Evolutionary diversification of Nepenthes (Nepenthaceae)

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Citation

Schwallier, R. M. (2016, December 12). *Evolutionary diversification of Nepenthes (Nepenthaceae)*. Retrieved from https://hdl.handle.net/1887/44867

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Title: Evolutionary diversification of Nepenthes (Nepenthaceae)
Issue Date: 2016-12-12

Chapter One

Diversity and Distributions (2016) 22: 97-110

Phylogenetic analysis of niche divergence reveals distinct evolutionary histories and climate implications for tropical carnivorous plants

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To analyze the underpinnings of historical drivers of diversity and their contributions to current distributions and future roles in a changing climate, we studied the relationship between ecological niche divergence and phylogenetic signal in tropical carnivorous pitcher plants. Estimates of realized ecological niches were reconstructed and plotted along a newly created multilocus molecular phylogeny. Phylogenetic signal was analyzed by comparisons of calculated phylogenetic relatedness with ecological niche divergence. Current and projected future potentially suitable habitats were mapped for several species of plants with variable evolutionary histories and distributions. Highland and lowland species had distinct phylogenetic signals. Higher altitude species had significantly lower molecular divergence as compared with the lowland species, yet ecological niches with less overlap. When projected onto a future climate scenario, highland species lose a greater amount of potentially suitable habitat compared to lower altitude species, and the majority of studied higher altitude species will face an overall loss of future suitable habitat. We conclude that distinct phylogenetic signals not only unravel differing evolutionary histories but also show that the implications of species' tolerances to future changing climate vary. Over the past million years, historical climate change shaped the differing evolution and ecological niches of highland and lowland tropical pitcher plant species. Rapid, recent radiations of the higher altitude species are reflected in limited molecular divergence, which is in sharp contrast with the more gradually evolved and genetically distinct lower altitude species in our study. Our projections for future potentially suitable habitats show that on-going climate shifts will have detrimental effects on especially the higher altitude species due to a narrower niche tolerance and dramatic loss of potentially suitable habitat.

Keywords: climate change, ecological niche modeling, molecular divergence, *Nepenthes*, niche divergence, phylogenetic signal

Introduction

Understanding ecological similarity between organisms and its link with phylogenetic relatedness is a topic apropos for ecological and evolutionary biology. Rooted in the hypothesis from Darwin (1859) that taxa are more likely to share similar trait values when they are more closely related, the subject has seen a recent revival with many empirical studies showing evidence of either a positive correlation (Prinzing et al., 2001; Chazdon et al., 2003; Brandt et al., 2009; Blanchet et al., 2014), mixed results (Pearman et al., 2008; Jiang et al., 2010; Burns & Strauss, 2011; Best et al., 2013; Godoy et al., 2014), no significant relationship (Losos et al., 2003; Cavender-Bares et al., 2004; Silvertown et al., 2006; Losos, 2008) or a negative correlation (Blomberg et al., 2003) for the studied traits. These varied results fuel intrigue into how and why the classical assumptions of a phylogenetic signal coinciding with ecological similarity are often not met. Here, we focus specifically on niche similarity expressed as its projected reciprocal spatial distribution (Losos et al., 2003).

Several scenarios that explore the complex interactions between ecological similarity and evolutionary distance have been developed. Among these possible contributions to variation in phylogenetic signal are differences in evolutionary backgrounds, timing or changes in geology or climate (Losos, 2008). Ecological niche modeling (ENM) is a tool used to assess niche similarity, and the projected reciprocal spatial distributions produced can be used in conservation biology. A species' fundamental niche is defined by abiotic factors and biotic interactions, and defines the potential habitat that is in line with these needs of the organism. The degree to which the fundamental niche space is occupied depends on the species' dispersal ability, its biotic interactions, as well as contingencies of biogeography and evolution (Soberón & Peterson, 2005; Soberón & Nakamura, 2009). Species live and evolve in their realized niches – the regions of the fundamental niche to which the species is restricted by the limiting factors present in its habitat (i.e. prey availability, competition, seed dispersal barriers, etc.). Climate and soil parameters of the realized niche are typically sourced for ENMs, a more narrow niche that is inherently distorted by niche availability (Jackson & Overpeck, 2000; Colwell & Futuyma, 2014), but quantifiable in a way unlike the all-encompassing fundamental niche (Hutchinson, 1957). This correlative approach t modeling distribution changes due to a changing climate is a valuable tool as often the only data available are occurrence localities (Pacifici et al., 2015). Family and genus-scale ENMs signal the role of ecology in clade diversification (Graham et al., 2004; Kozak & Wiens, 2006), shed light on species delimitation (Raxworthy et al., 2007) and predict likely effects of habitat alteration (Carroll, 2010) and climate change (Pacifici et al., 2015) – all of which contribute to effective and responsible conservation management practices (Araújo et al., 2011).

Our study system, Nepenthaceae, is a carnivorous plant family containing the single genus *Nepenthes* L., which evolved liquid-filled modified tendril tips known as pitfall traps (Cheek & Jebb, 2001). Among the 140+ recognized *Nepenthes* species, there is much variation in geographical distribution patterns. Habitats range from lowland to highland and coastal marshes to mountain peaks, with distributions varying between narrowly endemic to widely distributed (McPherson, 2009). Species diversity is concentrated in the Malay Archipelago (Cheek & Jebb, 2001).

