Running head

Tree root inference from gene duplication events

Title

STRIDE: Species Tree Root Inference from Gene Duplication Events

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Abstract

The correct interpretation of a phylogenetic tree is dependent on it being correctly rooted. A gene duplication event at the base of a clade of species is synapamorphic, and thus excludes the root of the species tree from that clade. We present STRIDE, a fast, effective, and outgroup-free method for species tree root inference from gene duplication events. STRIDE identifies sets of well-supported gene duplication events from cohorts of gene trees, and analyses these events to infer a probability distribution over an unrooted species tree for the location of the true root. We show that STRIDE infers the correct root of the species tree for a large range of simulated and real species sets. We demonstrate that the novel probability model implemented in STRIDE can accurately represent the ambiguity in species tree root assignment for datasets where information is limited. Furthermore, application of STRIDE to inference of the origin of the eukaryotic tree

resulted in a root probability distribution that was consistent with, but unable to distinguish between, leading hypotheses for the origin of the eukaryotes. In summary, STRIDE is a fast, scalable, and effective method for species tree root inference from genome scale data.

Keywords

Phylogenetics; phylogenomics; gene duplication;

Introduction

"*Nothing in biology makes sense except in the light of evolution*" (Dobzhansky, T. 2013), "*nothing in evolution makes sense except in the light of phylogeny*" (Sytsma, K.J. and Pires, J.C. 2001), and nothing in a phylogeny makes sense except in the light of its root. For example, the phylogeny for four species (Fig. 1A) has five possible roots (Fig. 1B-F) and each of the different roots corresponds to a different hypothesis as to the evolutionary history of the species. For the presented tree, identifying a wrong branch as the root (for example Fig. 1E) would lead us to conclude that elephants are more closely related to fish and birds than they are to wolves, even though we are using a tree with the correct topology. A species tree only gives the correct evolutionary relationships when rooted correctly (Fig. 1B). Thus it is of critical importance to our interpretation of relationships, and the evolutionary history of life on earth, that we have accurate methods of inferring the root of species phylogenies.

Correct species tree rooting is also of critical importance for the inference of orthology relationships between genes. Given an unrooted gene tree (Fig. 2A), knowledge of the correct branching order of the species tree (Fig. 1B) is required to correctly root the gene tree (Fig. 2B). An incorrect rooting of the species tree (Fig. 1C-F) leads to an incorrect inference of the root of the gene tree (Fig. 2C-F), and thus incorrect identification of orthologous genes (Fig. 2G-H). Therefore, our ability to compare the biology of species,

through comparisons between orthologous genes, is reliant on accurate methods of inferring the root of species phylogenies.

Although correct root placement is essential for our ability to interpret phylogenies, almost all models of sequence evolution used for tree inference are time-reversible and produce unrooted phylogenetic trees. In order to identify the root of a phylogeny extra information is required, usually knowledge of an extra species that is a suitable (i.e. closely related) outgroup for the set of species for which the root is unknown. However, outgroup choice is a common source of error in phylogenetic tree inference, with distantly related outgroups leading to inaccurate root placement and distortion of the phylogeny due to long branch attraction (Felsenstein, J. 1981) (Berger, S.A., Krompass, D., et al. 2011). While time-irreversible models of sequence evolution exist, they do not implicitly provide a method for accurately inferring the direction of time in a tree (Huelsenbeck, J.P., Bollback, J.P., et al. 2002, Williams, T.A., Heaps, S.E., et al. 2015). To address this issue, methods have been developed that can simultaneously infer rooted species and gene trees (Boussau, B., Szollosi, G.J., et al. 2013). However, these methods are computationally expensive and do not scale well to moderate or large species sets.

"Duplicate gene rooting" has been proposed as an alternative method for rooting species trees (Donoghue, M.J. and Mathews, S. 1998, Simmons, M.P., Bailey, C.D., et al. 2000). The conceptual basis for this approach is that gene duplication events are time-irreversible, unlike character substitution, and thus infer the direction of time on the species tree. Specifically, every node in an unrooted, binary gene tree has three branches incident upon it. If the node is a speciation node then any of the three incident branches could be the edge in the direction of the root, with the other two being in the opposite direction. Thus, speciation nodes are uninformative about the direction of time along the tree. For a duplication node, however, the symmetry is broken. Two of the edges will correspond to the two copies of the gene post-duplication, while the third edge will

correspond to the gene pre-duplication and thus point towards the root of the tree (Fig. 2A, node marked '*D*'). In the case of this example tree, it can be inferred that the root of the species tree must be outside of the subtree containing elephant and wolf. In an idealised case (with no effects such as incomplete lineage sorting or lateral gene transfer) the two post-duplication branches can be distinguished from the pre-duplication branch as the post-duplication branches contain genes from overlapping species sets. Furthermore, these species sets will be identical if there has been no gene loss or horizontal gene transfer, and the topology of the duplicate subtrees will recapitulate the species tree topology. Thus, if gene duplication nodes can be accurately identified in an unrooted gene tree, then the direction of time can be ascertained for all branches in the post-duplication subtrees. The direction of time on these branches determines the direction of time on the corresponding branches of the species tree, and multiple gene duplication events can be aggregated to determine the direction of time across the whole species tree, thus revealing the location of the root.

Here we present STRIDE, a novel algorithm for Species Tree Root Inference from gene Duplication Events. STRIDE identifies sets of well-supported gene duplication events from cohorts of gene trees, and analyses these events to infer a probability distribution over an unrooted species tree for the location of the true root. We show that STRIDE correctly identifies the community-accepted root of the majority of species trees. Additionally, we demonstrate that STRIDE effectively captures uncertainty in root placement when data is limited or conflicting. Finally, we demonstrate the utility of STRIDE to challenging phylogenetic problems by providing an outgroup-free root analysis of the origin of the eukaryotes.

Methods

Problem definition and approach

A branch of an unrooted species tree corresponds to a bipartition that splits the tree's taxa into two blocks. The presence of a well-supported gene duplication that respects the topology of the species tree is a synapamorphy that stipulates that the block in which the duplicates are found is a monophyletic clade. This synapamorphy identifies the direction of time along the branches within this monophyletic clade. The single exception to this is the branch in the unrooted tree corresponding to the root in which time flows in both directions. This is because the branch that spans the root in the unrooted species tree corresponds to two branches in the rooted species tree and both of its corresponding blocks are monophyletic clades (Fig. 1A & B). The method presented here aims to identify this root branch by identifying and analysing a set of well-supported gene-duplication events. The method identifies the complete set of gene duplication events contained within a set of user-supplied gene trees and uses these to infer the location of the root of the species tree. To express uncertainty in the case of limited data or data conflict, the method uses a probabilistic model of gene-duplication events to calculate a probability distribution across the branches of the species tree for the location of the root.

Inference of Orthogroups and Gene Trees

For each species set, the protein sequence translations of representative gene models were downloaded from appropriate online databases. These protein sequences were then subject to orthogroup inference using OrthoFinder v1.1.4 (Emms, D.M. and Kelly, S. 2015). The resulting sets of protein sequence orthogroups were aligned using MAFFT L-INS-I v7.305b (Katoh, K. and Standley, D.M. 2013) and subject phylogenetic inference using IQTREE v1.5.3 (Nguyen, L.T., Schmidt, H.A., et al. 2015). All methods used their default settings. Parallelisation of MAFFT and IQTREE runs was done using GNU Parallel (Tange, O. 2011). Alignments were viewed using AliView (Larsson, A. 2014).

