DNA Barcoding to Enhance Conservation of Sunshine Coast Heathlands

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Abstract: Conservation priorities and decisions can be informed by understanding diversity patterns and the evolutionary history of ecosystems, and phylogenetic metrics can contribute to this. This project used a range of diversity metrics in concert to examine diversity patterns in the Sunshine Coast heathlands, an ecosystem under intense pressure. The species richness and composition of 80 heathland sites over nine regional ecosystems of heathland on the Sunshine Coast were enhanced with phylogenetic metrics, determined by barcoding 366 heath species of the region. The resulting data were added to an existing phylogeny of regional rainforest species. The diversity metrics for sites and regional ecosystems were compared using univariate and multivariate statistics. The phylogeny from this study, and the low phylogenetic diversity of the heathlands, is consistent with the theory that heath species evolved on the fringes on a wider Australian rainforest flora. Distinctive heathland communities were highlighted, and the existence of geographically scattered, but compositionally similar, phylogenetically even sites points to a possible “refugial environment”, characterised by moisture and instability. This suggests contrasting conservation implications: the protection of distinctive communities but also the management of the dynamic processes in other wet and alluvial “refugial environments”. The potential for more focused conservation priorities is enhanced.

Keywords: heathlands; phylogenetic diversity; barcoding; phylogeny; conservation; refugia

1. Introduction

Conservation obligations are an imperative with the Convention on Biological Diversity calling for the 30% of land and sea areas of signatory countries to be protected by 2030 [1]. Overall, Australia has achieved the Aichi target of 17% of land area conserved [2]. However, conservation based on area alone is insufficient to reduce biodiversity loss, with calls for the consideration of factors, including ecological representation, genetic diversity, connectivity, endangered ecosystems, and species [2–6]. We need to know more about diversity patterns in ecosystems we are aiming to protect in order to inform conservation decisions.

Species richness (SR) is a standard measure of diversity, but incorporating metrics, such as genetic diversity, species composition, centres of endemism, and phylogenetic diversity, are advocated for identifying priority areas of biodiversity now and into the future [7–14]. Species composition patterns can identify areas of distinctiveness [15–17]. Phylogenetic diversity has been considered a useful surrogate for a diversity of traits, which provide ongoing material for evolutionary processes [14,18,19]. Phylogenetic diversity metrics have also been used in determining centres of diversity and distinctiveness [20] and in planning for conservation gains [21,22].

In addition, broader diversity measures may provide insight into community assembly processes, contributing to conservation prioritization. Areas of distantly related species, or “phylogenetic evenness”, may indicate refugial areas of conservation significance, whilst...
areas of “phylogenetic clustering” could represent locations where species had been subject to environmental filtering, such as recently expanded communities [23,24]. Deep-past and biogeographical origins may impact on the observed phylogenetic structuring of communities [21,25–27].

However, phylogenetic metrics used in understanding community assembly may be used differently in conservation [14]. For example, low diversity “phylogenetically clustered” areas may suggest compelling and distinctive community assembly patterns, but these areas may then be viewed as unimportant for conservation. However, when considered together, patterns of diversity, including species richness and composition, along with phylogenetic diversity and structuring, could all contribute to understanding local ecological processes and history [28]. Can diversity metrics be used in concert to contribute to an understanding of the floristic history of a region and so inform the prioritization of conservation areas in a region? This study aimed to explore this question in a system under pressure in the south-east corner of Queensland, Australia.

For millennia, the heathlands of the Sunshine Coast have been a source of sustenance for indigenous people [29], as reflected in the many place names and indigenous words, such as “wallum” to describe Banksia aemula and associated plant communities [30,31]. The area is under development pressure as one of the fastest growing regions in Australia [32–34]. The heathlands are threatened by land clearing, forestry, urbanisation, inappropriate fire regimes, and climate change [35,36]. In the 1960s it was recognized that the heathland grew on low-nutrient soils, with terminology such as “depauperate” heath and sedge communities used to describe the “poverty” of these lowlands [36]. Indeed, it is theorised the Australian heath evolved in infertile, seasonally waterlogged soils on the fringes of rainforest, with sclerophyll being an adaptation to low nutrients [37–40]. Thus, it is predicted that the heathlands should have a lower phylogenetic diversity than the surrounding rainforests, but is this so? Previous phylogenetic work in the surrounding rainforests offers an opportunity to explore this [15–17,41].

Almost all heathland species in Australia are endemic to this continent and many are localised [35], including in the heaths of the south-east corner of Queensland [36,37,42]. A major centre of endemism and floristic distinctiveness, corresponding to the “wallum” landscapes, has been identified along this coastal fringe north of Brisbane [43]. This is echoed in the occurrences of other significant biota in these heaths, including the eastern ground parrot and the acid frogs [44,45]. These coastal heaths lie on a complex landscape of coastal sand plains and dunes (Holocene and Pleistocene), alluvium, decomposed sandstones, and volcanic basalts on hills and ranges [46,47]. Are the montane areas with heterogenous environments “phylogenetically even”, reflecting local genetic studies [48] and the broader ecological findings of increased diversity in topographical heterogenous areas [49–51]?

There remain areas of heathlands that still reflect the pre-European, or “pre-clearing” conditions. In Queensland, vegetation communities are classified into “regional ecosystems” (RE). This framework, instigated in 1999, is used by private and government land managers for biodiversity and conservation assessment and management [52]. On the Sunshine Coast, the variation in the heath communities is reflected in nine heathland regional ecosystems. All have a predominant sclerophyllous shrub layer with a restiad or sedge ground-layer, growing either on wet and waterlogged substrates or on dry sands or rocky peaks [53]. The regional ecosystem classification, along with the beginnings of a barcode library for the local heath flora, provides an opportunity to assess the patterns of diversity and distinctiveness and the conservation significance of the heathland types.

**Aims of This Study**

With a goal towards exploring conservation priorities, this research plans to quantify the diversity and the distinctiveness of the Sunshine coast heathlands and to specifically address the questions:
1. Is there variation in species richness, phylogenetic diversity, and composition among heath regional ecosystems of the Sunshine Coast, which may be important for assessing conservation priorities?

2. Is there any evidence in the diversity metrics to inform on the ecological and evolutionary history of the heaths on the Sunshine Coast?

