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To cite this version:

Peter M Hollingsworth, Roseli Pellens, Philippe Grandcolas, Antje Ahrends. Assessing Hotspots of Evolutionary History with Data from Multiple Phylogenies: An Analysis of Endemic Clades from New Caledonia. Biodiversity Conservation and Phylogenetic Systematics - Preserving our evolutionary heritage in an extinction crisis, pp.237-262, 2016, 10.1007/978-3-319-22461-9_12. hal-02565187

HAL Id: hal-02565187
https://hal.science/hal-02565187
Submitted on 6 May 2020

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Assessing Hotspots of Evolutionary History with Data from Multiple Phylogenies: An Analysis of Endemic Clades from New Caledonia

Roseli Pellens, Antje Ahrends, Peter M. Hollingsworth, and Philippe Grandcolas

Abstract  The great bulk of the present knowledge of the Tree of Life comes from many phylogenies, each with relatively few tips, but with lots of diversity concerning taxa and characters sampled and methods of analysis used. For several biodiversity hotspots this is the kind of data available and ready to be used to have a better understanding on the evolutionary patterns and to identify areas with remarkable evolutionary history. But relying on data coming from independent studies raises some methodological challenges of standardization, comparability and assessments of bias to make the best use of the currently available information. To bring light to this subject here we analyzed the distribution of phylogenetic diversity in New Caledonia, a biodiversity hotspot characterized by strong rates of regional and internal endemicity. We used a dataset with 18 phylogenies distributed in 16 study sites, and based our analysis on the measure Ws sum. Our study comprises the analysis of (1) the role of the number of phylogenies on site’ scores and a strategy of standardization of the dataset by the number of phylogenies; (2) the influence of species richness on site scores and the design of the measure Ws ranks to focus on the most divergent species of each phylogeny; (3) an assessment of the influence of individual phylogenies; (4) a resampling strategy using multiple phylogenies to verify the results’ stability.

Keywords  Ws • Resampling • Rarefaction • Meta-analysis

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R. Pellens, P. Grandcolas (eds.), Biodiversity Conservation and Phylogenetic Systematics, Topics in Biodiversity and Conservation 14, DOI 10.1007/978-3-319-22461-9_12
**Introduction**

Opening wide the sampling window in biodiversity studies is a major goal today, and this leads to two major research challenges. On the one hand is the difficulty of dealing with big data, as, for example, those from entire genomes. Now that the main barriers to obtain enormous sequences seem to be broken, and that this kind of data is becoming easy and much cheaper to be obtained, the main constraint is the analysis of such huge datasets. On the other hand are the difficulties associated with synthesizing evidences produced by several independent studies where, by definition, the sampling protocols are not standardized. Both issues are at the core of the analysis of phylogenetic diversity for conservation and deserve more attention if we are to produce sound guidelines for conservation. However, in this chapter we will focus only on the last one.

Our interest in this problem is due to the fact that the great bulk of present knowledge of the Tree of Life does not result from a comprehensive analysis with standardized samples of taxa and characters. Instead, the greatest part of published works comprises studies at the level of families or genera, with lots of diversity concerning taxon and characters sampling and methods of analysis. But the increased facility of molecular sequencing and phylogenetic analysis observed in the recent years has led to a substantial increase in available phylogenies. As a consequence, for some biodiversity hotspots, an important number of detailed phylogenetic studies for several distinct groups are now available. The data from these independent studies, associated with a greater accuracy and availability of species occurrence records, provide a rich material that can enhance biodiversity conservation decisions. This allows for detecting evolutionary patterns across a broader sample of the Tree of Life and, ultimately, for detecting hotspots of evolutionary history within these biodiversity Hotspots. Obviously, the higher the diversity of groups covered by the set of phylogenies the finer the picture of the Tree of Life in the region and the more reliable the contribution of phylogenetic information to the conservation planning (Rodrigues et al. 2005).

Although the possibility of integrating results from different phylogenies has been studied for a while (see Posadas et al. 2001, 2004; Faith et al. 2004; López-Osorio and Miranda Esquivel 2010), we are only starting to explore the implications of different sampling effort and imperfect knowledge on studies of phylogenetic diversity for assessing areas for conservation (see Nipperess and Matsen 2013 and Nipperess, chapter “The Rarefaction of Phylogenetic Diversity: Formulation, Extension and Application” and Miranda-Esquivel, chapter “Support in Area Prioritization Using Phylogenetic Information”). In order to shed light to this problem here we propose some solutions when assessing hotspots for conservation within New Caledonia.
**Assessing Hotspots of Evolutionary Distinctiveness in New Caledonia**

New Caledonia is a Pacific Ocean island located some 1450 km east of Australia (Fig. 1). It is about 500 km long and 50 km wide and is classed as a globally significant biodiversity hotspot (Myers et al. 2000; Grandcolas et al. 2008; Kier et al. 2009). The island’s biological diversity is threatened by activities associated with large-scale opencast nickel mining, an increased frequency of fires, and by ecological displacements caused by invasive species (Bouchet et al. 1995; Beauvais et al. 2006; Pascal et al. 2008; Pellens and Grandcolas 2010).

