Ecography (Cop.). Author manuscript; available in PMC 2014 July 28.

Published in final edited form as:
Ecography (Cop.). 2014 June 1; 37(6): 572–580. doi:10.1111/j.1600-0587.2013.00442.x.

Directional biases in phylogenetic structure quantification: a Mediterranean case study

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Abstract

Recent years have seen an increasing effort to incorporate phylogenetic hypotheses to the study of community assembly processes. The incorporation of such evolutionary information has been eased by the emergence of specialized software for the automatic estimation of partially resolved supertrees based on published phylogenies. Despite this growing interest in the use of phylogenies in ecological research, very few studies have attempted to quantify the potential biases related to the use of partially resolved phylogenies and to branch length accuracy, and no work has examined how tree shape may affect inference of community phylogenetic metrics. In this study, using a large plant community and elevational dataset, we tested the influence of phylogenetic resolution and branch length information on the quantification of phylogenetic structure; and also explored the impact of tree shape (stemminess) on the loss of accuracy in phylogenetic structure quantification due to phylogenetic resolution. For this purpose, we used 9 sets of phylogenetic hypotheses of varying resolution and branch lengths to calculate three indices of phylogenetic structure: the mean phylogenetic distance (NRI), the mean nearest taxon distance (NTI) and phylogenetic diversity (stdPD) metrics. The NRI metric was the less sensitive to phylogenetic resolution, stdPD showed an intermediate sensitivity, and NTI was the most sensitive one; NRI was also less sensitive to branch length accuracy than NTI and stdPD, the degree of sensitivity being strongly dependent on the dating method and the sample size. Directional biases were generally towards type II errors. Interestingly, we detected that tree shape influenced the accuracy loss derived from the lack of phylogenetic resolution, particularly for NRI and stdPD. We conclude that well-resolved molecular phylogenies with accurate branch length information are needed to identify the underlying phylogenetic structure of communities, and also that sensitivity of phylogenetic structure measures to low phylogenetic resolution can strongly differ depending on phylogenetic tree shape.

Introduction

Detailed phylogenetic information regarding community composition is crucial for a comprehensive understanding of evolutionary and ecological mechanisms driving

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biodiversity (Losos 1996, Webb et al. 2002), effective conservation planning of the evolutionary potential of extant lineages (Forest et al. 2007), and accurate comprehension of ecosystem functioning (Hillebrand and Matthiessen 2009). During the last decade, the increasing effort to incorporate phylogenetic information on community assembly studies has triggered the emergence of a new discipline known as community phylogenetics (Webb et al. 2002; Cavender-Bares et al. 2009). This effort has been enhanced by the rapid increase of available molecular data, published phylogenies and major advances in computational methods. Nowadays, accurate phylogenies with evolutionary meaningful branch lengths can be obtained for a large number of taxa with supermatrices of molecular data (De Queiroz and Gatesy, 2007), which can be built either by retrieving sequences available on public databases or by sequencing informative molecular markers on sampled taxa.

Despite phylogenies based on supermatrices of molecular data provide detailed evolutionary information, they are still rarely built for ecological studies (but see Kress et al. 2010; Allan et al. 2012; Baraloto et al. 2012) probably because ecologists are generally unfamiliar with phylogenetic reconstruction methods. This hurdle has been partly mitigated thanks to the appearance of specialized software for the automatic estimation of partially resolved supertrees (i.e. phylogenies constructed by assembling published phylogenies; Bininda-Emonds et al. 2002) such as Phylomatic (Webb and Donoghue 2005). Phylomatic has been mostly used for plant studies, because it was initially released with a family level phylogeny for angiosperms based on updated consensus knowledge (e.g. Soltis et al. 2000; Davies et al. 2004; Bremer et al. 2009). It allows one obtain a supertree by assembling species lists of vascular plants (mammals are also available since recently) by matching sample taxa names to a master phylogeny, which is mainly resolved to the family level. This means that the resultant tree incorporates species grouped in polytomies at the family level. However, a higher degree of resolution can be attained in some cases by manually resolving the polytomies on the basis of published phylogenies. The main drawbacks of supertrees are that usually phylogenetic relationships among species are only partially resolved, and that branch length information is missing. A widely used approximation to obtain branch lengths for supertrees is to apply the Branch Length Adjuster algorithm (BLADJ), which produces a pseudo-chronogram by assigning an estimated age to major nodes (e.g. obtained from other publications) and distributing undated nodes evenly. This tool is currently implemented in Phylocom 4.2 software (Webb et al. 2008).

