Quest for Orthologs Entails Quest for Tree of Life: In Search of the Gene Stream

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Abstract

Quest for Orthologs (QfO) is a community effort with the goal to improve and benchmark orthology predictions. As quality assessment assumes prior knowledge on species phylogenies, we investigated the congruency between existing species trees by comparing the relationships of 147 QfO reference organisms from six Tree of Life (ToL)/species tree projects: The National Center for Biotechnology Information (NCBI) taxonomy, Opentree of Life, the sequenced species/species ToL, the 16S ribosomal RNA (rRNA) database, and trees published by Ciccarelli et al. (Ciccarelli FD, et al. 2006. Toward automatic reconstruction of a highly resolved tree of life. Science 311:1283–1287) and by Huerta-Cepas et al. (Huerta-Cepas J, Marcet-Houben M, Gabaldon T. 2014. A nested phylogenetic reconstruction approach provides scalable resolution in the eukaryotic Tree Of Life. PeerJ PrePrints 2:223) Our study reveals that each species tree suggests a different phylogeny: 87 of the 146 (60%) possible splits of a dichotomous and rooted tree are congruent, while all other splits are incongruent in at least one of the species trees. Topological differences are observed not only at deep speciation events, but also within younger clades, such as *Hominidae*, *Rodentia*, *Laurasiatheria*, *or rosids*. The evolutionary relationships of 27 archaea and bacteria are highly inconsistent. By assessing 458,108 gene trees from 65 genomes, we show that consistent species topologies are more often supported by gene phylogenies than contradicting ones. The largest concordant species tree includes 77 of the QfO reference organisms at the most. Results are summarized in the form of a consensus ToL (http://swisstree.vital-it.ch/species_tree) that can serve different benchmarking purposes.

Key words: Tree of Life, species tree, gene tree support.

Introduction

Because important applications in the Life Sciences rely on orthology inference, scientists—among whom many authors of orthology databases—joined a community effort named "Quest for Orthologs (QfO)," one of whose goals is to compare and improve orthology predictions (Gabaldon et al. 2009; Dessimoz et al. 2012; Sonnhammer et al. 2014). The evaluation of such inference depends on the thorough understanding of the evolution of gene families and taxa. To generate reference gene trees (Trachana et al. 2011, 2014; Sonnhammer et al. 2014), it is crucial to find a core set of species whose relative histories are known to the best of our knowledge. After realizing the incongruence of Trees of Life (ToL) at the third Quest for Orthologs (QfO3) conference in Lausanne (Switzerland) in 2013 (http://questfororthologs.org/meetings#quest_for_orthologs_3, last accessed July 7, 2015), a QfO species tree working group was initiated to survey the status of species phylogenies as well as to establish contacts between experts of the ToL community. Within the same year, exchanges were initiated between the two research communities at the Biodiversity Information Standards conference (Taxonomic Databases Working Group) in 2013 in Florence, Italy (http://www.slideshare.net/suzi.lewis/q4-o-at-tdwg-2013, last accessed July 7, 2015).

ToL links inferred species histories on a global level. Although there is an ongoing debate on tree or network-based evolution—particularly in prokaryotes where horizontal gene transfer (HGT) can be massive (Koonin et al. 2001; Treangen and Rocha 2011)—the concept of a ToL is an important pillar in evolutionary biology. In the context of quality assessment for orthology predictions, species trees are key in reconciling species and gene histories and decoding discordance between the two trees. Yet, understanding to which extent species trees are robust recapitulations of the evolution of life and to which extent they depend on method and data set contingencies is challenging. Initially, morphological and anatomical observations led to the assumption that species are related to each other, and species were grouped and classified accordingly. Molecular phylogenetics revolutionized the inference of species phylogenies, thanks to its ability to trace the evolution of proteins, genes, and genomes. Be it for predicting species phylogenies or understanding the evolutionary relationships of genes, the underlying analyses are alike (Whelan et al. 2001). As a first step, genes related to each other (homologs) are identified, followed by in-depth analysis on how the genes are related, that is, whether they diverged following a speciation event (ortholog), gene duplication (paralog), or were laterally transferred between organisms (xenolog). In other words, the prediction of gene relationships is based on knowledge of the species tree, and the species tree can be inferred from the study of gene relationships. Hence, there is a risk of circularity when inferring orthology predictions from ortholog-derived species trees.

In this study, we address the question of the current knowledge and reliability of species relationships by assessing the congruence among the most inclusive ToLs and species trees. To do this, we compared tree topologies for 147 species that were selected for the QfO reference data set 2013: 120 eukaryotes, 7 archaea, and 20 bacteria (hereafter named QfO reference organisms; taxa are listed in supplementary table S1, Supplementary Material online). The prediction of their evolutionary relationship differs in many ways which are all able to influence the tree topology (compare supplementary table S2, Supplementary Material online). To begin with, the size of the six analyzed species phylogenies ranges from 191 to 2,227,481 taxa. Taxon sampling has a strong impact on tree reconstruction. As an example, high species coverage has been shown to avoid systematic bias and long branch attraction (LBA) in phylogenetic analysis of molecular sequences (Philippe et al. 2000; Heath et al. 2008). Of importance is also the type of data: In contrast to noncoding genes, coding genes can be analyzed at the level of both nucleotide sequences and amino acid sequences, which can lead to complementary resolution at different levels in the tree space. The analysis of a single gene presumes that the gene history reflects that of the species, as opposed to the study of multiple marker genes. The latter allows the comparison of all gene tree topologies with each other, which helps to identify topological incongruence such as nonvertical gene traffic, which is especially relevant to the study of prokaryotic relationships. Phylogenetic signals can also be obtained from domain occurrence in proteomes, and the analysis of the binary matrix constitutes a fast tree-building approach for regular revision. Another fundamental difference is that not all species trees are built from scratch: Some ToLs use an existing species classification as the initial tree, to which phylogenetic information is added or to guide a phylogenetic analysis. In this case, species trees are not independent, and are thus likely to share higher topological congruence. Last but not the least, some species trees are rooted, while others are not, thus influencing the analysis as well as the interpretation of the tree topology.

