1 **Quartet Sampling distinguishes lack of support from conflicting support**

2 **in the plant tree of life[1]**

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13 **Short Title: Quartet Sampling of discordance in the plant tree of life**

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ABSTRACT

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15   ***Premise of the Study—*** Phylogenetic support has been difficult to evaluate within the plant

16   tree of life partly due to the inability of standard methods to distinguish conflicted versus poorly

17   informed branches. As phylogenomic and broad-scale datasets continue to grow, support

18   measures are needed that are more efficient and informative.

19   ***Methods—*** We describe the Quartet Sampling (QS) method that synthesizes several

20   phylogenetic and genomic analytical approaches into a quartet-based evaluation system. QS

21   rapidly characterizes discordance in large-sparse and genome-wide datasets, overcoming issues of

22   sparse alignment and distinguishing strong conflict from weak support. We test this method with

23   simulations and recent plant phylogenies inferred from variously sized datasets.

24   ***Key Results—*** QS scores decrease in variance with increasing replicates and are not strongly

25   affected by branch depth. Patterns of QS support from different phylogenies leads to a coherent

26   understanding of ancestral branches defining key disagreements, including *Ginkgo*+cycad,

27   magnoliids+eudicots (excluding monocots), and mosses+liverworts. The relationships of ANA

28   grade angiosperms, major monocot groups, and bryophytes and fern families are found to likely

29   be the result of discordant evolutionary histories, rather than poor information. Also, analyses of

30   phylogenomic data show QS can detect discordance due to introgression.

31   ***Conclusions—*** The QS method represents an efficient and effective synthesis of phylogenetic

32   tests that offer more comprehensive and specific information on branch support than conventional

33   measures. The QS method corroborates growing evidence that phylogenomic investigations that

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34   incorporate discordance testing are warranted to reconstruct the complex evolutionary histories

35   surrounding in particular ANA grade angiosperms, monocots, and non-vascular plants.

36   **Key words:** bootstrap; branch support; discordance; introgression; lineage sorting;

37   phylogenetics; phylogenetic methods; phylogenomics; plant tree of life; quartet sampling

38   INTRODUCTION

39   Discordance and uncertainty have emerged as a consistent feature throughout the history of our

40   evolving model of the plant tree of life (Crane, 1985; Chase et al., 1993; Palmer et al., 2004;

41   Soltis et al., 2011; Wickett et al., 2014). Furthermore, key points of uncertainty and contention

42   occur at pivotal transitions in the evolution of plant life on earth, such as the development of

43   vascular tissue (Pryer et al., 2001; Steemans et al., 2009; Banks et al., 2011), the rise of

44   seed-bearing plants (Chase et al., 1993; Chaw et al., 1997; Bowe et al., 2000; Qiu et al., 2006;

45   Jiao et al., 2011), and the explosive radiation of flowering plants (Crane, 1985; The Amborella

46   Genome Project, 2013; Goremykin et al., 2015; Taylor et al., 2015; Simmons, 2016; Edwards

47   et al., 2016). Modern phylogenomic datasets, rather than quelling these disagreements, have now

48   repeatedly shown that these phylogenetic conflicts are not only caused by methodological error or

49   data bias, but also are often the result of genuine biological processes. These forces most

50   commonly include incomplete lineage sorting (ILS), introgressive hybridization, and paralog

51   duplication-loss (e.g., Zhong et al., 2013b; Wickett et al., 2014; Zwickl et al., 2014; Yang et al.,

52   2015; Eaton et al., 2016; Pease et al., 2016b; Goulet et al., 2017; Walker et al., In Press). While

53   several methods have been proposed to deal with these issues in the context of species tree

54   inference (e.g., Zwickl and Hillis, 2002; Ogden and Rosenberg, 2006; Shavit Grievink et al.,

55   2010; Aberer et al., 2012; Anderson et al., 2012; Roure et al., 2012; Hinchliff and Roalson, 2013;

56   Mirarab et al., 2014), we still lack a generalized framework to quantify phylogenetic uncertainty

57   (specifically branch support) that distinguishes poorly informed branches from the increasingly

58   common case of multiple strongly supported, but mutually exclusive, phylogenetic histories.

59   Therefore, the development of systems of phylogenetic evaluation designed to utilize and describe

60   discordance (rather than minimize or control for it as error) offers strong promise to provide a

61   more holistic picture of the current state of the plant tree of life.

62   Quantification of branch support for molecular phylogenies has been proposed under many

63   methods over the last few decades (Felsenstein, 1985; Farris et al., 1996; Larget and Simon, 1999;

64   Anisimova and Gascuel, 2006; Anisimova et al., 2011; Ronquist et al., 2012; Larget, 2013).

65   Historically, one of the most commonly used tests has been the non-parametric bootstrap (NBS;

66   Felsenstein, 1985), which, along with recent variants like the rapid bootstrap (RBS; Stamatakis

67   et al., 2008), resamples the original data with replacement assuming that aligned sites are

68   independent and identically distributed (i.i.d.) samples that approximate the true underlying

69   distribution (Efron, 1992; Felsenstein, 1985). In practice, the assumptions of NBS (in particular

70   site independence) may rarely be met and can deteriorate under a variety of conditions

71   (Felsenstein, 1985; Felsenstein and Kishino, 1993; Hillis and Bull, 1993; Sanderson, 1995;

72   Andrews, 2000; Alfaro, 2003; Cummings et al., 2003). More recently the UltraFast bootstrap

73   approximation (UFboot) method, utilizing a likelihood-based candidate tree testing, was proposed

74   to address speed and score interpretation issues for NBS (Minh et al. (2013); and see comparison

75   in Simmons and Norton (2014)).

