RESEARCH ARTICLE

An Ancient Divide in a Contiguous Rainforest: Endemic Earthworms in the Australian Wet Tropics

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Abstract

Understanding the factors that shape current species diversity is a fundamental aim of ecology and evolutionary biology. The Australian Wet Tropics (AWT) are a system in which much is known about how the rainforests and the rainforest-dependent organisms reacted to late Pleistocene climate changes, but less is known about how events deeper in time shaped speciation and extinction in this highly endemic biota. We estimate the phylogeny of a species-rich endemic genus of earthworms (Terrisswalkerius) from the region. Using DEC and DIVA historical biogeography methods we find a strong signal of vicariance among known biogeographical sub-regions across the whole phylogeny, congruent with the phylogeography of less diverse vertebrate groups. Absolute dating estimates, in conjunction with relative ages of major biogeographic disjunctions across Australia, indicate that diversification in Terrisswalkerius dates back before the mid-Miocene shift towards aridification, into the Paleogene era of isolation of mesothermal Gondwanan Australia. For the Queensland endemic Terrisswalkerius earthworms, the AWT have acted as both a museum of biological diversity and as the setting for continuing geographically structured diversification. These results suggest that past events affecting organismal diversification can be concordant across phylogeographic to phylogenetic levels and emphasize the value of multi-scale analysis, from intra- to interspecies, for understanding the broad-scale processes that have shaped geographic diversity.

Introduction

Geologic and climatic oscillation events are likely to have profound effects on both rates of speciation and current distributions of taxa. The processes and timelines involved have long intrigued biologists [1] [2] [3] [4]. Moreover, there has been a long-standing attempt to explain
and understand the high species richness of the tropical rainforests [5] [6] [7] [8] and whether the rainforests act as a museum or cradle for biological diversity [9] [10] [11] [12]. More generally, there is growing appreciation of how long-term processes of landscape change and phyloge- netic evolution shape regional species pools and geographically structured diversity [11] [15]. Explanations for the high biological diversity of the tropics and the current distributions of species have included biotic and abiotic factors with no single variable accounting for the patterns we observe. One suite of related factors that have been proposed to explain some of the observed patterns are the effects of Pleistocene or older climate change in the tropics, the resulting refugia, and the impacts of these events on speciation [14] [15], though such refuge-based models have proved controversial [16] [17]. In this paper we explore whether climate-driven vicariance, as measured by biogeographic structuring (ancestral ranges) of sister clades within the phylogeny, has shaped long-term speciation in a system with a well-characterized history of late Quaternary contraction and refugia: The rainforests of the Australian Wet Tropics.

The Australian Wet Tropics (AWT) are the largest surviving remnant of Gondwanan rainforest, which once dominated the continent of Australia [18]. Climatic modeling and paleoecological data [19] [20] [21] [22] [23] and previous research on montane taxa [24] [25] [26] have shown that the currently near continuous rainforest was sundered into disconnected refugia as a result of late Quaternary climatic processes. These late Quaternary processes, especially in the mid- late Pleistocene [20] [27], had profound effects on current patterns of species richness and phylogeographic diversity [21] [25] [26] [28]. Based on the growing body of research from this region, there is support for the hypothesis that these refugia are the result of late Quaternary climate changes [24] [29] [30], perhaps undergoing extreme contraction, and for many of the groups that are found in these regions, large-scale extinction. Not only have phyl- ogeographic studies provided evidence of Pleistocene climatic events leaving discrete genetic signatures on modern populations, there is mounting evidence that the major “breaks” in genetic diversity (including across the Black Mountain Corridor) typically predate the Last Glacial Maximum ([31]: late-Miocene or early Pliocene; [32]: Pliocene; [33]: pre-Pleistocene; [28]: Late Pliocene or Early Pleistocene; [34]: pre-Pleistocene; [26]: Pliocene 2.3–4.6 Ma). Interestingly, historic biogeographic features within the AWT may not have the same impact on related taxa, as was found for two species of closely related rainforest trees with overlapping habitats and differing signature of the impact of geographic features and genetic diversity [34]. These previous studies highlight variation among species in the spatial and temporal scale of response.

The habitat contractions of the AWT are thought to be due to the long-term aridification of the Australian continent, which began during the mid-Miocene, through the Pliocene and continued with the high amplitude oscillation of the mid-late Pleistocene [18] [35]. The resulting isolation and local reduction of rainforest tracts doubtlessly had major effects on the biological diversity of this region. The contractions of the forests would clearly have cascading effects for taxa that had close ecological and evolutionary relationships with the rainforest themselves [36] [37] [38]. The escarpments and montane areas that harbor the endemic fauna of the Aus- tralian Wet Tropics are, in general, an ancient land formation. In this context, it is possible that rainforest contractions much deeper in time (e.g. mid-Miocene) have shaped current patterns of diversity. If true, how does this affect deeper phylogenetic splits? If climate driven contractions of rainforest over Plio-Pleistocene (or even mid- late Miocene or older) affected speciation processes, then phylogenies of species-rich endemic genera should show geographic structure at these deeper evolutionary scales analogous to phylogeographic studies observed within species. For most vertebrate genera unraveling the geography of speciation associated with longer time scales has not been possible because few, closely related clades of species are
found within the AWT and most genera have low species diversity [39]. A prominent exception is microhylid frogs in the genus *Cophixalus*, for which spatial phylogenetic pattern is congruent with geography [40].