3. Do the species composition and phylogenetic metrics provide insights into the community assembly dynamics of the heath; are the regional ecosystems operating as distinct and discreet communities or is there overlap in species composition?

4. Is there evidence of sites of “refugia” or centres of diversity where are these located, and do they warrant consideration of enhanced protection?

2. Materials and Methods

2.1. Design and Data Collection

For this study, the Sunshine Coast heathlands were defined as extending from Cooloola in the north, 150 km south to Bribie Island, and west up to 30 km to Mapleton and the Glasshouse Mountains. These heathlands fall within the council areas of Gympie, Noosa, Sunshine Coast, and Moreton Bay (Figure 1). The current extent and protection status of the heathlands is shown in Supplementary Figure S1.

The Queensland regional ecosystem framework is based on bioregions (broadscale landscape patterns), land zones (based on geology, soils and landforms), and vegetation (described in terms of structure and floristics) and are locally mapped at a scale of 1:50,000 [52]. To capture the diversity across the heath and to ensure the representativeness of heath types, sampling was undertaken of the mapped nine regional ecosystem (RE) heath types [53]. The Queensland Herbarium CORVEG database contained an existing dataset of thirteen sites from five regional ecosystems [54]. This dataset was expanded so that each regional ecosystem was sampled over at least six sites, although the limited extent of RE 12.12.10 meant that only three sites were sampled (Table 1). Sites were chosen which had been unaffected by fire for at least 12 months to reduce this confounding factor. There are also areas along the coast with special significance or were sites of “conservation battles” in the past [34], and some of these “iconic” areas were included to assess their diversity. To address the stratified sampling for other aspects of this project, a further 67 sites were sampled from across the Sunshine Coast study area, resulting in data for a total of 80 sites over nine regional ecosystems.

Site were selected using ArcGis 10.5: [55]: shape files of the Regional Ecosystem mapping of remnant vegetation for south-east Queensland [56] were overlaid with the Protected Area Status [57] to enable selection of sites within the Protected Area Estate. As site data were being added to the CORVEG database, collection methods were consistent with the Queensland Herbarium CORVEG techniques with a standard proforma and plot size of 1000 m² [58]. Data collection occurred during late winter, spring, and early summer, from July 2016 to December 2018. At each site, GPS bearings (10 m accuracy) were taken, and a complete native species list was recorded. The comprehensiveness of species lists was maximised by visiting each site twice, with at least one site visit aimed to be during the spring flowering peak [59]. Orchids were noted but not included in species lists, as their cryptic nature meant they were unable to be recorded consistently; likewise, ferns and bryophytes were noted but their inclusion was beyond the scope of this study. Species were identified on site using a field herbarium prepared and developed by comparing samples with Queensland Herbarium samples and by using field handbooks and keys [60,61]. Any species not clearly identified on site were collected for later verification by Queensland Herbarium botanists.
Table 1. Description and diversity metrics of the heathland regional ecosystems on the Sunshine Coast, Queensland.

| Regional Ecosystem | Short Description                                                                 | Biodiversity Status | No. Sites | SR     | PD     | MPD     | MNTD    | NRI    | NTI   |
|--------------------|-------------------------------------------------------------------------------------|---------------------|-----------|--------|--------|--------|--------|--------|-------|
| 12.2.9             | Banksia aemula low open woodland on dunes and sand plains, usually deeply leached soils | No concern at present | 12        | 121    | 3632.73 * | 208.48 | 32.94  | 0.63   | 2.91 *H |
| 12.2.12            | Closed heath on seasonally waterlogged sand plains                                   | Of concern          | 22        | 151    | 4258.21 * | 209.17 | 33.10  | 0.09   | 2.24 *H |
| 12.2.13            | Open or dry heath on dunes and beaches                                              | Endangered          | 6         | 61     | 2376.66 * | 203.59 | 45.79  | 2.41 C | 1.80 *H |
| 12.3.13            | Closed heathland on seasonally waterlogged alluvial plains usually near coast        | No concern at present | 7         | 89     | 3004.52 * | 210.71 | 38.66  | −0.76  | 2.36 *H |
| 12.3.14            | Banksia aemula low woodland on alluvial plains usually near coast                    | Of concern          | 7         | 95     | 3153.69 * | 209.56 | 39.56  | −0.16  | 1.94 *H |
| 12.5.9             | Sedgeland to heathland in low lying areas on complex of remnant Tertiary surface and Tertiary sedimentary rocks | Of concern          | 6         | 114    | 3564.05 * | 208.40 | 39.14  | 0.60   | 1.50   |
| 12.8.19            | Heath and rock pavement with scattered shrubs or open woodland on Cainozoic igneous hills and mountains | Of concern          | 9         | 106    | 3295.21 * | 207.88 | 36.92  | 0.88   | 2.36 *H |
| 12.9-10.22         | Closed sedgeland/shrubland on sedimentary rocks, generally coastal                   | Of concern          | 8         | 123    | 3849.04 * | 208.68 | 38.48  | 0.41   | 1.49   |
| 12.12.10           | Shrubland of rocky peaks on Mesozoic to Proterozoic igneous rocks                    | Of concern          | 3         | 61     | 2743.24  | 205.66 | 63.85  | 1.52   | −0.88  |

Regional Ecosystem, the species richness (SR), phylogenetic diversity (PD), mean phylogenetic distance (MPD), mean nearest taxon distance (MNTD), net relatedness index (NRI), and nearest taxon index (NTI) are given. Values that were significantly different from random ($p = 0.05$) are indicated with an asterisk. Superscript letters indicate whether these values are higher (*H) or lower (*L) than expected or whether they are significantly or clustered (*C). No Regional Ecosystems were significantly even. Biodiversity status is that given by Queensland Herbarium assessments in 2018 [53].

For each species, a herbarium voucher and DNA sample in silica consisting of approximately 5 g of healthy, fresh, clean leaf material (permit numbers WITK 17429716 and WITK 18628117) were collected with vouchers lodged at Queensland Herbarium or the University of the Sunshine Coast Herbarium. Taxonomy used was the same as for the Queensland Flora Census [62]. A total of 255 vouched DNA samples was collected.