Although some authors suggest that the method used for phylogenetic reconstruction does not alter qualitative conclusions on phylogenetic structure (Cadotte et al. 2008, 2009), there is some evidence that results relying upon phylogenies containing many unresolved nodes are not consistent with those obtained with resolved molecular phylogenies (Kress et al. 2009, Pei et al. 2011). Despite some simulation works have been conducted with the aim to explicitly quantify potential biases on estimates of phylogenetic structure (Swenson 2009) and on phylogenetic signal (Davies et al. 2009) due to lack of resolution in phylogenies, potential biases on estimates of phylogenetic structure related to the method of phylogenetic reconstruction have only recently started to be assessed with real world data (Kress et al. 2009, Pei et al. 2011). Whereas these studies have focused on phylogenetic resolution, the influence of branch length information on phylogenetic structure estimators remains unexplored.

Recently, Davies and Buckley (2012) showed that phylogenetic pairwise distances describe phylogenetic tree topology quite well, which suggests that tree stemminess (i.e. the relative distribution of inter-nodal distances, that is, whether nodes are more concentrated towards the root or the tips) could affect the degree of mismatch between poorly and further resolved phylogenies when quantifying phylogenetic structure. To our knowledge, no work has been done to explore how tree shape may impact community phylogenetic metrics.

In this paper, we use a large scale dataset embracing a diverse biogeographic area (Sierra de las Nieves, a Mediterranean mountain range) with the aim to: (1) test how strong is the influence of branch length information and phylogenetic resolution on phylogenetic structure estimators; (2) explore the impact of tree shape (focusing on stemminess) on the loss of accuracy in phylogenetic structure quantification due to phylogenetic resolution.

Materials and methods

Study area and plant datasets

In order to assemble two classes of floristic data matrices, we used a dataset of vegetation surveys across the mountain range Sierra de las Nieves (Cabezudo et al. 1998), and we also extracted the altitudinal range of all angiosperms present in this area from the detailed Flora Vascular de Andalucía Oriental (Blanca et al. 2009). Sierra de las Nieves is a mainly calcareous Mediterranean mountainous range, located in South Iberian Peninsula (Andalusia), in the western end of the Baetic Range (Fig. 1). Its altitudinal range varies from 300 m to 1919 m above sea-level, and it covers an area of 201.63 km^2.

First, we assembled a community-scale data matrix by retrieving a total of 121 vegetation surveys of different cover, composition and size (from 0.8 to 300 m^2) including all elevational and lithological arrays of the Sierra. We removed gymnosperms, ferns and non-vascular plants from the plots, and did not considerate any survey where the removed taxa were dominant. Second, in order to assemble an elevational floristic data matrix of Sierra de las Nieves according to the elevational range information in Blanca et al. (2009), we retrieved all species sampled within each belt of 100 m along the altitudinal gradient of the Sierra from 300 m above the sea level to the top, resulting in a community data matrix of 17 plots. Finally, we removed the taxa belonging to Dipsacaceae and Valerianaceae from our datasets (1.4% and 0.8% of taxa sampled in the elevational and community data matrices respectively), because the phylogenetic positions of both families are still controversial. The community-scale data matrix includes 485 taxa (species and subspecies) representing 59 families and 267 genera, whereas the floristic data matrix comprises 1165 taxa, representing 82 families and 483 genera. These two datasets obtained are very different in number of species per plot: the community plots have a taxa richness of 18.26 ± 1.66 (mean ± se), whereas elevational belts have a taxa richness of 758.06 ± 183.86.

Generation of phylogenetic hypotheses

We generated different phylogenetic trees differing in topological resolution and/or branch length, in order to assess the impact of both factors in the quantification of phylogenetic structure. The phylogenies comprise all species and subspecies of angiosperm plants that

occur in Sierra de las Nieves following Cabezudo et al. (1998). We did not include non-native and doubtful species, and did not considerate any taxonomical categories below subspecies level. Despite their putative importance in some regions and relevance for plant biodiversity accounts, hybrids were not considered because of lack of comprehensive information about their constancy.

We obtained a molecular phylogeny resolved to the genus level, which was inferred with a supermatrix built with DNA sequences available in Genbank following the pipeline of Roquet et al. (2013). Node support was estimated using bootstrap values (BS). Nodes with BS less than 50% were collapsed into soft polytomies (see Appendix for full details on the phylogenetic procedure). We retrieved sequences for more than 99% of the genera in the dataset. In the few cases where there were no sequences available, we inserted these genera a posteriori in the corresponding family node. We generated a chronogram dating the molecular phylogeny with penalized-likelihood as implemented in r8s (Sanderson 2003) and used a wide range of fossil data (see Table S1 in Appendix for references) to calibrate the chronogram (hereafter called “D1 phylogeny”).

