EukProt: a database of genome-scale predicted proteins across the diversity of eukaryotic life

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Database Availability

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Abstract

EukProt is a database of published and publicly available predicted protein sets and unannotated genomes selected to represent eukaryotic diversity, including 742 species from all major supergroups as well as orphan taxa. The goal of the database is to provide a single, convenient resource for studies in phylogenomics, gene family evolution, and other gene-based research across the spectrum of eukaryotic life. Each species is placed within the UniEuk taxonomic framework in order to facilitate downstream analyses, and each data set is associated with a unique, persistent identifier to facilitate comparison and replication among analyses. The database is currently in version 2, and all versions will be permanently stored and made available via FigShare. We invite the community to provide suggestions for new data sets and new annotation features to be included in subsequent versions, with the goal of building a collaborative resource that will promote research to understand eukaryotic diversity and diversification.

Introduction

Over the past 15 years, the discovery of diverse novel microbial eukaryotes, coupled with methods to reconstruct phylogenies based on hundreds of protein-coding genes (known as phylogenomics (Eisen, 2003)) have led to a remarkable reshaping in our understanding of the eukaryotic tree of life, the creation of new supergroups and the placement of enigmatic lineages in known supergroups (Brown et al., 2018; Burki et al., 2012, 2020; Gawryluk et al., 2019; Janouškovec et al., 2017; Kamikawa et al., 2014; Lax et al., 2018; Strassert et al., 2019; Yabuki et al., 2015). The phylogenomic approach has also been used to investigate branching patterns within eukaryotic supergroups, including those with implications for the early evolutionary events in the three major eukaryotic lineages: land plants (Wickett et al., 2014), animals (King & Rokas, 2017) and fungi (Kiss et al., 2019). Furthermore, analyses in diverse eukaryotes have the potential to reveal new genes, pathways and mechanisms of function for biological processes currently characterized only in these three well-studied lineages and their parasites (del Campo et al., 2014; Richter & Levin, 2019). In the ocean alone, planetary-scale metagenomics studies across the full spectrum of life from viruses to animals (Bork et al., 2015; Tara Oceans Coordinators et al., 2020) have already unveiled an extreme diversity

of eukaryotic genes (Carradec et al., 2018) whose phylogenetic origin and ecological function are mostly unknown.

A critical prerequisite to all of these studies is the underlying database of predicted proteins from which orthologs are extracted or other sequence analyses are performed. Because no single website stores the protein predictions for the complete set of eukaryotic taxa that have been sequenced at a genomic scale, each study has needed to assemble their own set, reducing reproducibility among analyses (due to the inclusion of different taxa, or different data sets for the same taxon), and producing a significant barrier to new researchers entering the field. In addition, because the large majority of the protein data sets from diverse eukaryotes are not included in major databases (see Table 1), researchers cannot easily access them via standard tools such as NCBI BLAST (Sayers et al., 2020) in order to find diverse eukaryotic homologs of a protein of interest, nor are valuable annotations such as protein domains (e.g., Pfam (El-Gebali et al., 2019), Interpro (Mitchell et al., 2019)) or gene ontology (The Gene Ontology Consortium, 2019) easily accessible for searching.

To address this gap, we gathered the predicted proteins from a comprehensive set of species representing known eukaryotic diversity. We placed each species within a unified taxonomic framework, UniEuk (Berney et al., 2017), in order to ensure that the evolutionary relationships among data sets are accurately and consistently described. Our database is designed to prioritize ease of use, with unique, persistent identifiers assigned to each data set and a standard system of nomenclature to facilitate repeatability of analyses.

The EukProt Database

The current version of EukProt (version 2) contains 742 eukaryotic species from 5 different types of sequence data (Figure 1): genome (240 species), single-cell genome (25), transcriptome (453), single-cell transcriptome (7) and expressed sequence tag (EST; 17). The data sets were downloaded from 30 different sources (Table 1), with the two principal sources being NCBI (Sayers et al., 2020) and the Moore Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) (Keeling et al., 2014). The representation among eukaryotic lineages is highly uneven, which is due to the difficulty of discovering, culturing or sequencing species from many lineages, as well as a bias towards sequencing either macroscopic, multicellular species or unicellular species that are parasites (e.g., Apicomplexa), photosynthetic (such as diatoms, which are part of the Ochrophyte lineage), or are otherwise economically important (del Campo et al., 2014).