Earthworms have been largely overlooked in studies of historical biogeography and phylogeography and have rarely been subjected to molecular analysis until recently [41] [42] [43] [44] [45] [46] [47] [48] [49] [50] [51] [52] [53]. As a diverse, Australian Wet Tropics endemic and rainforest-restricted group, earthworms of the genus *Terrisswalkerius* provide a promising system with which to address questions of long-term historical processes in the AWT (Fig 1). Like terrestrial snails that have been highly informative for phylogeography [25] [54], the earthworms are expected to have limited dispersal ability and to have been less prone to late Pleistocene local extinctions because of their large overall distributions and diversity. As inhabitants of moist soils and rotten logs with presumed environmental buffering, their biogeographic history may provide a rich view of the long-term historical processes that have shaped the region. *Terrisswalkerius* earthworms are striking due to their size (ranging from 24mm-317mm) and often bright colors. The 25 species of *Terrisswalkerius* are distributed in the wet
tropical rainforests of eastern North Queensland, from the Paluma Range, near Townsville north to the isolated rainforest of the McIlwraith Range in the Cape York Peninsula. Jamieson [42] reviewed the biology, distribution, and morphology of all known species of Terrisswalkerius and wide-scale molecular phylogenetic analyses [44][53] confirmed that the genus is a member of the annelid family Megascolecidae. An unpublished new species is included in the present study and a further species, T. leichhardtii, was described by Jamieson et al. [55].

As these endemic earthworm species are intimately associated with forest environments, they may be expected to track this long continuous history of decimation of the Gondwanan mesothermal rainforests that once dominated Australia, of which the Wet Tropics is considered to be the largest surviving remnant. Thus broadly, the current endemic species distributions of Terrisswalkerius may reflect a balance of 1) re-structuring of old, geographically dispersed diversity into persistent and much reduced regions; and 2) biogeographic speciation among regions. This balance might be weighed by assessing (by ancestral range reconstruction) the signal of vicariant speciation consistent with Australian Wet Tropics biogeography, compared to more complex idiosyncratic patterns expected from restructuring of ancestral diversity.

Here we estimate the phylogenetic structure of the AWT endemic earthworm lineage, Terrisswalkerius, overlaying this with current species distributions and ancestral range reconstruction to infer the effect of known barriers deduced from previous phylogeographic studies on long-term speciation processes. We also provide a provisional estimate for the timing of the diversification of the genus and the results are compared to recent phylogenetic analyses of other endemic but lower diversity taxa.

Materials and Methods

We amplified and sequenced DNA fragments from 16 species of Terrisswalkerius (34 specimens) and an additional 34 megascolecid outgroup taxa (68 specimens total). The nine species of Terrisswalkerius that were not included in this study are known only from the type collections and were not preserved in a manner likely to yield useful DNA sequences, excepting T. oculatus, although known from three localities, was not obtainable for sequencing. All collections were made under the auspices of the Department of the Environment (Queensland, Australia) in which one of the authors (KRM) was an officer and no additional permissions were required. The species studied are not on any threatened or endangered lists but we hope that this paper will encourage their protection. All worms were humanely euthanized in dilute ethanol following all protocols of the Department of the Environment (Queensland, Australia). All included specimens were sequenced for the mitochondrial (mt) 12S rRNA gene, with nested coverage for the following additional genes: mt 16S rRNA, mt DNA COII, and nuclear 28S rRNA [see S1 File for sequencing details and S1 Table for GenBank accession numbers].

Phylogenetic inference

The final concatenated data matrix comprised 695 sites of nuclear 28SrDNA, 315 and 435 sites of mitochondrial 12S and 16S rDNA respectively, and 558 sites of mitochondrial COII. Phylogenetic analyses were conducted on this data matrix using maximum parsimony, and maximum likelihood and Bayesian inference using partitioned GTR+Γ+I models for each gene region (Modeltest 3.06: [56]). Parsimony searches were performed with PAUP* 4.0b10 [57] using the random stepwise addition option of the heuristic search for 500 replicates with tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, and equal weighting of all characters, with bootstrap analyses (bs) using the closest stepwise addition of the heuristic search for 500 replicates. Maximum likelihood phylogenetic inference was
conducted with RAxML v7.2.6 [58] using 200 fast bootstraps. Bayesian analyses were performed with MrBayes v3.1.1 [59], using four 20 million generation Markov chains, sampled every 100 generations after a burn-in of 100,000 generations for Bayesian posterior probability (bpp) estimation. Convergence of chains was confirmed in all Bayesian analyses by examination of the average standard deviation of split frequencies. Additional analyses were also conducted with BEAST v1.4.8, as describe below, and GARLI v0.951 [60] (see SI for details).

To test alternative hypotheses for the monophyly of Terrisswalkerius a constraint tree search was implemented and the Shimodaira-Hasegawa test [61] was executed to investigate significant differences in tree lengths. This test was performed using RELL with 10,000 bootstrap replicates, and the results evaluated as a one-tailed test.

**Biogeographic reconstruction**

We employed biogeographic history DEC-LAGRANGE [62] [63] and S-DIVA [64] methods to infer the ancestral ranges through the phylogeny of Terrisswalkerius. In addition to individual node ancestral state reconstruction we summarized the relative amount of vicariance across the whole phylogeny. In LAGRANGE (version 20110117) this was done by summing the relative probabilities of ancestral node solutions that split into two mutually exclusive regions. For example, if the ancestral range is ABC and the daughter lineages ranges are AB and C. This is similar to the approach of Beaulieu et al. [65]. In S-DIVA nodes were scored as vicariance according to the most likely reconstruction. For the purposes of these summary biogeographic analyses we focused on a sub-tree of 23 nodes representing all the species and major lineages. Eight biogeographic regions were defined. Tests of maximum range limits 2–3 and dispersal constraints had only minor effects on the overall result (cf. [66]) and we use the results for the analysis presented in full in SI.