A complete list of Sunshine Coast heath plants comprising 366 species was compiled for later analysis, which included the species encountered on site, along with other species listed in local guidebooks [60,61,63,64] and confirmed through the Australian Virtual Herbarium [65].
Figure 1. Map of Queensland [66], Australia indicating the Sunshine Coast study area. In the expanded box, the Sunshine Coast area showing the location of the 80 sites. Sites are marked by their NRI results: phylogenetically clustered, random, or even.
2.2. DNA Barcoding and Sequence Alignment

The set of silica dried samples used for this study comprised 255 samples and 130 samples from other collections made from a variety of previous collections undertaken by the Shapcott lab and held at the University the Sunshine Coast [16,17,67]. DNA was extracted from 385 samples following the methods used by Shapcott [17]. The PCR amplification and sequencing of three accepted plastid DNA barcode markers, rbcL, matK, and psbA-trnH, used established methods [68]. The PCR product was purified with ExoSap, and forward and reverse primers were used along with the Big Dye Terminator v3.1 cycle sequencing kit (ThermoFisher Scientific, Waltham, MA, USA) in a cycle sequence reaction to attach dyes in preparation for sequencing [68]. This was followed by a sephadex purification and rehydration with HiDi formamide to prepare samples for sequencing on an AB3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) at the University of the Sunshine Coast. Any unsuccessful samples were reprocessed. Contigs were made using the forward and reverse sequences in Geneious version 10.2.6 (Biomatters, Auckland, New Zealand) (https://www.geneious.com (accessed on 27 March 2022) and were edited for accuracy and checked for quality and length. Contigs were exported to consensus sequences under the following quality control guidelines: a HQ score of a minimum of 65%, a sequence length of a minimum of 300 base pairs, and a minimal number of ambiguous base calls. Alignments of rbcL were completed using MUSCLE and the matK alignment was performed using MAFFT, in Geneious. The psbA-trnH makers were aligned using SATe [69]. All alignments were examined and manually adjusted to correct for homologies. Preliminary Trees were constructed in Geneious 10.2.6 for each marker to check the phylogenetic placement of species and any species that were clearly incorrectly placed on the tree were discarded, either as being contaminated DNA or a misidentification. In rare instances, sequences of less than HQ 65%, or less than 300 base pairs, were kept where they were placed correctly on the phylogenetic tree and there was no alternative sequence to use. Some samples were sequenced again for one or more loci to improve quality. For each plant species, at least two makers were used to construct the “barcode”. Missing sequences were retrieved from the public database GENBANK (www.ncbi.nlm.nih.gov/genbank/ (accessed on 27 March 2022)). In the few instances where no markers were procured for the species, a congeneric was used.

To further improve the robustness of the phylogeny, the data for the 366 heath species were aligned with an existing dataset of south-east and central Queensland rainforest species using the same three gene markers [16]. The final alignments for rbcL, matK, and psbA-trnH were trimmed and concatenated to create a three gene alignment for the heath and rainforest species of south-east Queensland, resulting in a dataset for 1576 species.

2.3. Phylogenetic Reconstruction

To enforce taxonomic relationships based on the global dataset from the Angiosperm Phylogeny Group III [70], a constraint tree using the R20120829 tree was built in Phylomatic version 3 [71]. In Mesquite [72], the tree was edited so each family was a polytomy and that the barcode data could then be used to infer the relationships of the species below the level of family, consistent with the methods of Shapcott [17].

The 3-marker alignment for the 1576 heath and rainforest species and constraint tree were uploaded to the CIPRES portal [73], and the RAxML-HPC2onXSEDE tool was used with a mixed partition model to search for the best scoring ML tree. This was repeated for eight runs and the best tree with the smallest likelihood score was selected. This tree was rooted in Geneious and dated in PATHd8 [74], with the ages of nine orders found using fossil dates and an age for angiosperms of 250 mya after the methods of Shapcott [16,17] to produce a final dated tree with calibrated molecular branch lengths for the 1576 heath and rainforest species. This tree was pruned in PICANTE in R to produce a dated tree for the 366 regional heath species of the Sunshine Coast [75].
2.4. Diversity Measures and Analyses

Summary data of the numbers of species (SR), genera (GR), and families (FR) were generated for the Sunshine Coast heath community of 366 species. Each of the species from the Sunshine Coast heath taxa was represented by a barcode identifier displayed on the dated heath phylogeny and these were used to create community lists of species found in each regional ecosystem, based on the field work data. Species were assigned to each of the nine regional ecosystems on a presence or absence basis, to be able to make broad comparisons of the heath regional ecosystems in terms of diversity measures. Additionally, community lists were developed for each of the 80 sites on a presence or absence basis to enable diversity measures to be generated for each site, to be used to statistically investigate variation between sites grouped by Regional Ecosystems.

The dated phylogeny, the complete Sunshine Coast heath community file, the community lists for individual sites and the individual regional ecosystems were used to derive phylogenetic metrics, and all analyses used R software [76]. Phylogenetic diversity (PD), mean phylogenetic distance (MPD), and mean nearest taxon (MNTD) were calculated for each regional ecosystem, as well as for each site using PICANTE [24,75]. A randomised null model, using the whole Sunshine Coast heath taxa and shuffling the taxa labels across the tips of the phylogeny, was used to calculate the probability of the phylogenetic diversity measures deviating significantly from random distributions. PICANTE calculates a standardised size effect (ses) and this figure multiplied by $-1$ gives the net relatedness ness index (NRI) for MPD and a nearest taxon index (NTI) for MNTD. A NRI has a value of 0 for a completely random community, increases as the community becomes more clustered, and decreases as a community becomes more even, with the NTI following a similar pattern [23]. The NRI and NTI were tested for significance using a randomised null model in PICANTE [75]. All these diversity measures were obtained for each of the nine regional ecosystems and for each of the 80 sites.

Individual site diversity measures were used to test for differences in PD, SR, FR, GR, MNTD, MPD, NRI, and NTI as well as between structural data (maximum and minimum heights and percentage cover of vegetation layers) between sites grouped by regional ecosystems, using the Kruskal–Wallis test followed by a Dunn’s post hoc test with a Bonferroni correction in the “stats” package and PMCNR packages [77]. Relationships between SR, GR, FR, and PD were tested using Spearman’s rank correlation tests in the “stats” package [76]. The significance of the observed frequency of phylogenetically even and phylogenetically clustered sites in each regional ecosystem was tested using Pearson’s chi-squared test in the gmodels package [78].