The six species trees under comparison are the following: 1) The NCBI taxonomy is probably the most widely used species classification in molecular phylogenetics because it is the repository for standardized nomenclature and taxonomic identifiers for international sequence databases (Federhen 2011). As such, it is often implicitly used as the guide for classification. However, the NCBI taxonomy database is not an authoritative source for nomenclature or classification (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Root, last accessed July 7, 2015). It currently stores data from 971,052 species and 1,254,947 taxonomic nodes (NCBI taxonomy browser; www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=statistics, February 19, 2015). The species classification, based on expert knowledge, includes a large number of multifurcating nodes that represent yet unknown species phylogenies. 2) The Open Tree of Life (Opentree) strives to capture the spectrum of known biodiversity. Initially based on the NCBI taxonomy, published species phylogenies are stepwise mapped on the species classification (http://biorxiv.org/content/early/2014/12/15/012260, last accessed July 7, 2015). With over 2.3 million taxa, it is the largest of the six species trees. 3) The sequenced species Tree of Life (sToL) is an NCBI taxonomy-guided, bifurcating likelihood tree based on the protein domain composition predicted for cellular organisms with complete proteomes (Fang et al. 2013). 4) Huerta-Cepas, Marcet-Houben, et al. (2014) developed a nested, hierarchical approach to maximize phylogenetic signals from the sequence data used for tree reconstruction (hereafter named Tree-HC). The species tree thus analyzed includes 216 eukaryotic species and no prokaryotes. 5) Cicarelli et al. (2006) published a species tree based on 31 concatenated universal proteins from 191 species, of which 23 are eukaryotes, 18 archaea, and 150 bacteria (hereafter named ToL-C). Although this tree has been updated, thanks to an improved HGT detection methodology (Creevey et al. 2011) and the possibility of careful data selection from a considerably increased collection of completely sequenced genomes, we decided to oppose this early version of a species tree—constructed by an automated procedure—to more recent and larger ToLs. 6) The 16S rRNA project is probably the greatest attempt for the detailed analysis of a gene history (Munoz et al. 2011; Yilmaz et al. 2013). In 1977, the study of this gene by Woese and Fox (1977) resulted in the identification of Archaea as a third domain of life. Today, the expert-curated multiple sequence alignment of ribosomal RNA sequences consists of data from over 10,000 taxa, and the 16S rRNA has become an important marker gene for metagenomics. However, more than one copy of this gene has been observed in genomes (Case et al. 2007). As the gene could be subject to HGT, the gene tree can differ from the actual species phylogeny. Consequently, alternative markers have been proposed to determine bacterial biodiversity (Case et al. 2007; Lang et al. 2013; Mende et al. 2013).

In this study, we show that—for the six species trees—the tree topology of the 147 QfO reference organisms is congruent at 87 of the 146 possible internal nodes of a rooted binary tree; all other nodes are incongruent in at least one of the species trees. In addition, we assessed the discordance of the different species trees with individual gene trees by using available phylomes for QfO species available at PhylomeDB (Huerta-Cepas, Capella-Gutierrez, et al. 2014). For benchmarking in the context of the QfO activities, we propose the use of an annotated consensus tree (ToL$_c$-147) whose internal branches can be multifurcated at different levels of consistency. We provide a first version here.

Material and Methods

Terms

The terms to describe tree relationships and properties have been used in different ways in publications. In this document, the general notion congruency (antonym: incongruency) refers to identical relationships of taxa in independent gene phylogenies or species phylogenies, as well as for splits obtained from these trees. The term concordance (antonym: discordance) is used to describe congruence between a gene tree and a species tree, likewise when trees were pruned to sets of common taxa. A tree topology—or part of it—is consistent, if identical relationships of taxa or clades have been reconstructed based on different data sets.

Species Tree Comparison

The NCBI taxonomic classification tree was generated at http://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi (July 14, 2014) based on the taxonomic identifiers for the species of the QfO reference proteomes. The sToL tree was constructed at http://supfam.org/SUPERFAMILY/cgi-bin/genome_names.cgi (July 8, 2014). Twenty-five species differed in their strain and could not be mapped based on the taxonomic identifiers. These species were mapped based on the species names (“relaxed species mapping”). Opentree provided a draft of the species tree (April 16, 2014) and the corresponding species mapping to taxonomic identifiers. The 216 eukaryotic species tree published by Huerta-Cepas (peerj.com/preprints/223/) was provided, including NCBI taxonomic identifiers and annotated branch support values (aLRT SH). Seventy-one species were mapped by taxonomic identifiers, 27 by species name. The ToL by Ciccarelli et al. (ToL-C) was copied from http://itol.embl.de/ (July 14, 2014). Forty-four QfO reference organisms were identified by their scientific names. The 16S rRNA-based species phylogeny of the all-species living tree project was obtained from http://www.arb-silva.de/projects/ (LTPs115). Twenty-two archaea and bacteria were mapped to the QfO data set by the species name. Species not matching the QfO reference proteomes were pruned from the trees using the Newick utilities (Junier and Zdobnov 2010); species names were standardized using Perl scripts. Robinson–Foulds (RF) distances between pairs of species trees—except for the two complementing species trees that have no taxa in common—were calculated with ETE v2.2 (Huerta-Cepas et al. 2010).

The species consensus tree (ToL$_c$-147) was constructed manually and consistency levels annotated for multifurcation at different levels of topological congruence. As a first approach, consistency values were assigned for each node and each species tree, according to the following instances: +1, node is congruent with the ToL$_c$-147 topology; +2, same as +1, with significant branch support; 0, multifurcating node; −1, alternative topology (incongruent with ToL$_c$-147); −2, alternative topology with significant branch support (supplementary table S3, Supplementary Material online). The average consistency value was calculated for each node, and results classified into four levels: Level L90 (>1), level L70 (0.75–1), level L50 (>0 to <0.75), and level L10 (≤0). The assigned levels correspond to the annotated consistency values (90, 70, 50, 10) in ToL$_C$-147, so nodes can be multifurcated at different levels with existing tree visualization tools. Further evidence for the evolutionary relationship of species was obtained from the literature, and levels adapted according to findings in a similar way as described above; for instance, when published clade-specific analyses significantly supported nodes of the consensus tree. Finally, a fifth level was introduced for practical reasons, for nodes with conflicting results which await further classification (L30).