Analysis of genetic diversity in relation to realized ecological niches can be used for estimations of current and future *Nepenthes* distributions. Characteristics within the genus, such as reproductive constraints and high endemism, suggest that its ecological niche may be conserved. All *Nepenthes* are dioecious, which minimizes colonization abilities because dispersal of both male and female propagules is necessary to create new populations (Baker, 1955). Perhaps resultant of this feature, a considerable number of *Nepenthes* species are narrow endemics (Robinson et al., 2009; Nongrum et al., 2012), suggesting that at least some species have constrained habitat requirements or are strongly limited in seed dispersal. Two-thirds of the 103 *Nepenthes* species documented on the IUCN Red List of Threatened Species (IUCN, 2015) are categorized as vulnerable, endangered or critically endangered, with primary threats including habitat loss and over-collecting (Jennings & Rohr, 2011). The limited distribution of a number of *Nepenthes* species further predisposes them to be at higher risk of extinction (Yesson & Culham, 2006).

In this study, our main goal was to determine how phylogenetic proximity interacts with ecological niche similarity by reconstructing realized ecological niches for a selection of *Nepenthes* species and plotting these on a multilocus molecular phylogeny. *Nepenthes* highland and lowland species have distinct distribution patterns, with higher altitude species often exhibiting narrow endemism and lower altitude species having wider ranges throughout the more proximally close islands within the Southeast Asian archipelago (Cheek & Jebb, 2001). Analysis of phylogenetic signal might unveil if these different distributional patterns are resultant of differing evolutionary backgrounds and speciation modes or timing (Losos, 2008). Based on earlier detected variation in geographical constraints between highland and lowland species and its effect on variation in niche conservatism (Kozak & Wiens, 2006), we hypothesize that there will be distinct phylogenetic signals for highland and lowland *Nepenthes* species. We therefore assess (i) if highland and lowland species' realized niche and phylogenetic signals are distinct and (ii) what implications these results might have on future distributions in a changing climate.

Methods

Plant sampling and DNA extraction: We sampled a total of five species of *Nepenthes* for DNA extraction from both the living collection of the Hortus botanicus in Leiden, the Netherlands, and field collections (Table S1). Total genomic DNA was extracted from 50 mg of fresh or silica dried young leaf tissue by grinding the material using a Retsch mill (Retsch MM200; GmbH, Haan, Germany) and further processed using either a Qiagen DNeasy®Plant Mini Kit or a modified CTAB protocol (Doyle & Doyle, 1990).

PCR and Sanger sequencing: Previous molecular studies of *Nepenthes* used the nuclear peptide transferase (PTR1), nuclear ribosomal transcribed spacers (nrITS1-5.8S-nrITS2) and plastid *trnK* intron (Meimberg et al., 2000; Meimberg & Heubl, 2006; Alamsyah & Ito, 2013). To mine existing sequence data in NCBI GenBank, we focused on these markers. The nrITS1-5.8S-nrITS2 region was amplified using ITS1, ITS2, ITS3 and ITS4 (White et al., 1990) and *trnK* using 2-trnK-3914F, Nep16-1270R, Nep2-1060F and 16-trnK-2R (Meimberg et al., 2000). Thermal cycling was carried out on a PTC 200 DNA engine (MJ Research, St. Bruno, Canada) in 25 lL reaction volume. Each reaction contained 5 ng of genomic DNA, 0.1 lM of each primer, 100 lM of each dNTP (Bioline, Londen, UK), Phire.

PCRbuffer(Finnzymes),1.5mMMgCl2,0.3mgmL-1BSA(PromegaCorporation, Madison, WI, USA), and 1 unit of Phire polymerase (Finnzymes). Positive and negative controls were included simultaneously with all amplifications to check for contamination. The thermal cycling profile started with a 5-min denaturation step at 95 °C, followed by 40 cycles of 20-sec denaturation at 94 °C, 20-sec ramped annealing at 50–54 °C and 20-sec elongation at 72 °C, with a final 5-min extension step at 72 °C. The PCR products were purified using the Wizard SV and PCR Clean-up systems (Promega). DNA sequencing was done using a 96-capillary 3730xl DNA Analyzer automated sequencer 3730XL (Applied Biosystems, Inc., Foster City, CA, USA) using standard dye-terminator chemistry (Macrogen Inc., Amsterdam, the Netherlands).

Sequence alignment and phylogenetic analyses: Contigs of trace files were compiled using Sequencher v.5.2.3 (Gene Codes Corporation, USA). Sequences were aligned automatically using MAFFT (Katoh et al., 2002) and inspected visually using Mesquite (Maddison & Maddison, 2011). All DNA sequences generated in this study were submitted to NCBI GenBank (Table S1). Maximum Likelihood (Felsenstein, 1973) tree searches and bootstrapping (Felsenstein, 1985) of the combined data were performed using RAxML v.8.1.11 (Stamatakis et al., 2008) on the CIPRES cluster (Miller et al., 2010). RAxML searches relied on the GTR+Γ+I model (six general time-reversible substitution rates, assuming gamma rate heterogeneity and a proportion of invariant sites) with maximum likelihood estimates of model parameters estimated during runs. Analyses were run with a model that specified all markers as separate partitions. Independent RAxML analyses per marker, and for combined cpDNA vs. nrDNA markers were tested for topological congruence using the de Vienne Congruence Index (de Vienne et al., 2007). Taxa of possible hybrid origin were pruned from the data matrix. We constructed a phylogeny under Maximum Likelihood (ML) optimization and ML bootstrap support values of >60 are included below the nodes (Fig. 3). More dense taxon sampling generally improves phylogenetic estimates, and the relative contribution of gene number and taxon number enhances phylogenetic accuracy (Zwickl & Hillis, 2002; Rokas & Carroll, 2005). The enhanced fully concatenated dataset and trees were submitted to TreeBASE (www.treebase.org; study number 162333).

