Article - Method

Title

SHOOT: phylogenetic gene search and ortholog inference

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Abstract

Determining the evolutionary relationships between genes is fundamental to comparative biological research. Here we present the phylogenetic search, SHOOT. SHOOT searches a user query sequence against a database of phylogenetic trees and returns a tree with the query sequence correctly placed within it. We show that SHOOT performs this analysis with comparable speed to a BLAST search. We demonstrate that SHOOT phylogenetic placements are as accurate as conventional tree inference and it can identify orthologs with

high accuracy. In summary, SHOOT is a fast and accurate tool for phylogenetic analyses of novel query sequences. It is available online at www.shoot.bio.

Background

Resolving the phylogenetic relationships between biological sequences provides a framework for inferring sequence function, and a basis for understanding the diversity and evolution of life on Earth. The entry point to such phylogenetic analyses is provided by algorithms that either align or identify regions of local similarity between pairs of biological sequences. The first implementations of such algorithms utilised global alignments to provide a basis to score similarity between sequences [1]. Later, faster local alignment methods were developed [2], followed by the FASTA heuristic database search [3] and culminating with the development of the BLAST algorithm and statistical methods for homology testing [4] in the 1990s. Since then, BLAST and other local alignment methods [5-7] have provided a critical foundation of biological science research and form the entry point to the majority of biological sequence analyses.

One feature of the problem that is under-utilised in BLAST and related local alignment search tools is the transitive nature of homology. Because local alignment searching methods do not store the relationships between sequences, a search of a query gene against a large database will involve carrying out many needless pairwise local alignments against numerous closely related homologs. An alternative approach would be to infer the relationships between all database sequences ahead of time using phylogenetic inference methods. These phylogenetic relationships can then be stored as part of the database, facilitating the use of lighter-weight search approaches or sparse reference databases with relationships already computed. Existing methods that take these kind of approaches include TreeFam for genes within the Metazoa [8] and TreeGrafter for annotating protein sequences using annotated phylogenetic trees [9].

Although local similarity searches such as BLAST are the primary entry point to the sequence analysis, a frequent end-goal of such analyses is to identify orthologs of the query sequence in other species. The use of phylogenetic methods is the canonical method for assessing gene relationships. Phylogenetic methods for estimating sequence similarity are more accurate than using local pairwise alignments, and critically they provide contextual information about the place of the query gene within its gene family. This includes the identification of orthologs, paralogs, and gene gain and loss within each clade in of the resultant phylogenetic tree. Although the similarity scores returned by local alignment methods can be used to approximate phylogenetic trees [10], they are not accurate and can be limited by only having alignments against a single query gene rather than alignments between sequences already in the database [11]. Moreover, even when all pairwise similarity scores are calculated the accuracy of phylogenetic trees inferred from these scores is limited [10]

Here we present SHOOT, a software tool for rapidly searching a phylogenetically partitioned and structured database of biological sequences. There are a number of advantages to taking a phylogenetic approach to sequence searching. We show that by grouping homologous genes in the database, a gene can then be rapidly assigned to its homology group, irrespective of the number of homologous genes. Further, false negatives are unlikely since complete homology groups can be identified securely ahead of time. This helps avoid the reduced sensitivity that results from local sequence similarity database search algorithm heuristics used to determine which sequences to consider aligning [6]. Phylogenetic inference methods can then be used to rapidly and accurately assign the gene to its correct position within the otherwise pre-computed gene tree for its homology group [12]. This avoids the need to evaluate gene-relatedness using e-values, which are a measure of the certainty that a pair of genes are homologous, rather than a direct evaluation of the phylogenetic relationship between genes [13]. In summary, SHOOT efficiently and

accurately places query sequences directly into phylogenetic trees. In this way the phylogenetic history of the query sequence and its orthologs can be immediately visualised, interpreted, and retrieved. SHOOT is provided for use at www.shoot.bio.

Results

Pre-computed databases of phylogenetic trees allow ultra-fast phylogenetic orthology analysis of novel gene sequences

The conventional procedure for sequence orthology analysis is to first assemble a group of gene sequences which share similarity and then perform phylogenetic tree inference on this group to infer the relationships between those genes. The SHOOT algorithm was designed to make such a phylogenetic analysis feasible as a real-time search using a two-stage approach. The first stage comprises the ahead-of-time construction of a SHOOT phylogenetic database and the second stage implements the SHOOT search for a query sequence (Figure 1). The database preparation phase includes multiple automated steps including homology group inference, multiple sequence alignment, phylogenetic tree inference, and homology group profiling (see Methods). Thus, prior to database searching the phylogenetic relationships between all genes in the database are already established. Subsequent SHOOT searches exploit the fact that the alignments and trees have already been computed to enable the use of accurate phylogenetic methods for placement of query genes within pre-computed gene trees with little extra computation required.

The median time for a complete a SHOOT search of a database containing 984,137 protein sequences from 78 species was 5.5 seconds using 16 cores of an Intel Xeon E5-2683 CPU for (Figure 2A). This compared with 1.19 seconds for a conventional BLAST search of the same sequence set (Figure 2A). However, unlike BLAST (or similar) sequence search methods, the output of a SHOOT search is not an ordered list of similar sequences but is instead a maximum likelihood phylogenetic tree with bootstrap support values inferred from

a multiple sequence alignment with the query gene embedded within it. SHOOT also computes the orthologs of the query gene using phylogenetic methods.

SHOOT is more accurate than BLAST in identifying the closest related gene sequence

A leave-one-out analysis was conducted to test SHOOT's ability to find the most closely related gene sequence in a given database. Here a set of 1000 test cases was randomly sampled from the UniProt Reference Proteomes database. Each test case consisted of a pair of genes sister to each other with at least 95% bootstrap support in a maximum likelihood gene tree. One member of the test pair was arbitrarily designated the "query sequence" and the other gene was designated "the expected closest gene" i.e. the gene that should be identified by a search method as the most similar gene in the database. To provide a comparison, BLAST [11] was also tested on the same dataset. The set of query genes were searched against the database and each method was scored on whether or not the closest/best scoring gene in each search result was "the expected closest gene". The tests showed that SHOOT identified "the expected closest gene" as the most closely related gene in 94.2% of cases (Figure 2A). For comparison, BLAST correctly identified the "the expected closest gene" as the most similar gene sequence in 88.4% of cases. To put this in context, there is a 1 in 9 chance that the top hit returned by BLAST is not the most closely related sequence in the database while there is a 1 in 17 chance that the same is true for SHOOT. Thus, SHOOT is better able to identify the closest related gene to a given query gene in a given database and can be used as an alternative to BLAST for this purpose.

SHOOT gives evolutionary context of a query gene's position within its gene family

Although for many users knowledge of the closest related gene as described above may be sufficient, in many instances there will be more than one gene that is equally closely related to the query gene in a given species. Thus, to generalise the "best hit" analysis above for larger gene sets the "Mean Average Precision at k" score [14] was calculated, to quantify the precision at which the k closest homologs identified by SHOOT or BLAST correspond to

the k expected closest homologs in maximum likelihood gene trees. This analysis was conducted for values of k between 1 (equivalent to the “best hit” analysis above) and 50 (Figure 2B). As k increased, MAP@k for BLAST fell to 71.8%. i.e. there was a 71.8% agreement between the closest homologs identified using BLAST and those identified using phylogenetic methods. In contrast, the use of phylogenetic methods in the database construction stage of SHOOT coupled with the accurate placement of genes within the database trees (Figure 2A), resulted in MAP@50 for SHOOT of 90.3%. Thus, both the list of most closely related genes and their rank order of relationship to the query gene is substantially more accurate for SHOOT than for BLAST.

SHOOT has high accuracy in identifying orthologs of the query gene

A frequent goal of sequence similarity searches is to identify orthologs of the query gene in other species. As stated above, local similarity search tools such as BLAST do not do this. Instead, they return a list of genes that should be subject to multiple sequence alignment and phylogenetic inference in order to infer the orthology relationships between genes. The phylogenetic tree returned by SHOOT provides the evolutionary relationships between genes inferred from multiple sequence alignment and maximum likelihood tree inference allowing orthologs and paralogs to be identified. SHOOT also automatically identifies orthologs and colours the genes in the tree according to whether they are orthologs or paralogs (Supplementary Figure 1), as identified using the species overlap method [15, 16], which has been shown to be an accurate method for automated orthology inference [17]. The tree viewer also supports a zoom functionality to view a progressively larger or smaller clade of genes around the query gene. An image of the tree can be downloaded, the tree can also be exported in Newick format, and the FASTA file of protein sequences in the tree can be downloaded to support further downstream analyses.