Supertrees provide information on the evolutionary relationships but lack branch length information. This lack of quantitative data of evolutionary relatedness is usually dealt producing a pseudo-chronogram with the BLADJ algorithm (Webb et al. 2008), which fixes the age of some nodes provided by the user (typically node ages derived from Wikström et al. 2001) and distributes evenly the other nodes. It is important to note that the dated tree of Wikström et al. (2001) was obtained with the fossil calibration of a single point, which has been shown to lead to biased estimates (e.g. Sauquet et al. 2012).

In order to disentangle the relative influence of branch length information and/or phylogenetic resolution on phylogenetic structure estimators, we generated a set of different pseudo-chronograms from the molecular phylogeny. To do so, we ultrametrized the topology of the molecular phylogeny following two strategies. First, we used BLADJ assigning ages based on the same range of fossil data as in the phylogeny dated with penalized-likelihood (D2 phylogeny). Second, we used BLADJ assigning node ages based on Wikström et al. (2001) (D3 phylogeny). The BLADJ-dated phylogenies allowed us to test the influence of branch length information on phylogenetic structure estimators.

We generated two less resolved phylogenies from the molecular phylogeny dated with penalized-likelihood. These less resolved phylogenies were generated following two different strategies. First, we collapsed all nodes below family level into soft polytomies (full polytomy phylogeny, FP). Second, we randomly collapsed 50% of nodes sustented by families accounting for more than 10 genera (i.e. Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Fabaceae, Lamiaceae, Orchidaceae, Poaceae and Rosaceae) (medium polytomy phylogeny, MP). These less resolved phylogenies allowed us to test the influence of phylogenetic resolution on phylogenetic structure estimators. Finally, we generated another four less resolved phylogenies by collapsing nodes of D2 and D3 following the above strategies, in order to test the combined influence of branch length information and phylogenetic resolution on phylogenetic structure estimators. In order to account for the possible influence of intra-genus phylogenetic uncertainty on phylogenetic

structure metrics (Davies et al. 2009), we randomly resolved genus level polytomies of each phylogenetic hypothesis by applying a Yule-Harding branching process with constant birth rates (N = 100). The algorithm assigns to each node the same probability of splitting in two lineages, resulting in a balanced topology (Nee 2006). Subspecies were constrained to split within their respective species.

Measures of phylogenetic structure

We assessed the impact of the different phylogenetic hypotheses on the estimates of three commonly used metrics of phylogenetic structure: the net relatedness index (NRI); the nearest taxon index (NTI); and the standardized phylogenetic diversity (stdPD). These three metrics are respectively the standardized effect size versions of the following indices: the mean phylogenetic distance (MPD), which measures the mean phylogenetic distance between each of the sampled taxa and every other terminal in the sample; the mean nearest taxon distance (MNTD), which measures the mean distance between each of the sampled taxa and its own most closely related terminal taxon in the sample (Webb et al. 2002); and the phylogenetic diversity (PD, also known as Faith's Index), which is the sum of the minimum spanning path in the phylogenetic tree connecting all species found in a local plot (Faith 1992). The indices used here were calculated as:

$$NRI = MPD_{obs} - MPD_{exp} / \, sd.MPD_{exp}$$

$$NTI = MNTD_{obs} - MNTD_{exp} / \, sd.MNTD_{exp}$$

$$stdPD = PD_{obs} - PD_{exp} / \, sd.PD_{exp}$$

where $sd.MPD_{exp}$, $sd.MNTD_{exp}$ and $sd.PD_{exp}$ are the standard deviations of 999 MPD (MPD_{exp}), MNTD ($MNTD_{exp}$) and PD (PD_{exp}) values generated by random draws of the same number of species from the same pool phylogeny.

We calculated NRI, NTI and stdPD values within the different plots using the nine sets of phylogenetic hypotheses (N=100 phylogenies per set). Random assemblages were drawn from the Sierra de las Nieves pool (N=1165) and from plants sampled in the community plots (N=485) respectively. Finally, we obtained a Gaussian distribution of possible phylogenetic structure values for each plot and index, from which we extracted the arithmetic mean as an approximation to the "true" phylogenetic structure values.