Trees were viewed using Dendroscope (Huson, D.H. and Scornavacca, C. 2012) and drawn using the ETE library (Huerta-Cepas, J., Serra, F., et al. 2016).

Identification of Putative Duplications

Gene-duplication events are considered informative if they occur on any branch other than a terminal branch in the species phylogeny, as a duplicated gene that occurs only in a single species is not informative to the position of the root of the tree. To identify informative gene duplication events a novel tree analysis algorithm was developed (Fig. 3). Prior to analysis of the gene trees, the unrooted species tree was analysed to determine the species sets in which genes would be expected to occur following a gene duplication event along any branch of the species tree. For each direction along each branch the sets of species in the child clades (X & Y) and in the grandchild clades (x_1, x_2, y_1 & y_2) immediately following that branch were identified (Fig. 3A). Then each gene tree was analysed in turn to identify well-supported gene duplication events within the gene tree as follows: each node, n, in the tree was considered in turn, if the node was an unresolved polytomy it was excluded as such nodes correspond to either a higher-order multiplication events (e.g. triplication) or an unresolved event in the gene tree (e.g. an amalgamation of several weakly supported bipartitions). Each analysed node therefore had three branches incident on it, and any pair of branches could potentially represent duplicate genes (Fig. 3B). For each pair of branches, b_1 and b_2, the sets of species, S_1 and S_2, below each branch were used to identify the locations in the species tree corresponding to these branches in the gene tree. This was done by identifying the smallest block, B_i in the species tree that contains all the species in S_i (i=1,2), thus making the method robust in the case of subsequent gene loss (Fig. 3B). If there was more than one block satisfying this criteria, each of these possible blocks were considered. A node, n, was considered as a putative gene duplication event if B_1=B_2.

Nodes that were identified as putative gene duplication events were further examined to reduce the possibility that their existence or location had been misidentified due to errors in gene tree inference. The criteria were: 1) There must be at least one gene from each of the expected grandchild clades in both S_1 and S_2 (Fig. 3C). 2) The branching structure immediately after the gene duplication event on branches b_1 and b_2 must match the expected branching structure (Fig. 3D), i.e. the first node for each duplicate split the descendent species into the expected sets X and Y, or subsets thereof. Note that it would not be valid to check the topology to the level of grandchild clades in step 2 since this would fail to identify gene duplication events if there were also a subsequent gene duplication event one branch lower in the species tree. In this case, the observed grandchild clades would both be subsets of one of the expected child clades rather than grandchild clades. Steps 1 and 2 check that the observed clades are subsets of the expected clades (rather than requiring they be equal to) as this is necessary to make the method robust to subsequent gene loss events.

Identifying the Maximum-Parsimony Root of the Species Tree

As discussed above, a gene duplication on a bipartition of an unrooted species tree i stipulates the direction of time for all branches of the subtrees derived from that bipartition. Given a set of gene duplication events, the branch in the species tree that violates the fewest gene duplication events is identified as the maximum parsimony root. If multiple such branches exist then they are each identified as equally parsimonious.

Probability model for the root of the species tree

For any given set of gene-trees, it is possible that errors in gene-tree inference will lead to false positive inference of gene duplication events that past the filtration criteria. To account for this, a probability model was developed for the location of the root of the tree given the set of (potentially conflicting) putative gene duplication events identified. The model consisted of two parts. The first part, the branch-level model, calculated the

probability that a branch was the root given only the duplications observed in either direction along that branch. The second part, the tree-level model, aggregated all duplications observed across all branches of tree to give the final probability distribution for the location of the root taking into account all information obtained from all gene duplication events observed across the tree.

At the branch-level, the set of putative gene duplication events identified on that branch are modelled by two Poisson processes, one giving rise to true positive gene duplications and the other to false positive duplications. On a given branch, *i*, of a species tree, m_i duplications are observed that support time flowing in one direction along the branch, $\rightarrow$, and n_i duplications supporting time flowing in the opposite direction, $\leftarrow$. The set of duplications on branch *i* is then written, $d_i = \binom{m_i}{n_i}$, and *D* is the set of putative duplications observed on all branches of the species tree, $D = \{d_1, d_2, \ldots, d_b\}$.

The final tree-level probability distribution $P(i = root|D)$ takes into account the complete set of duplications, D, observed on all branches of the tree rather than just the duplications, d_i, observed on a single branch:

$$P(o_i = root|D) = \frac{\prod_j P\left(o_j^{(i)}|d_j\right)}{\sum_k \prod_j P\left(o_j^{(k)}|d_j\right)} \quad (1)$$

where $o_j^{(i)} \in \{\rightarrow, \leftarrow, \text{root}\}$ is the orientation of the branch *j* that would be implied by the root of the tree being branch *i*. That is, the probability distribution for the root given all the gene duplication events on the tree can be expressed in terms of the probabilities for the orientation of each branch given only the gene duplications on that branch; $P(\rightarrow|d_i)$, $P(\leftarrow|d_i)$ and $P(\text{root}|d_i)$.

196 ***Poisson Model for Gene Duplications***

197 To calculate $P(o_i|d_i)$ the duplications observed on a branch are modelled as arising
198 from two Poisson processes. One process describes the number of true positive
199 duplications (corresponding to the actual direction of time along the branch) and the other
200 describes the number of false positive duplications. Let α be a parameter giving the
201 relative frequency of false positives to true positives across all branches of the tree. Then
202 $m \sim Po(\lambda)$ and $n \sim Po(\alpha\lambda)$, where λ is the expected number of true positives on the branch.
203 We set the total expected number of duplications on the branch from the two Poisson
204 processes to match the actual number observed, N. Thus $\lambda = N/(1+\alpha)$. The relative rate
205 of false positives to true positives across the whole tree can be estimated from the number
206 conflicting duplications given the maximum parsimony root of the tree. So as not to over-
207 penalise false-positive duplications, we take α to be one tenth of the ratio of conflicting to
208 non-conflicting duplications of the maximum parsimony root.