To evaluate the accuracy of ortholog inference 6 species were chosen at increasing time since divergence from human. These query species comprised Mouse, Chicken, Zebrafish,

the Tunicate *Ciona intestinalis*, fruit fly, and the yeast *Saccharomyces cerevisiae* (Figure 3A). Orthologs between these species and Human were determined from OrthoFinder on the 2020 Quest for Orthologs benchmark dataset [13, 17]. For each query species 100 query genes were selected, creating a test set of 600 genes in total. For these 600 genes SHOOT was evaluated on its accuracy in identifying the orthologs in human. For comparison BLAST best hit (BH) and reciprocal best hit (RBH) were likewise evaluated (Figure 3B). SHOOT was between 11% (Mouse) and 47% (*S. cerevisiae*) more accurate than either method using BLAST and the difference was greatest for more diverged species (Figure 3B). The greatest difference between SHOOT and BLAST was in the percentage of orthologs that were recovered (Recall, Figure 3C). For all species, the ortholog recall for SHOOT was >79%. Whereas the ortholog recall for BLAST RBH was for 37% for *S.* cerevisiae, the most distant species from human in the analysis (Figure 3C). The precision of SHOOT orthologs was intermediate between BLAST RBH and BH (Figure 3D). Thus, SHOOT ortholog assignments are more accurate than performing a “top hit” or “reciprocal best BLAST hit” analysis for identification of orthologs.

Curated databases place the gene in the context of model species and key events in the gene’s evolution

The initial release of SHOOT includes phylogenetic databases for Metazoa, Fungi, Plants, Bacteria & Archaea, and also the UniProt Quest for Orthologs (QfO) reference proteomes, which cover all domains of cellular life (Supplementary Tables 1-5). To maximise the utility of the gene trees to a wide range of researchers, the species within the databases have been chosen to contain model species, species of economic or scientific importance, and species selected because of their key location within the evolutionary history covered by the database. Each database also contains multiple outgroup species to allow robust rooting of the set of gene trees. As an example, Supplementary Figure 2 shows the phylogeny for the metazoan database, highlighting the taxonomic groups of the included species. Although a

number of databases are provided on the SHOOT webserver, the SHOOT command line tool has been designed so that databases can be compiled from any species set.

Discussion and Conclusions

SHOOT is a phylogenetic search engine for analysis of biological sequences. It has been designed to take a user-provided query sequence and return a phylogenetic analysis of that sequence using a database of reference organisms. We show that SHOOT can perform this search and analysis with comparable speed to a typical sequence similarity search and thus SHOOT is provided as a phylogenetically informative alternative to BLAST, and as a general-purpose sequence search algorithm for analysis and retrieval of related biological sequences.

Local similarity or profile-based search methods such as BLAST [11], DIAMOND [5] or MMseqs [18] have a wide range of uses across the biological and biomedical sciences. The near-ubiquitous utility of these methods has led to them being referred to as the Google of biological research. However, one of the most frequent use cases of these searches is to identify orthologs of a given query sequence. Due to the frequent occurrence of gene duplication and loss, orthologs are often indistinguishable from paralogs in the results of local similarity searches. This is because a given query sequence can have none, one, or many orthologs in a related species. Accordingly, the sequences identified by local similarity searching methods will be an unknown mixture of orthologs and paralogs [19]. The problem of distinguishing orthologs from paralogs can be partially mitigated by a reciprocal best hit search, but with low recall [19]. Phylogenetic methods are required to correctly distinguish orthologs from paralogs as they are readily able to distinguish sequence similarity (branch length) and evolutionary relationships (the topology of the tree).

SHOOT was designed to provide the accuracy and information of a phylogenetic analysis with the speed and simplicity of a local sequence similarity search. By pre-computing the

within-database sequence relationships, SHOOT can perform an individual search in a comparable time to BLAST. However, instead of a returning a list of similar sequences SHOOT provides a full maximum-likelihood phylogenetic tree as a result enabling immediate phylogenetic interrogation of the sequence search results. A phylogenetic tree provides the best representation available of the evolutionary history of a gene family. The tree allows the identification of speciation and gene duplication events and thus the identification of orthologs and paralogs. While, SHOOT identifies orthologs and paralogs algorithmically the phylogenetic tree can and should also be examined by a user to gain an understanding of how the gene family has evolved, using the orthology assignment by SHOOT as a guide.

A standard phylogenetic approach to identifying orthologs of a query gene is to begin a local sequence similarity search or profile search (HMMER [20], MMseqs [18]). Frequently, an e-value cut-off is applied to identify a set of similar sequences for subsequent phylogenetic analysis. Because e-values (and their constituent bit-scores) are imperfectly correlated with evolutionary relatedness, the set of similar sequences meeting the search threshold will often be missing some genes as well as often including genes that should not be present. A systematic study using HMMER found that for all n genes from an orthogroup clade to pass an e-value threshold, on average the threshold would have to be set such that 1.8n genes in total met the threshold [21]. i.e. an additional 80% of genes needed to be included, on average, to ensure the orthogroup was complete [21]. Thus, unless a very lenient search is used, genes will be incorrectly absent from the final tree. This can lead to incorrect rooting and subsequent mis-interpretation even by phylogenetic experts [21]. Thus, even for bespoke phylogenetic analyses, it is better to use phylogenetic methods to first select the clade of genes of interest. SHOOT supports this by inferring the tree for the entire family of detectable homologs. The use of trees for complete sets of homologs, together with the use of OrthoFinder's robust tree-rooting algorithm [13], avoids the problem of mis-rooting and misinterpretation of a tree inferred for a more limited set of genes. Also, by using OrthoFinder

clustering approach [13, 22], hits missed for a single sequence are also corrected by multiple hits identified for its homologs. This “phylogenetic gene selection workflow” is supported by SHOOT’s web interface, which allows a clade of genes to be selected and the protein sequences for just this clade to be downloaded for downstream user analyses.

In summary, SHOOT was designed to be as easy to use as BLAST, but to provide phylogenetically resolved results in which the query sequence is correctly placed in a phylogenetic tree. In this way the phylogenetic history of the query sequence and its orthologs can be immediately visualised, interpreted, and retrieved.

Materials and Methods

Database preparation

SHOOT consists of a database preparation program and a database search program. The database preparation program takes as input the results of an OrthoFinder [13] analysis of a set of proteomes.

To prepare phylogenetic databases for the SHOOT website, the OrthoFinder version 3.0 option, “-c1”, was used to cluster genes into groups consisting of all homologs, rather than the default behaviour which is to split homologous groups at the level of orthogroups. The advantage of the creating complete homologous groups is that their gene trees show an expanded evolutionary history of those genes, including ancient gene duplication events linking gene families, rather than only reaching back to the last common ancestor of the included species. This differs from a default OrthoFinder orthogroup analysis, for which the partitioning of genes into taxonomically comparable orthogroups groups is the priority. OrthoFinder-inferred rooted gene trees for these homolog groups are computed using MAFFT [23] and IQ-TREE [24] by using the additional options “-M msa -A mafft -T iqtree -s species_tree.nwk”, where “species_tree.nwk” was the rooted species tree for the included

252 species. For IQ-TREE, the best fitting evolutionary model was tested for using “-m TEST”
253 and bootstrap replicates performed using “-bb 1000”.

254 The OrthoFinder results were converted to a SHOOT database in two steps: splitting of large
255 trees and creation of the DIAMOND profiles database for assigning novel sequences to their
256 correct gene tree. Large trees are split since the time requirements for adding a sequence
257 to an MSA for a homologous group and for adding a sequence to its tree can grow super-
258 linearly in the size of the group, leading to needlessly long runtimes. It was found that
259 DIAMOND could instead be used to assign a gene to its correct subtree and then
260 phylogenetic placement could be applied to assign the gene to its correct position within the
261 subtree (Figure 4).