76   The other most common branch support metric has been the Bayesian posterior probability

77   (PP). PP scores are typically calculated from posterior distributions of trees generated using a

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78 Markov chain Monte Carlo (MCMC) sampler and then summarized using a majority-rule

79 consensus tree (e.g, Larget and Simon, 1999; Drummond and Rambaut, 2007; Ronquist et al.,

80 2012; Larget, 2013). The interpretation of PP values is arguably more straightforward than

81 bootstrap proportions, as PP values represent the simple probability that a clade exists in the

82 underlying tree (conditioned on the model of evolution employed, and assuming the data have

83 evolved in a treelike fashion), and do not involve data resampling. The individual and relative

84 performance of PP has been well-documented and generally favorable (Wilcox et al., 2002;

85 Alfaro, 2003; Cummings et al., 2003; Huelsenbeck and Rannala, 2004). However, it has been

86 recognized that in some scenarios (e.g., oversimplified substitution models) PP may give liberal

87 support (Suzuki et al., 2002; Douady et al., 2003; Nylander et al., 2004), and there are indications

88 that PP also may fail under a multi-species coalescent framework with conflicting phylogenies

89 (e.g., Reid et al., 2013). Some studies have noted the disproportionate effects of a few genes in

90 tipping the scales in the case of low information in large datasets (Brown and Thomson, 2016;

91 Shen et al., 2017).

92 Modern phylogenetic datasets being assembled to model the plant tree of life are currently

93 trending in two directions. Ongoing efforts to expand genetic sampling to as many plant species

94 as possible have produced increasingly species-rich, but data-sparse, alignments (i.e., large-sparse

95 matrices). Meanwhile, the accelerating accretion of new genomes and transcriptomes will

96 continue to deepen genome-wide datasets with millions of aligned sites. Both types of data

97 present particular challenges to both the tractability and interpretation of phylogenetic branch

98 support methods. NBS scores are known to perform poorly for large-sparse matrices (Wiens and

99 Morrill, 2011; Smith et al., 2011; Roure et al., 2012; Hinchliff and Roalson, 2013; Hinchliff and

100 Smith, 2014b), where the sampling procedure generates uninformative pseudo-replicates that

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101   mostly omit informative sites (or consist of mostly missing data). The problem of uninformative

102   pseudo-replicates also applies to alignment jackknifing used on sparse alignments, where

103   alignments are sampled without replacement. Therefore, alignment jackknifing also does not

104   offer a functional solution for large alignments with missing data.

105   Quantifying phylogenetic support from complete datasets brings unique challenges. By

106   "complete" dataset, we refer to any molecular data that is not a smaller subset of some larger

107   whole, which means not only whole genomes, but also whole transcriptomes, and even whole

108   plastid/mitochondrial genomes. PPs provide an appropriate testing framework and

109   straightforward interpretation, but available Bayesian methods of analysis and computational

110   speeds do not scale to typical phylogenomic datasets. Resampling methods (including NBS)

111   gauge how well the resampled dataset replicate the initial result, but complete datasets are not

112   samples of any larger whole. PP and NBS scores therefore both appear unsuitable for use on large

113   datasets, the former due to feasibility and the latter due to its assumption (Smith et al., 2009;

114   Hinchliff and Smith, 2014b).

115   A relatively well-studied, but less frequently applied, family of tests are the likelihood ratio

116   tests, such as the approximate likelihood ratio test (aLRT), the Shimodaira–Hasegawa [SH]-like

117   aLRT (SH-aLRT), and the Bayesian-like LRT (bLRT) (Anisimova and Gascuel, 2006; Guindon

118   et al., 2010; Anisimova et al., 2011). These tests conduct an approximated LRT of the alternative

119   nearest-neighbor interchange (NNI) moves at each branch of an optimized tree topology.

120   SH-aLRT has been shown to perform well (Anisimova et al., 2011; Simmons and Norton, 2014;

121   Simmons and Randle, 2014) and is computationally efficient enough to be run on large datasets in

122   large part because it does not require generation of multiple topology replicates (as in NBS).

123   Despite multiple implementations in PhyML and RAxML, and being the default support measure

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

125   2010; Stamatakis, 2014).

126   As phylogenomics has developed over the last decade, a variety of methods have been

127   introduced to factor the increased data and inherent gene tree-species tree conflict. These methods

128   measure the concordance of gene trees (broadly referring to a phylogeny from any sub-sampled

129   genomic region), including the internode and tree certainty scores (IC/TC; Rokas et al., 2003;

130   Salichos et al., 2014; Kobert et al., 2016), Bayesian concordance factors (Ané et al., 2006), and

131   other concordance measures (Allman et al., 2017). These various certainty scores are developed

132   around the central concept of a branch support statistic that measures concordance of various

133   trees with a particular tree hypothesis. This perspective on phylogenetic evaluation offers much in

134   terms of partitioning phylogenetic discordance and analyzing larger alignments more rapidly in a

135   phylogenomic coalescent-based framework, but these methods that address discordance using

136   gene trees may not be as suitable for large-sparse alignments.

137   Finally, quartet methods—in particular quartet puzzling methods—have been examined

138   extensively in phylogenetics, especially in relation to phylogenetic reconstruction (Strimmer

139   et al., 1997; Strimmer and von Haeseler, 1997; Ranwez and Gascuel, 2001; Chifman and

140   Kubatko, 2014; Mirarab et al., 2014). A quartet support method called "reliability values," which

141   correlates with NBS values, is calculated as a side effect of the quartet puzzling procedure

142   (Strimmer et al., 1997; Strimmer and von Haeseler, 1997). More recently, quartet procedures have

143   been explored to facilitate sampling of large-sparse alignments (Misof et al., 2013) and as part of

144   coalescent-based quartet inference methods (Gaither and Kubatko, 2016; Sayyari and Mirarab,

145   2016). These quartet methods benefit not only from the speed advantages of a smaller alignment,

146   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

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170   alignments (10,000–30,000 tips with >80% missing data), datasets for which Bayesian analyses

171   are simply not tractable. We also describe how QS enhances analysis of both genome-wide

172   datasets and smaller-scale multi-gene data sets conventionally used in systematics.

173   In this study, we will (1) describe the features, parameters, and interpretation of the QS

174   method, (2) validate the QS method with simulations that demonstrate its effectiveness, and (3)

175   evaluate and discuss the plant tree of life by testing the QS method on recently published

176   large-sparse and phylogenomic datasets at timescales spanning from Viridiplantae to sub-generic

177   clades. The goal of the re-analysis of these phylogenies is not to compare the methods of tree

178   inference or dataset composition, but instead to use the QS method to collectively analyze these

179   data as a diverse set of alternative phylogenetic hypotheses of the plant trees of life. We show

180   consistently through these datasets that the QS method acts as a more conservative test of branch

181   support that provides greater discrimination between highly supported and poorly supported

182   branches than other support measures. We also show that the QS method can identify branches in

183   a phylogeny where biological conflict is likely occurring, which can inform targeting of more

184   detailed investigations within a clade. Finally, we show several cases where low support for the

185   given phylogenetic relationship and strong counter-support for an alternative relationship together

186   indicate a coherent consensus. In other cases, QS specifically indicate the likely presence of

187   alternative phylogenetic histories, as distinct from low branch support. We hope this study

188   encourages additional discussion, testing, and innovation of new phylogenetic evaluation

189   methods. We also hope it contributes to the broader discussion about moving the plant tree of life

190   beyond the increasingly difficult goal of resolving a single unified 'Species Tree' (Hahn and

191   Nakhleh, 2015; Smith et al., 2015), and into a future where the complex "multiverse" of

192   phylogenetic relationships that is manifest throughout the plant tree of life are more fully

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193 explored and appreciated.

