Quartet Sampling distinguishes lack of support from conflicting support in the plant tree of life[1]

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ABSTRACT

Premise of the Study— Phylogenetic support has been difficult to evaluate within the plant tree of life partly due to the inability of standard methods to distinguish conflicted versus poorly informed branches. As phylogenomic and broad-scale datasets continue to grow, support measures are needed that are more efficient and informative.

Methods— We describe the Quartet Sampling (QS) method that synthesizes several phylogenetic and genomic analytical approaches into a quartet-based evaluation system. QS rapidly characterizes discordance in large-sparse and genome-wide datasets, overcoming issues of sparse alignment and distinguishing strong conflict from weak support. We test this method with simulations and recent plant phylogenies inferred from variously sized datasets.

Key Results— QS scores decrease in variance with increasing replicates and are not strongly affected by branch depth. Patterns of QS support from different phylogenies leads to a coherent understanding of ancestral branches defining key disagreements, including *Ginkgo*+cycad, magnoliids+eudicots (excluding monocots), and mosses+liverworts. The relationships of ANA grade angiosperms, major monocot groups, and bryophytes and fern families are found to likely be the result of discordant evolutionary histories, rather than poor information. Also, analyses of phylogenomic data show QS can detect discordance due to introgression.

Conclusions— The QS method represents an efficient and effective synthesis of phylogenetic tests that offer more comprehensive and specific information on branch support than conventional measures. The QS method corroborates growing evidence that phylogenomic investigations that

incorporate discordance testing are warranted to reconstruct the complex evolutionary histories surrounding in particular ANA grade angiosperms, monocots, and non-vascular plants.

Key words: bootstrap; branch support; discordance; introgression; lineage sorting; phylogenetics; phylogenetic methods; phylogenomics; plant tree of life; quartet sampling

INTRODUCTION

Discordance and uncertainty have emerged as a consistent feature throughout the history of our evolving model of the plant tree of life (Crane, 1985; Chase et al., 1993; Palmer et al., 2004; Soltis et al., 2011; Wickett et al., 2014). Furthermore, key points of uncertainty and contention occur at pivotal transitions in the evolution of plant life on earth, such as the development of vascular tissue (Pryer et al., 2001; Steemans et al., 2009; Banks et al., 2011), the rise of seed-bearing plants (Chase et al., 1993; Chaw et al., 1997; Bowe et al., 2000; Qiu et al., 2006; Jiao et al., 2011), and the explosive radiation of flowering plants (Crane, 1985; The Amborella Genome Project, 2013; Goremykin et al., 2015; Taylor et al., 2015; Simmons, 2016; Edwards et al., 2016). Modern phylogenomic datasets, rather than quelling these disagreements, have now repeatedly shown that these phylogenetic conflicts are not only caused by methodological error or data bias, but also are often the result of genuine biological processes. These forces most commonly include incomplete lineage sorting (ILS), introgressive hybridization, and paralog duplication-loss (e.g., Zhong et al., 2013b; Wickett et al., 2014; Zwickl et al., 2014; Yang et al., 2015; Eaton et al., 2016; Pease et al., 2016b; Goulet et al., 2017; Walker et al., In Press). While several methods have been proposed to deal with these issues in the context of species tree inference (e.g., Zwickl and Hillis, 2002; Ogden and Rosenberg, 2006; Shavit Grievink et al.,

2010; Aberer et al., 2012; Anderson et al., 2012; Roure et al., 2012; Hinchliff and Roalson, 2013; Mirarab et al., 2014), we still lack a generalized framework to quantify phylogenetic uncertainty (specifically branch support) that distinguishes poorly informed branches from the increasingly common case of multiple strongly supported, but mutually exclusive, phylogenetic histories. Therefore, the development of systems of phylogenetic evaluation designed to utilize and describe discordance (rather than minimize or control for it as error) offers strong promise to provide a more holistic picture of the current state of the plant tree of life.

Quantification of branch support for molecular phylogenies has been proposed under many methods over the last few decades (Felsenstein, 1985; Farris et al., 1996; Larget and Simon, 1999; Anisimova and Gascuel, 2006; Anisimova et al., 2011; Ronquist et al., 2012; Larget, 2013). Historically, one of the most commonly used tests has been the non-parametric bootstrap (NBS; Felsenstein, 1985), which, along with recent variants like the rapid bootstrap (RBS; Stamatakis et al., 2008), resamples the original data with replacement assuming that aligned sites are independent and identically distributed (i.i.d.) samples that approximate the true underlying distribution (Efron, 1992; Felsenstein, 1985). In practice, the assumptions of NBS (in particular site independence) may rarely be met and can deteriorate under a variety of conditions (Felsenstein, 1985; Felsenstein and Kishino, 1993; Hillis and Bull, 1993; Sanderson, 1995; Andrews, 2000; Alfaro, 2003; Cummings et al., 2003). More recently the UltraFast bootstrap approximation (UFboot) method, utilizing a likelihood-based candidate tree testing, was proposed to address speed and score interpretation issues for NBS (Minh et al. (2013); and see comparison in Simmons and Norton (2014)).

The other most common branch support metric has been the Bayesian posterior probability (PP). PP scores are typically calculated from posterior distributions of trees generated using a

Markov chain Monte Carlo (MCMC) sampler and then summarized using a majority-rule consensus tree (e.g, Larget and Simon, 1999; Drummond and Rambaut, 2007; Ronquist et al., 2012; Larget, 2013). The interpretation of PP values is arguably more straightforward than bootstrap proportions, as PP values represent the simple probability that a clade exists in the underlying tree (conditioned on the model of evolution employed, and assuming the data have evolved in a treelike fashion), and do not involve data resampling. The individual and relative performance of PP has been well-documented and generally favorable (Wilcox et al., 2002; Alfaro, 2003; Cummings et al., 2003; Huelsenbeck and Rannala, 2004). However, it has been recognized that in some scenarios (e.g., oversimplified substitution models) PP may give liberal support (Suzuki et al., 2002; Douady et al., 2003; Nylander et al., 2004), and there are indications that PP also may fail under a multi-species coalescent framework with conflicting phylogenies (e.g., Reid et al., 2013). Some studies have noted the disproportionate effects of a few genes in tipping the scales in the case of low information in large datasets (Brown and Thomson, 2016; Shen et al., 2017).

Modern phylogenetic datasets being assembled to model the plant tree of life are currently trending in two directions. Ongoing efforts to expand genetic sampling to as many plant species as possible have produced increasingly species-rich, but data-sparse, alignments (i.e., large-sparse matrices). Meanwhile, the accelerating accretion of new genomes and transcriptomes will continue to deepen genome-wide datasets with millions of aligned sites. Both types of data present particular challenges to both the tractability and interpretation of phylogenetic branch support methods. NBS scores are known to perform poorly for large-sparse matrices (Wiens and Morrill, 2011; Smith et al., 2011; Roure et al., 2012; Hinchliff and Roalson, 2013; Hinchliff and Smith, 2014b), where the sampling procedure generates uninformative pseudo-replicates that

mostly omit informative sites (or consist of mostly missing data). The problem of uninformative pseudo-replicates also applies to alignment jackknifing used on sparse alignments, where alignments are sampled without replacement. Therefore, alignment jackknifing also does not offer a functional solution for large alignments with missing data.

Quantifying phylogenetic support from complete datasets brings unique challenges. By "complete" dataset, we refer to any molecular data that is not a smaller subset of some larger whole, which means not only whole genomes, but also whole transcriptomes, and even whole plastid/mitochondrial genomes. PPs provide an appropriate testing framework and straightforward interpretation, but available Bayesian methods of analysis and computational speeds do not scale to typical phylogenomic datasets. Resampling methods (including NBS) gauge how well the resampled dataset replicate the initial result, but complete datasets are not samples of any larger whole. PP and NBS scores therefore both appear unsuitable for use on large datasets, the former due to feasibility and the latter due to its assumption (Smith et al., 2009; Hinchliff and Smith, 2014b).

A relatively well-studied, but less frequently applied, family of tests are the likelihood ratio tests, such as the approximate likelihood ratio test (aLRT), the Shimodaira–Hasegawa [SH]-like aLRT (SH-aLRT), and the Bayesian-like LRT (bLRT) (Anisimova and Gascuel, 2006; Guindon et al., 2010; Anisimova et al., 2011). These tests conduct an approximated LRT of the alternative nearest-neighbor interchange (NNI) moves at each branch of an optimized tree topology. SH-aLRT has been shown to perform well (Anisimova et al., 2011; Simmons and Norton, 2014; Simmons and Randle, 2014) and is computationally efficient enough to be run on large datasets in large part because it does not require generation of multiple topology replicates (as in NBS). Despite multiple implementations in PhyML and RAxML, and being the default support measure

in FastTree, this test has rarely been used in published studies (Guindon et al., 2010; Price et al., 2010; Stamatakis, 2014).

As phylogenomics has developed over the last decade, a variety of methods have been introduced to factor the increased data and inherent gene tree-species tree conflict. These methods measure the concordance of gene trees (broadly referring to a phylogeny from any sub-sampled genomic region), including the internode and tree certainty scores (IC/TC; Rokas et al., 2003; Salichos et al., 2014; Kobert et al., 2016), Bayesian concordance factors (Ané et al., 2006), and other concordance measures (Allman et al., 2017). These various certainty scores are developed around the central concept of a branch support statistic that measures concordance of various trees with a particular tree hypothesis. This perspective on phylogenetic evaluation offers much in terms of partitioning phylogenetic discordance and analyzing larger alignments more rapidly in a phylogenomic coalescent-based framework, but these methods that address discordance using gene trees may not be as suitable for large-sparse alignments.

Finally, quartet methods—in particular quartet puzzling methods—have been examined extensively in phylogenetics, especially in relation to phylogenetic reconstruction (Strimmer et al., 1997; Strimmer and von Haeseler, 1997; Ranwez and Gascuel, 2001; Chifman and Kubatko, 2014; Mirarab et al., 2014). A quartet support method called "reliability values," which correlates with NBS values, is calculated as a side effect of the quartet puzzling procedure (Strimmer et al., 1997; Strimmer and von Haeseler, 1997). More recently, quartet procedures have been explored to facilitate sampling of large-sparse alignments (Misof et al., 2013) and as part of coalescent-based quartet inference methods (Gaither and Kubatko, 2016; Sayyari and Mirarab, 2016). These quartet methods benefit not only from the speed advantages of a smaller alignment, but also from the statistical consistency of quartet trees, which avoid complex lineage sorting

147 issues that occur with more speciose phylogenies (Rosenberg, 2002; Degnan and Salter, 2005).

148 Few measures of support (except concordance methods) explicitly operate with the
149 expectation of multiple histories, or fail to distinguish different causes of poor support for a
150 branch in the phylogeny. In practice, this means when a phylogenetic relationship shows poor
151 support under NBS or PP, the specific cause is not apparent since these tests do not distinguish
152 between the case of multiple supported-but-conflicting phylogenetic relationships and the case of
153 simply low information. Being able to distinguish among these causes of low phylogenetic
154 support and to identify branches that have a strong consensus and a strong secondary evolutionary
155 history would provide valuable insight into the plant tree of life (among others; and see also
156 Brown and Lemmon, 2007).

157 Here, we describe the Quartet Sampling (QS) method (Fig. 1 and Table 1), which blends
158 aspects from many of the evaluation methods described above and leverages the efficiency of
159 quartet-based evaluation. The goal of the QS method is to evaluate phylogenies (particularly
160 large-sparse and genome-wide) by dissecting phylogenetic discordance to distinguish among (1)
161 differences in phylogenetic branch support due to lack of information (the general goal of
162 bootstrap or Bayesian posteriors), (2) poor support due to discordance as a result of lineage
163 sorting or introgression (as in concordance measures), and (3) low support due to particular taxa
164 with poor or conflicted information (i.e., "rogue taxa"; Wilkinson, 1996; Aberer et al., 2012).
165 These various causes of discordance are frequently surveyed separately in many modern
166 phylogenetic and particularly phylogenomic studies (e.g., Xi et al., 2014a; Wickett et al., 2014;
167 Yang et al., 2015; Pease et al., 2016b; Walker et al., In Press), but the QS method provides a
168 unified method for their execution, interpretation, and reporting. Additionally, the QS method
169 offers a viable means to describe branch support in large phylogenies built from sparse

alignments (10,000–30,000 tips with >80% missing data), datasets for which Bayesian analyses are simply not tractable. We also describe how QS enhances analysis of both genome-wide datasets and smaller-scale multi-gene data sets conventionally used in systematics.

In this study, we will (1) describe the features, parameters, and interpretation of the QS method, (2) validate the QS method with simulations that demonstrate its effectiveness, and (3) evaluate and discuss the plant tree of life by testing the QS method on recently published large-sparse and phylogenomic datasets at timescales spanning from Viridiplantae to sub-generic clades. The goal of the re-analysis of these phylogenies is not to compare the methods of tree inference or dataset composition, but instead to use the QS method to collectively analyze these data as a diverse set of alternative phylogenetic hypotheses of the plant trees of life. We show consistently through these datasets that the QS method acts as a more conservative test of branch support that provides greater discrimination between highly supported and poorly supported branches than other support measures. We also show that the QS method can identify branches in a phylogeny where biological conflict is likely occurring, which can inform targeting of more detailed investigations within a clade. Finally, we show several cases where low support for the given phylogenetic relationship and strong counter-support for an alternative relationship together indicate a coherent consensus. In other cases, QS specifically indicate the likely presence of alternative phylogenetic histories, as distinct from low branch support. We hope this study encourages additional discussion, testing, and innovation of new phylogenetic evaluation methods. We also hope it contributes to the broader discussion about moving the plant tree of life beyond the increasingly difficult goal of resolving a single unified ‘Species Tree’ (Hahn and Nakhleh, 2015; Smith et al., 2015), and into a future where the complex “multiverse” of phylogenetic relationships that is manifest throughout the plant tree of life are more fully

193 explored and appreciated.

194 MATERIALS AND METHODS

195 ***Quartet Sampling—*** The Quartet Sampling (QS) procedure outlined here was inspired by
196 aspects from several quartet-based and concordance methods, most particularly the process
197 originally outlined by Hinchliff and Smith (2014b). The QS method takes an existing
198 phylogenetic topology (which can be inferred by any method) and a molecular dataset (not
199 necessarily the one that generated the phylogeny) and separately evaluates one or more internal
200 branches on the given phylogeny. The QS method (Fig. 1) was designed to rapidly and
201 simultaneously assess the confidence, consistency, and informativeness of internal tree
202 relationships, concurrent with an analysis of the reliability of each terminal branch.

203 For a given phylogeny, each observed internal tree branch partitions the tree into four
204 non-overlapping subsets of taxa (Fig. 1A). These four sets of taxa can exist in three possible
205 relationships: the concordant relationship that matches the configuration in the given topology
206 and two alternative discordant configurations. The QS method repeatedly and randomly samples
207 one taxon from each of the four subsets and then evaluates the likelihood all three possible
208 phylogenies given the sequence data for the randomly selected quartet spanning that particular
209 branch.

210 For each quartet sampled for the focal branch, the likelihood is evaluated (using RAxML or
211 PAUP*; Stamatakis, 2014; Swofford and Sullivan, 2003) for all three possible topologies that
212 these four sampled taxa can take. The quartet topology with the best likelihood is then recorded
213 and tabulated across all replicates, generating a set of counts (across all replicates per branch) for

the concordant and each of the two discordant relationships. If a minimum alignment overlap is specified, then quartets must contain the minimum number of overlapping non-empty sites for all four taxa to be considered suitable for any calculations. Additionally, a parameter of a minimum likelihood differential may be set. If the most-likely topology (of the three) does not exceed the likelihood of the second-most-likely phylogeny by the set threshold then the quartet is considered "uninformative" and tabulated separately. Therefore, for a given internal branch, the QS method generates counts of the three possible topologies (and uninformative replicates) sampled from different quartets of taxa spanning the particular branch.

The QS method uses these resampled quartet tree counts to calculate three scores for each internal branch of the focal tree (Fig. 1B, Table 1, and Appendix S1; see Supplemental Data with this article). The QC (Quartet Concordance) score is an entropy-like measure (similar to the ICA score; Salichos et al. 2014) that quantifies the relative support among the three possible resolutions of four taxa. When the most commonly sampled topology is concordant with the input tree, then the QC takes positive values in the range (0,1]. Thus, QC=1 when all quartet trees are concordant with the focal branch. When one of the discordant topologies is the most commonly resampled quartet, QC takes negative values in the range [–1,0), approaching –1 when all quartet trees are one of the two discordant phylogenies. When support is evenly split among the three alternative topologies (or two if only two of the three possible are sampled), QC equals 0.

The QD (Quartet Differential) score uses the logic of the f- and D-statistics for introgression (Reich et al., 2009; Green et al., 2010; Durand et al., 2011; Pease and Hahn, 2015) and measures the disparity between the sampled proportions of the two discordant topologies (though with gene tree proportions, rather than site frequencies). The QD score does not specifically quantify introgression nor identify introgressing taxa, but does indicate that one alternative relationship is

sampled more often than the other. High values of QD report that there is one clearly preferred topology among the two discordant topologies, a potential indication of a biased biological process beyond background lineage sorting on the given branch, including introgression, strong rate heterogeneity, heterogeneous base compositions, etc. QD varies in the range [0,1] with a value of 0, meaning no skew in the proportions of discordant trees, and the extreme value of 1, which means all discordant trees sampled are only from one of the two possible alternative relationships.

The QU score (Quartet Uncertainty) quantifies the overall proportion of quartets for a given branch where the tree with the best likelihood value has a likelihood that is not higher than the next most likely tree by a given differential cutoff. This ensures that replicates are not counted as being concordant or discordant when the molecular data itself is effectively equivocal on the topology by all three options having nearly indistinguishable likelihood scores. QU is measured in the range [0,1], which indicates the proportion of sampled quartets that did not exceed of the cutoff. A QU of 0 means no "uncertain" quartets (i.e., highly informative data), while a value of 1 indicates 100% of quartets were uncertain (i.e., no information for the given branch). QU reports the informativeness of the branch, which in conjunction with QD and QC distinguishes between branches that have low information versus those with conflicting information (i.e., high discordance).

Finally, for each terminal taxon, a QF (Quartet Fidelity) score is calculated to report what proportion of the time inclusion of a given taxon in a quartet (across all branches) results in a discordant quartet topology. QF is therefore similar in approach to a "rogue taxon" test (Wilkinson, 1996; Aberer et al., 2012). For a given taxon, the QF score is measured in the range [0,1] as the proportion of quartet topologies involving the taxon that are concordant with the focal

tree branch. Therefore, QF=1 indicates a given taxon always produces concordant topologies when used in a quartet replicate. QF values approaching zero indicate mostly discordant topologies involving this taxon, and may indicate poor sequence quality or identity, a lineage-specific process that is distorting the phylogeny, or that the taxon is significantly misplaced in the given tree. Note that QF differs specifically from QC/QD/QU by being a taxon-specific test across internal branch tests rather than a branch-specific test.

Collectively, these four tests represent a means to distinguish the consistency of a branch (QC), the presence of a secondary evolutionary history (QD), the amount of information regarding a branch (QU), and the reliability of individual taxa in the tree (QF; Fig. 1B and see Table 1). Taken together these tests provide a means to disentangle these effects rather than have them conflated under a summary score as in standard measures of phylogenetic support. For a full technical description of the QS method, see Appendix S1.