262 The script “split_large_tree.py” was used to split any tree larger than 2500 genes into
263 subtrees of no more than 2500 genes each. Each subtree tree also contained an outgroup
264 gene, from outside the clade in the tree for that subtree, which was required for the later
265 sequence search stage. For each tree that was split into subtrees, a super-tree was also
266 created by the script of the phylogenetic relationships linking the subtrees. For each subtree,
267 the script extracted the sub-MSA for later use. This subtree size of 2500 genes was chosen
268 as it is the approximate upper limit tree size for which SHOOT could place a novel query
269 gene in the tree in 15 seconds. This was judged to be a reasonable wait for users of the
270 website to receive the tree for their query sequence. For the databases provided by the
271 SHOOT website, between 2 and 40 of the largest trees were split into subtrees.

272 The script “create_shoot_db.py” was used to create a DIAMOND database of “profiles” for
273 each unsplit tree or each subtree. A profile here refers to a set of representative sequences
274 that best describe the sequence variability within a homologous group. These profiles are
275 used to assign a novel query sequence to the correct tree or subtree. The representative
276 sequences for a gene tree are selected using k-means clustering applied to the MSA
277 corresponding to that (sub)tree using the python library Scikit-learn [25]. For each cluster,

the sequence closest to the centroid is chosen as a representative. For a homologous group of size N genes, k=N/10 representative sequences are used, with a minimum of min(20, N) representative sequences. This ensures that large and diverse homologous groups have sufficient representative sequences in the assignment database.

Database search

A query sequence is searched against the profiles database using DIAMOND [5] with default sensitivity and an e-value cut-off of 10^{-3}. If no hit is found, a second search is performed with the "--ultra-sensitive" setting. The top hitting sequence is used to assign the gene to the correct tree or subtree. The query gene is added to the pre-computed alignment using the MAFFT “--add” option and a phylogenetic tree is computed from this alignment using the precomputed tree for the reference alignment using EPA-ng [12] and gappa [26].

If the gene is added to a subtree then the tree is rooted on the outgroup sequence for that subtree. The outgroup is then removed from the subtree and the subtree is grafted back into the original larger tree, using the supertree to determine the overall topology. This method provides the accuracy of phylogenetic analysis to place the gene in its correct position within the subtree while at the same time providing the user with the full gene history for the complete homologous group given by the supertree, which was calculated in full in the earlier database construction phase. All tree manipulations by SHOOT are performed using the ETE Toolkit [27].

Curated databases

For the Plants database, the protein sequences derived from primary transcripts were downloaded from Phytozome [28]. The Uniport Reference Proteomes database was constructed using the 2020 Reference Proteomes [17]. For the Fungi and Metazoa databases the proteomes were downloaded from Ensembl [29] and the longest transcript variant of each gene was selected as a representative of that gene using OrthoFinder’s “primary_transcripts.py” script [13]. The Bacterial and Archaeal database proteomes were

downloaded from UniProt [30]. The parallelisation of tasks in the preparation of the databases was performed using GNU parallel [31].

Accuracy validation & performance

The UniProt Reference Proteomes database was used for validation of the SHOOT phylogenetic placements using a leave-one-out test. As this database covers the greatest phylogenetic range (covering all domains of life), its homologous groups contain the greatest sequence variability, and it provides the severest test of the accuracy of SHOOT. Test cases were constructed by selecting 1000 ‘cherries’ (pairs of genes sister to one another) with 95% bootstrap support from gene trees with median bootstrap support of at least 95%. The use of cherries allowed BLAST to be tested alongside SHOOT. This test was possible for BLAST since it would only have to identify a single closest gene, rather than having to identify a gene as the sister gene to a whole clade of genes (as SHOOT is designed to be able to do). The bootstrap support criteria ensured that the correct result was known with high confidence so that both methods could be assessed accurately. To ensure an even sampling of test cases, at most one test case was extracted from any one gene tree. Both the BLAST and SHOOT databases were completely pruned of the 1000 test cases. Each of the 1000 test cases was run using 16 cores of an Intel Xeon E5-2683 CPU and the runtime recorded (Figure 2).

To calculate the Mean Average Precision at k score, the expected trees were re-inferred using RAxML with the best-fitting model [32] so that a different method were used to that used in the SHOOT database construction. For each test gene the ordered list of closest homologs was calculated using branch length distance in the SHOOT results trees and e-values (with ties broken by bit score) for the BLAST results. These ordered homologs were compared to the expected ordered list of closest homologs from the expected RAxML trees to calculate the precision at each value of k from 1 to 50 and these precision scores were averaged over the 1000 test cases.

The ortholog prediction accuracy tests calculated the precision, recall and F-score for identifying orthologs in *Homo sapiens* for genes from *Mus musculus, Gallus gallus, Danio rerio, Ciona intestinalis, Drosophila melanogaster* and *Saccharomyces cerevisiae*. For each of these 6 species 100 genes were sampled at random. The expected orthologs were obtained from OrthoFinder 2020 Quest for Orthologs benchmark results, obtained from the benchmarking server: https://orthology.benchmarkservice.org. . For SHOOT, the orthologs were inferred using the species-overlap method [15] on the SHOOT results trees. For BLAST orthologs were predicted using the best hit (BH) method and the reciprocal best hit (RBH) method using the e-value scores.

SHOOT website

The tree visualisation is provided by the phylotree.js library [33]. The SHOOT website is implemented in JavaScript and Bootstrap and using the Flask web framework.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The SHOOT source code is available at https://github.com/davidemms/SHOOT. The code for the SHOOT webserver is available at https://github.com/davidemms/SHOOT_webserver. A compressed archive of all data is available at the Zenodo research data archive at https://doi.org/10.5281/zenodo.5602736 [34]. A webserver running SHOOT is available at https://shoot.bio.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DE and SK conceived and designed the project. DE developed the algorithms. DE and SK discussed the results and wrote the manuscript. All authors read and approved the final manuscript.

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Figure Legends

Figure 1. The workflow for the two separate stages of SHOOT: **A**) The database preparation stage. **B**) The sequence search stage. MSA, multiple sequence alignment. HG, homologous group. Individual shapes represent individual protein sequences.

Figure 2. Runtime and closest homologs identification accuracy for SHOOT and BLAST.. A) Violin plot of runtimes for 1000 searches of randomly sampled sequences against the same database of 984,137 protein sequences from 78 species. B) Accuracy at identifying the closest related database gene to a randomly selected query sequence. C) Mean Average Precision at k (MAP@k).

Figure 3. F-score, precision and recall at identifying orthologs in *Homo sapiens* for 100 query genes in each of *Mus musculus, Gallus gallus, Danio rerio, Ciona intestinalis, Drosophila melanogaster* and *Saccharomyces cerevisiae* for BLAST best hit (BH), BLAST reciprocal best hit (RBH) and SHOOT.