**Divergence estimation**

We performed a relaxed-clock dating using the combined data with a normal rate prior on the COII partition and two major calibration constraints. The analysis was run in BEAST v1.4.8 [67] with uncorrelated lognormal clock model, Yule speciation prior, and partitioned GTR+I+Γ models for each gene region. The analysis was calibrated using a truncated normal rate prior on the COII partition (min = 0.005; mean = 0.01; SD = 0.01 per lineage), and a minimum 50 million years ago (Ma) divergence for the Acanthodrilinae and 80 Ma for the Megascolecidae. These represent a summary of COII rates used for a range of invertebrates including oligochaetes [50] [68] [69], and major Earth biogeography limits for these widely distributed oligochaete groups [44] [70]. Two 20,000,000 step chains were run with 1/1000 sampling and 10% burn-in, leaving 18,000 posterior samples. All parameter ESS were greater than 200. Our results are comparable to previous oligochaete dating using similar data: Novo et al. [50], who estimated much the same age of ~70 Ma for the Lumbricidae (equivalent to our Criodrilus-Lumbricus split); and relative ages of Acanthodrilinae-Megascolecinae taxa by Buckley et al. [49]. The full relaxed-clock tree with calibration points and outgroups is shown in S1 Fig.

In addition to considering the absolute ages returned from the relaxed-clock dating, we also considered ages relative to several major disjunctions pertinent to deep Austral biogeography: the WT–Cape York Terrisswalkerius mcilwraithi split, the WT–SE Queensland (Qld) split in the sister group Fletcherodrilus, the SE Qld–Tasmania divergence in the Digaster-Diporochaeta, and the apparent absence of the group from New Guinea. These relate to the Miocene timescale of the major environmental shift to fragmentation and attenuation of mesic mesothermal environments [19] [20] [71], and age of New Guinean landmasses [72].
Results

All phylogenetic methods recovered similar topologies for the relationships within *Terrisswalkerius* and we present the BEAST consensus tree with ML and Bayesian support values in Fig 2 (see S1 Fig for the full tree with all distant outgroup taxa included). The major framework of the tree (Fig 2) is generally congruent between mitochondrial and nuclear gene data (see, however, [44], where 28S rDNA showed poor resolution compared with the combined nuclear and mitochondrial analysis; S3 and S4 Figs). *Terrisswalkerius* is recovered as monophyletic with the exception of *T. athertonensis*, the inclusion of this species being rejected by the SH test (delta lnL = 36.4, p = 0.0057). The position of *Didymogaster* Fletcher 1886 is somewhat equivocal with mtDNA being responsible for placement within *Terrisswalkerius sensu lato*, where *T. athertonensis* is included. All data unequivocally support *Fletcherodrilus* Michlaelsen 1891 as
the sister group of Terrisswalkerius, if T. athertonensis is excluded, as also shown in the maximum likelihood analysis of Jamieson et al. [44]. The present and previously published results [42] [44] suggest that T. athertonensis should be placed in a separate, cryptic genus. In a morphological cladistic analysis [73] it grouped with T. oculatus, embedded among Terrisswalkerius species but the morphological tree shows little agreement with molecular analyses. Terrisswalkerius Jamieson 1994 remains current pending further resolution and confirmation of the separate generic position of T. athertonensis. Terrisswalkerius s.s. is mostly well resolved with the exception of basal relationships involving T. macilwraithi and the T. nashi-liber lineage.

Although, in general, morphologically defined species are recovered as monophyletic, we do recover two paraphyletic species (T. canaliculatus and T. nashi), which may suggest that further taxonomic revision is needed in this diverse and understudied group. However, as implied by Jamieson [42], T. nashi would be monophyletic if T. liber, differing chiefly in approximation of genital pores, is included within T. nashi.

Biogeographic reconstruction

In examining the biogeographic structure of the Terrisswalkerius phylogeny, we find a clade containing three species (T. erici, T. millamilla, and T. phalacrus) found in the Atherton Uplands (AU) and Kirrama Uplands (KU), both south of the Black Mountain Corridor (although for some taxa the BMC split extends south of LU (Lamb Uplands); see [74], as sister to the remaining taxa, comprising both northern and southern taxa (Fig 2).

Summarized across 23 nodes representing all the species and major lineages, DEC-LA-GRANGE recorded 9 nodes as vicariance and S-DIVA 14 nodes (Fig 2; S2 Fig; S2 Table). For Terrisswalkerius s.s. (node A) the DEC marginal likelihood for vicariance was 0.53, which was also the most likely solution in S-DIVA. Overall, vicariance accounts for nearly half of the nodes, and not only among obvious sister tips but also through the depth of the phylogeny. This suggests that the regional biogeography of the Australian Wet Tropics has been an important component in diversification over an extended period of time.

Both DEC and DIVA reconstruct the most likely solution for node A (the ancestor of Terrisswalkerius) as a split between AU and either LU or CU (S2 Table). The extant species are then divided into two distinct clades with one clade found exclusively south of the BMC and the other with a wide distribution including all of the taxa north of the BMC and the extra-limital Cape York T. macilwraithi. Within this ‘northern’ clade the history is complex but with considerable signal of large-scale ‘north-south’ vicariance. Considering the biogeographic analyses and the known current distributions for species, we find what may have been expansions back across the BMC (although resolution is weak in this part of the topology) and this is probably the case for T. canaliculatus, T. erici, T. grandis, and T. terraereginae. We also find clear repeated diversification within individual regions of the AWT such as in the Atherton Uplands (AU; 13 species), Carbine Uplands (CU; eight species), Kirrama Uplands (KU; four species), Lamb Uplands (LU; six species), and Windsor Uplands (WU; five species). The overall pattern we recover in the AWT endemic Terrisswalkerius earthworms appears to be a combination of deep ‘north-south’ splits and more recent sub-regional diversification.