Guidelines for implementation of QS— We implemented the above procedure in `Python` in a program `quartetsampling` that samples an alignment randomly to generate many representative quartet topology replicates for each internal branch in a corresponding focal tree (https://github.com/fephyfofum/quartetsampling). This procedure has a number of advantages over NBS for larger datasets. First, alignment columns are not resampled (as in NBS and RBS), which allows even very sparse alignments to be used. Second, the number of likelihood calculations that are required is the number of internal branches in the tree multiplied by the number of replicates per branch multiplied by three possible topologies. Since computation time scales linearly with the number of taxa, individual replicates are fast, and the computations can be readily parallelized across processors and furthermore discretized across systems (with results

combined later). This allows QS to be efficiently applied to large alignments beyond the practical limits of NBS and PP. The most extensive computational time was for the Zanne et al. (2014b) 31,749 taxon dataset (see below), which we ran on the Wake Forest University DEAC high-performance cluster using 8 nodes with 16 CPU each. This analysis completed 200 replicates for the full tree in 13 hours. Smaller genome-wide datasets finished 1000 gene-tree replicates on quad-core desktops approximately 12 hours, and conventional multi-gene datasets took only a few minutes to a few hours to run on a standard desktop.

Although the SH-aLRT was by far the fastest method we consider here, the QS was fast enough for large scale analyses. QS can also be applied separately to only a few branches, allowing for more thorough exploration of particular branches of interest. Furthermore, the QS does not require the tree tested to be the maximum likelihood topology, a requirement for SH-aLRT. For our simulated data, we found that performing 200 QS replicates per branch was adequate to achieve low variance in QS score. As would be expected, more replicates per branch should generally be used for larger trees to sample a greater fraction of the total possible quartets.

Furthermore, some branches, especially in large trees, may be entirely unsupported by the alignment due to a lack of sampling overlap among appropriate taxa (i.e., no sites in the alignment contain data from each of the four subsets of taxa; Fig. 1A). Therefore, no phylogenetic information exists to inform the branch (i.e., are ‘‘‘indecisive” *sensu* Steel and Sanderson, 2010), and the QS procedure identifies these branches rather than discarding them or marking them as simply low support.

Guidelines for interpretation of QS support values— An important consideration with any measure used to ascertain confidence is the precise interpretation. In order to facilitate accurate

304 interpretation of the QS scores, we provide a concise visual description of the tests (Fig. 1) and a
305 table describing example scores and their interpretations (Table 1). Particularly notable as shown
306 in Table 1 and in the results, the QS method can not only "support" or "fail to support" a given
307 branch hypothesis, but also can offer "counter-support" for an alternative branch (as in the
308 IC/ICA scores; Salichos et al., 2014; Kobert et al., 2016). Therefore, even "inaccurate" branch
309 hypotheses can offer information in the form strength of the "counter-support" for an alternative
310 quartet topology (i.e., the degree of negativity of the QC score; for examples see Fig. 6).
311 The QS scores we have described calculate the sensitivity of the resolution of a particular
312 branch to different combinations of taxa sampled around that branch. Each QS replicate
313 calculates whether the four sampled taxa support the resolution of the branch found in the tree
314 over the alternative resolutions. This framework is similar to the interpretation made by those
315 using taxon jackknife analyses for outgroup sensitivity (e.g., Edwards et al., 2005) and the IC
316 score when used with incomplete trees (Kobert et al., 2016). We argue that this interpretation is
317 richer in information than the NBS, and in our simulations the QC score also appears to more
318 conservatively and accurately assign high support values to branches that are present in the true
319 tree (i.e., relatively low false positive rates, at least when the likelihood threshold is small, i.e., in
320 the range of $\sim$2 used here; Appendix S2). QC scores are particularly helpful in terms of clarifying
321 strength of support for branches with concordant tree frequencies not close to 1 (Appendix S3).

322 ***Generation and evaluation of simulated phylogenies—*** We first tested the method by
323 generating simulated phylogenies under the pure birth (birth = 1) model of evolution with 50,
324 100, and 500 tips using `pxbdsim` from the `phyx` toolkit (Brown et al., 2017). Using these trees,
325 we generated 1000 bp alignments (no indels) under the Jukes-Cantor model with INDELible v.

1.03 (Fletcher and Yang, 2009). Trees were scaled so that the average branch lengths were about 0.2, based on the observation that this generated reasonable trees with most branches recovered correctly from ML analyses. Using the same procedure, we also simulated trees with 500 tips and associated alignments with ten nucleotide partitions, each with 500 sites under the Jukes-Cantor model. We simulated both the full alignment with partitions and a modified randomly resampled sparse alignment to examine the behavior of QS in the presence of missing data (see Appendix S1 for details). These partitioned and sparse alignments had the same qualitative features as the full alignment.

Unlike the NBS method, which generates a set of trees from which branch support is estimated, the QS method requires only a single input topology for which branch support will be measured. We calculated QC, QD, QU, and QF scores for the true underlying tree as well as the ML tree generated by RAxML, but we focus on results for the ML tree. To examine how the number of replicates impacts the QS precision, we conducted simulations varying the number of replicates for randomly drawn branches in the simulated trees (Fig. 2A; Appendix S4). Based on these simulations, we elected to use 200 replicates per branch, since the variation in the QC score was generally low across all tree sizes when this many replicates were performed. We used RAxML and PAUP* to estimate the ML for the three alternative topologies for each QS replicate (using the `-f N` option and the GTRGAMMA model in RAxML). We also calculated branch-specific QC/QD/QU and taxon-specific QF scores using likelihood differential cutoffs of $\Delta L = 0$ (no filtering) and $\Delta L = 2.0$, which requires stronger conflicting signal to interpret branches in the input tree as unsupported.

Testing of Empirical Datasets— While the simulations show the general reliability of the method, our primary goal was to use this test to holistically evaluate recent large-scale plant phylogenies, and also to show the utility and versatility of this method in subgroup and even sub-genera analyses. Using published phylogenies and public datasets, we evaluated five recent large-scale phylogenies, including (1) an 103-transcriptome dataset spanning Viridiplantae from Wickett et al. (2014, abbreviated hereafter as WI2014), (2) two large-sparse phylogenies spanning land plants from Hinchliff and Smith (2014b, abbreviated HS2014) and Zanne et al. (2014b, abbreviated ZN2014), and (3) phylogenies spanning Magnoliophyta (angiosperms) with hundreds of genes from Xi et al. (2014a, abbreviated XI2014) and Cannon et al. (2015b, abbreviated CN2015). Additionally, to demonstrate the utility of this method at medium and short time scales, we evaluated two whole transcriptome datasets from the wild tomato clade *Solanum* sect. *Lycopersicon* from Pease et al. (2016b, abbreviated PE2016) and carnivorous plants from the order Caryophyllales from Walker et al. (In Press, abbreviated WA2017). Finally, we tested this method on a more typical medium-sized multi-locus dataset from Polypodopsida (ferns) from Pryer et al. (2016b, abbreviated PR2016), such as might appear in many phylogenetic studies of large subgroups. Data for these studies were obtained from datadryad.org and iplant.org (Hinchliff and Smith, 2014a; Matasci et al., 2014; Xi et al., 2014b; Zanne et al., 2014a; Cannon et al., 2015a; Pease et al., 2016a; Pryer et al., 2016a; Walker et al., 2017).

In addition, we analyzed the datasets using 200 individual gene trees for XI2014 and WA2017, and 1000 gene trees for PE2016 and WI2014. For these datasets, quartets are sampled as usual, but only the individual gene sequence alignments are assessed. These phylogenies were all evaluated using a minimum alignment overlap per quartet of 100 bp and a minimum likelihood differential of 2 (i.e., the optimal tree's log-likelihood must exceed the second-most likely tree by

a value of at least 2). We also calculated the phylogenies with and without partitioning in RAxML, but in all cases the partitioned datasets did not qualitatively differ from the results of the unpartitioned datasets. These data are provided as supplementary data, but are not shown here.

We also either re-calculated other measures of branch support or used values from the published studies for comparison to the QS method for each phylogeny, except HS2014 and ZN2014 where the size and sparseness of the datasets prohibited the calculation of other measures of support. For the datasets from CN2015, PR2016, WA2017, and XI2014 100 replicates each of RAxML NBS and SH-test were performed. Additionally, PP scores for PR2016 were calculated using MrBayes (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012), and IC scores for calculated for Walker et al. (In Press). For PE2016 and WI2014, RAxML NBS, MP-EST, or IC scores were taken from published values.

RESULTS AND DISCUSSION

Analysis of simulated and empirical datasets shows that QS components are consistent, convergent, and complementary— In order to examine the consistency and reliability of the Quartet Sampling method, we first tested QS on a set of simulated phylogenies. As expected, the QC score was generally correlated in a sigmoid fashion with the frequency of concordant trees (Appendix S5). In order to determine the appropriate number of testing replicates and demonstrate convergence, we conducted an analysis of the variance of the QS score by running the QS analysis 100 times on randomly selected branches with different numbers of per-branch replicates for trees with 50, 100, and 500 taxa. This analysis showed that the QC scores converge (with decreasing variance as expected) on a consistent mean value for each branch as the number

391 of replicates increased (Fig. 2A). Sampling 200 quartets per branch reduced the variance to less
392 than 0.003 in all cases. Similar patterns of convergence were also found for QD values, though
393 with high variance (Appendix S5). We also note that since QS is a branch-specific test (not a
394 tree-wide test), key branches of interest can be tested individually at much higher numbers of
395 replicates without the need to re-test the entire tree.

396 QC and QD both incorporate the frequencies of discordant quartets in different ways, but for
397 that same reason run the risk of redundancy. However, we observed in the Hinchliff and Smith
398 (2014b) dataset (abbreviated as HS2014) that QD can take on a range of values for QC (Appendix
399 S5). As QC goes to the limits of its range (–1,1), QD values tend to have more extreme values.
400 These extreme values for QD were either due to a lack of discordant trees that limits sampling
401 (when QC approaches 1) or a high amount of one discordant tree (when QC approaches –1). This
402 made QC and QD related, but not strictly correlated, measures. Overall, the mean QC for the
403 HS2014 (0.15; interquartile range (IQR) = [–0.13, 0.46]) and Zanne et al. (2014b) (abbreviated
404 ZN2014; 0.17; IQR = [–0.10, 0.63]) were low compared to the less speciose phylogenies (Fig.
405 2B; Appendix S5). Applying a minimum log-likelihood differential threshold to small trees
406 tended to push scores toward extremes, resulting in more 0s and 1s (Appendix S2). As expected,
407 given the sparsity of the matrices for ZN2014 and HS2014, the proportion of uninformative
408 quartets was high in both cases (mean QU of 0.65 and 0.85, respectively).

409 Notably, QS found that 33.4% and 29.8% of branches in HS2014 and ZN2014, respectively,
410 had QC values less than –0.05. This meant that about a third of the branches in these consensus
411 phylogenies reported not just “low support” for the given branch, went further to report
412 “counter-support” for one of the two alternative topological arrangements at that branch (see
413 Table 1). Many empirical cases have been documented where one anomalous lineage can distort

not only its own phylogenetic placement, but can exert a wide-ranging set of distortions on the topology (e.g., Fig. 8A, among many others). Resampling methods that infer a complete set of tips (like NBS) will produce replicates that may also be distorted and thus firmly support these relationships rather than highlight this discordance. Therefore, as pointed out by many other quartet methods (Mirarab et al., 2014; Sayyari and Mirarab, 2016), the use of quartets breaks down the problem in more manageable units that cannot be distorted by these complex lineage sorting effects.

QS indicates strong relationships within, but not among, major land plant lineages— While simulations and general characterizations of the QS method are necessary to demonstrate its effectiveness, the primary goal of this study was to use QS to reanalyze and compare several recent speciose and phylogenomic datasets to address ongoing debates of phylogenetic relationships in the plant tree of life. We used QS methods to evaluate two of the most speciose phylogenies of land plants presently assembled by Hinchliff and Smith (2014b) and Zanne et al. (2014b), and one of the most comprehensive phylogenies of Viridiplantae from Wickett et al. (2014) (abbreviated as WI2014). HS2014 spanned 13,093 taxa with a total alignment of 148,143 sites (Fig. 3), but extremely sparse overall coverage (96.4% missing characters). The alignment used for ZN2014 (Fig. 4) contained 31,749 taxa and an alignment length of 12,632 bp, but also with sparse coverage (82.3% missing characters). However, the QS method accommodates large, sparse matrices, by evaluating alignment overlap only for each replicate quartet without requiring complete occupancy for any partition of the alignment. Finally, the WI2014 (Fig. 5) contains only 80 taxa, but, as the pilot study for the 1000 Plants Project (1KP), spans the deepest time scale of these three (including Chlorophyta) and had a highly occupied alignment of 290,718 bp with only

436 10.7% missing data.

437 Most major plant groups showed strong support in HS2014 and ZN2014, including
438 angiosperms (QC=0.68 and 0.75, respectively), Anthocerotophyta (hornworts; QC=0.54,0.92),
439 Acrogymnospermae (gymnosperms; QC=0.37,0.32), and Polypodopsida (ferns; QC=0.23,0.46).
440 Marchantiophyta (liverworts), rosids, and asterids all showed low support in HC2014 (QC<0.2).
441 Monocots (both including and excluding *Acorus*) had poor support in both HS2014 and ZN2014
442 (–0.05<QC<0.05). In WI2014, all the major groups (i.e., labeled internal branches in Fig. 5)
443 showed high support. In contrast to the generally high support for these major established groups,
444 we frequently found low support on the "backbone" relationships among these groups, in a
445 manner consistent with most previous phylogenies of land plants.

446 The relationships among Marchantiophyta, Bryophyta (mosses), Anthocerotophyta,
447 lycophytes, and "euphyllophytes" (i.e., ferns and seed-bearing plants) has also been under
448 substantial ongoing debate (Shaw et al., 2011). HS2014 places mosses as sister to the remaining
449 land plants, followed successively by liverworts, hornworts, and lycophytes. However, HS2014
450 indicates QC=–0.04 for a branch defining liverworts + all other land plants the exclusion of
451 mosses (Figs. 3B, 6A), indicating that the most common quartet branch among the replicates was
452 not the branch shown in the tree. By contrast WI2014 shows strong support (QC=0.67) for the
453 branch leading to a moss+liverwort common ancestor (Fig. 5). While ZN2014 contains only
454 embryophytes, it shows weak support (QC=0.15) for the branch separating moss+liverwort from
455 the rest of land plants. WI2014 has hornworts (represented by two *Nothoceros*) as sister to a clade
456 of mosses and liverworts+tracheophytes (as in Renzaglia et al., 2000), with high support for all
457 relationships. However, note that WI2014 showed substantial counter-support against a common
458 mosses+liverworts+tracheophyte ancestor to the exclusion of hornworts. Coalescent analyses in

459 WI2014 also support this finding of a monophyletic Bryophyta+Marchantiophyta clade.
460 Therefore, while the topology of HS2014 was consistent with the order of many previous
461 phylogenies (Nickrent et al., 2000; Qiu et al., 2006; Chang and Graham, 2011), QS supported the
462 alternative configuration of mosses and liverworts as sister groups (as in WI2014 and ZN2014;
463 see also Zhong et al., 2013a).

464 In all three datasets, the monophyly of vascular plants was strongly maintained, despite the
465 inclusion of *Selaginella* that have unusual GC content (Banks et al., 2011). However, the branch
466 leading to *Selaginella* often was accompanied by a higher QD value, which could be a result of
467 the biased composition or indication of a secondary evolutionary history. The QF value for
468 *Selaginella* in all cases was moderate, indicating that overall it did not more often produce
469 discordant topologies compared to other lineages. We also observed substantial discordance and
470 counter-support for relationships tested among various bryophyte groups and key taxa in HS2014,
471 possibly indicative of substantially under-appreciated hybridization among mosses (Nylander
472 et al., 2004). The collective results indicated a (perhaps complicated) monophyly of liverworts
473 and mosses, inconsistent placement of hornworts, and strong support for tracheophytes.

474 ***QS analysis of ferns confirms close relationship to seed plants and shows QS is effective on***
475 ***smaller datasets—*** Another notable similarity of both HS2014 and ZN2014 was the poor score
476 at the base of "euphyllophytes" that placed ferns in a clade with seed plants. QC scores on this
477 branch were near zero in HS2104 and ZN2014 (0.02 and –0.06, respectively) and relatively low
478 for the WI2014 tree (QC=0.33); QD values were also relatively large (0.44–1). Within ferns the
479 arrangement of major clades in ZN2014 (Fig. 4E) was mostly consistent with the recently
480 published phylogeny by The Pteridophyte Phylogeny Group (PPG I, 2016). Those clades whose

481 relationships were counter-supported (Marratiales, Salviniales, Hymenophyllales) were
482 discordant with the PPG I consensus and other recent phylogenies (Pryer et al., 2004; Testo and
483 Sundue, 2016) demonstrating the utility of QS in highlighting suspect relationships. Some key
484 areas of known high uncertainty (e.g., *Saccoloma* and *Lindsaea*) were highlighted with low and
485 negative QC scores. Additionally, the debated position of *Equisetum* was also reflected by low
486 scores.

487 While QS was designed for large datasets, we also found that QS can perform well on smaller
488 multi-gene datasets conventionally used for systematics studies. Using the phylogeny and data
489 from Pryer et al. (2016b), we reanalyzed the 5778 bp alignment using QS, NBS, SH, and PP
490 scores (Fig. 7). The QS scores were more conservative, but confirmed the conclusions of Pryer
491 et al. (2016b) regarding the monophyly of maidenhair ferns (*Adiantum*) and its placement in a
492 clade with the Vittarioids. In terms of QC/QD/QU scores, ZN2014 also supported the monophyly
493 of *Adiantum* (0./1.0/0.83; 14 taxa), the Vittarioids (0.20/1/0.71; 8 taxa), and their common
494 ancestor (0.22/1/0.87). We also found that among different branches in PR2016 with fairly similar
495 NBS and PP values, QS offers a wide range of support values (including negative-QC
496 counter-support).

497 ***Gymnosperms are monophyletic under QS and support Ginkgo in a clade with cycads—***
498 Another question that has attracted substantial debate is the relationships among the major
499 gymnosperm lineages and angiosperms. Under QS evaluation, HS2014, ZN2014, XI2014, and
500 WI2014 all indicate strong support for monophyly of recognized gymnosperm lineages. However,
501 the relationships among cone-bearing lineages differ among these four phylogenies. ZN2014 and
502 WI2014 place *Ginkgo* in a clade with cycads (consistent with Qiu et al., 2006; Bowe et al., 2000;

503 Lee et al., 2011; Xi et al., 2013, ; Fig. 6B). While the HS2014 topology places cycads as sister to
504 the remaining gymnosperms (i.e., not monophyletic with *Ginkgo*), the QS evaluation
505 counter-supports this relationship. Therefore, even though HS2014/WI2014 and ZN2014 had
506 different topological arrangements of these taxa, the QS analyses of these datasets indicates a
507 consistent message of a *Ginkgo*+cycads clade separate from the rest of gymnosperms.
508 This pattern of disagreement in topology but consistent QS interpretation was observed again
509 in the placement of Gnetales relative to the conifer linages (Fig. 6C). ZN2014 indicates Gnetales
510 in a clade with a monophyletic Pinales (consistent with Lee et al. 2011) whereas HS2014 and
511 WI2014 show a Gnetales+Pinaceae ancestor distinct from other conifers (i.e., the "Gnepine"
512 hypothesis; Bowe et al., 2000; Xi et al., 2013). However, again the HS2014 and WI2014 QS
513 scores offer counter-support against the "Gnepine" relationship (QC=-0.19 and -0.67,
514 respectively) and show that one alternative was strongly preferred (QD=0.5 and 0.1). Here again,
515 in evaluating different competing topologies the QS method can offer consistent interpretations
516 that in this case indicates the monophyly of Pinales, but perhaps also offer some (albeit weak)
517 evidence that warrants further examination of possible gene flow between Gnetales and Pinales.
518 Finally, among the non-Pinaceae conifers, we observed conflicting patterns of poor backbone
519 support for monophyletic clades. In both ZN2014 and HS2014, *Amentotaxus* and *Torreya* form a
520 clade with the rest of Taxaceae to the exclusion of *Cephalotaxus*. Relationships between these
521 three Taxaceae lineages are both strongly counter-supported by QS. The high values of QD
522 (0.56–0.8) offer indication of possible introgression among these lineages. Therefore, again QS
523 scores highlight a part of the phylogenetic tree that may be mis-modeled by these consensus
524 phylogenies.

