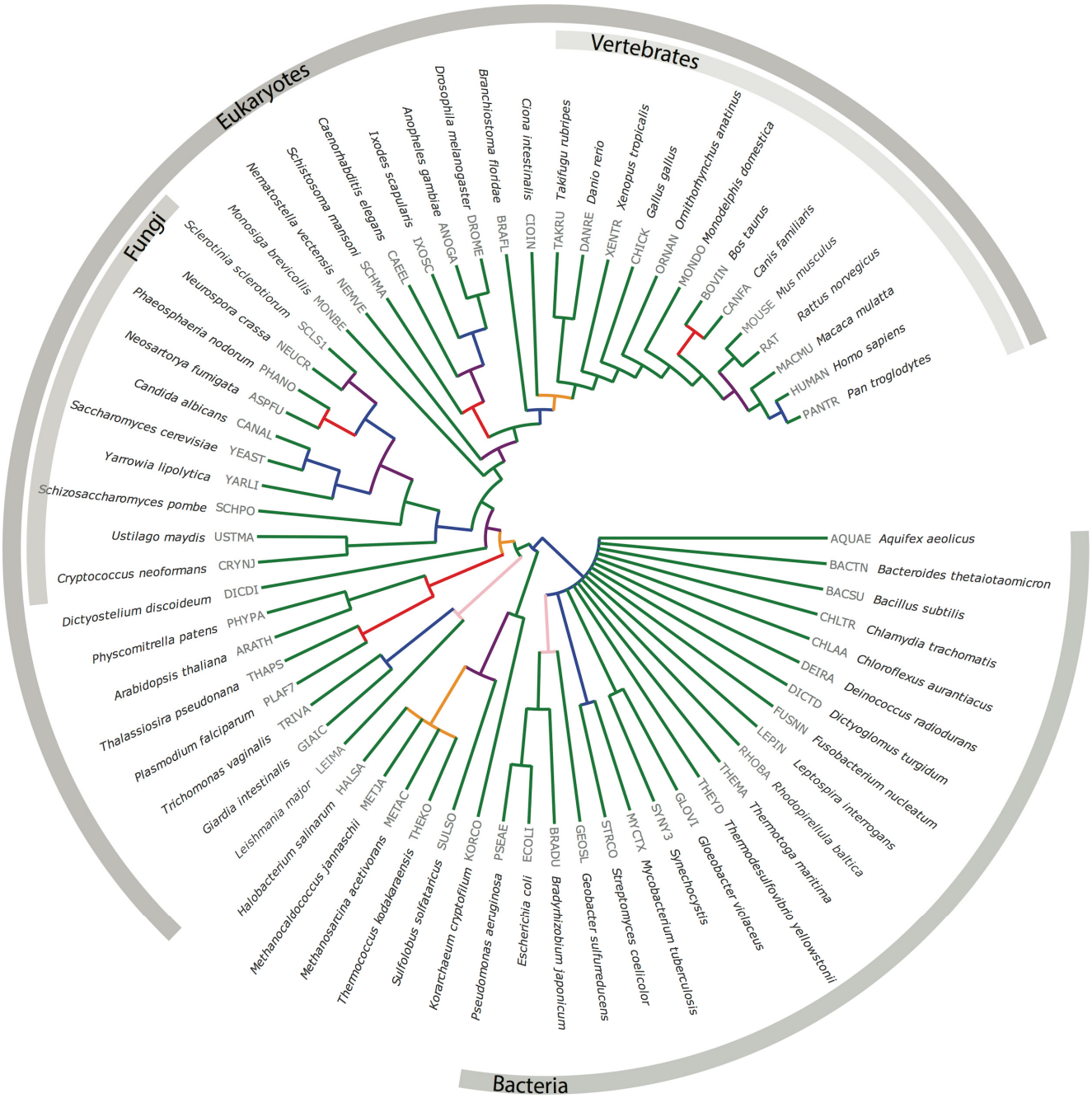
