This work is dedicated to Grace, for serious and riot reasons.

Evolutionary diversification of *Nepenthes* (Nepenthaceae)
Rachel M. Schwallier

Cover: *Nepenthes rafflesiana* silhouette on cyanotype.
Material kindly provided by Frederic Meijer Garden and Sculpture Park.

Layout design: Tiffany Neuman

This PhD research was made possible with financial support of:
Alberta Mennega Foundation
American Society of Plant Biologists
International Carnivorous Plant Society
P.E.O. Scholar Awards
Treub Foundation
Vernon I. Cheadle Foundation

Refer to web based depositions for all supplementary and extended data

Evolutionary diversification of *Nepenthes* (Nepenthaceae)

Proefschrift
ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op maandag 12 december 2016
klokke 10:00 uur

door

Rachel Marie Schwallier

At the still point of the turning world….
…Where past and future are gathered. Neither movement from nor towards,
Neither ascent nor decline. Except for the point, the still point,
There would be no dance, and there is only the dance.

From T.S. Elliot's Four Quartets

Promotor
Prof. dr. Erik F. Smets, Leiden University, Naturalis Biodiversity Center and KU Leuven

Co-promotors
Dr. Barbara Gravendeel, Leiden University, University of Applied Sciences Leiden and Naturalis Biodiversity Center
Dr. Niels Raes, Naturalis Biodiversity Center

Promotion committee
Prof.dr. Tinde van Andel, Wageningen University, Naturalis Biodiversity Center, Leiden University
Prof.dr. Peter M. van Bodegom, Leiden University
Dr. Steven B Janssens, Botanic Garden Meise (Belgium) and KU Leuven
Dr. Paul J.A. Kessler, Leiden University
Prof.dr. Menno Schilthuizen, Naturalis Biodiversity Center, Leiden University
Prof.dr. Marc S.M. Sosef, Botanic Garden Meise (Belgium)
Prof.dr. Herman P. Spaink, Leiden University

Table of Contents

Evolutionary diversification of *Nepenthes* (Nepenthaceae)

Rachel Marie Schwallier

General Introduction

On evolution towards insect eating plants

Botanical carnivory has long invoked curiosity and fascination. The horror and sometimes humor of flesh-eating plants peppers throughout Victorian Gothic and modern day kitsch with unnerving and enthralling splendor (Fig. 1a,b). Darwin himself called insect-digesting plants, 'one of the most wonderful plants in the world' (Darwin, 1875).

Over a century after Darwin's first experiments, the lure of carnivorous plants continues to entice researchers with questions of what exactly makes a carnivorous plant carnivorous to wider evolutionary history inquiries of biogeography and mechanics. Definitions of botanical carnivory vary. The most inclusive requires only that plant's leaves or roots absorb nutrients from decomposition of insects, leading to increased plant fitness (Chase et al., 2009). Other researchers propose that attraction, capture and digestion of prey must marry for carnivorous qualification (Ellison & Gotelli, 2009). With any interpretation, the carnivorous syndrome serves as a model of convergent evolution. Even the strictest qualification presents six independent appearances in disparate families of the angiosperm phylogeny (Ellison & Gotelli, 2001).

The driver of botanical carnivory unifies all families, whose insect propensity ignites from living in a habitat detrimentally low in nutrients. The insect trapping strategies for such novel nutrient acquisition, however, vary considerably with five distinct trapping mechanisms. The sticky mucilaginous flypaper traps like those of *Drosera* and the impetus snap traps of *Dionaea* are some of the most well known examples (Fig. 1c,d). Bladder traps, exclusive of *Utricularia,* and lobster pots of *Genlisea* force prey into inescapable digesting organs by suction or directionally encouraging hairs. The pitfall trapping genera *Nepenthes* (Nepenthaceae), *Cephalotus* (Cephalotaceae), *Sarracenia*, *Darlingtonia* and *Heliamphora* (Sarraceniaceae), use liquid-filled modified leaves that trap and digest insect prey in their enzyme rich slurry.

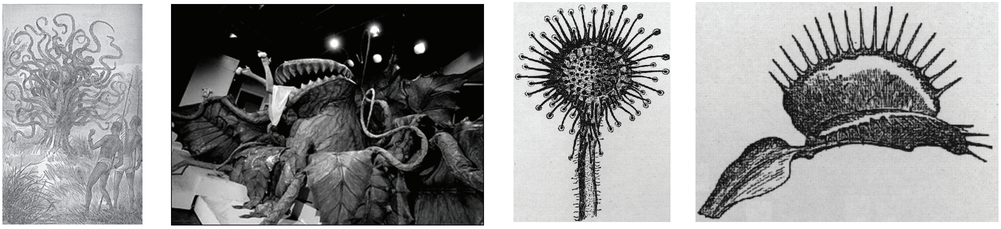

FIG 1a *FIG 1b* *FIG 1c* *FIG 1d*

FIGURE 1. a) Cryptid carnivorous man-eating plant described to occur in Africa and Central America in J.W. Buel's *Sea and Land*. b) The intergalactic plant from the 1960's musical *Little Shop of Horrors*. c) Flypaper trap of *Drosera rotundifolia* and d) snap trap of *Dionaea muscipula* (Darwin, 1875).

The iconic pitcher plants of Southeast Asia

The sole genus in the carnivorous plant family Nepenthaceae, *Nepenthes* L., includes around 140 species, several of which were described in the past five years (McPherson, 2009). Linnaeus (1937) coined the name *Nepenthes* over a century after its first record from Madagascar, and the name is as potent as the digestive fluid inside. 'Nepenthe' refers to the magical potion given to Helen in Homer's Odyssey to ease her grief and sorrow with forgetfulness. Upon discovery, Linnaeus thought the plants were so 'wonderful' and 'admirable' that they would quell the past ills of any botanist who encountered it.

Rightfully so, because *Nepenthes* has impressive, liquid-filled pitchers in spectacular shapes and forms used to attract, trap and digest insect prey (Fig. 2). The pitchers are the most recognizable and distinguishing characteristic of the plant and are formed from the tendril tip of the formed leaf. The dimorphic traps are prefaced as 'upper' and 'lower' pitchers based on the location on the plant and are morphologically and functionally distinguishable from each other (Moran, Booth, & Charles, 1999; Bonhomme et al., 2011; Bauer et al., 2012). Foraging, flying or crawling insects such as flies, ants or termites are attracted to the traps by visual lures such as anthocyanin pigments (Moran et al., 1999), extrafloral nectar bribes (Merbach et al., 2002) and olfactory cues (Di Giusto et al., 2010). Some species have remarkable prey specializations like *N. lowii,* which extracts nutrients from the feces of small mammals and

FIGURE 2. *Nepenthes lowii*, *Nepenthes ampullaria* and mosquito larva in the pitcher fluid of *Nepenthes rajah*. First photograph by R. Schwallier and the remaining two courtesy Christian Ziegler.

N. ampullaria, which acquires much of its nutrition from decomposing leaf litter that has fallen within its trap (Fig. 2) (Pavlovič et al., 2011).

Pitcher plants provide habitat to roosting bats (Bauer et al., 2011), frogs and snails along with their eggs (Das & Haas, 2010) and other aquatic invertebrates (Fig. 2) (Clarke & Wong, 1997). Additionally, there are some interesting relationships in which they provide food sources to animals. Ants (Bonhomme et al., 2011a) and spiders (Rembold et al., 2012) feed off prey scraps that fall into the pitchers, and small mammals eat nectar produced in the lid of some extraordinary, high mountain species (Chin et al., 2010).

Ecological and morphological diversity in *Nepenthes* were key motivators for the direction of studies in this thesis. The basic growth forms of *Nepenthes,* for example, range from a self-supporting rosette shrub to climbing and scrambling lianas (McPherson, 2009), growing in areas of almost perpetual moisture to those with menacing drought stress. Pollen fossils date the genus as far back as the Eocene from the then humid tropics of what is now France (Krutzsch, 1988), but current distribution is concentrated in the Malay Archipelago where populations are often small and hiding deep within the mountains. More minor extensions dot the coastlines of Madagascar, stretch into the far north of India and fleck pockets of Australia, Cambodia, India, Laos, Sri Lanka, Thailand and Vietnam (Cheek & Jebb, 2001; Meimberg & Heubl, 2006).

With the past unfolds the future

Evolutionary history and biodiversity are inexorably linked. The phylogenetic position of Nepenthaceae within the non-core Caryophyllales is supported by both nuclear and plastid gene sequences (Cuénoud et al. 2002; Brockington et al. 2009; Schäferhoff et al. 2009) in a monophyletic clade together with three other carnivorous plant families: Droseraceae, Drosophyllaceae and Dioncophyllaceae. The complex geological history of Southeast Asia, together with its tropical climate, was the perfect precursor to high species richness and endemism (Sodhi et al., 2010). In fact, Southeast Asia is identified as one of the most important regions to concentrate conservation efforts because of its ramping habitat destruction and huge species loss potential (Sodhi et al., 2010; Pacifici et al., 2015). The region fits the paradigm of a rapidly developing world, with agriculturally motivated deforestation and expanding urban spread. Southeast Asia makes up only 4% of the world's land area, but houses over 20% of the world's plant and animal diversity (Myers et al., 2000). Tacitly accepting these loses would be devastating on a global scale.

Although a recognized biodiversity hotspot and top priority for conservation, there are relatively few studies on the effects of anthropogenic climate change on the biota of Southeast Asia (Pacifici et al., 2015). The Intergovernmental Panel on Climate Change (IPCC) predicts that the climate of Southeast Asia will face unprecedented extremes in precipitation within this century (IPCC, 2014). *Nepenthes'* unique ecology and charismatic beauty make them ideal flagship organisms for conservation efforts, with potential to activate social awareness on both an economic and ecological level (Walpole & Leader-Williams, 2002). The plants are highly desired for hobbyists and botanical gardens worldwide, and generate valuable tourism income in their native areas. In addition to economic motivations for conservation, preservation of pitcher plant populations may also ensure stability of other mutualistically-associated organisms. *Nepenthes* are icons, with national parks and government organizations proudly displaying their pitchers on logos and highlighting them when local conservation efforts are touted.