Figures

Figure 1

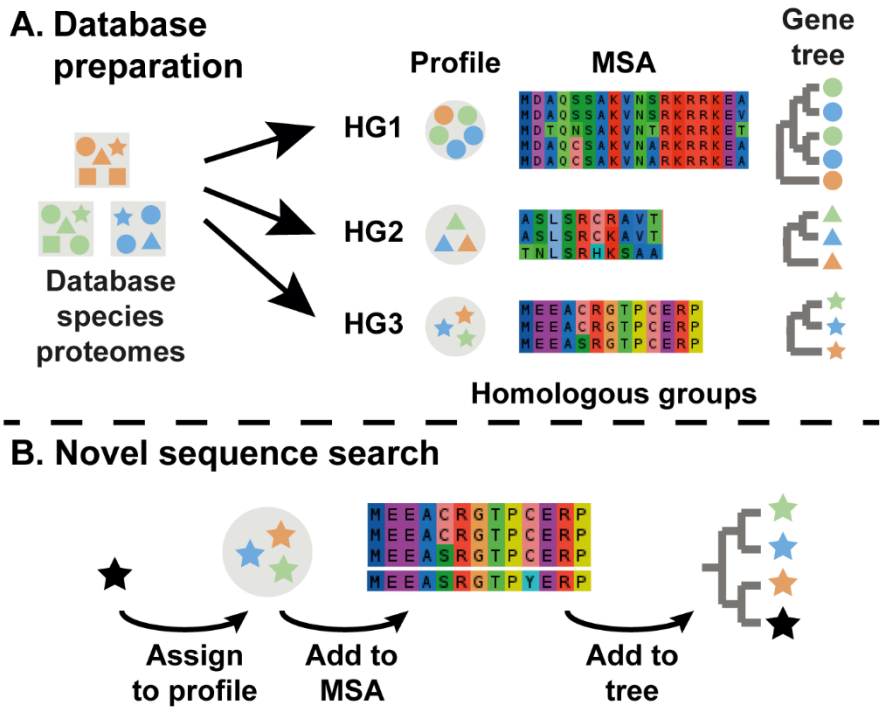
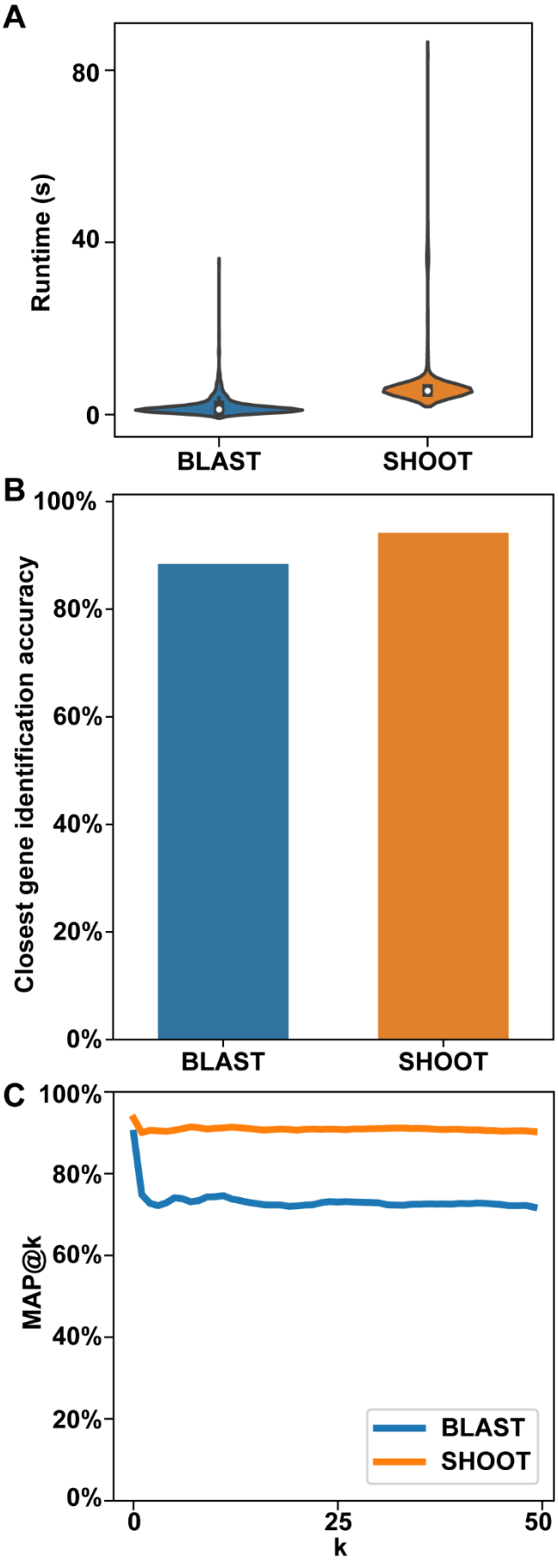
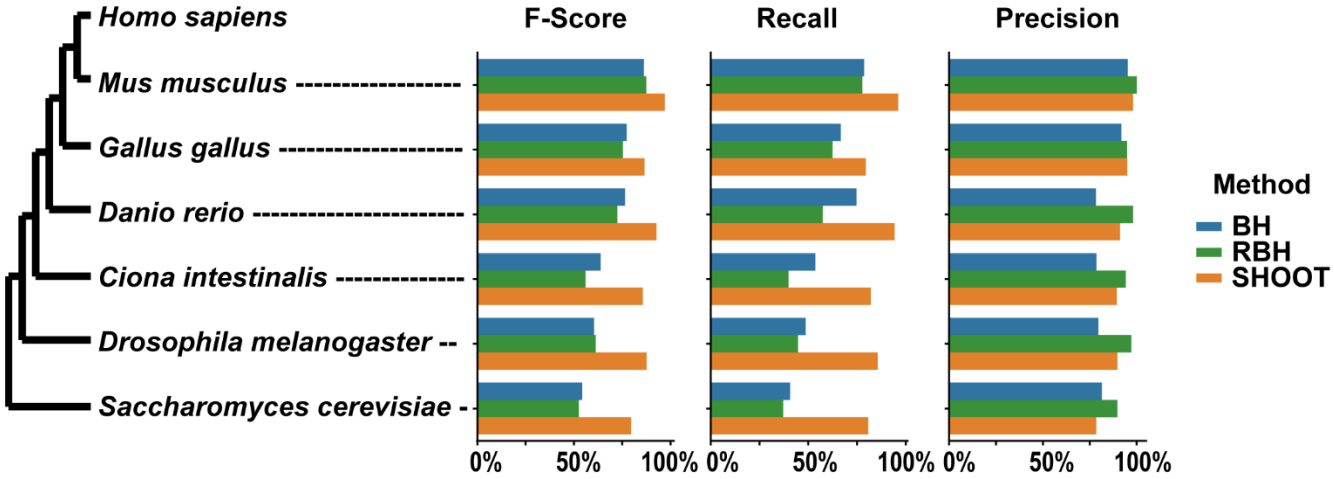


Figure 2



472 ***Figure 3***



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475 **Supplementary Table 1: UniProt 2020 Reference Proteomes – Species list**

Domain	Species
Archaea	*Halobacterium salinarum*
Archaea	*Korarchaeum cryptofilum*
Archaea	*Methanocaldococcus jannaschii*
Archaea	*Methanosarcina acetivorans*
Archaea	*Nitrosopumilus maritimus*
Archaea	*Saccharolobus solfataricus*
Archaea	*Thermococcus kodakarensis*
Bacteria	*Aquifex aeolicus*
Bacteria	*Bacillus subtilis*
Bacteria	*Bacteroides thetaiotaomicron*
Bacteria	*Bradyrhizobium diazoefficiens*
Bacteria	*Chlamydia trachomatis*
Bacteria	*Chloroflexus aurantiacus*
Bacteria	*Deinococcus radiodurans*
Bacteria	*Dictyoglomus turgidum*
Bacteria	*Escherichia coli*
Bacteria	*Fusobacterium nucleatum*
Bacteria	*Geobacter sulfurreducens*
Bacteria	*Gloeobacter violaceus*
Bacteria	*Helicobacter pylori*
Bacteria	*Leptospira interrogans*
Bacteria	*Mycobacterium tuberculosis*
Bacteria	*Mycoplasma genitalium*
Bacteria	*Neisseria meningitidis*
Bacteria	*Pseudomonas aeruginosa*
Bacteria	*Rhodopirellula baltica*
Bacteria	*Streptomyces coelicolor*
Bacteria	*Synechocystis sp.*
Bacteria	*Thermodesulfovibrio yellowstonii*
Bacteria	*Thermotoga maritima*
Eukaryota	*Anopheles gambiae*
Eukaryota	*Arabidopsis thaliana*
Eukaryota	*Batrachochytrium dendrobatidis*
Eukaryota	*Bos taurus*
Eukaryota	*Branchiostoma floridae*
Eukaryota	*Caenorhabditis elegans*
Eukaryota	*Candida albicans*
Eukaryota	*Canis lupus familiaris*
Eukaryota	*Chlamydomonas reinhardtii*
Eukaryota	*Ciona intestinalis*
Eukaryota	*Cryptococcus neoformans*
Eukaryota	*Danio rerio*
Eukaryota	*Dictyostelium discoideum*
Eukaryota	*Drosophila melanogaster*
Eukaryota	*Gallus gallus*
Eukaryota	*Giardia intestinalis*
Eukaryota	*Gorilla gorilla gorilla*

