------|-------------------|----------|----------------------|-------------------|---------|
| 1  | Q1Q0T2     | Hydrazine synthase             | <i>Kuenenia stuttgartiensis</i>    | 11         | 11                | 6.36     | 19.25                | 8.79              | 1.82    |
| 2  | Q1PZX1     | Signal peptide                 | <i>Kuenenia stuttgartiensis</i>    | 8          | 6                 | 6.63     | 21.08                | 10.11             | 1.61    |
| 3  | Q1Q0T4     | Hydrazine synthase             | <i>Kuenenia stuttgartiensis</i>    | 7          | 7                 | 13.29    | 18.29                | 5.08              | 3.99    |
| 4  | I3II48     | Uncharacterized protein        | <i>Candidatus Jettenia caeni</i>   | 6          | 4                 | 10.90    | 17.96                | 5.27              | 4.04    |
| 5  | Q1PX48     | Hydrazine oxidase              | <i>Kuenenia stuttgartiensis</i>    | 5          | 5                 | 5.16     | 26.93                | 7.4               | 1.25    |
| 6  | I3IHC5     | Electron transfer activity     | <i>Candidatus Jettenia caeni</i>   | 2          | 2                 | 4.40     | 20.45                | 7.96              | 1.73    |
| 7  | Q1PZD8     | Nitrite oxidoreductase         | <i>Kuenenia stuttgartiensis</i>    | 4          | 4                 | 12.09    | 9.96                 | 10.33             | 3.46    |
| 8  | Q1PW30     | Hydrazine dehydrogenase        | <i>Kuenenia stuttgartiensis</i>    | 3          | 3                 | 3.25     | 38.29                | 5.68              | 0.93    |
| 9  | Q1Q1D0     | Integral component of membrane | <i>Kuenenia stuttgartiensis</i>    | 1          | 1                 | 13.30    | 20.87                | 9.19              | 5.03    |
| 10 | I3IQB7     | Hydroxylamine oxidoreductase   | <i>Candidatus Jettenia caeni</i>   | 1          | 1                 | 7.91     | 20.12                | 7.4               | 1.78    |
| 11 | A0A0M2V157 | Hydrazine synthase subunit     | <i>Candidatus Brocadia fulgida</i> | 2          | 2                 | 5.93     | 26.72                | 8.62              | 1.34    |
| 12 | Q1Q0T9     | Electron transfer activity     | <i>Kuenenia stuttgartiensis</i>    | 1          | 1                 | 7.73     | 65.53                | 7.03              | 1.63    |

|    |            |  |  |   |   |       |        |      |      |
|----|------------|--|--|---|---|-------|--------|------|------|
| 13 | I3IPV9     | Putative formate dehydrogenase cytochrome b    | <i>Candidatus Jettenia caeni</i>         | 3 | 3 | 17.31 | 90.19  | 6.71 | 4.68 |
| 14 | I3IHD7     | Heme-binding protein                           | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 17.10 | 41.88  | 6.8  | 8.17 |
| 15 | I3IM93     | DNA-binding protein                            | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 6.67  | 41.47  | 6.47 | 1.68 |
| 16 | Q1Q5F8     | Bacterioferritin                               | <i>Kuenenia stuttgartiensis</i>          | 2 | 2 | 3.27  | 66.25  | 7.23 | 0.54 |
| 17 | I3IPV8     | Respiratory electron transport chain           | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 3.41  | 39.58  | 5.33 | 0.90 |
| 18 | Q1PZE4     | Carbohydrate binding                           | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 3.75  | 131.65 | 8.44 | 1.10 |
| 19 | Q1PW92     | Protein HflC, regulation of peptidase activity | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 3.90  | 47.92  | 8.35 | 0.77 |
| 20 | A0A1V4AVC2 | S-adenosylmethionine synthase                  | <i>Candidatus Brocadia caroliniensis</i> | 1 | 1 | 5.46  | 58.17  | 7.58 | 0.61 |
| 21 | Q1Q048     | Electron transfer activity                     | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 5.42  | 27.54  | 8.91 | 1.31 |
| 22 | Q1Q138     | 50S ribosomal protein                          | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 4.82  | 27.63  | 5.55 | 1.27 |
| 23 | Q1Q315     | Bacterioferritin                               | <i>Kuenenia stuttgartiensis</i>          | 2 | 2 | 1.92  | 42.18  | 7.64 | 0.87 |
| 25 | Q1PZD5     | Nitrite oxidoreductase                         | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 1.24  | 84.26  | 8.95 | 0.39 |
| 26 | Q1Q5N7     | Similar to hydroxylamine oxidoreductase haO    | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 18.88 | 61.11  | 4.74 | 5.10 |

