Published online 29 October 2018 *Nucleic Acids Research, 2019, Vol. 47, Database issue* **D271–D279**
doi: 10.1093/nar/gky1009

Ancestral Genomes: a resource for reconstructed ancestral genes and genomes across the tree of life

Xiaosong Huang[1,2], Laurent-Philippe Albou[2], Tremayne Mushayahama[2], Anushya Muruganujan[2], Haiming Tang[2] and Paul D. Thomas[2,*]

[1]School of Life Sciences, Guangzhou University, Guangzhou 510006, China and [2]Division of Bioinformatics, Department of Preventive Medicine, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA 90033, USA

Received August 16, 2018; Revised September 27, 2018; Editorial Decision October 09, 2018; Accepted October 10, 2018

ABSTRACT

A growing number of whole genome sequencing projects, in combination with development of phylogenetic methods for reconstructing gene evolution, have provided us with a window into genomes that existed millions, and even billions, of years ago. Ancestral Genomes (http://ancestralgenomes.org) is a resource for comprehensive reconstructions of these 'fossil genomes'. Comprehensive sets of protein-coding genes have been reconstructed for 78 genomes of now-extinct species that were the common ancestors of extant species from across the tree of life. The reconstructed genes are based on the extensive library of over 15 000 gene family trees from the PANTHER database, and are updated on a yearly basis. For each ancestral gene, we assign a stable identifier, and provide additional information designed to facilitate analysis: an inferred name, a reconstructed protein sequence, a set of inferred Gene Ontology (GO) annotations, and a 'proxy gene' for each ancestral gene, defined as the least-diverged descendant of the ancestral gene in a given extant genome. On the Ancestral Genomes website, users can browse the Ancestral Genomes by selecting nodes in a species tree, and can compare an extant genome with any of its reconstructed ancestors to understand how the genome evolved.

INTRODUCTION

The availability of completely sequenced genomes from organisms across the tree of life provides us with unprecedented opportunities to reconstruct the deep evolutionary past. Protein sequences can remain highly conserved over extended periods of time, indicating the effects of negative selection in removing deleterious mutations from populations. Clusters of orthologous groups—genes from different organisms that are inferred to derive from a single gene in the common ancestor of those organisms—have been used to infer the gene content of ancient ancestral genomes, such as that of the last universal common ancestor (1) (~4 billion years ago) and the last eukaryotic common ancestor (2) (~1.8 billion years ago). More recently, the development of tree reconciliation methods opens the door to ancestral genome reconstruction at any branch point in the tree of life (3,4).

Reconciled trees combine the information in the gene tree, usually obtained from protein sequences of related genes in different organisms, with prior knowledge of the species tree that relates those organisms. Because of this property, each node in a reconciled gene tree can be labeled with the evolutionary 'event' type that separated related genes: speciation, gene duplication, and horizontal gene transfer. Each speciation event can be labeled by the taxonomy designation of that group, such as Eukaryota (eukaryotes), or Metazoa (animals). A speciation event also represents a gene that is inferred to have been present in an ancestral genome, specifically a gene in the last common ancestor of two or more extant species. Thus, given a comprehensive set of gene trees (covering essentially all the genes in a set of extant species), we can infer the set of protein-coding genes that were present in the genome of each common ancestor among these extant species.

The Ancestral Genomes resource provides access to reconstructed genomes for the set of 78 last common ancestors, among a set of 112 different extant species, from bacteria to archaea to eukaryotes. The reconstructions are based on over 15,000 gene trees, covering over 1 million protein-coding genes. Resources currently exist for orthologs (see (5) for examples) and for phylogenetic trees (e.g. PANTHER (6), PhylomeDB (7), and TreeFam (8). However, unlike any other resource of which we are aware, Ancestral Genomes includes not only genes from extant genomes, but represents ancestral genes and genomes explicitly: each ancestral gene is given a stable identifier and reconstructed protein sequence, and is linked to a specific ancestral organ-

[*]To whom correspondence should be addressed. Tel: +1 323 442 7975; Fax: +1 323 442 7995; Email: pdthomas@usc.edu

ism (cross-referenced where possible to multiple resources including NCBI Taxonomy). Here, we describe the overall reconstruction process, and the tools available at http://ancestralgenomes.org for exploring those reconstructions.

ANCESTRAL GENOME RECONSTRUCTION PROCESS

Ancestral Genomes leverages the extensive development of the PANTHER resource for the past 20 years (6,9). As previously described (10), complete sets of protein coding genes are obtained from the UniProt resource (11), for 112 completely sequenced genomes. These genomes span the tree of life, and include 35 bacteria, 8 archaea, and 69 eukaryotes (covering 9 plants, 37 animals, 16 fungi and 9 protists). Genes are clustered into families based on sequence similarity and human curation, with extensive ongoing feedback from the broader community. This iterative process results in improvements with each successive release. The current version of PANTHER (version 13.1) comprises 15 524 families. A multiple sequence alignment is created for each family. After this stage, however, the information in Ancestral Genomes diverges from that in PANTHER.

The gene trees in AncestralGenomes are reconstructed using a modified version of the GIGA algorithm (12) that is used in PANTHER. The modification adds explicit inferences of gene deletion events in evolutionary history. These inferences are made using the standard evolutionary reconstruction criterion of maximum parsimony: whenever one or more genes are missing from a family tree (i.e. they are expected on the basis of the species tree) the algorithm assumes the smallest number of deletion events needed to explain the missing genes. For example, if a gene tree contains one rat gene, one mouse gene and one gorilla gene, but no gene in either chimpanzee or human, the most parsimonious scenario is that the gene was lost prior to the human-chimp common ancestor, rather than having been present in the human-chimp common ancestor, and subsequently lost independently twice (once in the chimpanzee lineage and once in the human lineage). As a result of this additional processing, the gene trees in Ancestral Genomes also contain an explicit tree node for every inferred ancestral gene, even if it is not a last common ancestor of extant genes due to gene deletions.

Ancestral Genomes maintains stable identifiers for all ancestral genes inferred to be present in each of the last common ancestors of the 112 extant genomes. The PANTHER identifiers for nodes in the tree (identifiers starting with PTN) are used whenever possible, to enable users to access information already available in PANTHER. However, Ancestral Genomes also includes additional stable identifiers for the explicit ancestral genes that are absent from PANTHER due to gene deletion.

Ancestral Genomes provides a reconstruction of the protein sequence encoded by each ancestral gene. Currently, sequences are reconstructed using a local maximum parsimony method. This method works from the leaves toward the root of the tree. We have implemented a simple voting procedure:

- If a plurality of an ancestral node's descendants have the same amino acid at a given site, the ancestor is inferred to conserve that same amino acid.
- If there is a tie vote among descendants, then the nearest outgroup sequence(s) are added to the votes.
- If there is still no plurality after considering the outgroups, then an unknown amino acid symbol ('X') is inferred at that site.

One advantage of this simple approach is that insertions and deletions can be treated the same way, and unlike other sequence reconstruction approaches such as maximum likelihood under an evolutionary model (13), we can also infer the occurrence of insertions and deletions of amino acids during evolution. However, we recognize that for the actual amino acid inferences, a maximum likelihood approach would be much better, and we are currently working on integrating the maximum likelihood marginal state reconstructions from Zhang (13,14).