Absolute and relative divergence dating

Relaxed-clock dating estimated the age of monophyletic members of Terrisswalkerius (labeled “A” in Fig 2; S1 Fig) to be 52 Ma (with 95% CI of 31–84 Ma), the separation of the Cape York species, T. macilwraithi at 40 Ma (21–58 Ma), the North-South Queensland split in Fletcherodrilus at 36 Ma (12–46) and the Qld-Tasmania split in the Diporochaeta group at 36 Ma (19–62...
Ma). There is considerable uncertainty in these estimates due to limited calibration information and high level of molecular change in the mitochondrial genes. However, relative dating should be less sensitive to these limitations [49] [75]. Significantly, the Cape York *T. mcillwraithi*, North-South Queensland *Fletcherodrilus* and the Qld-Tasmania *Diporoachaeta* splits are all younger than *Terrisswalkerius* s.s. (node A), and this pattern is consistent considering mitochondrial or nuclear data alone (Fig 3). It is also worth noting that the clade is around five times older than typical intra-specific phylogeography (Fig 2).

This inference places extant monophyletic *Terrisswalkerius* well into the Paleogene era of isolation of mesothermal Gondwanian Australia [19] [71] [76], and even the lower confidence interval (31 Ma) places a substantial proportion of the Australian Wet Tropics endemic diversity pre-dating the mid-Miocene major shift towards fragmentation and aridification [20] [71]. This can be further put in context by considering the relative age of phylogenetic splits spanning deep Austral biogeography (Fig 2). While each of these splits may not directly relate to historically driven eco-geographic divergence processes, they set a maximum (e.g. an overestimation due to extinction or under-sampling) and therefore emphasize the age of the AWT endemic *Terrisswalkerius* diversity as likely pre-dating the mid-Miocene major shift towards fragmentation and aridification. This can also explain their apparent absence from any New Guinean landmass, which (at ca. 10–15 Ma; [72]) likely post-dates these major historical eco-geographic divisions.

**Discussion**

Geologic and climatic oscillation events are expected to have profound effects including attenuation and extinction on less mobile organisms; the genetic signatures of these events are

![Fig 3. Ages of various groups relative to monophyletic *Terrisswalkerius* (node A). Quartile and 95% CI BEAST posterior distributions. *Digaster-Diporoachaeta* (node D) represents taxa spread from SE Queensland to Tasmania; *Fletcherodrilus* (node C) represents taxa spread across the Burdekin and St. Lawrence Gaps from the AWT to SE Qld, Cape York *T. mcillwraithi* represents the Laura Gap. Estimates use three different data matrices: mtDNA only, 28SrDNA only, and combined (all) (see SI for further details).](https://doi.org/10.1371/journal.pone.0136943.g003)
apparent in the endemic Australian Wet Tropics earthworms. The substantial component of geographical speciation between regions—vicariance—estimated across the phylogeny as a whole and fairly apparent in sister tip lineage phylogeography (Fig 2), indicates that the biogeographical structure of the AWT rainforests has played a significant role in diversification of *Terrisswalkerius*.

In the case of these earthworms, morphological conservatism hides the deep evolutionary history of the lineage, as there are few morphological characters to distinguish these species, which lack the genital markings that show high specificity in other megascolecid genera. Although we infer a middle Paleogene age (ca. 52 Ma) for the genus *Terrisswalkerius*, dating divergence times for lineages can be challenging in the absence of fossil or biogeographic information, but clearly molecular divergences are high. Relatively, *Terriwalkerius* is five times older than typical intra-specific phylogeography (Fig 2) and older than major biogeographic divisions (Fig 2; S2 Fig; S3 Table), consistent with it being of at least Olio-Miocene age.

Our finding of the signature of an ancient north–south division in *Terrisswalkerius* suggests the ‘Black Mountain Corridor’ may be a much older biogeographic barrier than had been previously proposed and played a role in AWT diversification prior to the Pleistocene-era phylogeography of previous vertebrate studies. There is now mounting evidence from phylogenetic analyses that the BMC barrier predates the Last Glacial Maximum [32] [33] [74] and our findings significantly push back the date for this barrier and add to this growing body of work. The distant species of the northern whole group, all suggest a complex history coinciding with the drying of the Australian continent, which is thought to have begun with the major ice-sheet expansion of the late-Paleogene [76] and continued from the mid-Miocene [18]. Thus for *Terrisswalkerius*, in situ AWT endemic processes continued all the while the greater mesic mesothermal Gondwanian world shrank and fragmented around it, leaving the isolated ‘island world’ of the Australian Wet Tropics.

Although the AWT are a very well studied region, the area to the north is less explored and the effect of long-term aridification and Pleistocene cycles on the isolated rainforest patches of the Cape York peninsular region are not well known. In addition the biological diversity of the Cape York (CY) area is understudied, but available evidence suggests that this region contains phylogenetic relics i.e. deep branch taxa [39]; this is the case in *Cophixalus* microhyd frogs [40], leaf-tailed geckos [77], camaenid snails [34], dung beetles [78] and now within the *Terrisswalkerius* earthworms (*T. mcilwraithi*: Fig 2). Our data recover the northern Cape York species, *T. mcilwraithi*, as an early lineage separating at ~ 26 Ma, within the primarily northern clade which is suggestive of long-term isolation and/or extinction of species within this clade. The hiatus in occurrence of *Terrisswalkerius* between CL and CY appears to be real as extensive collecting by KRM in the region between CL and *T. mcilwraithi* at CY yielded no specimens of the genus. The type-locality of *T. mcilwraithi* is, thus, almost two degrees of latitude north of previous records for the genus. Collection records by Dyne and Wallace [79] did not extend North of Cooktown. Biological diversity of the Cape York Peninsula is understudied and our results suggest that biologists interested in understanding phylogenetic, biogeographic, and evolutionary patterns of taxa of the AWT should include taxa from this region when they are known.