A key feature of the New Caledonian biota is its high level of endemism. The geographical isolation of the island and its ultramafic soils have all been proposed as factors promoting high levels of endemicity. This endemity exists at the level of the island, but also at finer geographical scales, and within New Caledonia microendemism is common with many species restricted to individual mountains, mountain slopes, valleys, watercourses or edaphic ‘islands’ (e.g., Murienne et al. 2005; Sharma and Giribet 2009; Espeland and Johanson 2010b; Pillon et al. 2010; Nattier et al. 2012, 2013).

![Localization of New Caledonia in the southern Pacific and the 16 study areas in New Caledonia’s mainland](image-url)
This high level of endemism has attracted considerable scientific interest in the evolutionary history of the island (see the review in Pellens and Grandcolas 2010) and phylogenies for several groups are now available. There have also been macro-analyses of the distribution of micro-endemic species (Wulff et al. 2013). However, to date there has been no systematic evaluation of the distribution of biodiversity in the context of evolutionary distinctiveness within the island, comparing multiple taxonomic groups over multiple geographical locations. In the current paper we tackle this topic with the aim to identify sites with high levels of phylogenetic diversity.

This type of study raises some methodological challenges. In an ideal world, one would design a sampling strategy involving equal sampling effort (or at least quantified sampling effort) at multiple sites for multiple sets of taxa, sampled for a common and comparable set of characters, and with the data analyzed in a common and comparable analytical framework. Unfortunately such a dataset does not exist presently for New Caledonia. Instead, we have taken an approach to make the best use of the currently available information by mining the literature for tree topologies, and then developing an analytical framework that copes with the shortcomings of the extant dataset.

Specifically we have pulled together the available data consisting of multiple phylogenies from different groups of organisms, of different levels of species richness, built from different character sets, different analytical methods and partially overlapping geographical locations. Our framework aims to standardize the contributions of these different datasets in a meta-analysis, and also to quantify the inevitably high-levels of uncertainty and variance in the range of possible conclusions that comes from dealing with (a) a complex biological system, and (b) imperfect data.

Material and Methods

Data and Sampling

We included all available phylogenetic studies up to 2010 that satisfied the three following conditions: (1) having a monophyletic group from New Caledonia with three species or more; (2) having extensive coverage of the geographic distribution of the group within New Caledonia’s mainland; (3) having species represented in at least three out of the 16 selected geographical areas (see below). This resulted in 18 phylogenies encompassing both terrestrial and freshwater organisms (Table 1). The monophyletic clades in which New Caledonian species were found ranged from 3 to 59 species (mean 14.9, median 10.5) and in total these phylogenies included 269 species, all endemic to New Caledonia. They included organisms as diverse as insects, harvestmen, gastropods, vertebrates (geckos – Squamata), and vascular plants.
We considered 16 areas (sites) within New Caledonian mainland. This set of sites includes the very great majority of areas with remaining native forests and are distributed throughout the length of the island. These areas correspond to geographical entities with discrete boundaries, such as isolated mountains, or parts of large ridge systems or lowlands separated from the adjacent one by main valleys, rivers or lakes (Fig. 1). The basic condition for including an area in this analysis was the availability of at least five phylogenetic studies containing species represented at the site, and a minimum of ten species studied. Distributional data were collected from the original phylogenetic studies from the literature cited therein, and from the specialists working on the group in the region (Table 1). Species richness in this paper