Interpretation of an ancestral genome

For most of the Ancestral Genomes we have reconstructed, specifically the eukaryotic Ancestral Genomes that are not too ancient (<1000 million years ago, mya), the interpretation is straightforward: these genes were likely present in most individuals of an ancestral species, defined as a population of interbreeding individuals. However, the interpretation is less clear for prokaryotes, and for Ancestral Genomes where the species tree of life is not fully resolved into bifurcating speciation events (e.g. the last eukaryotic common ancestor, that has four distinct lineages descending from it, whose branching order is not yet established). While horizontal gene transfer appears to be relatively rare among eukaryotes (15), it is common among prokaryotes (16). When horizontal transfer is relatively recent, and between species that are distantly related overall, the event can be identified from the gene trees. However, when the transfer is more ancient, or occurred between ancestral species that are not from highly divergent lineages in the species tree, these events will go undetected. As a result, ancestral prokaryotic genomes are likely to represent what Puigbo *et al.* (17) have referred to as a 'supergenome' of individuals from different species that could exchange genes via horizontal transfer, so no one organism carried the entire set of genes in the ancestral genome. Even in the absence of horizontal transfer, an ancestral genome will also have a complex interpretation whenever the species tree is multifurcated (non-binary), or speciation is so rapid that incomplete lineage sorting is common. In these cases as well, an ancestral genome is likely to contain more genes than were present in the genome of any single individual. Notable multifurcations in the species tree occur at eubacteria, eukaryota, and eumetazoa. The most extreme example in our reconstruction is the eubacterial ancestral supergenome, which is likely to be affected by both multifurcation, and undetected horizontal transfer following divergence from a common ancestor. We infer ∼6200 genes for the eubacterial supergenome, while the descendant ancestral supergenomes contain only ∼1000 to 5800 genes. This trend has been found in other reconstructions, such as that of Puigbo *et al.* (17), who concluded that high rates of gene loss are a valid interpretation, and in fact the prevailing mode of bacterial evolution.

COMPARING RECONSTRUCTIONS TO PREVIOUS STUDIES

To assess our reconstructions, we compared them to several published studies from independent groups. Unlike our reconstructions, none of these studies covers the entire tree of life, but they focus on different branches, which together provide a good sampling of the species tree in Ancestral Genomes. Overall, our reconstructions are consistent with the previous findings, including those of Puigbo *et al.* (17) for eubacteria described above.

In the eukaryotes, a number of studies have been carried out that involve gene family evolutionary reconstructions at large scale. These previous studies involve thousands of gene families but are not as comprehensive and complete as the reconstructions in PANTHER, so the most meaningful way to compare their results is to calculate the ratio between a given event inferred in PANTHER, to that in another study. Assuming a study samples randomly from the larger set of families reconstructed by PANTHER, the ratios calculated for different events from the same study should be similar. Zhou *et al.* (18) estimated the history of eukaryotic gene evolution using genes from three plants species, three animals and two fungi. They clustered the genes into families, and found 2600 that included genes from at least two kingdoms. Of these, they found that between 10–20% had good support for gene duplication occurring on the branch prior to the last eukaryotic common ancestor (LECA), depending on the bootstrap or likelihood ratio test threshold. In our reconstruction (PANTHER version 9), we find that of 7178 PANTHER families with the same phylogenetic distribution, 1781 (18%) contain duplications occur before LECA, which is within the range of the reconstructions from Zhou *et al.* Blomme *et al.* (19) reconstructed the history of gene duplication in a large sample of gene families constructed among seven vertebrate genomes. For internal branches of the species tree, we find PANTHER version 9 estimates of duplication events to be in a consistent ratio of about 2 with those of Blomme *et al.*: 4775:2972, about 1.6, for the branch prior to the last common ancestor (LCA) of vertebrates; 1099:545, about 2.0, for the branch prior to the LCA of zebrafish and pufferfish. For terminal branches, we find a substantially larger ratio (1613:363, about 4.4, for the pufferfish branch; 8621:1269, about 6.8, for the zebrafish branch; 3127:217, about 14.4, for the frog branch). We note that terminal branches, compared to internal branches, are much more dependent on genome assembly quality, as low quality can result in apparent recent duplications and deletions. Wyder *et al.* (20) estimated the extent of gene loss during insect evolution, for a sampling of gene families. Again, the ratios comparing different events from this study to our reconstruction method are roughly similar, at roughly 2: 1093:583, about 1.9, for the branch from the LCA of bees and flies to the LCA of flies; 580:306, about 1.9, for the branch prior to the LCA of mosquitoes; 1117:506, about 2.2, for the fruit fly branch). Our estimates of losses along other terminal branches are somewhat higher (666:206, about 3.2, for the yellow fever mosquito branch; 1641:423, about 3.9, for the malaria mosquito branch; 3700:582, about 6.4, for the honeybee branch) but the relationships hold (e.g. more losses in the yellow fever mosquito compared to the malaria mosquito).

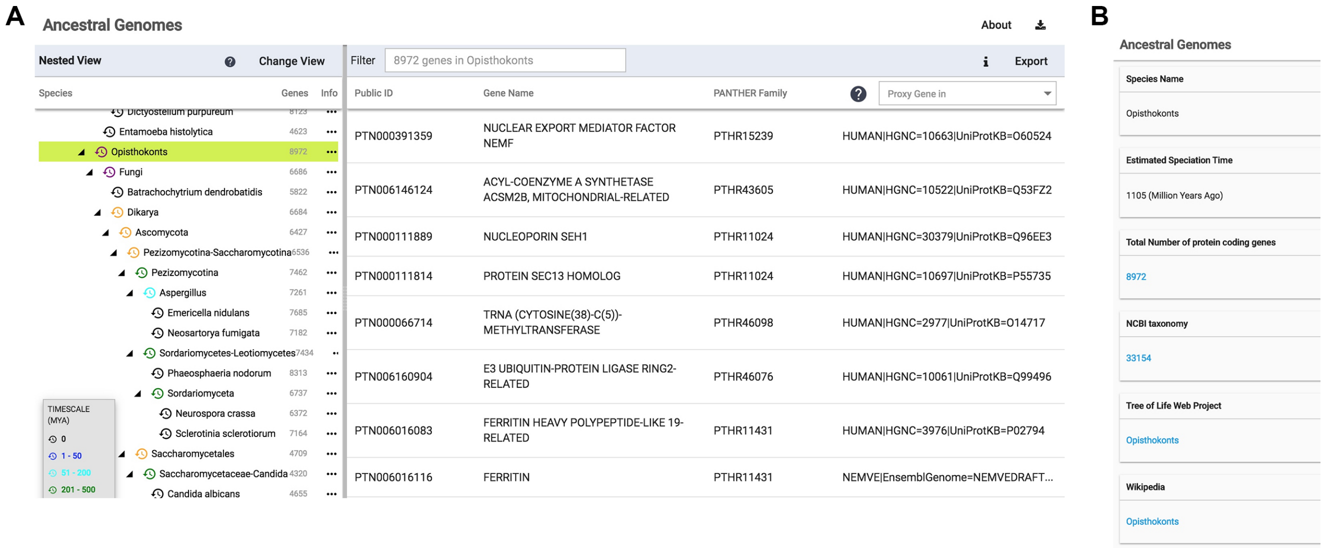
NAVIGATING ANCESTRAL GENOMES

The primary navigation method uses the species tree to select a common ancestral genome. For instance, clicking on ‘Opisthokonts’ will select the genome for the most recent common ancestor of all animals and fungi (Figure 1A), and retrieve a list of genes reconstructed for that ancestor. Users can then search/filter for specific genes by typing in the search field above the list of genes, download the list of genes and associated information, or get more information about that ancestor (Figure 1B). We chose to use a hierarchical, nested view of the species tree by default, rather than the phylogenetic tree view that is more traditional in phylogenetics. The reason for this choice is that the hierarchical view takes much less horizontal screen real estate, and allows users to simultaneously view the list of ancestral genes for the selected genome, and rapidly change their view to a different ancestral genome. The hierarchical view will be familiar to users, as it is used in computer interfaces to navigate file folder hierarchies. However, we also provide a phylogenetic Species Tree View (Figure 2) for navigating and selecting an ancestral genome. In both the Nested View and the Tree View, we use color coding to help orient the user as to the age of each ancestral genome, with the oldest genomes (>2000 mya) in red, and the newest (<50 mya) in blue. Extant genomes are in black.