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

214   the concordant and each of the two discordant relationships. If a minimum alignment overlap is

215   specified, then quartets must contain the minimum number of overlapping non-empty sites for all

216   four taxa to be considered suitable for any calculations. Additionally, a parameter of a minimum

217   likelihood differential may be set. If the most-likely topology (of the three) does not exceed the

218   likelihood of the second-most-likely phylogeny by the set threshold then the quartet is considered

219   "uninformative" and tabulated separately. Therefore, for a given internal branch, the QS method

220   generates counts of the three possible topologies (and uninformative replicates) sampled from

221   different quartets of taxa spanning the particular branch.

222   The QS method uses these resampled quartet tree counts to calculate three scores for each

223   internal branch of the focal tree (Fig. 1B, Table 1, and Appendix S1; see Supplemental Data with

224   this article). The QC (Quartet Concordance) score is an entropy-like measure (similar to the ICA

225   score; Salichos et al. 2014) that quantifies the relative support among the three possible

226   resolutions of four taxa. When the most commonly sampled topology is concordant with the input

227   tree, then the QC takes positive values in the range (0,1]. Thus, QC=1 when all quartet trees are

228   concordant with the focal branch. When one of the discordant topologies is the most commonly

229   resampled quartet, QC takes negative values in the range [–1,0), approaching –1 when all quartet

230   trees are one of the two discordant phylogenies. When support is evenly split among the three

231   alternative topologies (or two if only two of the three possible are sampled), QC equals 0.

232   The QD (Quartet Differential) score uses the logic of the f- and D-statistics for introgression

233   (Reich et al., 2009; Green et al., 2010; Durand et al., 2011; Pease and Hahn, 2015) and measures

234   the disparity between the sampled proportions of the two discordant topologies (though with gene

235   tree proportions, rather than site frequencies). The QD score does not specifically quantify

236   introgression nor identify introgressing taxa, but does indicate that one alternative relationship is

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237   sampled more often than the other. High values of QD report that there is one clearly preferred

238   topology among the two discordant topologies, a potential indication of a biased biological

239   process beyond background lineage sorting on the given branch, including introgression, strong

240   rate heterogeneity, heterogeneous base compositions, etc. QD varies in the range [0,1] with a

241   value of 0, meaning no skew in the proportions of discordant trees, and the extreme value of 1,

242   which means all discordant trees sampled are only from one of the two possible alternative

243   relationships.

244   The QU score (Quartet Uncertainty) quantifies the overall proportion of quartets for a given

245   branch where the tree with the best likelihood value has a likelihood that is not higher than the

246   next most likely tree by a given differential cutoff. This ensures that replicates are not counted as

247   being concordant or discordant when the molecular data itself is effectively equivocal on the

248   topology by all three options having nearly indistinguishable likelihood scores. QU is measured

249   in the range [0,1], which indicates the proportion of sampled quartets that did not exceed of the

250   cutoff. A QU of 0 means no "uncertain" quartets (i.e., highly informative data), while a value of 1

251   indicates 100% of quartets were uncertain (i.e., no information for the given branch). QU reports

252   the informativeness of the branch, which in conjunction with QD and QC distinguishes between

253   branches that have low information versus those with conflicting information (i.e., high

254   discordance).

255   Finally, for each terminal taxon, a QF (Quartet Fidelity) score is calculated to report what

256   proportion of the time inclusion of a given taxon in a quartet (across all branches) results in a

257   discordant quartet topology. QF is therefore similar in approach to a "rogue taxon" test

258   (Wilkinson, 1996; Aberer et al., 2012). For a given taxon, the QF score is measured in the range

259   [0,1] as the proportion of quartet topologies involving the taxon that are concordant with the focal

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260   tree branch. Therefore, QF=1 indicates a given taxon always produces concordant topologies

261   when used in a quartet replicate. QF values approaching zero indicate mostly discordant

262   topologies involving this taxon, and may indicate poor sequence quality or identity, a

263   lineage-specific process that is distorting the phylogeny, or that the taxon is significantly

264   misplaced in the given tree. Note that QF differs specifically from QC/QD/QU by being a

265   taxon-specific test across internal branch tests rather than a branch-specific test.

266   Collectively, these four tests represent a means to distinguish the consistency of a branch

267   (QC), the presence of a secondary evolutionary history (QD), the amount of information

268   regarding a branch (QU), and the reliability of individual taxa in the tree (QF; Fig. 1B and see

269   Table 1). Taken together these tests provide a means to disentangle these effects rather than have

270   them conflated under a summary score as in standard measures of phylogenetic support. For a full

271   technical description of the QS method, see Appendix S1.

272   ***Guidelines for implementation of QS—*** We implemented the above procedure in `Python` in

273   a program `quartetsampling` that samples an alignment randomly to generate many

274   representative quartet topology replicates for each internal branch in a corresponding focal tree

275   (https://github.com/fephyfofum/quartetsampling). This procedure has a number of advantages

276   over NBS for larger datasets. First, alignment columns are not resampled (as in NBS and RBS),

277   which allows even very sparse alignments to be used. Second, the number of likelihood

278   calculations that are required is the number of internal branches in the tree multiplied by the

279   number of replicates per branch multiplied by three possible topologies. Since computation time

280   scales linearly with the number of taxa, individual replicates are fast, and the computations can be

281   readily parallelized across processors and furthermore discretized across systems (with results

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282   combined later). This allows QS to be efficiently applied to large alignments beyond the practical

283   limits of NBS and PP. The most extensive computational time was for the Zanne et al. (2014b)

284   31,749 taxon dataset (see below), which we ran on the Wake Forest University DEAC

285   high-performance cluster using 8 nodes with 16 CPU each. This analysis completed 200

286   replicates for the full tree in 13 hours. Smaller genome-wide datasets finished 1000 gene-tree

287   replicates on quad-core desktops approximately 12 hours, and conventional multi-gene datasets

288   took only a few minutes to a few hours to run on a standard desktop.