| Key | Family       | Genus       | Reference                                  |
|-----|--------------|-------------|--------------------------------------------|
| 1   | Blattaria    | Angustonicus| Murienne (2006)                             |
| 2   | Blattaria    | Lauresilpha | Murienne et al. (2008)                      |
| 3   | Heteroptera  | Cephalidiosus Nobarnus | Murienne et al. (2009)                      |
| 4   | Orthoptera   | Agnoteceous | Desutter-Grandcolas and Robillard (2006)    |
| 5   | Trichoptera  | Xanthochorema| Espeland et al. (2008)                      |
| 6   | Trichoptera  | various     | Espeland and Johanson (2010a)              |
| 7   | Trichoptera  | Agmina      | Espeland and Johanson (2010b)              |
| 8   | Coleoptera   | Rhantus     | Balke et al. (2007)                        |
| 9   | Opiliones    | Troglosironidae | Troglosiro | Sharma and Giribet (2009) |
| 10  | Gastropoda   | various     | Haase and Bouchet (1998)                   |
| 11  | Squamata     | Marmorospha | Sadlier et al. (2009)                      |
| 12  | Squamata     | various     | Sadlier et al. (2004)                      |
| 13  | Squamata     | Dierogekko  | Bauer et al. (2006)                        |
| 14  | Squamata     | Eurydactylodes | Bauer et al. (2009)                      |
| 15  | Squamata     | Rhacodactylus| Good et al. (1997) and Bauer (1990)       |
| 16  | Ericales     | Planchonella| Swenson et al. (2007) and Munzinger and Swenson (2009) |
| 17  | Ericales     | various     | Munzinger and Swenson (2009), Swenson et al. (2008) and Swenson and Munzinger (2009, 2010a, b, c) |
| 18  | Ericales     | Diospyros   | Duangjai et al. (2009)                     |

Key = the reference number that will be used in Tables 2 and 3 when referring to these studies.
refers to the number of species from the 18 phylogenies occurring in each area. A species was considered a microendemic if it was recorded in an area and nowhere else. In total, our data set consists of 523 records of occurrence of a given species from a phylogeny at one of these 16 sites.

**Metric and Corrections for Bias**

We calculated evolutionary distinctiveness using the topology based metric, the Ws index from Posadas et al. (2001), which is derived from the Taxonomic Distinctness index conceived by Vane-Wright et al. (1991). We chose this metric for three reasons. (1) It assigns higher values to species with fewer and more distant relatives than to species with more and closer relatives, allowing for a better identification of areas with more phylogenetically divergent species (Redding et al. 2008). (2) It is designed for combining phylogenetic information from different cladograms, independently of the kind of characters (morphological, molecular, etc.) or reconstruction method, since it is a topology based metric. This way, we were able to integrate data from phylogenies of taxa as different as plants, reptiles, molluscs and arthropods to study the evolutionary distinctiveness of different areas in New Caledonia. (3) Each phylogeny contributes with the same amount of information, independently of its total species’ number, as the Ws values for the species in any given phylogeny sum to one.

The traditional procedure is to sum Ws of all species present in each area and rank areas according to this sum (Posadas et al. 2001; Lehman 2006; McGoogan et al. 2007; López-Osorio and Miranda Esquivel 2010). However, this practice often leads to strong correlations with species richness (see López-Osorio and Miranda Esquivel 2010), having the possibility of masking important evolutionary divergence in sites with less species, or less phylogenies. Secondly, as Ws is bound between 0 and 1 for a given phylogeny, it is sensitive to the number of sampled species in each phylogeny. Although this will in part be driven by species richness, it is also simply affected by the scope of the study selected by the investigator (e.g. family level or genus level). Thus the wider the phylogenetic breadth of a study (the more species included), the lower the overall maximum value for any one species. Thirdly, in the absence of exhaustive location-based sampling, the data available on the evolutionary diversity of a given site will simply reflect the taxa that happen to have been sampled for individual research projects. If this bias is not corrected for, it will be hard to see the phylogenetic content, as the number of phylogenies and the number of species in each site might drive the result.

In order to address these shortcomings, we designed a method to highlight sites containing the most divergent taxa from each of the phylogenies. We firstly calculated Ws for each species in each phylogeny, and placed the species in order from the highest to the lowest Ws value. We then awarded “points” to the most divergent species in each phylogeny and compared the resulting scores among sites. As we were interested in the ‘front-runners’ from each phylogeny – we firstly took the top
three species, i.e. the most ‘basal’ species from each phylogeny, assigning them a score of 1 (for most basal) 0.67 (second place) and 0.33 (for third place). However, we latterly truncated this to scores for 1st and 2nd place (1 and 0.67) to emphasise the most divergent species. In the case of ties for the most divergent species, the total score of 1.67 was divided by the number of species that tied. Where there is a unique first place score, but ties for second place, the ‘second prize’ of 0.67 was ‘shared’ amongst the species which tied. The scores were then summed for all phylogenies at each site.

