## Raw HiSeq sequencing data 250 bp PE sequencing, 2 lanes Pre-processing • FastQC, demultiplexing (R), PE merging incl. • Remove primers (Cutadapt), if needed R.C. & trimming singletons Quality filtering (max expected errors = 1) • Min max length (±10), dereplication (keep singletons) size>3 Clustering • Merge all files, minuniquesize = 3 cluster\_otus (includes chimera removal) Data filtering OTU table • Map samples against OTUs, merge lanes Discard OTUs with <0.01% in all samples + remapping</li> Taxonomy assignment BOLD and NCBI reference databases (R) Statistical analysis (R)

**Figure S5**: Detailed overview of the bioinformatic processing of the Illumina hight throughput sequencing data. Raw sequence data (**A**) is demultiplexed and pre processed (PE merging, remove primers, trimming, reverse complement, removal of low quality reads (**B**). The processed sequences are then pooled and demultiplexed with a minimum size of 3, to reduce noise by sequencing errors in clustering (**C**). Reads from all samples are then compared against the generated OTUs and OTUs with a minimum of 0.01% of sequences assigned in at least one sample, are discarded (data from both lanes is merged at this point) (**D**). All reads are again mapped against the OTU subset to generate the final OTU table, with taxonomy being assigned to each centroid using NCBI and BOLD (**E**).