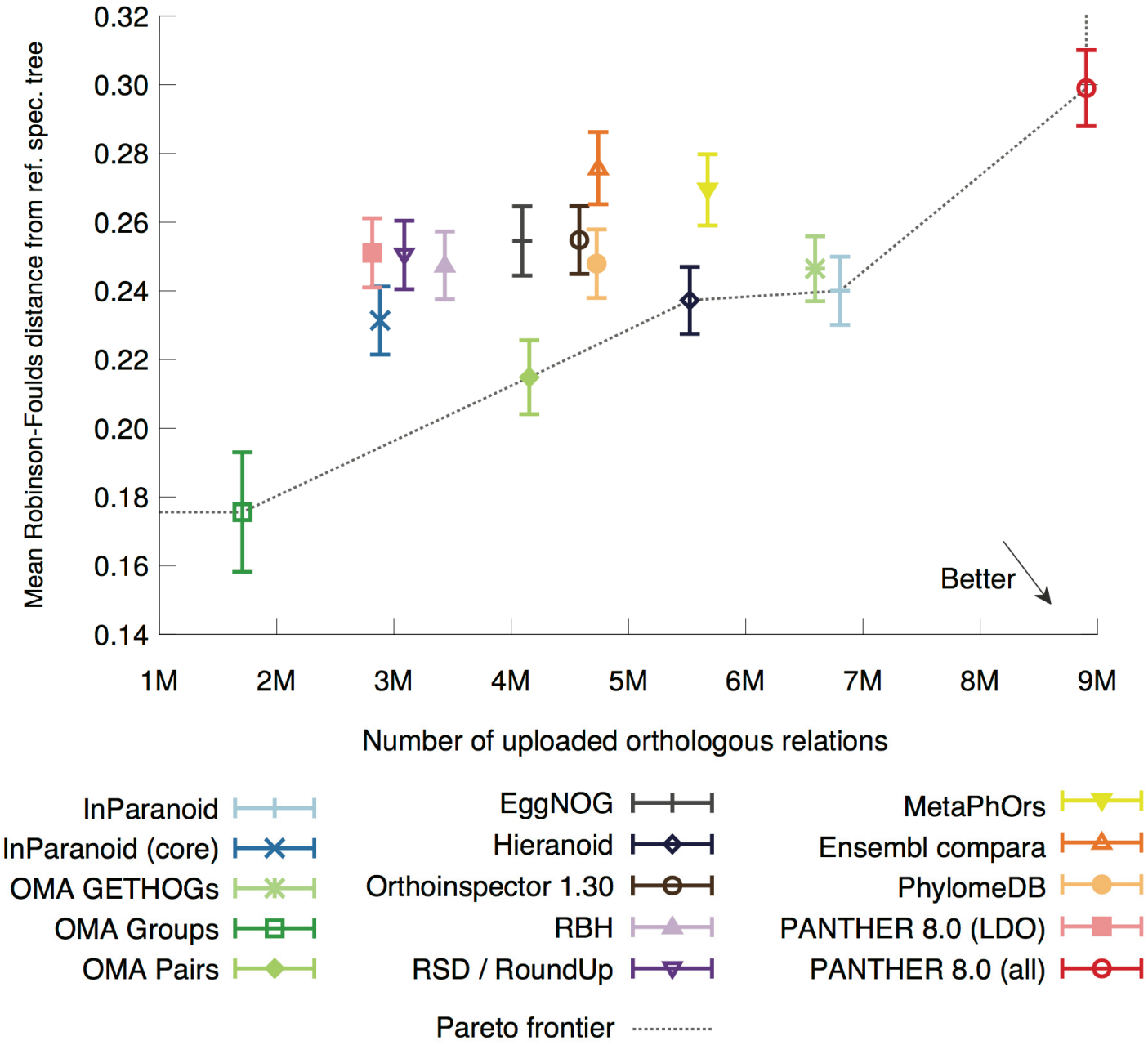