This method ensures that each phylogeny contributes a directly equal total score, and we are simply assessing in each case where the most divergent species are. The downside of using first and second ranked species, is that it discards information from all of the other species in each data set. To accommodate this, we also continue to report the (more conventional) sum of Ws values, also standardised by the number of phylogenies present at a given site.

Resampling Analysis

Our data set is constrained by the number of phylogenies that were available. To assess whether our findings are sensitive to the composition of the sample of phylogenies we have, we designed two tests. The first was through assessing the changes associated with the exclusion of a single phylogeny (single drops, a.k.a. Jackknifing). This is to see if the findings are being driven by a single influential phylogeny. Secondly, we undertook a resampling (or rarefaction) procedure, by defining subsets of 1, 2, 3… 15 phylogenies in a site and then calculating the mean and standard deviation of site’s scores with all possible combination of phylogenies with species occurring in it. This was to establish whether the results are stable with respect to the number of phylogenies we have available.

The R codes for these analyses are available from A.Ahrends@rbge.org.uk on request.

Results

The Role of the Number of Phylogenies on Site Scores

In our dataset the number of phylogenies with species occurring at a site ranged between 5 and 16 (mean and median=11). So, the first point that we investigated was the role of the number of phylogenies in site’s scores. This showed that over 75 % of the site’s ranking with Ws sum was explained by the number of phylogenies with species in the site (Regression model: Sum Ws = −2.13 + 0.555 number of phylogenies; F=41.75; DF=14; p=0.000; R²=0.75). With Ws ranks the influence of the number of phylogenies is a bit smaller but still important (Regression model:
Ws ranks = −1.03 + 0.259 number of phylogenies; F = 26.75; DF = 14; p = 0.000; R² = 0.66).

Based on it, we decided to standardize by dividing total Ws sum or total Ws ranks in the site by the number of phylogeny occurring in it. As expected, this came to a result where much less of the site’s ranking is explained by the number the phylogenies with species occurring in the site, but the number of phylogenies still explains a substantial proportion of the variance (Regression model: Ws sum/number of phylogenies = 0.04 + 0.0105 number of phylogenies; F = 8.9; DF = 14; p = 0.01; R² = 0.39; and Ws ranks/ number of phylogenies = 0.082 + 0.0237 number of phylogenies; F = 6.29; DF = 14; p = 0.025; R² = 0.31). In both cases, the standardized and non-standardized values are still correlated (Spearman r = 0.9, p < 0.01; and r = 0.83, p < 0.01 for Ws sum and Ws ranks, respectively). But ranking priorities change, putting in evidence the phylogenetic distinctiveness of some groups occurring in sites with less phylogeny (Figs. 2 and 3).

The Influence of Species Richness on Site Scores

The number of species in the 16 sites varied between 10 and 68 (mean = 33; median = 31), and over 80% of variation in the sum of Ws is explained by species richness. When Ws sums are standardized by the number of phylogenies, 70% of the variation is still explained by species richness – sites with more species have greater chances of accumulating high Ws sums (Fig. 4a, b).

The analysis with Ws ranks shows that all sites had at least one top or second ranking species (1–14 per site, mean and median = 7). The influence of species richness on Ws ranks is lower than Ws sums with just over 50% of the variation in Ws ranks explained by species richness. When Ws ranks were standardized by the number of phylogenies, the influence of species richness became much lower (32%), although still significant (Fig. 5a, b).

Influence of Individual Phylogenies

Tables 2 and 3 show the relative levels of evolutionary distinctiveness among sites when each of the 18 phylogenies is excluded from the analysis. It shows that some sites consistently have high levels of evolutionary distinctiveness, some have consistently lower levels, whereas some others show intermediate values and their ranking positions are more sensitive to the inclusion of any one phylogeny.

The sum of absolute difference in ranks when each of phylogeny was dropped synthesize this result (Figs. 6 and 7). It shows that several phylogenies contribute to site’s ranking, refuting the hypothesis that site’s ranking could be highly influenced by phylogenies with more species, or by a subset of phylogenies with more widespread species.
Fig. 2  Summed Ws values. The main figure shows the values standardized (= divided) by the number of phylogenies present at the site. The numbers on top of each bar give the number of species and phylogenies (in brackets and italics) at each site. The small figure at the bottom shows the non-standardized values.
**Fig. 3** Summed site scores for species on top and second ranks. The main figures shows the values standardized (= divided) by the number of phylogenies present at the site. The numbers on top of each bar give the number of scoring species and phylogenies (in brackets and italics) at each site. The small figure at the bottom shows the non-standardized values.
Fig. 4  Over 80% of the variation in the sites’ Ws sums is explained by species richness (upper figure part). There is still a strong relationship between species richness and the WS sums when standardised by the number of phylogenies, suggesting that species rich sites also have more species with high WS values (lower figure part).
Fig. 5 A little over 50% of the variation in the sites’ top and second top scores is explained by species richness (upper figure part). There is also still a dependency between species richness and the site scores when these are standardised by the number of phylogenies, suggesting that species rich sites also have more species with high WS values (lower figure part).
Values in red and bold are ‘real’ drops, i.e. the dropped phylogeny was indeed present at the site.