525 ***Quartet Sampling indicates biased conflict among the ANA grade angiosperms—*** Few
526 issues in angiosperm evolution have garnered more recent debate than the relationship among the
527 so-called "ANA grade" angiosperms, which include *Amborella*, Nymphaeales, and
528 Austrobaileyales. Of the four datasets where this phylogenetic relationship was testable, only
529 WI2014 inferred the "*Amborella*-first" hypothesis (Figs. 5, 6D; Qiu et al., 1999; Stefanović et al.,
530 2004; Qiu et al., 2006; Moore et al., 2007; Soltis et al., 2011; The Amborella Genome Project,
531 2013). ZN2014 shows a "Nymphaeales-first" relationship (Fig. 4B), and HS2014 and XI2014
532 indicate "*Amborella*+Nymphaeales"-first (Fig. 3B; Appendix S6). Regardless of the resolution
533 inferred in the consensus phylogenies from each dataset, the branches surrounding the
534 ANA-grade were all counter-supported (QC$<$0) and biased in their discordance (QD$>$0.8; Fig.
535 6D). ZN2014 offers weak support for *Amborella*+Nymphaeales, while XI2014 counter-supports
536 this relationship. In HS2014, WI2014, and ZN2014, the scores on the branch that placed
537 Austrobaileyales as the closest relative group to the remainder of angiosperms were strongly
538 QC-counter-supported and QD-biased.

539 Two questions surround the relationships of the ANA grade angiosperms. First, what was the
540 relationship among these lineages? Second, are the longstanding disagreements in inference of
541 these relationships the result of genuine biological conflict (i.e., introgression, horizontal transfer,
542 etc.), limitations in the data, or a methodological artifact arising from the depth of this branch, the
543 monotypic status of *Amborella*, and/or the rapidity of the angiosperm radiation? On the first
544 question, QS lacks support for "Nymphaeales-first". Whether there more support exists for
545 *Amborella*+Nymphaeales or "*Amborella*-first" (as found also by The Amborella Genome Project,
546 2013) is unclear. The results we present suggest that both relationships have support in the data,
547 depending on changes in the dataset composition (as also found by Wickett et al. 2014).

548 On the second question, however, the strong QD values indicate a biased conflict that suggests
549 a secondary evolutionary history conflicting with the primary tree in a way that has so far evaded
550 comprehensive characterization. Demonstrations that bryophyte mitochondrial sequences are
551 present in *Amborella* (Rice et al., 2013; Taylor et al., 2015) indicate that these lineages have
552 experienced ancient introgressive events that might create competing evolutionary narratives in
553 the manner indicated by the QS results shown here (and see also Shen et al., 2017). Given the
554 intensity of effort to address these relationships without a broad community consensus and the
555 specific evidence of long-range introgression in *Amborella*, a greater understanding of
556 ANA-grade evolution likely lies in an examination of complex evolutionary histories rather than
557 in a continuation of the debate over appropriate sampling or models.

558 ***QS supports a magnoliid+eudicot ancestor and raises questions about the placement of the***
559 ***Chloranthaceae and Ceratophyllaceae—*** The timings and order of the relationships among the
560 three "core angiosperm" lineages (eudicots, monocots, and magnoliids) represent the evolution of
561 three clades that have transformed the biosphere. ZN2014, WI2014, and XI2014 all indicate the
562 existence of a magnoliid+eudicot clade (Figs. 4B, 5 Fig. 6E, Appendix S6; also in Qiu et al.,
563 2006; Burleigh et al., 2009; Lee et al., 2011). An alternative monocot+eudicot clade appears in
564 HS2014 (Fig. 3B), which has been suggested by many other past phylogenies (Jansen et al., 2007;
565 Moore et al., 2007; Qiu et al., 2010; Soltis et al., 2011). However, in HS2014 the QS scores
566 counter-support both eudicot+monocots and magnoliids/monocot+eudicot clades. Here again,
567 despite disagreement among the topologies of the three large-scale phylogenies, the supporting
568 and counter-supporting QS scores indicate a common evolutionary history.
569 In addition to the three major core angiosperm groups, the Chloranthaceae have frequently

been placed in a clade with magnoliids (as in Jansen et al. 2007; Moore et al. 2007; Soltis et al. 2011; Group et al. 2016, and Fig. 3 in Wickett et al. 2014). However, both HS2014 and ZN2014 place Chloranthaceae in a clade with magnoliids, monocot, and eudicots, while WI2014 places it in a clade with just eudicots (excluding monocots and magnoliids) that is, however, counter-supported by the sampled quartets (QC=–0.54, QD=0.95).

Ceratophyllum, often placed as the most closely related group to eudicots (Jansen et al., 2007; Soltis et al., 2011), in HS2014 appears in a clade with the Chloranthaceae among the prior to monocot/magnoliid/eudicot splits. However, the branch placing Chloranthaceae+*Ceratophyllum* lineage in a clade with magnoliids+monocots+eudicots was counter-supported (Fig. 3B) with a strongly biased QD (0.81). ZN2014 supports *Ceratophyllum* as separate from the core angiosperms. Given that this question is inextricably linked with the relationships of the three core angiosperm groups, a consensus was not reached by this information alone. However, this evidence reopens the question of the relationship of Chloranthaceae and Ceratophyllaceae, both to each other and to the ANA grade angiosperm lineages (see discussion in Eklund et al., 2004).

Relatively consistent topology but poor support found within monocots— Within monocots, *Acorus* was weakly but consistently supported as a separate clade from the rest of monocots in HS2014, ZN2014, and WI2014 (Figs. 3B, 4B, 5). In general, the arrangement of monocot orders in both HS2014 (Fig. 3C) and ZN2014 (Fig. 4C) agreed with recent consensus phylogenies (Givnish et al., 2010; Soltis et al., 2011; Barrett et al., 2015; Givnish et al., 2016; McKain et al., 2016).

One exception was the placement of Liliales, which has varied in consensus trees and, here, either was sister to commelinids with weak support (HS2014) or was sister to Asparagales

(ZN2014). The commelinid orders were resolved inconsistently between studies. The commelinid stem branch was counter-supported in both ZN2014 and HS2014 with high QD values. In HS2014, Arecales were separate from the rest of the commelinids, but the ancestral commelinid branches both including and excluding Arecales were counter supported. In ZN2014, Arecales was supported in a clade with Zingiberales and Commelinales, but the ancestral branch for these three orders has a QD=0.79. This arrangement was consistent with previous phylogenies (e.g., Givnish et al. (2010)) that have poor support to these relationships. From the QS results, we would cautiously infer that (1) the relationships among the commelinids are still unknown, (2) there may be uncharacterized secondary evolutionary history distorting the phylogenetic placement of these groups, and (3) likely the variable data from both Liliales and Arecales together have a joint effect that is causing inconsistency in the phylogenetic inference.

Finally, in Poaceae, Quartet Sampling made clear the well-characterized discordance and complex relationships (e.g., Washburn et al., 2015; McKain et al., 2016). The "BOP" clade (Bambusoideae, Oryzeae, Pooideae) was counter-supported in HS2014 and both paraphyletic and counter-supported in ZN2014. Within the "PACMAD" clade, many of the named subgroupings were counter-supported with negative QC values. There were particularly high QU values in both HS2014 and ZN2014 for this clade indicating not only complex support but also low information. Therefore, even if someone were unfamiliar with the controversies surrounding monocot phylogenetics (as a whole and within groups), the QS evaluation framework clearly highlights this group as one with consistently high conflict and counter-support values for the consensus phylogenies in HS2014 and ZN2014.

613 ***Non-rosid/asterid eudicot lineages show substantial disagreement and rogue taxa—*** Within
614 eudicots, Ranunculales were supported as a separate clade from the rest of eudicots by WI2014,
615 HS2014, XI2014, and CN2015, or among the non-rosid/asterid eudicots in ZN2014 (Figs. 3B,
616 4B, 5; Appendix S6, Appendix S7). Notably in both ZN2015 and HS2014, *Nelumbo* (sacred
617 lotus) was placed sister to a highly supported Proteales clade, but support for the
618 Proteales+*Nelumbo* clade was strongly counter-supported by the QC and QD scores. This again
619 demonstrates a situation where QS effectively highlights this lineage's conflicted relationship
620 with the Proteales in a way that raises questions about a possible genuine biological (rather than
621 artifactual) cause of phylogenetic discordance.
622 *Vitis vinifera* (common grape vine) has also had variable inferred positions throughout
623 eudicots. WI2014, XI2014, and CN2015 all place *Vitis* as a "superrosid" and most closely related
624 clade to the rosids, but with widely ranging support and skew (QC=–0.13 to 0.63, QD=0.56 to
625 1.0) consistent with the conflict noted by Cannon et al. (2015b) (abbreviated CN2015; Appendix
626 S7). HS2014 (which sampled 64 Vitales) supported a Dilleniales+Gunnerales+Vitales clade,
627 while ZN2014 places Vitales+*Tetrastigma* among the rosids in a Vitales+malvid clade to the
628 exclusion of fabids. In the case of the grape vine, the high support in WI2014 (analyzed by gene
629 trees) accompanied by a high QD value (1.0) gives strong indication that while Vitales may
630 belong among the superrosids, an investigation of a secondary evolution history for grapes might
631 be warranted.
632 For the relationships among the superasterid groups (Caryophyllales, Berberidopsidales,
633 Santalales, and asterids), a common pattern was found in HS2014, ZN2015, WI2014, and XI2014
634 of near-zero QC values (–0.03 to 0.08), modest QD values (0–0.03), and high QU values
635 (0.49-0.86). This led to a consensus QS interpretation of low phylogenetic information, likely as a

result of the rapid radiation of these lineages. Generally, these phylogenies tended to support weakly the controversial placement of Caryophyllales as most closely related to the eudicot ancestor.

QS shows strong support for the consensus rosid phylogeny, but substantial discordance both within and among asterid clades— HS2014 (Fig. 3D) find similar resolutions among rosid lineages (Wang et al., 2009; Moore et al., 2010; Soltis et al., 2011; Zhao et al., 2016) (the lone exception was the Sapindales+Huertales clade). Among the branches leading to major groups, all were supported and most of the backbone branches were either supported or only weakly negative. The QS scores also correctly identified a poorly supported relationship in HS2014 between *Cynomorium* and Cucurbitales (QC=–0.31). *Cynomorium*, a non-photosynthetic parasitic plant with unusual morphology, has been placed tenuously and variably in groups as diverse as Rosales (Zhang et al., 2009) and Saxifragales (Nickrent et al., 2005), so its poor score here was expected and confirms the QS method's ability to detect poorly placed species. This "rogue" status was corroborated by the below-average QF score of QF=0.18 (mean 0.21 for HS2014). This means that for quartets that include *Cynomorium* as a randomly sampled taxon, only 18% produced a quartet topology concordant with the HS2014 tree.

In contrast to the analysis of the rosid phylogeny from HS2014, the asterid phylogeny from ZN2014 (Fig. 4D) appears to have substantial discordance and disagreement with most published phylogenies (Soltis et al., 2011; Beaulieu et al., 2013; Refulio-Rodriguez and Olmstead, 2014). ZN2014 QS scores supported the unusual hypothesis of a common Ericales+Cornales ancestor, weakly support the campanulid clade, and counter-support a common lamiid ancestor. The arrangement of families within Asterales either roughly conforms to Soltis et al. (2011) and

658 Beaulieu et al. (2013), or counter-supports branches (QC<0) that do not agree with these
659 consensus phylogenies. However, most of the branches that define the relationships among asterid
660 orders in ZN2014 were counter-supported by the data, though most have QC and QD values close
661 to zero. This indicates a scenario of a rapid radiation rather than hybridization (though these are
662 not mutually exclusive). The lamiid groups show strong support for the monophyly of the
663 *Lamianae* core orders, though in an order different from Stull et al. (2015). Among the asterids
664 there were also several notably low QF scores for singular genera like *Oncotheca*, *Vahlia*, and
665 *Pentaphragma* (all QF<0.16). Thus, the QS method in asterids does not necessarily add clarity to
666 this group, but perhaps more concretely puts into focus the combinations of discordance and low
667 information occurring in this group.

668 ***QS of shallow-timescale phylotranscriptomic datasets—*** So far, we have demonstrated the
669 utility of quartet sampling on large, sparse alignments which are often computationally intractable
670 with other support measures. We have also shown, in the case of WI2014, that a relatively large
671 and full occupied matrix from deep-timescale transcriptomic data can also be evaluated by QS.
672 However, the QS method can be used to rapidly evaluate phylogenetic support on genome-wide
673 datasets with little missing data for shorter evolutionary timescales. We tested the QS method on
674 two phylotranscriptomic datasets for the wild and domesticated tomato clade *Solanum* sect.
675 *Lycopersicon* (Fig. 8A; Pease et al., 2016b) and carnivorous plants spanning the Caryophyllales
676 (Fig. 8B; Walker et al., In Press).

677 The *Solanum* phylogeny from Pease et al. (2016b) was inferred from coding sequence
678 alignment of 33,105,168 nucleotide sites for 30 populations spanning all 13 wild and
679 domesticated tomato species, and two outgroup species. As described in Pease et al. 2016b, this

680 dataset contains a high level of phylogenetic discordance, but had a consensus phylogeny with
681 100% NBS support at all but two branches (occurring within a large species complex). However,
682 gene tree analysis of this group clearly showed evidence of massive phylogenetic discordance.
683 When we applied QS to this phylogeny using the entire alignment, scores for many branches were
684 also perfect (i.e., 1/0/0; Table 1). However, several of the other branches in the "Peruvianum
685 group" species complex had lower QS scores in the full alignment (Fig. 8A). When gene trees
686 were used (both a quartet of taxa and a gene alignment were randomly chosen for 1000 QS
687 replicates), all branches had QC$<$1 in a manner consistent with the gene tree discordance found
688 previously in this clade. We also observed the presence of high QD values within the major
689 subgroups reported for this clade, while nodes defining these groups showed high QC and low
690 QD values. This accurately captures the results found by Pease et al. (2016b) in terms of the low
691 discordance between groups versus high discordance within the major groups.
692 Most notably, the tree shown in Fig. 8A includes *S. huaylasense* accession LA1360. This
693 accession has been known (both from this and other datasets) to mostly likely be a hybrid
694 between populations from the green-fruited and red-fruited lineages (essentially those accessions
695 above and below LA1360, respectively, in Fig. 8A). Thus, the inclusion of this putative hybrid
696 lineage distorted the phylogeny as tree inference methods tried to cope with inherited and
697 introgressed alleles from two separate groups to place this accession in a consensus location on
698 the tree. While NBS scores were high for the branches surrounding the placement of LA1360, QS
699 showed negative QC scores and high QD scores (QD=1 for full alignment). This supports the
700 presence of the alternative phylogenetic history that has been previously corroborated by other
701 studies (see additional discussion in the Supplementary Results of Pease et al. 2016b). These data
702 show clearly that QS was able to distinguish between consistently supported relationships and

703 branches known to have conflict due to introgression (whereas NBS does not).

704 An analysis of transcriptomes of carnivorous plants from Caryophyllales (Fig. 8B; Walker
705 et al. In Press) showed QC scores consistent with IC scores. The QS scores for the ancestor of
706 "core Caryophyllales" were near zero and the high QD value when gene trees were used
707 (QD=0.68) supported the hypothesis of Walker et al. (In Press) that introgressive gene flow may
708 have occurred among these lineages. The evidence for placing *Drosophyllum* among the
709 carnivorous Caryophyllales has always been tenuous. The QS analysis here showed not only a
710 low QF value (QF=0.76, average for WA2017 was 0.89) for this taxon, but also low-QC/high-QD
711 values for the two branches that form the clade with *Ancistrocladus* and *Nepenthes*. As with the
712 tomato example above, this example demonstrates how QS scores can highlight an entire region
713 that may be distorted by the inclusion of a taxon with a strong potential for a secondary
714 evolutionary history (i.e., possible introgression).

715 CONCLUSION

716 In this study, our goal was to reanalyze several key and long-contested conflicts in the plant tree
717 of life by embracing the apparently complex relationships in the plant tree of life. The Quartet
718 Sampling method that we have described and demonstrated here synthesizes a blend of
719 phylogenetic and phylogenomic methods to provide a coherent framework for distinguishing
720 several causes of low phylogenetic branch support. QS also provides a tractable means to analyze
721 sparse datasets with tens of thousands of taxa but poor sequence overlap. Bootstrap and posterior
722 probability values have been shown to exhibit irregular behavior or to report uniformly high
723 confidence scores for large-scale datasets, despite substantial underlying conflict. Even when

724 conventional support measures do report low support, the specific cause of the low support is not
725 indicated, leaving it open to broad interpretation and speculation. Despite the decades of
726 documented concerns about the limitations of these support measures, bootstrap proportions
727 remain the dominant support measure in systematics. Our results, as well as those reported in
728 many other studies cited here, demonstrate the need for continuing discussion about the nature of
729 phylogenetic disagreement itself and how best to quantify this complexity. While much testing
730 and refinement of the QS method is undoubtedly left to be done, we find it provides a key
731 function that has been missing from other support measures, namely the ability to distinguish
732 among different causes of low support that commonly occur in modern molecular phylogenies.
733 The application of the QS method to the plant tree of life has also yielded some intriguing
734 results. The comparison of several recent consensus phylogenies showed that they differ at
735 several key, disputed relationships. However, when QS scores were compared, only one topology
736 was supported with the alternatives showing weak support, conflicted support, or even
737 counter-support (e.g., *Ginkgo* placed in a clade with cycads, Gnetales in a clade with a common
738 conifer ancestor, monocots separate from a magnoliid+eudicot clade, and Caryophyllales sister to
739 asterids). QS scores for some other relationships indicated the existence of multiple conflicting
740 but supported evolutionary histories (e.g., the placement of *Amborella*, possible widespread gene
741 flow in the monocots, and notoriously difficult-to-place genera like *Haplomitrium*, *Nelumbo*, and
742 *Cynomorium*). The artist Man Ray once remarked that "We have never attained the infinite variety
743 and contradictions that exist in nature." Overall, the picture painted by QS is one of substantial
744 contradiction, but this conflict can be a richly informative (not just confounding) illustration of
745 the interwoven evolutionary histories contained within the plant tree of life.

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LITERATURE CITED

Aberer, A. J., D. Krompass, and A. Stamatakis. 2012. Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. *Systematic Biology* 62: 162–166.

Alfaro, M. E. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* 20: 255–266.

Allman, E. S., L. S. Kubatko, and J. A. Rhodes. 2017. Split scores: a tool to quantify phylogenetic signal in genome-scale data. *Systematic Biology* .

Anderson, C. N. K., L. Liu, D. Pearl, and S. V. Edwards. 2012. Tangled trees: the challenge of

763 inferring species trees from coalescent and noncoalescent genes. *In* Methods in Molecular
764 Biology. Springer Science Business Media, 3–28.