Supplementary Figure 1

SwissTree reference species tree

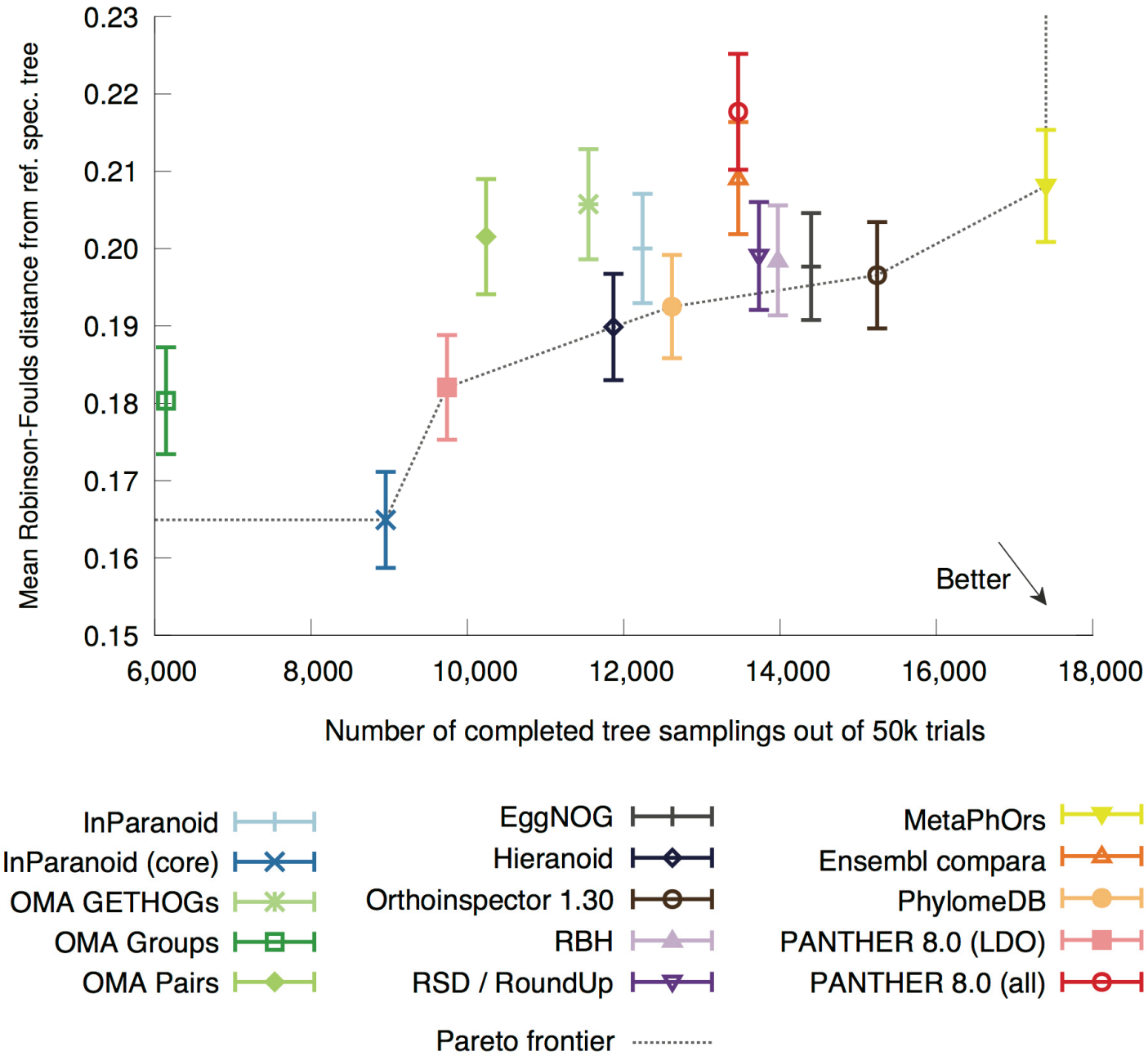
Internal branches are coloured according to their confidence levels: L90+≥10myr: green, L90+<10myr: purple, L70: blue, L50: orange, L30: pink and L10: red. For the species tree discordance test we collapse all branches below L90 or having shorter branches as 10myr based on estimates from the timetree resource[44]. The tree can be retrieved in electronic format from http://swisstree.vital-it.ch/species_tree.