Statistical analyses

First, we used the scores of the phylogenetic structure metrics to determine the relationship between the values obtained from the use of a chronogram and different pseudo-chronograms derived from the same molecular phylogeny. All regressions were assessed by fitting ordinal least squares models, and the coefficient of determination values (R^2) were extracted as an estimate of the strength of the relationships. Second, we determined whether

pseudo-chronograms tend to generate Type I errors (overestimation of phylogenetic structure) or Type II errors (underestimation) as compared to the chronogram following the method proposed by Swenson (2009). We compared pseudo-chronograms ~ chronogram regressions to the null hypothesis of a perfect correlation between them (regression slope = 1). We forced regression lines to the origin and recorded the slopes of the new regressions. Slopes greater than one indicate a bias towards overestimation of community phylogenetic structure (Type I errors), whereas slopes less than one suggest a bias towards underestimation or random pattern (Type II errors). This method has the advantage of detecting general directional biases when quantifying phylogenetic structure regardless of the statistical performance of the different null models commonly used to assess the significance of phylogenetic structure values (Hardy 2008).

Tree shape simulations

We explored the impact of tree shape (stemminess) on phylogenetic structure quantification comparing the values of the metrics derived from the use of a partially resolved phylogeny and a further resolved phylogeny. To do so, we transformed branch lengths of the chronogram (D1 phylogeny) using Pagel's δ (Pagel 1999), generating a range of trees with different degrees of stemminess (N=26), and characterised the stemminess of the resultant trees using the γ statistic (Pybus and Harvey's 2000). The stemminess of the simulated trees ranged from $\gamma = -15.22$ to $\gamma = 16.64$, corresponding to the trees with the longest inter-nodal distances towards the tips ("tippy") and the root ("stemmy") of the phylogeny respectively. In other words, negative values of γ indicate that nodes cluster near the root, while positive values indicate that nodes cluster near the tips. We generated a less resolved tree from each simulated phylogeny by collapsing all below family nodes into soft polytomies, and calculated NRI, NTI and stdPD within each plot in the community-scale data matrix using both sets of phylogenetic hypotheses. We regressed the scores of phylogenetic structure matrices derived from each simulated tree to those derived from their less resolved homologous, forcing regression lines to the origin. Finally, we compared the slopes of each comparison to the value of the γ statistic associated to each simulated tree. For this analysis, species and subspecies were inserted as polytomies in the corresponding genus node.

Results

Phylogenetic structure

For each metric of phylogenetic structure, we quantified the relationship between the values obtained using a chronogram (D1 phylogeny) and those obtained with pseudo-chronograms of varying degree of resolution and/or branch length information (Table 1), as well as the trend of pseudo-chronograms to make directional biases (Table 2, Fig. 2). When directional biases occured, they were generally towards type II errors, except for stdPD in elevational belts, where we also detected type I errors. The size of community plots did not affect the output of the analyses, since there was no correlation between NRI scores and plot size (e.g. NRI derived from the chronogram; $R^2 = -0.005$, p-value = 0.546). We assume here that the chronogram is closer to the true evolutionary relationships, as a high percentage of nodes obtained a high statistical support (70% of nodes showed a bootstrap support greater than 70).

Regarding the impact of phylogenetic resolution, phylogenetic structure estimations were more accurate and less biased when calculated with the MP phylogeny than with the less resolved FP phylogeny, except for NTI in elevational belts, where the use of FP yielded less biased estimations compared to MP (Table 2), despite the NTI values obtained with MP yield a higher correlation with the values derived from the chronogram than FP (Table 1). The estimation of stdPD in elevational belts suffered of equal biases with MP and FP but in opposite directions, showing a trend towards type II errors with MP and towards type I errors with FP (Table 2). Overall, NRI and NTI were the less and the most sensitive metrics to phylogenetic resolution, respectively.

In regard to the influence of the dating method, directional biases were stronger in elevational belts and overall towards type II errors, except for stdPD, for which the D3 phylogeny yielded type I statistical errors (Table 2). The D3 phylogeny generated less biased estimates of the metrics, except for NRI in elevational belts, where the D2 phylogeny performed better. In general, the D3 phylogeny yielded more accurate values for all estimators (Table 1), except for NTI at community-scale and NRI in elevational belts. Equally accurate estimates were obtained with D2 and D3 for stdPD at community-scale and NTI in elevational belts.

