Sequence analysis
PhyloMagnet: fast and accurate screening of short-read meta-omics data using gene-centric phylogenetics

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Abstract

Motivation: Metagenomic and metatranscriptomic sequencing have become increasingly popular tools for producing massive amounts of short-read data, often used for the reconstruction of draft genomes or the detection of (active) genes in microbial communities. Unfortunately, sequence assemblies of such datasets generally remain a computationally challenging task. Frequently, researchers are only interested in a specific group of organisms or genes; yet, the assembly of multiple datasets only to identify candidate sequences for a specific question is sometimes prohibitively slow, forcing researchers to select a subset of available datasets to address their question. Here, we present PhyloMagnet, a workflow to screen meta-omics datasets for taxa and genes of interest using gene-centric assembly and phylogenetic placement of sequences.

Results: Using PhyloMagnet, we could identify up to 87% of the genera in an in vitro mock community with variable abundances, while the false positive predictions per single gene tree ranged from 0 to 23%. When applied to a group of metagenomes for which a set of metagenome assembled genomes (MAGs) have been published, we could detect the majority of the taxonomic labels that the MAGs had been annotated with. In a metatranscriptomic setting, the phylogenetic placement of assembled contigs corresponds to that of transcripts obtained from transcriptome assembly.

Availability and implementation: PhyloMagnet is built using Nextflow, available at github.com/maxemil/PhyloMagnet and is developed and tested on Linux. It is released under the open source GNU GPL licence and documentation is available at phylomagnet.readthedocs.io. Version 0.5 of PhyloMagnet was used for all benchmarking experiments.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

High-throughput DNA sequencing has revolutionized biology, opening up new fields of research and enabling new fundamental insights in the life sciences. During the past decades, several sequencing technologies have been developed, each differing significantly in sequence read length, quality and throughput (Mardis, 2017). Applications comprise DNA shotgun sequencing as well as RNA sequencing of complex microbial communities, termed metagenomics and metatranscriptomics, respectively (Mitchell et al., 2018).

Large environmental sequencing initiatives like the Tara Oceans project (Sunagawa et al., 2015) have provided researchers with enormous amounts of metagenome data. Using recently developed genome-resolved or genome-centric metagenomic approaches, draft genomes or metagenome assembled genomes (MAGs) of uncultured taxa can be assembled for the first time from shotgun metagenomic sequencing data of microbial communities (Alneberg et al., 2014; Eren et al., 2015). In order to apply those tools, however, metagenome assembly needs to be performed, which is computationally demanding and introduces additional challenges compared to single genome assembly such as the uneven coverage of contigs (contiguous sequences) from different organisms or the presence of microdiversity (Quince et al., 2017). Together with the ever-growing sequencing capacity, it becomes increasingly demanding to identify which of the available datasets (publicly deposited or locally generated sequence datasets) actually contain sequence data of a given taxon or gene of interest.

Instead of assembling short reads into longer contigs, the taxonomic composition of a metagenomic or metatranscriptomic dataset can be assessed using microbiome profilers that classify reads directly.
In general, these tools base their classification on the comparison of reads to reference sequences with a known taxonomy, and either work similar to the BLAST algorithm (e.g. Huson et al., 2016; Truong et al., 2015) or use exact k-mer matches to such reference sequences to classify reads (e.g. Ounit et al., 2015; Wood and Salzberg, 2014). Development in this area is continuing in order to increase analysis speed while reducing memory footprint. Currently, DIAMOND is one of the fastest local aligners that has a sensitivity comparable to BLAST (Buchfink et al., 2015), and MetaCache is one of the fastest and most memory efficient k-mer based classifiers, using only a discriminatory subset of available k-mers (Muller et al., 2017). All of these approaches, however, are based on sequence similarity, which can be incongruent with the true phylogenetic relationship of sequences (Smith and Pease, 2017).