209 Bayes' rule gives

210 $$P(o_i) = \frac{P(d_i|o_i)P(o_i)}{P(d_i)}$$

211 where $P(d_i) = \sum_{o\in\{\rightarrow,\leftarrow,r\}} P(d_i|o)P(o)$. The priors are given by $P(r) = {}^{1}/_{b}$ and $P(\rightarrow) =$
212 $P(\leftarrow) = {}^{b-1}/_{2b}$, where b=2t-3 is the number of branches on an unrooted tree with t taxa.
213 The probability mass function for the Poisson distribution immediately gives $P(d|\rightarrow)$ and
214 $P(d|\leftarrow)$:

215 $$P(d|\rightarrow) = Po(m;\lambda)Po(n;\alpha\lambda)$$

216 $$= \frac{\lambda^m e^{-\lambda}}{m!}\frac{(\alpha\lambda)^n e^{-\alpha\lambda}}{n!}$$

217 and,

218 $$P(d|\leftarrow) = Po(n;\lambda)Po(m;\alpha\lambda)$$

$$= \frac{\lambda^n e^{-\lambda}}{n!} \frac{(\alpha\lambda)^m e^{-\alpha\lambda}}{m!}$$

The branch with the root is more complicated since it actually corresponds to two branches on the rooted tree we are attempting to recover. On these two branches time flows in opposite directions, away from a central root that separates them. We must allow for the $\binom{m}{n}$ duplications on the branch to actually correspond to $\binom{m-s}{t}$ duplications on one of the two branches and $\binom{n-t}{s}$ on the other branch (with opposite orientation to the first). The number of false positive duplications, *s* and *t*, are unknown and therefore must be summed over. Similarly, the location of root could fall at any point along the length of the original branch. If the root were a fraction, *x*, along the length of the branch then the expected rate of false positive and true positive duplications on that fraction of the branch would be $x\lambda$ and $x\alpha\lambda$ respectively whereas on the other branch the rates would be $(1-x)\lambda$ and $(1-x)\alpha\lambda$. Thus, integrating over the position of the root along the branch and summing over the distribution of the $\binom{m}{n}$ putative duplications between true positives and false positives on the two resulting branches, we find:

$$P(d|r) = \sum_{s=0}^{m}\sum_{t=0}^{n}\int_0^1 Po^T(m-s;x\lambda)Po^F(t;x\alpha\lambda)Po^T(n-t;(1-x)\lambda)Po^F(s;(1-x)\alpha\lambda)\,dx$$

$$= \sum_{s=0}^{m}\sum_{t=0}^{n} B(m-s+t+1, n-t+s+1)\frac{\lambda^{m-s}e^{-\lambda}}{(m-s)!}\frac{(\alpha\lambda)^{n-t}e^{-\alpha\lambda}}{(n-t)!}\frac{\lambda^{s+t}\alpha^{s+2t-n}}{s!\,t!}$$

Where *B(,)* is the beta function.

The duplications observed in just one species are uninformative as to the location of the root and so should not affect the root probabilities produced by the model. As such, the branch model for terminal branches is modified to only model the number of inward duplications (those supporting the tree minus the species on the terminal branch as a monophyletic clade). The rates $\lambda_{Term,TP}$ and $\lambda_{Term,FP}$ are the observed true positive and

241 false positive rates for inward duplications on the terminal branches for the maximum
242 parsimony root. For the terminal branches, the branch model is:

243 $$P^{Term}(d|\rightarrow) = \mathrm{Po}\left(m; \lambda_{Term,FP}\right)$$

244 and

245 $$P^{Term}(d|\mathrm{r}) = \mathrm{Po}\left(m; \lambda_{Term,TP}\right).$$

246

247 The branch-level model takes into account only the duplications observed on a
248 single branch and these probabilities feed into the tree-level model to give the final
249 probabilities for the position of the root (Fig. 4). The behaviour of the branch model is in
250 good agreement with an intuitive understanding of the probabilities that should be
251 assigned to the three possible orientations for a branch given the number of putative
252 duplications observed in either direction (Fig. 4A-C). The probability of time flowing to the
253 left/right increases monotonically with the number of putative duplications supporting it.
254 The probability of a branch being a root is highest when the number of putative gene
255 duplications in both directions is the same. Finally, the probability of a branch being a root
256 remains significantly above zero if there is any number of gene duplications in both
257 directions (Fig. 4B & C). This reflects the fact that putative gene duplications supporting
258 the monophyletic nature of both blocks of a bipartition support that bipartition being the
259 root. The fact that there could be a large difference in the number of gene duplications in
260 one direction compared to the other due to different branch lengths on the two sides of the
261 root is accounted for by integrating over the position of the root along the original root
262 branch. Thus, the probability of a branch being a root is > 30% when there are 20
263 duplications in one direction compared to 5 in the opposite direction (Fig. 4C). For
264 comparison, the probability of the orientation of the branch being in the direction of the 5
265 duplications is vanishingly small (~10^{-13}). The branch-level probability model thus gives

266 probabilities for each branch taking into account only the duplications observed on that
267 branch. The final probabilities for the root of the tree, taking into account all duplications
268 across the tree are then given by the tree-level model (Equation 1, Fig. 4D & E).

269 ***Algorithm implementation and availability***

270 STRIDE is implemented in python. Further information, use instructions, an example
271 dataset, and a standalone implementation of the algorithm is available under the University
272 of Oxford Academic Use Licence at https://github.com/davidemms/STRIDE. The complete
273 set of gene trees and species trees required to replicate this analysis are provided for
274 download form the Zenodo research data archive at
275 https://doi.org/10.5281/zenodo.581360.

276 ## *Results*

277 ***STRIDE identifies the correct root of species trees given simulated gene tree***
278 ***datasets***

279 The ability of STRIDE to correctly infer the root of a known species tree was tested
280 using three published, simulated gene tree datasets. The first dataset consisted of 2000
281 simulated gene trees from 40 species with heterogeneous rates of gene duplication and
282 loss within trees (Boussau, B., Szollosi, G.J., et al. 2013). The second and third datasets
283 consisted of 12000 gene trees from 12 species and 7500 gene trees from 17 species,
284 respectively. These two datasets were similar to the first dataset but also incorporated
285 incomplete lineage sorting generated using a range of effective population sizes (Wu,
286 Y.C., Rasmussen, M.D., et al. 2014). Since incomplete lineage sorting can lead to
287 misidentification of gene duplication and loss events these latter two datasets provided a
288 good test of STRIDE's robustness in the face of gene-tree/species-tree incongruence. For
289 all three simulated datasets, STRIDE correctly inferred the root of the species tree and

assigned it a probability of 100% (Table 1, Supplementary File 1. Fig. S1-S3). Thus for these simulated datasets the method performed well.

Application of STRIDE to real species datasets

Simulated datasets generally do not capture all the nuances and difficulties seen in real biological datasets. These nuances include errors in orthogroup inference, alignment inference and gene tree inference. Thus to demonstrate the utility of STRIDE, a diverse range of groups of species were sampled from throughout the eukaryotic domain (Table 1). This included every group of eukaryotes on Ensembl Genomes containing more than 4 genera (Yates, A., Akanni, W., et al. 2016). To expand this group of tests, additional sets of genomes were obtained for 47 Birds (Jarvis, E.D., Mirarab, S., et al. 2014), 42 Green Plants (Goodstein, D.M., Shu, S.Q., et al. 2012) and 16 Kinetoplastids (Aslett, M., Aurrecoechea, C., et al. 2010). In total, this gave 12 species groups with varying levels of taxon sampling and with estimated divergence times ranging from c. 56 million years for the Primates (dos Reis, M., Donoghue, P.C.J., et al. 2014) to c. 1500 million years for the Green Plants (Parfrey, L.W., Lahr, D.J.G., et al. 2011). These species sets thus provided a diverse group with which to test the utility of STRIDE. Furthermore, for each of these species sets, there is an accepted consensus on the topology and location of the root of the species tree (Supplemental File 1). In all cases these topologies and root branches were assumed to be true when STRIDE's performance was assessed. On average, across each of the simulated and real dataset in this analysis STRIDE took ~18 seconds to run using four cores of an Intel Core i7-4770 3.4GHz CPU.