Ecological niche modeling: All collection data of the genus *Nepenthes* were downloaded from the Global Biodiversity Information Facility (GBIF; < April 16, 2014>) from L, NY, US, KEP, NBC, SI and SING herbaria records. This dataset included 735 records covering 69 species. We pruned the sourced presence records (Table S2) to remove replicate species' data records at five arc-minute spatial raster cell resolution, and removed species with fewer than the five unique localities needed for ecological niche modeling (Pearson et al., 2007). This yielded 15 *Nepenthes* species for study, which were categorized as lower altitude when average occurrence records were below 750 m altitude (Miettinen et al., 2012) and as higher altitude when above this threshold. Some species within this categorization have occurrence records within a wide-ranged elevation, which may be partially caused by the wide latitudinal range necessitating the elevation range to stay within its preferred habitat, like is the case with *Nepenthes ampullaria* Jack. For consistency, we refer to these on average lower and higher altitude categorization as 'lowland' and 'highland' species. Occurrence record availability, due to either narrow endemism or low number of collection records, limited a wider scale analysis of the genus *Nepenthes*.

To build the ENMs, we combined species' locality data with soil data derived from International Soil Reference and Information Centre (ISRIC) (Batjes, 2012) and data on current climate from WorldClim (http://www.worldclim.org/) at a spatial resolution of 5 arc-minutes. To predict the impact of global climate change on the future distributions of *Nepenthes* species, we selected the global climate model from the Met Office Hadley Centre HadGEM2-AO, which includes Earth system components such as ocean biology and terrestrial carbon cycling in the model predictions (The HadGEM2 Development Team et al., 2011). This model involves an estimated 1.4 °C increase in temperature by 2070 within Southeast Asia, and ranks the highest in predictive performance for our study area (Baek et al., 2013). This model and the Intergovernmental Panel on Climate Change report (IPCC, 2013) estimate that Southeast Asia will face extremes in precipitation – with increase in monsoon duration, intensity and subsequent overall increase in precipitation, along with more drought susceptibility during the months of July–October. Regional studies (Alamgir et al., 2015; Scriven et al., 2015) show this climate scenario to have future diversity distributions comparable to other temperature predictions. The bioclimatic datasets for both present and future HadGEM2-AO conditions for the year 2070 at 5 arc-minute spatial resolution were downloaded from worldclim.org (Hijmans et al., 2005). To avoid issues with multicollinearity (Dormann et al., 2013), we selected uncorrelated climatic and soil data from a target background sample of all botanical collection localities from the study area (92°E-165°E, -15°S-22°N). From groups of correlated variables ($|$Spearman rho$| > 0.7$), we retained the most ecologically meaningful variables (i.e. the ability of carnivorous plants to thrive in nitrogen poor soil, for example) (Table 1).

We selected MaxEnt version 3.3.3k (Phillips et al., 2006; Elith et al., 2011) to model the potentially suitable habitat of the 15 *Nepenthes* species. MaxEnt uses presence-only data to identify potentially suitable species' distributions based on a probability distribution of maximum entropy, an approach that has been shown to outperform other modeling algorithms (Elith et al., 2006, 2011; Aguirre-Gutiérrez et al., 2013) especially when the sample size is small (Wisz et al., 2008). To account for collection bias, we applied the target background approach using all botanical collection localities from the study area (92°E-165°E, -15°S-22°N) as the target-background sample (Phillips et al., 2009). It should be noted that some areas within our study region, especially within the highlands, might have historically been or are currently inaccessible for collecting due to political or logistical restriction. The target background approach used minimizes this effect. We trained all ENMs using all presence records and 10,000 records sampled from the target background sample from the study area. To avoid model over-fitting, we set MaxEnt to use linear and quadratic modeling features only; hinge, product and threshold features were excluded following Merow et al. (2013). We did not partition the presence data into a training and test partition, because we tested the ENMs for significant deviation from random expectation against a bias-corrected null model (Raes & ter Steege, 2007). Testing against a null-distribution avoids the subjective interpretation of AUC values when applied to presence-only data and a background sample (Lobo et al., 2008) and closely resembles standard significance tests. We also report the AUC values for comparison purposes. The calibrated ENMs were subsequently projected on the current climatic conditions of the entire study area. The projected ENMs were used to assess the pairwise niche overlap between *Nepenthes* species using Schoener's *D* and Hellinger's *I* metric of niche overlap (Warren et al., 2008).