Traditional phylogenetic tools on the other hand offer several robust evolutionary models for both nucleic and amino acids that theoretically allow for a more reliable taxonomic assignment of sequences, but are slow compared to similarity-based methods, usually prohibiting their application to large metagenome datasets. In addition, short reads generally do not provide enough phylogenetic signal, leading to artefactual inferences (Matsen et al., 2010). Several tools have been developed to overcome these barriers by instead placing fragmentary sequences (particularly from amplicon sequencing data) onto a phylogenetic reference tree (Barbera et al., 2019; Berger et al., 2011; Matsen et al., 2010).

Shotgun metagenomic or metatranscriptomic data is often analysed with a focus on gene rather than genome reconstruction, and is then usually called gene-centric. In this approach, the short reads or the assembled sequences are partitioned according to their affiliation to gene families. These methods can be used to determine which genes are present or actively transcribed in a sample, and can be combined with assemblers to reconstruct full-length sequences for a gene of interest. There exist several gene-centric targeted assemblers that perform de-novo reconstruction, e.g. via an overlap graph of candidate reads (Gruber-Vodicka et al., 2019; Kucuk et al., 2017; Pericard et al., 2018; Steinegger et al., 2019). While several of those only reconstruct the 16S rRNA gene or are limited to transcriptomic data, the MEGAN gene-centric assembler reconstructs contigs based on the alignment of reads to any reference protein sequence (Huson et al., 2017).

A recently published tool, GraftM, uses the ideas of phylogenetic placement and gene-centric metagenomics to taxonomically classify sequences of genes within metagenomes (Boyd et al., 2018). It is capable of placing either short-read sequences or pre-assembled metagenomic contigs onto a single reference tree at a time, but does not perform gene-centric assembly, which would increase phylogenetic signal of query sequences. Additionally, its reference trees can only be inferred using the extremely fast but less accurate maximum-likelihood-based tree inference programme FastTree (Price et al., 2010; Zhou et al., 2018). Here, we present PhyloMagnet, an efficient workflow system for parallel handling of both references and queries, gene-centric assembly, and robust phylogenetic inference, and show that it outperforms GraftM in terms of runtime and classification precision and sensitivity.

The goals of the work presented here were to:

1. Create a computational workflow that could determine the presence of taxa of interest in large short-read datasets based on gene-centric assembly and robust phylogenetic inference, especially with the objective of selecting good candidate datasets for metagenomic assembly and genome-resolved metagenomics.
2. Create a workflow that uses state-of-the-art methods and is versatile and fast enough to accommodate a broad range of applications, while being modular in order to easily incorporate new approaches.
3. Compare the workflow’s performance in terms of computational footprint and sensitivity/precision to GraftM, another recently published tool with a similar application.

2 Implementation

PhyloMagnet exploits the idea of gene-centric assembly (Huson et al., 2017) to efficiently screen sequence datasets of short reads for target genes, and to taxonomically classify assembled gene sequences using phylogenetic placement. Below is a description of the analysis steps employed by the pipeline (see also Fig. 1), which requires the following inputs:

1. One or several query short-read sequence data files in FASTQ or FASTA format (potentially ‘raw’, untrimmed reads, see Section 2.3), corresponding to the metagenomic or transcriptomic data(s) to query [Fig. 1(1)].
2. One or several homologous groups of reference proteins, each sequence annotated with its taxonomic affiliation (in the EggNOG format, containing NCBI’s taxonomy ID and a unique identifier, e.g. ‘70448.Q0P3H7’).

2.1 Alignment and tree reconstruction of references

For each input group of reference sequences a multiple sequence alignment is computed using either MAFFT (Katoh and Standley, 2013) or PRANK (Löytynoja and Goldman, 2010), without applying any filtering or trimming methods. Then a reference tree is reconstructed using any of IQ-TREE (Nguyen et al., 2015), RAxML-NG (Kozlov et al., 2019; Stamatakis, 2014) or FastTree (Price et al., 2010), making it possible to choose the appropriate method for a specific analysis. This way the user can make a trade-off between speed and quality of the reference tree and choose the appropriate evolutionary model. Reference alignments and trees can be precomputed (e.g. on a local machine) and then provided to PhyloMagnet as a compressed reference package (e.g. on an HPC cluster). This also increases reproducibility, as such reference packages can be released alongside results.