Supplementary Figure 2

Generalised Species Tree Discordance test with alternative recall measure

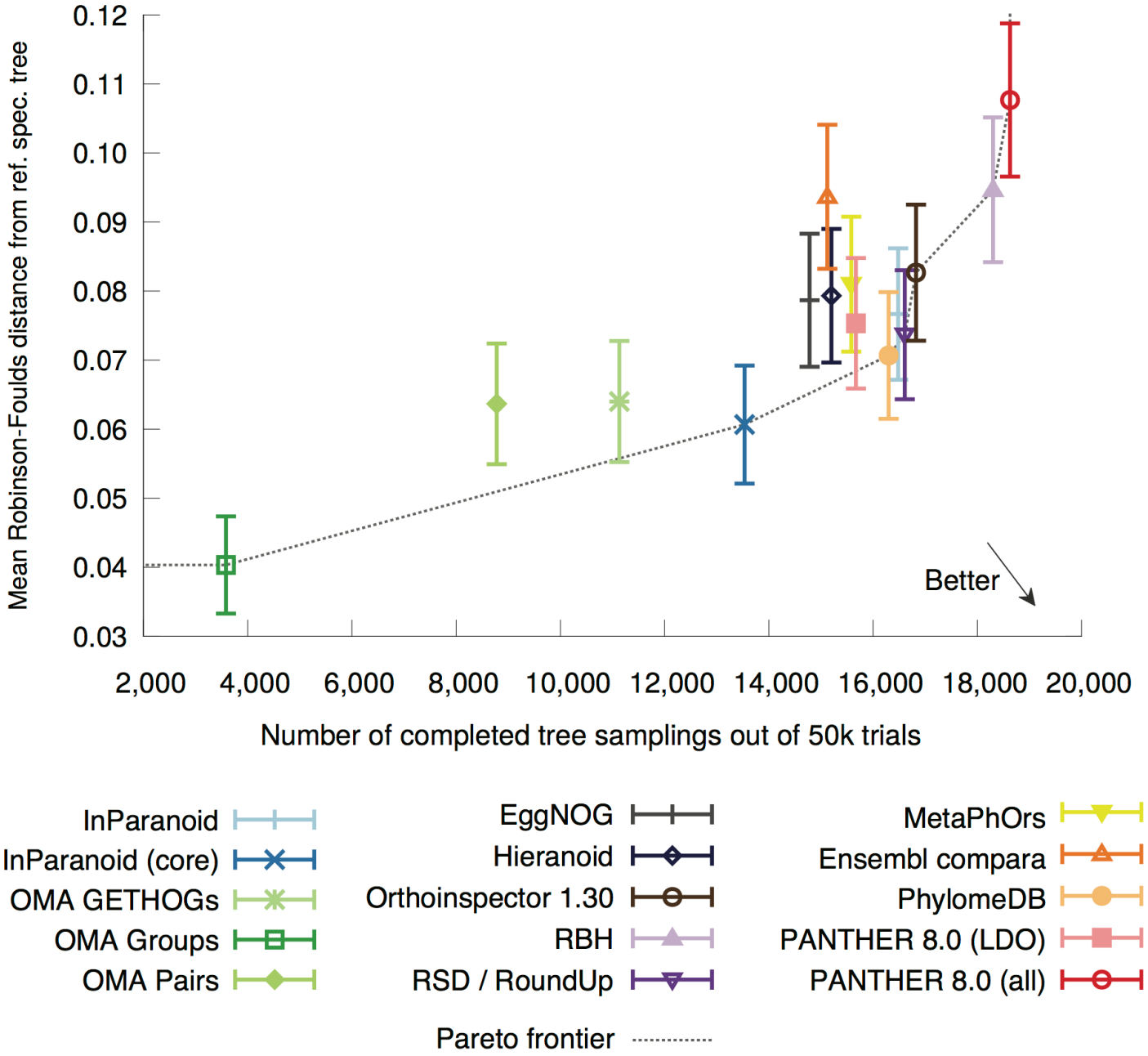
Same as main Figure 2, but with number of orthologous relationships on the *x*-axis instead of number of complete tree samplings: the Generalised Species Tree Discordance test assesses the congruence of inferred orthologs with a trusted reference tree, focusing on eukaryotes. A trade-off between precision (measured in terms of tree error in the *y*-axis) and recall (measured in number of orthologous relationships in the *x*-axis) can be observed. Only high-confidence branches of the reference tree (L90, see Methods), at least 10 myr long, are considered. Results for other clades are provided in Supplementary Figure 3-5. Error bars indicate 95% confidence intervals.