765 Andrews, D. W. K. 2000. Inconsistency of the bootstrap when a parameter is on the boundary of
766 the parameter space. *Econometrica* 68: 399–405.

767 Ané, C., B. Larget, D. A. Baum, S. D. Smith, and A. Rokas. 2006. Bayesian estimation of
768 concordance among gene trees. *Molecular Biology and Evolution* 24: 412–426.

769 Anisimova, M. and O. Gascuel. 2006. Approximate likelihood-ratio test for branches: a fast,
770 accurate, and powerful alternative. *Systematic Biology* 55: 539–552.

771 Anisimova, M., M. Gil, J.-F. Dufayard, C. Dessimoz, and O. Gascuel. 2011. Survey of branch
772 support methods demonstrates accuracy, power, and robustness of fast likelihood-based
773 approximation schemes. *Systematic Biology* 60: 685–699.

774 Banks, J. A., T. Nishiyama, M. Hasebe, J. L. Bowman, M. Gribskov, C. dePamphilis, V. A.
775 Albert, et al. 2011. The *Selaginella* genome identifies genetic changes associated with the
776 evolution of vascular plants. *Science* 332: 960–963.

777 Barrett, C. F., W. J. Baker, J. R. Comer, J. G. Conran, S. C. Lahmeyer, J. H. Leebens-Mack, J. Li,
778 et al. 2015. Plastid genomes reveal support for deep phylogenetic relationships and extensive
779 rate variation among palms and other commelinid monocots. *New Phytologist* 209: 855–870.

780 Beaulieu, J. M., D. C. Tank, and M. J. Donoghue. 2013. A Southern Hemisphere origin for
781 campanulid angiosperms, with traces of the break-up of Gondwana. *BMC Evolutionary*
782 *Biology* 13: 80.

783 Bowe, L. M., G. Coat, and C. W. dePamphilis. 2000. Phylogeny of seed plants based on all three
784 genomic compartments: extant gymnosperms are monophyletic and Gnetales' closest relatives
785 are conifers. *Proceedings of the National Academy of Sciences of the United States of America*
786 97: 4092–4097.

787 Brown, J. M. and A. R. Lemmon. 2007. The importance of data partitioning and the utility of
788 Bayes factors in Bayesian phylogenetics. *Systematic Biology* 56: 643–655.

789 Brown, J. M. and R. C. Thomson. 2016. Bayes factors unmask highly variable information
790 content, bias, and extreme influence in phylogenomic analyses. *Systematic Biology* : syw101.

791 Brown, J. W., J. F. Walker, and S. A. Smith. 2017. Phyx: phylogenetic tools for unix.
792 *Bioinformatics* .

793 Burleigh, J. G., K. W. Hilu, and D. E. Soltis. 2009. Inferring phylogenies with incomplete data
794 sets: a 5-gene, 567-taxon analysis of angiosperms. *BMC Evolutionary Biology* 9: 61.

795 Cannon, S. B., M. R. McKain, A. Harkess, M. N. Nelson, S. Dash, M. K. Deyholos, Y. Peng,
796 et al. 2015a. Data from: Multiple polyploidy events in the early radiation of nodulating and
797 non-nodulating legumes. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.ff1tq.

798 Cannon, S. B., M. R. McKain, A. Harkess, M. N. Nelson, S. Dash, M. K. Deyholos, Y. Peng,
799 et al. 2015b. Multiple polyploidy events in the early radiation of nodulating and nonnodulating
800 legumes. *Molecular Biology and Evolution* 32: 193–210.

801 Chang, Y. and S. W. Graham. 2011. Inferring the higher-order phylogeny of mosses (Bryophyta)
802 and relatives using a large, multigene plastid data set. *American Journal of Botany* 98:
803 839–849.

804 Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall,
805 et al. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid
806 gene rbcL. *Annals of the Missouri Botanical Garden* 80: 528.

807 Chaw, S. M., A. Zharkikh, H. M. Sung, T. C. Lau, and W. H. Li. 1997. Molecular phylogeny of
808 extant gymnosperms and seed plant evolution: analysis of nuclear 18S rRNA sequences.
809 *Molecular Biology and Evolution* 14: 56–68.

810 Chifman, J. and L. Kubatko. 2014. Quartet inference from SNP data under the coalescent model.
811 *Bioinformatics* 30: 3317–3324.

812 Crane, P. R. 1985. Phylogenetic analysis of seed plants and the origin of angiosperms. *Annals of*
813 *the Missouri Botanical Garden* : 716–793.

814 Cummings, M. P., S. A. Handley, D. S. Myers, D. L. Reed, A. Rokas, and K. Winka. 2003.
815 comparing bootstrap and posterior probability values in the four-taxon case. *Systematic*
816 *Biology* 52: 477–487.

817 Degnan, J. H. and L. A. Salter. 2005. Gene tree distributions under the coalescent process.
818 *Evolution* 59: 24–37.

819 Douady, C. J., F. Delsuc, Y. Boucher, W. F. Doolittle, and E. J. P. Douzery. 2003. Comparison of
820 Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molecular*
821 *Biology and Evolution* 20: 248–254.

822 Drummond, A. J. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling
823 trees. *BMC Evolutionary Biology* 7: 214.

824 Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. Testing for ancient admixture
825 between closely related populations. *Molecular Biology and Evolution* 28: 2239–2252.

826 Eaton, D. A. R., E. L. Spriggs, B. Park, and M. J. s. Donoghue. 2016. Misconceptions on missing
827 data in RAD-seq phylogenetics with a deep-scale example from flowering plants. *Systematic*
828 *Biology* : syw092.

829 Edwards, E. J., R. Nyffeler, and M. J. Donoghue. 2005. Basal cactus phylogeny: implications of
830 *Pereskia* (Cactaceae) paraphyly for the transition to the cactus life form. *American Journal of*
831 *Botany* 92: 1177–1188.

832 Edwards, S. V., Z. Xi, A. Janke, B. C. Faircloth, J. E. McCormack, T. C. Glenn, B. Zhong, et al.
833 2016. Implementing and testing the multispecies coalescent model: a valuable paradigm for
834 phylogenomics. *Molecular Phylogenetics and Evolution* 94: 447–462.

835 Efron, B. 1992. Bootstrap methods: another look at the jackknife. *In* Springer Series in Statistics.
836 Springer Science Business Media, 569–593.

837 Eklund, H., J. A. Doyle, and P. S. Herendeen. 2004. Morphological phylogenetic analysis of
838 living and fossil Chloranthaceae. *International Journal of Plant Sciences* 165: 107–151.

839 Farris, J. S., V. A. Albert, M. Kallersjo, D. Lipscomb, and A. G. Kluge. 1996. Parsimony
840 jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

841 Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap.
842 *Evolution* 39: 783–791.

843 Felsenstein, J. and H. Kishino. 1993. Is there something wrong with the bootstrap on
844 phylogenies? a reply to Hillis and Bull. *Systematic Biology* 42: 193–200.

845 Fletcher, W. and Z. Yang. 2009. INDELible: a flexible simulator of biological sequence
846 evolution. *Molecular Biology and Evolution* 26: 1879–1888.

847 Gaither, J. and L. Kubatko. 2016. Hypothesis tests for phylogenetic quartets, with applications to
848 coalescent-based species tree inference. *Journal of Theoretical Biology* 408: 179–186.

849 Givnish, T. J., M. Ames, J. R. McNeal, M. R. McKain, P. R. Steele, C. W. dePamphilis, S. W.
850 Graham, et al. 2010. Assembling the tree of the monocotyledons: plastome sequence
851 phylogeny and evolution of Poales. *Annals of the Missouri Botanical Garden* 97: 584–616.

852 Givnish, T. J., A. Zuluaga, I. Marques, V. K. Y. Lam, M. S. Gomez, W. J. D. Iles, M. Ames, et al.
853 2016. Phylogenomics and historical biogeography of the monocot order Liliales: out of
854 Australia and through Antarctica. *Cladistics* 32: 581–605.

855 Goremykin, V. V., S. V. Nikiforova, D. Cavalieri, M. Pindo, and P. Lockhart. 2015. The root of
856 flowering plants and total evidence. *Systematic Biology* 64: 879–891.

857 Goulet, B. E., F. Roda, and R. Hopkins. 2017. Hybridization in plants: old ideas, new techniques.
858 *Plant Physiology* 173: 65–78.

859 Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, et al.
860 2010. A draft sequence of the neandertal genome. *Science* 328: 710–722.

861 Group, T. A. P., M. W. Chase, M. J. M. Christenhusz, M. F. Fay, J. W. Byng, W. S. Judd, D. E.

862 Soltis, et al. 2016. An update of the Angiosperm Phylogeny Group classification for the orders
863 and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.

864 Guindon, S., J.-F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New
865 algorithms and methods to estimate maximum-likelihood phylogenies: assessing the
866 performance of PhyML 3.0. *Systematic Biology* 59: 307–321.

867 Hahn, M. W. and L. Nakhleh. 2015. Irrational exuberance for resolved species trees. *Evolution*
868 70: 7–17.

869 Hillis, D. M. and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing
870 confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.

871 Hinchliff, C. E. and E. H. Roalson. 2013. Using supermatrices for phylogenetic inquiry: an
872 example using the sedges. *Systematic Biology* 62: 205–219.

873 Hinchliff, C. E. and S. A. Smith. 2014a. Data from: Some limitations of public sequence data for
874 phylogenetic inference (in plants). Dryad Digital Repository.
875 http://dx.doi.org/10.5061/dryad.450qq.

876 Hinchliff, C. E. and S. A. Smith. 2014b. Some limitations of public sequence data for
877 phylogenetic inference (in plants). *PLoS ONE* 9: e98986.

878 Huelsenbeck, J. and B. Rannala. 2004. Frequentist properties of Bayesian posterior probabilities
879 of phylogenetic trees under simple and complex substitution models. *Systematic Biology* 53:
880 904–913.

881 Jansen, R. K., Z. Cai, L. A. Raubeson, H. Daniell, C. W. dePamphilis, J. Leebens-Mack, K. F.

882 Muller, et al. 2007. Analysis of 81 genes from 64 plastid genomes resolves relationships in
883 angiosperms and identifies genome-scale evolutionary patterns. *Proceedings of the National*
884 *Academy of Sciences of the United States of America* 104: 19369–19374.

885 Jiao, Y., N. J. Wickett, S. Ayyampalayam, A. S. Chanderbali, L. Landherr, P. E. Ralph, L. P.
886 Tomsho, et al. 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature* 473:
887 97–100.

888 Kobert, K., L. Salichos, A. Rokas, and A. Stamatakis. 2016. Computing the internode certainty
889 and related measures from partial gene trees. *Molecular Biology and Evolution* 33: 1606–1617.

890 Larget, B. 2013. The estimation of tree posterior probabilities using conditional clade probability
891 distributions. *Systematic Biology* 62: 501–511.

892 Larget, B. and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian
893 analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750–759.

894 Lee, E. K., A. Cibrian-Jaramillo, S.-O. Kolokotronis, M. S. Katari, A. Stamatakis, M. Ott, J. C.
895 Chiu, et al. 2011. A functional phylogenomic view of the seed plants. *PLoS Genetics* 7:
896 e1002411.

897 Matasci, N., L.-H. Hung, Z. Yan, E. J. Carpenter, N. J. Wickett, S. Mirarab, N. Nguyen, et al.
898 2014. Data access for the 1,000 Plants (1KP) project. *GigaScience* 3.

899 McKain, M. R., H. Tang, J. R. McNeal, S. Ayyampalayam, J. I. Davis, C. W. dePamphilis, T. J.
900 Givnish, et al. 2016. A phylogenomic assessment of ancient polyploidy and genome evolution
901 across the Poales. *Genome Biology and Evolution* : evw060.

902 Minh, B. Q., M. A. T. Nguyen, and A. von Haeseler. 2013. Ultrafast approximation for
903 phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1188–1195.

904 Mirarab, S., R. Reaz, M. S. Bayzid, T. Zimmermann, M. S. Swenson, and T. Warnow. 2014.
905 ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* 30:
906 i541–i548.

907 Misof, B., B. Meyer, B. von Reumont, P. Kück, K. Misof, and K. Meusemann. 2013. Selecting
908 informative subsets of sparse supermatrices increases the chance to find correct trees. *BMC*
909 *Bioinformatics* 14: 348.

910 Moore, M. J., C. D. Bell, P. S. Soltis, and D. E. Soltis. 2007. Using plastid genome-scale data to
911 resolve enigmatic relationships among basal angiosperms. *Proceedings of the National*
912 *Academy of Sciences of the United States of America* 104: 19363–19368.

913 Moore, M. J., P. S. Soltis, C. D. Bell, J. G. Burleigh, and D. E. Soltis. 2010. Phylogenetic analysis
914 of 83 plastid genes further resolves the early diversification of eudicots. *Proceedings of the*
915 *National Academy of Sciences of the United States of America* 107: 4623–4628.

916 Nickrent, D. L., J. P. Der, and F. E. Anderson. 2005. Discovery of the photosynthetic relatives of
917 the "Maltese mushroom" *Cynomorium*. *BMC Evolutionary Biology* 5: 38.

918 Nickrent, D. L., C. L. Parkinson, J. D. Palmer, and R. J. Duff. 2000. Multigene phylogeny of land
919 plants with special reference to bryophytes and the earliest land plants. *Molecular Biology and*
920 *Evolution* 17: 1885–1895.

921 Nylander, J., F. Ronquist, J. Huelsenbeck, and J. Nieves-aldrey. 2004. Bayesian phylogenetic
922 analysis of combined data. *Systematic Biology* 53: 47–67.

923 Ogden, T. H. and M. S. Rosenberg. 2006. Multiple sequence alignment accuracy and
924 phylogenetic inference. *Systematic Biology* 55: 314–328.

925 Palmer, J. D., D. E. Soltis, and M. W. Chase. 2004. The plant tree of life: an overview and some
926 points of view. *American Journal of Botany* 91: 1437–1445.

927 Pease, J. B., D. C. Haak, M. W. Hahn, and L. C. Moyle. 2016a. Data from: Phylogenomics
928 reveals three sources of adaptive variation during a rapid radiation. Dryad Digital Repository.
929 http://dx.doi.org/10.5061/dryad.182dv.

930 Pease, J. B., D. C. Haak, M. W. Hahn, and L. C. Moyle. 2016b. Phylogenomics reveals three
931 sources of adaptive variation during a rapid radiation. *PLoS Biology* 14: e1002379.

932 Pease, J. B. and M. W. Hahn. 2015. Detection and polarization of introgression in a five-taxon
933 phylogeny. *Systematic Biology* 64: 651–662.

934 PPG I. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of*
935 *Systematics and Evolution* 54: 563–603.

936 Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2–approximately maximum-likelihood
937 trees for large alignments. *PLoS ONE* 5: e9490.

938 Pryer, K. M., L. Huiet, F.-W. Li, C. J. Rothfels, and E. Schuettpelz. 2016a. Data from:
939 Maidenhair ferns, *Adiantum*, are indeed monophyletic and sister to shoestring ferns, vittarioids
940 (Pteridaceae). Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.4m6s6.

941 Pryer, K. M., L. Huiet, F.-W. Li, C. J. Rothfels, and E. Schuettpelz. 2016b. Maidenhair ferns

(*Adiantum*) are indeed monophyletic and sister to shoestring ferns, vittarioids (Pteridaceae). *Systematic Botany* 41: 17–23.

Pryer, K. M., H. Schneider, A. R. Smith, R. Cranfill, P. G. Wolf, J. S. Hunt, and S. D. Sipes. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* 409: 618–622.

Pryer, K. M., E. Schuettpelz, P. G. Wolf, H. Schneider, A. R. Smith, and R. Cranfill. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. *American Journal of Botany* 91: 1582–1598.

Qiu, Y.-L., J. Lee, F. Bernasconi-Quadroni, D. E. Soltis, P. S. Soltis, M. Zanis, E. A. Zimmer, et al. 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402: 404–407.

Qiu, Y.-L., L. Li, B. Wang, Z. Chen, V. Knoop, M. Groth-Malonek, O. Dombrovska, et al. 2006. The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences of the United States of America* 103: 15511–15516.

Qiu, Y.-L., L. Li, B. Wang, J.-Y. Xue, T. A. Hendry, R.-Q. Li, J. W. Brown, et al. 2010. Angiosperm phylogeny inferred from sequences of four mitochondrial genes. *Journal of Systematics and Evolution* 48: 391–425.

Ranwez, V. and O. Gascuel. 2001. Quartet-based phylogenetic inference: improvements and limits. *Molecular Biology and Evolution* 18: 1103–1116.

Refulio-Rodriguez, N. F. and R. G. Olmstead. 2014. Phylogeny of Lamiidae. *American Journal of Botany* 101: 287–299.

963 Reich, D., K. Thangaraj, N. Patterson, A. L. Price, and L. Singh. 2009. Reconstructing Indian
964 population history. *Nature* 461: 489–494.

965 Reid, N. M., S. M. Hird, J. M. Brown, T. A. Pelletier, J. D. McVay, J. D. Satler, and B. C.
966 Carstens. 2013. Poor fit to the multispecies coalescent is widely detectable in empirical data.
967 *Systematic Biology* 63: 322–333.

968 Renzaglia, K. S., R. J. Duff, D. L. Nickrent, and D. J. Garbary. 2000. Vegetative and reproductive
969 innovations of early land plants: implications for a unified phylogeny. *Philosophical*
970 *Transactions of the Royal Society B: Biological Sciences* 355: 769–793.

971 Rice, D. W., A. J. Alverson, A. O. Richardson, G. J. Young, M. V. Sanchez-Puerta, J. Munzinger,
972 K. Barry, et al. 2013. Horizontal transfer of entire genomes via mitochondrial fusion in the
973 angiosperm *Amborella*. *Science* 342: 1468–1473.

974 Rokas, A., B. L. Williams, N. King, and S. B. Carroll. 2003. Genome-scale approaches to
975 resolving incongruence in molecular phylogenies. *Nature* 425: 798–804.

976 Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under
977 mixed models. *Bioinformatics* 19: 1572–1574.

978 Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Hohna, B. Larget, et al.
979 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large
980 model space. *Systematic Biology* 61: 539–542.

981 Rosenberg, N. A. 2002. The probability of topological concordance of gene trees and species
982 trees. *Theoretical Population Biology* 61: 225–247.

983 Roure, B., D. Baurain, and H. Philippe. 2012. Impact of missing data on phylogenies inferred
984 from empirical phylogenomic data sets. *Molecular Biology and Evolution* 30: 197–214.

985 Salichos, L., A. Stamatakis, and A. Rokas. 2014. Novel information theory-based measures for
986 quantifying incongruence among phylogenetic trees. *Molecular Biology and Evolution* 31:
987 1261–1271.

988 Sanderson, M. J. 1995. Objections to bootstrapping phylogenies: a critique. *Systematic Biology*
989 44: 299.

990 Sayyari, E. and S. Mirarab. 2016. Fast coalescent-based computation of local branch support
991 from quartet frequencies. *Molecular Biology and Evolution* : msw079.

992 Shavit Grievink, L., D. Penny, M. D. Hendy, and B. R. Holland. 2010. Phylogenetic tree
993 reconstruction accuracy and model fit when proportions of variable sites change across the tree.
994 *Systematic Biology* 59: 288–297.

995 Shaw, A. J., P. Szövényi, and B. Shaw. 2011. Bryophyte diversity and evolution: windows into
996 the early evolution of land plants. *American Journal of Botany* 98: 352–369.