2.2 Alignment to reference protein sequences

Identifiers from the EggNOG database, containing orthologous groups of protein sequences from all domains of life with functional annotations (Huerta-Cepas et al., 2016b), can be specified to be used as input reference sequences. Alternatively, sets of homologous protein sequences curated by the user in FASTA format can be used. In order to check for the potential presence of homologues encoding...
these proteins of interest in the query metagenomes or metatranscriptomes, each of the short-read datasets given as input [see Section 2(1)] is then aligned to the collection of reference protein sequences using the DIAMOND aligner in blastX mode [Fig. 1(2); Buchfink et al., 2015].

2.3 Gene-centric assembly of reads
In a subsequent step, PhyloMagnet uses the gene-centric assembler implemented in MEGAN (Huson et al., 2016, 2017) to assemble reads into contigs [Fig. 1(3)]. The assembly is performed independently for each orthologous group of reference proteins, and the available alignments of reads to the protein reference sequences of a group is used to infer overlaps between reads, thereby concatenating them into contigs. As only the aligned part (core) of each read is used for the assembly, no pre-processing such as adapter clipping or quality trimming is needed. The results are written to a FASTA file per orthologous group if any contig in that group passes the cut-off for the minimum length (200 bp, can be adjusted if needed) that the gene-centric assembler uses. The assembled contigs are already in-frame and are subsequently translated into amino acid sequences using the standard genetic code.

2.4 Phylogenetic placement of reconstructed protein sequences
Next, the assembled and translated contigs are aligned to the alignments of each homologous reference group (maintaining the columns of the previously computed reference alignment), using the phylogeny-aware alignment tool PaPaRa [Fig. 1(4); Berger and Stamatakis, 2011]. This alignment of reference sequences and contigs is then used to place the contigs onto the reference tree using the evolutionary placement algorithm (EPA-ng) [Fig. 1(5); Berger et al., 2011; Barbera et al., 2019]. In a final stage, the tool gappa is used to annotate the internal branches of the reference tree and assign taxonomic labels to the translated contigs based on the likelihood weights of the placement (Czech and Stamatakis, 2019). Then a summary list of taxonomic labels is created.

2.5 ‘Magnetizing’ trees and identifying candidate datasets
The user can choose to specify taxonomic names (e.g. ‘Escherichia’) that should be used to filter (‘magnetize’) the list of all labels, specify a taxonomic rank (e.g. ‘family’) or a combination of both. The occurrences of the chosen taxonomic labels are summarized per reference group and metagenomic or transcriptomic datasets in order to assist manual decision of candidate datasets [Fig. 1(6)]. The information of how many trees were positive for a taxon of interest can be used as an approximation of coverage (see Section 4). The user could, e.g. select datasets that display differential coverage for subsequent genome extraction, which often relies on such differences to group genome contigs together (Albertsen et al., 2013; Alneberg et al., 2014).

2.6 Availability
PhyloMagnet is an open source software package and released under a GPLv3 licence. It is written as a Nextflow (Di Tommaso et al., 2017) script and available on github (github.com/maxemil/PhyloMagnet). Several functions and utilities are implemented either in python or bash (Dalk et al., 2009; Huerta-Cepas et al., 2016a; McKinney, 2010). All needed dependencies are available as a singularity (Kurtz et al., 2017) container (singularity-hub.org/containers/978) and the documentation can be found on ReadTheDocs (phylo magnet.readthedocs.io).

3 Benchmarking
To evaluate the performance of the PhyloMagnet workflow and exemplify its potential uses, we performed three benchmark experiments using an in vitro mock community as well as environmental metagenomic and metatranscriptomic sequencing datasets. We chose the datasets such that we could compare the results produced by PhyloMagnet to reference genome mapping data (Singer et al., 2016), genomes extracted from metagenomes with taxonomic annotation (Delmont et al., 2018) and an assembled metatranscriptome (Frazier et al., 2017), respectively. For details on command line parameters, see Supplementary Material.