To investigate patterns and similarities among site communities, the presence/absence matrices of species composition were used to calculate pairwise dissimilarity matrices using Végdist and the Bray-Curtis method in the Vegan package [79]. A dissimilarity matrix between sites was calculated based on PD using Unifrac, a measure of phylogenetic distance between sites, within PICANTE [80,81].

These distance matrices were used in non-metric multidimensional scaling (NMDS) to visualise relationships among sites using Vegan [79]. Northings and eastings data for each site were used to calculate geographic distance matrices using Végdist and the euclidian method in Vegan. All the dissimilarity matrices were tested for correlation using the Mantel test and the Spearman method in Vegan [82].

To visualise and assist the interpretation of the regional ecosystem data, labelled phylogenetic trees were produced using the iTOL program [83].

3. Results

3.1. Phylogenetic Position

The Sunshine Coast heath taxa list comprised 366 species, excluding ferns and orchids, covering 26 orders, 73 families, and 201 genera. Ten of these families contained 10 or more species, and included Myrtaceae (42 species), Cyperaceae (34 species), Poaceae (29 species), Fabaceae (27 species), Ericaceae (22 species), Mimosaceae and Proteaceae (19 species each),
Restionaceae and Rutaceae (12 species each), and Laxmanniaceae (10 species). Over the 80 sites of data collection, 280 species were encountered, which represented 76.6% of the Sunshine coast heath taxa of 366 species. These 280 species came from 157 genera and 56 families, with some of the families more frequently encountered on sites than others; Myrtaceae, Cyperaceae, Proteaceae, Xanthorrhoeaceae, and Fabaceae were each encountered on more than 70 of the 80 sites.

Whilst the south-east and central Queensland rainforest and heath species share some orders, the heath species are not represented by any unique orders. The heath taxa of 366 species are grouped within the larger south-east Queensland and central Queensland rainforest and heath phylogeny (Figure 2). Some orders, such as the Magnoliales, Pandanales, and Piperales, are not represented within the heath, many orders were poorly represented, including the Laurales, Solanales, and the Sapindales, whilst orders such as the Poales (including the families Restionaceae and Cyperaceae) and the Ericales are richly represented.

Figure 2. Phylogenetic position of the Sunshine Coast heath species (indicated in red) within the wider south-east Queensland and central Queensland rainforest phylogeny.
3.2. Species Richness and Phylogenetic Diversity Metrics

While the total combined species richness of the regional ecosystems ranged from 61 for RE 12.12.10 (3 sites) to 151 species for RE 12.2.12 (22 sites in total), reflecting in part the differential sampling across regional ecosystems, the mean species richness per site for each regional ecosystem varied from 28.7 to 43.3 species/1000 m$^2$ and was not significantly different (Table 2). The phylogenetic diversity of the individual nine regional ecosystems ranged from 2376 to 4258, with a total PD for the Sunshine Coast heath taxa of 8156 (Table 1). All regional ecosystems had a lower PD than expected in the context of the Sunshine Coast heath regional species pool ($p < 0.05$), except for the shrublands of rocky peaks, RE 12.12.10 (Table 1). The endangered dry open heath on dunes, RE 12.2.13 was the only heath type identified as “phylogenetically clustered” with a significant NRI ($p < 0.05$), with all other regional ecosystems identified as “phylogenetically random” (Table 1, Figure 3). Six of the nine regional ecosystems had a mean nearest taxon index (NTI) higher than expected by chance ($p < 0.05$), meaning that the species in these communities are more closely related at the terminal nodes (Table 1). Furthermore, when sites were grouped by regional ecosystems and compared, the NRI was found to be significantly higher (more clustered) in the heaths compared to the alluvial closed heath: the NRI in RE 12.2.9 and in RE 12.2.13 were higher compared with RE 12.3.13 (Kruskal Wallis chi-squared = 20.912, $p = 0.0074$) (Table 2). There were no other significant differences in PD, MPD, MNTD and NTI, SR, GR, or FR between sites grouped by regional ecosystem (Table 2).

### Table 2. Summary of mean diversity values of the Sunshine Coast heath sites grouped by regional ecosystem.

| RE     | No. Site | SR     | GR     | FR     | PD       | MPD     | MNTD    | NRI     | NTI     |
|--------|-----------|--------|--------|--------|----------|---------|---------|---------|---------|
| 12.12.10 | 3         | 30.3 (4.2) | 27.0 (3.7) | 18.3 (3.1) | 1616.9 (126.7) | 206.8 (0.7) | 72.3 (7.5) | 0.61 (0.20) | ab 0.48 (0.51) |
| 12.2.12 | 22        | 34.5 (12.1) | 30.7 (10.0) | 17.5 (5.0) | 1754.6 (402.0) | 211.1 (3.8) | 68.7 (14.5) | −0.45 (0.94) | ab 0.67 (0.64) |
| 12.2.13 | 6         | 36.3 (2.7) | 32.0 (1.4) | 17.8 (1.0) | 1771.3 (71.7) | 206.0 (2.2) | 56.1 (6.5) | 0.87 (0.60) | a 1.48 (0.52) |
| 12.2.9  | 12        | 30.1 (11.2) | 26.1 (9.2) | 15.5 (4.3) | 1570.0 (390.6) | 205.5 (6.9) | 70.8 (25.9) | 0.92 (1.29) | a 0.99 (0.77) |
| 12.3.13 | 7         | 31.0 (7.9) | 27.7 (6.7) | 17.0 (3.7) | 1652.2 (344.6) | 213.5 (2.6) | 66.2 (9.3) | −1.08 (0.74) | b 0.90 (0.89) |
| 12.3.14 | 7         | 28.7 (4.3) | 26.4 (4.0) | 14.9 (2.9) | 1464.3 (251.3) | 208.3 (8.4) | 60.4 (15.5) | 0.09 (1.79) | a 1.47 (1.15) |
| 12.5.9  | 6         | 43.3 (7.0) | 37.3 (5.8) | 21.2 (3.8) | 1923.3 (302.0) | 208.2 (5.2) | 55.2 (5.8) | 0.41 (1.62) | ab 1.28 (0.79) |
| 12.8.19 | 9         | 31.2 (16.5) | 27.9 (14.2) | 16.7 (8.7) | 1564.9 (756.5) | 206.6 (9.6) | 61.9 (9.3) | −0.09 (1.34) | ab 1.24 (1.10) |
| 12.9-10.22 | 8     | 42.5 (12.3) | 37.1 (11.2) | 21.0 (5.9) | 1991.1 (514.2) | 208.9 (5.1) | 62.9 (10.2) | −0.12 (1.05) | ab 0.48 (1.16) |

For each regional ecosystem (RE), the species richness (SR), genus richness (GR), family richness (FR), phylogenetic diversity (PD), mean phylogenetic distance (MPD), mean nearest taxon distance (MNTD), net relatedness index (NRI), and nearest taxon index (NTI) are given. Values that were significantly different in Kruskal–Wallis tests ($p < 0.05$) are indicated with letters. Means sharing the same letter are not significantly different. Please note, the higher the NRI or NTI, the more closely the taxa in the community are related. Standard deviations are shown in brackets.