Assessment of Gene Tree Support for Species Topologies

For each phylome reconstructed for the QfO project (phylDs 500–542), the trees were downloaded from phylogenomic database phylomeDB (Huerta-Cepas, Capella-Gutierrez, et al. 2014). These gene trees were constructed with a methodology similar to the one used to infer the species phylogeny of Tree-HC, although based on a different set of proteomes. Because gene tree reconstruction was not guided by a species tree and because this experiment is not a quality assessment, this collection of gene phylogenies is suitable to estimate gene tree support for our consensus tree and alternative topologies. Each tree in each phylome was first compared with a given species tree. If they overlapped in less than three species, the tree was discarded. Trees were then pruned so that they contained only species that were present in the species tree. Trees were then rooted by the species placed closest to the root according to the species tree. The tree was then split into orthologous trees following the methodology explained previously (Marcet-Houben and Gabaldon 2011) as implemented in ETE v2.2. Briefly, all duplication nodes were detected in the tree by using a species overlap algorithm (Huerta-Cepas et al. 2007). All the possible combinations of the duplicated parts of the tree were done and then the tree was pruned as many times as combinations of duplicated nodes were found. These pruned trees are called orthologous trees as all the leaves in them are orthologous to each other. Only trees with less than 100 associated orthologous trees were considered to reduce computing time. A support measure was then calculated as follows: Species trees and orthologous trees were pruned so that they contained exactly the same set of species. Then, for each node in the remaining species tree, we searched each orthologous tree for the presence of the node. The number of trees that contained the node was divided by the total amount of trees that contained the species derived from the node, whether they were monophyletic or not. If a tree resulted in more than one orthologous tree, then each orthologous tree was checked for the presence of the node and then divided by the number of orthologous trees that contained the species derived from the node. This was done to ensure that each tree contributed equally to the final result.

Results and Discussion

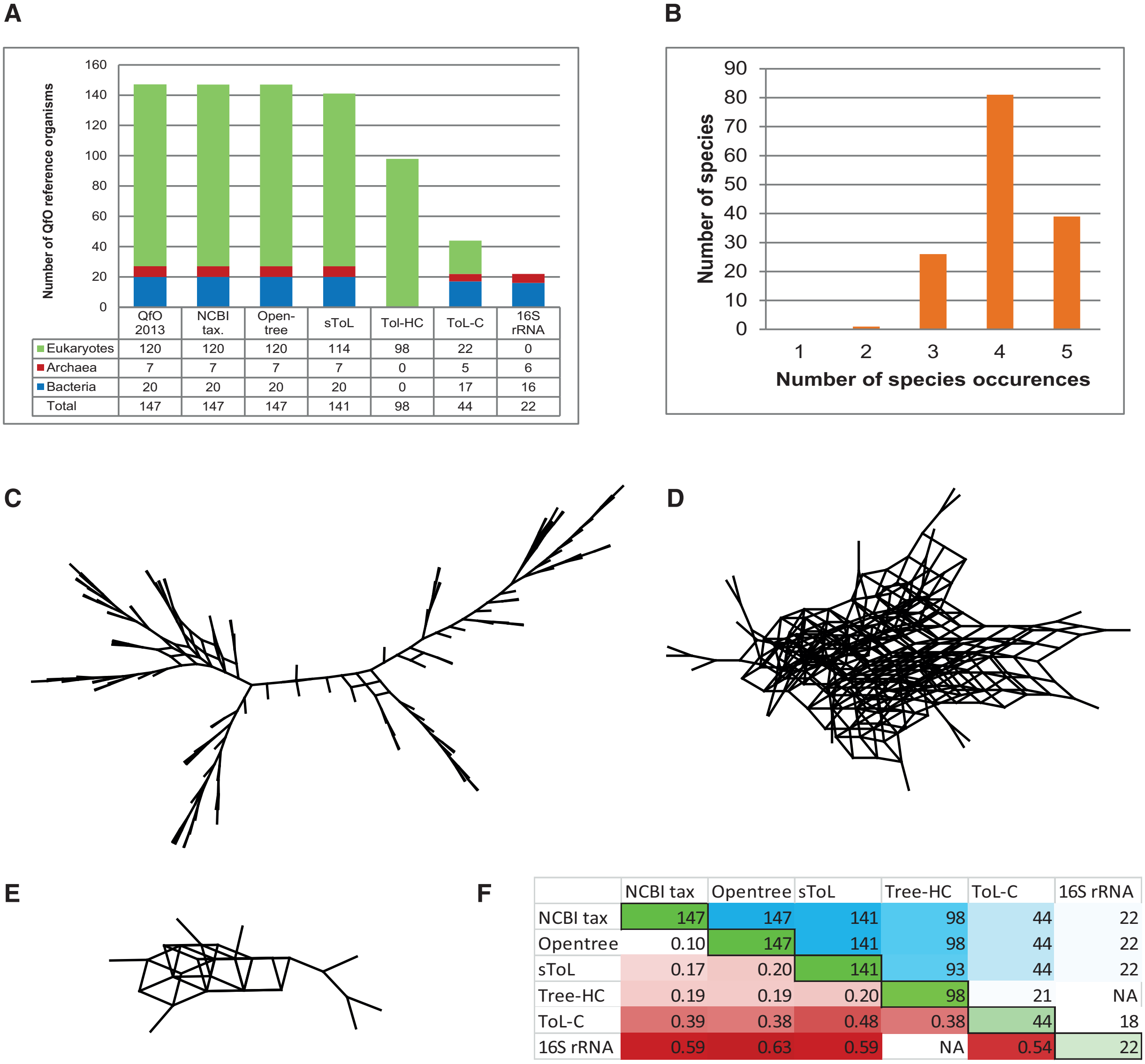
Species Supernetworks: Survey on the Topological Congruence of Species Trees

Six well-known broad species phylogenies were compared: The NCBI taxonomic classification, the Opentree of Life, the sequenced sToL, the 16S rRNA-based species tree, and the species phylogenies published by Ciccarelli et al (ToL-C) and Huerta-Cepas et al (Tree-HC). All these trees were generated by applying different methodologies and using different databases, data sets, and sources of phylogenetic signals (supplementary table S1, Supplementary Material online), which makes the search for a consensus species tree especially meaningful. Nonrelevant taxa were pruned from all trees, resulting in cladograms of up to 147 QfO reference organisms (fig. 1*A* and supplementary file S1, Supplementary Material online). Four of the trees cover species from all the three domains of life (ToLs), and the two complementing species trees consist of prokaryotes and eukaryotes, respectively. Thus, each species can be found in the analyzed data sets—at the most five times—and the average frequency was 4.1 (fig. 1*B*).