There is mounting evidence for a pre-Pleistocene climatic effect on flora and fauna across the Black Mountain Corridor of the Australian Wet Tropics but we are still in the early stages of understanding the factors that may have shaped this pattern. Aridification of the Australian continent undoubtedly led to contractions of the forest and may have driven species into relictual habitats thus resulting in contracted ranges and/or massive extinctions. Diversification in isolation would have been possible during these periods of forest contractions and our data are
suggestive of this. Studies of species across many taxonomic scales in this region suggests that vicariance events may be geographically, but not necessarily temporally congruent, which poses the question how common is this in other geographic regions?

Our data show that for the endemic Terrisswalkerius earthworms, the rainforests of the AWT have acted not only as a museum to preserve biological diversity, but also have promoted continued and rapid diversification of the genus. Interestingly we see deep divergences among species of the endemic Terrisswalkerius earthworms, with many lineages restricted to distinct biogeographic regions (Fig 1), but we also recover paraphyletic species suggesting recent separation without sufficient evolutionary time for ancestral polymorphisms to sort. Hybridization cannot be ruled out for the two pairs of paraphyletic species \( T. \ canaliculatus \) and \( T. \ terrareginae \) overlap in their distributional ranges in the Atherton Uplands (AU) and Bellenden-Ker/Bartle-Frere (BK); \( T. \ nashi \) and \( T. \ liber \) overlap in their distributional range in the Mount Finnigan Uplands (FU). However, it is more probable that \( T. \ liber \) has evolved within \( T. \ nashi \) by reduction of the distance between the male pores and that reproductive isolation is not complete. In other lineages with overlapping distributions we see clear genetic differentiation such as in the Carbine Uplands (CU) where eight species are found co-occurring and the Atherton Uplands (AU) with twelve species. The results presented here show that for the Terrisswalkerius earthworms, the AWT are the center for ancient and ongoing diversification.

Although many vertebrate and invertebrate taxa have been examined from the Australian Wet Tropics UNESCO World Heritage Site, the Terrisswalkerius earthworms are the most diverse radiation that has been examined to date and the results presented here highlight the need for continued investigations across diverse groups of species and with varying depths of evolutionary history in the AWT, but also in other geographic arenas. Generally, vertebrates requiring large areas may have been subject to extinction, but when smaller vertebrates (i.e. frogs: [40] [80] [81] and invertebrate radiations have been examined [25] [28] [33] [54] [78], these data provide a richer picture of the evolutionary history of this region and suggest that these taxa had limited dispersal abilities and/or were buffered in smaller areas. The Australian Wet Tropics, which are biologically and topologically complex [82], provide an exceptional opportunity to study not only short-term evolutionary processes resulting from the Last Glacial Maximum (LGM), but also long-term processes, which affected the diversification and redistribution of biodiversity on a larger timescale. This is not unique to the AWT, with parallel questions being asked in other biologically diverse and geographically old regions such as the African tropics [83], Neotropics [84], and Madagascar [85]. Our results highlight the need for continuing evolutionary studies in this and other geographically old regions to include data from multiple levels, from individuals to higher taxonomic scales, across diverse groups of organisms thus allowing independent replicates of evolution in order to more fully unravel the complex history of the planet’s biological diversity.

**Supporting Information**

S1 Fig. BEAST relaxed-clock maximum clade credibility phylogeny, showing BEAST posterior probabilities followed by RAxML bootstrap supports (PP/BS as percent; some terminal nodes show only PP to avoid clutter) based on the combined data matrix with all outgroups. Terrisswalkerius taxa north of the Black Mountain Corridor (BMC) in grey. Node circles indicate biogeographic analyses: grey and black filled circles indicate inferred ‘vicariance’ by respectively either or both DEC and DIVA methods. Divergence scale in mya. See Jamieson et al. [44] and Buckley et al. [49] for more details on higher classification. Additional biogeographic calibration constraint nodes Megascolecidae (E) and Acanthodrilinae (B) indicated. (TIF)
S2 Fig. Phylogeny and branches labeled as implemented in DEC and DIVA biogeographic analyses. Branch labels correspond to values in S3 Table. (TIF)

S3 Fig. BEAST v1.4.8 analysis of COII data. Maximum clade credibility chronogram from 10 million steps 10% burn-in, showing median age and 95% CI age bars (in millions of years). Labeling of groups follows Fig 2 in main text. (TIF)

S4 Fig. BEAST v1.4.8 analysis of 28S rRNA data. Maximum clade credibility chronogram from 10 million steps 10% burnin, showing 95% CI age bars (in millions of years). Labeling of groups follows Fig 2 in main text. (TIF)

S1 File. Supporting Information. Additional Materials and Methods. (DOC)

S1 Table. List of all specimens, collection accession numbers and GenBank accession numbers. “X” denotes missing sequence information for taxon. (DOC)

S2 Table. Biogeographic analysis constraint matrix for DEC and DIVA. (DOC)

S3 Table. Summary of DEC and DIVA biogeographic analyses following labeling on S2 Fig. (DOC)

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Author Contributions

Conceived and designed the experiments: CSM AFH KRM BGMJ CM. Performed the experiments: CSM AFH KRM BGMJ CM. Analyzed the data: CSM AFH. Contributed reagents/materials/analysis tools: CSM AFH KRM BGMJ CM. Wrote the paper: CSM AFH KRM BGMJ CM.