Across the 80 sites, diversity, as measured by species richness, varied widely, from 6–58 species per 1000 m$^2$, whilst genus richness ranged from 6–51 genera per 1000 m$^2$, suggesting few instances of congeneric species in each site (Table 3). Indeed, species richness was positively correlated with genus richness (Spearman rho = 0.986, $p < 0.001$) and family richness (Spearman rho = 0.9256, $p < 0.001$) as well as PD (Spearman rho = 0.994, $p < 0.001$). Moreover, 19 of the individual sites had a lower PD than expected by chance: across the individual 80 sites, PD ranged from 443 to 2585, with no sites having a higher PD than expected ($p < 0.05$) (Table 3). The NTI showed that 17 sites contained species significantly more clustered on the terminal nodes than expected by chance ($p < 0.05$) (Table 3). Based on the NRI, seven of the 80 sites were “phylogenetically even” ($p < 0.05$), and these sites were found in RE 12.2.12, 12.3.13, and 12.3.14, ecosystems of alluvial or seasonally waterlogged areas (Table 3). Six sites were “phylogenetically clustered” ($p < 0.05$), and both these “clustered” and “even” sites were scattered across the Sunshine
Coast (Figure 1). Phylogenetically clustered sites were found in regional ecosystems 12.2.9, 12.3.14, 12.8.19, and 12.5.9, with the Banksia aemula heath of the dunes (12.2.9) found to have more phylogenetically clustered sites than expected by chance, and the closed heathland on alluvial plains (12.3.13) found to contain more phylogenetically even sites than expected ($\chi^2 = 28.76, p = 0.026$).

Figure 3. Phylogenetic position of the Regional Ecosystems represented against the phylogenetic tree of the Sunshine Coast heath site taxa.

Table 3. Phylogenetic metrics of each of the 80 study sites, grouped by regional ecosystem.

| Regional Ecosystem | Site | SR  | GR  | FR  | PD    | MPD | MNTD | NRI  | NTI  |
|--------------------|------|-----|-----|-----|-------|-----|------|------|------|
| **RE 12.2.9**      |      |     |     |     |       |     |      |      |      |
| **Banksia aemula, low open woodland on dunes and sand plains, usually deeply leached soils** |      |     |     |     |       |     |      |      |      |
| 15248              | 34   | 32  | 19  | 1866.9 | 212.0 | 69.1 | −0.72 | 0.41 |
| 15250              | 9    | 9   | 8   | 824.5  | 214.5 | 143.7 | −0.41 | −0.7 |
| 15621              | 20   | 17  | 9   | 1061.0 | 189.5 | 60.9  | 3.56  | 1.90 |
| 16488              | 28   | 25  | 16  | 1687.7 | 207.2 | 76.7  | 0.42  | 0.27 |
| 16491              | 25   | 22  | 13  | 1433.6 | 211.8 | 75.0  | −0.49 | 0.65 |
| BI29-63            | 37   | 33  | 18  | 1774.9 | 208.9 | 58.9  | 0.13  | 1.12 |
| BI29-65            | 16   | 15  | 11  | 1122.2 | 197.5 | 91.2  | 1.8   | 0.51 |
Table 3. Cont.