These plants represent intrigue, curiosity and pride in diversity. With such worldwide interest and daunting habitat loss, *Nepenthes* are some of the most vulnerable plants in the world. Two-thirds of *Nepenthes* species documented on the IUCN Red List of Threatened Species are categorized as vulnerable, endangered or critically endangered (IUCN, 2015). Despite this high level of protection, very little is known about the evolutionary mechanisms driving diversity in *Nepenthes*, an important key to understanding the future resilience of the genus and protecting this culturally important plant. Because several *Nepenthes* species endemically occur in small numbers on high altitude populations (McPherson, 2012), extinction is a realistic outcome if these plants cannot adapt, tolerate or track to their preferred habitat. *Nepenthes*' ability to adapt to a drastically different climate is directly related to their anatomy; however, surprisingly little is known about the anatomical detail of the genus beyond its predatory structures. This work explores the diversity-invoking evolutionary history of the culturally rich plant family, *Nepenthes*, in order to motivate and gain insight into how peeking into the past can help guide the future.

Outline of this PhD thesis

The interplay between evolution and its role in diversification of *Nepenthes* binds the individual studies of this thesis. How evolutionary mechanisms and timing relate to molecular divergence and phylogenetic signal in the genus *Nepenthes* were investigated here. This in conjunction with distribution modeling and mapping anatomical characters – an approach that led to a broad understanding of why and how best to protect specific geographical areas for conservation of *Nepenthes*. All work was framed with an interdisciplinary approach, each chapter furthering exploration of the connective ties in how past evolutionary history and its impact on diversity helps us to predict future diversity. Each chapter is further described below.

Contributions towards understanding ecological niche diversity and phylogenetic signal in *Nepenthes*: In *Chapter 1*, we analyzed the relationship between ecological niche and genetic similarity from a molecular phylogenetic perspective. We asked (i) if highland and lowland species' realized niche and phylogenetic signals were distinct and (ii) what implications these results might have on future distributions in a changing climate.

Revealing the evolutionary mechanisms that created the endemic icons on Mt. Kinabalu: Our contribution to the large collaborative work in *Chapter 2* involved untangling the evolutionary origins of the *Nepenthes* species of Mt. Kinabalu. This was the first application of multi-taxon molecular phylogenetics for an entire tropical montane biodiversity hotspot. Our results considerably deepened the understanding of the evolution of endemism in general, as well as uncovered the origin of Borneo's biodiversity - of which *Nepenthes* plays a significant role.

Expanding the knowledge of *Nepenthes* wood anatomical diversity placed in a phylogenetic context: *Chapter 3* analyzed the relationships between anatomical wood characters, habit, abiotic preferences and phylogenetics. With the current pace of anthropogenic change, understanding the pliability and evolutionary response to shifting abiotic conditions is urgently important. Our work offered the most extensive anatomical work of this iconic genus to date and incorporated evolutionary perspective through character mapping at the genus and Caryophyllales order level.

Contributions in understanding the development of the uniquely dimorphic carnivorous traps of Nepenthaceae: Dimorphic leaf pitcher traps in carnivorous plants have evolved only once. In *Chapter 4,* we studied the ontogeny of upper and lower pitcher types based on 3D geometric-morphometrics and microscopic microstructure analysis. We investigated whether (i) these were overlapping forms throughout the progression of dimorphic pitcher development or distinct morphologically throughout development, (ii) if microstructures corresponded with progression in pitcher development and (iii) whether microstructure development was different in the two pitcher types.

Calling attention to the value of preserving *Nepenthes* for its cultural tradition: *Chapter 5* bridges the previous chapter's 'how to conserve' with an intimate story of tradition on 'why to conserve'. The practice of cooking a sticky rice snack inside a *Nepenthes* pitcher was called to our attention during fieldwork in Malaysia for research during previous chapters, and a new study was born on the spot. The work aimed to uncover and preserve the knowledge involved in the preparation and history of a luring carnivorous plant trap snack.

General discussion: The final piece in this work combines the most pertinent conclusions of each chapter. A reflective summary of the findings is given along with suggestions for future directions.

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

Rachel Schwallier, Niels Raes, Hugo J. de Boer, Rutger A. Vos, Rogier R. van Vugt and Barbara Gravendeel

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.

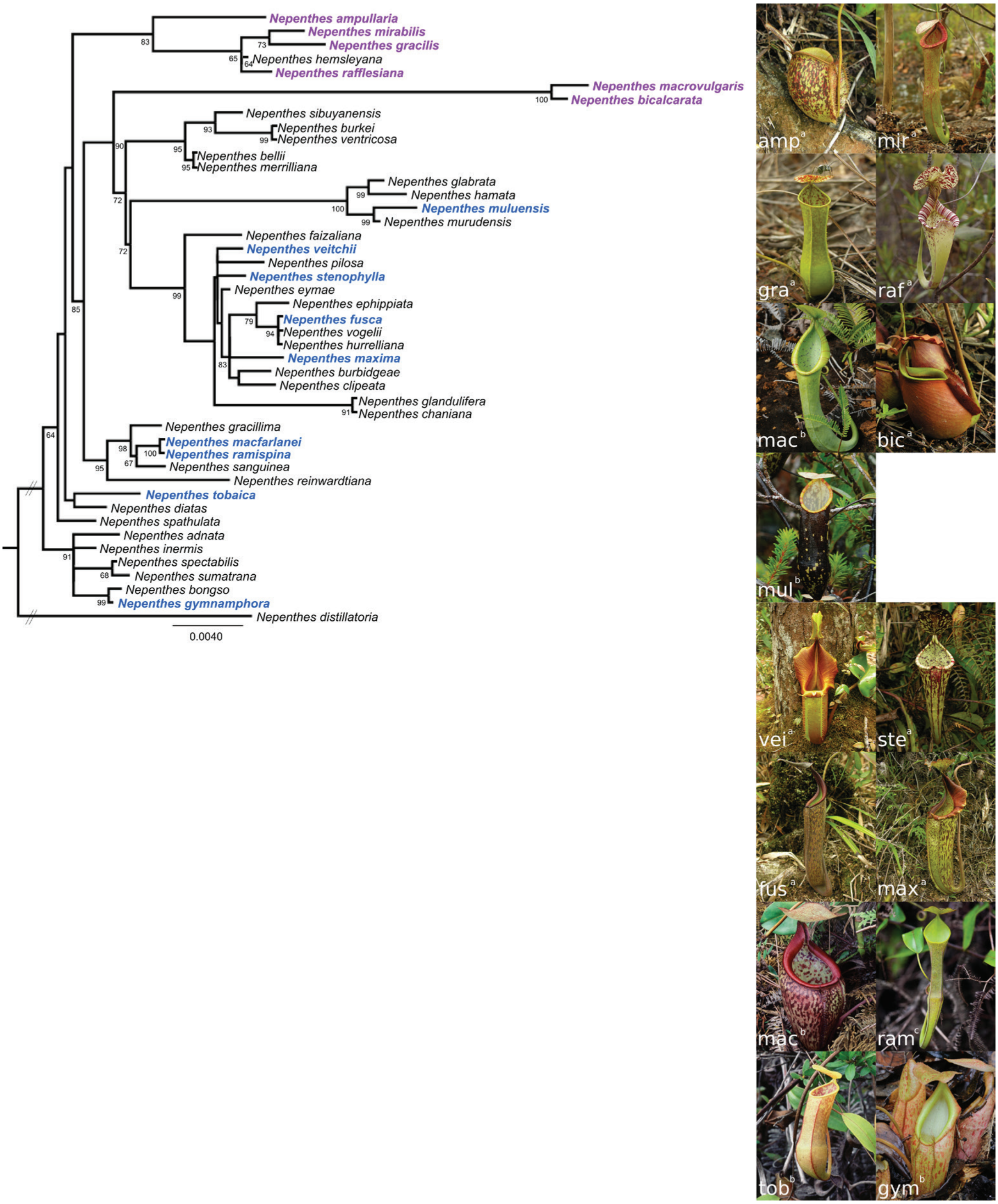

FIGURE 3. Pruned Maximum Likelihood phylogeny of *Nepenthes* based on a concatenated three-gene nuclear (nrITS and PTR1) and plastid (*trnK*) dataset. ML support values of >60 are shown below the corresponding nodes. Photos on the right illustrate pitcher morphology of lower altitude (pink) and higher 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.

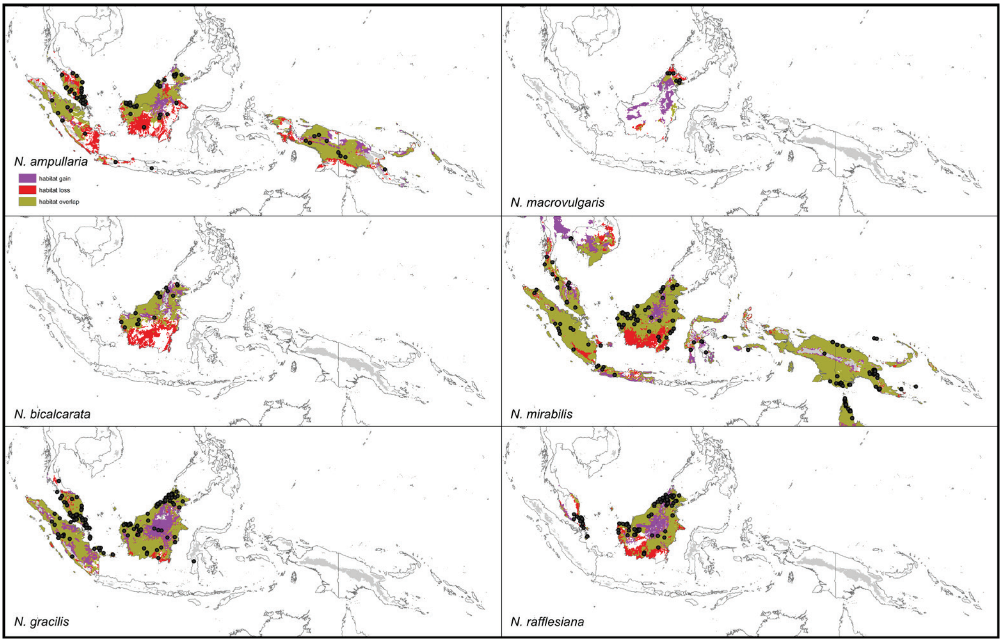

FIGURE 4. Predictive ecological niche models of lower altitude *Nepenthes* species inferred from presence localities (circles) and current environmental data, and then projected to the future (2070, HadGEM2-AO hd60bi70). Potentially suitable habitat projected on unit areas (van Welzen et al., 2005) already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