ANCESTRAL GENE INFORMATION

Selecting an ancestral gene will bring up the gene page for that gene, that contains information about the gene, including the stable identifier and reconstructed sequence as described above (Figure 3). This page also shows gene function information (Gene Ontology terms) that has been inferred for each ancestral gene, by expert curators in the Gene Ontology Phylogenetic Annotation project (21). This project uses the phylogenetic tree, as well as experimentally supported GO annotations, to infer ancestral gain and loss of function. In Ancestral Genomes, we use this information to propagate GO terms from more ancient, to more recent ancestral genes in the tree. In addition, to facilitate browsing and analysis, we also assign a protein name to each ancestral gene whenever possible. Protein names are selected based on an extension of the subfamily naming convention used in PANTHER, as described in Mi *et al.* (10). In brief, whenever a new gene was created by gene duplication (and is present in at least two different genomes, as confirmation of its presence), one of the duplicates is named for its descendant in a well-studied, extant genome. This name is then inherited by its descendants in other extinct genomes, unless another duplication occurs that requires the naming rule to be re-applied.

For each ancestral gene, we also provide a list of its proxy genes in extant genomes. From a practical standpoint, proxy genes provide a way to analyze ancestral genes.



Ancestral Genomes

Nested View ? Change View

Species	Genes	Info
Dictyostelium purpureum	8123	•••
Entamoeba histolytica	4623	•••
◢ Opisthokonts	8972	•••
◢ Fungi	6686	•••
Batrachochytrium dendrobatidis	5822	•••
◢ Dikarya	6684	•••
◢ Ascomycota	6427	•••
◢ Pezizomycotina-Saccharomycotina	6536	•••
◢ Pezizomycotina	7462	•••
◢ Aspergillus	7261	•••
Emericella nidulans	7685	•••
Neosartorya fumigata	7182	•••
◢ Sordariomycetes-Leotiomycetes	7434	••
Phaeosphaeria nodorum	8313	•••
◢ Sordariomyceta	6737	•••
Neurospora crassa	6372	•••
Sclerotinia sclerotiorum	7164	•••
◢ Saccharomycetales	4709	•••
◢ Saccharomycetaceae-Candida	4320	•••
Candida albicans	4655	•••

TIMESCALE (MYA)
0
1 - 50
51 - 200
201 - 500

Filter: 8972 genes in Opisthokonts

Public ID	Gene Name	PANTHER Family	Proxy Gene in
PTN000391359	NUCLEAR EXPORT MEDIATOR FACTOR NEMF	PTHR15239	HUMAN|HGNC=10663|UniProtKB=O60524
PTN006146124	ACYL-COENZYME A SYNTHETASE ACSM2B, MITOCHONDRIAL-RELATED	PTHR43605	HUMAN|HGNC=10522|UniProtKB=Q53FZ2
PTN000111889	NUCLEOPORIN SEH1	PTHR11024	HUMAN|HGNC=30379|UniProtKB=Q96EE3
PTN000111814	PROTEIN SEC13 HOMOLOG	PTHR11024	HUMAN|HGNC=10697|UniProtKB=P55735
PTN000066714	TRNA (CYTOSINE(38)-C(5))-METHYLTRANSFERASE	PTHR46098	HUMAN|HGNC=2977|UniProtKB=O14717
PTN006160904	E3 UBIQUITIN-PROTEIN LIGASE RING2-RELATED	PTHR46076	HUMAN|HGNC=10061|UniProtKB=Q99496
PTN006016083	FERRITIN HEAVY POLYPEPTIDE-LIKE 19-RELATED	PTHR11431	HUMAN|HGNC=3976|UniProtKB=P02794
PTN006016116	FERRITIN	PTHR11431	NEMVE|EnsemblGenome=NEMVEDRAFT...

Ancestral Genomes

Species Name: Opisthokonts

Estimated Speciation Time: 1105 (Million Years Ago)

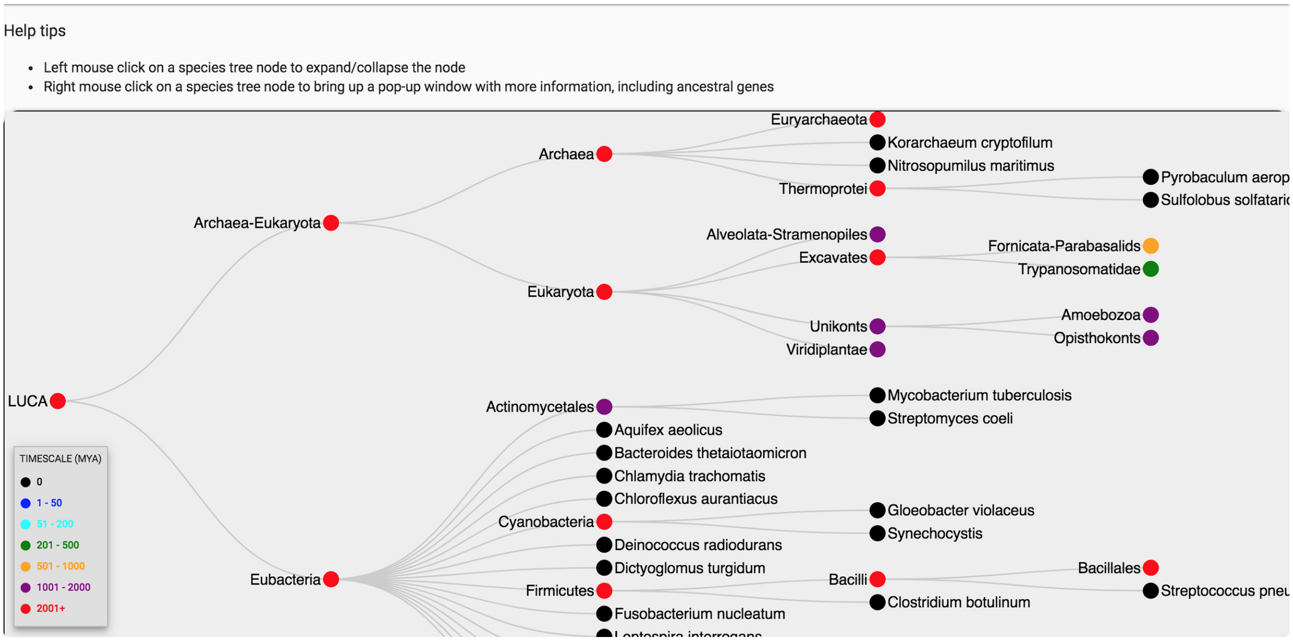
Total Number of protein coding genes: 8972

NCBI taxonomy: 33154

Tree of Life Web Project: Opisthokonts

Wikipedia: Opisthokonts

Figure 1. Browsing and selecting an ancestral genome. (**A**) Users can browse the available genomes on the left panel. Clicking on a genome (e.g. Opisthokonts) brings up the list of genes in the right panel. (**B**) Clicking on the '…' (info) button in the left panel in (A) brings up more information about that ancestral organism, including links to information in other resources, and the estimated age of that ancestor (in millions of years ago) as reported in the TimeTree (30) resource.