References

1. Dobzhansky T (1941) Genetics and the origin of species. Columbia University Press, New York, NY, USA. 364 p.
2. Endler JA (1982) Problems in distinguishing historical from ecological factors in biogeography. Amer Zool 22: 441–452.
3. Mayr E, O’Hara RJ (1986) The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. Evolution 40: 55–67.
4. Ricklefs RE, Schlüter D (1993) Species diversity in ecological communities: Historical and geographical perspectives. University of Chicago Press, Chicago, IL, USA. 414 p.
5. Wallace AR (1878) Tropical Nature and Other Essays. Macmillan and Co. Press, London, England. 384 p.
6. Fischer AG (1960) Latitudinal variations in organic diversity. Evolution 14: 64–81.
7. Pianka ER (1966) Latitudinal gradients in species diversity: a review of concepts. Am Nat 100: 33–46.
8. Janzen DH (1967) Why mountain passes are higher in the tropics. Am Nat 101: 233–249.
9. Stebbins GL (1974) Flowering plants: evolution above the species level. The Belknap Press of Harvard University Press, Cambridge, MA, USA. 480 p.
10. Jablonski D, Roy K, Valentine JW (2006) Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. Science 314: 102–106. PMID: 17023653
11. Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. Trends Ecol Evol 19: 639–644. PMID: 16701326
12. Moreau CS, Bell CD (2013) Testing the museum versus cradle biological diversity hypothesis: Phylogeny, diversification, and ancestral biogeographic range evolution of the ants. Evolution 67: 2240–2257. doi: 10.1111/evo.12105 PMID: 23888848
13. Ricklefs RE (2005) Phylogeographic perspectives on patterns of regional and local species richness. In: Tropical rainforests: past, present, and future (eds. Bermingham E., Dick C. W. and Moritz C.), The University of Chicago Press, Chicago, IL, USA. pp. 313–321.
14. Haffer J (1969) Speciation in Amazonian forest birds. Science 165: 131
15. VanZolini PE, Williams EE (1970) South American anoles: the geographic differentiation and evolution of the Anolis chrysolepis species group (Sauria: Iguanidae). Arq Zool São Paulo 19: 1–298.
16. Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. Ann Rev Ecol Syst 31: 533–563.
17. Bush MB, Silman MR (2004) Observations on Late Pleistocene cooling and precipitation in the lowland Neotropics. J Quaternary Sci 19: 677–684.
18. Greenwood DR, Christophel DC (2005) The origins and Tertiary history of Australian “tropical” rainforests. In: Tropical rainforests: past, present, and future (eds. Bermingham E., Dick C. W. and Moritz C.), The University of Chicago Press, Chicago, IL, USA. pp. 336–373.
19. Nix HA (1991) Biogeography: pattern and processes. In: Rainforest animals: atlas of vertebrates (eds. Nix H. A. & Switzer M. A.), Australian Nature Conservation Agency, Canberra, Australia. pp. 11–40.
20. Kershaw AP, Moss PT, Wild R (2005) Patterns and causes of vegetation change in the Australian wet tropics region over the last 10 million years. In: Tropical rainforests: past, present, and future (eds. Bermingham E., Dick C. W. and Moritz C.), The University of Chicago Press, Chicago, IL, USA. pp. 374–400.
21. Graham CH, Moritz C, Williams SE (2006) Habitat history improves prediction of biodiversity in rainforest fauna. Proc Natl Acad Sci USA 103: 632–636. PMID: 16407139
22. Hilbert DW, Graham A, Hopkins MS (2007) Glacial and interglacial refugia within a long-term rainforest refugium: the Wet Tropics bioregion of NE Queensland, Australia. Palaeoecogr Palaeoclimatol Palaeoecol 251: 104–118.
23. VanDerWal J, Shoo LP, Williams SE (2009) New approaches to understanding late Quaternary climate sluations and refugial dynamics in Australian wet tropical rain forests. J Biogeogr 36: 291–301.
24. Williams SE, Pearson RG (1997) Historical rainforest contractions, localized extinctions and patterns of vertebrate endemism in the rainforests of Australia’s wet tropics. Proc R Soc Lond B 264: 709–716.
25. Hugall A, Moritz C, Moussalli A, Stanisic J (2002) Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail Gnarosophia bellendenkerensis (Brazier 1875). Proc Natl Acad Sci USA 99: 6112–6117. PMID: 11972064
26. Bell RC, Parra JL, Tonione M, Hoskin CJ, Mackenzie JB, Williams SE, et al. (2010) Patterns of persistence and isolation indicate resilience to climate change in montane rainforest lizards. Mol Ecol 19: 2531–2544. doi: 10.1111/j.1365-294X.2010.04676.x PMID: 20497322
27. Hocknull SA, Zhao J, Feng Y, Webb GE (2007) Responses of Quaternary rainforest vertebrates to climate change in Australia. Earth Planet Sci Lett 264: 317–331.
28. Bell KL, Moritz C, Moussalli A, Yeates DK (2007) Comparative phylogeography and speciation of dung beetles from the Australian Wet Tropics rainforest. Mol Ecol 16: 4984–4998. PMID: 17927709
29. Avise JC, Walker D John GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc R Soc Lond B 265: 1707–1712.
30. Bermingham E, Dick CW, Moritz C (2005) Tropical rainforests: past, present, and future. The University of Chicago Press, Chicago, IL, USA. 672 p.
31. Schneider CJ, Cunningham M, Moritz C (1998) Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Mol Ecol 7: 487–498.