Supplementary Figure 3

Generalised Species Tree Discordance test on vertebrates

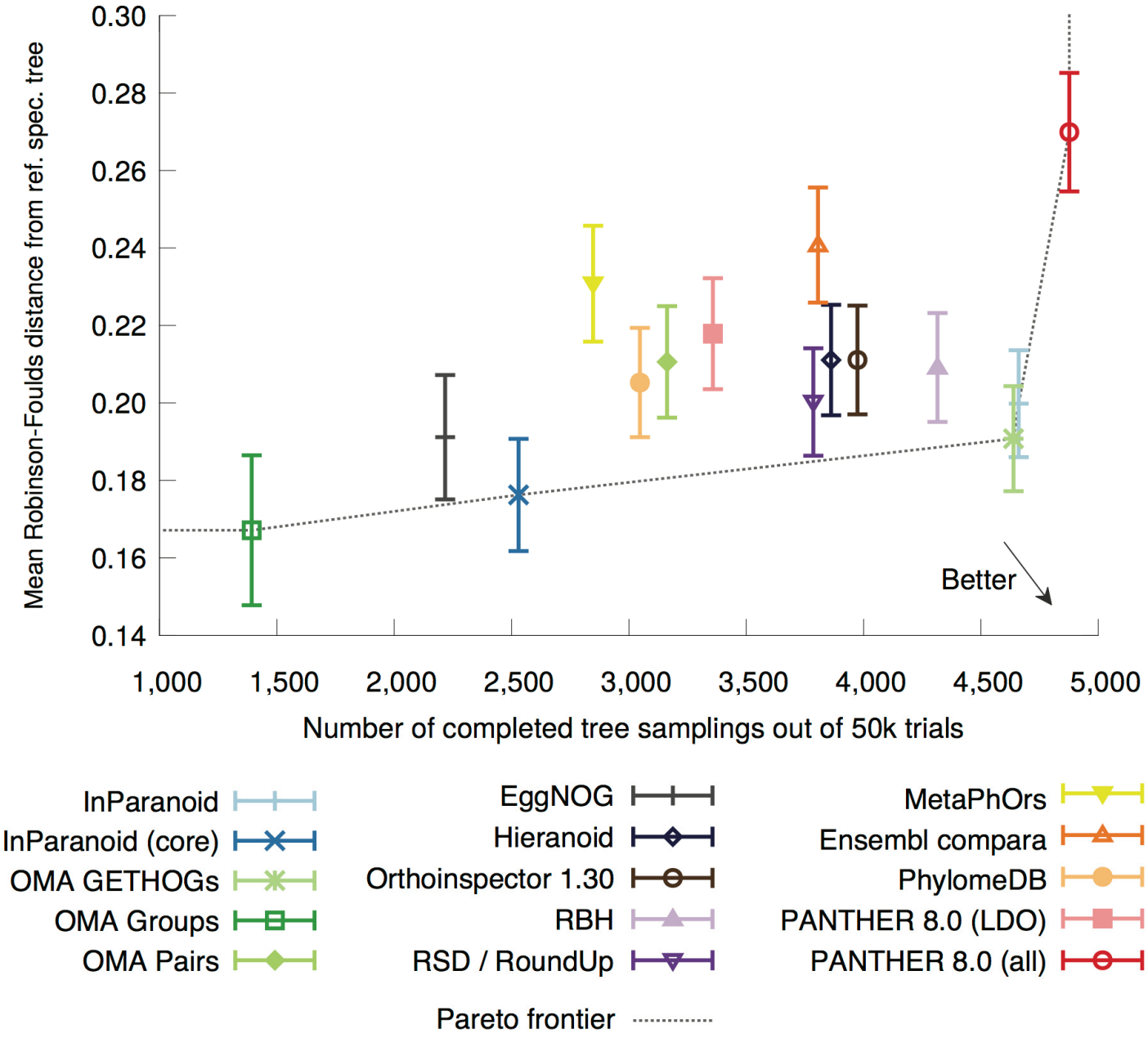
Same as main Figure 2, but covering vertebrate genomes only.