289   Although the SH-aLRT was by far the fastest method we consider here, the QS was fast

290   enough for large scale analyses. QS can also be applied separately to only a few branches,

291   allowing for more thorough exploration of particular branches of interest. Furthermore, the QS

292   does not require the tree tested to be the maximum likelihood topology, a requirement for

293   SH-aLRT. For our simulated data, we found that performing 200 QS replicates per branch was

294   adequate to achieve low variance in QS score. As would be expected, more replicates per branch

295   should generally be used for larger trees to sample a greater fraction of the total possible quartets.

296   Furthermore, some branches, especially in large trees, may be entirely unsupported by the

297   alignment due to a lack of sampling overlap among appropriate taxa (i.e., no sites in the

298   alignment contain data from each of the four subsets of taxa; Fig. 1A). Therefore, no

299   phylogenetic information exists to inform the branch (i.e., are ''"indecisive" *sensu* Steel and

300   Sanderson, 2010), and the QS procedure identifies these branches rather than discarding them or

301   marking them as simply low support.

302   ***Guidelines for interpretation of QS support values—*** An important consideration with any

303   measure used to ascertain confidence is the precise interpretation. In order to facilitate accurate

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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.

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326 1.03 (Fletcher and Yang, 2009). Trees were scaled so that the average branch lengths were about

327 0.2, based on the observation that this generated reasonable trees with most branches recovered

328 correctly from ML analyses. Using the same procedure, we also simulated trees with 500 tips and

329 associated alignments with ten nucleotide partitions, each with 500 sites under the Jukes-Cantor

330 model. We simulated both the full alignment with partitions and a modified randomly resampled

331 sparse alignment to examine the behavior of QS in the presence of missing data (see Appendix S1

332 for details). These partitioned and sparse alignments had the same qualitative features as the full

333 alignment.

334   Unlike the NBS method, which generates a set of trees from which branch support is

335 estimated, the QS method requires only a single input topology for which branch support will be

336 measured. We calculated QC, QD, QU, and QF scores for the true underlying tree as well as the

337 ML tree generated by RAxML, but we focus on results for the ML tree. To examine how the

338 number of replicates impacts the QS precision, we conducted simulations varying the number of

339 replicates for randomly drawn branches in the simulated trees (Fig. 2A; Appendix S4). Based on

340 these simulations, we elected to use 200 replicates per branch, since the variation in the QC score

341 was generally low across all tree sizes when this many replicates were performed. We used

342 RAxML and PAUP* to estimate the ML for the three alternative topologies for each QS replicate

343 (using the $-\mathtt{f}$ $\mathtt{N}$ option and the GTRGAMMA model in RAxML). We also calculated

344 branch-specific QC/QD/QU and taxon-specific QF scores using likelihood differential cutoffs of

345 $\Delta L = 0$ (no filtering) and $\Delta L = 2.0$, which requires stronger conflicting signal to interpret

346 branches in the input tree as unsupported.

347   ***Testing of Empirical Datasets—***   While the simulations show the general reliability of the

348   method, our primary goal was to use this test to holistically evaluate recent large-scale plant

349   phylogenies, and also to show the utility and versatility of this method in subgroup and even

350   sub-genera analyses. Using published phylogenies and public datasets, we evaluated five recent

351   large-scale phylogenies, including (1) an 103-transcriptome dataset spanning Viridiplantae from

352   Wickett et al. (2014, abbreviated hereafter as WI2014), (2) two large-sparse phylogenies spanning

353   land plants from Hinchliff and Smith (2014b, abbreviated HS2014) and Zanne et al. (2014b,

354   abbreviated ZN2014), and (3) phylogenies spanning Magnoliophyta (angiosperms) with hundreds

355   of genes from Xi et al. (2014a, abbreviated XI2014) and Cannon et al. (2015b, abbreviated

356   CN2015). Additionally, to demonstrate the utility of this method at medium and short time scales,

357   we evaluated two whole transcriptome datasets from the wild tomato clade *Solanum* sect.

358   *Lycopersicon* from Pease et al. (2016b, abbreviated PE2016) and carnivorous plants from the

359   order Caryophyllales from Walker et al. (In Press, abbreviated WA2017). Finally, we tested this

360   method on a more typical medium-sized multi-locus dataset from Polypodopsida (ferns) from

361   Pryer et al. (2016b, abbreviated PR2016), such as might appear in many phylogenetic studies of

362   large subgroups. Data for these studies were obtained from datadryad.org and iplant.org

363   (Hinchliff and Smith, 2014a; Matasci et al., 2014; Xi et al., 2014b; Zanne et al., 2014a; Cannon

364   et al., 2015a; Pease et al., 2016a; Pryer et al., 2016a; Walker et al., 2017).

365   In addition, we analyzed the datasets using 200 individual gene trees for XI2014 and

366   WA2017, and 1000 gene trees for PE2016 and WI2014. For these datasets, quartets are sampled

367   as usual, but only the individual gene sequence alignments are assessed. These phylogenies were

368   all evaluated using a minimum alignment overlap per quartet of 100 bp and a minimum likelihood

369   differential of 2 (i.e., the optimal tree's log-likelihood must exceed the second-most likely tree by

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370 a value of at least 2). We also calculated the phylogenies with and without partitioning in

371 RAxML, but in all cases the partitioned datasets did not qualitatively differ from the results of the

372 unpartitioned datasets. These data are provided as supplementary data, but are not shown here.

373   We also either re-calculated other measures of branch support or used values from the

374 published studies for comparison to the QS method for each phylogeny, except HS2014 and

375 ZN2014 where the size and sparseness of the datasets prohibited the calculation of other measures

376 of support. For the datasets from CN2015, PR2016, WA2017, and XI2014 100 replicates each of

377 RAxML NBS and SH-test were performed. Additionally, PP scores for PR2016 were calculated

378 using MrBayes (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012), and IC scores for

379 calculated for Walker et al. (In Press). For PE2016 and WI2014, RAxML NBS, MP-EST, or IC

380 scores were taken from published values.