Eukaryota	*Helobdella robusta*
Eukaryota	*Homo sapiens*
Eukaryota	*Ixodes scapularis*
Eukaryota	*Leishmania major*
Eukaryota	*Lepisosteus oculatus*
Eukaryota	*Monodelphis domestica*
Eukaryota	*Monosiga brevicollis*
Eukaryota	*Mus musculus*
Eukaryota	*Nematostella vectensis*
Eukaryota	*Neosartorya fumigata*
Eukaryota	*Neurospora crassa*
Eukaryota	*Oryza sativa subsp. japonica*
Eukaryota	*Oryzias latipes*
Eukaryota	*Pan troglodytes*
Eukaryota	*Paramecium tetraurelia*
Eukaryota	*Phaeosphaeria nodorum*
Eukaryota	*Physcomitrella patens*
Eukaryota	*Phytophthora ramorum*
Eukaryota	*Plasmodium falciparum*
Eukaryota	*Puccinia graminis*
Eukaryota	*Rattus norvegicus*
Eukaryota	*Saccharomyces cerevisiae*
Eukaryota	*Schizosaccharomyces pombe*
Eukaryota	*Sclerotinia sclerotiorum*
Eukaryota	*Thalassiosira pseudonana*
Eukaryota	*Tribolium castaneum*
Eukaryota	*Trichomonas vaginalis*
Eukaryota	*Ustilago maydis*
Eukaryota	*Xenopus tropicalis*
Eukaryota	*Yarrowia lipolytica*
Eukaryota	*Zea mays*

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478 **Supplementary Table 2: Fungi species list**

Agaricus bisporus	*Cryptococcus neoformans*	*Rhizoctonia solani*
Amanita muscaria	*Encephalitozoon intestinalis*	*Rhizopus delemar*
Aspergillus fumigatus	*Enterocytozoon bieneusi*	*Saccharomyces cerevisiae*
Aspergillus nidulans	*Fusarium oxysporum*	*Schizosaccharomyces pombe*
Batrachochytrium salamandrivorans	*Magnaporthe oryzae*	*Sclerotinia sclerotiorum*
Blumeria graminis	*Mortierella elongata*	*Spizellomyces punctatus*
Botrytis cinerea	*Neurospora crassa*	*Ustilago maydis*
Candida albicans	*Phaeosphaeria nodorum*	*Yarrowia lipolytica*
Colletotrichum graminicola	*Puccinia graminis*	*Zymoseptoria tritici*

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480 **Outgroup**

Caenorhabditis elegans	*Homo sapiens*	*Dictyostelium discoideum*
Drosophila melanogaster	*Monosiga brevicollis*	

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Supplementary Table 3: Metazoan species list

Amphimedon queenslandica	*Danio rerio*	*Octopus bimaculoides*
Anolis carolinensis	*Daphnia magna*	*Oncorhynchus mykiss*
Anopheles gambiae	*Drosophila melanogaster*	*Ornithorhynchus anatinus*
Apis mellifera	*Gadus morhua*	*Oryzias latipes*
Astatotilapia calliptera	*Gallus gallus*	*Pan troglodytes*
Bombyx mori	*Glossina morsitans*	*Petromyzon marinus*
Bos taurus	*Helobdella robusta*	*Phascolarctos cinereus*
Branchiostoma lanceolatum	*Homo sapiens*	*Poecilia formosa*
Bubo bubo	*Ixodes scapularis*	*Rattus norvegicus*
Caenorhabditis elegans	*Latimeria chalumnae*	*Schistosoma mansoni*
Callithrix jacchus	*Lepisosteus oculatus*	*Strongylocentrotus purpuratus*
Callorhinchus milii	*Leptobrachium leishanense*	*Tetraodon nigroviridis*
Canis familiaris	*Mnemiopsis leidyi*	*Thelohanellus kitauei*
Chrysemys picta	*Monodelphis domestica*	*Trichinella spiralis*
Ciona intestinalis	*Mus musculus*	*Trichoplax adhaerens*
Corvus moneduloides	*Nematostella vectensis*	*Xenopus tropicalis*
Amphimedon queenslandica	*Danio rerio*	*Octopus bimaculoides*

Outgroup

Dictyostelium discoideum	*Phaeosphaeria nodorum*	*Schizosaccharomyces pombe*
Monosiga brevicollis	*Saccharomyces cerevisiae*	

Supplementary Table 4: Plants species list

Amborella trichopoda	*Glycine max*	*Picea glauca*
Anthoceros punctatus	*Gossypium raimondii*	*Pinus sylvestris*
Aquilegia coerulea	*Hordeum vulgare*	*Prunus persica*
Arabidopsis thaliana	*Manihot esculenta*	*Selaginella moellendorffii*
Azolla filiculoides	*Marchantia polymorpha*	*Setaria italica*
Brassica oleracea	*Micromonas spRCC299*	*Solanum lycopersicum*
Chara braunii	*Musa acuminata*	*Spirodela polyrhiza*
Chlamydomonas reinhardtii	*Oryza sativa*	*Triticum aestivum*
Eucalyptus grandis	*Ostreococcus lucimarinus*	*Volvox carteri*
Gingko biloba	*Physcomitrella patens*	*Zea mays*

Outgroup

Chondrus crispus	*Chondrus crispus*	*Chondrus crispus*

493 **Supplementary Table 5: Bacterial & Archaeal strains list**

UniProt proteome	NCBI taxon	Name in SHOOT	Selection
UP000000425	122586	Neisseria_meningitidis	QfO UniProt ref. prot.
UP000000429	85962	Helicobacter_pylori	QfO UniProt ref. prot.
UP000000431	272561	Chlamydia_trachomatis	QfO UniProt ref. prot.
UP000000536	69014	Thermococcus_kodakarensis	QfO UniProt ref. prot.
UP000000554	64091	Halobacterium_salinarum	QfO UniProt ref. prot.
UP000000557	251221	Gloeobacter_violaceus	QfO UniProt ref. prot.
UP000000577	243231	Geobacter_sulfurreducens	QfO UniProt ref. prot.
UP000000625	83333	Escherichia_coli	QfO UniProt ref. prot.
UP000000718	289376	Thermodesulfovibrio_yellowstonii	QfO UniProt ref. prot.
UP000000792	436308	Nitrosopumilus_maritimus	QfO UniProt ref. prot.
UP000000798	224324	Aquifex_aeolicus	QfO UniProt ref. prot.
UP000000805	243232	Methanocaldococcus_jannaschii	QfO UniProt ref. prot.
UP000000807	243273	Mycoplasma_genitalium	QfO UniProt ref. prot.
UP000001025	243090	Rhodopirellula_baltica	QfO UniProt ref. prot.
UP000001408	189518	Leptospira_interrogans	QfO UniProt ref. prot.
UP000001414	226186	Bacteroides_thetaiotaomicron	QfO UniProt ref. prot.
UP000001425	1111708	Synechocystis_Kazusa	QfO UniProt ref. prot.
UP000001570	224308	Bacillus_subtilis	QfO UniProt ref. prot.
UP000001584	83332	Mycobacterium_tuberculosis	QfO UniProt ref. prot.
UP000001686	374847	Korarchaeum_cryptofilum	QfO UniProt ref. prot.
UP000001973	100226	Streptomyces_coelicolor	QfO UniProt ref. prot.
UP000001974	273057	Saccharolobus_solfataricus	QfO UniProt ref. prot.
UP000002008	324602	Chloroflexus_aurantiacus	QfO UniProt ref. prot.
UP000002438	208964	Pseudomonas_aeruginosa	QfO UniProt ref. prot.
UP000002487	188937	Methanosarcina_acetivorans	QfO UniProt ref. prot.
UP000002521	190304	Fusobacterium_nucleatum	QfO UniProt ref. prot.
UP000002524	243230	Deinococcus_radiodurans	QfO UniProt ref. prot.
UP000002526	224911	Bradyrhizobium_diazoefficiens	QfO UniProt ref. prot.
UP000007719	515635	Dictyoglomus_turgidum	QfO UniProt ref. prot.
UP000008183	243274	Thermotoga_maritima	QfO UniProt ref. prot.
UP000000265	272620	Klebsiella_pneumoniae	Highly cited
UP000000579	71421	Haemophilus_influenzae	Highly cited
UP000000580	262316	Mycolicibacterium_paratuberculosis	Highly cited
UP000000584	243277	Vibrio_cholerae	Highly cited
UP000000586	171101	Streptococcus_pneumoniae	Highly cited
UP000000588	242619	Porphyromonas_gingivalis	Highly cited
UP000000609	272624	Legionella_pneumophila	Highly cited
UP000000799	192222	Campylobacter_jejuni	Highly cited
UP000000813	176299	Agrobacterium_fabrum	Highly cited
UP000000815	632	Yersinia_pestis	Highly cited
UP000000817	169963	Listeria_monocytogenes	Highly cited
UP000000818	195102	Clostridium_perfringens	Highly cited
UP000001006	623	Shigella_flexneri	Highly cited
UP000001014	99287	Salmonella_typhimurium	Highly cited
UP000001978	272563	Clostridioides_difficile	Highly cited
UP000002196	272623	Lactococcus_lactis	Highly cited
UP000002256	395491	Rhizobium_leguminosarum	Highly cited