To estimate potentially suitable habitat of *Nepenthes* species, we accounted for dispersal limitation by projecting the ENMs onto the phytogeographical region(s) defined by van Welzen et al. (2005) in which each species is known to occur from its collection records. The nine phytogeographical sub-regions include the Malay Peninsula, Borneo, Sumatra, Java, Philippines, Sulawesi, Lesser Sunda Islands, Moluccas and New Guinea. The Australian Cape York and Indochinese Peninsulas were included as potential suitable habitat range for *Nepenthes mirabilis* (Lour.) Druce as its presence localities were also recorded for these regions. The calibrated ENMs were projected on both current and future abiotic (climate and soil) conditions for islands on which they are known to occur. For pictoral representation of the overlapping potentially suitable habitats of the current and future time scenarios, the continuous logistic MaxEnt predictions ranging from zero to one were converted in discrete presence–absence predictions using the 10 percentile training presence threshold. This threshold forces 10% of the presence records outside the predicted presence area. This is a conservative threshold that accounts for potential errors in the identification and georeferencing of specimens. Non-thresholded potentially suitable habitat maps of each species are provided in the supplementary material (Figs S1 and S2).

Genetic diversity and ecological divergence: To analyze ecological niche divergence, we calculated niche overlap in R with the phyloclim package (Heibl, 2011) using non-thresholded ENMs for the entire study area. This package calculates the statistical measure Schoener's *D* (Schoener & Gorman, 1968) and the similarity metric Hellinger's *I* (Hellinger, 1909). The corresponding estimates of evolutionary divergence, or Pairwise Genetic Distance Matrix, was made in MEGA 5.2.2 (Tamura et al., 2011) using pairwise distance estimation, nucleotide substitution including both transitions and transversions, p-distance method, uniform rates and pairwise deletion of gaps/missing data, including all codon positions. The analysis involved nucleotide sequences of 45 different taxa translated into 1st, 2nd and 3rd coding and noncoding codon positions and excluding all ambiguous positions, for a total of 4784 final characters.