381 **RESULTS AND DISCUSSION**

382 *Analysis of simulated and empirical datasets shows that QS components are consistent,*

383 *convergent, and complementary—* In order to examine the consistency and reliability of the

384 Quartet Sampling method, we first tested QS on a set of simulated phylogenies. As expected, the

385 QC score was generally correlated in a sigmoid fashion with the frequency of concordant trees

386 (Appendix S5). In order to determine the appropriate number of testing replicates and

387 demonstrate convergence, we conducted an analysis of the variance of the QS score by running

388 the QS analysis 100 times on randomly selected branches with different numbers of per-branch

389 replicates for trees with 50, 100, and 500 taxa. This analysis showed that the QC scores converge

390 (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

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414 not only its own phylogenetic placement, but can exert a wide-ranging set of distortions on the

415 topology (e.g., Fig. 8A, among many others). Resampling methods that infer a complete set of

416 tips (like NBS) will produce replicates that may also be distorted and thus firmly support these

417 relationships rather than highlight this discordance. Therefore, as pointed out by many other

418 quartet methods (Mirarab et al., 2014; Sayyari and Mirarab, 2016), the use of quartets breaks

419 down the problem in more manageable units that cannot be distorted by these complex lineage

420 sorting effects.

421 ***QS indicates strong relationships within, but not among, major land plant lineages—*** While

422 simulations and general characterizations of the QS method are necessary to demonstrate its

423 effectiveness, the primary goal of this study was to use QS to reanalyze and compare several

424 recent speciose and phylogenomic datasets to address ongoing debates of phylogenetic

425 relationships in the plant tree of life. We used QS methods to evaluate two of the most speciose

426 phylogenies of land plants presently assembled by Hinchliff and Smith (2014b) and Zanne et al.

427 (2014b), and one of the most comprehensive phylogenies of Viridiplantae from Wickett et al.

428 (2014) (abbreviated as WI2014). HS2014 spanned 13,093 taxa with a total alignment of 148,143

429 sites (Fig. 3), but extremely sparse overall coverage (96.4% missing characters). The alignment

430 used for ZN2014 (Fig. 4) contained 31,749 taxa and an alignment length of 12,632 bp, but also

431 with sparse coverage (82.3% missing characters). However, the QS method accommodates large,

432 sparse matrices, by evaluating alignment overlap only for each replicate quartet without requiring

433 complete occupancy for any partition of the alignment. Finally, the WI2014 (Fig. 5) contains only

434 80 taxa, but, as the pilot study for the 1000 Plants Project (1KP), spans the deepest time scale of

435 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

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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

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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;

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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.

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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).

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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

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570   been placed in a clade with magnoliids (as in Jansen et al. 2007; Moore et al. 2007; Soltis et al.

571   2011; Group et al. 2016, and Fig. 3 in Wickett et al. 2014). However, both HS2014 and ZN2014

572   place Chloranthaceae in a clade with magnoliids, monocot, and eudicots, while WI2014 places it

573   in a clade with just eudicots (excluding monocots and magnoliids) that is, however,

574   counter-supported by the sampled quartets (QC=–0.54, QD=0.95).

575   *Ceratophyllum*, often placed as the most closely related group to eudicots (Jansen et al., 2007;

576   Soltis et al., 2011), in HS2014 appears in a clade with the Chloranthaceae among the prior to

577   monocot/magnoliid/eudicot splits. However, the branch placing Chloranthaceae+*Ceratophyllum*

578   lineage in a clade with magnoliids+monocots+eudicots was counter-supported (Fig. 3B) with a

579   strongly biased QD (0.81). ZN2014 supports *Ceratophyllum* as separate from the core

580   angiosperms. Given that this question is inextricably linked with the relationships of the three

581   core angiosperm groups, a consensus was not reached by this information alone. However, this

582   evidence reopens the question of the relationship of Chloranthaceae and Ceratophyllaceae, both to

583   each other and to the ANA grade angiosperm lineages (see discussion in Eklund et al., 2004).

584   ***Relatively consistent topology but poor support found within monocots—*** Within monocots,

585   *Acorus* was weakly but consistently supported as a separate clade from the rest of monocots in

586   HS2014, ZN2014, and WI2014 (Figs. 3B, 4B, 5). In general, the arrangement of monocot orders

587   in both HS2014 (Fig. 3C) and ZN2014 (Fig. 4C) agreed with recent consensus phylogenies

588   (Givnish et al., 2010; Soltis et al., 2011; Barrett et al., 2015; Givnish et al., 2016; McKain et al.,

589   2016).

590   One exception was the placement of Liliales, which has varied in consensus trees and, here,

591   either was sister to commelinids with weak support (HS2014) or was sister to Asparagales

592   (ZN2014). The commelinid orders were resolved inconsistently between studies. The

593   commelinid stem branch was counter-supported in both ZN2014 and HS2014 with high QD

594   values. In HS2014, Arecales were separate from the rest of the commelinids, but the ancestral

595   commelinid branches both including and excluding Arecales were counter supported. In ZN2014,

596   Arecales was supported in a clade with Zingiberales and Commelinales, but the ancestral branch

597   for these three orders has a QD=0.79. This arrangement was consistent with previous phylogenies

598   (e.g., Givnish et al. (2010)) that have poor support to these relationships. From the QS results, we

599   would cautiously infer that (1) the relationships among the commelinids are still unknown, (2)

600   there may be uncharacterized secondary evolutionary history distorting the phylogenetic

601   placement of these groups, and (3) likely the variable data from both Liliales and Arecales

602   together have a joint effect that is causing inconsistency in the phylogenetic inference.

603   Finally, in Poaceae, Quartet Sampling made clear the well-characterized discordance and

604   complex relationships (e.g., Washburn et al., 2015; McKain et al., 2016). The "BOP" clade

605   (Bambusoideae, Oryzeae, Pooideae) was counter-supported in HS2014 and both paraphyletic and

606   counter-supported in ZN2014. Within the "PACMAD" clade, many of the named subgroupings

607   were counter-supported with negative QC values. There were particularly high QU values in both

608   HS2014 and ZN2014 for this clade indicating not only complex support but also low information.

609   Therefore, even if someone were unfamiliar with the controversies surrounding monocot

610   phylogenetics (as a whole and within groups), the QS evaluation framework clearly highlights

611   this group as one with consistently high conflict and counter-support values for the consensus

612   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

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636   result of the rapid radiation of these lineages. Generally, these phylogenies tended to support

637   weakly the controversial placement of Caryophyllales as most closely related to the eudicot

638   ancestor.