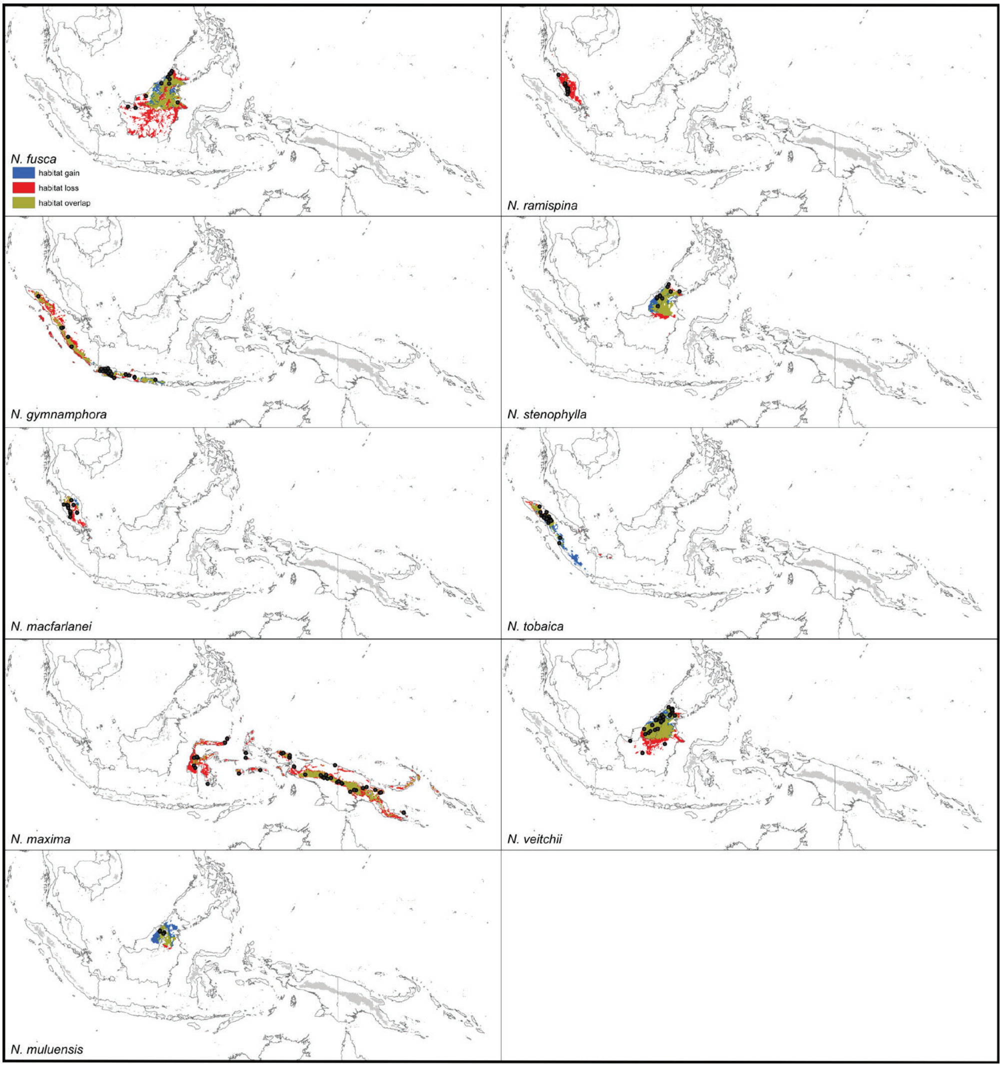

FIGURE 5. Predictive ecological niche models of higher altitude *Nepenthes* species inferred from presence localities (circles) and current environmental data, and then projected to the future (2070, HadGEM2-AO hd60bi70). Potentially suitable habitat projected on unit areas (van Welzen et al., 2005)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

Chapter Two

Modified from NATURE (2015) 524: 347-350

Evolution of endemism on a young tropical mountain

Vincent S. F. T. Merckx, Kasper P. Hendriks, Kevin K. Beentjes, Constantijn B. Mennes, Leontine E. Becking, Katja T. C. A. Peijnenburg, Aqilah Afendy, Nivaarani Arumugam, Hugo de Boer, Alim Biun, Matsain M. Buang, Ping-Ping Chen, Arthur Y. C. Chung, Rory Dow, Frida A. A. Feijen, Hans Feijen, Cobi Feijen-van Soest, Jo´zsef Geml, René Geurts, Barbara Gravendeel, Peter Hovenkamp, Paul Imbun, Isa Ipor, Steven B. Janssens, Merlijn Jocqué, Heike Kappes, Eyen Khoo, Peter Koomen, Frederic Lens, Richard J. Majapun, Luis N. Morgado, Suman Neupane, Nico Nieser, Joan T. Pereira, Homathevi Rahman, Suzana Sabran, Anati Sawang, Rachel M. Schwallier, Phyau-Soon Shim, Harry Smit, Nicolien Sol, Maipul Spait, Michael Stech, Frank Stokvis, John B. Sugau, Monica Suleiman, Sukaibin Sumail, Daniel C. Thomas, Jan van Tol, Fred Y. Y. Tuh, Bakhtiar E. Yahya, Jamili Nais, Rimi Repin, Maklarin Lakim & Menno Schilthuizen

Tropical mountains are hotspots of biodiversity and endemism, but the evolutionary origin of their unique biotas are poorly understood. Mountains may either act as “museums” where older lineages persist through evolutionary time, or as “cradles” where new species continue to be generated. We traced the evolutionary origins of endemism in 28 genera of plants, animals, and fungi on Mount Kinabalu (Malaysian Borneo), one of the richest biodiversity hotspots on earth. We demonstrate that most of its unique biota is younger than the mountain itself and evolved from a mixture of immigrant and local lowland ancestors. According to the molecular clock analyses, *Nepenthes* show centric endemism. Speciation of the four *Nepenthes* species endemic to Mount Kinabalu, *N. edwardsiana, N. rajah, N. villosa* and *N. x kinabaluensis*, appears to coincide with the timing of mountain formation.

Keywords: Nepenthes systematics, tropical ecology.

Tropical mountains are hot spots of biodiversity and endemism (Korner & Spehn, 2002; Chen et al., 2009; Graham et al., 2014), but the evolutionary origins of their unique biotas are poorly understood (Graham et al., 2014). In varying degrees, local and regional extinction, long-distance colonization, and local recruitment may all contribute to the exceptional character of these communities (Cadena et al., 2011). Also, it is debated whether mountain endemics mostly originate from local lowland taxa, or from lineages that reach the mountain by long-range dispersal from cool localities elsewhere (Rodriguez-Castaneda et al., 2010). Here we investigate the evolutionary routes to endemism by sampling an entire tropical mountain biota on the 4,095-metre-high Mount Kinabalu in Sabah, East Malaysia. We discover that most of its unique biodiversity is younger than the mountain itself (6 million years), and comprises a mix of immigrant pre-adapted lineages and descendants from local lowland ancestors, although substantial shifts from lower to higher vegetation zones in this latter group were rare. These insights could improve forecasts of the likelihood of extinction and 'evolutionary rescue' (Schiffers et al., 2013) in montane biodiversity hot spots under climate change scenariosIn mountainous areas of the humid tropics, steep environmental gradients coincide with high primary productivity and relative climatic stability to sustain large numbers of species, often with striking degrees of endemism at higher elevations (Korner & Spehn, 2002; Hoorn et al., 2013). It has therefore been recognized that tropical mountains are biodiversity hot spots of great conservation value (Korner & Spehn, 2002), especially because endemics on mountain tops are vulnerable to becoming trapped and then annihilated as a result of global warming (Chen et al., 2009; La Sorte & Jetz, 2010).

The evolutionary origins of these unique biotas, however, are poorly understood (Graham et al., 2014). Like other insular habitats (Warren et al., 2015), the endemic biota of an isolated mountain results from complex dynamics among colonization, *in situ* speciation, and local extinction. Each of these factors is dependent on the age and size of the habitat, and on the environmental contrast between the insular habitat and its matrix (Watson, 2002). In the case of a tropical mountain top, an added complication is the fact that climate fluctuations may have widened and restricted the geographic range over which the montane conditions have extended in the past, meaning that parts of the endemic biota may be relicts, and other components may be novel in character (Cadena et al., 2011; Graham et al., 2014).

Disentangling these possibilities for a single tropical montane biodiversity hot spot requires molecular phylogenetic study of a large number of fauna and flora elements. However, with only few exceptions (Madriñán et al., 2013; Ornelas et al., 2013), evolutionary studies in such hot spots have been limited to single taxa (Barkman & Simpson, 2001; Gawin et al., 2014). This precludes broad understanding of the evolutionary and biogeographic origins of an endemic biota as a whole (Graham et al., 2014).

We investigated the evolutionary routes to endemism by sampling an entire tropical mountain biota on the UNESCO World Heritage site of Gunung Kinabalu in Sabah, East Malaysia. Included in this are the iconic carnivorous plant genera *Nepenthes* (Table 3 & Figure 6). We demonstrate that most of its unique biodiversity is younger than the mountain itself and comprises a mix of immigrant pre-adapted lineages as well as descendants from local lowland ancestors.

TABLE 3. *Nepenthes* collected from the Kinabalu Park and Crocker Range Park expedition stations and their distribution.

Species	Distribution
Nepenthes chaniana	Borneo
Nepenthes edwardsiana	Endemic to Mount Kinabalu & neighboring Mount Tambyukon
Nepenthes fusca	Borneo
Nepenthes gracilis	Wide distribution across SE Asia
Nepenthes lowii	Borneo
Nepenthes mirabilis	Wide distribution across SE Asia
Nepenthes rajah	Endemic to Mount Kinabalu & neighboring Mount Tambyukon
Nepenthes tetaculata	Wide distribution across Borneo & Sulawesi
Nepenthes villosa	Endemic to Mount Kinabalu & neighboring Mount Tambyukon
Nepenthes x kinabaluensis	Endemic to Mount Kinabalu & neighboring Mount Tambyukon

FIGURE 6. *Nepenthes* endemic to Mount Kinabalu & neighboring Mount Tambyukon. From left to right, *N. edwardsiana, N. rajah, N. villosa, N. x kinabaluensis.* Photos courtesy of Rogier van Vugt.