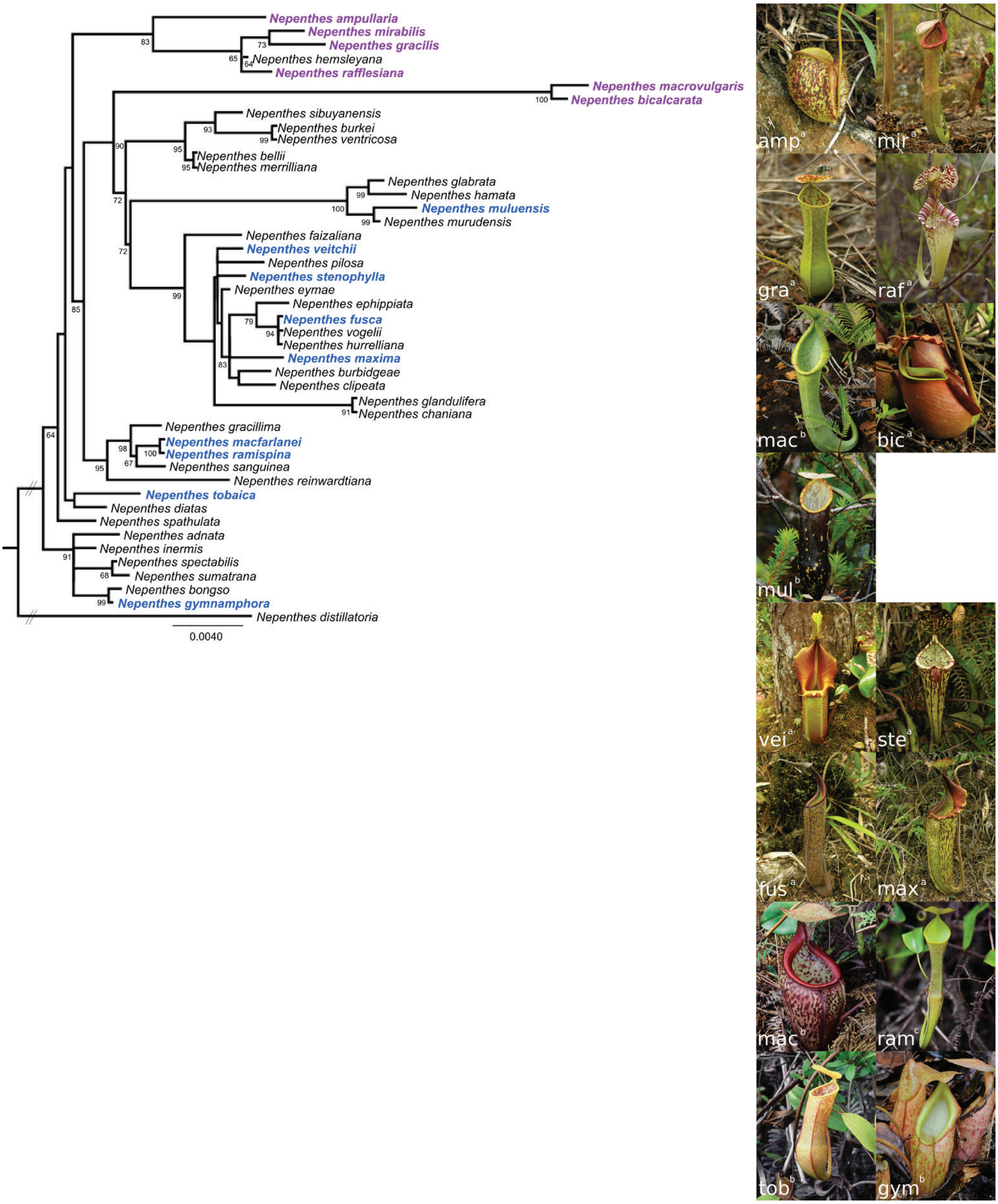

FIGURE 3. Pruned Maximum Likelihood phylogeny of *Nepenthes* based on a concatenated three-gene nuclear (nrITS and PTR1) and plastid (*trnK*) dataset. ML support values of >60 are shown below the corresponding nodes. Photos on the right illustrate pitcher morphology of lower altitude (pink) and higher altitude (blue) species and are represented by the first three letters of the species name (i.e. *Nepenthes ampullaria* is 'amp'). Pictures courtesy of Stewart McPherson[a], Chi'en Lee[b] and Alastair S. Robinson[c].

TABLE 1. Environmental variables used in ecological niche models.

Variable	Source	Description
Bio01	WorldClim	Max temperature of warmest month
Bio02	WorldClim	Temperature annual Range
Bio05	WorldClim	Precipitation of coldest quarter
Bio06	WorldClim	Min Temperature of Coldest Month
Bio13	WorldClim	Precipitation of Wettest Month
Bio15	WorldClim	Precipitation Seasonality (Coefficient of Variation)
Bio18	WorldClim	Precipitation of Warmest Quarter
Bio19	WorldClim	Precipitation of Coldest Quarter
ASLAT	ISRIC-WISE	Aluminum saturation (as % of effective CEC)
CECc	ISRIC-WISE	Cation exchange capacity of clay size fraction (cmolckg-1)
CFRAG	ISRIC-WISE	Course fragments % (>2mm)
CNrt	ISRIC-WISE	Carbon/nitrogen ratio
ELCO	ISRIC-WISE	Electrical conductivity (dS m-1)
ECEC	ISRIC-WISE	Effective cation exchange capacity
PHAQ	ISRIC-WISE	Soil reaction (phh20)
STPC	ISRIC-WISE	Silt mass (%)
TAWC	ISRIC-WISE	Total available water capcity. (from -10 to -1500kPa) (mm)
TOTN	ISRIC-WISE	Total nitrogen (% mass)

Results

Molecular phylogeny: The expanded and combined dataset increased resolution (i.e. discovered less polytomous species' relationships) from previously published single marker phylogenies (Meimberg et al., 2001; Heubl et al., 2006; Alamsyah & Ito, 2013) and encompassed all 15 species for which we had sufficient locality records for ecological niche modeling (Fig. 3). These species were sampled throughout the phylogenetic diversity of the genus, and the phylogenetic signal showed clustering of higher altitude and lower altitude clades. Our specimen altitude range coincided well with the referenced ranges of McPherson (2009). All major higher and lower altitude clades used in our study are well supported (ML bootstrap support values >64).

Current and future *Nepenthes* distributions: All 15 *Nepenthes* ENMs tested significantly better than random expectation with significantly non-random distribution ($P < 0.05$) and were used in the further analyses. We created potentially suitable habitat maps of *Nepenthes* species generated from MaxEnt predictions for both current and future climatic conditions (Figs 4 and 5). In general, higher altitude species have a much more narrow distribution compared to lower altitude species (Table 2). Not only is there simply less total highland surface area compared to lowland, as indicated by the grey-shaded highland regions in the corresponding figures, but highland *Nepenthes* species are also occurring only narrowly within the available highland area. Two-thirds of the higher altitude species were projected to lose suitably available habitat under future climatic conditions, losing on average 17% by 2070 (Table 2). In contrast, lowland species gained an average of 17% in potentially suitable future habitat (Table 2). Lowland species also have a higher proportion of areas available with shared potentially suitable habitat in the present and future than those in the higher altitude clade (Table 2, Figs 4 & 5).

Genetic vs. ecological divergence: We found significantly less ecological niche overlap among the higher altitude species than among the lower altitude species for both Schoener's *D* ($t = 4.42$, $P = 0.00003$) and Hellinger's *I* ($t = 4.14$, $P = 0.00007$). The average pairwise Schoener's *D* niche overlap between higher altitude species was 32% as compared with 55% for lowland species. Similarly, average Hellinger's *I* among higher altitude species was 54%, while the lowland species had an average of 78% overlap. Genetic divergence also varied significantly between higher altitude and lowland species comparisons ($t = 3.51$, $P = 0.00049$). Higher altitude species had higher molecular similarity to each other, on average 0.010 nucleotide differences per site, while comparisons among the lowland species had an average of 0.018 nucleotide differences per site.

We found a high Mantel correlation between genetic similarity and our estimates of the niche overlap among higher altitude species for both Schoener's *D* ($r = 0.55$, $P = 0.0016$) and Hellinger's *I* ($r = 0.61$, $P = 0.0001$). There was less support for the same positive Mantel correlation among lowland species for genetic similarity and Schoener's *D* ($r = 0.34$, $P = 0.09$) and for genetic divergence and Hellinger's *I* ($r = 0.34$, $P = 0.098$). When combining higher and lower altitude species into one dataset, the Mantel test indicated no statistical support for a correlation between genetic divergence and niche overlap. The complete matrices for our estimates of niche overlap and genetic divergence can be found in the supplementary Tables S3 & S4.