Tree shape simulations

The degree of stemminess of the simulated trees strongly affected the extent of the statistical errors produced by poorly resolved phylogenies compared to their further resolved homologous when quantifying phylogenetic structure. The slope of the regressions increased with the γ value of the trees, particularly in the range between $-15 > \gamma < 5$. (Fig. 3). From $\gamma = 5$ to 15, NRI stabilized, whereas stdPD values slightly diminished for $\gamma = 5$ - 9 and then increased abruptly. NTI was the metric less affected by changes in tree stemminess.

Discussion

Recent years have seen an increasing effort to incorporate phylogenetic hypotheses to the study of community assembly processes. Two main approaches have been followed to do so: either to obtain molecular phylogenies, or to generate supertrees based on previously published data, which usually contain a notable number of unresolved nodes and lack branch length information. Despite this growing interest in the use of phylogenies in ecological research, very few studies have attempted to quantify the potential biases related to the use of partially resolved phylogenies and to branch length accuracy with real data (but see Kress et al. 2009, Pei et al. 2011). Moreover, it has not been previously examined, to our knowledge, how tree shape may affect inference of community phylogenetic metrics.

Recently, a simulation study (Swenson 2009) showed that the lack of phylogenetic resolution can be a potential source of type II statistical errors when quantifying phylogenetic structure. Later, two case studies (Kress et al. 2009 and Pei et al. 2011) tested the directional biases in the quantification of phylogenetic structure with real world data with the metrics NRI and NTI. Both studies compared the scores derived from a supertree assembled with Phylomatic, to those derived from a fully resolved supermatrix phylogeny. The Phylomatic-generated phylogeny in Kress et al. (2009) was ultrametrized using BLADJ

with ages from Wikström et al. (2001), whereas Pei et al. (2011) did not specify this information. Again, their results suggested that the use of poorly resolved supertrees biased community phylogenetic structure analyses, tending to fail to detect non-random phylogenetic structuring (type II errors).

Our results only partially agree with the general trend towards false negatives for NRI and NTI observed in these previous case studies. We found that, with our datasets, NRI was quite resilient to the loss of phylogenetic resolution (m = 1.003 and 0.953 for MP and FP phylogenies, respectively). Furthermore, at community scale, NRI estimates derived from the FP + D3 phylogeny (which would be equivalent to a phylogeny generated with Phylomatic and ultrametrized using BLADJ with ages from Wikström et al. (2001), the typical approach in community phylogenetics research) were not biased compared to the NRI values derived from the chronogram (m = 1.004, Fig. 2). The resilience detected here for NRI, particularly in the community dataset, cannot be explained by differences in species richness as it is only slightly lower (18.26 ± 1.66), to the one reported in Pei et al. (2011) (27.57 ± 0.48 in intermediated species richness habitats).

In relation to this point, we have shown that the impact of poor phylogenetic resolution on phylogenetic structure quantification depends, to a greater or lesser extent, on the stemminess of the tree. The differences in sensibility found between our study and the studies of Pei et al. (2011) and Kress et al. (2009) in regard to NRI could be rather explained by the different shape of the regional phylogenetic trees, as this metric was the most affected by stemminess. Tropical and subtropical taxa sampled in the surveys of Kress et al. (2009) and Pei et al. (2011) included only trees and shrubs lineages, which are probably older than those included in our Mediterranean community matrix (Hawkins et al. 2011). Moreover, despite a similar family richness was reported in the community phylogeny pool in the three studies, the genus per family ratio is clearly higher in our community matrix (4.53 for our dataset vs. 3.18 for Kress et al. 2009 and 2.16 for Pei et al. 2011). Thus, it is probable that the shape of the phylogenies used in the two mentioned studies is considerably different from ours, the later with longer inter-nodal distances towards the root of the phylogeny (≈ “stemmy” phylogeny). In fact, our phylogeny pool presents a $\gamma = 3.86$, consistent with a scenario of recent diversification within evolutionarily disparate lineages (Davies and Buckley 2012). Thus, we suggest that the differences in the trend of poorly resolved phylogenies to make statistical errors found between our study and the studies of Pei et al. (2011) and Kress et al. (2009) could be to a large part due to the different shape of the phylogenies used to derive phylogenetic structure values.