The EukProt database is organized around 5 guiding principles:

Breadth of species phylogenetic diversity: the objective of the database is to represent known eukaryotic diversity as fully as possible. In practice, this means that all species with available data sets are included for the majority of lineages. The exceptions are the land plants (Streptophyta), animals (Metazoa) and fungi, and their parasites/pathogens (members of Apicomplexa, Peronosporomycetes, Metakinetoplastina and Fornicata), for which we included a subset of species selected to represent phylogenetic diversity within each group. Within animals, we also emphasized the inclusion of marine planktonic species, as we anticipate these could serve as mapping targets for data from large-scale metagenomic and metatranscriptomic ocean sequencing projects (e.g., *Tara* Oceans (Carradec et al., 2018; Karsenti et al., 2011; Richter et al., 2019)).

Convenience of access: the full data set and its associated metadata can be downloaded from FigShare with a single click. For 501 of the 724 species in the database, publicly available protein sequences were ready to be included directly. For the remaining species, we processed the publicly available data to produce protein sequences for inclusion in the database. The most common actions

were merging independent sets of protein predictions for the same species (105 species), translating mRNA sequences from transcriptomes or EST projects (67), and *de novo* assembling and translating transcriptomes from raw read data (47; we also provide these assemblies as part of the database, as they are not publicly available). A full list of the different types of actions is described in the Methods, and the actions taken for each species are available in the database metadata. We note that 16 species in the database are represented by genomic data lacking accompanying protein annotations; 15 of these are single-cell genomes. We considered annotation of these genomes to be outside the scope of this release, so we include their nucleotide data in a separate file, which can be searched for proteins of interest using translated homology search software (e.g., tblastn (Altschul, 1997) or BLAT (Kent, 2002)).

A unified taxonomic framework: we placed all species into a unified taxonomic framework, UniEuk (Berney et al., 2017), which is based on the most recent consensus eukaryotic taxonomy (Adl et al., 2019). This will maximize interoperability of the database with other resources built upon to the UniEuk taxonomic framework (e.g., EukBank and EukMap (Berney et al., 2017), EcoTaxa (https://ecotaxa.obs-vlfr.fr), and EukRibo (Berney et al., in prep)), and ensure that results arising from the database will be able to use a common set of identifiers to describe analyses at different taxonomic levels. For example, this might include labeling groups in a phylogenetic tree, or summarizing read placement data by taxonomic group. Knowledge of the taxonomy can also facilitate the detection of mis-identified gene sequences within a data set, via comparison of the topology of gene trees to the topology of the taxonomy. In addition to the full taxonomic lineage, we provide for each species the “supergroup”, a very high-level taxonomic grouping, and “taxogroup”, a more fine-grained grouping into evolutionarily or ecologically relevant lineages (see Methods). Finally, for each species we include a list of names it was previously known by, to prevent issues resulting from revisions to species names or lineages, which have occurred frequently with improvements in sequencing and phylogenetic techniques and the discovery of new eukaryotic organisms.

Appropriate references to publications and data sources: to ensure that the researchers who generated and provided each data set receive appropriate credit, we provide the DOI of the publication describing each data set as well as the URL from which it was downloaded. The list of URLs should also allow users of the database to download the original sequences for each data set (although it is not possible to guarantee that the URLs for all data providers will be permanently available).

Reusability, persistence and replicability: the database will be released in successive versions, each of which will be permanently stored and accessible at FigShare. In that way, analyses using the database will need only to specify which version was used, enabling follow-up analyses or replications to begin with the identical database. In addition, each individual data set within the database is assigned a unique, permanent identifier. When a new data set becomes available for a given species, it is assigned a new unique identifier (and the identifier of the data set it replaces, if any, is indicated in the database metadata). These and all other changes between versions of the database will be logged as appropriate.