At 4,095 m, Kinabalu is the tallest mountain between the Himalayas and New Guinea. It is a solitary 'sky island', having emerged during the Pliocene and early Pleistocene as a granite pluton within the surrounding sandstone of the Crocker Range, the latter having formed much earlier, between the Eocene and the early Miocene (Cottam et al., 2013). Because of the area's tectonic activity, as well as Pleistocene sea level changes, the exact historical progression of its elevation above sea level is not known, but it is likely that a major rise, even beyond today's elevation, of Kinabalu, as well as the central spine of the Crocker Range, took place between 6 million years ago and today (for more geological background see Methods). Since the early days of its exploration (Whitehead, 1893), Kinabalu has been famous for its extremely high biological diversity, especially its richness in endemic species, with endemism proportions reaching 25–30% for some taxa (Wong, 1996).

To unravel the origins of the exceptionally rich Kinabalu biota, we mounted a Malaysian–Dutch expedition in which 47 taxonomists worked at 37 localities, spanning the full range of elevations (Fig. 7). We used Sanger sequencing to sequence one or more fast-evolving loci for 1,852 individuals, belonging to 18 genera representing gastropods, annelids, insects, arachnids, vertebrates, pteridophytes, bryophytes, and angiosperms. We also obtained 3.7 million basidiomycete and glomeromycete ITS2 rDNA sequences from soil cores with ion semiconductor sequencing. In addition, we retrieved data from eight previously published single-taxon studies on vascular plants.

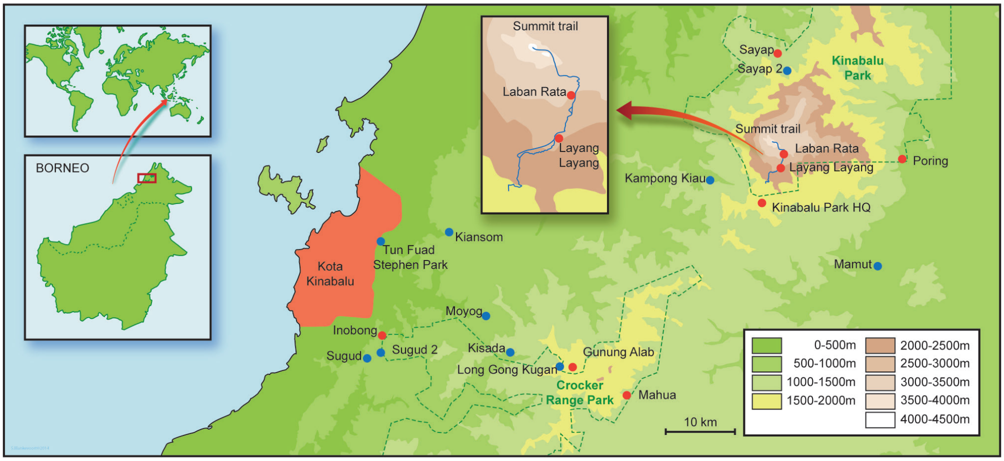

FIGURE 7. Map of the study area. Inset left, location of the study area in the World and in Borneo. Inset middle, detail of the summit trail in Kinabalu Park. The eight expedition stations in Kinabalu Park and Crocker Range Park are indicated with red markers, ten additional sampling sites with blue markers. Not indicated separately are 15 sites along the summit trail, and four sites very close to Mahua, Gunung Alab, and Inobong.

We analyzed all data within a phylogenetic framework to estimate the times of origin of endemic species, and to determine whether endemic species had descended from local or distant congeners (Methods, see web based publication online Fig. 2, Figs 8-10). Note that we define 'endemic' as restricted to the area in which our expedition took place. Although the present study offers the most comprehensive evolutionary analysis of any mountain biota to date, the taxa covered are, by necessity, an uneven and fragmentary sampling of the full diversity. Nonetheless, we expect that our results are representative for the Kinabalu biota as a whole, as our selected taxa encompass organisms with a wide variety of phylogenetic backgrounds, ecologies, and life history traits. Similar to Mesoamerican endemic cloud forest seed plants and vertebrates (Ornelas et al., 2013), our molecular dating results show that the estimated mean stem-node ages of 33 endemic species span a wide range, from 1.12 million years to 14.6 million years (Figs. 8,9 and web-based publication Extended data Figs. 7-9). However, 76% of these fall within the past 6 million years, the time span during which Kinabalu is likely to have reached its present elevation. Only two endemics, the frog *Kalophrynus baluensis* and the flowering plant *Ilex kinabaluensis*, are markedly older than the mountain itself. These may be explained as artefactual if we failed to identify the closest non-endemic sister lineage, thereby inflating their reconstructed age, or if these species are actually not endemics, but more widespread. Alternatively, they may truly be old endemics that evolved during cooler periods at lower elevations in Borneo before Kinabalu's formation.

Our phylogenetic and biogeographic analyzes (Extended Data Online Table 1 and Figs 1–4, 7–9) suggest the existence of two categories of endemics (van Steenis, 1964): 'eccentric' (12 taxa) and 'centric' (25 taxa). The eccentric type of endemic has sister taxa that occur either in temperate climates (seven cases) or in other tropical mountains outside of Borneo (five cases). To this group belong all bryophytes, pteridophytes, some of the fungal lineages and also the endemics in the flowering plant groups *Hedyotis*, *Ilex*, *Impatiens*, *Ranunculus* and *Euphrasia*, and the animals *Coeliccia* and *Tritetrabdella*. Eccentric endemics predominantly occur at high elevations (mean lower elevational boundary, 2,212 m; s.d., 837 m), they are strict Kinabalu endemics (they do not occur on nearby, lower mountains), and are further characterized by high dispersal capacities (one, two and seven clades with eccentric endemism have small, medium and large dispersal, respectively).

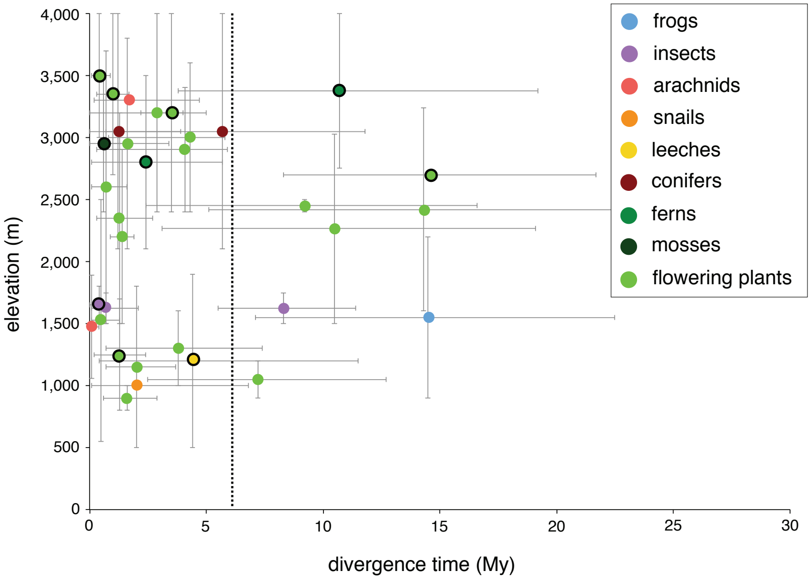

FIGURE 8. Elevations and ages for endemic species. Elevations (mid-points, minima and maxima) and dates of origination derived from molecular dating (averages and 95% credible intervals) for endemic species; eccentric species (see main text) are indicated with a black circle. The vertical dashed line indicates the oldest possible date for Kinabalu to have reached its current elevation. For details, see Extended Data Online Figs 5 and 6.