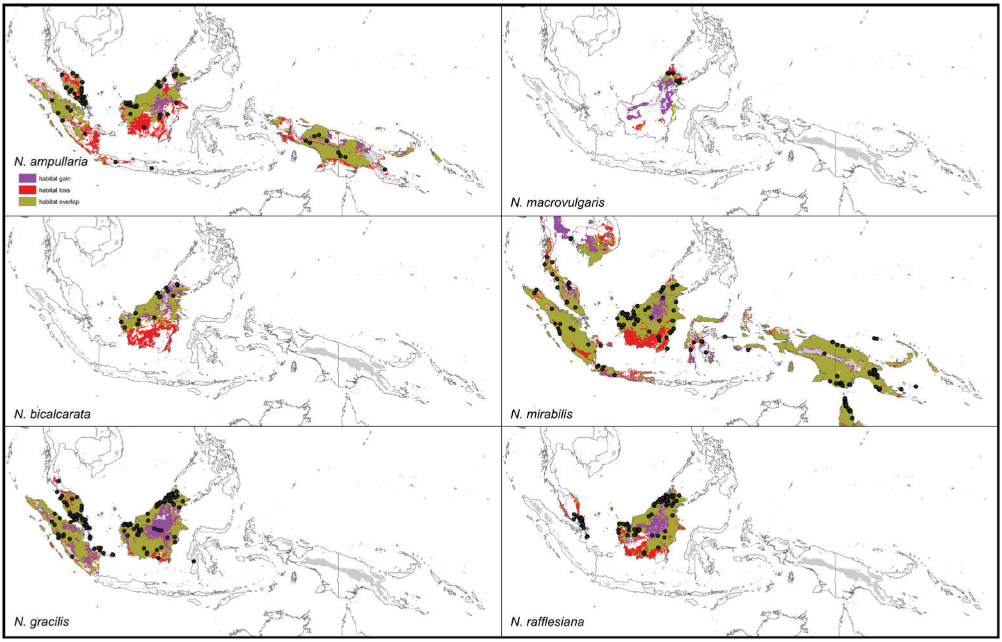

FIGURE 4. Predictive ecological niche models of lower altitude *Nepenthes* species inferred from presence localities (circles) and current environmental data, and then projected to the future (2070, HadGEM2-AO hd60bi70). Potentially suitable habitat projected on unit areas (van Welzen et al., 2005) already occupied by each species. Grey shading represents altitude above 750 m.