997 Shen, X.-X., C. T. Hittinger, and A. Rokas. 2017. Contentious relationships in phylogenomic
998 studies can be driven by a handful of genes. *Nature Ecology & Evolution* 1: 0126.

999 Simmons, M. P. 2016. Mutually exclusive phylogenomic inferences at the root of the
1000 angiosperms: *Amborella* is supported as sister and Observed Variability is biased. *Cladistics* .

1001 Simmons, M. P. and A. P. Norton. 2014. Divergent maximum-likelihood-branch-support values
1002 for polytomies. *Molecular Phylogenetics and Evolution* 73: 87–96.

1003 Simmons, M. P. and C. P. Randle. 2014. Disparate parametric branch-support values from
1004 ambiguous characters. *Molecular Phylogenetics and Evolution* 78: 66–86.

1005 Smith, S. A., J. M. Beaulieu, and M. J. Donoghue. 2009. Mega-phylogeny approach for
1006 comparative biology: an alternative to supertree and supermatrix approaches. *BMC*
1007 *Evolutionary Biology* 9: 37.

1008 Smith, S. A., J. M. Beaulieu, A. Stamatakis, and M. J. Donoghue. 2011. Understanding
1009 angiosperm diversification using small and large phylogenetic trees. *American Journal of*
1010 *Botany* 98: 404–414.

1011 Smith, S. A., M. J. Moore, J. W. Brown, and Y. Yang. 2015. Analysis of phylogenomic datasets
1012 reveals conflict, concordance, and gene duplications with examples from animals and plants.
1013 *BMC Evolutionary Biology* 15.

1014 Soltis, D. E., S. A. Smith, N. Cellinese, K. J. Wurdack, D. C. Tank, S. F. Brockington, N. F.
1015 Refulio-Rodriguez, et al. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal*
1016 *of Botany* 98: 704–730.

1017 Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
1018 large phylogenies. *Bioinformatics* 30: 1312–1313.

1019 Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML
1020 Web servers. *Systematic Biology* 57: 758–771.

1021 Steel, M. and M. J. Sanderson. 2010. Characterizing phylogenetically decisive taxon coverage.
1022 *Applied Mathematics Letters* 23: 82–86.

1023 Steemans, P., A. L. Herisse, J. Melvin, M. A. Miller, F. Paris, J. Verniers, and C. H. Wellman.
1024 2009. Origin and radiation of the earliest vascular land plants. *Science* 324: 353–353.

1025 Stefanović, S., D. W. Rice, and J. D. Palmer. 2004. Long branch attraction, taxon sampling, and
1026 the earliest angiosperms: *Amborella* or monocots? *BMC Evolutionary Biology* 4: 35.

1027 Strimmer, K., N. Goldman, and A. von Haeseler. 1997. Bayesian probabilities and quartet
1028 puzzling. *Molecular Biology and Evolution* 14: 210–211.

1029 Strimmer, K. and A. von Haeseler. 1997. Likelihood-mapping: a simple method to visualize
1030 phylogenetic content of a sequence alignment. *Proceedings of the National Academy of*
1031 *Sciences of the United States of America* 94: 6815–6819.

1032 Stull, G. W., R. D. de Stefano, D. E. Soltis, and P. S. Soltis. 2015. Resolving basal lamiid
1033 phylogeny and the circumscription of Icacinaceae with a plastome-scale data set. *American*
1034 *Journal of Botany* 102: 1794–1813.

1035 Suzuki, Y., G. V. Glazko, and M. Nei. 2002. Overcredibility of molecular phylogenies obtained
1036 by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences of the United*
1037 *States of America* 99: 16138–16143.

1038 Swofford, D. L. and J. Sullivan. 2003. The phylogenetic handbook, volume 7, chapter Phylogeny
1039 inference based on parsimony and other methods using PAUP*. Cambridge University Press.
1040 ISBN 0521730716, 160–206.

1041 Taylor, Z. N., D. W. Rice, and J. D. Palmer. 2015. The complete moss mitochondrial genome in
1042 the angiosperm *Amborella* is a chimera derived from two moss whole-genome transfers. *PLoS*
1043 *ONE* 10: e0137532.

1044 Testo, W. and M. Sundue. 2016. A 4000-species dataset provides new insight into the evolution of
1045 ferns. *Molecular Phylogenetics and Evolution* 105: 200–211.

1046 The Amborella Genome Project. 2013. The *Amborella* genome and the evolution of flowering
1047 plants. *Science* 342: 1241089–1241089.

1048 Walker, J. F., Y. Yang, M. J. Moore, J. Mikenas, A. Timoneda, S. F. Brockington, and S. A. Smith.
1049 2017. Data from: Widespread paleopolyploidy, gene tree conflict, and recalcitrant relationships
1050 among the carnivorous Caryophyllales. Dryad Digital Repository. In press.

1051 Walker, J. F., Y. Yang, M. J. Moore, J. Mikenas, A. Timoneda, S. F. Brockington, and S. A. Smith.
1052 In Press. Widespread paleopolyploidy, gene tree conflict, and recalcitrant relationships among
1053 the carnivorous Caryophyllales. *American Journal of Botany* .

1054 Wang, H., M. J. Moore, P. S. Soltis, C. D. Bell, S. F. Brockington, R. Alexandre, C. C. Davis,
1055 et al. 2009. Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of*
1056 *the National Academy of Sciences of the United States of America* 106: 3853–3858.

1057 Washburn, J. D., J. C. Schnable, G. Davidse, and J. C. Pires. 2015. Phylogeny and photosynthesis
1058 of the grass tribe Paniceae. *American Journal of Botany* 102: 1493–1505.

1059 Wickett, N. J., S. Mirarab, N. Nguyen, T. Warnow, E. Carpenter, N. Matasci, S. Ayyampalayam,
1060 et al. 2014. Phylotranscriptomic analysis of the origin and early diversification of land plants.
1061 *Proceedings of the National Academy of Sciences of the United States of America* 111:
1062 E4859–E4868.

1063 Wiens, J. J. and M. C. Morrill. 2011. Missing data in phylogenetic analysis: reconciling results
1064 from simulations and empirical data. *Systematic Biology* 60: 719–731.

1065 Wilcox, T. P., D. J. Zwickl, T. A. Heath, and D. M. Hillis. 2002. Phylogenetic relationships of the
1066 dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support.
1067 *Molecular Phylogenetics and Evolution* 25: 361–371.

1068 Wilkinson, M. 1996. Majority-rule reduced consensus trees and their use in bootstrapping.
1069 *Molecular Biology and Evolution* 13: 437–444.

1070 Xi, Z., L. Liu, J. S. Rest, and C. C. Davis. 2014a. Coalescent versus concatenation methods and
1071 the placement of *Amborella* as sister to water lilies. *Systematic Biology* 63: 919–932.

1072 Xi, Z., L. Liu, J. S. Rest, and C. C. Davis. 2014b. Data from: Coalescent versus concatenation
1073 methods and the placement of *Amborella* as sister to water lilies. Dryad Digital Repository.
1074 http://dx.doi.org/10.5061/dryad.qb251.

1075 Xi, Z., J. S. Rest, and C. C. Davis. 2013. Phylogenomics and coalescent analyses resolve extant
1076 seed plant relationships. *PLoS ONE* 8: e80870.

1077 Yang, Y., M. J. Moore, S. F. Brockington, D. E. Soltis, G. K.-S. Wong, E. J. Carpenter, Y. Zhang,
1078 et al. 2015. Dissecting molecular evolution in the highly diverse plant clade Caryophyllales
1079 using transcriptome sequencing. *Molecular Biology and Evolution* 32: 2001–2014.

1080 Zanne, A. E., D. C. Tank, W. K. Cornwell, J. M. Eastman, S. A. Smith, R. G. FitzJohn, D. J.
1081 McGlinn, et al. 2014a. Data from: Three keys to the radiation of angiosperms into freezing
1082 environments. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.63q27.2.

1083 Zanne, A. E., D. C. Tank, W. K. Cornwell, J. M. Eastman, S. A. Smith, R. G. FitzJohn, D. J.
1084 McGlinn, et al. 2014b. Three keys to the radiation of angiosperms into freezing environments.
1085 *Nature* 506: 89–92.

1086 Zhang, Z.-H., L. I. Chun-Qi, and L. I. Jianhua. 2009. Phylogenetic placement *Cynomoriumin* in
1087 Rosales inferred from sequences of the inverted repeat region of the chloroplast genome.
1088 *Journal of Systematics and Evolution* 47: 297–304.

1089 Zhao, L., X. Li, N. Zhang, S.-D. Zhang, T.-S. Yi, H. Ma, Z.-H. Guo, et al. 2016. Phylogenomic
1090 analyses of large-scale nuclear genes provide new insights into the evolutionary relationships
1091 within the rosids. *Molecular Phylogenetics and Evolution* 105: 166–176.

1092 Zhong, B., L. Liu, Z. Yan, and D. Penny. 2013a. Origin of land plants using the multispecies
1093 coalescent model. *Trends in Plant Science* 18: 492–495.

1094 Zhong, B., Z. Xi, V. V. Goremykin, R. Fong, P. A. Mclenachan, P. M. Novis, C. C. Davis, et al.
1095 2013b. Streptophyte algae and the origin of land plants revisited using heterogeneous models
1096 with three new algal chloroplast genomes. *Molecular Biology and Evolution* 31: 177–183.

1097 Zwickl, D. J. and D. M. Hillis. 2002. Increased taxon sampling greatly reduces phylogenetic
1098 error. *Systematic Biology* 51: 588–598.

1099 Zwickl, D. J., J. C. Stein, R. A. Wing, D. Ware, and M. J. Sanderson. 2014. Disentangling
1100 methodological and biological sources of gene tree discordance on *Oryza* (Poaceae)
1101 chromosome 3. *Systematic Biology* 63: 645–659.

Table 1: Quartet Sampling (QS) score interpretation. See text for details.

Example QS Score (QC/QD/QU)*	**Interpretation**
1.0/-/0.0	**Full support:** All sampled quartet replicates support the focal branch (QC=1) with no uncertainty when likelihood cutoffs are used (QU=0).
0.5/0.02/0.03	**Strong support:** A strong majority of quartets support the focal branch (QC=0.5) and the low skew in discordant frequencies (QD≈0) indicate no alternative history is favored.
0.7/0.9/0.03	**Strong support with discordant skew:** A strong majority of quartets support the focal branch (QC=0.7), but the skew in discordance (QD=0.9) indicates the possible presence of a supported secondary evolutionary history.
0.05/0.04/0.03	**Weak support:** Only a weak majority of quartets support the focal branch (QC=0.05), and the frequency of all three possible topologies are similar (QD≈0).
0.1/0.9/0.03	**Weak support with discordant skew:** Only a weak majority of quartets support the focal branch (QC=0.1), and the skew in discordance (QD=0.9) indicates the possible presence of a supported secondary evolutionary history.
-0.5/0.9/0.07	**Counter-support:** A strong majority of quartets support one of the alternative discordant quartet arrangements history (QC<0; QD expected to be high).
1/0.03/0.95	**Poorly informed:** Despite supportive QC/QD values, 95% of quartets failed the likelihood cutoff (QU=0.95), likely indicating few informative sites.
0.0/0.0/0.0	**Perfectly conflicted:** The (unlikely) case where the frequency of all three possible trees was equal with no uncertainty, indicating a rapid radiation or highly complex conflict.

Notes: * QC = Quartet Concordance; QD = Quartet Differential; QU = Quartet Uncertainty.

FIGURE LEGENDS

Fig. 1. Description of the Quartet Sampling method. (A) The focal branch "*b*" divides the phylogeny into four subclades $\{S_1, S_2, S_3, S_4\}$ from which tips (A–J) are sampled. Two replicates with different sampled tips for the given branch are shown with the three possible unrooted topologies (one concordant and two discordant). (B) Each internal branch is labeled with a set of three scores (QC/QD/QU), which offer different, but complementary, information. Terminal branches are evaluated by the QF score, which reports the frequency of a taxon generating concordant topologies. (See Materials and Methods for full details and Supplementary Methods for a technical description.)

Fig. 2. Results of Simulation Testing of the Quartet Sampling Method. (A) QC values converge on a central value with increasing numbers of replicates from randomly selected branches from simulated trees with 50, 100, and 500 taxa. (B) Distributions of QC, QU, and QF values for HS2014 (black), ZN2014 (dotted black), and XI2014/CN2015/PR2016/WA2017 (similar distributions; gray solid). (C) Mean QC values (diamond) with 5%ile to 95%ile (whiskers) for branches in HS2015 binned by the number of subtending taxa (i.e., moving root-ward in the tree left-to-right). Overall mean is shown with horizontal dotted line.

Fig. 3. Phylogeny from Hinchliff and Smith (2014b). (A) Full phylogeny with heat map coloration of branches by QC scores for internal branches: dark green (QC>0.2), light green (0.2≤QC>0), light orange (0≤QC≤–0.05, or dark orange (QC>–0.05). (B) QC/QD/QU scores (200 replicates of full alignment) for major plant groups and key orders within angiosperms. QC/QD/QU scores after group names are for the ancestral branch (i.e., the "stem" branch), and a single QF score is shown for monotypic tips. Major subgroups groups are highlighted with vertical labels. (C) QS scores for monocots (excluding *Acorus*). (D,E,F) QS scores for rosids, Bryophyta, and gymnosperms. Abbreviations: Acro, Acrogymnospermae; ANA, ANA grade; Aru, Arundinoideae; Bry, Bryophyta, Chl, Chloridoideae; Dan, Danthonioideae; Mar, Marchantiophyta; Poly, Polypodopsida.

Fig. 4. Phylogeny from Zanne et al. (2014b). (A) Full phylogeny with heat map coloration of branches by QC scores

for internal branches using same color scheme as (Fig. 3). (B) QC/QD/QU scores (200 replicates of full alignment) for major plant groups and key orders within angiosperms, using same color scheme as (Fig. 3). (C) QS scores shown for monocots (except *Acorus*). (D) QS scores for asterids. (E) QS scores for fern lineages and (F) QS scores for gymnosperm lineages respectively. Abbreviations: Alseu, Alseuosmiaceae; ANA, ANA grade angiosperms; Argo, Argophyllaceae; Aster, Asteraceae; Bory, Boryaceae; Caly, Calycanthaceae; Eriach, Eriachneae; Good, Goodeniaceae; gym, gymnosperms; Hypox, Hypoxidaceae; Isach, Isachneae; Phell, Phellinaceae; Poly, Polypodopsida.

Fig. 5. Maximum likelihood phylogeny spanning Viridiplantae from Fig. 2 in Wickett et al. (2014) with QC/QD/QU scores for 200 replicates of the full alignment. Nodes are colored according to QC score using same color scheme as (Fig. 3). Bootstrap values (italicized in square brackets) from Wickett et al. (2014) are shown for comparison. Missing QS or bootstrap values indicate a perfect score. The three taxa with the lowest QF values are highlighted. Species names have been excluded or abbreviated in the case where two congeners are included.

Fig. 6. Key phylogenetic disagreements with QC scores using compared across various datasets. Branches for HS2014 and ZN2014 were resampled with 10000 replicates. Branches for WI2014 and XI2014 were exhaustively sampled (>1000 replicates). Highlighting on QC values follows the same colors as Fig. 3. "Conifers-II" refers to a hypothesized clade comprising the non-Pinales orders in Pinidae. Abbreviations: Gnet, Gnetidae; Pin, Pinidae.

Fig. 7. Phylogeny of Pteridaceae ferns from Pryer et al. (2016b) with QC/QD/QU scores for 200 replicates of the full alignment. Nodes are colored according to QC score using same color scheme as (Fig. 3). Bootstrap/SH-test/posterior probability values (italicized in square brackets) are shown for comparison. Omitted values indicate a perfect score. The three taxa with the lowest QF values are highlighted. Abbreviations: *Pityro*, *Pityrogramma*.

Fig. 8. QS scores for phylogenies from whole-transcriptome data. Omitted values indicate a perfect score. Nodes are colored according to QC score using same color scheme as (Fig. 3). (A) Phylogeny of *Solanum* sect. *Lycopersicon*

1156 from Pease et al. (2016b) Bootstrap values (italicized in square brackets) are shown for comparison. (B) Phylogeny

1157 of Caryophyllales from Walker et al. (In Press) IC scores (light grey) are shown for comparison (all bootstrap and

1158 SH-test scores were 100). The three taxa with the lowest QF values are highlighted.

APPENDICES

Appendix S1. Supplementary Methods providing a technical description of the QS method

Appendix S2. Comparison of QC and bootstrap ICA (information criterion-all; Salichos, et al. 2014) scores on trees reconstructed from 100 simulated datasets with 50 taxa with 1,000 base pairs under a Jukes-Cantor model of evolution. Blue circles represent branches in the true tree, with the size of the circle proportional to the log of the number of substitutions. Red triangles represent branches not in the true tree.

Appendix S3. Comparison of the rapid bootstrap and quartet sampling on the ML/PP consensus tree. For each branch, the RBS, QS (raw concordant frequency (Freq1), QC score), SH, and PP scores are presented (clockwise from top left in each legend). Black dots identify clades that are not in the true tree.

Appendix S4. Shows the consistency of the frequency of concordant quartets (f_1), QC, and QD toward a central value with increasing number of per-branch replicates for a randomly selected branch. Trees with 50 taxa (left), 100 taxa (center), and 500 taxa (right) are shown. Boxes show median $\pm$ IQR. Whiskers show 5^{th}–95^{th} percentile, with values outside this range shown as circle points.

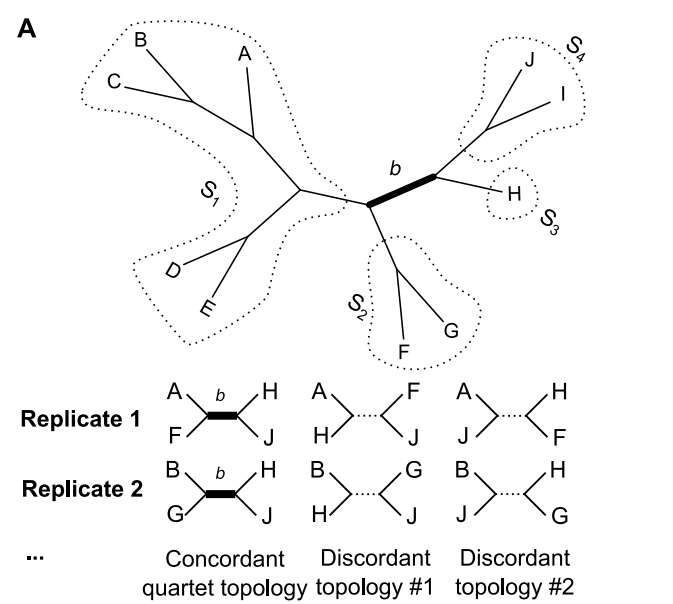
Appendix S5. Histograms (top row) showing the distributions of QC (left), QU (middle), and QF (right) values for the HS2014 dataset (green), ZN2014 (black), and smaller dataset (XI2014, CN2015, PR2016, WA2017) with similar distributions (orange). Scatter plots (bottom row) showing the close (but non-linear) relationship between QC and raw concordant quartet frequency (f_1; left), bounded but otherwise uncorrelated relationships between QC and QD (middle), and QC and QU (right). See main text for dataset abbreviations.

Appendix S6. Phylogeny of angiosperms from Xi et al. (2014a) with QC/QD/QU scores for 200 replicates of the full alignment and for 200 replicates from individual gene trees (in parentheses). Nodes are colored according to QC score using same color scheme as (Fig. 3). MrBayes PP/RAxML NBS values (italicized in square brackets) from Xi

et al. (2013). are shown for comparison. Perfect scores for any given test are omitted or shown as '*' indicates bootstrap of 100, while '-' indicates a missing value. The three taxa with the lowest QF values are highlighted.