UP000006381	272621	Lactobacillus_acidophilus	Highly cited
UP000007477	871585	Acinetobacter_calcoaceticus	Highly cited
UP000008319	529507	Proteus_mirabilis	Highly cited
UP000008816	93061	Staphylococcus_aureus	Highly cited
UP000014594	1260356	Enterococcus_faecalis	Highly cited
UP000075229	140	Borrelia_hermsii	Highly cited
UP000198289	615	Serratia_marcescens	Highly cited
UP000028936	1528098	Rickettsiales_bacterium	Mitochondrion relative
UP000180235	1188229	Gloeomargarita_lithophora	Chloroplast relative
UP000000543	279808	Staphylococcus_haemolyticus	Phylo. sampling
UP000000547	167879	Colwellia_psychrerythraea	Phylo. sampling
UP000000645	232721	Acidovorax_JS42	Phylo. sampling
UP000001169	272569	Haloarcula_marismortui	Phylo. sampling
UP000001361	883	Desulfovibrio_vulgaris	Phylo. sampling
UP000001362	243159	Acidithiobacillus_ferrooxidans	Phylo. sampling
UP000001961	64471	Synechococcus_CC9311	Phylo. sampling
UP000002011	471854	Dyadobacter_fermentans	Phylo. sampling
UP000002139	448385	Sorangium_cellulosum	Phylo. sampling
UP000002145	203119	Hungateiclostridium_thermocellum	Phylo. sampling
UP000002148	388919	Streptococcus_sanguinis	Phylo. sampling
UP000002208	546414	Deinococcus_deserti	Phylo. sampling
UP000002257	395965	Methylocella_silvestris	Phylo. sampling
UP000002386	471223	Geobacillus_WCH70	Phylo. sampling
UP000002457	521011	Methanosphaerula_palustris	Phylo. sampling
UP000002495	235279	Helicobacter_hepaticus	Phylo. sampling
UP000003277	742743	Dialister_succinatiphilus	Phylo. sampling
UP000003415	469616	Fusobacterium_mortiferum	Phylo. sampling
UP000003446	661087	Olsenella_F0356	Phylo. sampling
UP000003855	665956	Subdoligranulum_4-3-54A2FAA	Phylo. sampling
UP000003981	621372	Paenibacillus_D14	Phylo. sampling
UP000004073	1105031	Clostridium_MSTE9	Phylo. sampling
UP000004090	428127	Absiella_dolichum	Phylo. sampling
UP000004259	246199	Ruminococcus_albus	Phylo. sampling
UP000004478	1225176	Cecembia_lonarensis	Phylo. sampling
UP000004870	638300	Cardiobacterium_hominis	Phylo. sampling
UP000005262	768704	Desulfosporosinus_meridiei	Phylo. sampling
UP000006229	1131455	Mycoplasma_canis	Phylo. sampling
UP000006415	857290	Scardovia_wiggsiae	Phylo. sampling
UP000006556	370438	Pelotomaculum_thermopropionicum	Phylo. sampling
UP000006743	557723	Haemophilus_parasuis	Phylo. sampling
UP000007271	1185325	Lactobacillus_coryniformis	Phylo. sampling
UP000007753	452662	Sphingobium_japonicum	Phylo. sampling
UP000007995	997888	Bacteroides_finegoldii	Phylo. sampling
UP000008204	41431	Rippkaea_orientalis	Phylo. sampling
UP000008212	243275	Treponema_denticola	Phylo. sampling
UP000008308	263358	Micromonospora_maris	Phylo. sampling
UP000008701	290317	Chlorobium_phaeobacteroides	Phylo. sampling
UP000009044	634177	Komagataeibacter_medellinensis	Phylo. sampling
UP000009154	1112204	Gordonia_polyisoprenivorans	Phylo. sampling
UP000011615	1230457	Haloterrigena_limicola	Phylo. sampling
UP000011728	931276	Clostridium_saccharoperbutylacetonicum	Phylo. sampling

UP000013232	1123367	Thauera_linaloolentis	Phylo. sampling
UP000017993	1262970	Subdoligranulum_CAG314	Phylo. sampling
UP000018014	1262708	Bacillus_CAG988	Phylo. sampling
UP000018042	1262875	Eggerthella_CAG209	Phylo. sampling
UP000018237	1262989	Firmicutes_bacterium	Phylo. sampling
UP000018329	1262693	Alistipes_CAG268	Phylo. sampling
UP000018361	1263102	Prevotella_copri	Phylo. sampling
UP000018415	1341679	Acinetobacter_indicus	Phylo. sampling
UP000019028	1239307	Sodalis_praecaptivus	Phylo. sampling
UP000019082	1302241	Cutibacterium_acnes	Phylo. sampling
UP000019222	1224164	Corynebacterium_vitaeruminis	Phylo. sampling
UP000019267	1276246	Spiroplasma_culicicola	Phylo. sampling
UP000020878	1454005	Candidatus_Accumulibacter	Phylo. sampling
UP000028780	156978	Corynebacterium_imitans	Phylo. sampling
UP000028875	1462526	Virgibacillus_massiliensis	Phylo. sampling
UP000029622	1156417	Caloranaerobacter_azorensis	Phylo. sampling
UP000030960	561184	Mameliella_alba	Phylo. sampling
UP000031057	1348853	Novosphingobium_malaysiense	Phylo. sampling
UP000031627	1410383	Candidatus_Tachikawaea	Phylo. sampling
UP000032279	1335616	Paucilactobacillus_wasatchensis	Phylo. sampling
UP000032287	137591	Weissella_cibaria	Phylo. sampling
UP000033511	43662	Pseudoalteromonas_piscicida	Phylo. sampling
UP000036114	1628212	Chromobacterium_LK11	Phylo. sampling
UP000036921	1581033	Bacillus_FJAT-21945	Phylo. sampling
UP000037530	171383	Vibrio_hepatarius	Phylo. sampling
UP000037870	1592329	Actinobacteria_bacterium	Phylo. sampling
UP000044377	1109412	Brenneria_goodwinii	Phylo. sampling
UP000050971	1736540	Aeromicrobium_Root472D3	Phylo. sampling
UP000051467	1736232	Arthrobacter_Leaf69	Phylo. sampling
UP000051585	1736381	Aureimonas_Leaf454	Phylo. sampling
UP000051643	270918	Salegentibacter_mishustinae	Phylo. sampling
UP000051802	676599	Stenotrophomonas_panacihumi	Phylo. sampling
UP000053086	1700846	Lysinibacillus_F5	Phylo. sampling
UP000054024	146536	Streptomyces_curacoi	Phylo. sampling
UP000054457	1685377	Microbulbifer_ZGT114	Phylo. sampling
UP000057134	1766	Mycolicibacterium_fortuitum	Phylo. sampling
UP000058305	412690	Microterricola_viridarii	Phylo. sampling
UP000061489	1420916	Marinobacter_similis	Phylo. sampling
UP000065824	1702325	Chelatococcus_CO-6	Phylo. sampling
UP000070463	1698267	Candidate_MSBL1-archaeon	Phylo. sampling
UP000077018	683316	Frankia_EI5c	Phylo. sampling
UP000077275	47311	Methanobrevibacter_cuticularis	Phylo. sampling
UP000077319	1822215	Erythrobacter_HI00D59	Phylo. sampling
UP000093220	189873	Bradyrhizobium_LMTRsp-3	Phylo. sampling
UP000093585	319501	Brevibacillus_WF146	Phylo. sampling
UP000094329	1891921	Piscirickettsia_litoralis	Phylo. sampling
UP000094487	1888892	Sphingomonas_turrisvirgatae	Phylo. sampling
UP000094689	1842539	Bosea_RAC05	Phylo. sampling
UP000095256	762845	Enterococcus_rivorum	Phylo. sampling
UP000176615	1739315	Globicatella_HMSC072A10	Phylo. sampling
UP000182624	43305	Butyrivibrio_proteoclasticus	Phylo. sampling