3.1 Reference sequences
To assess the general taxonomic composition of datasets we used a set of 16 ribosomal proteins (rp16) that are thought to represent reliable phylogenetic markers, as they should be vertically inherited throughout evolution and present in a single copy in most organisms (Brown et al., 2015). For this, we downloaded the corresponding sets of unaligned homologous sequences from the EggNOG database v4.5.1 (Huerta-Cepas et al., 2016b).
As a second set of reference protein sequences, we used the set of 12 protein coding genes known to be present in chloroplast genomes of Dinophyceae ( Howe et al., 2008). This phylum of single-celled algae can be found in a wide range of aquatic environment and notably contains coral symbionts within the genus Symbiodinium (Gomez, 2012). For each of the genes we downloaded all available curated chloroplast encoded protein sequences for all phyla from UniProt (Apweiler et al., 2004) as well as all available proteins from the Dinophyceae from the same database.
All reference groups were aligned using MAFFT E-INS-i (Katoh and Standley, 2013) and reference trees were reconstructed using IQ-TREE (under the LG + G + F model; Nguyen et al., 2015).

3.2 Datasets
The first dataset we selected was the MBARC-26 (Mock Bacteria ARCeha Community), an in vitro mock community of 23 bacterial and 3 archaeal strains (in 24 genera) with finished reference genomes that were pooled and sequenced on an Illumina HiSeq instrument (Singer et al., 2016). As the taxonomic classification is dependent on the reference sequences, we added orthologous sequences to the EggNOG rp16 references for those genera missing from the original EggNOG datasets. To avoid using identical sequences as references, we used available genomes from related species within the same genera to expand the rp16 references. The orthologous proteins were identified by performing HMMEHMMv3.1b2; Eddy, 2011) searches of the EggNOG rp16 reference alignments against the additional proteomes (see Supplementary Material). These extended rp16 references were then used as references in PhyloMagnet in order to classify the MBARC-26 short-read data.
As a second dataset we used several metagenomic datasets from the geographic region ‘Southern Ocean’ that are part of the metagenomic datasets of the Tara Oceans Initiative, as defined by Delmont et al. (2018). We used the EggNOG rp16 references to assess taxonomic composition in those datasets and compared the results with the taxonomic classification of the MAGs reconstructed by Delmont et al. (2018). The authors extracted 375 genome bins from these datasets, but only presented detailed information, including taxonomy, for 13 ‘non-redundant’ MAGs that passed several quality and completeness filters. To be able to compare our classification results to a more extensive set of extracted genome bins, we inferred taxonomic labels for those bins that were not part of the 13 non-redundant MAGs using the tool sourmash that uses k-mer matches to taxonomically classify genomes (Tinus Brown and Ibrer, 2016).
Finally, we analysed the metatranscriptomes published by Frazier et al. (2017), who sequenced mRNA from both healthy corals and such that are affected by the so-called ‘bleaching’, a stress response in which Symbiodinium symbionts are expelled (Howe et al., 2008). We used the chloroplast protein references to search for Dinophyceae (and especially Symbiodinium) sequences in the metatranscriptome data. We then compared the assembled sequences with their placement in the sequences from the metatranscriptomic assembly available at NCBI’s GEO database (Barrett et al., 2012). Similar to how the assembly was
generated, we combined all of the 27 individual datasets by Frazier et al. (2017) into a single dataset for this analysis. In order to identify the transcripts in the assembly, we performed a tblastn search, querying the reference sequences against a database of the quality-filtered transcripts. The identified sequences were then, analogous to how sequences are classified in PhyloMagnet, placed onto the reference tree with EPA-ng.

3.3 Comparison with GraftM

We compared the performance of PhyloMagnet with that of the recently published tool GraftM (Boyd et al., 2018, v.0.11.1). GraftM also places sequences (either unassembled reads or pre-assembled contigs) onto a reference phylogeny using the tool pplacer, for which EPA-ng represents a scalable replacement that is able to handle larger amounts of data. We created GraftM reference packages (gpkg; containing the reference alignment, tree and the taxonomic annotation) from each of the extended rp16 references using the create command (see Supplementary Material). We then used each gpkg to analyse the MBARC-26 dataset and recovered taxonomic classifications of the query sequences. For both tools, we counted the number of genera that were correctly identified in each tree (true positives) as well as the number of genera that were identified even though they were not present in the MBARC-26 mock community (false positives). We also assessed the runtime and memory consumption of both tools for analysis of the full MBARC-26 dataset (50 Gb) as well as for subsamples of 1 and 10% (0.5 and 5 Gb, respectively).