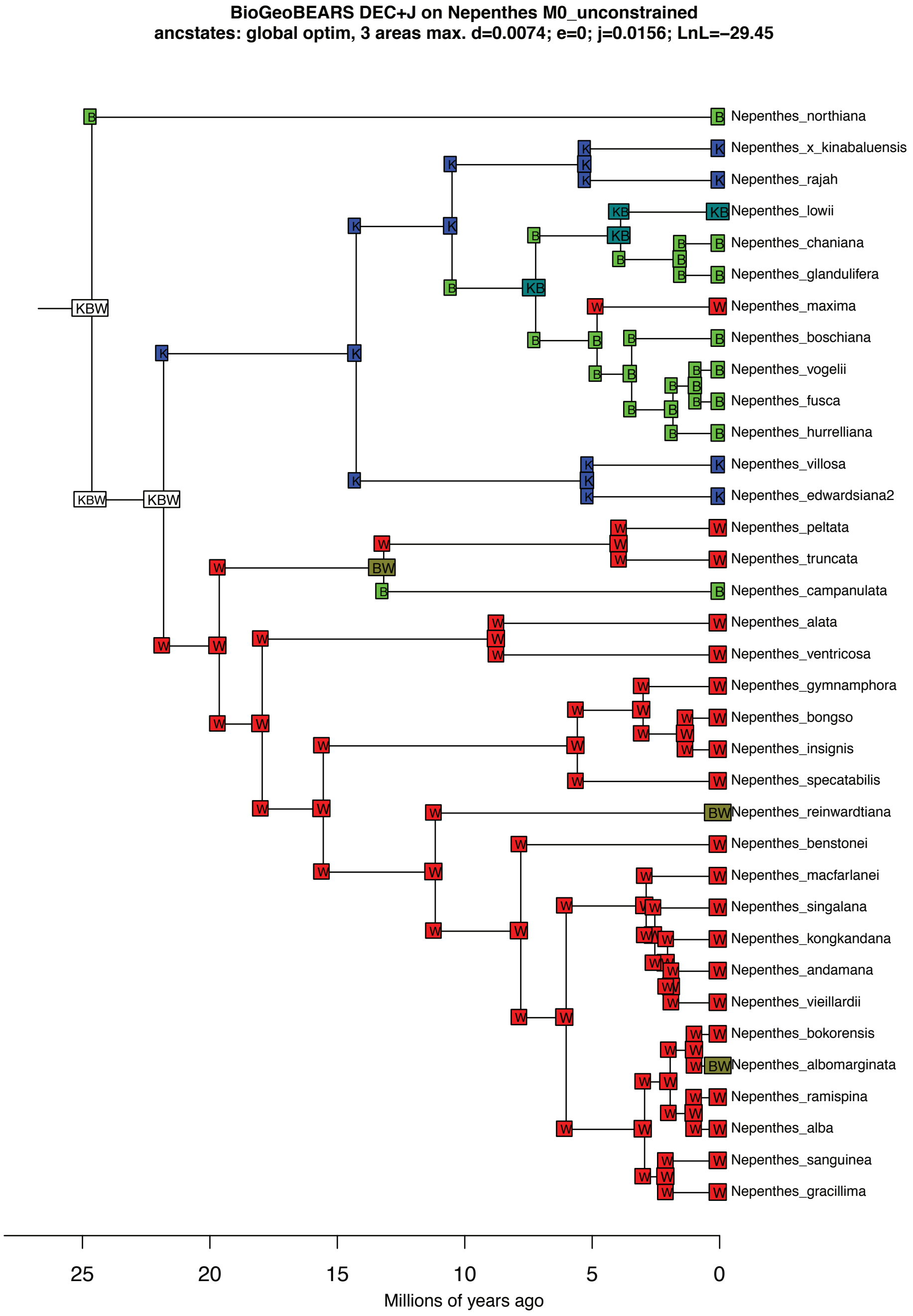

FIGURE 9. Ancestral range estimations for *Nepenthes*.

The centric type of endemic has sister taxa that occur locally, in the Borneo lowlands. Six out of the eight endemic animal species, some of the fungal lineages, the conifers, and 17 of the 20 flowering plants belong to this type. Centric endemics occur on average at lower elevations (mean lower elevational boundary, 1,724 m; s.d., 728 m) and tend to have lower dispersal capacities than the eccentric endemics (eight, seven and ten clades with centric endemism have small, medium and large dispersal, respectively). Some centric endemics, including *Nepenthes*, are not strict Kinabalu endemics, as they are also found on other mountains in Kinabalu's vicinity. Our ancestral state reconstructions (Supplementary Table 6 online) confirm a pattern of altitudinal bottom-up cladogenesis in the centric endemics, with 8 out of 11 species ranging partially or entirely outside of the 95% credibility interval for the reconstructed elevation of the most recent common ancestor, and the remaining 3 out of 11 falling entirely within this interval. When we performed the same analysis with Kitayama's (1992) seven vegetation zones, rather than elevation, we found that only three of these eight elevation shifts may represent a shift towards a higher vegetation zone. This suggests that, even in high-elevation centric endemics, substantial niche shifts are rare. Niche conservatism is probably even underestimated, since the Massenerhebung effect causes an

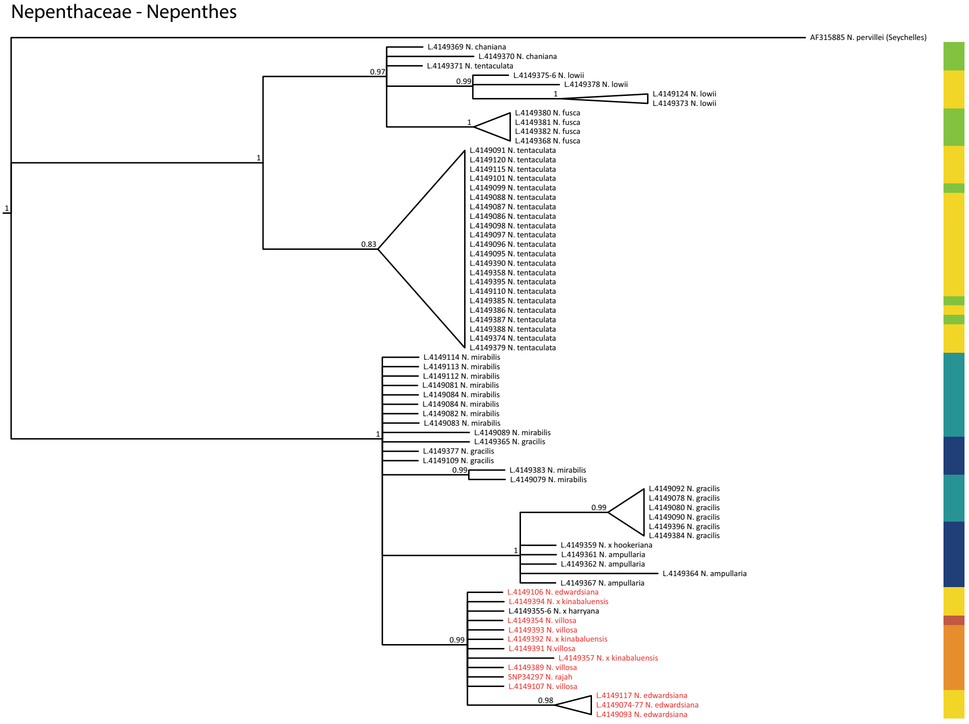

FIGURE 10. Phylogenetic reconstructions for Nepenthaceae.

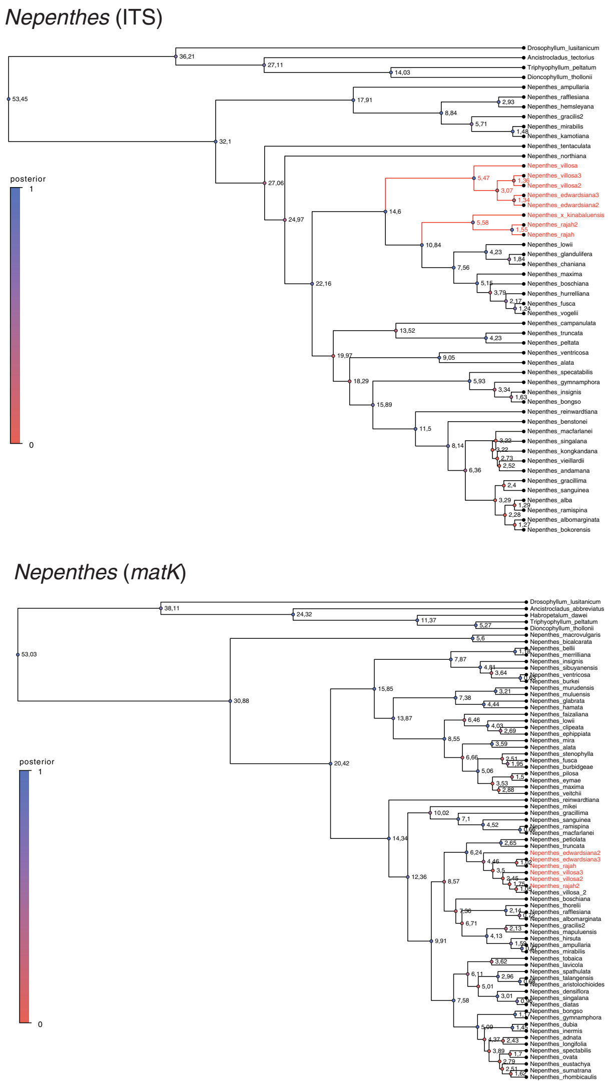

FIGURE 11. Chronograms for *Nepenthes*. Node colors represent posterior probabilities. Endemic species printed in red.

upward shift of vegetation zones on Kinabalu compared with other Bornean mountains (Grubb, 1971; Gawin et al., 2014). Niche conservatism indeed occurs in *Nepenthes*. The high altitude species *N. villosa*, occurs in the alpine zone and moss forest zone, and seems to be derived from clades of species occurring in the moss forest and submontane zones. Those ancestral clades in turn seem derived from species occurring in lowland forest in the separate nrITS and matK and combined analyses (Figs. 10, 11).Our multi-taxon study shows that Kinabalu's biodiversity hot spot is of recent origin. This means the mountain is an evolutionary cradle, accumulating neo-endemics, as has been suggested for other young, high-elevation biodiversity hot spots at low latitudes (Favre et al., 2014), such as the Tibetan plateau (Schwery et al., 2015), the Andean highlands (Hoorn et al., 2013), and Afrotropical volcanoes (Price et al., 2014). However, probably as a consequence of the rapid emergence of the mountain (Cottam et al., 2013) and its unique alpine summit conditions (Kitayama, 1992), many of these neo-endemics have not evolved by drastic niche shifts from local ancestors, but rather by immigration of pre-adapted propagules from elsewhere. This explains the multiple independent colonization events in some taxa (for example, *Glomus*, *Rhododendron* and *Coeliccia*). In addition, local lowland taxa have also generated montane species, but although some of these have reached vegetation zones above 2,000 m, most do not show substantial niche shifts away from their ancestral niche. The niches of *Nepenthes* species endemic to Mount Kinabalu and neighborying Mount Tambyukon appear to be conserved, coincide with the timing of mountain formation and are show a pattern of centric endemism. The fact that the endemic biota of Kinabalu appears to be composed largely of pre-adapted (eccentric) species and locally derived (centric), ecologically conserved endemics is in line with niche conservatism (Crisp et al., 2009).

We suggest that our novel approach of molecular dating of multiple clades be applied to larger communities in this and other tropical montane biodiversity hot spots (Culmsee & Leuschner, 2013). In combination, such information should allow a detailed dissection of the relative roles of ecological speciation, colonization and habitat filtering in the formation of endemic biotas in this and other tropical mountains. Moreover, such understanding could improve predictions of the likelihood of extinction and evolutionary rescue of endemic species experiencing changing climate conditions (Schiffers et al., 2013).