Orthogroups for each species set were inferred using OrthoFinder (Emms, D.M. and Kelly, S. 2015), and gene trees for each orthogroup were inferred using IQTREE v1.5.3 (Nguyen, L.T., Schmidt, H.A., et al. 2015) from a multiple sequence alignment generated using MAFFT L-INS-I v7.305b (Katoh, K. and Standley, D.M. 2013). For each species set, STRIDE was run with a published unrooted species tree (without branch

316 lengths) and the complete set of gene trees inferred from all orthogroups identified by
317 OrthoFinder. The number species, gene trees, informative duplications and other details
318 are provided in Table 1.

319 In all 12 test cases, there is a single maximum parsimony root. In 9 of the 12 tests
320 this root agreed with the accepted root of the species set (Table 1). Figures 5 to 7 present
321 the results of the STRIDE analysis applied to the plant, fungi, and bird datasets. These
322 datasets correspond to the largest, median and smallest number of informative
323 duplications per species identified by STRIDE. The results for the remaining datasets can
324 be found in Supplemental File 1 Figures S4-S12. For the plant dataset, sufficient gene-
325 duplication events were identified for the probability model to assign a probability of 100%
326 to the accepted root separating the algae from the land plants (Ruhfel et al. BMC
327 Evolutionary Biology201414:23) (Fig. 5). A probability of 100% was also assigned for the
328 correct root in the fungi, even though fewer informative gene duplication events were
329 identified (Fig. 6, Table 1). In both the plant and fungal datasets, STRIDE also identified
330 substantial numbers of gene duplication events that support sub-clades within both
331 species trees (Fig. 5 and Fig. 6).

332 While STRIDE identified the community-accepted root in 75% of the datasets, it
333 failed to identify this root for the bird (Fig. 7), rodent and Laurasiatheria (Supplementary
334 File 1 Fig. S11 & S12) datasets. These three datasets had the smallest, second smallest
335 and fourth smallest number of informative gene duplication events per species respectively
336 (Table 1). In addition, while there were no conflicting gene duplication events in the bird
337 dataset, the rodent and Laurasiatheria datasets had the highest and fifth highest ratio of
338 conflicting to informative duplications (Table 1). Consistent with these observations,
339 analysis of the factors affecting the accuracy of STRIDE revealed that root probability
340 assignment was positively correlated with the number of informative duplications per
341 species (R^2=0.17, Supplementary File 1 Fig. S13A) and negatively correlated with the

proportion of duplications which were in conflict (R^2=0.24, Supplementary File 1 Fig. S13B). Furthermore, the proportion of conflicting duplications was negatively correlated with the number of species (R^2=0.36, Supplementary File 1 Fig. S13C), suggesting increased taxon sampling facilitated more accurate identification of gene duplication events. Thus the ability of STRIDE to detect the true root is affected by taxon sampling and the number of gene duplication events detected in the dataset.

STRIDE Provides Evidence for Location of the Root of the Eukaryotic Tree

Given the performance of stride on the datasets outlined above it was assessed whether STRIDE could provide insight into one of the most contentious and difficult tree rooting problems in biology, the root of the eukaryotic tree (Burki, F. 2014). Here, a set of 45 species that were distributed across the eukaryotic tree were selected. These were subject to orthogroup and gene tree inference as before and the complete set of 16770 gene trees were submitted for analysis by STRIDE. This identified 2316 putative gene duplication events excluding the root from (and supporting the monophyly of) major clades within the eukaryotes including the opisthokonta, fungi, metazoa, and achiplastida (Fig. 8A). Duplication events supporting further subclades within these major groupings were also abundant (Fig. 8A). In contrast, other major sub-clades including amoebazoa, the SAR supergroup, and the excavata, did not receive support from gene duplication events (Fig. 8A). This lack of gene duplication events meant that STRIDE could not exclude the root of the species tree from the basal branches of these groups and thus could not provide evidence for or against the five most popular placements for the root of the eukaryotic tree (Burki, F. 2014). This ambiguity in root assignment is represented effectively in the probabilities assigned to all putative root-spanning branches (Fig. 8B).

Discussion

STRIDE is an automated method for identifying and analysing gene duplication events to infer the root of species trees. Through analysis of simulated and real datasets,

368 we show how the performance of STRIDE is affected by data quantity, data conflict, and
369 taxon sampling. Furthermore, we demonstrate that STRIDE is effective in identifying the
370 root of species trees for the majority of species datasets and effectively captures the
371 ambiguity in root assignment given the input data.

372 The aim of STRIDE is to infer a probability distribution over an entire species tree
373 for the location of its root. This aim is different from algorithms that attempt to reconcile
374 gene trees with species trees (Szollosi, G.J., Tannier, E., et al. 2015) or model duplication
375 and loss processes on a tree (Gorecki, P. and Eulenstein, O. 2014). STRIDE identifies and
376 utilises well-supported gene duplication events and does not evaluate gene loss events for
377 the following reasons. First, gene trees can distinguish parallel duplication events on
378 adjacent branches from a single shared duplication event, which is not possible for gene
379 loss events. Second, the topology of the gene tree post-duplication genes can be
380 compared with the species tree to confirm the accuracy of the inference, this cannot be
381 done with gene loss events. Third, most genomes are incomplete and vary considerably in
382 the quality of their annotation leading to high rates of false positive gene loss (Veeckman,
383 E., Ruttink, T., et al. 2016, Dunne, M.P. and Kelly, S. 2017).

384 A major advantage of using STRIDE is that sets of species can be analysed without
385 the inclusion on an outgroup. This is potentially advantageous in situations where inclusion
386 of an outgroup can effect the topology of gene trees inferred for the ingroup species
387 (Berger, S.A., Krompass, D., et al. 2011). Moreover, if the outgroup is distantly related to
388 the ingroup species then additional problems of long branch attraction can lead to incorrect
389 root placement (Philippe, H., Brinkmann, H., et al. 2011, Kuck, P., Mayer, C., et al. 2012,
390 Salichos, L. and Rokas, A. 2013). STRIDE is also suitable for large dataset analysis and
391 for situations where appropriate outgroups are not available. Although STRIDE as
392 presented is a method for identifying the root of an unrooted species tree, the output from
393 STRIDE can provide a wealth of useful information. For example, STRIDE maps high

394 confidence gene duplication events to branches in a species tree. These gene duplication
395 events provide strong evidence for monophyly of the species that share the gene
396 duplication event. Thus STRIDE can be used to provide additional support to branches in
397 a species tree that might be weakly supported by molecular sequence data. In this context,
398 it is worth noting that STRIDE could also be used to evaluate support for alternative
399 species-tree topologies by providing support for clades from gene duplication events.

400 The application of STRIDE to the eukaryotes was able to exclude the root of the
401 eukaryotes from the opisthokonts and from a number of other groups, however STRIDE
402 was unable to uniquely place the root as there were insufficient gene duplication events
403 identified that could exclude the root from other portions of the tree. It is likely that poor
404 taxon sampling for some of the groups (e.g. the amoebozoa and excavata), coupled with
405 genome reduction associated with adaptation to parasitism in many of these species,
406 impeded the discovery of these gene duplication events. With improved taxon sampling
407 STRIDE may ultimately be able to provide further insight as to the location of the root of
408 the eukaryotic tree. Furthermore, as STRIDE produces branch-level probabilities these
409 could be combined with probabilities obtained from other analyses to perform a multi-data-
410 type analysis of the origin of the eukaryotes.