At first, the degree of incongruence between the predicted species phylogenies was explored through supernetworks for each of the three domains of life (fig. 1*C*–*E*). In a phylogenetic supernetwork, identical topologies are merged into a tree structure and alternative topologies are combined into a network (Huson et al. 2004). Considerably higher topological congruence is observed in the eukaryotic clade compared with the two prokaryotic clades. One known major factor disturbing phylogenetic reconstruction in prokaryotes is the pervasiveness of HGT in these domains (Bapteste et al. 2004). Rates of HGT have been estimated to lie in the range of 1.6–32.6% (Koonin et al. 2001) and higher (McDaniel et al. 2010), and a constant rate of interspecies gene traffic was reported for universally single copy genes (Trachana et al. 2014).

Quantification of Topological Congruence between Species Trees

There are basically two approaches for the quantification of differences in tree topologies: 1) Tree incompatibility, which is calculated from contradicting splits and does not take into account multifurcation, and 2) tree dissimilarity, which is determined from all nonidentical splits, thus measuring incongruent branching patterns including multifurcation. At first, the former sounds more suitable when aiming to identify a consensus, however it does not take into account unresolved (multifurcating) nodes, and thus equates a star-like tree with the perfect tree. This is why we decided to measure the dissimilarity between species phylogenies by applying the RF



	QfO 2013	NCBI tax.	Open-tree	sToL	Tol-HC	ToL-C	16S rRNA
🟩 Eukaryotes	120	120	120	114	98	22	0
🟥 Archaea	7	7	7	7	0	5	6
🟦 Bacteria	20	20	20	20	0	17	16
Total	147	147	147	141	98	44	22

	NCBI tax	Opentree	sToL	Tree-HC	ToL-C	16S rRNA
NCBI tax	147	147	141	98	44	22
Opentree	0.10	147	141	98	44	22
sToL	0.17	0.20	141	93	44	22
Tree-HC	0.19	0.19	0.20	98	21	NA
ToL-C	0.39	0.38	0.48	0.38	44	18
16S rRNA	0.59	0.63	0.59	NA	0.54	22

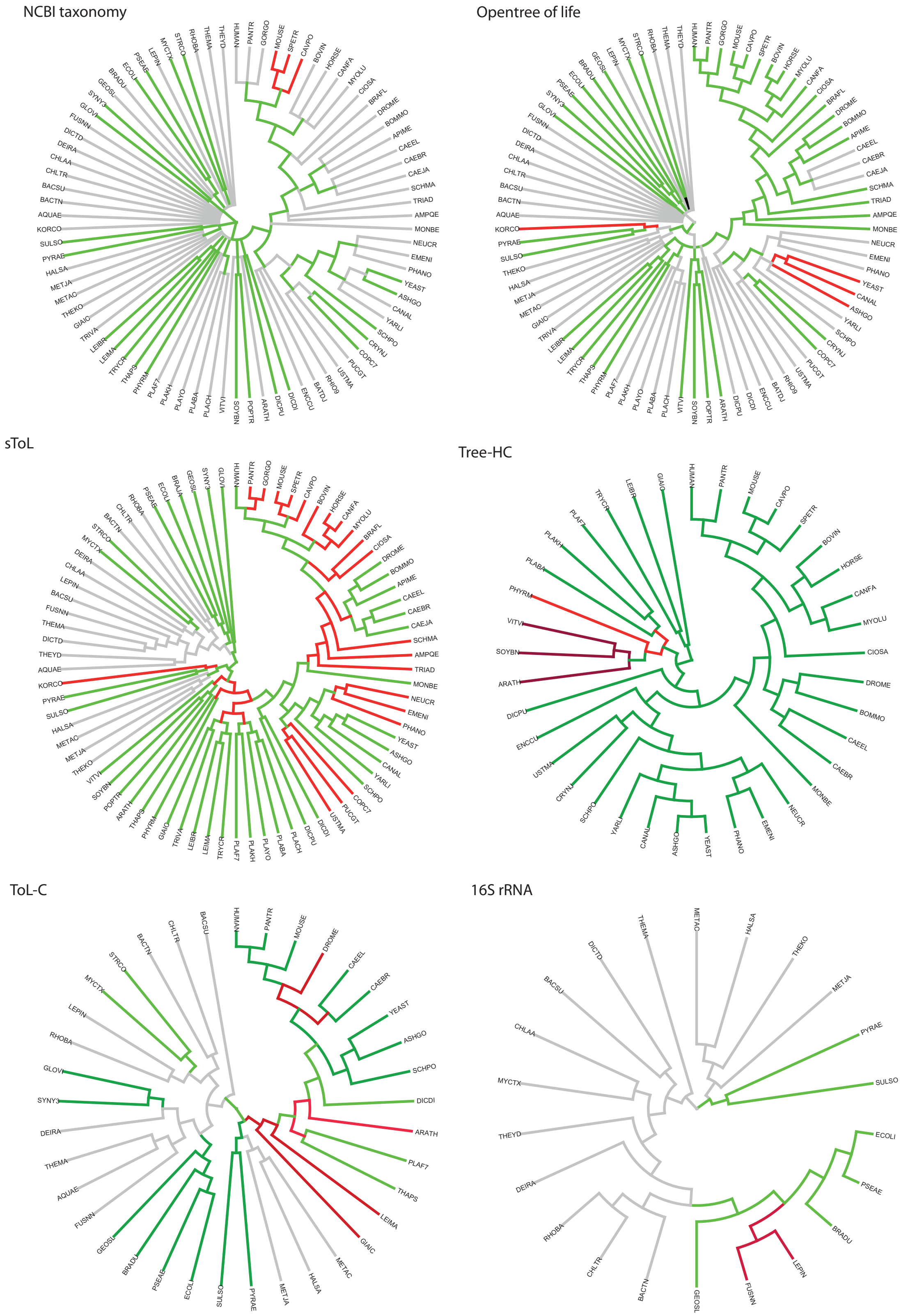
Fig. 1.—Comparison of the six species trees. (*A*) Coverage of QfO species in the analyzed ToLs/species trees: Stacked bar chart of species from the Quest for Orthologs reference proteome set 2013 mapped to the species trees, color-coded by domains of life. The far left column presents the QfO reference organisms. (*B*) Frequency of QfO reference organisms in the analyzed ToLs/species trees. On average, each QfO reference organism occurred in the data set about 4.1 times; represented only twice are the amoeba *Polysphondylium_pallidum* (NCBI TaxId: 13642), the fungi *Rhizopus delemar* (TaxId: 246409) and *Batrachochytrium_dendrobatidis* (NCBI TaxId: 684364). Supernetwork of the eukaryote (*C*), bacterial (*D*), and archaeal (*E*) clade visualize topological congruence and incongruence between ToLs/species trees. (*F*) RF distances between ToLs/species trees: For each tree, the table shows the number of species in common with the species of the QfO reference data set (green cells), the number of QfO reference organisms shared by two trees (blue), and the average RF distances per node between trees (red).