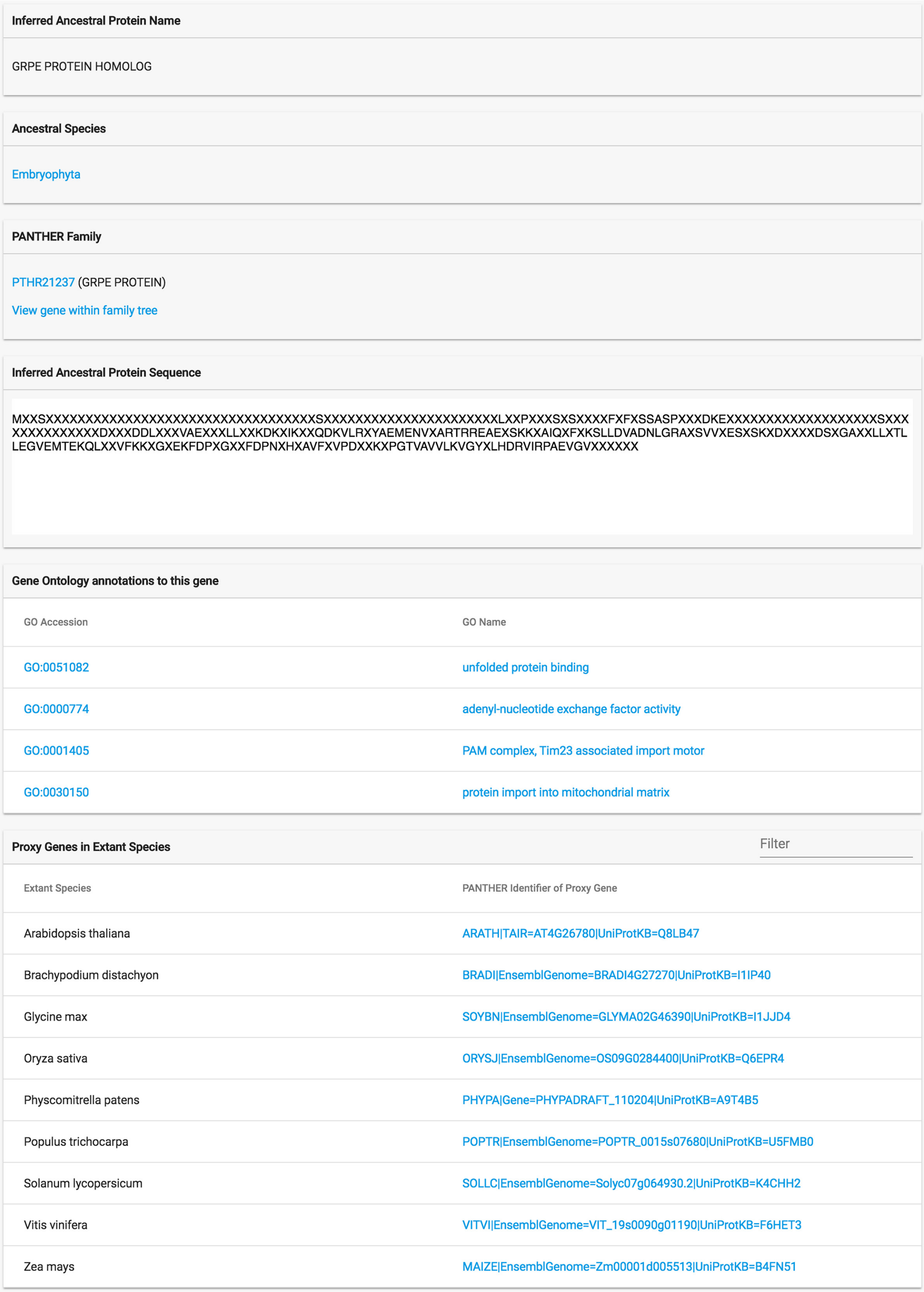


Help tips

- Left mouse click on a species tree node to expand/collapse the node
- Right mouse click on a species tree node to bring up a pop-up window with more information, including ancestral genes

Figure 2. Species Tree Browser. We also provide a species tree view of the genomes in Ancestral Genomes. This view is much less compact than the default nested hierarchical view, but it is a more standard view in phylogenetics. Right-clicking on a node will bring up the ancestral genome overview page (Figure 1B) for that genome, from which users can bring up the list of genes inferred for that genome.

Because the ancestral genes themselves are extinct, they have not been studied in the lab. But their descendants in extant genomes may have been studied, particularly if they are from a well-studied 'model organism' (22) such as the bacterium *E. coli*, yeast (budding or fission), the plant *A. thaliana*, fruit fly, nematode worm *C. elegans*, mouse, rat or zebrafish. More formally, proxy genes are simply an extension of the well-known comparative biology concept of orthologs, which are widely used to infer properties of an uncharacterized gene in one organism, from a related, experimentally characterized gene in another organism (23). Orthology is a relationship between two extant genes, via the gene that was their last common ancestor. Fitch defined orthologs as two genes in different extant species that can be traced to the same gene in the common ancestor genome of those organisms (24). Proxy genes describe the relationship between an ancestral gene and its extant descendants. We define a proxy gene as the most closely related descendant of that ancestral gene in an extant organism. One could in principle define 'closest' in different ways, but for conve-



Inferred Ancestral Protein Name

GRPE PROTEIN HOMOLOG

Ancestral Species

Embryophyta

PANTHER Family

PTHR21237 (GRPE PROTEIN)

View gene within family tree

Inferred Ancestral Protein Sequence

```
MXXSXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXSXXXXXXXXXXXXXXXXXXXXXXLXXPXXXSXSXXXXFXFXSSASPXXXDKEXXXXXXXXXXXXXXXXXXXSXXX
XXXXXXXXXXXDXXXDDLXXXVAEXXXLLXXKDKXIKXXQDKVLRXYAEMENVXARTRREAEXSKKXAIQXFXKSLLDVADNLGRAXSVVXESXSKXDXXXXDSXGAXXLLXTL
LEGVEMTEKQLXXVFKKXGXEKFDPXGXXFDPNXHXAVFXVPDXXKXPGTVAVVLKVGYXLHDRVIRPAEVGVXXXXXX
```

Gene Ontology annotations to this gene

GO Accession	GO Name
GO:0051082	unfolded protein binding
GO:0000774	adenyl-nucleotide exchange factor activity
GO:0001405	PAM complex, Tim23 associated import motor
GO:0030150	protein import into mitochondrial matrix

Proxy Genes in Extant Species

Extant Species	PANTHER Identifier of Proxy Gene
Arabidopsis thaliana	ARATH|TAIR=AT4G26780|UniProtKB=Q8LB47
Brachypodium distachyon	BRADI|EnsemblGenome=BRADI4G27270|UniProtKB=I1IP40
Glycine max	SOYBN|EnsemblGenome=GLYMA02G46390|UniProtKB=I1JJD4
Oryza sativa	ORYSJ|EnsemblGenome=OS09G0284400|UniProtKB=Q6EPR4
Physcomitrella patens	PHYPA|Gene=PHYPADRAFT_110204|UniProtKB=A9T4B5
Populus trichocarpa	POPTR|EnsemblGenome=POPTR_0015s07680|UniProtKB=U5FMB0
Solanum lycopersicum	SOLLC|EnsemblGenome=Solyc07g064930.2|UniProtKB=K4CHH2
Vitis vinifera	VITVI|EnsemblGenome=VIT_19s0090g01190|UniProtKB=F6HET3
Zea mays	MAIZE|EnsemblGenome=Zm00001d005513|UniProtKB=B4FN51

Figure 3. Ancestral gene information page. This page has information about the ancestral gene, including stable identifier, protein name, reconstructed sequence, inferred GO functions and proxy genes.