Previous case studies that assessed potential biases in the detection of phylogenetic structure of real communities linked to lack of phylogenetic resolution only focused on the metrics NRI and NTI. Here, we have also investigated potential biases on the widely used metric stdPD. Our estimates of stdPD showed an intermediate resilience between NRI and NTI. Although all these metrics are derived from the same internode distances, these are traversed only once when calculating stdPD, whereas for NRI they are taken in account as many times as the number of pairwise distances defined by such internodes. Deep nodes defining phylogenetic relationships between different families and orders are fully and equally resolved in both the chronogram and the pseudo-chronograms, and are expected to hold

more weight on phylogenetic structure estimates compared to intra-family internodes because they are simply defining broader distances. Because NRI traverses deep internodes much more times than NTI and stdPD, the effect of phylogenetic uncertainty is mitigated. In constrast, NTI estimates relies mainly on intra-family internodes, and thus, the effect of phylogenetic uncertainty is expected to be maximal for this metric. The fact that all internodes are equally weighted when calculating stdPD would explain why the effect of phylogenetic uncertainty on stdPD estimations is intermediate compared to NTI and NRI.

In regard to the influence of the dating approach, differences in phylogenetic structure quantification with pseudo-chronograms compared to the chronogram could be related to the accuracy of calibrations and the method used. Which ultrametrizing approach was better depended both on the metric and the dataset. The D2 and D3 pseudo-chronograms differ in the type of calibration data and also in the number of ages provided. The phylogeny D3 probably yielded overall better results than D2 because it was ultrametrized by fixing a higher number of deep nodes (extracted from Wikström et al. 2001), which are expected to hold more weight on phylogenetic structure estimates than shallower nodes. In fact, differences in the yield between D3 and D2 in regards with NTI were smaller as compared to those with NRI or stdPD, since NTI relies mainly on shallower nodes. However, althought the D3 phylogeny generated similar results to those generated by the chronogram at community scale, it also made directional biases in elevational belts. It should be noted that the procedure of BLADJ, which places undated nodes evenly between calibrated nodes, is not evolutionarily realistic in many cases; for instance, for angiosperms it has been shown that the deepest branches are very short (e.g. Fiz-Palacios et al. 2011). Dating molecular phylogenies with relaxed molecular clock methods such as penalized-likelihood (r8s) yield more evolutionarily realistic chronograms as they date based on the amount of genetic differences and can account for heterogeneity in the rates of DNA evolution. This is the reason why the molecular phylogeny dated with r8s and the phylogeny D2 yielded different results when estimating phylogenetic structure, despite both phylogenies were calibrated with the same fossil ages. Thus, if the obtention of a molecular phylogeny is not possible, and given the procedure used by BLADJ, it is highly recommendable to provide BLADJ as much node ages as possible; and more importantly, these ages should be extracted from reliable and updated dating studies in which the phylogeny has been calibrated with a wide range of fossils (e.g. Fiz-Palacios et al. 2011).

Our results also suggest that there is some interaction between phylogenetic resolution and branch length accuracy. For example, phylogenetic resolution was critical when quantifying stdPD at both spatial scales, the FP phylogeny generating less accurated estimates than MP (Table 1); however, FP + D3 generated more accurated stdPD estimates than MP + D3, particularly in elevational belts. This result suggests that the accuracy of phylogenetic structure quantification based on BLADJ-dated phylogenies will not necessarily be improved by manually resolving polytomies on the basis of published phylogenies, which is indeed a widespread procedure in community phylogenetic research.

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Conclusions

Our results support previous evidence that well-resolved molecular phylogenies are needed to identify the underlying phylogenetic structure of communities, that may otherwise be underestimated. However, the bias detected with our data in phylogenetic structure quantification related to the phylogenetic data quality differs in some particular cases from previous studies performed in other biogeographical regions with disparate evolutionary assemblages (Kress et al. 2009, Pei et al. 2011). We show that differences in tree shape (stemminess) for species pool phylogenies from different biogeographical regions can notably affect the degree of mismatch between poorly and further resolved phylogenies when quantifying phylogenetic structure.