4 Results

4.1 Classification of ribosomal proteins in the MBARC-26 dataset

We evaluated the performance of PhyloMagnet and GraftM to detect the presence of the 24 MBARC genera (23 of those detectable, as Nocardiopsis was part of the pooled community but not present in the sequence data) from Singer et al. (2016) in the metagenomic dataset (Supplementary Fig. S1 and Table S1). The number of correctly detected as well as falsely reported genera are displayed in Figure 2. PhyloMagnet correctly identified up to 20 (87%) of the MBARC genera and up to 7 (with an average of 2) false positive genera in all of the 16 trees. In contrast, GraftM identified a maximum of 9 (39%) of the correct MBARC genera while giving up to 14 (with an average of 4) false positives for each tree (Fig. 2). Some of the reported false positive and false negative errors of both PhyloMagnet and GraftM could be attributed to closely related and possibly unresolved taxonomic groups such as Escherichia/Salmonella, Thermobacillus/Paenibacillus or possibly Clostridium/Ruminiclostridium. Another confounding factor might be the well-known disagreement between phylogeny and taxonomy in some cases (e.g., Escherichia/Salmonella; Retchless and Lawrence, 2010). Some taxa with very low abundance in the data (e.g., Corynebacterium and Clostridium) were picked up by GraftM but not PhyloMagnet, which is likely due to the fact that there are not enough reads to reconstruct longer contigs for these taxa, impeding an identification by PhyloMagnet as we used the default cut-off implemented in the gene-centric assembler. In general we observe a correspondence between the percentage of mapped reads (Singer et al., 2016) and the number of trees a genus was detected in Supplementary Figure S2, suggesting that we can use the number of trees as a rough proxy for the abundance of a taxon in a dataset. When comparing results for the full dataset and the subsampled datasets, PhyloMagnet seems to profit immensely from the additional data, likely because the assembler can connect more reads and thus reconstruct more contigs above the length threshold. In terms of runtime, PhyloMagnet is twice as fast as GraftM when using 10 threads, making more efficient use of available computational resources. It uses, however, significantly more memory due to the requirements of MEGAN that performs the memory intensive sequence assembly, which GraftM does not include (see Supplementary Fig. S1).

We tested the performance of PhyloMagnet in a scenario where no sequences from the correct genus or family were available by removing the respective sequences from the rp16 references. PhyloMagnet was then run on each such reference dataset and we assessed whether the correct family or order was recovered. Our results show that if not the right genus (or family) is present but only other sequences from the corresponding family (or order), the right taxon could be recovered in 30 and 20% or the reference trees (see Supplementary Fig. S3). To further compare the performance of PhyloMagnet with that of a k-mer based metagenome profiling tool, we used Kraken2 (Wood et al., 2019; Wood and Salzberg, 2014) to classify the MBARC-26 dataset. Kraken performs the classification at a significantly higher speed and with a very good recovery rate of true positives, but also predicts a significant amount of false positive labels (see Supplementary Fig. S4).