A: ranks based on standardised values. B: ranks based on not standardised values. For a key to the phylogenies see Table 1

**Table 2** Site ranks (based on $W_s$) if a given phylogeny is dropped

|                | Drop... |          | Drop... |          |
|----------------|---------|----------|---------|----------|
|                | 1       | 2        | 3        | 4        | 5        | 6        | 7        | 8        | 9        | 10       | 11       | 12       | 13       | 14       | 15       | 16       | 17       | 18       | none     |
| GrandSud       | 1       | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        |
| LaFoaCanala    | 3       | 2        | 2        | 3        | 4        | 5        | 2        | 3        | 2        | 3        | 2        | 3        | 1        | 2        | 3        | 2        | 2        | 1        | 2        |
| RiviereBleue   | 2       | 3        | 4        | 2        | 2        | 3        | 3        | 2        | 3        | 4        | 2        | 3        | 3        | 3        | 4        | 4        | 6        | 2        | 3        |
| MtsKoghis      | 4       | 4        | 6        | 4        | 4        | 5        | 5        | 4        | 4        | 6        | 4        | 2        | 3        | 3        | 4        | 4        | 6        | 2        | 3        |
| MtsDzumac      | 5       | 5        | 3        | 5        | 8        | 4        | 6        | 5        | 5        | 8        | 6        | 4        | 5        | 5        | 8        | 6        | 5        | 5        | 8        |
| GrandNord      | 6       | 6        | 5        | 6        | 6        | 4        | 4        | 6        | 7        | 4        | 11       | 5        | 5        | 7        | 7        | 5        | 6        | 6        | 4        |
| MtAoupinose     | 7       | 9        | 7        | 7        | 4        | 6        | 7        | 8        | 7        | 8        | 7        | 8        | 8        | 6        | 8        | 7        | 8        | 7        | 8        |
| MtMandjella     | 8       | 7        | 8        | 7        | 7        | 9        | 10       | 9        | 6        | 8        | 6        | 8        | 8        | 7        | 8        | 9        | 6        | 8        | 7        |
| FarinoUnio      | 9       | 8        | 10       | 9        | 10       | 9        | 8        | 9        | 9        | 8        | 9        | 9        | 9        | 8        | 9        | 8        | 9        | 9        | 8        |
| MTPanie         | 10      | 10       | 10       | 9        | 10       | 9        | 10       | 10       | 10       | 10       | 10       | 10       | 10       | 10       | 10       | 10       | 10       | 10       | 10       |
| AtoufTchingou   | 11      | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       | 11       |
| MTHumboldt      | 12      | 12       | 13       | 12       | 12       | 12       | 15       | 13       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       |
| MtKaala         | 13      | 14       | 14       | 13       | 14       | 14       | 15       | 14       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       |
| MtMou           | 14      | 13       | 12       | 14       | 13       | 14       | 15       | 14       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       | 15       |
| NinguForetSailles | 15   | 15       | 15       | 14       | 13       | 13       | 13       | 14       | 13       | 13       | 13       | 13       | 13       | 13       | 13       | 13       | 13       | 13       | 13       |
| ColRoussettes   | 16      | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       | 16       |
Table 3  Site ranks (based on *Ws ranks*) if a given phylogeny is dropped