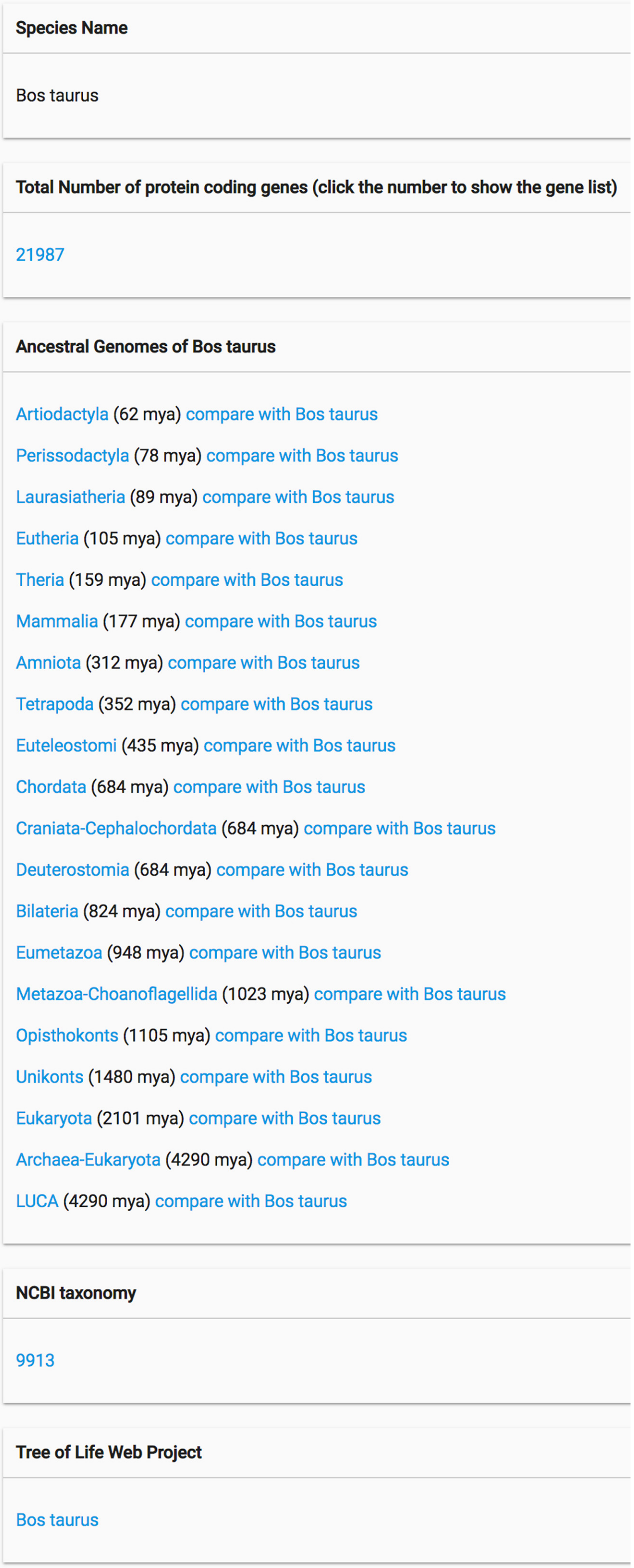
nience we define it in terms of protein sequence divergence, as this quantity is easily calculated from our trees as total branch length in the path from an ancestral gene to an extant (leaf) descendant.

COMPARING AN EXTANT GENOME WITH ITS ANCESTRAL GENOMES

From an extant genome information page, users can view the available ancestral reconstructions in that lineage, leading back to the last universal common ancestor (Figure 4). A link is available to a web page that compares the extant genome to the selected ancestral genome. The comparison page has four separate sections (Figure 5). The first section displays the genes that were inherited in the extant genome from the selected ancestral genome. For each ancestral gene, the table lists the extant gene(s) that can be traced back to that ancestral gene. Note that an ancestral gene can have more than one descendant in the extant genome, if one or more gene duplications have occurred during the time between the ancestor and its descendant. The second section displays the genes in the ancestral genome that were lost during the time between the ancestor and its descendant. The third section displays the genes gained by horizontal transfer or potential '*de novo*' gene evolution: genes that were gained in the extant genome relative to the ancestor, but could not be traced to a reconstructed gene in that ancestor. Note that it is possible that some potential *de novo* gene gains can in fact be traced to an ancestral gene, but for various biological (e.g. high degree of sequence divergence) or technical (e.g. how PANTHER has divided up genes into families) reasons, were not able to be traced. Finally, we include a fourth section that lists the extant genes whose histories have not yet been reconstructed. These are generally genes with few homologs among the >100 species in the PANTHER trees, and therefore represent genes that can only be traced back to relatively recent ancestral genomes.

GENE TREE VIEWER WITH EXPLICIT REPRESENTATION OF GENE LOSS EVENTS

The gene trees in Ancestral Genomes include explicit representations of inferred gene loss events, a feature that, to our knowledge, is not available in any other resource. At http://ancestralgenomes.org, we provide a gene tree viewer that enables visualization of these gene loss events (Figure 6). Gene losses appear as branches in the tree along which a gene was lost, and are labeled with the first ancestral species that is inferred to have been missing that gene. Because of this visualization convention, the gene trees in Ancestral Genomes also contain an explicit tree node for every inferred ancestral gene, even if it is not a last common ancestor of extant genes due to gene deletions. Note that the Gene Tree Viewer at Ancestral Genomes includes features that enable users to explore protein sequence evolution throughout the tree. The gene trees can be accessed from the ancestral gene information page (Figure 3), and will highlight the selected gene. The Gene Tree Viewer uses a nested hierarchical view ('Detailed') by default, which allows the display of table view with information about each ancestral (and extant) gene. Users can toggle back to the standard 'Phylogenetic' view with one click. Users can select the multiple



Species Name

Bos taurus

Total Number of protein coding genes (click the number to show the gene list)