411 In summary, STRIDE is a fast and effective method for genome scale phylogenetic
412 analysis that can be used both to identify high confidence gene duplication events and
413 identify the root of species trees without the requirement for an outgroup.

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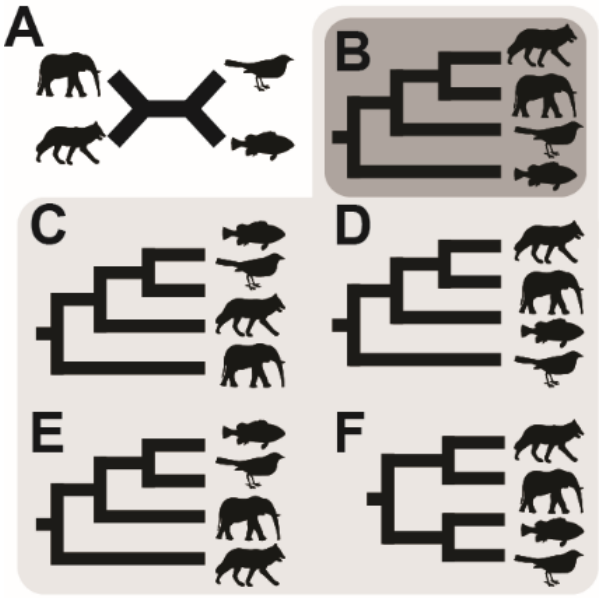
487

Figures

Figure 1



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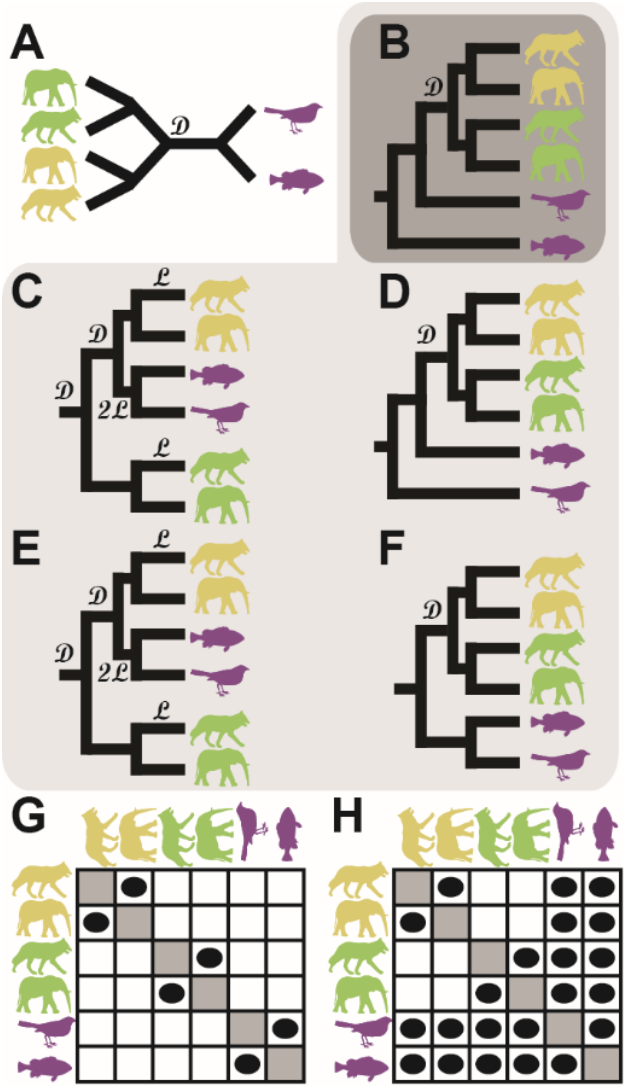

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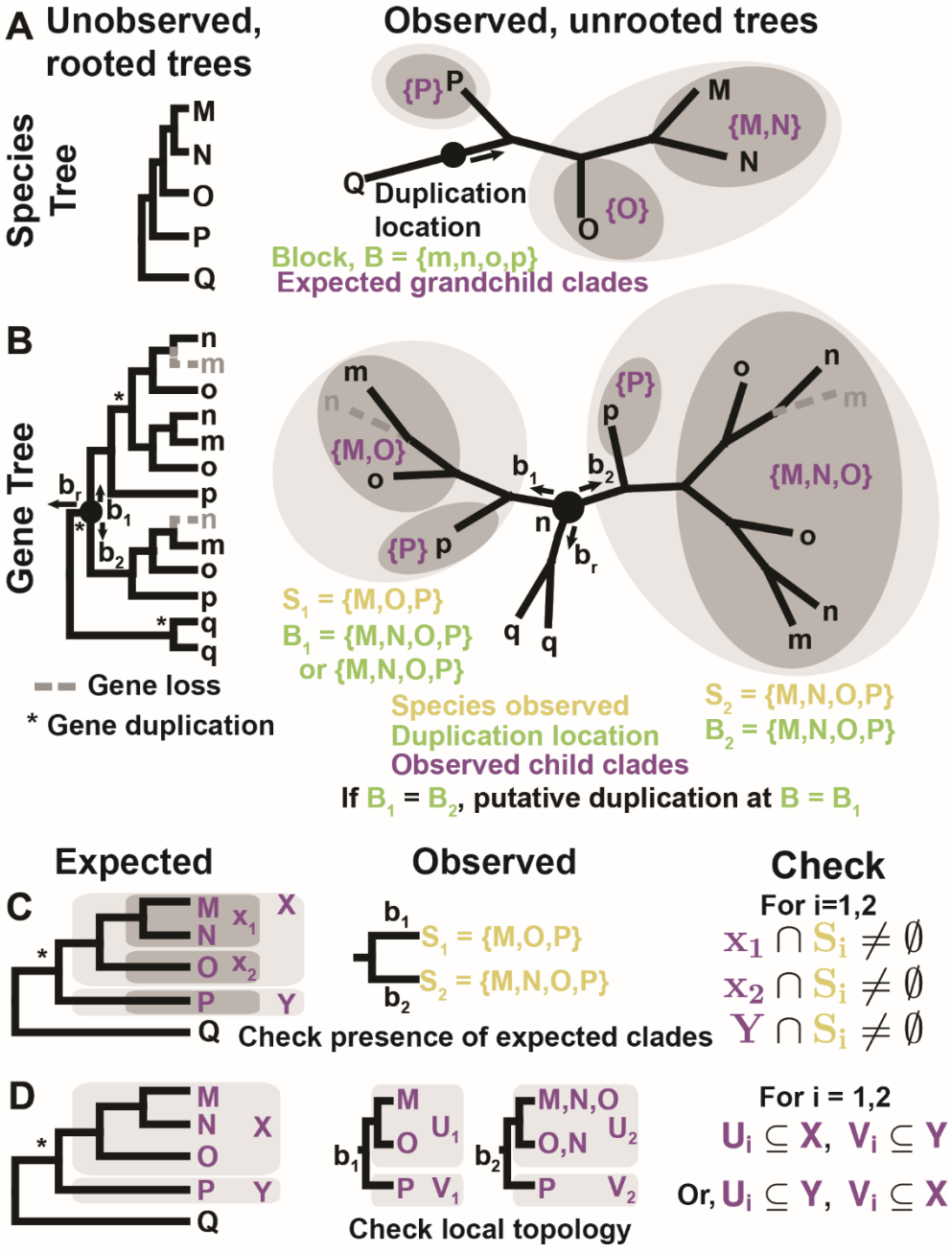