metric (Robinson and Foulds 1981) averaged over the number of nodes common to a tree pair (fig. 1*F*). The resulting normalized RF distances between pairs of species trees range from 10% to 63%, thus confirming that none of the species phylogenies are congruent. Distances are shortest between trees which include a high fraction of eukaryotic species, while they increase according to the fraction of prokaryotic species. This result indicates once again high dissimilarity in bacterial and archaeal phylogenies and is thus in agreement with the above described supernetworks. The smallest distance between ToLs was calculated for the NCBI taxonomy and Opentree. This is not surprising, as Opentree is based on the NCBI taxonomic classification. Both trees still share a relatively large number of unresolved nodes, but Opentree synthesizes the taxonomic hierarchy with published species phylogenies, which results in a higher resolution in *Homininae* or early diverging metazoans (*Amphimedon*, *Trichoplax*, *Bilateria*) for instance. The RF distance is the most commonly used dissimilarity measure for pairs of tree topologies, regardless of its high susceptibility to certain topological differences which can result in maximum distance values, even for the interchange of a single leaf (Lin et al. 2011). Thus one needs to exercise caution and avoid overinterpreting the results. It should also be recalled that—at this stage—it is not possible to assess the quality of inferred species trees for many reasons, most important of all the lack of knowledge on the true tree. Thus, low distances do not indicate high accuracy nor do high distances indicate failure. The goal of this study is to identify topological incongruence between species phylogenies and, to this end, we summarize node consistency based on the comparison of all six species phylogenies: 87 of the 146 possible splits of a bifurcating, rooted tree are congruent in the compared species trees; all other splits are incongruent in at least one of the six trees (supplementary table S3, Supplementary Material online). Noteworthy, NCBI reflects well topological incongruence in the form of multifurcating nodes, but misses some of the highly supported nodes, such as the consistent node for Diptera/*Bombyx mori*, Metazoa/Monosiga, or Opisthokonta.

In eukaryotes, 67% (80/119) of the internal nodes of a rooted binary tree are congruent, 79% (94/119) are congruent or compatible (multifurcating), and alternative phylogenies are suggested for 21% (25/119) of the internal nodes. Various reasons can be given for the topological differences within the eukaryotic clade. Tree reconstruction artifacts are frequently explained by the lack of a phylogenetic signal or the failing to discriminate a phylogenetic signal from noise. The former is commonly observed in closely related species, species which diverged in a short interval from a common lineage (short common branch length) or in genes under strong structural and functional constraints, thus evolving at a low rate and lacking shared traits. When homoplasy prevails synapomorphy in genes of fast-evolving species, taxa tend to be grouped by mistake—an artifact known as LBA (Schulmeister 2004). For the 147 QfO reference organisms, such knowledge can, for instance, help explain topological incongruence observed for discrete representatives of invertebrate clades sampled along the human lineage as well as for species which diverged early from major clades such as ecdysozoans. Differing phylogenies are not only observed at deep nodes, but also at recent speciation events (figs. 2 and 3*A*). The phylogeny of *Homininae* is an example: The topology is unresolved in the NCBI classification, and a monophyletic origin of chimp and gorilla is suggested by sToL. Opentree implemented a resolved but differing species history—human being more closely related to chimp than to gorilla—based on relevant published phylogenies. One of the underlying studies is a recent phylogenetic analysis of complete mitochondrial primate genomes that provides significant support for a common ancestor of human and chimp after the divergence of gorilla, which is in agreement with many other clade-specific analyses (Pozzi et al. 2014). Within the mammalian branch, we note two further clades with contradicting phylogenies: *Glires* and *Laurasiatheria*. Within the first group, the rodents *Cavia porcellus* and *Spermophilus tridecemlineatus* show an interchanged divergence order, and each of the two topologies is suggested by two of the four species trees that include the relevant QfO reference organisms. Tree-HC provides significant branch support for a monophyletic origin of *Murinae* and *C. porcellus*, and Opentree has implemented supporting results for the same topology. For *Laurasiatheria*, none of the species trees suggest the same phylogeny. For the same clades, incongruence is also observed in various published phylogenies, thus indicating that phylogenetic signals which are derived from the different data sets are ambiguous.

In nonvertebrate eukaryotes, differing bifurcating tree topologies are observed within chordates for species of the genus *Ciona* and *Branchiostoma floridae*, for the platyhelminth *Schistosoma mansoni*, for early divergence groups within ecdysozoans, for the metazoans *Trichoplax adhaerens* and *Amphimedon queenslandica*; within fungi for the ascomycetes *Ashbya gossypii*, *Candida albicans*, and *Phaeosphaeria nodorum*, and for the basidiomycete *Puccinia graminis*; within *Viridiplantae* for the *rosids* *Arabidopsis thaliana* and *Vitis vinifera*, and within the genus *Plasmodium* for *Plasmodium berghei*. At deep nodes, incongruent phylogenies concern the groups *Halvaria*, *AH/SAR*, the recently suggested clade *Neozoa* (*Unikonta* and *AH/SAR*; He et al. 2014), and *Excavata*.