Growing the EukProt Database with Community Involvement

The core functionality of the database is the distribution of genome-scale protein sequences across the diversity of eukaryotic life and within the UniEuk framework. However, we anticipate that numerous other features might be useful to the community, and we hope to involve the community in suggesting and adding new features in successive versions. These may include information or analyses on full data sets, such as the sequencing technology that was used (e.g., Illumina, PacBio), the 18S ribosomal DNA sequence corresponding to the data set, the completeness of the data set as

estimated by software such as BUSCO (Simão et al., 2015), or the estimation of potential contamination levels with non-target species, as inferred using systematic sequence homology searches. Additionally, we could consider adding features on the level of individual protein sequences, such as protein domains from Pfam (El-Gebali et al., 2019)/Interpro (Mitchell et al., 2019), gene ontology (The Gene Ontology Consortium, 2019)/eggNOG (Huerta-Cepas et al., 2019), or the assignment of unique identifiers to individual protein sequences (currently, FASTA files are distributed as is, without modification to headers/identifiers).

As new genome-scale eukaryotic protein data sets become available, we plan to add them to the database. As yet, we do not have a formal mechanism to accomplish this, and will instead depend on monitoring the literature and assistance from the community. We also plan to add any data sets we may have inadvertently overlooked when building the current version of the database.

In the longer term, we hope the standardization of our database provides a path towards including all data sets in a major sequence repository such as NCBI/EBI/DDBJ, so that they can be more broadly accessible and integrated into the suites of tools available at these repositories.

Methods

Species and strain identity

We determined species and strain identities by reading the publications that described the data sets, consulting the literature for naming revisions, and comparing 18S ribosomal DNA sequences to reference sequence databases. For species that were previously known by other names, we recorded them in the metadata for the data set, except in cases where a species was originally assigned to a genus but not identified to the species level (e.g., *Goniomonas* sp., now identified as *Goniomonas avonlea*, is not listed as a previous name).

Supergroups and taxogroups from UniEuk

Unlike the taxonomic system used in some other resources, the full taxonomic lineages we provide for all species (which follow the framework developed in the UniEuk project) are not based on a fixed number of ranks, but on a free, unlimited number of taxonomic levels, in order to match phylogenetic evidence as closely as possible. This provides end-users more information and flexibility, but could also make it more difficult to summarize results of downstream analyses. Therefore, we provide two additional fields (“supergroup” and “taxogroup”) to help end-users whenever it is useful to distribute eukaryotic diversity into a fixed number of taxonomic categories of equivalent phylogenetic depth or ecological relevance. The 42 “supergroups” (of which 38 are included in EukProt) consist of strictly monophyletic, deep-branching eukaryotic lineages of a phylogenetic depth equivalent to the “classic” Alveolata, Rhizaria, and Stramenopiles. They are therefore highly variable in relative diversity, ranging from clades consisting of a single, orphan genus (e.g. *Ancoracysta*, *Mantamonas*, *Palpitomonas*), to Metazoa as a whole. The “taxogroup” level allows further subdivision of large supergroups into lineages of relatively equivalent evolutionary or ecological relevance, based on current knowledge. This level is more arbitrarily defined; it can include paraphyletic groupings in highly diversified supergroups to accommodate minor lineages of similar ecology or phylogenetic depth. As illustrative examples, diatoms are in the Diatomeae taxogroup within the Stramenopiles supergroup, and coccolithophores are in the Prymnesiophyceae taxogroup within the Haptophyta supergroup. Small, ecologically and morphologically homogeneous supergroups are not subdivided further; in such cases the “taxogroup” level is the same as the “supergroup” level. The same approach will be used in