21987

Ancestral Genomes of Bos taurus

Artiodactyla (62 mya) compare with Bos taurus

Perissodactyla (78 mya) compare with Bos taurus

Laurasiatheria (89 mya) compare with Bos taurus

Eutheria (105 mya) compare with Bos taurus

Theria (159 mya) compare with Bos taurus

Mammalia (177 mya) compare with Bos taurus

Amniota (312 mya) compare with Bos taurus

Tetrapoda (352 mya) compare with Bos taurus

Euteleostomi (435 mya) compare with Bos taurus

Chordata (684 mya) compare with Bos taurus

Craniata-Cephalochordata (684 mya) compare with Bos taurus

Deuterostomia (684 mya) compare with Bos taurus

Bilateria (824 mya) compare with Bos taurus

Eumetazoa (948 mya) compare with Bos taurus

Metazoa-Choanoflagellida (1023 mya) compare with Bos taurus

Opisthokonts (1105 mya) compare with Bos taurus

Unikonts (1480 mya) compare with Bos taurus

Eukaryota (2101 mya) compare with Bos taurus

Archaea-Eukaryota (4290 mya) compare with Bos taurus

LUCA (4290 mya) compare with Bos taurus

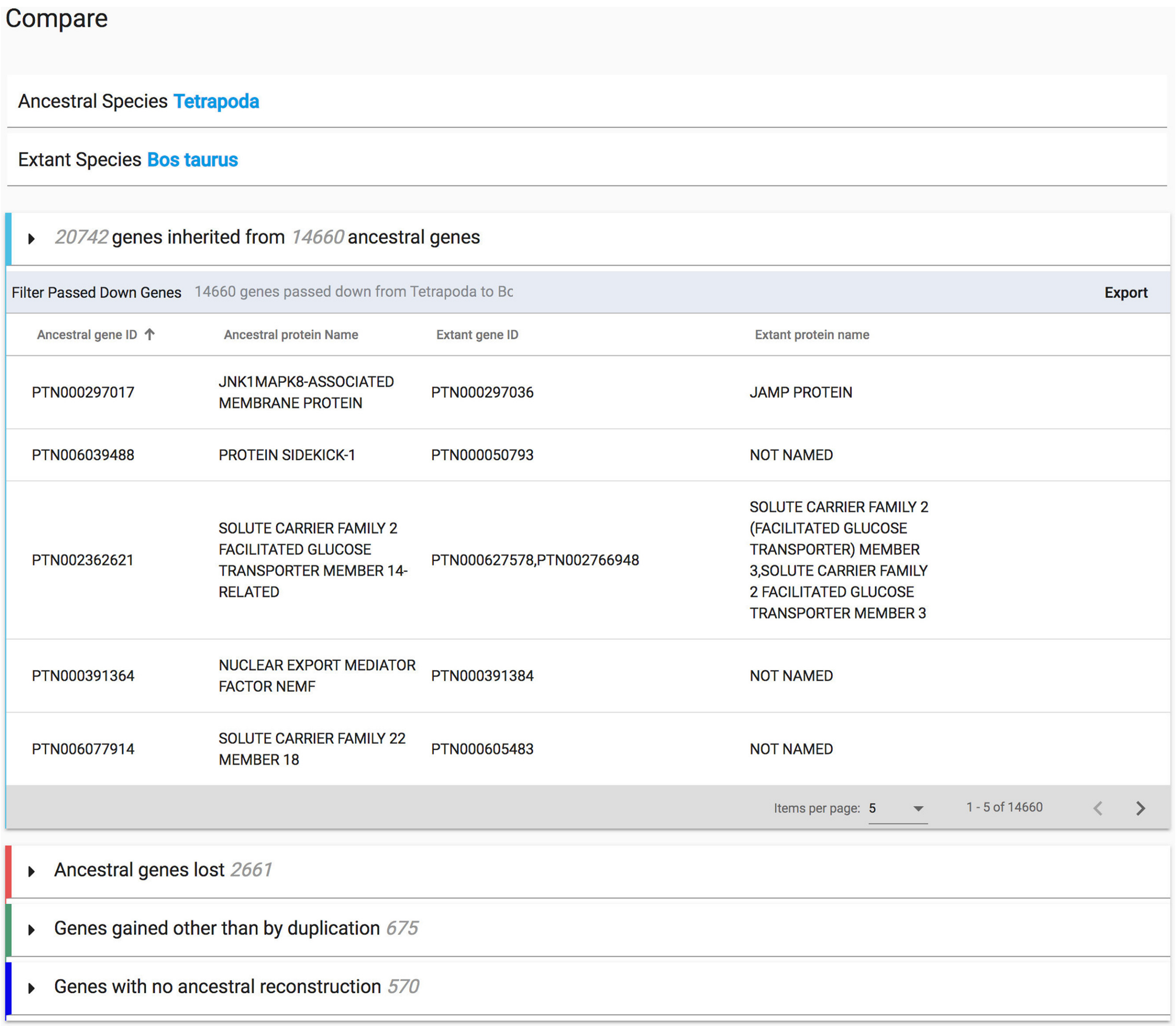
NCBI taxonomy

9913

Tree of Life Web Project

Bos taurus

Figure 4. Extant species information page. This page has information about the extant species, including species name, total number of protein coding genes, its ancestral species and their speciation times. Each ancestral species is followed by a link to the genome comparison page.



Compare

Ancestral Species **Tetrapoda**

Extant Species **Bos taurus**

▸ *20742* genes inherited from *14660* ancestral genes

Filter Passed Down Genes 14660 genes passed down from Tetrapoda to Bc Export

Ancestral gene ID ↑	Ancestral protein Name	Extant gene ID	Extant protein name
PTN000297017	JNK1MAPK8-ASSOCIATED MEMBRANE PROTEIN	PTN000297036	JAMP PROTEIN
PTN006039488	PROTEIN SIDEKICK-1	PTN000050793	NOT NAMED
PTN002362621	SOLUTE CARRIER FAMILY 2 FACILITATED GLUCOSE TRANSPORTER MEMBER 14-RELATED	PTN000627578,PTN002766948	SOLUTE CARRIER FAMILY 2 (FACILITATED GLUCOSE TRANSPORTER) MEMBER 3,SOLUTE CARRIER FAMILY 2 FACILITATED GLUCOSE TRANSPORTER MEMBER 3
PTN000391364	NUCLEAR EXPORT MEDIATOR FACTOR NEMF	PTN000391384	NOT NAMED
PTN006077914	SOLUTE CARRIER FAMILY 22 MEMBER 18	PTN000605483	NOT NAMED