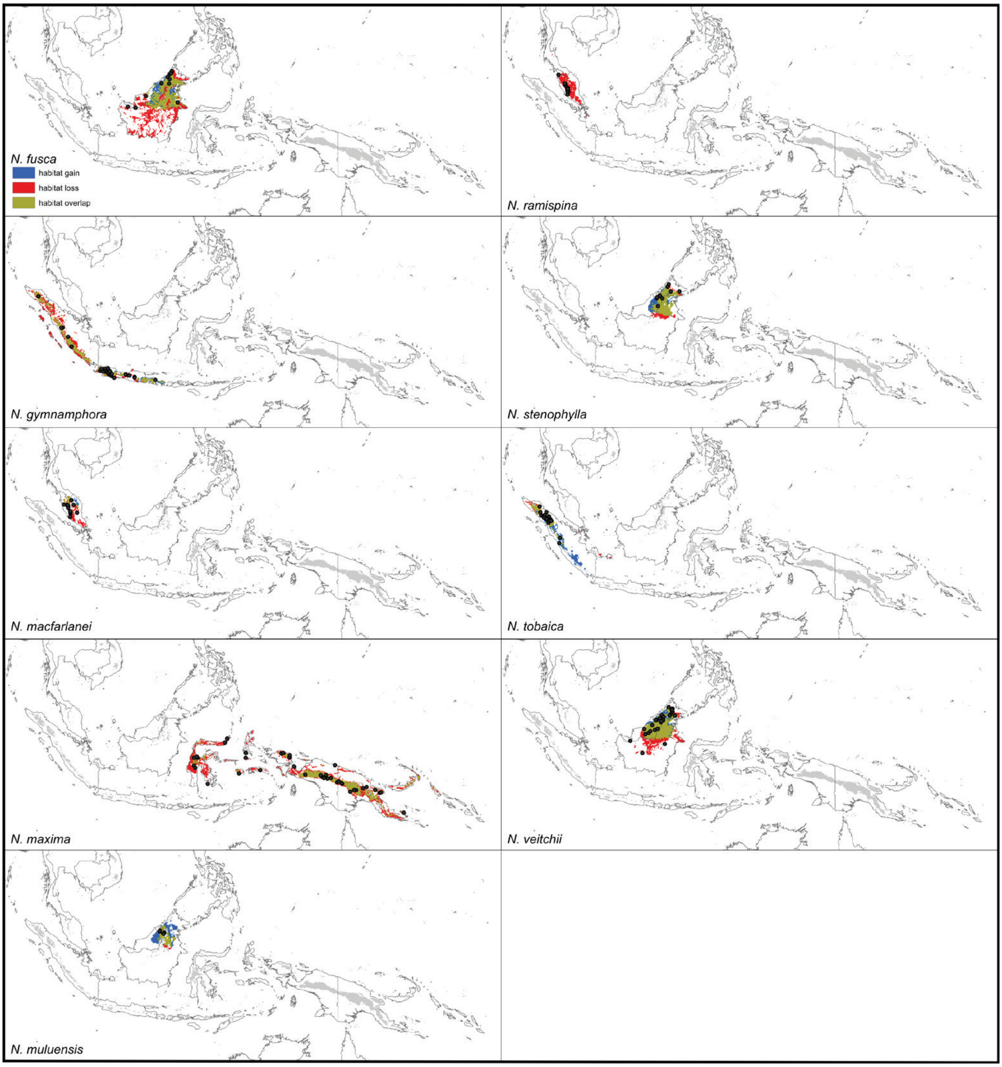

FIGURE 5. Predictive ecological niche models of higher altitude *Nepenthes* species inferred from presence localities (circles) and current environmental data, and then projected to the future (2070, HadGEM2-AO hd60bi70). Potentially suitable habitat projected on unit areas (van Welzen et al., 2005) already occupied by each species. Grey shading represents altitude above 750m.

TABLE 2. Potentially suitable habitat based on ecological niche models for *Nepenthes* in present and future climate (2070, HadGEM2-AO hd60bi70).

Species	Suitable present habitat (km^2)	Suitable future habitat (km^2)	Suitable habitat gain/loss (-) %	Suitable habitat overlap %
Lowland				
N. ampullaria	164630	137,300	-17	72
N. bicalcarata	52050	40,870	-21	63
N. gracilis	116390	142,200	22	94
N. macrovulgaris	7810	17,130	119	60
N. mirabilis	289570	293,350	0	88
N. rafflesiana	65390	65,630	0	76
Highland				
N. fusca	38910	22,600	-42	49
N. gymnamphora	24080	14,820	-38	56
N. macfarlanei	5380	2,340	-57	36
N. maxima	43770	20,080	-54	46
N. muluensis	4760	9,630	102	91
N. ramispina	7880	230	-97	3
N. stenophylla	14900	15,490	4	82
N. tobaica	6290	9,790	56	80
N. veitchii	27030	20,820	-23	69

Discussion

This is the first study that compares molecular proximity and ecological niche divergence in carnivorous pitcher plants. We found that molecular phylogenetic relatedness within our respective lower altitude and higher altitude clades of *Nepenthes* coincided with our estimates of ecological niche similarity. Lower altitude clades were more genetically diverged, but had greater overlapping ecological niches than those of the higher altitude, thus giving a unique phylogenetic signal for each clade. Differing rates and/or mechanisms of diversification might explain the positive correlations found in separate highland and lowland comparisons and the lack of phylogenetic signal in relation to ecological niche for the genus of *Nepenthes* as a whole. The lowlands having less topographical variation and more dispersion potential may play a contributing role in the distinguishable signals between the higher and lower altitudinal signals, along with the resolution scale and accuracy. Previous molecular studies favour the hypothesis that higher altitude species evolved from lowland species (Meimberg & Heubl, 2006). Liew et al. (2009) also found that the lowland land snails of Mt. Kinabalu historically migrated up the mountain, resulting in the formation of many highland endemics. We suggest that the relatively recent orogenesis of many Southeast Asian mountain chains (Hall, 1998) such as the Crocker range, which encompasses Mt. Kinabalu (Beaman et al., 2005), presented newly available niche space, and thus triggered a radiation of highland *Nepenthes* species (Merckx et al., 2015).

The rapid filling of unoccupied niches allows opportune diversifications, or adaptive radiations. The Hawaiian silver sword alliance (Baldwin et al., 1991) and Macaronesian flora (Francisco-Ortega et al., 1996) are two classic examples of how reduced competition, reproductive isolation and new environmental opportunities influence plant specializations. It can be inferred from the phylogenetic analyses presented (Fig. 3) that the lowland species are ancestral. A study of the evolution of the endemic *Nepenthes* of Mount Kinabalu (Merckx et al., 2015) and the following arguments support this. As similarly found in higher altitude *Nepenthes* species, the tall mountains of eastern Africa have acted as 'skyislands' in the rapid speciation of *Dendrosenecio* (Knox & Palmer, 1995). The higher altitude *Nepenthes* species echo the small genetic distance pattern displayed in the relatively recent, rapid radiations of the silverswords (Baldwin et al., 1998) and the New Zealand and Australian genus *Microseris* (Vijverberg et al., 2000). Also suggestive of this rapid and recent evolution are the short branch lengths of the higher altitude clade containing the '*Nepenthes maxima* Reinw. complex (Robinson et al., 2011) comprised of the morphologically and genetically similar species of *N. maxima*, *Nepenthes stenophylla* Mast. and *Nepenthes fusca* Danser (Fig. 3). As individuals with recent common ancestors have more common genes (Goodman, 1981), the lowland *Nepenthes* species is likely evolved in a more gradual manner and much longer ago, promoting more molecular divergence (Givnish, 1997). Figure 3 shows two notable patterns. The first is that higher and lower altitude clades branch off from one another. For example, the clade rooted on *N. ampullaria*, which includes taxa of lower average elevation, is followed by the branching off of the clade rooted on *N. reinwardtiana*, which includes higher altitude species from the Malay Peninsula. Subsequently, the lowland species *N. macrovulgaris* J.R. Turnbull & A.T. Middleton and *Nepenthes bicalcarata* Hook.f. branch off followed by a number of clades including higher altitude species from Borneo. If we do not assume a past, elevated connection between these highlands (there is no geological evidence that tells us we should), this suggests repeated colonization of highland habitats. The second notable pattern is that many clades that include higher altitude species have very short internodes and terminal branches. This suggests rapid diversification. In combination, these patterns are consistent with an underlying process of adaptive radiations that track orogenesis.