4.2 Classification of taxa in the Tara Southern Ocean dataset

The PhyloMagnet workflow could identify 65 taxa (families) over the 8 datasets and 16 reference trees, whereas 21 were found in at least 4 trees for at least 1 dataset (Fig. 3). These taxa cover all but one of the taxonomic groups for which genomic bins could be identified by Delmont et al. (2018) (marked with an asterisk in Fig. 3). Most noticeable, the authors of that study recovered 6 non-redundant high-quality MAGs for the family Flavobacteriaceae which could be identified in every single tree for each dataset here as well.
Furthermore, the original authors identified one MAG each within the Alteromonadaceae, the Rickettsiales and the Alphaproteobacteria as well as two within Gammaproteobacteria. All of these taxonomic groups were detected by PhyloMagnet except for the Rickettsiales, who could have been mis-identified as Pelagibacterales in the phylogenetic placement. Alternatively, the genomic bins could have been mis-labelled as Rickettsiales and actually belong to the Pelagibacterales, as those two lineages commonly artefactually branch together in phylogenetic trees due to convergent genome streamlining, resulting in a similar sequence composition bias (Marriott et al., 2018; Rodriguez-Ezepeleta and Embley, 2012; Roger et al., 2017; Viklund et al., 2013). It is very likely that the MAGs that were labelled as Gammaproteobacteria by Delmont et al. (2018) are actually members of the Piscirickettsiaceae or Portiococaceae, which were both detected by PhyloMagnet in several individual datasets and the majority of single gene trees. Here, we also recovered the additional taxonomic labels (Portiococaceae, Rhodobacteraceae, Pelagibacteraceae, Cryomorphaceae) that could be assigned to raw genomic bins (which were not included in the original analyses as they did not pass quality and/or completeness thresholds) from the same study (Supplementary Table S2). Some of the labels we recovered were not represented by any MAGs/Bins, indicating either false positive classification of contigs or a low abundance of the genomic DNA, such that no genome bins could be reconstructed by Delmont et al. (2018).

Fig. 4. Phylogenetic placement of contigs and transcripts. As an example of the coral bleaching metatranscriptome analyses, the tree of the plastid gene psbB is shown. The sequences reconstructed by PhyloMagnet (left) and the assembled and quality-filtered transcripts from Frazier et al. (2017) (right) were placed onto the reference tree. All sequences were placed on branches sister to two Symbiodinium sequences (U6EFR9 and A0A0A0N1X1)
4.3 Identification of chloroplast genes in the coral bleaching dataset

Using PhyloMagnet contigs were reconstructed from the pooled coral bleaching dataset of Frazier et al. (2017). Using the Phylogenetic placement workflow, contigs classified as Symbiodiaceae could be identified in 10 out of the 12 chloroplast gene reference trees. The number of contigs that were reconstructed for each gene from the pooled sequencing data of 23 datasets ranged from 2 (psbE) to as many as 169 (psbA), whereas we could identify either one or two transcripts from the corresponding published transcriptome assembly for 9 out of the 12 genes. The two genes for which no contigs could be reconstructed were psbD and petD, both missing in the assembled transcriptome as well, which is likely due to two distinct issues. First, the psbH gene is only around 30 amino acid residues long, making contigs shorter than the default length cut-off of 200 bp implemented in the gene-centric assembler. Besides, psbH has never been identified, experimentally or computationally, in any Symbiodinium species, but the identification within the Dinophyceae comes from the species *Amphidinium operculatum* (Barbrook et al., 2014; Nisbet et al., 2004). Second, it seems that the transcription level of petD is quite low, so that very few reads would have been sequenced, making an assembly of contigs or transcripts virtually impossible (Nisbet et al., 2008). In those cases where transcripts could be identified, they were generally placed on the same branches or very close within the reference tree as were all of the corresponding contigs (Fig. 4).

5 Conclusion

We have shown that by applying phylogenetic placement methods to protein sequences that were reconstructed from short-read sequencing data, our PhyloMagnet workflow can accurately identify short-read sequence datasets that contain sequences for genes and taxa of interest. We compared PhyloMagnet to a similar tool that does not rely on using a gene-centric assembly approach and demonstrated that PhyloMagnet is faster and has a higher precision and sensitivity (at the price of consuming more memory).

PhyloMagnet allows researchers to explore the microbial diversity of a specific clade, or to specifically assess the presence of a metabolic pathway of interest. For example, PhyloMagnet was able to identify several lineages from single gene trees that match the results of a genome-resolved metagenomic study, showcasing how our tool could be used to screen the contents of a metagenomic dataset before applying metagenome assembly and binning methods.

Finally, we have also shown that the gene-centric phylogenetic approach of PhyloMagnet can be successfully used to efficiently detect expressed genes of taxa of interest in metatranscriptomic datasets.

Hence, PhyloMagnet represents a powerful tool that will enable researchers to pre-screen large metagenomic and metatranscriptomic datasets prior to engaging in time and resource consuming computational analyses in their research.