639   ***QS shows strong support for the consensus rosid phylogeny, but substantial discordance***

640   ***both within and among asterid clades—*** HS2014 (Fig. 3D) find similar resolutions among rosid

641   lineages (Wang et al., 2009; Moore et al., 2010; Soltis et al., 2011; Zhao et al., 2016) (the lone

642   exception was the Sapindales+Huertales clade). Among the branches leading to major groups, all

643   were supported and most of the backbone branches were either supported or only weakly

644   negative. The QS scores also correctly identified a poorly supported relationship in HS2014

645   between *Cynomorium* and Cucurbitales (QC=–0.31). *Cynomorium*, a non-photosynthetic

646   parasitic plant with unusual morphology, has been placed tenuously and variably in groups as

647   diverse as Rosales (Zhang et al., 2009) and Saxifragales (Nickrent et al., 2005), so its poor score

648   here was expected and confirms the QS method's ability to detect poorly placed species. This

649   "rogue" status was corroborated by the below-average QF score of QF=0.18 (mean 0.21 for

650   HS2014). This means that for quartets that include *Cynomorium* as a randomly sampled taxon,

651   only 18% produced a quartet topology concordant with the HS2014 tree.

652   In contrast to the analysis of the rosid phylogeny from HS2014, the asterid phylogeny from

653   ZN2014 (Fig. 4D) appears to have substantial discordance and disagreement with most published

654   phylogenies (Soltis et al., 2011; Beaulieu et al., 2013; Refulio-Rodriguez and Olmstead, 2014).

655   ZN2014 QS scores supported the unusual hypothesis of a common Ericales+Cornales ancestor,

656   weakly support the campanulid clade, and counter-support a common lamiid ancestor. The

657   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

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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

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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

33

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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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.

1102   *Notes:* * QC = Quartet Concordance; QD = Quartet Differential; QU = Quartet Uncertainty.

FIGURE LEGENDS

1103

1104 **Fig. 1.** Description of the Quartet Sampling method. (A) The focal branch "*b*" divides the phylogeny into four

1105 subclades $\{S_1, S_2, S_3, S_4\}$ from which tips (A–J) are sampled. Two replicates with different sampled tips for the

1106 given branch are shown with the three possible unrooted topologies (one concordant and two discordant). (B) Each

1107 internal branch is labeled with a set of three scores (QC/QD/QU), which offer different, but complementary,

1108 information. Terminal branches are evaluated by the QF score, which reports the frequency of a taxon generating

1109 concordant topologies. (See Materials and Methods for full details and Supplementary Methods for a technical

1110 description.)

1111

1112 **Fig. 2.** Results of Simulation Testing of the Quartet Sampling Method. (A) QC values converge on a central value

1113 with increasing numbers of replicates from randomly selected branches from simulated trees with 50, 100, and 500

1114 taxa. (B) Distributions of QC, QU, and QF values for HS2014 (black), ZN2014 (dotted black), and

1115 XI2014/CN2015/PR2016/WA2017 (similar distributions; gray solid). (C) Mean QC values (diamond) with 5%ile to

1116 95%ile (whiskers) for branches in HS2015 binned by the number of subtending taxa (i.e., moving root-ward in the

1117 tree left-to-right). Overall mean is shown with horizontal dotted line.

1118

1119 **Fig. 3.** Phylogeny from Hinchliff and Smith (2014b). (A) Full phylogeny with heat map coloration of branches by

1120 QC scores for internal branches: dark green (QC>0.2), light green (0.2≤QC>0), light orange (0≤QC≤–0.05, or

1121 dark orange (QC>–0.05). (B) QC/QD/QU scores (200 replicates of full alignment) for major plant groups and key

1122 orders within angiosperms. QC/QD/QU scores after group names are for the ancestral branch (i.e., the "stem"

1123 branch), and a single QF score is shown for monotypic tips. Major subgroups groups are highlighted with vertical

1124 labels. (C) QS scores for monocots (excluding *Acorus*). (D,E,F) QS scores for rosids, Bryophyta, and gymnosperms.

1125 Abbreviations: Acro, Acrogymnospermae; ANA, ANA grade; Aru, Arundinoideae; Bry, Bryophyta, Chl,

1126 Chloridoideae; Dan, Danthonioideae; Mar, Marchantiophyta; Poly, Polypodopsida.

1127

1128 **Fig. 4.** Phylogeny from Zanne et al. (2014b). (A) Full phylogeny with heat map coloration of branches by QC scores

54

1129   for internal branches using same color scheme as (Fig. 3). (B) QC/QD/QU scores (200 replicates of full alignment)

1130   for major plant groups and key orders within angiosperms, using same color scheme as (Fig. 3). (C) QS scores shown

1131   for monocots (except *Acorus*). (D) QS scores for asterids. (E) QS scores for fern lineages and (F) QS scores for

1132   gymnosperm lineages respectively. Abbreviations: Alseu, Alseuosmiaceae; ANA, ANA grade angiosperms; Argo,

1133   Argophyllaceae; Aster, Asteraceae; Bory, Boryaceae; Caly, Calycanthaceae; Eriach, Eriachneae; Good,

1134   Goodeniaceae; gym, gymnosperms; Hypox, Hypoxidaceae; Isach, Isachneae; Phell, Phellinaceae; Poly,

1135   Polypodopsida.

1136

1137   **Fig. 5.** Maximum likelihood phylogeny spanning Viridiplantae from Fig. 2 in Wickett et al. (2014) with QC/QD/QU

1138   scores for 200 replicates of the full alignment. Nodes are colored according to QC score using same color scheme as

1139   (Fig. 3). Bootstrap values (italicized in square brackets) from Wickett et al. (2014) are shown for comparison.

1140   Missing QS or bootstrap values indicate a perfect score. The three taxa with the lowest QF values are highlighted.

1141   Species names have been excluded or abbreviated in the case where two congeners are included.

1142

1143   **Fig. 6.** Key phylogenetic disagreements with QC scores using compared across various datasets. Branches for

1144   HS2014 and ZN2014 were resampled with 10000 replicates. Branches for WI2014 and XI2014 were exhaustively

1145   sampled (>1000 replicates). Highlighting on QC values follows the same colors as Fig. 3. "Conifers-II" refers to a

1146   hypothesized clade comprising the non-Pinales orders in Pinidae. Abbreviations: Gnet, Gnetidae; Pin, Pinidae.

1147

1148   **Fig. 7.** Phylogeny of Pteridaceae ferns from Pryer et al. (2016b) with QC/QD/QU scores for 200 replicates of the full

1149   alignment. Nodes are colored according to QC score using same color scheme as (Fig. 3).

1150   Bootstrap/SH-test/posterior probability values (italicized in square brackets) are shown for comparison. Omitted

1151   values indicate a perfect score. The three taxa with the lowest QF values are highlighted. Abbreviations: *Pityro*,

1152   *Pityrogramma*.