|                | Drop... | Drop none |
|----------------|---------|-----------|
|                | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 none |
| GrandSud       | 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 |
| LaFoaCanala    | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| RiviereBleue   | 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 |
| MtsKoghis      | 4 5 4 4.5 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| MtsDzumac      | 11 12 10 12 12 12 11 9 11 10 12 12 12 11 11 10 11 11 |
| GrandNord      | 10 10 9 11 10 9 8 10 12 11 11 14 11 9 12 10 12 11 |
| MtAoupinie     | 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 |
| MtMandjelia    | 12 7 11 7 11 7 11 7 7 7 7 7 7 7 7 7 7 7 |
| FarinoUnio     | 5 4 6 4.5 4 4 5 3 4 5 5 4 5 4 5 4 4 4 4 |
| MtPanie        | 7.5 11 12 11 8.5 10 13 7 8 8.5 8.5 8.5 8.5 9.5 7.5 7.5 7.5 8 8.5 |
| AteouTchingou  | 7.5 8 7 8 8.5 8 9 11 12 8.5 8.5 8.5 9.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5 12 |
| MtHumboldt     | 13 13 13 13 13 13 13 13 13 13 13 13 13 13 13 13 13 13 13 13 |
| MtKaala        | 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 |
| MtMou          | 9 9 9 9 11 11 11 10 9 11 10 10 10 10 10 10 10 10 10 |
| NinguaForetSailles | 14 14 15 14 14 14 14 14 14 14 14 14 14 14 14 14 14 14 14 14 |
| ColRoussettes  | 16 16 16 16 16 16 16 16 16 16 16 16 16 16 16 16 16 16 16 16 |

Values in red and bold are ‘real’ drops, i.e. the dropped phylogeny was indeed present at the site. A: ranks based on standardised values. B: ranks based on not standardised values. For a key to the phylogenies see caption Table 1.
Fig. 6 Sum over all sites of absolute differences in site ranks (based on \( W_{s\ sum} \)) if the phylogeny (x axis) is dropped. The main figures shows the values standardised (= divided) by the number of phylogenies present at the site when a phylogeny is dropped. The small figure at the bottom shows the nonstandardised values.
Fig. 7 Sum over all sites of absolute differences in site ranks (based on \(W_s\) ranks) if the phylogeny (x axis) is dropped. The main figure shows the values standardised (= divided) by the number of phylogenies present at the site when a phylogeny is dropped. The small figure at the bottom shows the non-standardised values.
Resampling Multiple Phylogenies: How Stable Are the Results?

This resampling procedure is based on all possible combinations of phylogenies present at a site (from 1 to N, where N is the number of phylogenies with species in the site). Although there is (a) considerable overlap in the relative evolutionary divergence of sites in this resampling scheme and (b) the standard deviations are high, there are still some differences that emerge. For instance, when only 50% of the phylogenies are used in the resampling (n=9), the standard deviations of the top scoring sites do not overlap with those from the least phylogenetically diverse sites (Figs. 8 and 9). Thus with only nine phylogenies one can separate the four top scoring sites from the six least phylogenetically diverse ones when using Ws sum; and the two top sites and three bottom sites when Ws ranks are employed.

Consideration of Individual Sites

When the data set is evaluated using Ws sums, the site harbouring the greatest phylogenetic divergence is Grand Sud, and to a lesser degree La Foa Canala and Riviere Bleue. Grand Sud never drops in rank when individual phylogenies are dropped, and La Foa Canala and Riviere Bleue never below the 6th rank. The lower bound Ws (mean – SD) for Grand Sud still ranks 4th compared to the mean value of all others sites when all possible combinations of phylogenies are rarified to the smallest number of phylogenies present at any one site (n=5) (the lower bound Ws for La Foa Canala and Riviere Bleue drop to ranks 9 and 10). The lowest scoring site is Col des Roussettes, and low phylogenetic diversity is also found in Ningua Foret Sailles, Mt Mou, Mt Kaala, and Mt Humboldt. These sites never move above the 12th rank when individual phylogenies are dropped, and their upper bound (mean + SD) ranks in the lowest two thirds compared to the mean value of all other sites when all possible combinations of phylogenies are rarified to the smallest number of phylogenies present at any one site.

When the same data set is evaluated using Ws ranks (summing the scores for the first and second most divergent species for each phylogeny), the sites harbouring the greatest phylogenetic divergence are also La Foa Canala and Grand Sud. These sites never drop below the 3rd rank when individual phylogenies are dropped, and their lower bound (mean – SD) still ranks in the upper half compared to the mean value of all others sites when all possible combinations of phylogenies are rarified to the smallest number of phylogenies present at any one site (n=5). The lowest scoring sites are Col des Roussettes, Mt Kaala and Ningua Forest Sailles. These sites never move above the 13th rank when individual phylogenies are dropped, and their upper bound (mean + SD) ranks in the lowest quarter compared to the mean value of all other sites when all possible combinations of phylogenies are rarified to the smallest number of phylogenies present at any one site.
Fig. 8 Mean and standard deviation for the sites’ summed $W_s$ values, resampled over all possible combinations of phylogenies present at the given site. The main figure shows the values standardised (= divided) by the number of phylogenies present at the site. The small figure at the bottom shows the non-standardised values.
Fig. 9  Mean and standard deviation for the sites’ summed site scores ($W_s$ ranks), resampled over all possible combinations of phylogenies present at the given site. The main figures shows the values standardised (= divided) by the number of phylogenies present at the site. The small figure at the bottom shows the nonstandardised values.
Discussion