As for prokaryotes, 27% (7/26, archaea: 50% [3/6], bacteria: 21% [4/19]) of the internal nodes are congruent, 38% (10/26, archaea: 50% [3/6], bacteria: 37% [7/19]) are congruent or compatible (multifurcating), and 62% (16/26, archaea: 50% [3/6], bacteria 63% [12/19]) are incongruent. At the deepest nodes in the ToL, all relevant species trees agree on the monophyly of bacteria, and that of archaea when rooted with bacteria. The representative group of archaeal QfO reference organisms include both crenarchaeotes and euryarchaeotes, and their monophyly is still disputed (Wolf et al. 2001). Noteworthy, the tree topology for five archaeal species common to Tol-C and the 16S rRNA species tree is congruent and in agreement with a recently published archaeal phylogeny based on over 200 marker genes (Petitjean et al. 2015). Consistent clades are observed within *Archaea* for the class *Thermoprotei* (*Pyrobaculum aerophilum*, *Sulfolobus solfataricus*) and the phylum *Euryarchaeota* (*Halobacterium salinarum*, *Methanosarcina acetivorans*, *Methanocaldococcus jannaschii*, *Thermococcus kodakaraensis*), within bacteria for the phylum *Cyanobacteria* (*Gloeobacter violaceus*, *Synechocystis* sp.), the order *Actinomycetales* (Mycobacterium tuberculosis, Streptomyces coelicolor), the class *Gammaproteobacteria* (*Escherichia coli*, *Pseudomonas aeruginosa*), and the phylum *Proteobacteria* (*Bradyrhizobium diazoefficiens*, *Geobacter sulfurreducens*, and *Gammaproteobacteria*). Other nodes within prokaryotes are unresolved or conflicting.



NCBI taxonomy

Opentree of life

sToL

Tree-HC

ToL-C

16S rRNA

Fig. 2.—Overview of critical spots in reconstructed species phylogenies. For lack of space, all species trees were pruned to include only species which illustrate yet unresolved phylogenies and contradicting topologies. Color codes: Light green = topologies supporting the consensus tree (fig. 3); dark green = topologies supporting the consensus tree with significant support; red = topologies differ from the consensus tree; dark red = topologies with significant support differ from the consensus tree; light gray = unresolved and/or unknown topologies.