Appendix S7. Phylogeny from Cannon et al. (2015b) with QC/QD/QU scores for 200 replicates of the full alignment. Nodes are colored according to QC score using same color scheme as (Fig. 3). Bootstrap values (italicized in square brackets) are shown for comparison. Perfect scores for any given test are omitted or shown as '*' indicates bootstrap of 100, while "–" indicates a missing value. The three taxa with the lowest QF values are highlighted.

Appendix S8. Relationship between QC and frequencies of the three possible alternative quartet topologies from QS runs on simulated data. Points represent branches in the trees, with the "test topology" axis identifying the frequency at which the topology consistent with the tree was recovered across all QS replicates for that branch, and the "alt n" axes identifying the frequencies of the two alternative (conflicting) topologies.



B **Quartet Sampling Internal Node Scores** = **0.52** / **0.04** / **0.01**

Quartet Concordance (QC)
How often is the concordant quartet inferred over both discordant quartets?

QC=1 → all concordant
QC=0 → equivocal conc./disc.
QC<0 → disc. > concordant

Quartet Differential (QD)
Are discordant #1 and #2 frequencies equal or skewed?

QD=0 → all concordant
QD=0.3 → skewed
QD=1 → all #1 or #2

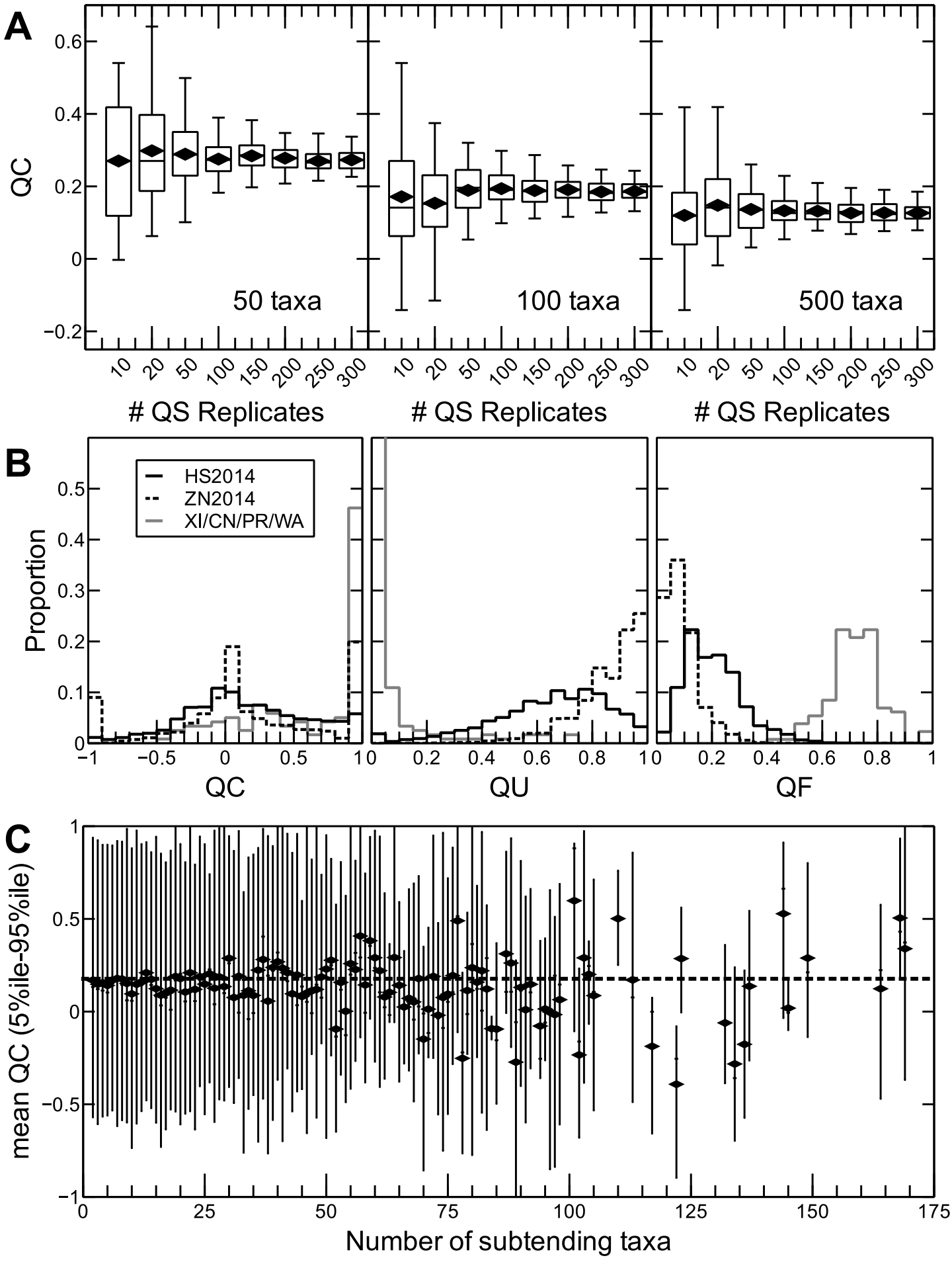
Quartet Uncertainty (QU)
What proportion of replicates were uninformative? (failed likelihood differential cutoff)

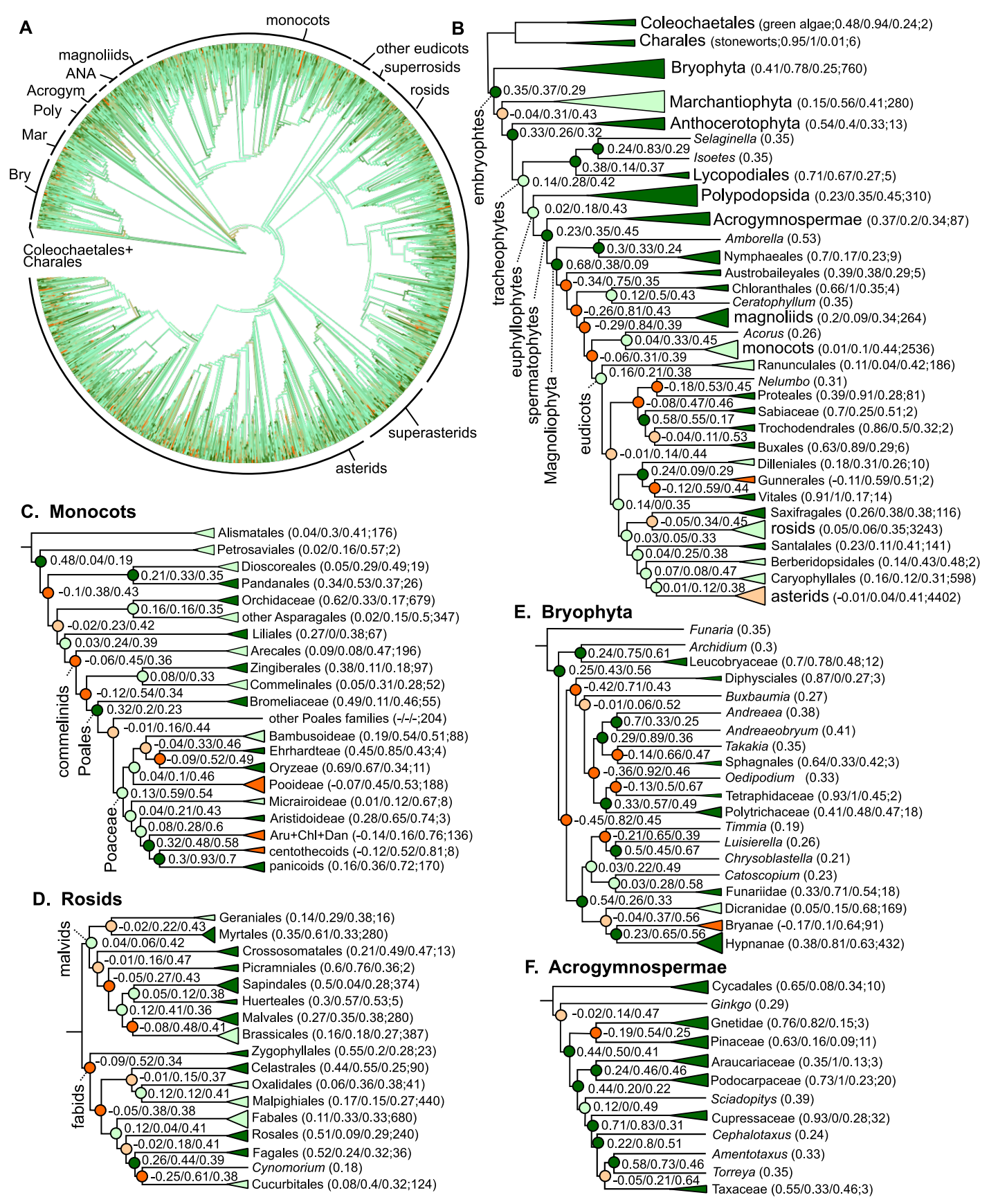
Examples:
QU=0 → no uninformative
QD=0.3 → 30% uninform.
QD=1 → none informative

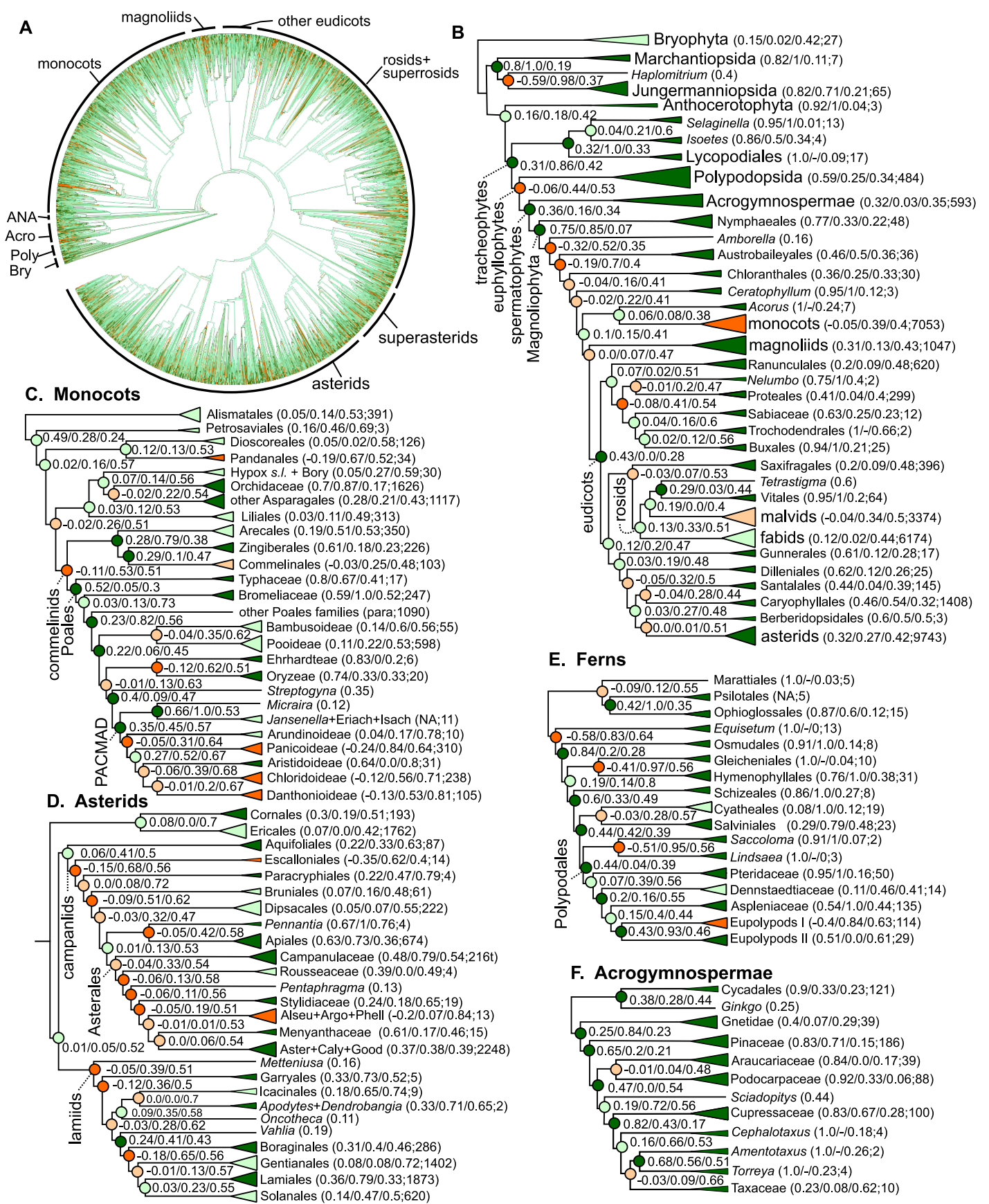
Quartet Sampling Terminal Node Scores = **(0.52)**

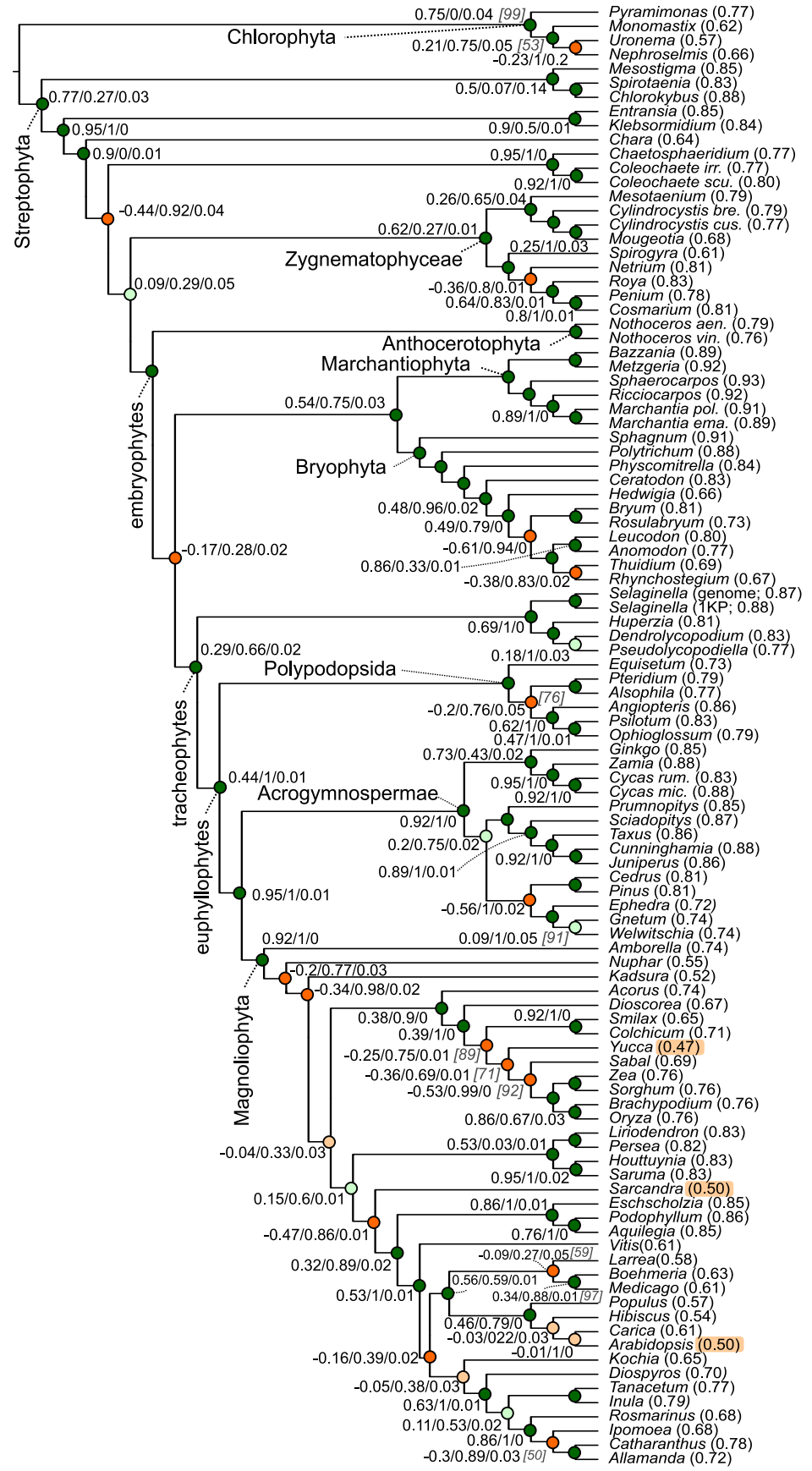
Quartet Fidelity (QF)
When this taxon is sampled, how often does it produce a concordant topology?