UP000184455	1855338	Nitrosospira_Nsp11	Phylo. sampling
UP000184520	634436	Marisediminitalea_aggregata	Phylo. sampling
UP000186096	58117	Microbispora_rosea	Phylo. sampling
UP000186602	1261634	Roseburia_sp499	Phylo. sampling
UP000187327	1883416	Halomonas_sp1513	Phylo. sampling
UP000187995	1805827	Rhodococcus_MTM3W5	Phylo. sampling
UP000190286	745368	Gemmiger_formicilis	Phylo. sampling
UP000191905	1873176	Pseudaminobacter_manganicus	Phylo. sampling
UP000192042	1325564	Nitrospira_japonica	Phylo. sampling
UP000193006	199441	Alkalihalobacillus_krulwichiae	Phylo. sampling
UP000193136	1969733	Geothermobacter_EPR-M	Phylo. sampling
UP000194216	1985172	Sphingomonas_IBVSS2	Phylo. sampling
UP000194221	1635173	Tenacibaculum_holothuriorum	Phylo. sampling
UP000195076	1932621	Nostoc_T09	Phylo. sampling
UP000195161	1929267	Flavobacterium_FPG59	Phylo. sampling
UP000195529	1965622	Megasphaera_An286	Phylo. sampling
UP000195781	1232426	Collinsella_massiliensis	Phylo. sampling
UP000197446	431059	Pelomonas_puraquae	Phylo. sampling
UP000198589	1798228	Blastococcus_DSMsp-46838	Phylo. sampling
UP000198953	46177	Nonomuraea_pusilla	Phylo. sampling
UP000199067	1780377	Coriobacteriaceae_bacterium	Phylo. sampling
UP000199242	1141221	Chryseobacterium_taihuense	Phylo. sampling
UP000199432	1882749	Opitutus_GAS368	Phylo. sampling
UP000199671	332524	Actinomyces_ruminicola	Phylo. sampling
UP000199705	551996	Mucilaginibacter_gossypii	Phylo. sampling
UP000199768	1881066	Phyllobacterium_YR620	Phylo. sampling
UP000199802	1965654	Lachnoclostridium_An76	Phylo. sampling
UP000202922	1524263	Confluentimicrobium_lipolyticum	Phylo. sampling
UP000215509	554312	Paenibacillus_rigui	Phylo. sampling
UP000216308	1383851	Halorubrum_halodurans	Phylo. sampling
UP000217076	83401	Roseospirillum_parvum	Phylo. sampling
UP000217289	1294270	Melittangium_boletus	Phylo. sampling
UP000221394	442709	Flavimobilis_soli	Phylo. sampling
UP000222106	638953	Georgenia_soli	Phylo. sampling
UP000230810	2049589	Pseudomonas_HLS-6	Phylo. sampling
UP000232878	2058137	Polaribacter_ALD11	Phylo. sampling
UP000232889	1250229	Ulvibacter_MAR-2010-11	Phylo. sampling
UP000235352	2029108	Bacillus_UMB0899	Phylo. sampling
UP000236356	2067550	Clostridium_chh4-2	Phylo. sampling
UP000236731	797291	Sphingobacterium_lactis	Phylo. sampling
UP000238164	75385	Micropruina_glycogenica	Phylo. sampling
UP000238375	1469603	Spirosoma_oryzae	Phylo. sampling
UP000243063	1245526	Pseudomonas_guangdongensis	Phylo. sampling
UP000243494	2020948	Romboutsia_maritimum	Phylo. sampling
UP000244224	589035	Gemmobacter_caeni	Phylo. sampling
UP000245108	2108523	Lawsonibacter_asaccharolyticus	Phylo. sampling
UP000245507	2201891	Nocardioides_silvaticus	Phylo. sampling
UP000245623	2173179	Microbacterium_4-13	Phylo. sampling
UP000245926	2202825	Methylobacterium_durans	Phylo. sampling
UP000247832	670078	Arthrobacter_livingstonensis	Phylo. sampling
UP000249065	2230885	Roseicella_frigidaeris	Phylo. sampling

UP000250434	1804986	Amycolatopsis_albispora	Phylo. sampling
UP000252733	989	Marinilabilia_salmonicolor	Phylo. sampling
UP000253318	1931232	Marinitenerispora_sediminis	Phylo. sampling
UP000254875	2211104	Paraburkholderia_lacunae	Phylo. sampling
UP000260665	2184758	Rhodoferax_IMCC26218	Phylo. sampling
UP000265971	1825976	Neorhizobium_NCHU2750	Phylo. sampling
UP000266860	1630648	Novosphingobium_MD-1	Phylo. sampling
UP000269803	2485200	Frondihabitans_PhB188	Phylo. sampling
UP000273083	1329262	Mobilisporobacter_senegalensis	Phylo. sampling
UP000275325	2495580	Sphingomonas_TF3	Phylo. sampling
UP000276437	1930071	Methylomusa_anaerophila	Phylo. sampling
UP000279089	1647451	Chitinophaga_barathri	Phylo. sampling
UP000282084	2072	Saccharothrix_australiensis	Phylo. sampling
UP000287188	2014872	Dictyobacter_kobayashii	Phylo. sampling
UP000287890	2507159	Clostridium_JN-9	Phylo. sampling
UP000288096	45657	Desulfonema_ishimotonii	Phylo. sampling
UP000288291	2495899	Lactobacillus_xujianguonis	Phylo. sampling
UP000288967	2501295	Dyella_M7H15-1	Phylo. sampling
UP000289784	2137479	Pseudoxanthomonas_composti	Phylo. sampling
UP000292120	2528630	Aquabacterium_KMB7	Phylo. sampling
UP000294096	2510646	Loktanella_IMCC34160	Phylo. sampling
UP000294498	1539049	Dinghuibacter_silviterrae	Phylo. sampling
UP000295707	1537524	Thiogranum_longum	Phylo. sampling
UP000297351	2561925	Brevundimonas_S30B	Phylo. sampling
UP000306069	2040651	Campylobacter_12-5580	Phylo. sampling
UP000307244	2571272	Pedobacter_RP-3-15	Phylo. sampling
UP000307467	343240	Thiotrophic_endosymbiont	Phylo. sampling
UP000307507	2565924	Flavobacterium_CC-CTC003	Phylo. sampling
UP000307657	2565367	Lacinutrix_CAUsp-1491	Phylo. sampling
UP000315440	2527991	Pseudobythopirellula_maris	Phylo. sampling
UP000316225	384678	Paracoccus_sulfuroxidans	Phylo. sampling
UP000316304	2528004	Novipirellula_galeiformis	Phylo. sampling
UP000318165	92402	Mycoplasma_equirhinis	Phylo. sampling
UP000318431	1036180	Massilia_lurida	Phylo. sampling
UP000318566	2768454	Streptomyces_SLBN-118	Phylo. sampling
UP000319173	713054	TM7_phylum	Phylo. sampling
UP000322791	2606448	Hymenobacter_KIGAM108	Phylo. sampling
UP000324880	1948890	Rhodobacterales_bacterium	Phylo. sampling
UP000325372	2613842	Wenzhouxiangella_W260	Phylo. sampling
UP000326711	2487892	Corynebacterium_LMM-1652	Phylo. sampling
UP000326944	2590022	Sulfurimonas_GYSZ1	Phylo. sampling
UP000437955	2653936	Tetrasphaera_F2B08	Phylo. sampling
UP000441772	2650774	Bifidobacterium_LMGsp-31471	Phylo. sampling
UP000462055	2650748	Actinomadura_LD22	Phylo. sampling
UP000474632	2710884	Parapusillimonas_SGNA-6	Phylo. sampling
UP000476210	343235	Methanotrophic_endosymbiont	Phylo. sampling
UP000477884	2703788	Edaphobacter_12200R-103	Phylo. sampling
UP000481552	2706104	Streptomyces_SID8455	Phylo. sampling
UP000500686	754515	Mycoplasma_ES2806-GEN	Phylo. sampling
UP000502894	2708020	Legionella_TUM19329	Phylo. sampling
UP000503441	2714933	Leucobacter_HDW9A	Phylo. sampling