Supplementary Figure 4

Generalised Species Tree Discordance test on fungi

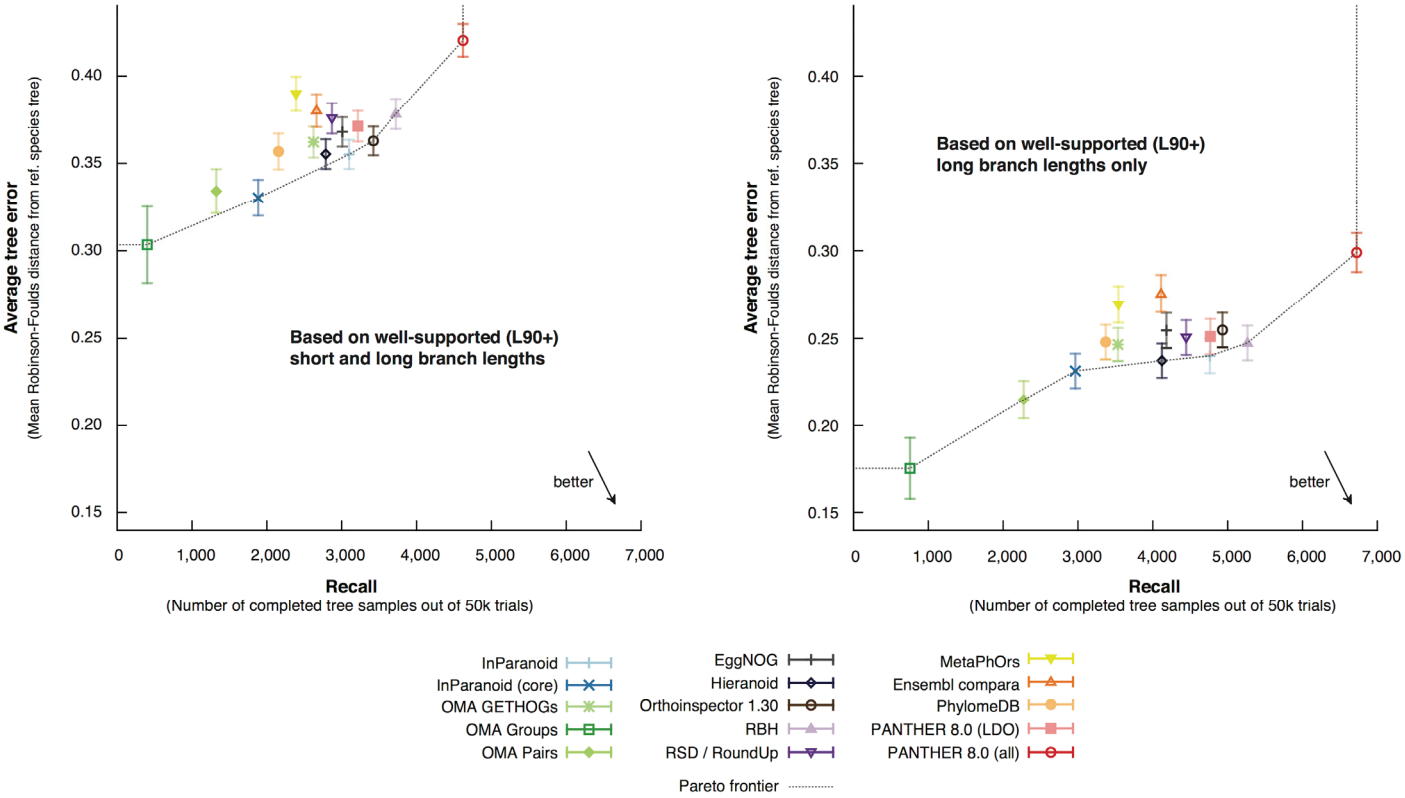
Same as Figure 2, but covering fungal genomes only.