Although phylogenetic resolution has been proved to be a source of directional bias on phylogenetic structure quantification, the accuracy of branch length information can be even more important than phylogenetic resolution in quantifying phylogenetic structure. The strength of the impact of branch lengths in phylogenetic structure quantification depends on the chosen estimators. For instance, a family level phylogeny dated with BLADJ using ages from Wikström et al. (2001) can be enough to calculate accurately NRI, but the same phylogeny can lead to spurious results with the indices NTI or stdPD, depending ultimately on the tree shape and the sample size. Anyhow, we confirm here that the choice between supermatrix and supertree approaches (the former lacking for branch length information) can be critical for the accurate detection of underlying patterns of phylogenetic structure of plant assemblages. The supertree approach has the clear advantage to be a faster, easier and more economic way to generate phylogenetic hypotheses than molecular methods, as only a few informatic notions are needed (Webb and Donoghue 2005, Webb et al. 2008). However, in the light of results found in the present study, we strongly suggest that sensibility analysis should be carried out when dealing with partially resolved phylogenies, and that efforts should be dedicated to obtain reliable and well-resolved phylogenetic hypotheses to work with.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Centre of Supercomputing of Galicia (CESGA) for their computational service. We are grateful to the reviewers for their useful comments on this manuscript. This work was supported primarily by the Spanish *Organismo Autónomo de Parques Nacionales* through the grant 296/2011, and secondarily by the Spanish *Ministerio de Ciencia e Innovación* and the Regional Government of Andalucía, through the grants HF-2008-0040 and P09-RNM-5280. RMV was financially supported by a pre-doctoral grant (FPU) from the Spanish *Ministerio de Educación, Cultura y Deporte*. CR received financial support from the European Research Council under the European Community's Seven Framework Programme FP7/2007-2013 Grant Agreement no. 281422.

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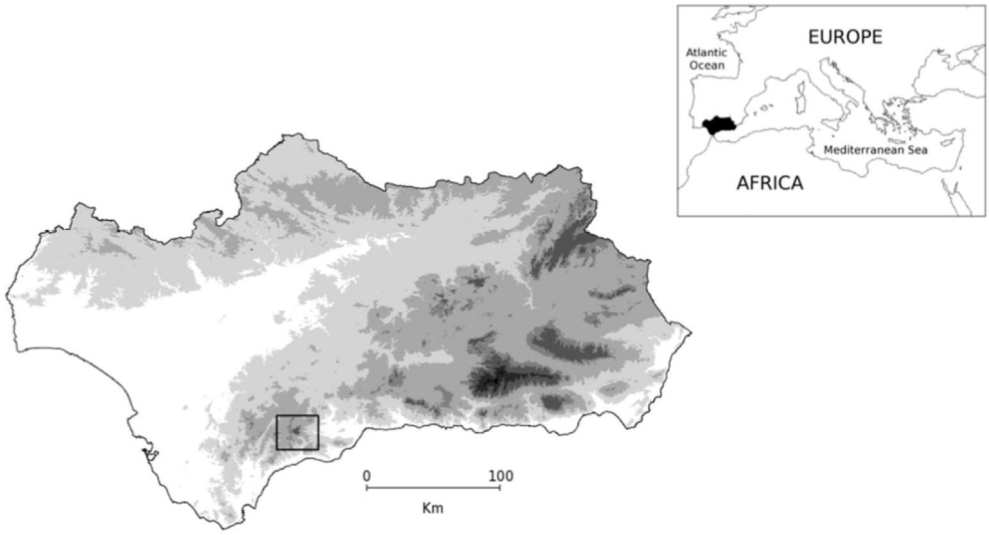

Figure 1. Map showing the location of the study area (Sierra de las Nieves) within Andalusia (South Spain)

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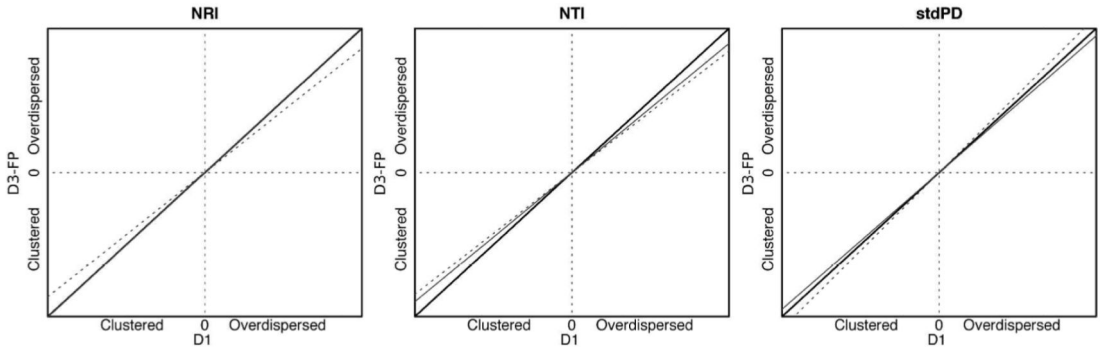

Figure 2. Graphical representation of the slope analysis showing directional biases in NRI, NTI and stdPD derived from the use of the molecular phylogeny dated with BLADJ using ages from Wikström et al. (2001) and with all nodes below family level collapsed into soft polytomies (D3-FP) instead of the same molecular phylogeny dated with penalized-likelihood and resolved to genus level (D1 chronogram).