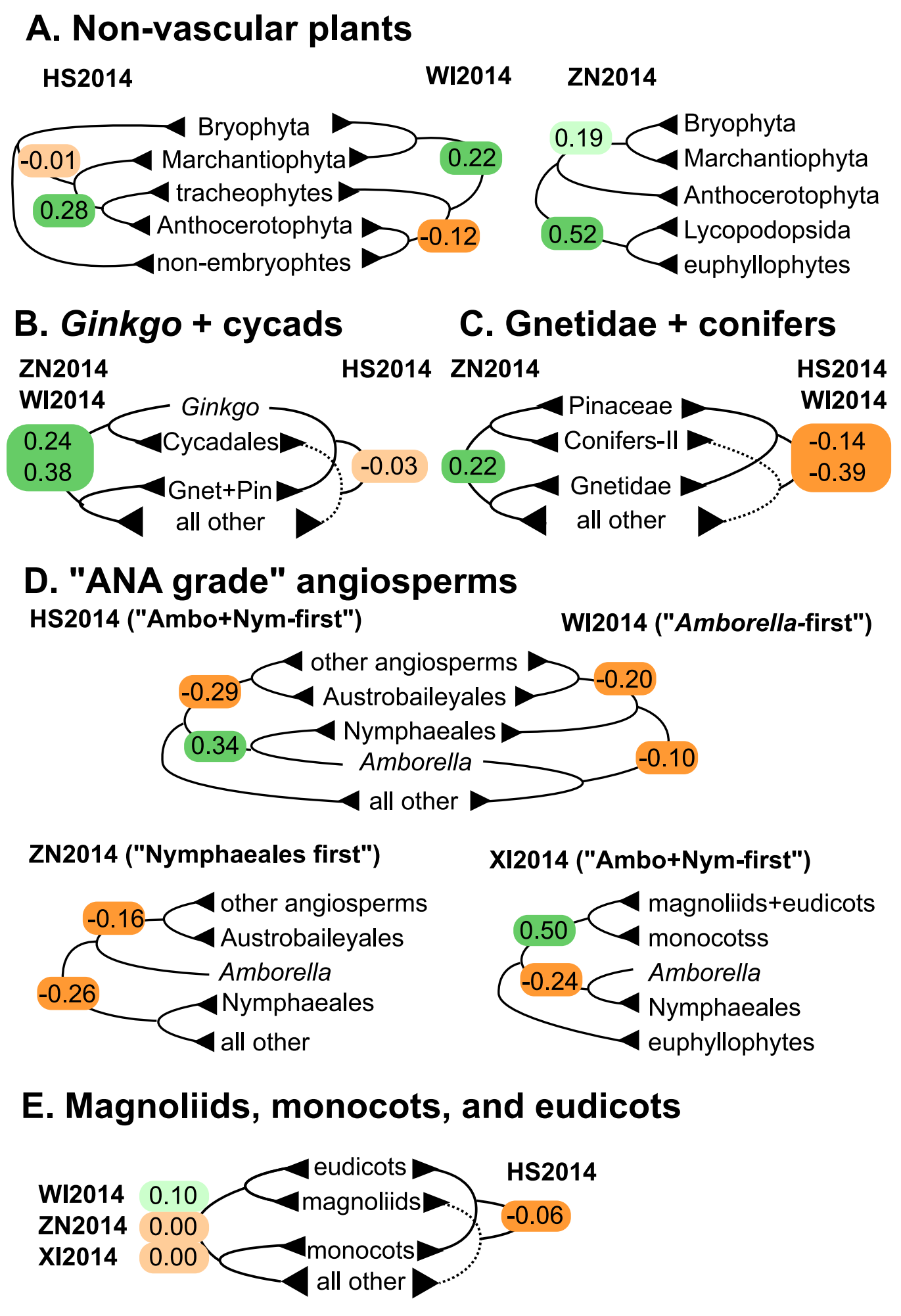
Examples:
QF=1 → all concordant
QF=0.1 → 10% concordant
QF=0 → none concordant

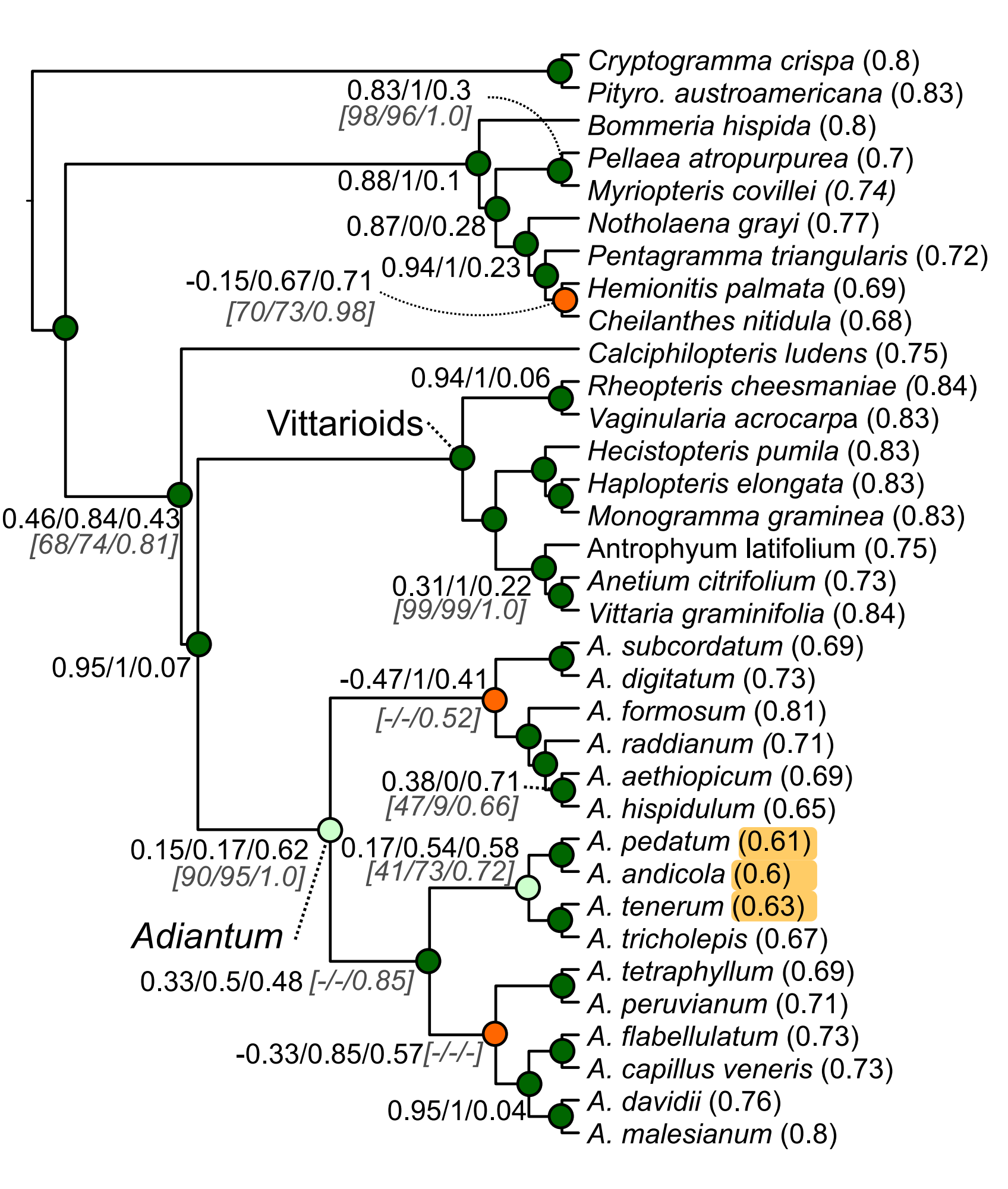

A
QC
0.6
0.4
0.2
0
−0.2
50 taxa
100 taxa
500 taxa
10
20
50
100
150
200
250
300
QS Replicates
B
HS2014
ZN2014
XI/CN/PR/WA
Proportion
0.5
0.4
0.3
0.2
0.1
0
−1
−0.5
0
0.5
1
0.2
0.4
0.6
0.8
QC
QU
QF
C
mean QC (5%ile-95%ile)
1
0.5
0
−0.5
−1
0
25
50
75
100
125
150
175
Number of subtending taxa

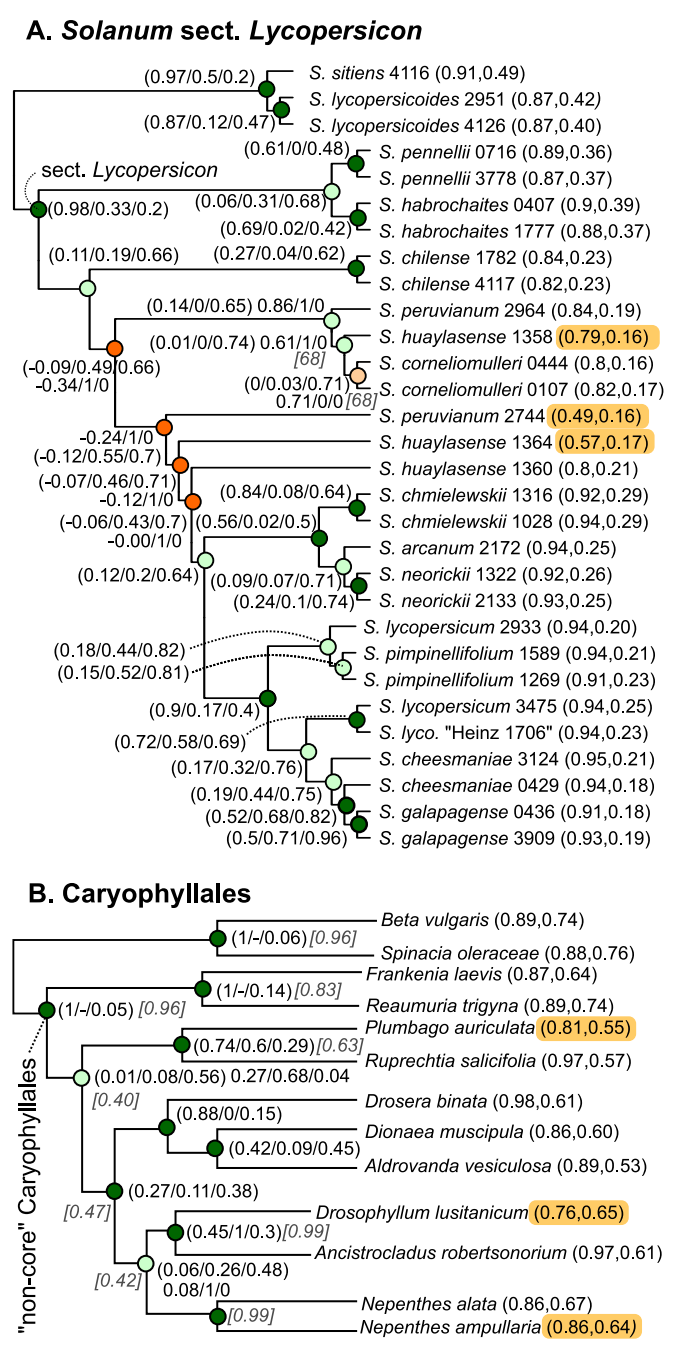

A
monocots
other eudicots
superrosids
rosids
magnoliids
ANA
Acrogym
Poly
Mar
Bry
Coleochaetales+
Charales
superasterids
asterids
B
Coleochaetales (green algae;0.48/0.94/0.24;2)
Charales (stoneworts;0.95/1/0.01;6)
Bryophyta (0.41/0.78/0.25;760)
0.35/0.37/0.29
Marchantiophyta (0.15/0.56/0.41;280)
-0.04/0.31/0.43
Anthocerotophyta (0.54/0.4/0.33;13)
0.33/0.26/0.32
Selaginella (0.35)
0.24/0.83/0.29
Isoetes (0.35)
0.38/0.14/0.37
Lycopodiales (0.71/0.67/0.27;5)
0.14/0.28/0.42
Polypodopsida (0.23/0.35/0.45;310)
0.02/0.18/0.43
Acrogymnospermae (0.37/0.2/0.34;87)
0.23/0.35/0.45
Amborella (0.53)
0.3/0.33/0.24
Nymphaeales (0.7/0.17/0.23;9)
0.68/0.38/0.09
Austrobaileyales (0.39/0.38/0.29;5)
-0.34/0.75/0.35
Chloranthales (0.66/1/0.35;4)
0.12/0.5/0.43
Ceratophyllum (0.35)
-0.26/0.81/0.43
magnoliids (0.2/0.09/0.34;264)
-0.29/0.84/0.39
Acorus (0.26)
0.04/0.33/0.45
monocots (0.01/0.1/0.44;2536)
-0.06/0.31/0.39
Ranunculales (0.11/0.04/0.42;186)
0.16/0.21/0.38
Nelumbo (0.31)
-0.18/0.53/0.45
Proteales (0.39/0.91/0.28;81)
-0.08/0.47/0.46
Sabiaceae (0.7/0.25/0.51;2)
0.58/0.55/0.17
Trochodendrales (0.86/0.5/0.32;2)
-0.04/0.11/0.53
Buxales (0.63/0.89/0.29;6)
-0.01/0.14/0.44
Dilleniales (0.18/0.31/0.26;10)
0.24/0.09/0.29
Gunnerales (-0.11/0.59/0.51;2)
-0.12/0.59/0.44
Vitales (0.91/1/0.17;14)
0.14/0/0.35
Saxifragales (0.26/0.38/0.38;116)
-0.05/0.34/0.45
rosids (0.05/0.06/0.35;3243)
0.03/0.05/0.33
Santalales (0.23/0.11/0.41;141)
0.04/0.25/0.38
Berberidopsidales (0.14/0.43/0.48;2)
0.07/0.08/0.47
Caryophyllales (0.16/0.12/0.31;598)
0.01/0.12/0.38
asterids (-0.01/0.04/0.41;4402)
embryophytes
tracheophytes
euphyllophytes
spermatophytes
Magnoliophyta
eudicots
C. Monocots
Alismatales (0.04/0.3/0.41;176)
Petrosaviales (0.02/0.16/0.57;2)
0.48/0.04/0.19
Dioscoreales (0.05/0.29/0.49;19)
0.21/0.33/0.35
Pandanales (0.34/0.53/0.37;26)
-0.1/0.38/0.43
Orchidaceae (0.62/0.33/0.17;679)
0.16/0.16/0.35
other Asparagales (0.02/0.15/0.5;347)
-0.02/0.23/0.42
Liliales (0.27/0/0.38;67)
0.03/0.24/0.39
Arecales (0.09/0.08/0.47;196)
-0.06/0.45/0.36
Zingiberales (0.38/0.11/0.18;97)
0.08/0/0.33
Commelinales (0.05/0.31/0.28;52)
-0.12/0.54/0.34
Bromeliaceae (0.49/0.11/0.46;55)
0.32/0.2/0.23
other Poales families (-/-/-;204)
-0.01/0.16/0.44
Bambusoideae (0.19/0.54/0.51;88)
-0.04/0.33/0.46
Ehrhardteae (0.45/0.85/0.43;4)
-0.09/0.52/0.49
Oryzeae (0.69/0.67/0.34;11)
0.04/0.1/0.46
Pooideae (-0.07/0.45/0.53;188)
0.13/0.59/0.54
Micrairoideae (0.01/0.12/0.67;8)
0.04/0.21/0.43
Aristidoideae (0.28/0.65/0.74;3)
0.08/0.28/0.6
Aru+Chl+Dan (-0.14/0.16/0.76;136)
0.32/0.48/0.58
centothecoids (-0.12/0.52/0.81;8)
0.3/0.93/0.7
panicoids (0.16/0.36/0.72;170)
commelinids
Poales
Poaceae
D. Rosids
Geraniales (0.14/0.29/0.38;16)
-0.02/0.22/0.43
Myrtales (0.35/0.61/0.33;280)
0.04/0.06/0.42
Crossosomatales (0.21/0.49/0.47;13)
-0.01/0.16/0.47
Picramniales (0.6/0.76/0.36;2)
-0.05/0.27/0.43
Sapindales (0.5/0.04/0.28;374)
0.05/0.12/0.38
Huerteales (0.3/0.57/0.53;5)
0.12/0.41/0.36
Malvales (0.27/0.35/0.38;280)
-0.08/0.48/0.41
Brassicales (0.16/0.18/0.27;387)
Zygophyllales (0.55/0.2/0.28;23)
-0.09/0.52/0.34
Celastrales (0.44/0.55/0.25;90)
-0.01/0.15/0.37
Oxalidales (0.06/0.36/0.38;41)
0.12/0.12/0.41
Malpighiales (0.17/0.15/0.27;440)
-0.05/0.38/0.38
Fabales (0.11/0.33/0.33;680)
0.12/0.04/0.41
Rosales (0.51/0.09/0.29;240)
-0.02/0.18/0.41
Fagales (0.52/0.24/0.32;36)
0.26/0.44/0.39
Cynomorium (0.18)
-0.25/0.61/0.38
Cucurbitales (0.08/0.4/0.32;124)
malvids
fabids
E. Bryophyta
Funaria (0.35)
Archidium (0.3)
0.24/0.75/0.61
Leucobryaceae (0.7/0.78/0.48;12)
0.25/0.43/0.56
Diphysciales (0.87/0/0.27;3)
-0.42/0.71/0.43
Buxbaumia (0.27)
-0.01/0.06/0.52
Andreaea (0.38)
0.7/0.33/0.25
Andreaeobryum (0.41)
0.29/0.89/0.36
Takakia (0.35)
-0.14/0.66/0.47
Sphagnales (0.64/0.33/0.42;3)
-0.36/0.92/0.46
Oedipodium (0.33)
-0.13/0.5/0.67
Tetraphidaceae (0.93/1/0.45;2)
0.33/0.57/0.49
Polytrichaceae (0.41/0.48/0.47;18)
-0.45/0.82/0.45
Timmia (0.19)
-0.21/0.65/0.39
Luisierella (0.26)
0.5/0.45/0.67
Chrysoblastella (0.21)
0.03/0.22/0.49
Catoscopium (0.23)
0.03/0.28/0.58
Funariidae (0.33/0.71/0.54;18)
0.54/0.26/0.33
Dicranidae (0.05/0.15/0.68;169)
-0.04/0.37/0.56
Bryanae (-0.17/0.1/0.64;91)
0.23/0.65/0.56
Hypnanae (0.38/0.81/0.63;432)
F. Acrogymnospermae
Cycadales (0.65/0.08/0.34;10)
Ginkgo (0.29)
-0.02/0.14/0.47
Gnetidae (0.76/0.82/0.15;3)
-0.19/0.54/0.25
Pinaceae (0.63/0.16/0.09;11)
0.44/0.50/0.41
Araucariaceae (0.35/1/0.13;3)
0.24/0.46/0.46
Podocarpaceae (0.73/1/0.23;20)
0.44/0.20/0.22
Sciadopitys (0.39)
0.12/0/0.49
Cupressaceae (0.93/0/0.28;32)
0.71/0.83/0.31
Cephalotaxus (0.24)
0.22/0.8/0.51
Amentotaxus (0.33)
0.58/0.73/0.46
Torreya (0.35)
-0.05/0.21/0.64
Taxaceae (0.55/0.33/0.46;3)