| Regional Ecosystem | Site         | SR | GR | FR | PD   | MPD | MNTD | NRI | NTI |
|--------------------|--------------|----|----|----|------|-----|------|-----|-----|
| RE 12.2.12         |              |    |    |    |      |     |      |     |     |
| Closed heath on seasonally waterlogged sand plains | COO29-44 | 40 | 34 | 19 | 1817.0 $^{*L}$ | 200.9 | 52.7 | 2.54 $^{*C}$ | 1.68 |
|                    | COO29-62 | 47 | 40 | 20 | 2081.9 | 205.9 | 49.9 | 1.11 | 1.69 $^{*H}$ |
|                    | LCOO29-43 | 40 | 34 | 19 | 1881.3 | 204.5 | 56.3 | 1.46 | 1.25 |
|                    | MCNP29-60 | 28 | 23 | 15 | 1458.6 | 207.4 | 61.0 | 0.5  | 1.49 |
|                    | NNP29-61 | 37 | 29 | 15 | 1830.7 | 205.7 | 54.2 | 1.13 | 1.63 $^{*H}$ |
| RE 12.2.13         |              |    |    |    |      |     |      |     |     |
| Open or dry heath on dunes and beaches | MHD213-3 | 32 | 30 | 17 | 1761.5 | 208.8 | 64.0 | 0.09 | 0.98 |
|                    | MHD213-35 | 38 | 34 | 19 | 1846.3 | 208.8 | 54.6 | 0.14 | 1.51 |
|                    | NNP213-13 | 38 | 32 | 17 | 1686.3 $^{*L}$ | 205.4 | 45.9 | 1.04 | 2.35 $^{*H}$ |
|                    | NNP213-14 | 38 | 33 | 18 | 1764.1 | 204.1 | 52.6 | 1.43 | 1.78 $^{*H}$ |
|                    | NNP213-32 | 34 | 32 | 17 | 1706.3 | 203.8 | 60.9 | 1.34 | 1.15 |
|                    | NNP213-47 | 38 | 31 | 19 | 1836.2 | 205.0 | 58.6 | 1.19 | 1.1  |
| RE 12.3.13         |              |    |    |    |      |     |      |     |     |
| Closed heathland on seasonally waterlogged alluvial plains, usually near coast | 16454 | 22 | 22 | 14 | 1316.3 | 211.3 | 69.1 | -0.38 | 1.32 |
|                    | BSA313-4 | 29 | 26 | 17 | 1588.2 | 214.9 | 73.3 | -1.25 | 0.43 |
|                    | COO313-17 | 25 | 21 | 13 | 1277.7 $^{*L}$ | 208.6 | 53.8 | 0.19 | 2.22 $^{*H}$ |
|                    | MRNP313-16 | 35 | 33 | 21 | 1939.0 | 215.3 | 74.0 | -1.63 $^{*E}$ | -0.14 |
|                    | MRNP313-59 | 26 | 22 | 13 | 1380.0 | 213.8 | 66.4 | -1.03 | 1.17 |
|                    | PV313-37 | 35 | 32 | 21 | 1959.8 | 215.3 | 74.1 | -1.60 $^{*H}$ | -0.15 |
|                    | TNP313-36 | 45 | 38 | 20 | 2104.3 | 215.2 | 52.8 | -1.85 $^{*H}$ | 1.49 |
| RE 12.3.14         |              |    |    |    |      |     |      |     |     |
| Banksia aemula, low woodland on alluvial plains, usually near coast | 15622 | 24 | 22 | 12 | 1405.8 | 196.6 | 81.7 | 2.65 $^{*C}$ | 0.21 |
|                    | BSA314-38 | 28 | 28 | 17 | 1610.3 | 215.9 | 76.0 | -1.53 $^{*E}$ | 0.27 |
|                    | BSA314-6 | 31 | 29 | 17 | 1702.5 | 215.6 | 66.0 | -1.54 $^{*E}$ | 0.9  |
|                    | BSA314-8 | 28 | 24 | 14 | 1385.5 $^{*L}$ | 215.2 | 48.0 | -1.37 $^{*H}$ | 2.38 $^{*H}$ |
|                    | MRNP314-15 | 23 | 21 | 10 | 962.8 $^{*L}$ | 197.3 | 38.0 | 2.27 $^{*C}$ | 3.34 $^{*H}$ |
|                    | MRNP314-57 | 35 | 31 | 17 | 1530.9 $^{*L}$ | 206.7 | 52.5 | 0.63 | 1.89 $^{*H}$ |
|                    | MRNP314-58 | 32 | 30 | 17 | 1652.0 | 211.1 | 60.9 | -0.45 | 1.28 |
| 12.5.9             |              |    |    |    |      |     |      |     |     |
| Sedgeland to heathland in low lying areas on complex of remnant Tertiary surface and Tertiary sedimentary rocks | COO59-19 | 46 | 40 | 23 | 2094.7 | 209.3 | 57.7 | 0.05 | 0.89 |
|                    | COO59-25 | 39 | 36 | 20 | 1891.7 | 212.6 | 59.7 | 0.97 | 1.01 |
|                    | COO59-26 | 32 | 28 | 16 | 1499.8 $^{*L}$ | 212.1 | 54.4 | -0.69 | 1.89 $^{*H}$ |
|                    | COO59-56 | 51 | 45 | 26 | 2264.9 | 208.7 | 59.0 | 0.19 | 0.37 |
|                    | LC59-37 | 49 | 40 | 24 | 2147.3 | 208.1 | 55.7 | 0.34 | 0.96 |
|                    | LC59-48 | 43 | 36 | 18 | 1641.3 $^{*L}$ | 198.3 | 44.2 | 3.55 $^{*C}$ | 2.54 $^{*H}$ |
### Table 3. Cont.

| Regional Ecosystem | Site          | SR | GR | FR | PD  | MPD     | MNTD    | NRI | NTI |
|--------------------|---------------|----|----|----|-----|---------|---------|-----|-----|
| 12.8.19 Heath and rock pavement with scattered shrubs or open woodland on Cainozoic igneous hills and mountains | 13962 | 6   | 6   | 4   | 443.7  | 203.2 | 66.1 | 0.34 | 2.46 eH |
|                    | MB1981-12     | 12  | 11  | 7   | 721.5  | 197.7 | 74.0 | 1.22 | 1.73 eH |
|                    | MNP819-40     | 44  | 38  | 23  | 2322.4 | 213.6 | 68.9 | −1.35 | −0.32 |
|                    | MNP819-41     | 38  | 33  | 20  | 1916.6 | 213.4 | 62.2 | −1.14 | 0.73 |
|                    | ME212-9       | 45  | 41  | 24  | 2121.7 | 211.6 | 58.4 | −0.76 | 0.84 |
|                    | ME819-1       | 34  | 31  | 21  | 1824.7 | 213.4 | 72.1 | −1.07 | 0.13 |
|                    | ME819-11      | 42  | 37  | 21  | 1961.2 | 212.5 | 52.4 | −1    | 1.66 |
|                    | ME819-30      | 48  | 42  | 25  | 2286.6 | 208.2 | 56.6 | 0.35  | 0.84 |
|                    | WHM819-9      | 12  | 12  | 5   | 573.8  | 185.4 | 46.4 | 2.60 eL | 3.11 eH |
| 12.9-10.22 Closed sedgeland/shrubland on sedimentary rocks, generally coastal | COO910-20     | 49  | 45  | 20  | 1975.0  | 210.7 | 46.9 | −0.48 | 1.97 eH |
|                    | COO910-54     | 45  | 40  | 23  | 2087.8 | 205.2 | 64.8 | 1.32  | 0.03 |
|                    | COO910-55     | 50  | 44  | 26  | 2458.4 | 211.8 | 69.3 | −0.94 | −0.88 |
|                    | COO910-70     | 44  | 41  | 22  | 2025.4 | 210.1 | 60.0 | −0.3  | 0.67 |
|                    | ES910-33      | 53  | 41  | 27  | 2402.4 | 210.2 | 60.5 | −0.4  | 0.08 |
|                    | ES910-71      | 50  | 44  | 23  | 2255.8 | 210.9 | 59.8 | −0.59 | 0.37 |
|                    | NNS910-34     | 16  | 12  | 8   | 818.2   | 198.1 | 59.6 | 1.67  | 2.34 eH |
|                    | NNS910-72     | 33  | 30  | 19  | 1906.3 | 214.5 | 82.6 | −1.29 | −0.74 |
| 12.12.10 Shrubland of rocky peaks on Mesozoic to Proterozoic igneous rocks | MNPI2100-30   | 27  | 25  | 15  | 1483.3 | 206.2 | 69.9 | 0.69  | 0.91 |
|                    | MNPI2100-66   | 29  | 25  | 19  | 1632.1 | 207.7 | 80.7 | 0.38  | −0.08 |
|                    | SP1210-31     | 35  | 30  | 21  | 1735.4 | 206.6 | 66.3 | 0.76  | 0.61 |

For each site, the species richness (SR), genus richness (GR), family richness (FR), phylogenetic diversity (PD), mean phylogenetic distance (MPD), mean nearest taxon distance (MNTD), net relatedness index (NRI), and nearest taxon index (NTI) are given. Values that were significantly different from random (p = 0.05) are indicated with an asterisk. Letters indicate whether these values are higher (*H*) or lower (*L*) than expected or whether they are significantly even (*E*) or clustered (*C*).