32. Ponniah M, Hughes JM (2004) The evolution of Queensland spiny mountain crayfish of the genus Euastacus. I. Testing vicariance and dispersal with interspecific mitochondrial DNA. Evolution 58: 1073–1085. PMID: 15212388

33. Shull HC, Pérez-Losada M, Blair D, Sewell K, Sinclair EA, Lawler S, et al. (2005) Phylogeny and biogeography of the freshwater crayfish Euastacus (Decapoda: Parastacidae) based on nuclear and mitochondrial DNA. Mol Phyol Evol 37: 249–263.

34. Rossetto M, Crayn D, Ford A, Ridgeway P, Rymer P (2007) The comparative study of range-wide genetic structure across related, co-distributed rainforest trees reveals contrasting evolutionary histories. Aust J Bot 55: 416–424.

35. Bowler JM (1982) Aridity in the late Tertiary and Quaternary of Australia. In: Evolution of the Flora and Fauna of Arid Australia (eds. Barker W. R. and Greenslade P. J. M.), Peacock Publications, Adelaide, Australia. pp. 35–45.

36. Farrell BD (1998) “Inordinate fondness” explained: why are there so many beetles? Science 281: 555–559. PMID: 9677197

37. Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallón S, Lupia R (2004) Ferns diversified in the shadow of angiosperms. Nature 428: 553–557. PMID: 15058303

38. Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NP (2006) Phylogeny of the ants: diversification in the age of angiosperms. Science 312: 101–104. PMID: 16601190

39. Moritz C, Hoskin C, Graham CH, Hugall A, Moussalli A (2005) Historical biogeography, diversity and conservation of Australia’s tropical rainforest herpetofauna. In: Phylogeny and Conservation (eds. Purvis A., Gittleman J. L. & Brooks T. M.), Cambridge University Press, London, England. pp. 243–266.

40. Hoskin CJ (2004) Australian microhylid frogs (Cophixalus and Austrochaperina): phylogeny, taxonomy, calls, distribution and breeding biology. Aust J Zool 52: 237–269.

41. Jamieson BGM (1981) Historical biogeography of Australian Oligochaeta. In: Ecological Biogeography of Australia. Keast, A. ed. Dr. W. Junk, The Hague, pp. 887–921.

42. Jamieson BGM (2000) Native earthworms of Australia (Megascolecidae, Megascolecinae). Science Publishers, Enfield, New Hampshire, USA (CD ROM); and Supplement (2001) http://barriemjamieson.com/Megascolecinae%20Supplement.pdf

43. Jamieson BGM (2006) Non-leech Clitellata. In: Reproductive Biology and Phylogeny of Annelida. Series Editor Jamieson B.G.M., Volume 4. (eds. Rouse G. and Pleijel F.), Science Publishers, Enfield, New Hampshire, U.S.A. Jersey, Plymouth, U.K. pp. 235–392.

44. Jamieson BGM, Tillier S, Tillier A, Justine J-L, Ling E, James S, et al. (2002) Phylogeny of the Megascolecidae and Crassiclitellata (Annelida, Oligochaeta): combined versus partitioned analysis using nuclear (28S) and mitochondrial (12S, 16S) rDNA. Zoosystema 24: 707–734.

45. Chang C-H, Lin S-M, Chen J-H (2008) Molecular systematics and phylogeography of the gigantic earthworms of the Metaphire formosae species group (Ciliatella, Megascolecidae). Mol Phy Ecol 49: 958–968.

46. King RA, Tibble AL, Symondson WOC (2008) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. Mol Ecol 17: 4684–4686. doi: 10.1111/j.1365-294X.2008.03931.x PMID: 18992008

47. Minamiya Y, Yokoyama J, Fukuda T (2009) A phylogeographic study of the Japanese earthworm, Metaphire sieboldi (Horst, 1883) (Oligochaeta: Megascolecidae): Inferences from mitochondrial DNA sequences. Eur J Soil Biol 45: 423–430.

48. Stürzenbaum SR, Andre J, Kille P, Morgan AJ (2009) Earthworm genomes, genes and proteins: the (re)discovery of Darwin’s worms. Proc R Soc B 276: 789–797. doi: 10.1098/rspb.2008.1510 PMID: 19129111

49. Buckley TR, James S, Allwood J, Bartlum S, Howitt R, Prada R (2011) Phylogenetic analysis of New Zealand earthworms (Oligochaeta: Megascolecidae) reveals ancient clades and cryptic taxonomic diversity. Mol Phylogen Evol 58: 85–96.

50. Novo M, Almodovar A, Fernandez R, Giribet G, Diaz Cosin DJ (2011) Understanding the biogeography of a group of earthworms in the Mediterranean basin-The phylogenetic puzzle of Hormogastridae (Ciliatella: Oligochaeta). Mol Phylogen Evol 61: 125–135.

51. Perez-Losada M, Breinholt JW, Porto PG, Aira M, Dominguez J (2011) An earthworm riddle: systematics and phylogeography of the Spanish Lumbricid Postandrilus. PLOS ONE 6: e28153. doi: 10.1371/journal.pone.0028153 PMID: 22140529
52. Sharma A, Sonah H, Deshmukh RK, Gupta NK, Singhand NK, Sharma TR (2011) Analysis of genetic diversity in earthworms using DNA markers. Zool Sci 28: 25–31. doi: 10.2108/zsj.28.25 PMID: 21186943

53. James SW, Davidson SK (2012) Molecular phylogeny of earthworms (Annelida: Crassiclitellata) based on 28S, 18S and 16S gene sequences. Invertebr Syst 26: 213–229.

54. Hugall A, Stanisic J, Moritz C (2003) The case of the Sphaerospira lineage and history of Queensland rainforests. In: The molecular systematics and phylogeny of mollusks (eds. Lydeard C. and Lindberg D.), Smithsonian Institution Press, Washington D.C., USA. pp. 270–301.