A
magnoliids
other eudicots
monocots
rosids+
superrosids
ANA
Acro
Poly
Bry
superasterids
asterids
B
Bryophyta (0.15/0.02/0.42;27)
Marchantiopsida (0.82/1/0.11;7)
0.8/1.0/0.19
Haplomitrium (0.4)
-0.59/0.98/0.37
Jungermanniopsida (0.82/0.71/0.21;65)
Anthocerotophyta (0.92/1/0.04;3)
0.16/0.18/0.42
Selaginella (0.95/1/0.01;13)
0.04/0.21/0.6
Isoetes (0.86/0.5/0.34;4)
0.32/1.0/0.33
Lycopodiales (1.0/-/0.09;17)
0.31/0.86/0.42
Polypodopsida (0.59/0.25/0.34;484)
-0.06/0.44/0.53
Acrogymnospermae (0.32/0.03/0.35;593)
0.36/0.16/0.34
Nymphaeales (0.77/0.33/0.22;48)
0.75/0.85/0.07
Amborella (0.16)
-0.32/0.52/0.35
Austrobaileyales (0.46/0.5/0.36;36)
-0.19/0.7/0.4
Chloranthales (0.36/0.25/0.33;30)
-0.04/0.16/0.41
Ceratophyllum (0.95/1/0.12;3)
-0.02/0.22/0.41
Acorus (1/-/0.24;7)
0.06/0.08/0.38
monocots (-0.05/0.39/0.4;7053)
0.1/0.15/0.41
magnoliids (0.31/0.13/0.43;1047)
0.0/0.07/0.47
Ranunculales (0.2/0.09/0.48;620)
0.07/0.02/0.51
Nelumbo (0.75/1/0.4;2)
-0.01/0.2/0.47
Proteales (0.41/0.04/0.4;299)
-0.08/0.41/0.54
Sabiaceae (0.63/0.25/0.23;12)
0.04/0.16/0.6
Trochodendrales (1/-/0.66;2)
0.02/0.12/0.56
Buxales (0.94/1/0.21;25)
0.43/0.0/0.28
Saxifragales (0.2/0.09/0.48;396)
-0.03/0.07/0.53
Tetrastigma (0.6)
0.29/0.03/0.44
Vitales (0.95/1/0.2;64)
0.19/0.0/0.4
malvids (-0.04/0.34/0.5;3374)
0.13/0.33/0.51
fabids (0.12/0.02/0.44;6174)
0.12/0.2/0.47
Gunnerales (0.61/0.12/0.28;17)
0.03/0.19/0.48
Dilleniales (0.62/0.12/0.26;25)
-0.05/0.32/0.5
Santalales (0.44/0.04/0.39;145)
-0.04/0.28/0.44
Caryophyllales (0.46/0.54/0.32;1408)
0.03/0.27/0.48
Berberidopsidales (0.6/0.5/0.5;3)
0.0/0.01/0.51
asterids (0.32/0.27/0.42;9743)
tracheophytes
euphyllophytes
spermatophytes
Magnoliophyta
eudicots
rosids
C. Monocots
Alismatales (0.05/0.14/0.53;391)
Petrosaviales (0.16/0.46/0.69;3)
0.49/0.28/0.24
Dioscoreales (0.05/0.02/0.58;126)
0.12/0.13/0.53
Pandanales (-0.19/0.67/0.52;34)
0.02/0.16/0.57
Hypox s.l. + Bory (0.05/0.27/0.59;30)
0.07/0.14/0.56
Orchidaceae (0.7/0.87/0.17;1626)
-0.02/0.22/0.54
other Asparagales (0.28/0.21/0.43;1117)
0.03/0.12/0.53
Liliales (0.03/0.11/0.49;313)
-0.02/0.26/0.51
Arecales (0.19/0.51/0.53;350)
0.28/0.79/0.38
Zingiberales (0.61/0.18/0.23;226)
0.29/0.1/0.47
Commelinales (-0.03/0.25/0.48;103)
-0.11/0.53/0.51
Typhaceae (0.8/0.67/0.41;17)
0.52/0.05/0.3
Bromeliaceae (0.59/1.0/0.52;247)
0.03/0.13/0.73
other Poales families (para;1090)
0.23/0.82/0.56
Bambusoideae (0.14/0.6/0.56;55)
-0.04/0.35/0.62
Pooideae (0.11/0.22/0.53;598)
0.22/0.06/0.45
Ehrhardteae (0.83/0/0.2;6)
-0.12/0.62/0.51
Oryzeae (0.74/0.33/0.33;20)
-0.01/0.13/0.63
Streptogyna (0.35)
0.4/0.09/0.47
Micraira (0.12)
0.66/1.0/0.53
Jansenella+Eriach+Isach (NA;11)
0.35/0.45/0.57
Arundinoideae (0.04/0.17/0.78;10)
-0.05/0.31/0.64
Panicoideae (-0.24/0.84/0.64;310)
0.27/0.52/0.67
Aristidoideae (0.64/0.0/0.8;31)
-0.06/0.39/0.68
Chloridoideae (-0.12/0.56/0.71;238)
-0.01/0.2/0.67
Danthonioideae (-0.13/0.53/0.81;105)
commelinids
Poales
PACMAD
D. Asterids
0.08/0.0/0.7
Cornales (0.3/0.19/0.51;193)
Ericales (0.07/0.0/0.42;1762)
0.06/0.41/0.5
Aquifoliales (0.22/0.33/0.63;87)
-0.15/0.68/0.56
Escalloniales (-0.35/0.62/0.4;14)
0.0/0.08/0.72
Paracryphiales (0.22/0.47/0.79;4)
-0.09/0.51/0.62
Bruniales (0.07/0.16/0.48;61)
-0.03/0.32/0.47
Dipsacales (0.05/0.07/0.55;222)
-0.05/0.42/0.58
Pennantia (0.67/1/0.76;4)
0.01/0.13/0.53
Apiales (0.63/0.73/0.36;674)
-0.04/0.33/0.54
Campanulaceae (0.48/0.79/0.54;216t)
-0.06/0.13/0.58
Rousseaceae (0.39/0.0/0.49;4)
-0.06/0.11/0.56
Pentaphragma (0.13)
-0.05/0.19/0.51
Stylidiaceae (0.24/0.18/0.65;19)
-0.01/0.01/0.53
Alseu+Argo+Phell (-0.2/0.07/0.84;13)
0.0/0.06/0.54
Menyanthaceae (0.61/0.17/0.46;15)
0.01/0.05/0.52
Aster+Caly+Good (0.37/0.38/0.39;2248)
-0.05/0.39/0.51
Metteniusa (0.16)
-0.12/0.36/0.5
Garryales (0.33/0.73/0.52;5)
0.0/0.0/0.7
Icacinales (0.18/0.65/0.74;9)
0.09/0.35/0.58
Apodytes+Dendrobangia (0.33/0.71/0.65;2)
-0.03/0.28/0.62
Oncotheca (0.11)
0.24/0.41/0.43
Vahlia (0.19)
-0.18/0.65/0.56
Boraginales (0.31/0.4/0.46;286)
-0.01/0.13/0.57
Gentianales (0.08/0.08/0.72;1402)
0.03/0.23/0.55
Lamiales (0.36/0.79/0.33;1873)
Solanales (0.14/0.47/0.5;620)
campanlids
Asterales
lamiids
E. Ferns
-0.09/0.12/0.55
Marattiales (1.0/-/0.03;5)
0.42/1.0/0.35
Psilotales (NA;5)
Ophioglossales (0.87/0.6/0.12;15)
Equisetum (1.0/-/0;13)
-0.58/0.83/0.64
Osmudales (0.91/1.0/0.14;8)
0.84/0.2/0.28
Gleicheniales (1.0/-/0.04;10)
-0.41/0.97/0.56
Hymenophyllales (0.76/1.0/0.38;31)
0.19/0.14/0.8
Schizeales (0.86/1.0/0.27;8)
0.6/0.33/0.49
Cyatheales (0.08/1.0/0.12;19)
-0.03/0.28/0.57
Salviniales (0.29/0.79/0.48;23)
0.44/0.42/0.39
Saccoloma (0.91/1/0.07;2)
-0.51/0.95/0.56
Lindsaea (1.0/-/0;3)
0.44/0.04/0.39
Pteridaceae (0.95/1/0.16;50)
0.07/0.39/0.56
Dennstaedtiaceae (0.11/0.46/0.41;14)
0.2/0.16/0.55
Aspleniaceae (0.54/1.0/0.44;135)
0.15/0.4/0.44
Eupolypods I (-0.4/0.84/0.63;114)
0.43/0.93/0.46
Eupolypods II (0.51/0.0/0.61;29)
Polypodales
F. Acrogymnospermae
Cycadales (0.9/0.33/0.23;121)
0.38/0.28/0.44
Ginkgo (0.25)
Gnetidae (0.4/0.07/0.29;39)
0.25/0.84/0.23
Pinaceae (0.83/0.71/0.15;186)
0.65/0.2/0.21
Araucariaceae (0.84/0.0/0.17;39)
-0.01/0.04/0.48
Podocarpaceae (0.92/0.33/0.06;88)
0.47/0.0/0.54
Sciadopitys (0.44)
0.19/0.72/0.56
Cupressaceae (0.83/0.67/0.28;100)
0.82/0.43/0.17
Cephalotaxus (1.0/-/0.18;4)
0.16/0.66/0.53
Amentotaxus (1.0/-/0.26;2)
0.68/0.56/0.51
Torreya (1.0/-/0.23;4)
-0.03/0.09/0.66
Taxaceae (0.23/0.08/0.62;10)


Streptophyta
0.77/0.27/0.03
0.95/1/0
0.9/0/0.01
-0.44/0.92/0.04
0.09/0.29/0.05
embryophytes
-0.17/0.28/0.02
0.29/0.66/0.02
tracheophytes
0.44/1/0.01
euphyllophytes
0.95/1/0.01
0.92/1/0
Magnoliophyta
-0.2/0.77/0.03
-0.34/0.98/0.02
-0.04/0.33/0.03
0.15/0.6/0.01
-0.47/0.86/0.01
0.32/0.89/0.02
0.53/1/0.01
-0.16/0.39/0.02
-0.05/0.38/0.03
0.63/1/0.01
0.11/0.53/0.02
0.86/1/0
-0.3/0.89/0.03 [50]
Chlorophyta
0.75/0/0.04 [99]
0.21/0.75/0.05 [53]
-0.23/1/0.2
0.5/0.07/0.14
0.9/0.5/0.01
0.95/1/0
0.92/1/0
Zygnematophyceae
0.26/0.65/0.04
0.62/0.27/0.01
0.25/1/0.03
-0.36/0.8/0.01
0.64/0.83/0.01
0.8/1/0.01
Anthocerotophyta
Marchantiophyta
0.54/0.75/0.03
0.89/1/0
Bryophyta
0.48/0.96/0.02
0.49/0.79/0
-0.61/0.94/0
0.86/0.33/0.01
-0.38/0.83/0.02
0.69/1/0
Polypodopsida
0.18/1/0.03
[76]
-0.2/0.76/0.05
0.62/1/0
0.47/1/0.01
Acrogymnospermae
0.73/0.43/0.02
0.95/1/0
0.92/1/0
0.92/1/0
0.2/0.75/0.02
0.89/1/0.01
0.92/1/0
-0.56/1/0.02
0.09/1/0.05 [91]
0.38/0.9/0
0.39/1/0
0.92/1/0
-0.25/0.75/0.01 [89]
-0.36/0.69/0.01 [71]
-0.53/0.99/0 [92]
0.86/0.67/0.03
0.53/0.03/0.01
0.95/1/0.02
0.86/1/0.01
0.76/1/0
-0.09/.27/0.05 [59]
0.56/0.59/0.01
0.34/0.88/0.01 [97]
0.46/0.79/0
-0.03/022/0.03
-0.01/1/0
0.64/0.83/0.01
0.8/1/0.01
0.89/1/0
0.69/1/0
0.62/1/0
0.47/1/0.01
0.95/1/0
0.92/1/0
0.92/1/0
0.92/1/0
0.09/1/0.05 [91]
0.92/1/0.01
0.39/1/0
0.53/0.03/0.01
0.95/1/0.02
0.86/1/0.01
0.76/1/0
0.56/0.59/0.01
0.34/0.88/0.01 [97]
0.46/0.79/0
0.63/1/0.01
0.11/0.53/0.02
0.86/1/0
Pyramimonas (0.77)
Monomastix (0.62)
Uronema (0.57)
Nephroselmis (0.66)
Mesostigma (0.85)
Spirotaenia (0.83)
Chlorokybus (0.88)
Entransia (0.85)
Klebsormidium (0.84)
Chara (0.64)
Chaetosphaeridium (0.77)
Coleochaete irr. (0.77)
Coleochaete scu. (0.80)
Mesotaenium (0.79)
Cylindrocystis bre. (0.79)
Cylindrocystis cus. (0.77)
Mougeotia (0.68)
Spirogyra (0.61)
Netrium (0.81)
Roya (0.83)
Penium (0.78)
Cosmarium (0.81)
Nothoceros aen. (0.79)
Nothoceros vin. (0.76)
Bazzania (0.89)
Metzgeria (0.92)
Sphaerocarpos (0.93)
Ricciocarpos (0.92)
Marchantia pol. (0.91)
Marchantia ema. (0.89)
Sphagnum (0.91)
Polytrichum (0.88)
Physcomitrella (0.84)
Ceratodon (0.83)
Hedwigia (0.66)
Bryum (0.81)
Rosulabryum (0.73)
Leucodon (0.80)
Anomodon (0.77)
Thuidium (0.69)
Rhynchostegium (0.67)
Selaginella (genome; 0.87)
Selaginella (1KP; 0.88)
Huperzia (0.81)
Dendrolycopodium (0.83)
Pseudolycopodiella (0.77)
Equisetum (0.73)
Pteridium (0.79)
Alsophila (0.77)
Angiopteris (0.86)
Psilotum (0.83)
Ophioglossum (0.79)
Ginkgo (0.85)
Zamia (0.88)
Cycas rum. (0.83)
Cycas mic. (0.88)
Prumnopitys (0.85)
Sciadopitys (0.87)
Taxus (0.86)
Cunninghamia (0.88)
Juniperus (0.86)
Cedrus (0.81)
Pinus (0.81)
Ephedra (0.72)
Gnetum (0.74)
Welwitschia (0.74)
Amborella (0.74)
Nuphar (0.55)
Kadsura (0.52)
Acorus (0.74)
Dioscorea (0.67)
Smilax (0.65)
Colchicum (0.71)
Yucca (0.47)
Sabal (0.69)
Zea (0.76)
Sorghum (0.76)
Brachypodium (0.76)
Oryza (0.76)
Liriodendron (0.83)
Persea (0.82)
Houttuynia (0.83)
Saruma (0.83)
Sarcandra (0.50)
Eschscholzia (0.85)
Podophyllum (0.86)
Aquilegia (0.85)
Vitis(0.61)
Larrea(0.58)
Boehmeria (0.63)
Medicago (0.61)
Populus (0.57)
Hibiscus (0.54)
Carica (0.61)
Arabidopsis (0.50)
Kochia (0.65)
Diospyros (0.70)
Tanacetum (0.77)
Inula (0.79)
Rosmarinus (0.68)
Ipomoea (0.68)
Catharanthus (0.78)
Allamanda (0.72)


A. Non-vascular plants
HS2014
WI2014
ZN2014
Bryophyta
Marchantiophyta
tracheophytes
Anthocerotophyta
non-embryophtes
-0.01
0.28
0.22
-0.12
0.19
0.52
Bryophyta
Marchantiophyta
Anthocerotophyta
Lycopodopsida
euphyllophytes
B. Ginkgo + cycads
ZN2014
WI2014
HS2014
Ginkgo
Cycadales
Gnet+Pin
all other
0.24
0.38
-0.03
C. Gnetidae + conifers
ZN2014
HS2014
WI2014
Pinaceae
Conifers-II
Gnetidae
all other
0.22
-0.14
-0.39
D. "ANA grade" angiosperms
HS2014 ("Ambo+Nym-first")
WI2014 ("Amborella-first")
other angiosperms
Austrobaileyales
Nymphaeales
Amborella
all other
-0.29
0.34
-0.20
-0.10
ZN2014 ("Nymphaeales first")
other angiosperms
Austrobaileyales
Amborella
Nymphaeales
all other
-0.16
-0.26
XI2014 ("Ambo+Nym-first")
magnoliids+eudicots
monocotss
Amborella
Nymphaeales
euphyllophytes
0.50
-0.24
E. Magnoliids, monocots, and eudicots
WI2014
ZN2014
XI2014
0.10
0.00
0.00
eudicots
magnoliids
monocots
all other
HS2014
-0.06


Cryptogramma crispa (0.8)
0.83/1/0.3
[98/96/1.0]
Pityro. austroamericana (0.83)
Bommeria hispida (0.8)
Pellaea atropurpurea (0.7)
0.88/1/0.1
Myriopteris covillei (0.74)
0.87/0/0.28
Notholaena grayi (0.77)
Pentagramma triangularis (0.72)
0.94/1/0.23
-0.15/0.67/0.71
[70/73/0.98]
Hemionitis palmata (0.69)
Cheilanthes nitidula (0.68)
Calciphilopteris ludens (0.75)
0.94/1/0.06
Rheopteris cheesmaniae (0.84)
Vaginularia acrocarpa (0.83)
Vittarioids
Hecistopteris pumila (0.83)
Haplopteris elongata (0.83)
0.46/0.84/0.43
[68/74/0.81]
Monogramma graminea (0.83)
Antrophyum latifolium (0.75)
Anetium citrifolium (0.73)
0.31/1/0.22
[99/99/1.0]
Vittaria graminifolia (0.84)
A. subcordatum (0.69)
0.95/1/0.07
-0.47/1/0.41
A. digitatum (0.73)
[-/-/0.52]
A. formosum (0.81)
A. raddianum (0.71)
0.38/0/0.71
[47/9/0.66]
A. aethiopicum (0.69)
A. hispidulum (0.65)
0.15/0.17/0.62
[90/95/1.0]
0.17/0.54/0.58
[41/73/0.72]
A. pedatum (0.61)
A. andicola (0.6)
A. tenerum (0.63)
Adiantum
A. tricholepis (0.67)
0.33/0.5/0.48 [-/-/0.85]
A. tetraphyllum (0.69)
A. peruvianum (0.71)
-0.33/0.85/0.57[-/-/-]
A. flabellulatum (0.73)
A. capillus veneris (0.73)
0.95/1/0.04
A. davidii (0.76)
A. malesianum (0.8)


A. Solanum sect. Lycopersicon
(0.97/0.5/0.2)
S. sitiens 4116 (0.91,0.49)
S. lycopersicoides 2951 (0.87,0.42)
(0.87/0.12/0.47)
S. lycopersicoides 4126 (0.87,0.40)
(0.61/0/0.48)
S. pennellii 0716 (0.89,0.36)
S. pennellii 3778 (0.87,0.37)
sect. Lycopersicon
(0.06/0.31/0.68)
S. habrochaites 0407 (0.9,0.39)
(0.98/0.33/0.2)
(0.69/0.02/0.42)
S. habrochaites 1777 (0.88,0.37)
(0.11/0.19/0.66)
(0.27/0.04/0.62)
S. chilense 1782 (0.84,0.23)
S. chilense 4117 (0.82,0.23)
(0.14/0/0.65) 0.86/1/0
S. peruvianum 2964 (0.84,0.19)
(0.01/0/0.74) 0.61/1/0
[68]
S. huaylasense 1358 (0.79,0.16)
S. corneliomulleri 0444 (0.8,0.16)
(-0.09/0.49/0.66)
-0.34/1/0
(0/0.03/0.71)
0.71/0/0 [68]
S. corneliomulleri 0107 (0.82,0.17)
S. peruvianum 2744 (0.49,0.16)
-0.24/1/0
(-0.12/0.55/0.7)
S. huaylasense 1364 (0.57,0.17)
S. huaylasense 1360 (0.8,0.21)
(-0.07/0.46/0.71)
-0.12/1/0
(0.84/0.08/0.64)
S. chmielewskii 1316 (0.92,0.29)
(-0.06/0.43/0.7)
(0.56/0.02/0.5)
S. chmielewskii 1028 (0.94,0.29)
-0.00/1/0
S. arcanum 2172 (0.94,0.25)
(0.12/0.2/0.64)
(0.09/0.07/0.71)
S. neorickii 1322 (0.92,0.26)
(0.24/0.1/0.74)
S. neorickii 2133 (0.93,0.25)
S. lycopersicum 2933 (0.94,0.20)
(0.18/0.44/0.82)
S. pimpinellifolium 1589 (0.94,0.21)
(0.15/0.52/0.81)
S. pimpinellifolium 1269 (0.91,0.23)
(0.9/0.17/0.4)
S. lycopersicum 3475 (0.94,0.25)
S. lyco. "Heinz 1706" (0.94,0.23)
(0.72/0.58/0.69)
S. cheesmaniae 3124 (0.95,0.21)
(0.17/0.32/0.76)
S. cheesmaniae 0429 (0.94,0.18)
(0.19/0.44/0.75)
S. galapagense 0436 (0.91,0.18)
(0.52/0.68/0.82)
S. galapagense 3909 (0.93,0.19)
(0.5/0.71/0.96)
B. Caryophyllales
Beta vulgaris (0.89,0.74)
(1/-/0.06) [0.96]
Spinacia oleraceae (0.88,0.76)
Frankenia laevis (0.87,0.64)
(1/-/0.14) [0.83]
Reaumuria trigyna (0.89,0.74)
(1/-/0.05) [0.96]
Plumbago auriculata (0.81,0.55)
(0.74/0.6/0.29) [0.63]
Ruprechtia salicifolia (0.97,0.57)
(0.01/0.08/0.56) 0.27/0.68/0.04
[0.40]
Drosera binata (0.98,0.61)
(0.88/0/0.15)
Dionaea muscipula (0.86,0.60)
(0.42/0.09/0.45)
Aldrovanda vesiculosa (0.89,0.53)
(0.27/0.11/0.38)
[0.47]
Drosophyllum lusitanicum (0.76,0.65)
(0.45/1/0.3) [0.99]
Ancistrocladus robertsonorium (0.97,0.61)
(0.06/0.26/0.48)
[0.42]
0.08/1/0
Nepenthes alata (0.86,0.67)
[0.99]
Nepenthes ampullaria (0.86,0.64)
"non-core" Caryophyllales