

Title: Supplementary Movie 1.

Description: Growth of *N. elongata*. Time-lapse of *N. elongata* cells grown in BSTSY at 37°C with 5% CO₂. This movie was used to create Supplementary Figure 2b.

Title: Supplementary Movie 2.

Description: Growth of *A. filiformis*. Time-lapse of *A. filiformis* cells grown in PY at 37°C. This movie was used to create the panels shown in Supplementary Figure 2c.

Title: Supplementary Movie 3.

Description: Growth of *S. muelleri*. Time-lapse of *S. muelleri* cells grown in meat extract at 37°C with 5% CO₂. This movie was used to create the panels shown in Supplementary Figure 2d.

Title: Supplementary Movie 4.

Description: Growth of *C. steedae*. Time-lapse of *C. steedae* cells grown in BSTSY at 37°C. This movie was used to create the panels shown in Supplementary Figure 2e.

Title: Supplementary Movie 5.

Description: Confocal microscopy-based 3D reconstruction of an *A. filiformis* filament subjected to triple FDAA labelling. Stills from this movie are shown in Figure 3d.

Title: Supplementary Movie 6.

Description: Confocal microscopy-based 3D reconstruction of a *C. steedae* filament subjected to triple FDAA labelling. Stills from this movie S6 are shown in Figure 4e.

Title: Supplementary Movie 7.

Description: Confocal microscopy-based 3D reconstruction of an *C. steedae* filament subjected to FDAA labelling (45 min with BADA). Stills from this movie are shown in Supplementary Figure 8.

Title: Supplementary Data 1.

Description: Genomes used for comparative genomics.

Title: Supplementary Data 2.

Description: Average Nucleotide Identity (ANI) between pairs of publicly available *Neisseriaceae* draft genomes.

Title: Supplementary Data 3.

Description: Genes used for the *Neisseriaceae* phylogeny.

Title: Supplementary Data 4.

Description: Table of identified muropeptides. Identity was confirmed by MS analysis. Short name, structure, theoretical, observed neutral mass in Da and retention time are indicated.

Title: Supplementary Data 5.

Description: Genes present or absent in MuLDi *Neisseriaceae* when *Kingella* spp. are excluded from the comparative genomic analysis presented in Figure 5a.

Title: Supplementary Data 6:

Description: Phylogenies raw data.