209 EukRibo, a database of reference ribosomal RNA gene sequences developed in parallel (Berney et
210 al., in prep) to help users link analyses of different types of genetic data.
211
212 *Merging strains from the same species*
213
214 In general, we only included data from a single strain/isolate per species. However, when only a
215 single transcriptome data set was available for a given strain of a species, and there were additional
216 published transcriptome data sets for other strains of the same species, we combined them using CD-
217 HIT (Li & Godzik, 2006) run with default parameter values, in order to guard against the possibility
218 that a single transcriptome might lack genes expressed only in one condition or experiment. When
219 multiple strains were merged to produce a species' data set (there were 25 such cases), this
220 information is indicated in the metadata for the data set.
221
222 *Processing steps applied to publicly available data*
223
224 EukProt metadata indicate the additional steps applied to each data set after downloading from the
225 data source (if any). All software parameter values were default, unless otherwise specified below or
226 in the metadata record for a given data set.
227
228 'assemble mRNA': *de novo* transcriptome assembly using Trinity v. 2.8.4, http://trinityrnaseq.github.io/
229 (Haas et al., 2013). We trimmed Illumina input reads for adapters and sequence quality using the
230 built-in '--trimmomatic' option. We trimmed 454 input reads prior to running Trinity with Trimmomatic v.
231 0.3.9, http://www.usadellab.org/cms/?page=trimmomatic (Bolger et al., 2014) with the directives
232 'ILLUMINACLIP:[454 adapters FASTA file]:2:30:10 SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5
233 MINLEN:25'.
234
235 'translate mRNA': *de novo* translation of mRNA sequences with Transdecoder v. 5.3.0,
236 http://transdecoder.github.io/. When the number of predicted protein sequences for a given species
237 was less than half of the input mRNA sequences, we reduced the minimum predicted protein length to
238 50 (from the default of 100).
239
240 'CD-HIT': clustering of protein sequences to produce a non-redundant data set using CD-HIT v. 4.6,
241 http://weizhongli-lab.org/cd-hit/ (Li & Godzik, 2006). We used this tool principally to combine protein
242 predictions for different strains of the same species, but also to reduce the size of very large predicted
243 protein sets (> 50,000 proteins) that showed evidence of redundancy.
244
245 'extractfeat', 'transeq', and 'trimseq': from the EMBOSS package v. 6.6.0.0,
246 http://emboss.sourceforge.net/ (Rice et al., 2000). We used extractfeat to produce coding sequences
247 (CDS) from genomes with gene annotations but without publicly available protein sequences. We
248 used transeq to translate CDS directly into proteins. We used trimseq to trim EST sequences before
249 translation with Transdecoder.
250
251 *The EukProt database distribution on FigShare*
252
253 The database is distributed in a single archive containing five files. One file contains 726 protein data
254 sets, for species with either a genome (239) or single-cell genome (10) with predicted proteins, a
255 transcriptome (453), a single-cell transcriptome (7), or an EST assembly (17). A second file contains
256 16 genomes lacking predicted protein annotations, for 15 species with single-cell genomes, and 1
257 species with a genome sequence. These can be queried for proteins of interest with translated
258 sequence homology search software. A third file contains assembled transcriptome contigs, for 53
259 species with publicly available mRNA sequence reads but no publicly available assembly. The
260 proteins predicted from these assemblies are included in the proteins file. Finally, the database

metadata are distributed as two files: one file for the data sets included in the current version of the database (742), and a second file for data sets not included (50), accompanied by the reason they were not included (for example, if the sequences are published but not publicly available or if they were replaced by a higher-quality data set for the same species).

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426 **Table 1.** Web hosts from which data sets were downloaded. The count for figshare.com includes 314
427 species from the MMETSP (Keeling et al., 2014) for which a procedure to remove cross-
428 contamination was applied (Marron et al., 2016).
429

Hostname	Number of Data Sets
figshare.com	351
ncbi.nlm.nih.gov	281
datadryad.org	32
ebi.ac.uk	16
rutgers.edu	9
genoscope.cns.fr	8
yadi.sk	6
oist.jp	5
jgi.doe.gov	3
genomics.cn	3
compagen.org	2
nrifs.fra.affrc.go.jp	2
obs-vlfr.fr	1
zenodo.org	1
licebase.org	1
ufl.edu	1
drive.google.com	1
liv.ac.uk	1
bioenergychina.org	1
algaegenome.org	1
giardiadb.org	1
nal.usda.gov	1
ovgu.de	1
umontreal.ca	1
ugent.be	1
bitbucket.org	1
davidadlergold.com	1
sciencemag.org	1
malab.cn	1
treegenesdb.org	1