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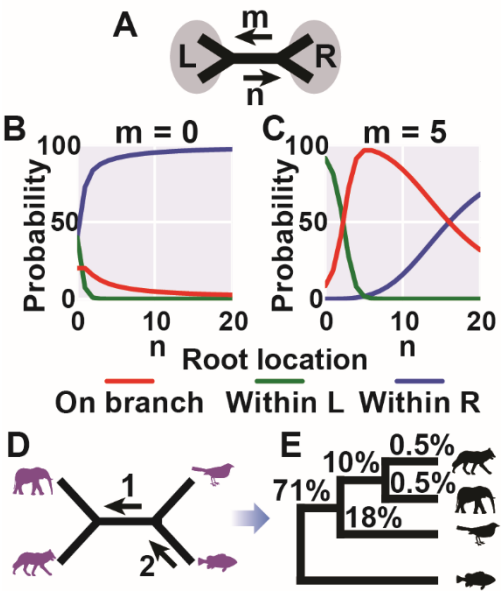

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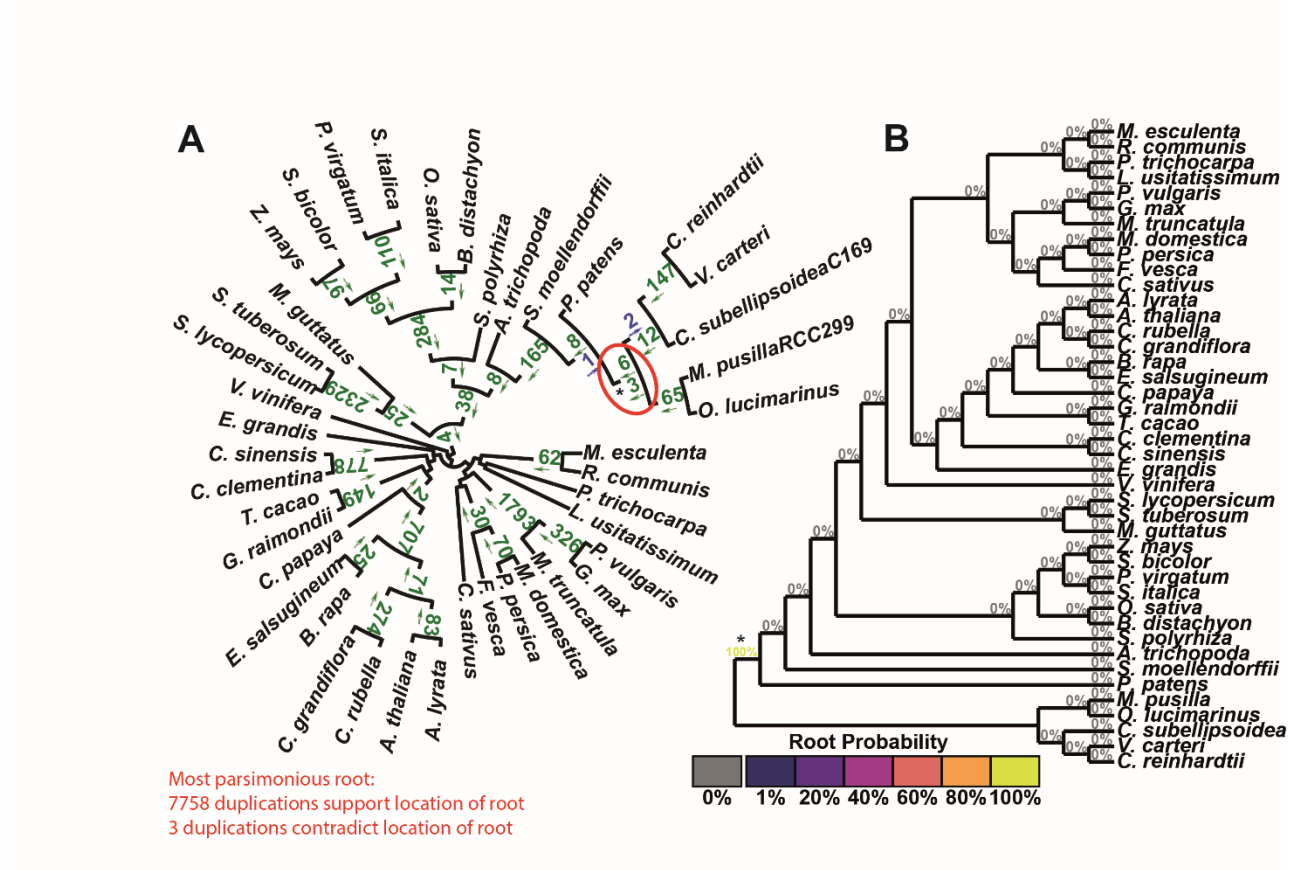
Figure 5



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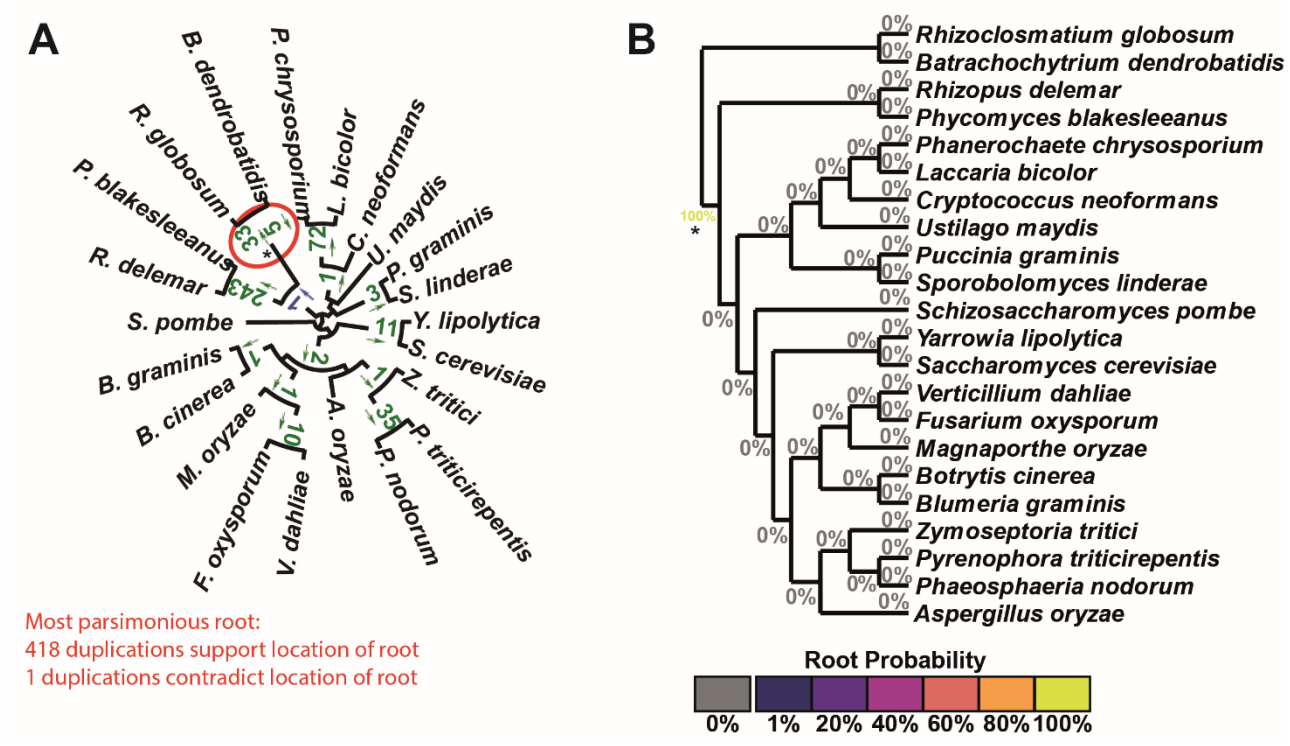