Chapter Three

Exploring wood anatomical diversity in *Nepenthes* and close Caryophyllales relatives

Rachel Schwallier, Barbara Gravendeel, Hugo de Boer, Stephan Nylinder, Bertie Joan van Heuven, Anton Sieder, Sukaibin Sumail, Rogier van Vugt and Frederic Lens

Nepenthes attracts wide attention with its spectacularly shaped carnivorous pitchers, cultural value and horticultural curiosity. Despite the plants' iconic intrigue, surprisingly little anatomical detail is known about the genus beyond its modified leaf tip traps. We explore the wood anatomical diversity of *Nepenthes* and assess it with a phylogenetic framework to investigate whether the wood characters within the genus are relevant from an evolutionary or ecological perspective, or rather depend on differences in developmental stages, growth habits, substrates or precipitation. Observations were performed using light microscopy (LM) and scanning electron microscopy (SEM). Ancestral states of selected wood and pith characters were reconstructed using an existing molecular phylogeny for *Nepenthes* and a broader Caryophyllales framework. Pairwise comparisons were assessed for possible relationships between wood anatomy and developmental stages, growth habits, substrates and ecology. Wood anatomy of *Nepenthes* is diffuse porous, with mainly solitary vessels showing simple, bordered perforation plates and alternate intervessel pits, fibres with distinctly bordered pits (occasionally septate), apotracheal axial parenchyma, and co-occurring uni- and multiseriate rays often including silica bodies. Abiotic conditions (soil type and precipitation) and growth habit (stem length) correlate with multiseriate ray height and width, vessel diameter and presence of silica grains. For Caryophyllales as a whole, silica grains, successive cambia, bordered perforation plates and helically banded idioblasts are the result of convergent evolution. Peculiar helical sculpturing patterns within various cell types occur uniquely within the insectivorous clade of non-core Caryophyllales. The wood anatomical variation in *Nepenthes* displays variation for some characters dependent on soil type, precipitation and stem length, but is largely conservative. The helical-banded fibre-sclereids that mainly occur idioblastically in pith and cortex are synapomorphic for *Nepenthes*. Other typical *Nepenthes* characters, such as silica grains and bordered perforation plates, evolved convergently in different Caryophyllales lineages.

Keywords: Ancestral state reconstruction, carnivorous plants, Caryophyllales, helically-banded idioblasts, *Nepenthes,* pitcher plants, silica grains, wood anatomy.

Introduction

Nepenthes L. is a monotypic genus of carnivorous woody plants including of 140+ species, with many described in just the last five years (McPherson 2012; http://www.ipni.org/, accessed 24 March 2016). Its centre of distribution is in the Malay Archipelago, but extends into Australia, Cambodia, India, Laos, Madagascar, Sri Lanka, Thailand and Vietnam (Cheek & Jebb, 2001; Meimberg & Heubl, 2006). This distribution range supports diverse growth habits, from robust lianas up to 20 meters tall to compact, woody rosette plants of only a few centimetres high (McPherson, 2009). *Nepenthes* are most widely recognized and identified by their impressive, liquid-filled pit-fall traps (Cheek & Jebb, 2001), whose main function is to lure, retain and digest insect prey. Some species have developed alternative feeding strategies, acquiring nitrogen from fallen leaf litter or the faeces of small mammals and birds (Moran et al., 2003; Chin et al., 2010; Greenwood et al., 2011). In spite of its iconic intrigue in the horticulture, tourism and research community, surprisingly little is known about the anatomical detail of the genus beyond its predatory structures. More information about the anatomical plant body of *Nepenthes*, whose dioecious character minimizes colonization potential (Baker, 1955), is desired in response to growing concern over the physiological pliability needed for plants with low ability to move along with a progressively changing climate gradient (Shaw & Etterson, 2012; IPCC, 2014; Merckx et al., 2015; Schwallier et al., 2016).

The Intergovernmental Panel on Climate Change (IPCC) predicts that the climate of Southeast Asia will face unprecedented extremes in precipitation within this century (IPCC, 2014). Consequently, information about drought tolerance of CITES protected species that grow as narrow endemics in very wet environments, like highland *Nepenthes*, is especially pertinent. Although no experimental studies on drought stress resistance have been carried out in the genus, it is to be expected that such narrow endemics are vulnerable to lethal levels of embolism formation in their water conducting cells when facing mild levels of drought stress (Choat et al. 2012). In combination with experimental studies, observations on wood anatomy could be integrated in mechanistic models to estimate survival in future climate scenarios, which is especially relevant to the narrowly endemic *Nepenthes* species that have range-confining abiotic and biotic-interaction variables (Clarke et al., 2009; Bonhomme et al., 2011a; Greenwood et al., 2011; Rembold et al., 2012; Merckx et al., 2015; van der Ent et al., 2015; Schwallier et al., 2016).

Anatomical studies of non-pitcher forming leaves, roots and stems of *Nepenthes* are available for only a very small number of species (Heinricher, 1906; Metcalfe & Chalk, 1950; Pant & Bhatnagar, 1977; Carlquist, 2010). One of the more interesting anatomical features observed in the genus are helical idioblasts (or 'spiral elements') in the leaves (Solereder, 1908; Metcalfe & Chalk, 1950), pith, cortex and rhizome rays (Metcalfe & Chalk, 1950; Carlquist, 2010) and in the stem cortex (Metcalfe & Chalk, 1950). The most seminal wood anatomical study of the genus investigated only three species, *N. ampullaria, N. lowii* and *N. x kinabaluensis* (Carlquist, 1981). With this, Carlquist reasoned that further investigation of additional species would not likely show more anatomical diversity, yet observation of just one additional species, *N. alata,* almost 30 years later (Carlquist, 2010), unveiled novel characters. In addition to this, *Nepenthes* species inhabit various elevations, climates and substrates throughout their distribution range (McPherson, 2012; Moran et al., 2013), all of which could reflect in variation of wood anatomy (Carlquist 1966, 1975; Baas 1976; Baas et al. 1983; Lens et al. 2011, 2013; Kidner et al. 2015). More thorough investigation of wood species spanning across the ecological and phylogenetic diversity is therefore desired.

Previous wood anatomical studies in other Caryophyllales families have revealed evolutionary informative characters, elucidating important taxonomical clarifications and insights in key innovations (Carlquist, 2010). The phylogenetic position of Nepenthaceae within the non-core Caryophyllales is supported by both nuclear and plastid gene sequences (Cuénoud et al., 2002; Brockington et al., 2009; Schäferhoff et al., 2009), in a monophyletic clade together with three other carnivorous plant families: Droseraceae, Drosophyllaceae and Dioncophyllaceae. This clade is characterized by a specific leaf habit with juvenile rosette forms elongating during maturation (Albert et al., 1992). Relationships within this carnivorous clade were poorly resolved in the first phylogenetic studies, but more recent multigene analyses indicate a potential sister group relationship between Nepenthaceae and Droseraceae, still with poor support (Schäferhoff et al., 2009; Soltis et al., 2011).

Here, we present a detailed wood anatomical survey of 40 *Nepenthes* species covering a wide range in altitude, life form and climatic/edaphic preferences, thereby increasing our anatomical knowledge of the genus significantly. In addition to these novel wood descriptions, our observations are confronted with an existing phylogenetic framework at the genus level and beyond to assess the evolutionary history of selected wood characters. Furthermore, we explore whether differences in developmental stages of the stem, growth habit and abiotic preferences have an impact on stem anatomical variation, as has been demonstrated in various woody angiosperms (Carlquist 1966, 1975; Baas 1976; Baas et al. 1983; van den Oever et al. 1981; Noshiro and Baas 2000; Lens et al. 2004, 2005, 2008, 2011; Olson et al. 2014; Kidner et al. 2016).

Methods and materials

In total, wood samples of 40 *Nepenthes* species were collected representing all major subclades within the genus based on the present phylogenetic knowledge (Heubl et al. 2006; Alamsyah and Ito 2013; Merckx et al. 2015; Schwallier et al. 2016). Specimens were derived from living plants as follows: five species were collected in the field in Borneo and one in Madagascar, and nine were sourced from the living collection of the Hortus botanicus in Leiden. Twenty-five samples were harvested from the dried herbaria material of Naturalis Biodiversity Center (n = 20) and the Sabah Parks Herbarium (n = 5) (Supplementary Data Table S1).

Wood from living plants was harvested at the base of mature plants. To increase our sampling, we also used herbarium material, which is most often collected further from the plant base. More juvenile herbarium branches/twigs, therefore, were the only available stems in these samples (Supplementary Data Table S1). Categorization of wood juvenilism was assessed for each species (Table 4) based on the amount of wood formed in each of the specimens. Since wood formation is never pronounced within *Nepenthes*, we considered a sample to be mature when there were at least 20 rows of wood cells, which clearly defined the herbarium samples from the more mature field/greenhouse samples. Our observations in sampling the entire stem of the mature *N. mirabilis, N. rafflesiana* and *N. reinwardtiana* showed a strikingly similar wood anatomy from the base towards the stem apex where upper pitchers were growing (100+ cm from base), which validated inclusion of juvenile samples into our assessment. *Nepenthes campanulata* and *N. clipeata*, the only two small herbaceous species within the genus that never form tendrils, are rare in cultivation and had to be excluded from the study due to the necessity of deleterious sampling.

Wood sections of 25 μm in thickness were made using a sledge microtome (Reichert, Germany). Preparation of sections and macerations follows Lens et al. (2005). Sections were observed using a Leica DM2500 light microscope and photographed with a Leica DFC-425C digital camera (Leica Microscopes, Wetzlar, Germany). Wood surfaces for SEM observations were platinum-palladium-coated with a sputter coater (Quorum Q150TS Quorum Technologies, Laughton, United Kingdom) and observed with a Jeol JSM-7600F field emission scanning electron microscope (JEOL Ltd., Tokyo, Japan). For this study, we use the wood anatomical terminology of the IAWA list of microscopic features for hardwood identification (IAWA Committee, 1989). In alignment with this, fibre-tracheids are defined as long, imperforate cells with more than one row of distinctly bordered pits in tangential and radial walls. Because of the combination of mainly solitary vessels and imperforate cells with many, large bordered pits, Carlquist (1981) calls these imperforate cells tracheids under the assumption that they are able to conduct water if a sufficient number of vessels embolize (Carlquist, 1984). Because hydraulic studies have not been carried out in the genus, we prefer to name the imperforate cells fibre-tracheids.