Likewise, genetic and ecological variation between the higher and lower altitude clades may also be attributed to different modes of speciation. Highland and lowland species may have undergone allopatric speciation within the higher altitude clade and sympatric speciation within the lower altitude clade. The former evolving due to stochastic processes that only require the absence of homogenizing gene flow, as caused by orogenesis, and the latter via adaptive processes in response to disruptive natural selection (Jakob et al., 2010; Couvreur et al., 2011). In addition to this, the extreme variability of micro-niches produced with the topographical diversity and elevational gradation of mountains (Takyu et al., 2003) might have provided montane species with higher ecological diversity as compared with lowland species - and therefore may have played a role in the lower niche overlap found among higher altitude species. Mountain formations play an important role in species diversification (Nürk et al., 2015). High niche diversity on mountains occurs within close distances, providing habitat potential for species with especially low dispersal ability such as *Nepenthes*. Mountain refugia during the rapidly changing Pleistocene environment (Gathorne-Hardy et al., 2002) may have promoted the broad elevation range of some lowland *Nepenthes* species (e.g. *N. ampullaria* and *N. mirabilis*), thus increasing accessibility to nearby habitat diversity. Along these elevation gradients, species richness often peaks at mid-elevations (Rahbek, 2004), with climatic variables largely influencing this variation (Rahbek, 1995; Lomolino, 2001). Bedrock and edaphic variability, which we found to be highly predictive of *Nepenthes* distribution, also peak at mid-elevation and are highly correlated with elevation and vegetation zones (Kitayama, 1992).

Our ENMs predicted potentially suitable habitat of *Nepenthes* species with significant confidence ($P < 0.05$). Their environment has contributed and continues to play an important role in determining geographical distributions within the genus (Clarke & Moran, 2011; Moran et al., 2013). We defined suitable habitat based on climate and soil characteristics and projected how the spatial distribution of suitable habitat may be altered by climate change. While persisting through major shifts in climate through the last 33 to 56 million years (Krutzsch, 1988), the distribution range for *Nepenthes* had a tumultuous and eventful geological history including many changes in sea level and climate within the Pleistocene and Paleocene epochs (Hall, 1998; Zachos et al., 2001). Our results show that the higher altitude species will lose a greater amount of potentially suitable habitat by 2070 compared to the lower species (Table 2). The majority of studied higher altitude species will face an overall loss of suitable habitat under the tested future climate scenario (Table 2) and will have a much smaller area of original habitat remaining in the future compared to lowland species. It is estimated that some habitats may disappear completely as a result of the current and predicted climate change (Williams et al., 2007). Thermal specializations in tropical species (Janzen, 1967; Huey & Webster, 1976) suggest further inabilities for highland *Nepenthes* to adjust to a changing environment as exposure to low variation in temperature reduces thermal tolerance (Addo-Bediako et al., 2000; Ghalambor et al., 2006; Araújo et al., 2013). In addition to this, higher altitude *Nepenthes* species are expected to be less drought tolerant based on more narrow vessel element widths (Carlquist, 1981) and more slowly growing compared to lowland species. The drought incapacities of higher altitude species could prove detrimental as shifts in precipitation are predicted to occur in the future (The HadGEM2 Development Team *et al.*, 2011). Tracking preferred habitat is a possible survival solution (Le Roux & McGeoch, 2008; Chen et al., 2009), although short distance escapes by lowland plants has been shown to be more restrictive than those in the more-closely graduated elevations of the highlands (Bertrand et al., 2011). If lowland *Nepenthes* species upscale the mountain to track preferred habitat, however, highland species face new competition. Survival of *Nepenthes* populations also depends on dispersal ability and many biotic interactions. Habitat shifts would need to account for interactions concerned with diet (Moran, 2001; Merbach et al., 2002; Clarke et al., 2009; Greenwood et al., 2011), symbiotic specializations (Rembold et al., 2010; Bonhomme et al., 2011b) and pollinators. Mobility and life history traits of animals and insects may contribute to arrival in their preferred habitat at an earlier stage, leaving gaps in the range shifts for these organisms and *Nepenthes*.

Potential suitable habitat occurring in both the present and the future scenario (Figs. 4 & 5) are likely to be the only areas supporting highland species' populations in the future as rapid migration within this genus is unlikely due to reproductive and dispersal constraints. Beyond changing climate, lowland species contend with disturbance potential because of greater accessibility and utility, and therefore more direct anthropogenic impact on habitat (Corlett, 2011). As assisted migration to newly established areas of preferred habitat remains controversial (Couvreur et al., 2011), we suggest that preservation of these overlap areas is critical for the conservation of highland species along with preservation of genetic diversity through live collections and seed saving.