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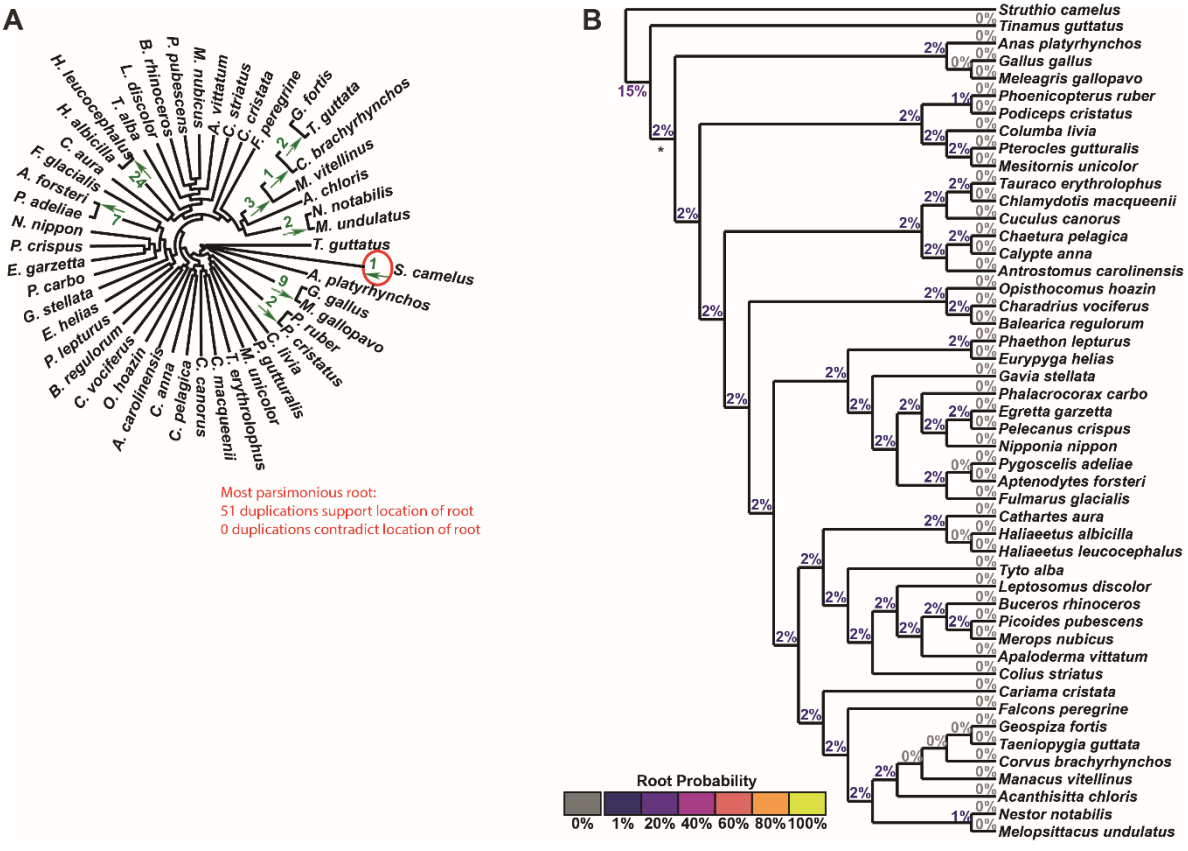

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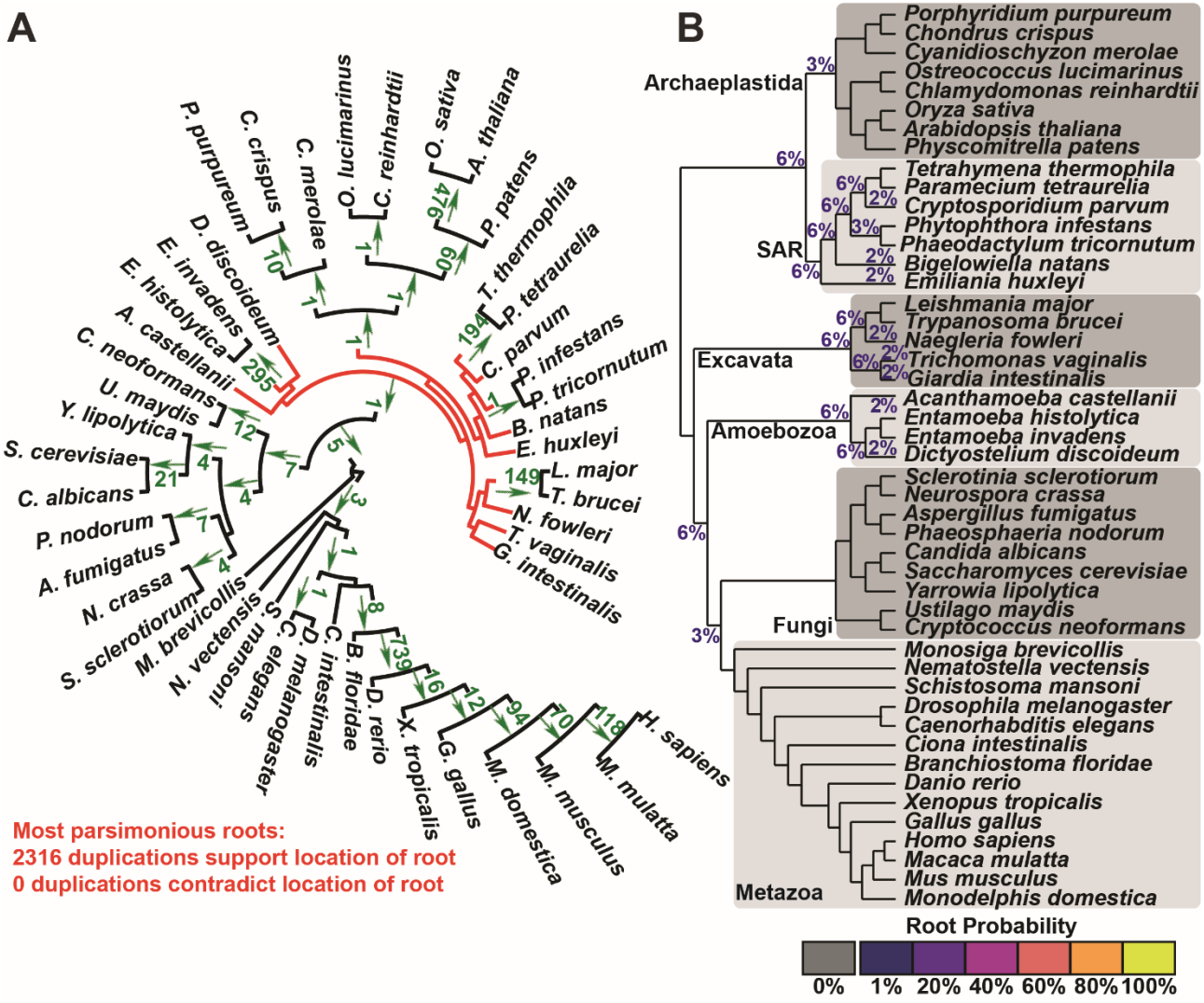