55. Jamieson BGM, McDonald KR, James SW (2013) A new species of Terrisswalkerius (Megascolecidae, Megascolecinae, Oligochaeta) from the Wet Tropics of Queensland. Mem Queensland Mus – Nature 58: 15–21.

56. Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818 PMID: 9918953

57. Swofford DL (2001) PAUP* Star (ver 4.0b10). Sinauer Associates, Sunderland, MA, USA.

58. Stamatakis A, Hoover P, Rougemont J (2008) A fast bootstrapping algorithm for the RAxML web-servers. Syst Biol 57: 758–771. doi: 10.1080/10635150802429642 PMID: 18853362

59. Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755. PMID: 11524383

60. Shimodaira H, Hasegawa H (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol Biol Evol 16: 1114–1116.

61. Shimaoka A, Hasegawa H (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol Biol Evol 16: 1114–1116.

62. Ree RH, Moore BR, Webb CO, Donoghue MJ (2005) A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59: 2299–2311. PMID: 16396171

63. Ree RH, Smith S (2008) Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst Biol 57: 4–14. doi: 10.1080/10635150701883881 PMID: 18253896

64. Yu Y, Harris AJ, He X-J (2010) S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. Mol Phylogenet Evol 56: 848–850.

65. Beaullieu JM, Tank DC, Donoghue MJ (2013) A Southern Hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. BMC Evol Biol 13: 80. doi: 10.1186/1471-2148-13-80 PMID: 23565668

66. Chacon J, Renner SS (2014) Assessing model sensitivity in ancestral area reconstruction using Lagrange: a case study using the Colchicaceae family. J Biogeogr 41: 1414–1427.

67. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7: 214. PMID: 17996036

68. Quek SP, Davies SJ, Ilton T, Pierce NE (2004) Codiversification of an ant-plant mutualism: the phylogeny of host use in Crematogaster (Formicidae) associates of Macaranga (Euphorbiaceae). Evolution 58: 554–570. PMID: 15119439

69. Fernández R, Novo M, Almodóvar A, Simancas B, Díaz Cosín DJ (2013) Adding complexity to the complex: New insights into the phylogeny, diversification and origin of parthenogenesis in the Aporrectodea caliginosa species complex (Oligochaeta, Lumbricidae). Mol Phylogenet Evol 64: 368–379.

70. Sanmartín I, Ronquist F (2004) Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst Biol 53: 216–243. PMID: 15205050

71. Byrne M, Steane DA, Joseph L, Yeates DK, Jordan GJ, Crayn D, et al. (2011) Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. J Biogeogr 38: 1635–1656.

72. Baldwin SL, Fitzgerald PG, Webb LE (2012) Tectonics of the New Guinea Region. Annu Rev Earth Pl Sc 40: 495–520.

73. Jamieson BGM (1994) Some earthworms from the wet tropics and from Bunya mountains, Queensland (Megascolecidae: Oligochaeta). Mem Queensland Mus 37: 157–181.

74. Moritz C, Hoskin CJ, MacKenzie JB, Phillips BL, Tonione M, Silva N, et al. (2009) Identification and dynamics of a cryptic suture zone in tropical rainforest. Proc R Soc Lond B 276: 1235–1244.

75. Loader SP, Pisani D, Cotton JA, Gower DJ, Day JJ, Wilkinson M (2007) Relative time scales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians. Biol Lett 3: 505–508. PMID: 17609171

76. McGowran B, Holdgate GR, Li Q, Gallagher SJ (2004) Cenozoic stratigraphic succession in southeastern Australia. Aust J Earth Sci 51: 459–496.
77. Hoskin CJ, Couper PJ, Schneider CJ (2003) A new species of Phyllurus (Lacertilia: Gekkonidae) and a revised phylogeny and key for the Australian leaf-tailed geckos. Aust J Zool 51: 153–164.

78. Bell KL, Yeates DK, Moritz C, Monteith GB (2004) Molecular phylogeny and biogeography of the dung beetle genus Temnoplectron Westwood (Scarabaeidae: Scarabaeinae) from Australia’s wet tropics. Mol Phy Evol 31: 741–753.

79. Dyne GR Wallace CC (1994) Biodiversity and conservation of the earthworm fauna of the wet tropics of Queensland’s World Heritage area. Mem Qld Mus 36 (1): 59–66.

80. Bell RC, MacKenzie JB, Hickerson MJ, Chavarria KL, Cunningham M, Williams M, et al. (2012) Comparative multi-locus phylogeography confirms multiple vicariance events in co-distributed rainforest frogs. Proc R Soc B 1730: 991–999.

81. Hoskin CJ, Tonione M, Higgie M, MacKenzie JB, Williams SE, VanDerWal J, et al. (2014) Persistence in peripheral refugia promotes phenotypic divergence and speciation in a rainforest frog. Am Nat 178: 561–578.

82. Williams SE, Pearson RG, Walsh PJ (1996) Distributions and biodiversity of the terrestrial vertebrates of Australia’s wet tropics: a review of current knowledge. Pacific Conserv Biol 2: 327–362.

83. Voelker G, Outlaw RK, Bowie RCK (2010) Pliocene forest dynamics as a primary driver of African bird speciation. Global Ecol Biogeogr 19: 111–121.

84. Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC (2009) Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. PLOS Biol 7: e1000056.

85. Vences M, Wollenberg KC, Vieites DR, Lees DC (2009) Madagascar as a model region of species diversification. Trends Ecol Evol 24: 456–465. doi: 10.1016/j.tree.2009.03.011 PMID: 19500874