1153

1154   **Fig. 8.** QS scores for phylogenies from whole-transcriptome data. Omitted values indicate a perfect score. Nodes are

1155   colored according to QC score using same color scheme as (Fig. 3). (A) Phylogeny of *Solanum* sect. *Lycopersicon*

55

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 5th–95th 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

57

1185   et al. (2013). are shown for comparison. Perfect scores for any given test are omitted or shown as '*' indicates

1186   bootstrap of 100, while '-' indicates a missing value. The three taxa with the lowest QF values are highlighted.

1187

1188   **Appendix S7.** Phylogeny from Cannon et al. (2015b) with QC/QD/QU scores for 200 replicates of the full alignment.

1189   Nodes are colored according to QC score using same color scheme as (Fig. 3). Bootstrap values (italicized in square

1190   brackets) are shown for comparison. Perfect scores for any given test are omitted or shown as '*' indicates bootstrap

1191   of 100, while "–" indicates a missing value. The three taxa with the lowest QF values are highlighted.

1192

1193   **Appendix S8.** Relationship between QC and frequencies of the three possible alternative quartet topologies from QS

1194   runs on simulated data. Points represent branches in the trees, with the "test topology" axis identifying the frequency

1195   at which the topology consistent with the tree was recovered across all QS replicates for that branch, and the "alt n"

1196   axes identifying the frequencies of the two alternative (conflicting) topologies.

A
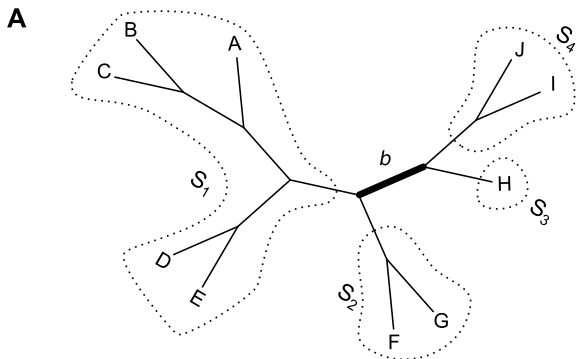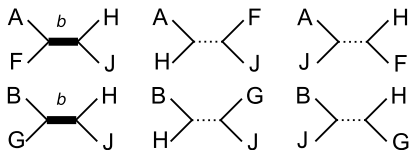
Replicate 1

Replicate 2

...
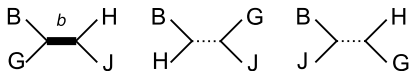
Concordant quartet topology

Discordant topology #1

Discordant topology #2

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

monocots
magnoliids
ANA
Acrogym
Poly
Mar
Bry

Coleochaetales+
Charales

other eudicots
superrosids
rosids

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)

embryophtes
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

QC

50 taxa | 100 taxa | 500 taxa

QS Replicates

B

Proportion

HS2014
ZN2014
XI/CN/PR/WA

QC | QU | QF

C

mean QC (5%ile–95%ile)

Number of subtending taxa

A

magnoliids   other eudicots

monocots

rosids+
superrosids

ANA
Acro
Poly
Bry

superasterids

asterids

B

Bryophyta (0.15/0.02/0.42;27)
0.8/1.0/0.19   Marchantiopsida (0.82/1/0.11;7)
-0.59/0.98/0.37   *Haplomitrium* (0.4)
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

Cornales (0.3/0.19/0.51;193)
0.08/0.0/0.7   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)
Metteniusa (0.16)
-0.05/0.39/0.51   Garryales (0.33/0.73/0.52;5)
-0.12/0.36/0.5   Icacinales (0.18/0.65/0.74;9)
0.0/0.0/0.7   *Apodytes*+*Dendrobangia* (0.33/0.71/0.65;2)
0.09/0.35/0.58   *Oncotheca* (0.11)
0.03/0.28/0.62   *Vahlia* (0.19)
0.24/0.41/0.43   Boraginales (0.31/0.4/0.46;286)
-0.18/0.65/0.56   Gentianales (0.08/0.08/0.72;1402)
-0.01/0.13/0.57   Lamiales (0.36/0.79/0.33;1873)
0.03/0.23/0.55   Solanales (0.14/0.47/0.5;620)

campanlids
Asterales
lamids

E. Ferns

Marattiales (1.0/-/0.03;5)
-0.09/0.12/0.55   Psilotales (NA;5)
0.42/1.0/0.35   Ophioglossales (0.87/0.6/0.12;15)
-0.58/0.83/0.64   *Equisetum* (1.0/-/0;13)
0.84/0.2/0.28   Osmudales (0.91/1.0/0.14;8)
-0.41/0.97/0.56   Gleicheniales (1.0/-/0.04;10)
0.19/0.14/0.8   Hymenophyllales (0.76/1.0/0.38;31)
0.6/0.33/0.49   Schizeales (0.86/1.0/0.27;8)
-0.03/0.28/0.57   Cyatheales (0.08/1.0/0.12;19)
0.44/0.42/0.39   Salviniales (0.29/0.79/0.48;23)
-0.51/0.95/0.56   *Saccoloma* (0.91/1/0.07;2)
0.44/0.04/0.39   *Lindsaea* (1.0/-/0;3)
0.07/0.39/0.56   Pteridaceae (0.95/1/0.16;50)
0.2/0.16/0.55   Dennstaedtiaceae (0.11/0.46/0.41;14)
0.15/0.4/0.44   Aspleniaceae (0.54/1.0/0.44;135)
0.43/0.93/0.46   Eupolypods I (-0.4/0.84/0.63;114)
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)
0.25/0.84/0.23   Gnetidae (0.4/0.07/0.29;39)
0.65/0.2/0.21   Pinaceae (0.83/0.71/0.15;186)
-0.01/0.04/0.48   Araucariaceae (0.84/0.0/0.17;39)
0.47/0.0/0.54   Podocarpaceae (0.92/0.33/0.06;88)
0.19/0.72/0.56   *Sciadopitys* (0.44)
0.82/0.43/0.17   Cupressaceae (0.83/0.67/0.28;100)
0.16/0.66/0.53   *Cephalotaxus* (1.0/-/0.18;4)
0.68/0.56/0.51   *Amentotaxus* (1.0/-/0.26;2)
-0.03/0.09/0.66   *Torreya* (1.0/-/0.23;4)
Taxaceae (0.23/0.08/0.62;10)

Phylogenetic tree with tip labels:

- *Cryptogramma crispa* (0.8)
- *Pityro. austroamericana* (0.83)
- *Bommeria hispida* (0.8)
- *Pellaea atropurpurea* (0.7)
- *Myriopteris covillei* (0.74)
- *Notholaena grayi* (0.77)
- *Pentagramma triangularis* (0.72)
- *Hemionitis palmata* (0.69)
- *Cheilanthes nitidula* (0.68)
- *Calciphilopteris ludens* (0.75)
- *Rheopteris cheesmaniae* (0.84)
- *Vaginularia acrocarpa* (0.83)
- *Hecistopteris pumila* (0.83)
- *Haplopteris elongata* (0.83)
- *Monogramma graminea* (0.83)
- Antrophyum latifolium (0.75)
- *Anetium citrifolium* (0.73)
- *Vittaria graminifolia* (0.84)
- *A. subcordatum* (0.69)
- *A. digitatum* (0.73)
- *A. formosum* (0.81)
- *A. raddianum* (0.71)
- *A. aethiopicum* (0.69)
- *A. hispidulum* (0.65)
- *A. pedatum* (0.61)
- *A. andicola* (0.6)
- *A. tenerum* (0.63)
- *A. tricholepis* (0.67)
- *A. tetraphyllum* (0.69)
- *A. peruvianum* (0.71)
- *A. flabellulatum* (0.73)
- *A. capillus veneris* (0.73)
- *A. davidii* (0.76)
- *A. malesianum* (0.8)

Node labels:

- 0.83/1/0.3 *[98/96/1.0]*
- 0.88/1/0.1
- 0.87/0/0.28
- 0.94/1/0.23
- -0.15/0.67/0.71 *[70/73/0.98]*
- 0.94/1/0.06
- Vittarioids
- 0.46/0.84/0.43 *[68/74/0.81]*
- 0.31/1/0.22 *[99/99/1.0]*
- 0.95/1/0.07
- -0.47/1/0.41 *[-/-/0.52]*
- 0.38/0/0.71 *[47/0/0.66]*
- 0.15/0.17/0.62 *[90/95/1.0]*
- 0.17/0.54/0.58 *[41/73/0.72]*
- *Adiantum*
- 0.33/0.5/0.48 *[-/-/0.85]*
- -0.33/0.85/0.57 *[-/-/-]*
- 0.95/1/0.04

A. Non-vascular plants

HS2014

- Bryophyta
- -0.01 — Marchantiophyta
- 0.28 — tracheophytes
- Anthocerotophyta
- non-embryophtes

WI2014

- 0.22
- -0.12

ZN2014

- 0.19 — Bryophyta
- Marchantiophyta
- Anthocerotophyta
- 0.52 — Lycopodopsida
- euphyllophytes

B. *Ginkgo* + cycads

ZN2014
WI2014

- 0.24
- 0.38

- *Ginkgo*
- Cycadales
- Gnet+Pin
- all other

HS2014

- -0.03

C. Gnetidae + conifers

ZN2014

- 0.22

- Pinaceae
- Conifers-II
- Gnetidae
- all other

HS2014
WI2014

- -0.14
- -0.39

D. "ANA grade" angiosperms

HS2014 ("Ambo+Nym-first")

- -0.29 — other angiosperms
- Austrobaileyales
- 0.34 — Nymphaeales
- *Amborella*
- all other

WI2014 ("*Amborella*-first")

- -0.20
- -0.10

ZN2014 ("Nymphaeales first")

- -0.16 — other angiosperms
- Austrobaileyales
- *Amborella*
- -0.26 — Nymphaeales
- all other

XI2014 ("Ambo+Nym-first")

- magnoliids+eudicots
- 0.50 — monocotss
- -0.24 — *Amborella*
- Nymphaeales
- euphyllophytes

E. Magnoliids, monocots, and eudicots

WI2014 — 0.10
ZN2014 — 0.00
XI2014 — 0.00

- eudicots
- magnoliids
- monocots
- all other

HS2014

- -0.06

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)

sect. *Lycopersicon*

(0.98/0.33/0.2)

(0.61/0/0.48) — *S. pennellii* 0716 (0.89,0.36)
S. pennellii 3778 (0.87,0.37)
(0.06/0.31/0.68)
(0.69/0.02/0.42) — *S. habrochaites* 0407 (0.9,0.39)
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 — *S. huaylasense* 1358 (0.79,0.16)
[68] — *S. corneliomulleri* 0444 (0.8,0.16)
(-0.09/0.49/0.66)
-0.34/1/0 (0/0.03/0.71) — *S. corneliomulleri* 0107 (0.82,0.17)
0.71/0/0 [68] — *S. peruvianum* 2744 (0.49,0.16)
-0.24/1/0 — *S. huaylasense* 1364 (0.57,0.17)
(-0.12/0.55/0.7)
(-0.07/0.46/0.71) — *S. huaylasense* 1360 (0.8,0.21)
-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)

(0.18/0.44/0.82) — *S. lycopersicum* 2933 (0.94,0.20)
(0.15/0.52/0.81) — *S. pimpinellifolium* 1589 (0.94,0.21)
— *S. pimpinellifolium* 1269 (0.91,0.23)
(0.9/0.17/0.4) — *S. lycopersicum* 3475 (0.94,0.25)
(0.72/0.58/0.69) — *S. lyco.* "Heinz 1706" (0.94,0.23)
(0.17/0.32/0.76) — *S. cheesmaniae* 3124 (0.95,0.21)
(0.19/0.44/0.75) — *S. cheesmaniae* 0429 (0.94,0.18)
(0.52/0.68/0.82) — *S. galapagense* 0436 (0.91,0.18)
(0.5/0.71/0.96) — *S. galapagense* 3909 (0.93,0.19)

B. Caryophyllales

Beta vulgaris (0.89,0.74)
(1/-/0.06) [0.96] — *Spinacia oleracea* (0.88,0.76)
Frankenia laevis (0.87,0.64)
(1/-/0.05) [0.96] (1/-/0.14) [0.83] — *Reaumuria trigyna* (0.89,0.74)
(0.74/0.6/0.29) [0.63] — *Plumbago auriculata* (0.81,0.55)
(0.01/0.08/0.56) 0.27/0.68/0.04 — *Ruprechtia salicifolia* (0.97,0.57)
[0.40]
(0.88/0/0.15) — *Drosera binata* (0.98,0.61)
(0.42/0.09/0.45) — *Dionaea muscipula* (0.86,0.60)
Aldrovanda vesiculosa (0.89,0.53)
(0.27/0.11/0.38)
[0.47]
(0.45/1/0.3) [0.99] — *Drosophyllum lusitanicum* (0.76,0.65)
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