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References

Albertsen, M. et al. (2013) Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. *Nat. Biotechnol.*, 31, 533–538.

Alen, J. et al. (2014) Binning metagenomic contigs by coverage and composition. *Nat. Methods*, 11, 1144–1146.

Apweiler, R. et al. (2004) UniProt: the Universal Protein knowledgebase. *Nucleic Acids Res.*, 32, 1150–1119.

Barbera, P. et al. (2019) EPA-ng: massively parallel evolutionary placement of genetic sequences. *Syst. Biol.*, 68, 365–369.

Barbrook, A.C. et al. (2014) The chloroplast genome of a Symbiondium sp. clade C3 isolate. *Protist*, 165, 1–13.

Barrett, T. et al. (2012) NCBI GEO: archive for functional genomics data sets–update. *Nucleic Acids Res.*, 41, D991–D995.

Berger, S.A. et al. (2011) Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. *Syst. Biol.*, 60, 291–302.

Berger, S.A. and Stamatakis, A. (2011) Aligning short reads to reference alignments and trees. *Bioinformatics*, 27, 2068–2075.

Boyd, J.A. et al. (2018) Graafm: A tool for scalable, phylogenetically informed classification of genes within metagenomes. *Nucleic Acids Res.*, 46, e59.

Brown, C.T. et al. (2013) Unusual biology across a group comprising more than 15% of domain bacteria. *Nature*, 523, 208–211.

Buchfink, B. et al. (2015) Fast and sensitive protein alignment using DIAMOND. *Nat. Methods*, 12, 59–60.

Czech, L. and Stamatakis, A. (2019) Scalable methods for analyzing and visualizing phylogenetic placement of metagenomic samples. *PLoS One*, 14, e0217030–50.

Dalke, A. et al. (2009) Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics*, 25, 1423–1423.

Delmont, T.O. et al. (2018) Nitrogen-fixing populations of Planktonicymetes and Proteobacteria are abundant in surface ocean metagenomes. *Nat. Microbiol.*, 3, 804–813.

Di Tommaso, P. et al. (2017) Nextflow enables reproducible computational workflows. *Nat. Biotechnol.*, 35, 316–319.

Eddy, S.R. (2011) Accelerated profile HMM searches. *PLoS Comput. Biol.*, 7, e1002195.

Eren, A.M. et al. (2015) Anvi'o: an advanced analysis and visualization platform for omics data. *PeerJ*, 3, e1319.

Frazier, M. et al. (2017) De novo metatranscriptome assembly and coral gene expression profile of *Montipora capitata* with growth anomaly. *BMC Genomics*, 18, 1–11.

Gomez, F. (2012) A quantitative review of the lifestyle, habitat and trophic diversity of dinoflagellates (*Dinoflagellata, Alveolata*). *Syst. Biodivers.*, 10, 267–275.

Gruber-Vodicka, H.R. et al. (2019) phyloFlash – rapid SSU rRNA profiling and targeted assembly from metagenomes. *bioRxiv*, 521922. doi: 10.1101/521922.

Huson, D.H. et al. (2008) The remarkable chloroplast genome of *dinoflagellates*. *J. Exp. Bot.*, 59, 1035–1045.

Huerta-Cepas, J. et al. (2016) ETE 3: reconstruction, analysis, and visualization of phylogenomic data. *Mol. Biol. Evol.*, 33, 1635–1638.

Huerta-Cepas, J. et al. (2016) EGGNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res.*, 44, D286–D293.

Huson, D.H. et al. (2016) MEGAN community edition - interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Comput. Biol.*, 12, e1004957.

Huson, D.H. et al. (2017) Fast and simple protein-alignment-guided assembly of orthologous gene families from microbiome sequencing reads. *Microbiome*, 5, 11.

Katoh, K. and Standley, D.M. (2013) MAFFT multiple sequence alignment software ver 7: improvements in performance and usability. *Mol. Biol. Evol.*, 30, 772–780.

Kozlov, A.M. et al. (2019) RAXML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*, 35, 4452–4455.

Kuck, E. et al. (2017) Kollector: transcript-informed, targeted de novo assembly of gene loci. *Bioinformatics*, 33, 2789–2789.