Items per page: 5 ▾ 1 - 5 of 14660 ‹ ›

▸ Ancestral genes lost *2661*

▸ Genes gained other than by duplication *675*

▸ Genes with no ancestral reconstruction *570*

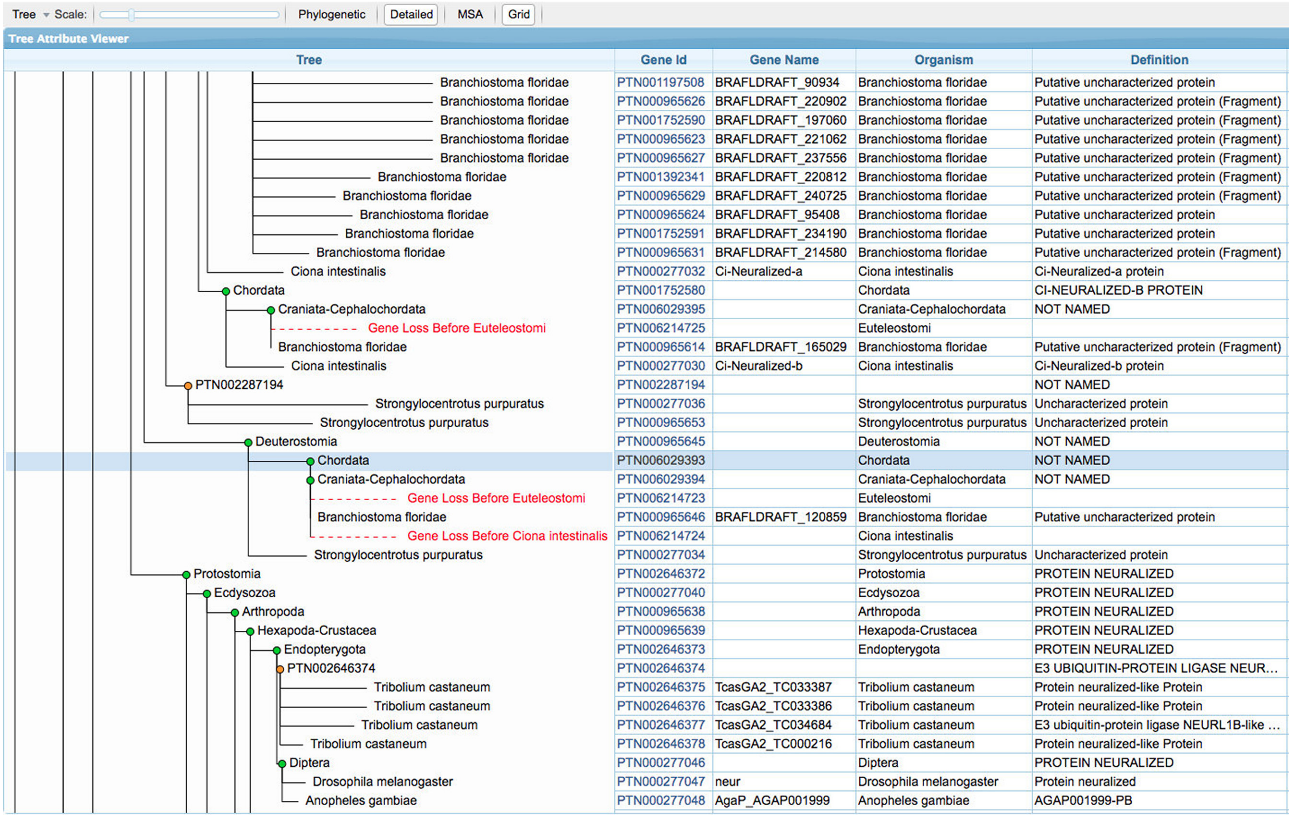
Figure 5. Extant and Ancestral Genomes comparison page. There are four collapsible sections on the page, each with a header displaying summarizing information about the changes in gene content from the ancestral genome to the extant genome. Each section can be expanded to list the genes in that category.

sequence alignment viewer to see the reconstructed amino acid sequences of ancestral genes, and explore how those sequences have evolved.

CONCLUSIONS

We have created a new resource for exploring reconstructed Ancestral Genomes across the tree of life. For the first time, the scientific community can easily access a current ‘best estimate’ as to the number of protein coding genes present in Ancestral Genomes across the tree of life, and can explore the reconstructed genes in terms of their extant descendants, and even their reconstructed protein sequences. Users should understand some of the caveats of these reconstructions. Sampling of genomes across the tree of life can affect the accuracy of reconstructions: branches that are under-sampled, such as bacteria and archaea, will be less accurate. Because reconstruction methods rely on detection of homology at the protein sequence level, if homologs diverge too much in sequence, their relationships will not be detected. As a result, we are likely to underestimate the gene content of some ancestral genomes, particularly those that are more ancient.

We recognize that ancestral genome reconstruction is an ongoing and active area of research, and we expect that the accuracy of our reconstructions will continue to improve over time. The reconstructions in the Ancestral Genomes resource will be updated yearly, in synchronization with the PANTHER database. Improvements in the sets of genes available from the UniProt Reference Proteomes data source will result in improved reconstructions. Improvements are also expected in the gene trees due to ongoing feedback from manual curation in the GO Phylogenetic Annotation project (21), the sharing of data between PANTHER and Ensembl Compara (25), and orthol-



Tree	Gene Id	Gene Name	Organism	Definition
Branchiostoma floridae	PTN001197508	BRAFLDRAFT_90934	Branchiostoma floridae	Putative uncharacterized protein
Branchiostoma floridae	PTN000965626	BRAFLDRAFT_220902	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Branchiostoma floridae	PTN001752590	BRAFLDRAFT_197060	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Branchiostoma floridae	PTN000965623	BRAFLDRAFT_221062	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Branchiostoma floridae	PTN000965627	BRAFLDRAFT_237556	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Branchiostoma floridae	PTN001392341	BRAFLDRAFT_220812	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Branchiostoma floridae	PTN000965629	BRAFLDRAFT_240725	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Branchiostoma floridae	PTN000965624	BRAFLDRAFT_95408	Branchiostoma floridae	Putative uncharacterized protein
Branchiostoma floridae	PTN001752591	BRAFLDRAFT_234190	Branchiostoma floridae	Putative uncharacterized protein
Branchiostoma floridae	PTN000965631	BRAFLDRAFT_214580	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Ciona intestinalis	PTN000277032	Ci-Neuralized-a	Ciona intestinalis	Ci-Neuralized-a protein
Chordata	PTN001752580		Chordata	CI-NEURALIZED-B PROTEIN
Craniata-Cephalochordata	PTN006029395		Craniata-Cephalochordata	NOT NAMED
Gene Loss Before Euteleostomi	PTN006214725		Euteleostomi	
Branchiostoma floridae	PTN000965614	BRAFLDRAFT_165029	Branchiostoma floridae	Putative uncharacterized protein (Fragment)
Ciona intestinalis	PTN000277030	Ci-Neuralized-b	Ciona intestinalis	Ci-Neuralized-b protein
PTN002287194	PTN002287194			NOT NAMED
Strongylocentrotus purpuratus	PTN000277036		Strongylocentrotus purpuratus	Uncharacterized protein
Strongylocentrotus purpuratus	PTN000965653		Strongylocentrotus purpuratus	Uncharacterized protein
Deuterostomia	PTN000965645		Deuterostomia	NOT NAMED
Chordata	PTN006029393		Chordata	NOT NAMED
Craniata-Cephalochordata	PTN006029394		Craniata-Cephalochordata	NOT NAMED
Gene Loss Before Euteleostomi	PTN006214723		Euteleostomi	
Branchiostoma floridae	PTN000965646	BRAFLDRAFT_120859	Branchiostoma floridae	Putative uncharacterized protein
Gene Loss Before Ciona intestinalis	PTN006214724		Ciona intestinalis	
Strongylocentrotus purpuratus	PTN000277034		Strongylocentrotus purpuratus	Uncharacterized protein
Protostomia	PTN002646372		Protostomia	PROTEIN NEURALIZED
Ecdysozoa	PTN000277040		Ecdysozoa	PROTEIN NEURALIZED
Arthropoda	PTN000965638		Arthropoda	PROTEIN NEURALIZED
Hexapoda-Crustacea	PTN000965639		Hexapoda-Crustacea	PROTEIN NEURALIZED
Endopterygota	PTN002646373		Endopterygota	PROTEIN NEURALIZED
PTN002646374	PTN002646374			E3 UBIQUITIN-PROTEIN LIGASE NEUR…
Tribolium castaneum	PTN002646375	TcasGA2_TC033387	Tribolium castaneum	Protein neuralized-like Protein
Tribolium castaneum	PTN002646376	TcasGA2_TC033386	Tribolium castaneum	Protein neuralized-like Protein
Tribolium castaneum	PTN002646377	TcasGA2_TC034684	Tribolium castaneum	E3 ubiquitin-protein ligase NEURL1B-like …
Tribolium castaneum	PTN002646378	TcasGA2_TC000216	Tribolium castaneum	Protein neuralized-like Protein
Diptera	PTN000277046		Diptera	PROTEIN NEURALIZED
Drosophila melanogaster	PTN000277047	neur	Drosophila melanogaster	Protein neuralized
Anopheles gambiae	PTN000277048	AgaP_AGAP001999	Anopheles gambiae	AGAP001999-PB

Figure 6. Gene Tree Viewer. Gene loss is shown explicitly in red, with text indicating the ancestral species prior to which the gene was lost.

ogy benchmarking in the Quest for Orthologs Consortium (5). Although we have used the PANTHER pipeline for our initial reconstructions, we recognize that other methods, such as using different methods for multiple alignment (26) or construction of reconciled gene trees under a deletion-transfer-loss model (27,28) may result in differences in the inferred ancestral gene content, as well as in the inferred ancestral gene sequences (29). We encourage community feedback on our reconstructions, and will aim to incorporate feedback in future releases.