### 3.3. Species and Phylogenetic Composition

Sunshine Coast heath sites varied in their species composition with some groupings based on regional ecosystems apparent in the NMDS analysis (Figure 4). The heaths of the old volcanic, Mesozoic to Proterozoic rocky peaks (RE 12.12.10) displayed similarities in species composition between sites. In contrast, the heaths of the rocky pavements of Cainozoic igneous rocks, RE 12.8.19, were divided in two, with the Glasshouse Mountains clustering as a group and the sites further north on Mt Coolum and Mt Emu forming a separate grouping (Figure 4). There were strong similarities in species composition between the heaths of the dry dunes, RE 12.2.13, and RE12.2.9, whilst the remainder of the heath types, which are heathlands of alluvial or seasonally waterlogged areas, showed considerable overlap in species composition (Figure 4). Phylogenetic composition showed a similar patterning to the vegetation composition, but it is striking that the heath of the dry dunes, RE 12.2.13 and 12.2.9, appears more phylogenetically similar to the heaths of the dry rocky peaks, 12.12.10 and 12.8.19, than do the other heath types (Figure 4). Mantel tests indicated a significant strong and positive correlation (r = 0.8563, p < 0.001) between species composition and phylogenetic distance, whereas geographic distance between sites was very weakly correlated with phylogenetic distance (r = 0.1319, p = 0.006) and species composition (r = 0.1284, p = 0.006).

Whilst the “even” and “clustered” sites were scattered across the region (Figure 1), the even sites showed distinct similarities in terms of species composition and phylogenetic distance (Figure 4).
Figure 4. Comparison of non-metric multidimensional scaling (NMDS) analyses for comparing relationships among 80 Sunshine Coast heathland sites. Vegetation composition using Bray–Curtis dissimilarity is shown on the (left) and phylogenetic UniFrac dissimilarity is on the (right). Sites have been coloured by their regional ecosystems (above) and by their NRI significance (below).

4. Discussion

4.1. Value of a Range of Diversity Metrics

Phylogenetic and species composition metrics from this study have provided deeper insight into the diversity and composition of the Sunshine Coast heathlands than species richness alone. This has resulted in a more nuanced characterization of community distinctiveness and ongoing ecological processes [84]. In this study, no regional ecosystem on the Sunshine Coast was significantly different in terms of species richness, family richness, genus richness, or phylogenetic diversity. It was expected from earlier studies in heath over south-eastern Australia that the wetter alluvial regional ecosystems of heath would be significantly lower in species richness than the drier heath regional ecosystems [37,85,86], raising the contention that these wetter systems are not a priority for conservation. However, in some coastal heath regions of New South Wales, no species richness differences were found between wet and dry heaths but, as was found in this study, compositional differences were apparent [87]. So, what do the compositional and phylogenetic data from this study suggest?

4.2. Data Consistent with Theory of Evolutionary History of Heath Flora

The phylogenetic tree from this study, and the lack of unique orders within the heath, is consistent with the theory that sclerophyll and heath vegetation evolved in the low nutrient and/or waterlogged areas within the extensive Australian Mesozoic rainforest [37]. Phylogenetic data in other studies, as well as the fossil record, have provided support for rainforest being the ancestral state of Australian flora, with the sclerophyllous component becoming more common after the Oligocene, with increasing climatic variability, seasonality, and aridity [38,88,89]. This resulted in the radiation of sclerophyll flora, including the Myrtaceae, Proteaceae, Ericaceae, and Casuarinaceae [90,91], all typical components of the heath flora. The overall phylogenetic diversity of the heath is considerably lower than
found in studies of corresponding rainforest areas [16,17,41]. All the heath ecosystems, except one, had a low phylogenetic diversity, yet they also contained ancient lineages such as Proteaceae and Restionaceae. These data support the possible impact of deep-past processes on the phylogenetic patterns seen in the heath; it may not only be a result of present-day assembly processes, such as environmental filtering [26]. The NTI for each regional ecosystem showed a stronger (higher) clustering than the NRI, which has been suggested as indicating more recent diversification events [92], further supporting recent speciation in the heath. This has implications in terms of conservation, as maximizing phylogenetic diversity alone, without historical interpretation, can have negative outcomes [27].

4.3. Phylogenetic Clustering

The Sunshine Cast heath data found phylogenetic clustering (a high NRI) on one of the nine regional ecosystems, in the dry, high, wind-buffeted, leached sand dunes, and along with a high NTI (clustered), which provides evidence for single clusters of taxa on the species pools, as opposed to several clusters evenly distributed around the tree [93]. This clustering, also found in global dune plant communities, leads to the conclusion that environmental filtering is at play [92]. This is supported by this regional ecosystem (RE 12.2.13) having a distinct community composition but overlapping in composition with a structurally taller system (RE 12.2.9), both growing in highly nutrient leached sands [94]. No other heath regional ecosystems were found to have a clustered NRI, but six had a clustered NTI, suggesting several clusters evenly distributed around the tree [93]. Phylogenetic clustering, due to the presence of closely related species, could indicate environmental filtering [23] and may be disadvantageous in terms of species competition. Yet, in long term heath studies in New South Wales, phylogenetic clustering became more pronounced over time since fire, in contrast to the expectation that increased competition would inhibit the coexistence of species with high niche overlap [95]. Furthermore, it is argued that closely related species may not necessarily compete more closely than distantly related ones, that filtering may not be the dominant mechanism, and that facilitative interactions may play a part in this [14,96,97]. The co-occurrence of closely related species may be beneficial, for example, when facilitated by mutualistic exchange, such as through mycorrhizal symbiosis shared by co-occurring members of a clade, increasing the host’s tolerance to environmental change [98]. Mycorrhizal strategies have been suggested as drivers of heath assemblages in Western Australia and Brazil, with well-known strategies used by the distinctive heath families Ericaceae, Orchidaceae, and Myrtaceae [99,100]. For the Sunshine Coast heaths, whose phylogeny is consistent with the general theory of sclerophyll and heath derivation, there are indicators that environmental filtering is a driver of their assembly but hints that facilitation and mutualism may be factors contributing to their community assembly and their general pattern of phylogenetic clustering.