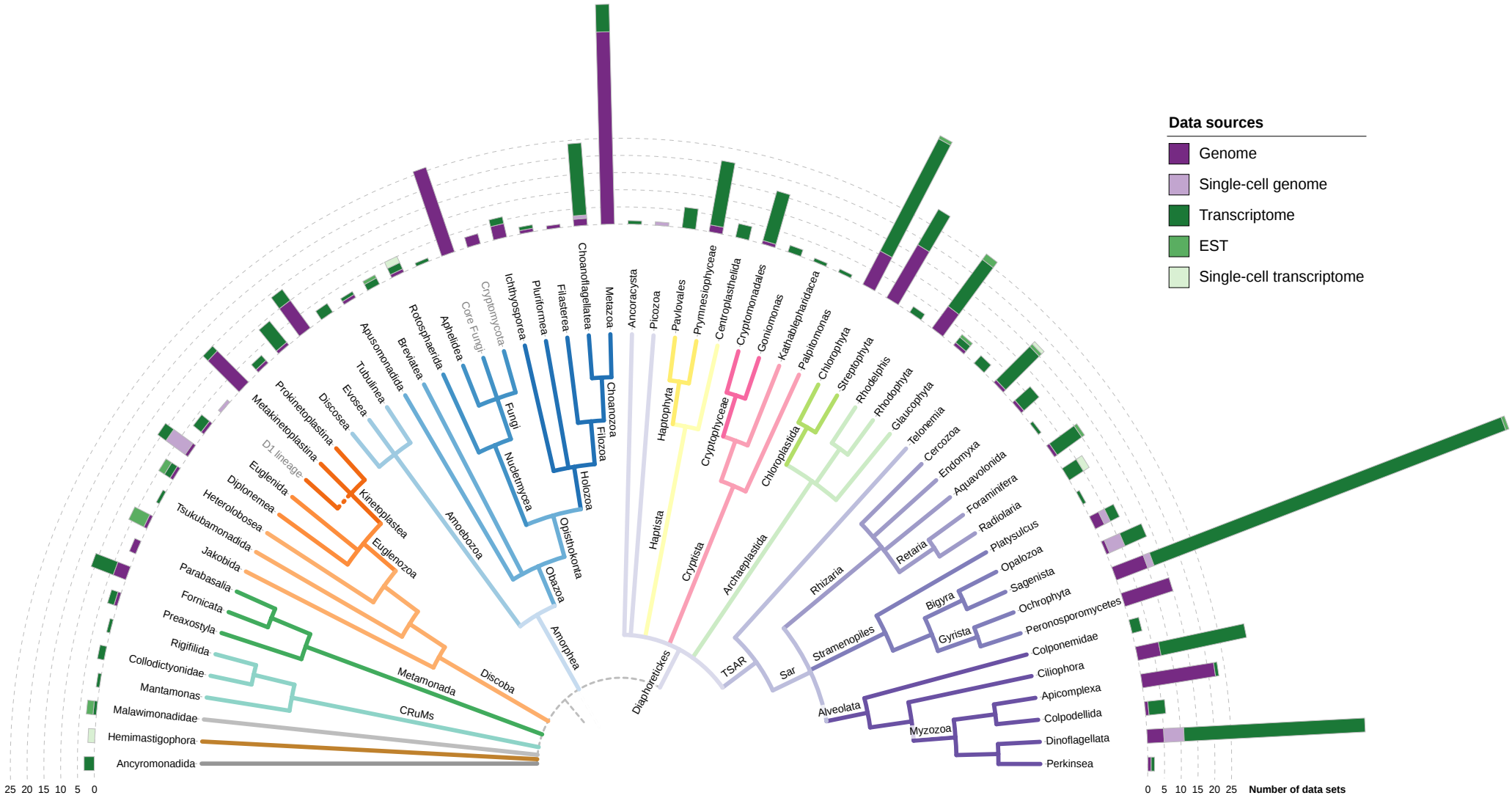

Figure 1. Distribution of 742 source data sets on the eukaryotic tree of life, separated by data set type, with taxonomy based on UniEuk (Adl et al., 2019; Berney et al., 2017). The position of the eukaryotic root, indicated with a dashed line, is currently unresolved. Group names shown in grey are not officially recognized: “D1 lineage” refers to a single-cell genome most closely related to Kinetoplastea (Wideman et al., 2019), whose affiliation with the group is indicated with a dashed line; “Cryptomycota” represents rozellids and microsporidia; “Core Fungi” encompasses all other fungal lineages from chytrids to Dikarya. The figure was created with iTOL (Letunic & Bork, 2019).