Nepenthes sequences of the nuclear ribosomal marker nrITS and the plastid marker *trnK-matK* were derived from previous studies and NCBI GenBank (Supplementary Data Table S2). A Caryophyllales alignment was obtained from Soltis et al. (2011) based on 17 genes representing the nucleus, plastid and mitochondrion genomes. Sequences were aligned automatically using MAFFT v.7.237 (Katoh et al., 2002) as implemented in AliView v.1.14 (Larsson, 2014). Character trait mapping and phylogenetic analyses were performed in two separate analyses, within *Nepenthes* and across selected genera within the Caryophyllales, using BEAST v.1.8.2 (Heled and Drummond, 2010; Drummond et al., 2012) on the CIPRES portal (Miller et al., 2010).

For the *Nepenthes* analysis, nrITS and *trnK-matK* were analyzed independently rather than concatenated due to the extensive levels of hybridization between *Nepenthes* species (Clarke & Wong, 1997; McPherson, 2009). For the independent analyses of nrITS and *trnK-matK* matrices, speciation patterns were described using a Birth-Death tree prior (Gernhard, 2008). Test for best fit substitution model was performed using PartitionFinder v1.1.1, only testing for models implemented in the BEAST software bundle, resulting in TN93 with equal base frequencies and gamma being selected for nrITS, and HKY with estimated base frequencies and gamma in *trnK-matK*. Markov chain Monte Carlo (MCMC) chains were run for 10 million generations, sampling parameters every 1000 generations. Tracer v.1.6 (Rambaut et al., 2014) was used to assess effective sampling sizes (ESS) for all parameters and to decide the percentage of burn-in for tree constructions. Two independent runs per marker were carried out in BEAST, and combined using LogCombiner v.1.8.2 (part of the BEAST software bundle). The combined set of posterior topologies were summarized as maximum clade credibility (MCC) tree using TreeAnnotator v.1.8.2 (also part of the BEAST software bundle).

Mesquite v.2.75 (Maddison & Maddison, 2011) was used to prune the topologies of species lacking wood data to create a set of empirical trees to use for the wood anatomy trait optimization. Three wood characters: axial parenchyma distribution, presence of septate fibres and silica presence in ray cells; the two pith characters: pith lignification and presence of medullary bundles; were added as five separate trait partitions to be optimized together with the topology as described above. The empirical trees created with the full species dataset were selected for in TreeAnnotator as the 'target tree' so that the inferred topology was based on the most robust dataset available. Character trees were visualized in FigTree v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

For the Caryophyllales level ancestral state reconstructions, the analyses were set up as described above but instead included wood anatomical characters more informative at the genus level, i.e. presence of silica bodies, type perforation plate border, successive cambia and spiral thickening presence and location referenced from literature (Supplementary Data Table S3). A trait was considered present if it was recorded in at least one species within each genus. To fit with character optimization, this alignment was pruned to only include genera with woody species that had wood characters described for at least two of the four characters of interest. The Soltis et al. (2011) molecular phylogeny included 31 of the 33 families of Caryophyllales, 24 of which were eventually included in our analysis. Based on model test results, substitution models were set to GTR with estimated base frequencies and gamma being selected, while remaining settings were identical to the previously described *Nepenthes* wood anatomy character optimization.

Pairwise comparisons of measured wood anatomical characters against precipitation variables, juvenile wood samples, referenced maximum stem length and occurrence on different soil types, were made using the Pearson correlation coefficient. To estimate potential of drought exposure, we extracted BIOCLIM variables (http://www.worldclim.org/) at 2.5 arc-minute spatial raster cell resolution for annual precipitation and mean temperature of driest month from a total of 930 localities for the species for which we have studied wood samples. Locality data were downloaded from the Global Biodiversity Information Facility (GBIF; < February 13, 2015>) from L, NY, US, KEP, NBC, SI and SING herbaria records. Extractions were made in QGIS v2.8 (<http://www.qgis.org/en/site/>). Referenced maximum stem length and soil type (whether occurring on ultramafic soil or not) was extracted from the descriptive texts of McPherson (2009) and the International Union for Conservation of Nature (IUCN) (2015) (Supplementary Data Table S4).

Results

Wood description: All values for the *Nepenthes* genus-wide wood description are provided as averages, with minimum and maximum values in parentheses. Detailed species-specific observations can be found in Table 4.

The diagnostic summary of the genus is as follows: Growth ring boundaries absent in all species, with the exception of an indistinct growth ring in *N. khasiana* (Fig. 12A) and *N. rajah*. Wood diffuse porous. Vessels almost exclusively solitary with simple perforation plates (Fig. 12C); vessel elements (15)–35–110–(170) μm in tangential diameter, (150)–215–490–(730) μm in length, and (8)–12–55–(64)/mm^2. Intervessel pits alternate (Fig. 12D), pits 5–7 μm in horizontal diameter. Gums occasionally present in *N. ampullaria, N. bokoriensis, N. chaniana, N. gymnamphora, N. khasiana, N. madagascariensis, N.*

rafflesiana, N. rajah, N. sanguinea and *N. villosa*. Sculpturing patterns on inside vessel walls absent. Fibre-tracheids thin- and thick-walled combination or thick-walled, (250)–415–770–(950) µm long with distinctly bordered pits of 5–6 µm in horizontal diameter in both tangential and radial vessels; scarce septate fibres in *N. ampullaria, N. hemsleyana, N. khasiana, N. lamii, N. lowii, N. mirabilis, N. pervellei, N. rajah, N. rhombicaulis, N. tentaculata* and the yet unnamed Thai *N.* sp. Axial parenchyma diffuse-in-aggregates, sometimes forming incomplete short bands of 1–2–(3–8) cells wide in *N. ampullaria, N. gracillima, N. gymnamphora, N. hirsuta, N. khasiana, N. madagascariensis, N. maxima, N. mirabilis, N. sanguinea, N. thorellii* and *N. tomariana*; clear banding pattern of 1–2–(3–10) cells wide observed in *N. bokoriensis, N. burbidgeae, N. chaniana, N. hemsleyana, N. lowii, N. rafflesiana, N. rajah, N. rhombicaulis, N. smilesii* (Fig. 12E)*, N. veitchii, N. ventricosa, N. villosa* and the yet unnamed Thai *N.* sp. Axial parenchyma strands of 2–3–(4) cells; *N. ampullaria* and *N. lowii* additionally included fusiform axial parenchyma; little axial parenchyma observed in *N. tobaica*; scarcely scanty paratracheal in several species. Rays exclusively uniseriate in *N. bicalcarata, N. burbidgeae, N. hirsuta, N. kerrii, N. muluensis, N. neoguinensis, N. pilosa, N. stenophylla, N. tentaculata, N. tobaica* and *N. veitchii*; 3–18 rays mm^{-1}, (100)–185–1090–(2600) µm long. Uniseriate and multiseriate rays present in the other species (Fig. 13A); multiseriate rays usually 2–(3–4) seriate, occasionally up to 14-seriate in *N. bokoriensis* and *N. tomariana*; (0)–1–6 rays mm^{-1}, (150)–190–1500–(3900) µm long. Rays usually composed of upright or square cells, sometimes in combination with procumbent cells. Silica in ray cells was found in most species studied (Fig. 13C–D) and additionally in the axial parenchyma of *N. rafflesiana*. Helical idioblasts present in the multiseriate rays of *N. gymnamphora, N. khasiana, N. lowii, N. rafflesiana* and *N. rajah*.

Stem parts outside wood cylinder: Pith composed of wider parenchyma cells in the centre, surrounded by an outer zone of narrower, lignified cells. The level of pith lignification varies (Table 4). *Nepenthes chaniana, N. madagascariensis, N. sanguinea* and *N. tentaculata* are barely lignified with few, thin-walled lignified cells. The majority of species have either slight pith lignification with many thin-walled lignified cells (n = 14) or markedly lignified pith with thin- to thick-walled cells (n = 15). The latter cells are intermediate between parenchyma cells and fibres, and are usually septate. This intermediary cell-type is also present in the four most markedly lignified, thick-walled pith cells of *N. macfarlanei, N. muluensis, N. stenophylla* and *N. tobaica*. Helically banded fibre-sclereids (Fig. 13E, F) are present in the pith in all species except *N. bokoriensis, N. edwardsiana, N. lamii* and *N. maxima*. Medullary bundles are present in the pith of *N. burbidgeae, N. macfarlanei, N. pilosa, N. reinwardtiana, N. sanguinea, N. stenophylla, N. tobaica* (Fig. 13G) and *N. veitchii*. Helical idioblasts were present in the cortex of all species for which we could section parts of the cortex (n = 14) (Fig. 13H). The helical idioblasts can be very thin- to very thick-walled, depending on the species. Silica grains were also observed in the secondary phloem of the species for which secondary phloem was sectioned. Crystal druses were found in pith cells of *N. rhombicaulis.*

Correlations with developmental stem stages, growth habit and abiotic preferences: Complete pairwise comparison data and results are presented in Tables S4 and S5 online with supported correlations described below. Juvenile wood specimens had higher pith lignification than mature specimens (r =0.27, N=39, $p < 0.05$) and had lower ray width (r =0.29, N=39, $p < 0.05$). Species referenced to grow on ultramafic soil had an average multiseriate ray height shorter than species not referenced to grow on this soil type (r =0.31, N=39, $p < 0.05$). Species with longer referenced stem lengths had larger multiseriate ray height maximums (r =0.27, N=39, $p < 0.05$). Maximum vessel diameter and ray width were greater when precipitation in the driest month of the year was higher (r =0.27, N=39, $p < 0.05$ and r = -0.26, N=39, $p < 0.05$, respectively). Multiseriate ray height average and maximum were higher with greater annual precipitation (r =0.28, N=39, $p < 0.05$ and r =0.30, N=39, $p < 0.05$, respectively)

Reconstruction of wood and pith ancestral states: The wood and pith characters optimized on the *Nepenthes* phylogeny are presented in Figs. 14 and 15. Posterior support values generated by the BEAST analyses are indicated on Figs. 14 and 15 as icons when Bayesian posterior probabilities (bpp) ≥ 0.80 and ≥ 0.90. There is no single wood character that defines one entire subclade. Silica grains (Figs. 14A and 15A), for example, are lost seven times throughout the *trnK-matK* phylogeny. Markedly lignified pith (Figs. 14B and 15B) is present in a number of independent clades in both *trnK-matK* and ITS. Likewise, presence of occasional septate fibres (Figs. 14C and 15C) is scattered throughout the phylogeny. Seven of the eight species with medullary bundles also have a marked lignification of the pith (Figs. 14C and 15C). Clear axial parenchyma bands (Fig. 14D and 15D) and medullary bundle presence in the pith (Fig. 14B and 15B) are derived features that evolved multiple times independently.