|    |            |   |                                    |   |   |       |       |       |      |
|----|------------|---|------------------------------------|---|---|-------|-------|-------|------|
| 27 | Q1Q5P2     | 2Fe-2S ferredoxin   | <i>Kuenenia stuttgartiensis</i>    | 1 | 1 | 2.79  | 37.19 | 8.9   | 0.98 |
| 28 | I3IJ16     | Putative K <sup>+</sup> -stimulated pyrophosphate-energized sodium pump | <i>Candidatus Jettenia caeni</i>   | 1 | 1 | 3.91  | 32.68 | 9.26  | 1.12 |
| 29 | A0A0M2UUG1 | Cellular component  | <i>Candidatus Brocadia fulgida</i> | 1 | 1 | 2.57  | 42.38 | 7.58  | 1.62 |
| 30 | I3IM87     | DNA-directed RNA polymerase subunit alpha                               | <i>Candidatus Jettenia caeni</i>   | 1 | 1 | 6.25  | 35.80 | 7.43  | 0.99 |
| 31 | A0A0M2UXD2 | Integral component of membrane  | <i>Candidatus Brocadia fulgida</i> | 1 | 1 | 9.89  | 60.20 | 7.25  | 3.53 |
| 32 | A0A0C9PJ87 | tRNA uridine 5'-carboxymethylaminomethyl modification enzyme            | <i>Candidatus Brocadia sinica</i>  | 1 | 1 | 1.54  | 66.64 | 7.74  | 0.54 |
| 33 | Q1PZD3     | Conserved hypothetical (monoheme) protein                               | <i>Kuenenia stuttgartiensis</i>    | 1 | 1 | 8.26  | 13.43 | 7.09  | 2.61 |
| 34 | A0A0M2UYY8 | Uncharacterized protein   | <i>Candidatus Brocadia fulgida</i> | 1 | 1 | 7.74  | 17.71 | 7.71  | 2.03 |
| 35 | Q1Q0M1     | Uncharacterized protein   | <i>Kuenenia stuttgartiensis</i>    | 1 | 1 | 10.68 | 11.07 | 10.27 | 3.06 |
| 36 | I3IRJ8     | Stage V sporulation protein   | <i>Candidatus Jettenia caeni</i>   | 1 | 1 | 5.65  | 19.78 | 6.57  | 1.78 |
| 37 | A0A0M2UQR9 | Putative bc1 protein  | <i>Candidatus Brocadia fulgida</i> | 1 | 1 | 3.13  | 68.14 | 8.41  | 0.52 |

|    |            |                                     |  |   |   |       |       |       |      |
|----|------------|-------------------------------------|--|---|---|-------|-------|-------|------|
| 38 | Q1PVQ5     | Similar to superoxide reductase     | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 12.21 | 61.96 | 4.65  | 3.40 |
| 39 | I3IL37     | Uncharacterized protein             | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 1.10  | 93.12 | 5.67  | 0.39 |
| 40 | I3IQB8     | Putative cytochrome c               | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 5.88  | 21.66 | 9.25  | 1.69 |
| 41 | A0A1V4AWL7 | Phosphorelay sensor kinase activity | <i>Candidatus Brocadia caroliniensis</i> | 1 | 1 | 4.13  | 26.47 | 7.53  | 1.45 |
| 42 | I3IK34     | Uncharacterized protein             | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 12.05 | 9.31  | 9.99  | 3.80 |
| 43 | I3IJV4     | Putative chemotaxis protein         | <i>Candidatus Jettenia caeni</i>         | 1 | 1 | 19.05 | 4.66  | 9.42  | 7.51 |
| 44 | Q1Q586     | Signal peptide                      | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 28.00 | 10.93 | 10.23 | 3.15 |
| 45 | Q1PVM6     | Thioredoxin peroxidase              | <i>Kuenenia stuttgartiensis</i>          | 1 | 1 | 2.88  | 29.98 | 4.81  | 1.13 |