Thin solid lines and dashed lines represent community-plot and elevational belt regression lines respectively. The thick solid lines represent the null hypothesis, a perfect 1:1 relationship through the origin. Slopes greater than one indicate a bias towards over-prediction of phylogenetic diversity or structure and vice versa for slopes less than one. Note that the regression line corresponding to NRI values at community scale perfectly matchs that of the null hypothesis.

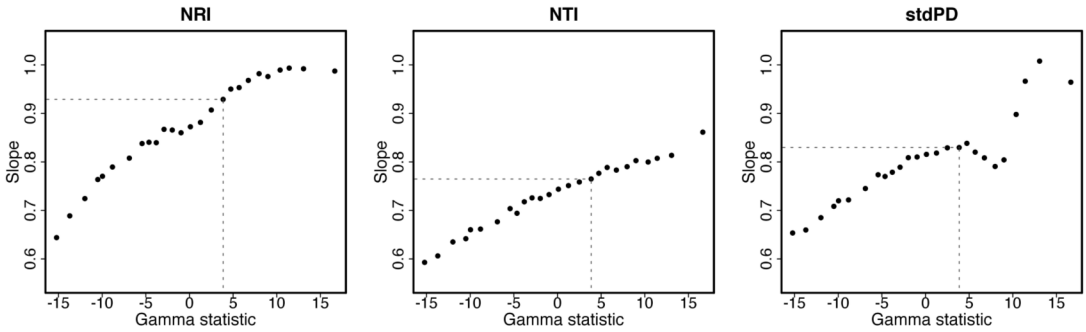

Figure 3. Graphical representation of the relationship between the stemminess and the yield of phylogenetic structure quantification with a range of poorly resolved trees (family level) versus their further resolved homologous (genus level).

The marked dots correspond to the real chronogram (D1) from which the range of tree with varying degrees ofstemminess was obtained by transforming branch lengths using Pagel's δ.

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Table 1

Coefficient of determination values (R^2) from regressing NRI, NTI, and stdPD scores derived using a molecular phylogeny resolved to genus level and dated with penalized-likelihood, onto the same indexes derived from different pseudo-chronograms of different resolution and/or branch length information.

All the pseudo-chronograms were generated from the molecular phylogeny. All regression values were significant at < 0.001, except for "*", where it was at < 0.01.

Metric	Dataset	Resolution		Dating method		Resolution + Dating method			
		MP	FP	D2	D3	MP + D2	FP + D2	MP + D3	FP + D3
NRI	Community	0.999	0.970	0.803	0.908	0.801	0.802	0.902	0.896
NTI	Community	0.988	0.872	0.853	0.775	0.838	0.792	0.734	0.803
stdPD	Community	0.992	0.901	0.829	0.828	0.816	0.790	0.785	0.856
NRI	Elevational belt	0.999	0.988	0.946	0.887	0.943	0.953	0.876	0.890
NTI	Elevational belt	0.997	0.940	0.947	0.948	0.797	0.888	0.815	0.934
stdPD	Elevational belt	0.976	0.939	0.869	0.939	0.416*	0.814	0.708	0.960

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Table 2

Slopes from regressing NRI, NTI and stdPD scores derived using a molecular phylogeny resolved to genus level and dated with penalized-likelihood, onto the same indexes derived from different pseudo-chronograms of different resolution and/or branch length information.

All the pseudo-chronograms were generated from the molecular phylogeny. All regression values were significant at < 0.001.

Metric	Dataset	Resolution		Dating method		Resolution + Dating method			
		MP	FP	D2	D3	MP + D2	FP + D2	MP + D3	FP + D3
NRI	Community	1.003	0.953	0.779	1.001	0.778	0.780	1.003	1.004
NTI	Community	1.028	0.847	0.910	0.971	0.917	0.823	0.981	0.896
stdPD	Community	1.027	0.900	0.717	1.007	0.715	0.666	1.017	0.949
NRI	Elevational belt	1.022	0.949	0.933	0.898	0.933	0.928	0.901	0.861
NTI	Elevational belt	0.876	0.914	0.877	0.921	0.697	0.773	0.714	0.844
stdPD	Elevational belt	0.934	1.067	0.760	1.037	0.580	0.694	0.854	1.084