Kurtzer, G.M. et al. (2017) Singularity: scientific containers for mobility of compute. *PLoS One*, 12, e0177459.

Loytynoja, A. and Goldman, N. (2010) webPRANK: a phylogeny-aware multiple sequence aligner with interactive alignment browser. *BMC Bioinformatics*, 11, 579.
Mardis,E.R. (2017) DNA sequencing technologies: 2006-2016. *Nat. Protocols*, 12, 213–218.

Martijn,J. et al. (2018) Deep mitochondrial origin outside the sampled alphaproteobacteria. *Nature*, 557, 101–105.

Matsen,Fa. et al. (2010) pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. *BMC Bioinformatics*, 11, 538.

Mckinney,W. (2010) Data structures for statistical computing in Python. In: van der Walt,S. and Millman,J. (eds) *Proceedings of the 9th Python in Science Conference, Austin, Texas*, pp. 51–56.

Mitchell,A.L. et al. (2018) EBI Metagenomics in 2017: enriching the analysis of microbial communities, from sequence reads to assemblies. *Nucleic Acids Res.*, 46, D726–D735.

Nguyen,L.T. et al. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.*, 32, 268–274.

Nisbet,R.E.R. et al. (2018) Transcript analysis of Dinoflagellate plastid gene minicircles. *Protist*, 159, 31–39.

Nisbet,R.E.R. et al. (2004) Novel plastid gene minicircles in the dinoflagellate *Amphidinium operculatum*. *Gene*, 331, 141–147.

Ounit,R. et al. (2015) CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. *BMC Genomics*, 16, 236.

Pericard,P. et al. (2018) MATAM: reconstruction of phylogenetic marker genes from short sequencing reads in metagenomes. *Bioinformatics*, 34, 585–591.

Price,M.N. et al. (2010) FastTree 2 - approximately maximum-likelihood trees for large alignments. *PLoS ONE*, 5, e9490.

Quince,C. et al. (2017) Shotgun metagenomics, from sampling to analysis. *Nat. Biotechnol.*, 35, 833–844.

Retchless,A.C. and Lawrence,J.G. (2010) Phylogenetic incongruence arising from fragmented speciation in enteric bacteria. *Proc. Natl. Acad. Sci. USA*, 107, 11453–11458.

Rodriguez-Ezpeleta,N. and Embley,T.M. (2012) The SAR11 group of alpha-proteobacteria is not related to the origin of mitochondria. *PLoS One*, 7, e30520.

Roger,A.J. et al. (2017) The origin and diversification of mitochondria. *Curr. Biol.*, 27, R1177–R1192.

Singer,E. et al. (2016) Next generation sequencing data of a defined microbial mock community. *Sci. Data*, 3, 160081.

Smith,S.A. and Pease,J.B. (2017) Heterogeneous molecular processes among the causes of how sequence similarity scores can fail to recapitulate phylogeny. *Brief. Bioinformatics*, 18, 451–457.

Stamatakis,A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.

Steinmeger,M. et al. (2019) Protein-level assembly increases protein sequence recovery from metagenomic samples manyfold. *Nat. Methods*, 16, 603.

Sunagawa,S. et al. (2015) Structure and function of the global ocean microbiome. *Science*, 348, 1261359.

Titus Brown,C. and Irber,L. (2016) sourmash: a library for MinHash sketching of DNA. *J. Open Source Softw.*, 1, 27.

Truong,D.T. et al. (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat. Methods*, 12, 902–903.

Viklund,J. et al. (2013) Comparative and phylogenomic evidence that the alphaproteobacterium HIMB59 is not a member of the oceanic SAR11 clade. *PLoS One*, 8, e78858.

Wood,D.E. et al. (2019) Improved metagenomic analysis with Kraken 2. *bioRxiv*, doi: 10.1101/762302.

Wood,D.E. and Salzberg,S.L. (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol.*, 15, R46.

Zhou,X. et al. (2018) Evaluating fast maximum likelihood-based phylogenetic programs using empirical phylogenomic data sets. *Mol. Biol. Evol.*, 35, 886–903.