4.4. Community Assembly

The diversity and composition patterns of the Sunshine Coast heaths are also intriguing against the background of contrasting theories of communities moving towards a climax assemblage [101], as opposed to random, temporary, and fluctuating assemblages of individual species responding to stochastic events [102]. Furthermore, this is a rejection of these two extreme views: ecosystem constraints and individual variability constantly interact, with the relative importance of each unsolved [103]. In the Sunshine Coast heaths this study found some distinct compositional communities as in the Glasshouse Mountains (RE 12.8.19), the montane heaths of the old volcanics (RE 12.12.10), and the dry sands (RE 12.2.9 and RE 12.2.13), with each of these communities also having their own unique species. However, this study also found the overlapping composition of the other five ecosystems, all found on the wetter sands, sediments, and alluviums. In this context, the observations of an early ecologist ring true—that the “wallum” vegetation of the coastal lowlands are a dynamic ecosystem with no climax and are subject to continual disturbance in the form of fire, wind, cyclones, periodic drought, storms, and flooding [36]. The vegeta-
tion was described as a continuum, as an oscillation of ecotones between floristic reservoirs, and consisting of flora of predominantly wide potential environment, with an infusion of species with much narrower environmental requirements [36]. Likewise, heaths of the Sydney sandstones have been described as dynamic “mosaics” over time [104] and that for Australian heathlands in general it is difficult to designate any one species as dominant, with proportions changing with microhabitat [105]. This mix of overlapping regional ecosystems in the wet and alluvial areas appears to be a dynamic system, where there is movement of species within the constantly changing environment, subject to extremes of moisture, fire, wind, and drainage. In terms of long-term conservation, it suggests the facilitation of this movement may be essential, and connectivity between these protected areas needs to be considered.

4.5. A Refugial Environment?

The phylogenetically even sites were all located in the wet and alluvial ecosystems, and despite being scattered across the coast, were similar in terms of species composition and phylogenetic distance. Conservation priorities have focused on refugial areas, which have facilitated survival of biota for millennia and are likely to do so into the future [106–109]. Phylogenetically even sites may be indicative of refugial areas [17,110], although it is essential to be mindful of historical evolutionary processes [12,27]. Moisture has been a factor that contributes to sites acting as refugia, with dated core samples from the wetlands of nearby Stradbroke Island suggesting the wetlands have acted as refugia from regional drying for over the last 100,000 years [111]. Waterways, mesic habitats, and riparian areas have been linked to relictual taxa [49,89,112–114]. Refugia act as buffers to extreme conditions, and it is important to understand the evolutionary history of sites and the processes being protected by them. It could be argued that these wet and alluvial heath areas, with overlapping composition, are dynamic “refugial environments” and conserving these areas is protecting these ongoing processes of change. It is possible that they are not so much fixed in space as reflecting the concept of “shifting refuges”, driven partly by stochastic events [115].

5. Conclusions

The Sunshine Coast heathlands are ecosystems under pressure from development, and this study aimed to use diversity measures, in concert, to inform conservation priorities. The phylogeny from this study is consistent with the theory that heath evolved on the fringes of a wider Australian rainforest flora, with the phylogenetic diversity being low in comparison with the surrounding rainforest flora. Whilst the heath regional ecosystems on the Sunshine Coast could not be discriminated by SR, GR, FR, or PD, composition and NRI highlighted the distinctive heath communities of the coastal high dunes and the montane areas. The wet and alluvial heaths of the coastal lowlands displayed an overlapping composition but also contained the phylogenetically even sites, possibly pointing to a “refugial environment” characterised by stochastic events and instability. This suggests contrasting conservation implications: the conservation of distinctive communities but also conservation of the dynamic processes in the wet and alluvial “refugial environments”. With this enhanced understanding, further examination of the remnant extent and protection status of each regional ecosystem of heath is warranted.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/d14060436/s1. Figure S1: Map of the study area showing the areas of heath that have been cleared (in black), the areas of heath currently protected in National Parks (in green), and the remaining areas of heath (in red) tenured privately, or in lower levels of protection. The large area of National Park at the top of the map is the Cooloola section of the Great Sandy National Park. The major population centers are in the mid to southern coastal sections of the map.
Author Contributions: Conceptualization, H.P. and A.S.; methodology, A.S., T.R., H.P., M.H. and Y.S.; investigation, H.P. and M.H.; software, A.S., H.P., M.H. and Y.S.; formal analysis, H.P. and A.S.; resources, H.P.; A.S. and T.R.; data curation, H.P., T.R., M.H. and A.S.; writing—original draft preparation, H.P.; writing—review and editing, H.P., A.S., T.R. and Y.S.; supervision, A.S., T.R. and Y.S.; project administration, A.S. and H.P.; funding acquisition, H.P. and A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Holsworth Wildlife Research Endowment—Equity Trustees Charitable Foundation & the Ecological Society of Australia, and also by the the Friends of the Maroochy Bushland Botanic Garden. Specimen collection and fieldwork on protected areas was conducted with permit numbers WITK 17429716 and WITK 18628117 issued by the Department of Environment and Heritage Protection, Queensland.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Site data in this study are openly available in the Queensland Herbarium CORVEG database [54]. Voucher specimens are stored at Queensland Herbarium and the University of the Sunshine Coast herbarium. All other data are held in the University of the Sunshine Coast research repository and can be made available following publication of the PhD thesis of the lead author.

Acknowledgments: Queensland Herbarium curators and technical staff are thanked for their assistance. The fieldwork assistance of Ann Moran and Allan Ward was deeply appreciated. Rachele Wilson, Brittany Elliott, and USC laboratory and technical staff are thanked for their training and support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

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