Methodological Considerations

Even using metrics based on the Ws, there are several ways of evaluating evolutionary distinctiveness. Ws gives information on the total distribution of evolutionary divergence in the entire data set. An advantage of this index is that each phylogeny has its scores scaled between 0 and 1 and thus phylogenetic diversity can be represented by many species with small values (from phylogenies with many species), or few species with large values (from phylogenies with few species). However, this feature also introduces a limitation. If there is high beta-diversity (differentiation among sites) in each phylogeny (e.g. if each species only occurs at a single site), then small phylogenies have the potential to dominate the ranking of individual sites (as the most divergent species in small phylogenies have higher Ws values than the most divergent species in large phylogenies). In contrast, if there is low beta-diversity, then phylogenies with many species will have many species at individual sites, and thus will be able to ‘compete’ with the smaller phylogenies by having Ws totals that reflect the sum of several co-occurring species. In this latter case (low beta-diversity), Ws will be strongly correlated with overall species richness of a phylogeny.

Using the sum of 1st and 2nd ranks circumvents these problems. The power of this metric is that it gets at a simple question – where are the most divergent two species from each phylogeny, summed across sites and phylogenies. The downside is that, of course, it does not include information from species below the 1st and 2nd ranks. Thus it is purely targeted at examining the distribution of phylogenetically basal species, rather than the total sum of phylogenetic diversity. This needs to be borne in mind in its interpretation.

Another promising application of Ws ranks is in the detection of places of recent diversification. This can be achieved by focusing on the inverse of the most phylogenetic divergent species as used here, i.e., through awarding first and second prizes for the most and second most recent species of the phylogeny. Likewise, the methods of standardization and rarefaction can be very helpful for dealing with diverse sampling protocols and identifying the influence of different phylogenies to the ranking. Although evolutionary potential is a factor that requires genetic studies to be formally tackled (see Mace and Purvis 2008; the analysis of Grandcolas and Trewick in chapter “What Is the Meaning of Extreme Phylogenetic Diversity? The Case of Phylogenetic Relict Species”), the identification of sites that accumulate species with recent diversification is a first step to set out future study projects and monitoring strategies for testing this hypothesis. So, the possibility of identifying these sites should not be neglected.

Both of these metrics can then be adjusted to focus on micro-endemics, by using the measure Wes from Posadas et al. (2001) and the approach of 1st and 2nd ranks of Wes as developed here for the Ws. Wes is simply the Ws divided by the number of sites (or any measure of spatial distribution) the species occurs. The use of Wes,
rather than Ws has the same issues with ‘sum’ versus 1st and 2nd ranks concepts as above. With Wes the Ws values are ‘diluted’ by being divided across each site that a species is recorded from and the main benefit is that sites will score more highly in proportion to the uniqueness of their species composition.

The resampling methods used here assure that ranking is not driven by a single or very small set of phylogenies, and the resampling with multiple drops indicates the tendency of sites remaining in similar ranking positions with the addition of phylogenies. To the best of our knowledge, this is the first time a set of phylogenetic studies are analysed this way (but see the proposition of Miranda-Esquivel, chapter “Support in Area Prioritization Using Phylogenetic Information”), and this seems to be a very promising way of integrating the problems of diversity of sampling effort.

Some Considerations About the Sites Prioritized

The results of both analyses put in evidence that a few sites – Grand Sud, La Foa-Canala and Rivière Bleue are always ranking high. This clearly documents that these sites harbour remarkable species from a phylogenetic point of view. If ever these sites would be affected by disturbances, some more original evolutionary history would be lost in New Caledonia. How does it fit the conservation planning in New Caledonia? This planning is rather opportunistic, with the definition of small protected areas with very different status and varied protection level. Given the amazing level of micro-endemicity, every mountain or river harbours a conspicuous number of endemics so that any prioritization is difficult even among different protected areas. In every province, communication or action emphasis is often put on emblematical and large and supposedly virgin forested areas out of mining priorities, such as Massif du Panié in the North, or Rivière Bleue in the South. Our results do not adjust perfectly with this situation. The three high-ranking sites are not all emblematical and targetted areas and the protected areas concerned have different status. Grand Sud and Rivière Bleue areas are including natural reserves with high protection level but a large part of these areas are also situated outside the reserves, potentially putting at risk some populations of endemics. These risks are also increased because of the metalliferous soils derived from ultramafic rocks that are widespread in these southern areas and which are potentially places for nickel mining. La Foa-Canala area is another with less direct disturbances but with reserves with lower protection level. The reserve of Col d’Amieu is a place for forest logging and traditional seasonal bat hunting and is generally not targeted as an emblematical area.