Supplementary Figure 5

Generalised Species Tree Discordance test on tree of life

Same as Figure 2, but covering all genomes in the reference genome dataset, including prokaryotes.



Supplementary Figure 6

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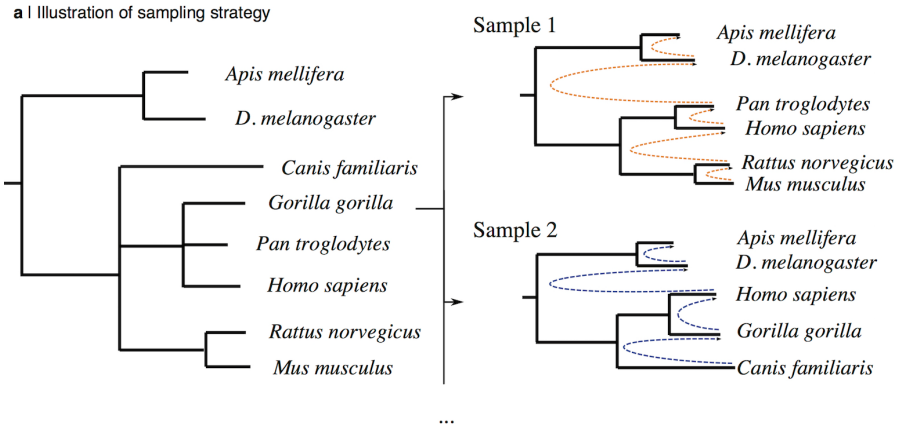
Generalised Species Tree Discordance test on eukaryotes from Figure 2, but also including short (L90+) branches—and therefore including potentially ILS (left-hand figure). The right hand figure depicts Figure 2 for comparison. The relative position of the methods does not substantially change, but the average discordance (y-axis) is considerably higher when including the short branches—due to a combination of stochastic error in the reconstruction of the gene trees and potential incomplete lineage sorting around short branches.

a | Illustration of sampling strategy



b | Pseudo code

```
Auxiliary functions:
    randomlyPruneMultifurcations(): randomly prunes multifurcation
        to yield a fully resolved binary tree.
    getSetOfOrthologs(m, p, s): returns the predicted orthologs of protein p
        in species s by method m.

Input:
    method: an orthology method,
    ReferenceTree: a reference species tree

function speciespathFromTree(tree:binary tree)
    if tree is a Leaf
        return tree.species
    else
        children = Shuffle(getchildren(tree))
        return speciespathFromTree(children[0]), speciespathFromTree(children[1])
    end if
end

function getOrthologsAlongSpeciesPath(cur_protein, remaining_species:list)
    candidates = getSetOfOrthologs(method, cur_protein, remaining_species[0])
    for ortholog in Shuffle(candidates)
        if length(remaining_species)==1:
            // we reached the end of the path
            return cur_protein, ortholog
        else
            res = getOrthologsAlongSpeciesPath(ortholog, remainign_species[1:])
            if res != NULL
                return cur_protein, res
            end if
        end if
    end for
    return NULL
end

NR_SAMPLED_TREES = 50000
SUBSAMPLE_TREE_SIZE = 3000

for k from 1 to NR_SAMPLED_TREES do
        T <- randomlyPruneMultifurcations(ReferenceTree)
        path <- speciespathFromTree(T)
        orthologs <- getOrthologsAlongSpeciesPath(path)
   if orthologs != NULL
       // a complete candidate dataset
       datasets.append(tree = T, orthologs = orthologs)
   end if
end for

for candidate in Shuffle(dataset)[0:SUBSAMPLE_TREE_SIZE]
   geneTree <- inferTree(candidate.orthologs)
   score.append(rfDistance(geneTree, candidate.tree)
end for

return mean(score), length(dataset)
```

Supplementary Figure 7

Generalised Species Discordance Test: diagram and pseudo code

Generalised species discordance test: (a) illustration and (b) pseudocode of the ortholog sampling strategy, with two potential paths along two possible bifurcating trees (orange and blue dashed lines).