FUNDING

National Science Foundation [1458808 to P.D.T.]; National Human Genome Research Institute of the National Institutes of Health [U41HG002273 to P.D.T.]; Funding for open access charge: National Science Foundation.

Conflict of interest statement. None declared.

REFERENCES

1. Mirkin,B.G., Fenner,T.I., Galperin,M.Y. and Koonin,E.V. (2003) Algorithms for computing parsimonious evolutionary scenarios for genome evolution, the last universal common ancestor and dominance of horizontal gene transfer in the evolution of prokaryotes. *BMC Evol. Biol.*, **3**, 2.
2. Fritz-Laylin,L.K., Prochnik,S.E., Ginger,M.L., Dacks,J.B., Carpenter,M.L., Field,M.C., Kuo,A., Paredez,A., Chapman,J., Pham,J. *et al.* (2010) The genome of Naegleria gruberi illuminates early eukaryotic versatility. *Cell*, **140**, 631–642.
3. Doyon,J.P., Ranwez,V., Daubin,V. and Berry,V. (2011) Models, algorithms and programs for phylogeny reconciliation. *Brief Bioinform.*, **12**, 392–400.
4. Bansal,M.S., Alm,E.J. and Kellis,M. (2012) Efficient algorithms for the reconciliation problem with gene duplication, horizontal transfer and loss. *Bioinformatics*, **28**, i283–i291.
5. Altenhoff,A.M., Boeckmann,B., Capella-Gutierrez,S., Dalquen,D.A., DeLuca,T., Forslund,K., Huerta-Cepas,J., Linard,B., Pereira,C., Pryszcz,L.P. *et al.* (2016) Standardized benchmarking in the quest for orthologs. *Nat. Methods*, **13**, 425–430.
6. Mi,H., Poudel,S., Muruganujan,A., Casagrande,J.T. and Thomas,P.D. (2016) PANTHER version 10: expanded protein families and functions, and analysis tools. *Nucleic Acids Res.*, **44**, D336–D342.
7. Huerta-Cepas,J., Capella-Gutierrez,S., Pryszcz,L.P., Marcet-Houben,M. and Gabaldon,T. (2014) PhylomeDB v4: zooming into the plurality of evolutionary histories of a genome. *Nucleic Acids Res.*, **42**, D897–D902.
8. Schreiber,F., Patricio,M., Muffato,M., Pignatelli,M. and Bateman,A. (2014) TreeFam v9: a new website, more species and orthology-on-the-fly. *Nucleic Acids Res.*, **42**, D922–D925.
9. Thomas,P.D., Campbell,M.J., Kejariwal,A., Mi,H., Karlak,B., Daverman,R., Diemer,K., Muruganujan,A. and Narechania,A. (2003) PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.*, **13**, 2129–2141.
10. Mi,H., Muruganujan,A. and Thomas,P.D. (2013) PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. *Nucleic Acids Res.*, **41**, D377–D386.
11. UniProt Consortium, T. (2018) UniProt: the universal protein knowledgebase. *Nucleic Acids Res.*, **46**, 2699.
12. Thomas,P.D. (2010) GIGA: a simple, efficient algorithm for gene tree inference in the genomic age. *BMC Bioinformatics*, **11**, 312.
13. Yang,Z. (1997) PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput Appl. Biosci.*, **13**, 555–556.

14. Stamatakis,A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.
15. Danchin,E.G. (2016) Lateral gene transfer in eukaryotes: tip of the iceberg or of the ice cube? *BMC Biol.*, **14**, 101.
16. Thomas,C.M. and Nielsen,K.M. (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat. Rev. Microbiol.*, **3**, 711–721.
17. Puigbo,P., Lobkovsky,A.E., Kristensen,D.M., Wolf,Y.I. and Koonin,E.V. (2014) Genomes in turmoil: quantification of genome dynamics in prokaryote supergenomes. *BMC Biol.*, **12**, 66.
18. Zhou,X., Lin,Z. and Ma,H. (2010) Phylogenetic detection of numerous gene duplications shared by animals, fungi and plants. *Genome Biol.*, **11**, R38.
19. Blomme,T., Vandepoele,K., De Bodt,S., Simillion,C., Maere,S. and Van de Peer,Y. (2006) The gain and loss of genes during 600 million years of vertebrate evolution. *Genome Biol.*, **7**, R43.
20. Wyder,S., Kriventseva,E.V., Schroder,R., Kadowaki,T. and Zdobnov,E.M. (2007) Quantification of ortholog losses in insects and vertebrates. *Genome Biol.*, **8**, R242.
21. Gaudet,P., Livstone,M.S., Lewis,S.E. and Thomas,P.D. (2011) Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Brief Bioinform.*, **12**, 449–462.
22. Oliver,S.G., Lock,A., Harris,M.A., Nurse,P. and Wood,V. (2016) Model organism databases: essential resources that need the support of both funders and users. *BMC Biol.*, **14**, 49.
23. Sonnhammer,E.L., Gabaldon,T., Sousa da Silva,A.W., Martin,M., Robinson-Rechavi,M., Boeckmann,B., Thomas,P.D., Dessimoz,C. and Quest for Orthologs, c. (2014) Big data and other challenges in the quest for orthologs. *Bioinformatics*, **30**, 2993–2998.
24. Fitch,W.M. (1970) Distinguishing Homologous from Analogous Proteins. *Syst. Zool.*, **19**, 99–113.
25. Herrero,J., Muffato,M., Beal,K., Fitzgerald,S., Gordon,L., Pignatelli,M., Vilella,A.J., Searle,S.M., Amode,R., Brent,S. *et al.* (2016) Ensembl comparative genomics resources. *Database (Oxford)*, **2016**, bav096.
26. Loytynoja,A. and Goldman,N. (2008) Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. *Science*, **320**, 1632–1635.
27. Stolzer,M., Lai,H., Xu,M., Sathaye,D., Vernot,B. and Durand,D. (2012) Inferring duplications, losses, transfers and incomplete lineage sorting with nonbinary species trees. *Bioinformatics*, **28**, i409–i415.
28. Jacox,E., Chauve,C., Szollosi,G.J., Ponty,Y. and Scornavacca,C. (2016) ecceTERA: comprehensive gene tree-species tree reconciliation using parsimony. *Bioinformatics*, **32**, 2056–2058.
29. Vialle,R.A., Tamuri,A.U. and Goldman,N. (2018) Alignment modulates ancestral sequence reconstruction accuracy. *Mol. Biol. Evol.*, **35**, 1783–1797.
30. Kumar,S., Stecher,G., Suleski,M. and Hedges,S.B. (2017) TimeTree: a resource for timelines, timetrees, and divergence times. *Mol. Biol. Evol.*, **34**, 1812–1819.