Therefore, a recommendation based on our analysis of phylogenetic diversity should consider that conservation planning in New Caledonia is modified in two ways. The small natural parks in the South should become larger or connect with several new reserves, and the Reserve du Col d’Amieu should be carefully considered with improvement of the protection level.
**Future Perspectives**

In this work we focused in one method already adjusted to deal with prioritization of areas based on the evolutionary distinctiveness, the $W_s$ (Posadas et al. 2001). The same procedure can be directly employed to any measure of evolutionary distinctiveness (ED), in which each species has a score related to its position in the phylogeny and the area ranks are assessed through the sum of the scores of the species occurring in it. So, it could be identically employed when using the EDGE or HEDGE measures, where ED is associated to threat status (Isaac et al. 2007; see also May-Collado et al. chapter “Global Spatial Analyses of Phylogenetic Conservation Priorities for Aquatic Mammals”), or in cases where ED is combined with geographical rarity, or with species abundance, as, for example, the AED from Cadotte and Davies (2010).

As shown by Faith et al. (2004) and Faith (chapter “The PD Phylogenetic Diversity Framework: Linking Evolutionary History to Feature Diversity for Biodiversity Conservation” this volume) PD could easily be used to assess site’s rank when using data from several phylogenies: in cases where phylogenies are based on different kinds of characters or method of analysis, PD can be employed on the simple basis of counting nodes. The great advantage is that PD (the sum of the minimum spanning path linking all the species in an area) is a group measure (see Hartman and Steel 2007) and takes in consideration the complementarity, which would result in avoiding redundancies. However, at the present state of knowledge the rarefaction as used here, or the standardization for number of phylogenies cannot be directly applied to group measures such as PD. As presented in the introduction of this chapter the rarefaction of PD is newly developed (Nipperess and Matsen 2013). Many solutions are designed in Nipperess’ (chapter “The Rarefaction of Phylogenetic Diversity: Formulation, Extension and Application”: the standardization of sampling effort; the calculation of phylogenetic evenness, phylogenetic beta diversity, and phylogenetic dispersion. So, an extension to the application of these solutions when using phylogenetic data from several phylogenies will complete this framework and provide more options about the measure to be employed.

Biodiversity conservation is a very complex issue, and conservation guidelines should take multiple variables in consideration. Ideally, the analysis should provide explicit information about the way each variable has been weighted and, as far as possible, a set of scenarios under different weights. In this perspective, complex frameworks for systematic conservation planning have been developed and are becoming to be employed more often. For example, the Zonation procedure (Moilanen 2007; Lehtomaki and Moilanen 2013) used by Arponen and Zupan (chapter “Representing Hotspots of Evolutionary History in Systematic Conservation Planning for European Mammals”), and the gap analysis (Ball and Possingham 2000) used in the study of Silvano et al. (chapter “Priorities for Conservation of the Evolutionary History of Amphibians in the Cerrado”). In these procedures, phylogenetic diversity is included as a weight along with other biological data like spe-
cies’ distribution area, threat status, or some economic variables, such as the cost for conservation.

Although the results presented in this study highly stand by themselves, they can also be integrated in this kind of analysis as weights according to site’s rank considering both Ws sums and Ws ranks amongst other variables. In this case, there is no doubt that the procedures conducted here will give a reliable picture of the phylogenetic distribution across this set of sites, and provide a better instrument to the conservation of the phylogenetic diversity.

To conclude, the analytical problems and need for the solutions outlined above will decrease as large-scale sequencing projects bring more directly comparable data together. However, until comprehensive and balanced sampling from common gene sets across taxa and sites are realized, the challenges of standardization, comparability and assessments of bias will remain relevant.

Acknowledgements This project was funded by the French Agency of Research, through the grant ANR Bioneocal to PG. We thank the authors of the phylogenies and in particular Ulf Swenson and Marianne Espeland, for giving access to their databases of species occurrences, and Hervé Jourdan (IRD Nouméa) for helping RP with the delimitation of the study areas. We also thank David Nipperess and Romain Nattier for their comments that helped to improve this manuscript.

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