UP000505377	2736640	Pseudonocardia_broussonetiae	Phylo. sampling

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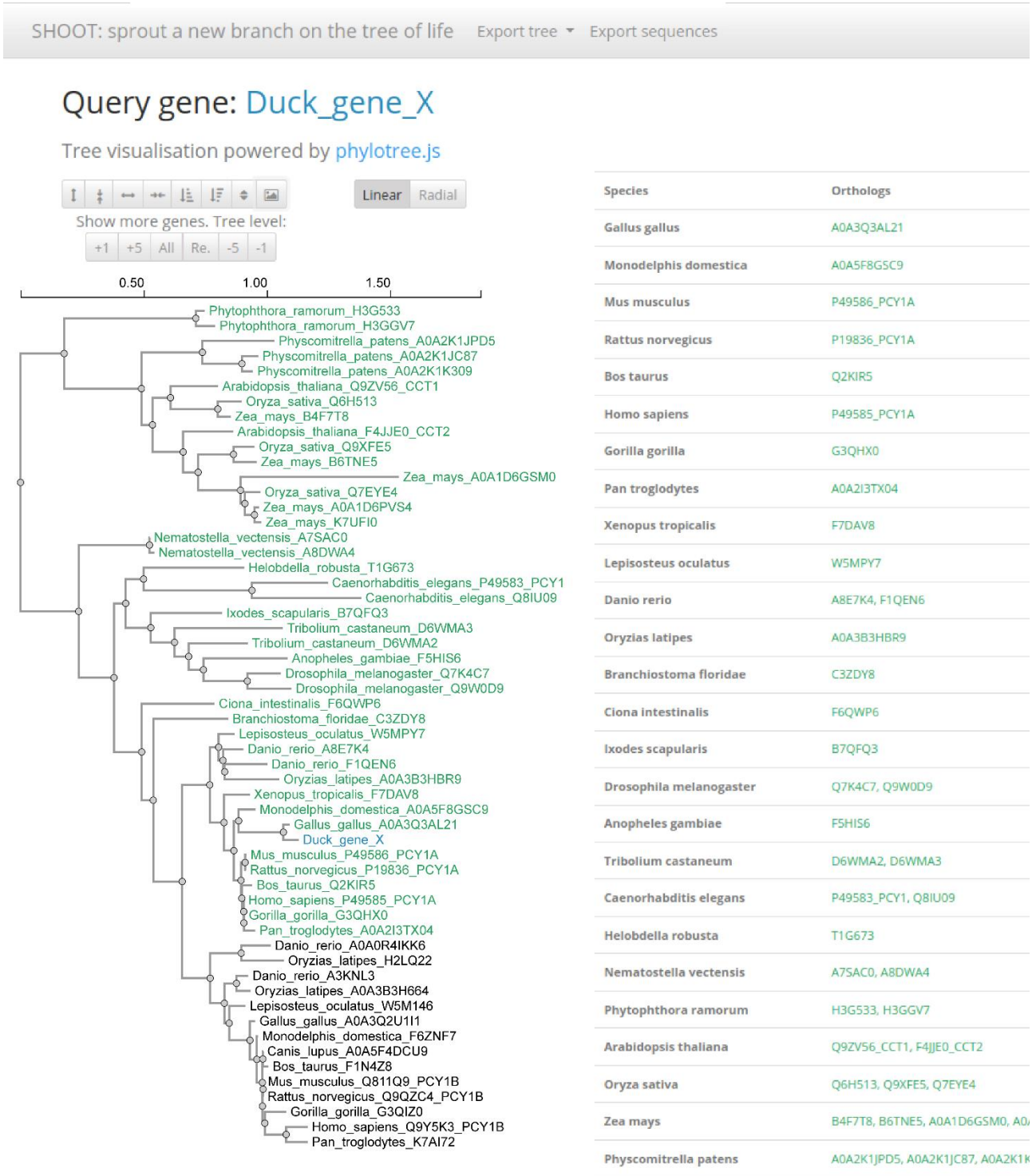
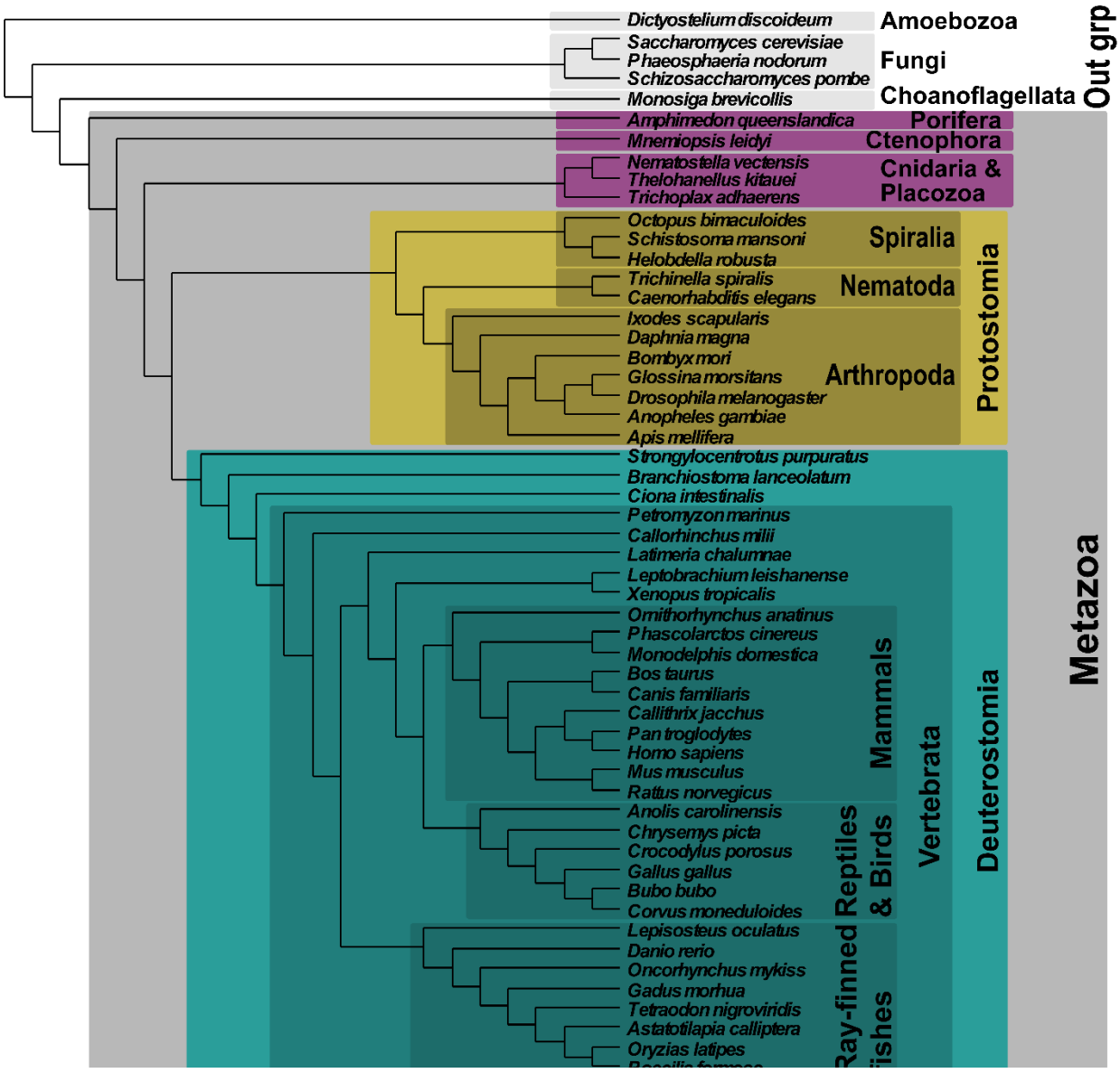
Supplementary Figures

Supplementary Figure 1



500 **Supplementary Figure 2**



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Supplementary Figure Legends

Supplementary Figure 1. An example gene tree and orthologs table returned by SHOOT. Here, the UniProt Reference Proteomes database was searched using a for a query gene sequence labelled “Duck_gene_X”. This corresponds to the Duck protein ENSAPLP00000002788, which is not included in the database.

Supplementary Figure 2. Phylogeny for the species in the Metazoan dataset.