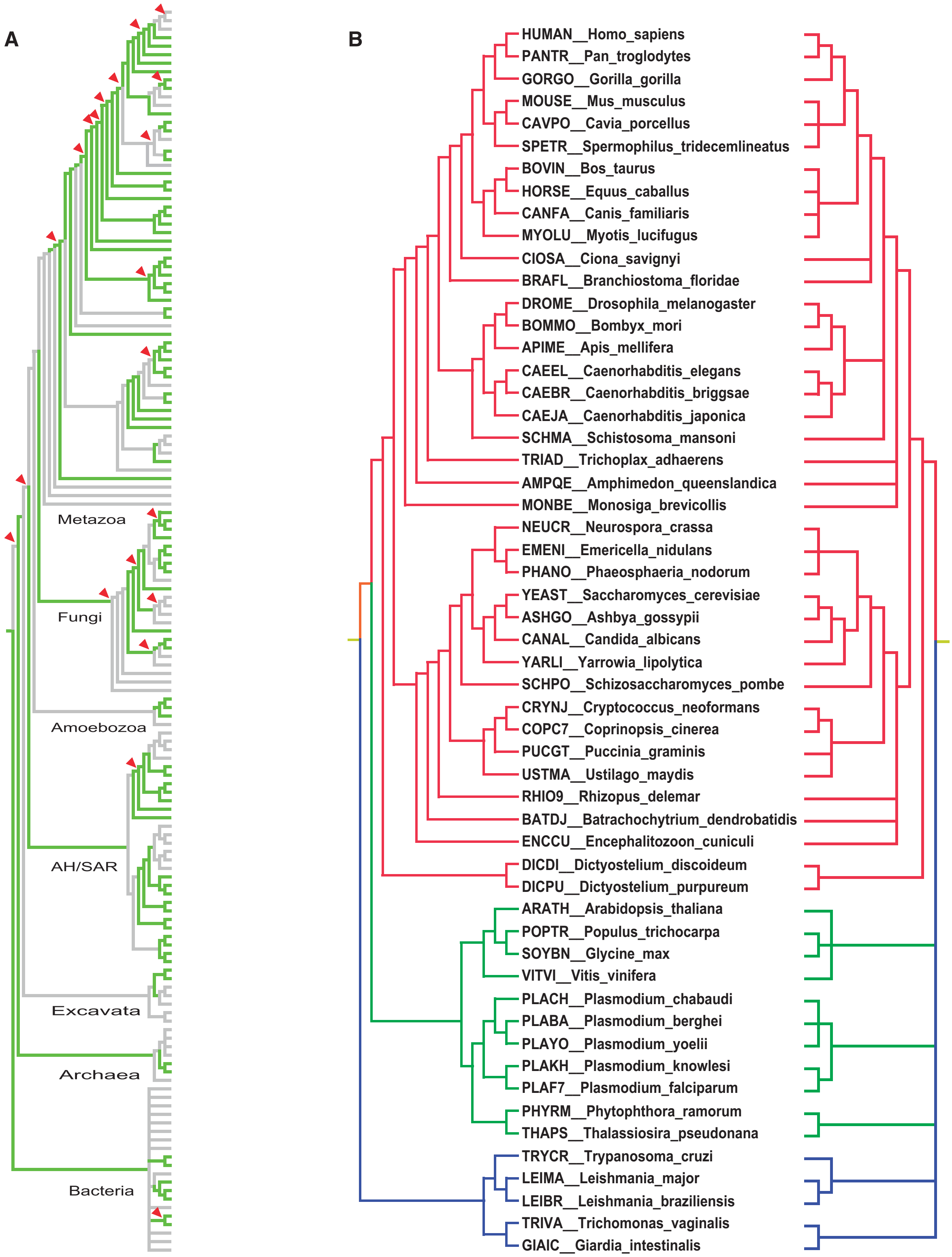

A
Metazoa
Fungi
Amoebozoa
AH/SAR
Excavata
Archaea
Bacteria
B
HUMAN__Homo_sapiens
PANTR__Pan_troglodytes
GORGO__Gorilla_gorilla
MOUSE__Mus_musculus
CAVPO__Cavia_porcellus
SPETR__Spermophilus_tridecemlineatus
BOVIN__Bos_taurus
HORSE__Equus_caballus
CANFA__Canis_familiaris
MYOLU__Myotis_lucifugus
CIOSA__Ciona_savignyi
BRAFL__Branchiostoma_floridae
DROME__Drosophila_melanogaster
BOMMO__Bombyx_mori
APIME__Apis_mellifera
CAEEL__Caenorhabditis_elegans
CAEBR__Caenorhabditis_briggsae
CAEJA__Caenorhabditis_japonica
SCHMA__Schistosoma_mansoni
TRIAD__Trichoplax_adhaerens
AMPQE__Amphimedon_queenslandica
MONBE__Monosiga_brevicollis
NEUCR__Neurospora_crassa
EMENI__Emericella_nidulans
PHANO__Phaeosphaeria_nodorum
YEAST__Saccharomyces_cerevisiae
ASHGO__Ashbya_gossypii
CANAL__Candida_albicans
YARLI__Yarrowia_lipolytica
SCHPO__Schizosaccharomyces_pombe
CRYNJ__Cryptococcus_neoformans
COPC7__Coprinopsis_cinerea
PUCGT__Puccinia_graminis
USTMA__Ustilago_maydis
RHIO9__Rhizopus_delemar
BATDJ__Batrachochytrium_dendrobatidis
ENCCU__Encephalitozoon_cuniculi
DICDI__Dictyostelium_discoideum
DICPU__Dictyostelium_purpureum
ARATH__Arabidopsis_thaliana
POPTR__Populus_trichocarpa
SOYBN__Glycine_max
VITVI__Vitis_vinifera
PLACH__Plasmodium_chabaudi
PLABA__Plasmodium_berghei
PLAYO__Plasmodium_yoelii
PLAKH__Plasmodium_knowlesi
PLAF7__Plasmodium_falciparum
PHYRM__Phytophthora_ramorum
THAPS__Thalassiosira_pseudonana
TRYCR__Trypanosoma_cruzi
LEIMA__Leishmania_major
LEIBR__Leishmania_braziliensis
TRIVA__Trichomonas_vaginalis
GIAIC__Giardia_intestinalis

FIG. 3.—Consensus tree. (*A*) Consensus phylogeny of the 147 QfO reference organisms. Green branches highlight congruent and bifurcating topologies, grey branches indicate topologies that are either multifurcating or incongruent in at least one of the species trees. Red triangles mark nodes that are supported by at least 75% of the gene trees (see also supplementary file S4, Supplementary Material online). (*B*) Eukaryotic clade of the consensus tree at highest (bifurcating, left handed) and lowest (L90, right handed) resolution, pruned to the species set as in figure 2. Bifurcation is not yet possible for most internal nodes in the archaeal and bacterial clades; the topologies are thus identical for both trees.

In summary, for the QfO reference organisms the topological congruence between species trees is more than 3-fold higher in the eukaryotic clade than in the bacterial clade. Discrepancies can be explained with tree reconstruction artifacts and differences in genome evolution. As HGT is prevalent in prokaryotes, inclusion of species or genes prominently subjected to it will heavily affect phylogeny (Trachana et al. 2014).

Consequences for Orthology Prediction and Benchmarking

Needless to say, a resolved evolutionary species history would be preferable for orthology prediction and benchmarking. The largest nonconflicting and bifurcating tree generated from the six species trees would, however, only include 77 species at the most (ToL_c-77; supplementary file S2, Supplementary Material online). Thus, we constructed a consensus tree composed of all 147 QfO reference organisms (ToL_c-147) taking into consideration both the degree of topological incongruence and the branch support, if available. A large fraction of consistent nodes of the eukaryotic clade present in Tree-HC was significantly supported. For yet unresolved and inconsistent nodes, high quality phylogenetic studies were conducted from the literature, because clade-specific analyses commonly possess a higher species density, more marker genes, and less ambiguous or missing characters that originate from distantly