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505 ***Tables***

506 **Table 1**

Group	Species	Gene Trees	Informative Duplications	Number of Conflicting Duplications	Number of MP Roots	Correct MP Root	Probability for MP Root	Probability for Correct Root
Metazoa + outgroup (simulated)	40	2000	664	0	1	yes	100.0%	100.0%
Drosophila (simulated)	12	12000	1360	1	1	yes	100.0%	100.0%
Primates (simulated)	17	7500	1593	0	1	yes	100.0%	100.0%
Birds (Aves)	47	14454	51	0	1	no	15.0%	2.0%
Flies (Diptera)	7	11688	279	11	1	yes	100.0%	100.0%
Fish	11	16520	650	7	1	yes	100.0%	100.0%
Fungi	21	9325	419	1	1	yes	100.0%	100.0%
Hymenoptera	5	9157	108	7	1	yes	100.0%	100.0%
Kinetoplastids	16	9731	76	4	1	yes	55.0%	55.0%
Laurasiatheria	14	15804	135	7	1	no	100.0%	0.0%
Metazoa	21	13017	2065	0	1	yes	48.0%	48.0%
Nematoda	7	8392	93	2	1	yes	100.0%	100.0%
Primates + outgroup	11	19096	117	11	1	yes	8.0%	8.0%
Rodents	7	15485	22	6	1	no	9.0%	0.5%
Plants	42	28356	7761	3	1	yes	100.0%	100.0%
Eukaryotes	45	16770	2316	0	25	-	-	-
Total simulated	69	21500	3617	1	-	3	-	-
Total real-world	254	187795	14092	59	-	9	-	-
Total	323	209295	17709	60	-	12	-	-

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Figure Legends

Figure 1

Possible roots for a four-taxa species tree. A) Unrooted species tree for four species: elephant, wolf, fish & bird. B) The correct rooting of the species tree. B-F) The five possible rooted species trees for the unrooted species tree in A.

Figure 2

Orthologues inferred from gene trees depend on the root. A) An unrooted gene tree corresponding to an orthogroup with a gene duplication event in the common ancestor of wolf and elephant. Genes from each species are represented by an image of the species. B-F) The most parsimonious rootings of the gene trees (fewest duplications and losses) for each of the five roots of the species tree, as shown in Figure 1B-F. $\mathcal{D}$ - gene duplication event, $\mathcal{L}$ - gene loss event. G) Orthologues inferred from the incorrect trees D & E. H) Orthologues inferred from the correctly rooted tree B and also the close to correctly rooted trees D & F.

Figure 3

Identification of well-supported gene duplication events. Upper case letters M,N,O,P & Q are species, lower case m,n,o,p & q are genes from the corresponding species. A) The unknown, rooted species tree (left) and the observed, unrooted species tree (right). Black dot and arrow show the location of a single hypothetical gene duplication event on a branch with time flowing in the direction indicated by the arrow. The branch location and direction is uniquely identified by the block, B, of species whose common ancestor would have inherited the duplicate genes. The expected species in the child clades (X & Y) and grandchild clades (x_1, x_2 & Y) after this hypothetical duplication are highlighted with light/dark grey ellipses respectively. B) The unknown, rooted gene tree (left) and the observed, unrooted gene tree (right) for a hypothetical gene family with three gene

duplication events (marked by *) and two gene loss events (grey, dotted line). The node currently being analysed is n and b_r is the current, tentative direction towards the root. For these n and b_r, b_1 and b_2 are analysed to see if they are well-supported gene duplication branches. Si is the set of species below branch b_i, B_i is the smallest block of a bipartition containing S_i (i=1,2) C) The check that genes from each of the expected grandchild clades are present on each duplicate branch D) The check that the local topology for each duplication branch agrees with expected topology. U_i and V_i are the observed child clades on branch b_i. The observed child clades should not contain genes from any species not in the expected child clades

Figure 4

The branch-level probability model employed by STRIDE. These branch-level probabilities are used by the tree probability model to give the overall probabilities for the location of the root of the species tree. A) A single branch in the tree with m/n duplications supporting L/R as monophyletic clades. B) Branch-level model probabilities for position of the root with respect to the branch when m=0 (the model only takes into account duplications on that branch). C) As for B with m=5. D) Hypothetical total number of gene duplication events on the 4 species phylogeny. One gene duplication event is shared by elephant and dog and 2 are shared by elephant, dog and bird. D) The final tree-level model probabilities for the location of the root calculated by STRIDE taking into account all the gene duplication events on all branches in D.

Figure 5

STRIDE analysis applied the set of plant gene trees. A) Numbers of identified gene duplication events are marked on the branches they are observed on and arrows indicate the direction in which the duplication occurred. Gene duplication events are in agreement with the maximum parsimony root of the tree if the arrow points away from the root, and

are in green. Those that disagree are in blue. The maximum parsimony root is circled in red and is in agreement with the correct root, marked with a *. B) The probabilities for the location of the root calculated by STRIDE.

Figure 6

STRIDE analysis applied the set of fungi gene trees. A) Numbers of identified gene duplication events are marked on the branches they are observed on and arrows indicate the direction in which the duplication occurred. Gene duplication events are in agreement with the maximum parsimony root of the tree if the arrow points away from the root, and are in green. Those that disagree are in blue. The maximum parsimony root is circled in red and is in agreement with the correct root, marked with a *. B) The probabilities for the location of the root calculated by STRIDE.

Figure 7

STRIDE analysis applied the set of Bird gene trees. A) Numbers of identified gene duplication events are marked on the branches they are observed on and arrows indicate the direction in which the duplication occurred. Gene duplication events are in agreement with the maximum parsimony root of the tree if the arrow points away from the root, and are in green. Those that disagree are in blue. The maximum parsimony root is circled in red and is in agreement with the correct root, marked with a *. B) The probabilities for the location of the root calculated by STRIDE, coloured according to the displayed heat map.

Figure 8

STRIDE analysis applied the set of Eukaryotic gene trees. A) Numbers of identified gene duplication events are marked on the branches they are observed on and arrows indicate which block of the bipartition the duplicate genes occur in. None of the gene duplication events contradict each other. The maximum parsimony roots have red branches, the

584 branches from which the root is excluded are black. B) The probabilities for the location of

585 the root calculated by STRIDE. Major groups of species are marked.