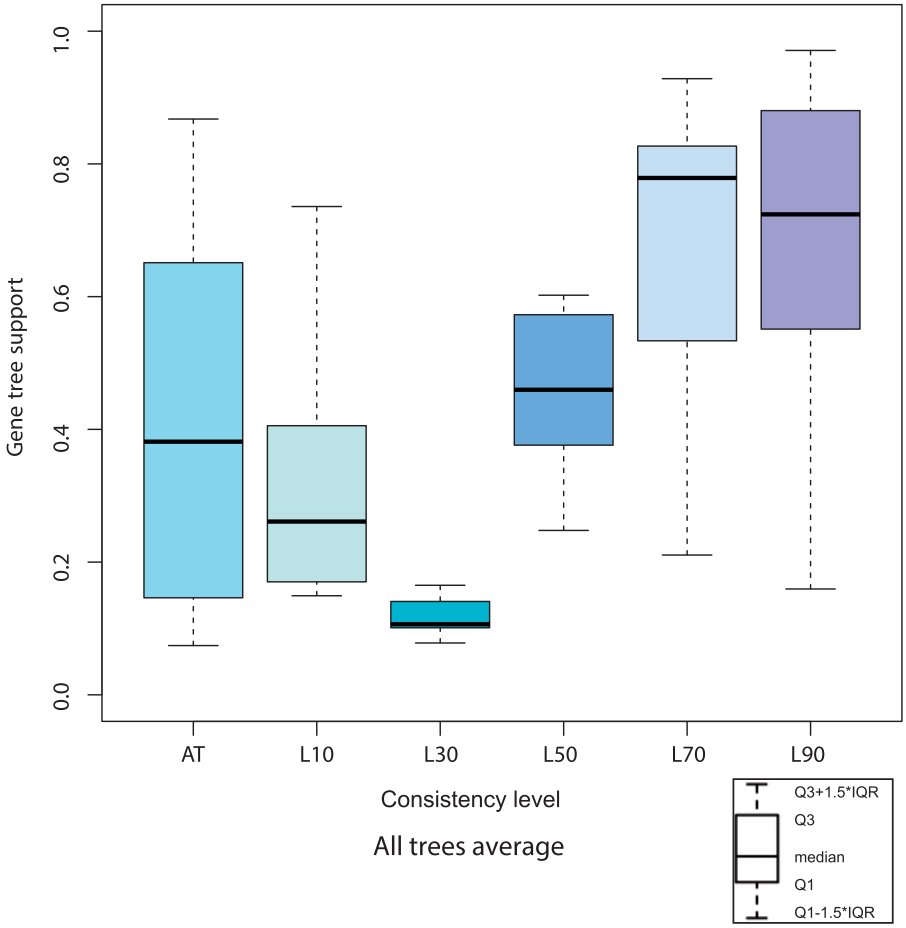


Fig. 4.—Box plot of gene tree fractions supporting species tree topologies at different consistency levels. Consistent species tree topologies with (L90) and without (L70) significant branch support are generally in compliance with the analyzed gene trees. The fraction of supporting gene trees drops considerably when species tree topologies are incongruent, once or more, between the species trees (L10, L30, L50). Consistency categories "L30" and AT were assigned for practical reasons. Level L30 is the default value for conflicting nodes prior to evaluation, and the two remaining nodes (*Excavata*, *Proteobacteria*) show on the one hand conflicting species topologies, on the other hand significant branch support in at least one of the species trees. Only a low fraction of our gene trees supports these speciation nodes. Category AT indicates alternative topologies suggested by the species trees, and results cover the range of conflicting levels (L10, L50); this makes sense because alternative topologies are incongruent with the consensus tree and between species trees. For each box plot, bottom of the box is the first quartile (Q1), top of the box is the third quartile (Q3), the middle bar is the median, whiskers represent the 1.5 interquartile range (IQR).

related species. So far, all studies that have been taken into account concern eukaryotic clades: Primates (Fabre et al. 2009; Pozzi et al. 2014), laurasiatherians (Zhou et al. 2011; Pozzi et al. 2014), chordates (Delsuc et al. 2006; Putnam et al. 2008), nonchordate metazoans (Halanych et al. 1995; Telford et al. 2005; Delsuc et al. 2006; Dunn et al. 2008; Srivastava et al. 2008; Nosenko et al. 2013; Misof et al. 2014), choanozoans (Shalchian-Tabrizi et al. 2008), fungi (Ebersberger et al. 2011; Medina et al. 2011; Capella-Gutierrez et al. 2012), amoebozoans (Lahr et al. 2011; Fiz-Palacios et al. 2013), plants (Wang et al. 2009; Burleigh et al. 2010; Zeng et al. 2014), halvarians (Simpson et al. 2006; Krief et al. 2010; Burki et al. 2012), and deep branches in eukaryotes (Simpson et al. 2006; Hampl et al. 2009; Burleigh et al. 2010). In contrast to eukaryotes, the predicted evolutionary relationships for bacteria and archaea are too divergent to generate a possible bifurcating tree. Consequently, for the time being we retain the polytomy of the NCBI taxonomic classification. As a result, orthology prediction for these domains of life has to be complemented with non–tree-based methods for HGT prediction such as the analysis of codon usage or oligonucleotide profiles. The proposed species consensus tree ToL$_c$-147 is presented in figure 3 and available at http://swisstree.vital-it.ch/species_tree (last accessed July 7, 2015). The assigned consistency values are annotated and can be used to multifurcate nodes at different levels of topological congruence between species trees.

However, even if there were a bifurcating, resolved species tree, there could be a risk in oversimplifying the view on genome evolution. Assuming that the main gene stream—inherited genes with full-length orthology—reflects species evolution, each genome is likely to contain a fraction of genes which is discordant with any species tree. This can be because of horizontal/endosymbiont gene transfer and pseudo-orthologs in accurate gene trees, but it can also be because of the lack of phylogenetic signal and noise resulting from incomplete lineage sorting, gene conversion, changes in domain architectures, wrong gene models, sequence errors, and so on. As an illustration, a recent analysis of the evolution of 48 bird species and the genes encoded in their genomes revealed that no single gene tree was fully congruent with the reconstructed species phylogeny (Jarvis et al. 2014). In a group of closely related vertebrate species where the role of HGT is negligible, such a result indicates that both incomplete lineage sorting and stochastic noise can create widespread discordance between gene trees and species trees. The intentional exploration of this nonsupporting gene fraction might provide advanced insight into genome and species evolution. Consequently, it is worthwhile reflecting the reliability of predicted gene relationships by means of confidence scores which allow experiment-specific data selection by database users. In addition, confidence scores could be used for improved orthology benchmarking, accounting for the sensitivity/specificity trade-off.

Assessment of Gene Tree Support for Species Tree Topologies

To assess the level of discordance between the different species trees and the individual gene trees, we compared each species tree node with 458,108 gene phylogenies built for proteins encoded in 65 QfO reference species (Huerta-Cepas, Capella-Gutierrez, et al. 2014). This analysis was performed on ToL$_c$-147 as well as on each species tree. By analyzing results according to the assigned consistency levels, we show that gene tree topologies coincide more often with consistent nodes (consistency levels L90 and L70) in species trees than with conflicting ones (consistency levels L10, L30, and L50) (fig. 4). This trend is also observed in the individual box plots for each project (supplementary file S3, Supplementary Material online). Interestingly, species trees which differ from the consensus tree (hereafter named "alternative species topologies" and assigned category "AT" for practical reasons) occur as the most dispersed group. The range of its gene tree support values corresponds to that determined for incongruent nodes. In fact, category AT includes many prokaryotic speciation nodes which are still polytomous in ToL$_c$-147 because of incongruent topologies in the species trees, thus explaining the comparatively tall box plot. Even when assuming ToL$_c$-147 to present the true tree, gene trees congruent with alternative species topologies can be correct, for instance, when containing xenologs or pseudo-orthologs. ToL$_c$-147 with annotated gene tree support is presented in supplementary file S4, Supplementary Material online.

Conclusions

This study sheds light on the current resolution of the species phylogeny for the 147 QfO reference proteomes. Open questions concern not only the true species tree, but likewise which type of phylogenetic signal is most informative for tree reconstruction at a particular level of depth and taxonomic range. As for sequence-based phylogenetic analysis, the selection of good marker genes and a higher and balanced species density in the tree space may help to stabilize the tree topology. A comparison of species trees obtained from the analysis of different types of characters, including morphological ones, can help to identify strengths and weakness of each approach in order to optimize species tree inference. Other issues worthwhile discussing between expert communities include taxon sampling and visualization of annotated evolutionary changes which result in the characteristics of extant species at any level of organization. We hope that this work will contribute to a lively exchange between the QfO and ToL communities.

For orthology benchmarking, we propose a species consensus tree with conservation values associated to each node for multifurcation at the desired consistency level. We provide here the first version of one such reference tree covering the QfO reference species.

Supplementary Material

Supplementary tables S1–S3 and supplementary files S1–S4 are available at Genome Biology and Evolution online (http://www.gbe.oxfordjournals.org/).

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