Character optimizations for a selection of woody genera in Caryophyllales are presented in Fig. 16. Posterior support values generated by the BEAST analyses are indicated on Fig. 16 as icons when Bayesian posterior probabilities (bpp) ≥ 0.80 and ≥ 0.90. The most striking evolutionary trend is the diversity of helical sculpturing patterns in the carnivorous clade, with helical idioblasts in pith and cortex (and occasionally the rays) of *Nepenthes* (Fig. 16B). Other typical *Nepenthes* features, such as the presence of silica grains, have evolved convergently within the order (Fig. 16A). Successive cambia (Fig. 16C) and non-bordered vessel perforation plates (Fig. 16D) have evolved in numerous Caryophyllales families independently as well.

TABLE 4. Overview of selected anatomical wood characters of Nepenthaceae. Values reported between hyphens are mean values with flanking min and max. Ray composition reported as (1) upright (U), (2) most upright, few square (Us), (3) most square, few upright (uS), (4) mixed upright and square (US) or (5) mainly upright with few square and procumbent cells (Usp).

Nepenthes species	Vessel diameter (µm)	Vessel density (mm^{-1})	Vessel element length (µm)	Gums in vessels	Fibre-tracheid length (µm)	Fibre-tracheids septate	Fibre-tracheids thick-walled	Distinct axial parenchyma bands	Width of axial parenchyma bands (nr of cells)	Scanty paratracheal axial parenchyma
*N. ampullaria^*	25-60-105	14-27-40	250-360-470	+	300-500-700	±	+	±	1-8	+
*N. bicalcarata**	40-98-160	8/14/25	300-460-590	-	450-620-780	-	+	-	/	-
N. bokorensis^*	40-68-100	14-24-44	200-325-500	+	410-590-820	-	-	+	1-2	+
*N. burbidgeae1**	50-78-100	22-25-30	200-320-500	-	500-650-750	-	-	+	1	-
*N. burbidgeae2**	50-76-120	13-19-24	250-380-525	-	625-770-900	-	-	+	1	-
N. chaniana^*	35-47-70	16-24-36	250-340-540	+	415-545-670	-	+	+	2	+
*N. distillatoria**	20-46-75	28-36-40	200-285-375	-	350-525-710	-	+	-	/	-
*N. edwardsiana**	30-51-70	22-43-52	270-380-550	-	300-515-710	-	-	-	/	-
N. gracilis	40-94-170	9-16-23	250-332-550	-	400-549-750	+	-	+	1-4	+
*N. gracillima**	25-37-60	20-31-40	240-315-405	-	250-430-600	-	+	±	1	-
N. gymnamphora	50-104-150	28-33-39	250-370-500	+	400-565-750	-	-	±	1-4	+
*N. hemsleyana**	25-37-50	20-24-28	210-360-450	-	310-445-600	±	+	+	1-2	+
*N. hirsuta**	15-38-50	32-45-60	280-390-500	-	300-850-625	-	+	±	1	-
N. kerrii^*	30-53-75	16-31-44	200-295-400	-	300-465-800	-	-	-	/	±
N. khasiana	20-61-90	45-52-64	200-260-340	+	350-515-700	±	-	±	1	-
*N. lamii**	35-60-105	16-37-48	200-420-710	-	300-505-740	+	-	-	/	±
N. lowii	45-80-115	21-22-30	250-450-730	-	270-465-600	±	-	+	1-7	+
*N. macfarlanei**	35-57-90	24-34-48	260-490-720	-	300-605-950	-	+	-	/	-
N. madagascariensis	25-54-120	16-29-40	200-265-360	+	300-450-710	-	-	±	4-6	+
N. maxima^*	25-43-95	18-33-46	205-340-570	-	360-565-750	-	-	±	3-4	-
N. mirabilis	30-78-150	20-23-34	210-370-710	-	350-520-700	±	-	±	1-4	+
*N. muluensis**	25-54-100	20-28-36	250-390-500	-	360-460-610	-	+	-	/	-
*N. neoguineensis**	40-88-125	20-27-40	250-320-425	-	320-440-600	-	±	-	/	±
*N. pervillei**	50-73-110	26-32-40	300-405-510	-	500-645-900	±	-	-	/	-
*N. pilosa**	50-94-140	22-26-32	250-400-600	-	600-680-850	-	-	-	2-3	-
*N. rafflesiana^*	25-78-125	18-22-29	275-390-540	+	400-555-740	-	+	+	1-10	+
N. rajah	50-65-90	8/13/20	150-256-400	+	450-580-700	±	-	+	2-6	-
*N. reinwardtiana**	40-52-70	28-36-44	210-350-540	-	320-525-700	-	+	-	/	-
*N. rhombicaulis^*	30-45-75	32-38-50	260-375-490	-	400-515-850	±	-	+	1-5	+
N. sanguinea	15-34-50	20-32-48	240-340-480	+	375-520-710	-	+	±	1-2	-
N. smilesii	20-38-50	40-55-64	155-215-325	-	275-420-530	-	±	+	1-4	-
*N. stenophylla**	60-84-100	14-23-32	200-340-450	-	650-740-850	±	-	-	2-3	-
*N. tentaculata**	25-48-100	16-30-36	270-355-500	-	300-460-700	-	+	-	/	-
N. thorelii	20-37-55	40-48-56	200-305-460	-	300-425-550	-	±	±	1-2	-
N. tobiaca	25-54-75	32-41-54	245-360-500	-	450-600-750	-	+	-	/	-
N. tomoriana	25-40-65	36-46-64	260-355-500	-	380-585-850	-	+	±	1-2	±
*N. veitchii**	75-107-130	28-35-48	200-304-450	-	500-640-700	ND	-	+	1-2	-
*N. ventricosa^*	40-65-105	15-20-30	200-315-500	-	355-490-605	-	-	+	1-2	+
N. villosa	30-53-75	36-49-61	290-380-490	+	400-625-900	-	-	+	1-2	+
N. sp. (Thai origin)	30-57-90	32-46-58	250-330-460	-	275-435-550	±	+	+	1-2	+

Pith lignification reported as (1) slightly lignified with few, thin-walled cells (-), (2) slightly lignified with many, thin-walled cells in outer zone of pith (±), (3) markedly lignified with thin-thick walled cells intermediate between axial parenchyma cells and fibers in large portion of outer pith (+) or (4) markedly lignified with thick-walled cells intermediate between axial parenchyma and fibers throughout entire pith (++). Cortex not available for analysis in species marked with /. Character not determinable in categories marked with ND. Juvenile wood indicated with *, greenhouse grown specimens indicated with ^.

Rays exclusively uniseriate	Ray width (nr of cells)	Height uniseriate rays (µm)	Height multiseriate rays (µm)	Density uniseriate rays (mm^{-1})	Density multiseriate rays (mm-1)	Ray compostition	Silica bodies in rays	Medullary bundles	Pith lignification	Helical idioblasts in pith	Helical idioblasts in multiseriate rays	Helical idioblasts in cortex
-	1(2-4)	170-760-2100	310-1090-1900	12-14	0-2	Usp	+	-	+	+	-	/
+	/	400-800-1800	/	6-10	0	US	-	-	+	+	/	/
-	1(2-4,10)	170-330-550	400-1140-3300	3-7	2-5	US	±	-	+	-	-	/
+	/	250-620-1000	/	12-14	0	U	±	+	+	+	/	+
-	1(2-3)	300-690-1500	700-1070-1700	11-14	0-1	Us	+	+	+	+	-	/
-	1(2)	250-390-750	560-935-1210	7-16	0-2	US	±	-	-	+	-	+
-	1(2,10)	170-490-1250	750-1350-1950	8-12	0-1	US	±	-	±	+	-	/
-	1(2)	260-580-1250	405-810-2250	8-13	0-3	Us	-	-	±	-	-	/
-	1,2-5(6-10)	ND	ND	ND	ND	Usp	+	-	+	+	/	+
-	1(2-3,10)	150-290-600	/	12-16	0	Us	±	-	±	+	/	/
-	1 (2)	400-755-1400	600-1365-2100	9-13	1-2	Usp	-	-	±	±	+	/
-	1(2)	210-690-1200	600-940-1250	10-13	0-2	Us	±	-	±	+	-	/
+	/	150-370-700	/	9-12	0	uS	+	-	+	+	/	+
+	/	245-710-1210	/	8-14	0	US	-	-	+	+	/	+
-	1(2-3,14)	170-485-710	230-765-1800	11-14	3-5	Us	-	-	±	+	+	/
-	1(5-8)	200-580-1350	450-640-900	7-15	0-1	Us	±	-	±	-	-	/
+	1(2-5)	120-470-950	250-660-1800	4-10	0-3	US	-	-	+	+	+	+
-	1(2-3)	400-805-1950	100-1500-2200	14-20	0-1	Us	-	+	++	+	-	/
-	1(6-12)	180-520-1600	300-1365-3900	3-10	1-6	Usp	±	-	-	+	-	/
-	1(2-3)	175-470-1150	550-1270-3400	7-12	2-6	uS	±	-	±	-	-	/
-	1(2)	300-1090-2200	450-1390-3200	9-13	0-2	Usp	±	-	±	+	-	+
+	/	150-400-900	/	6-14	0	Us	-	-	++	+	/	+
+	/	300-520-950	/	9-12	0	Us	-	-	+	+	/	/
-	1(2)	175-395-1100	450-535-600	7-15	0-1	US	±	-	+	+	-	/
+	/	350-770-1100	/	10-15	0	U	+	+	+	+	/	+
-	1(2-4)	200-500-1100	1000-1475-2300	7-10	2-3	Usp	+	-	±	+	+	/
-	1(2)	150-183-350	150-192-250	10-13	0-1	Usp	±	-	±	+	+	+
-	1(2-4)	150-510-790	400-955-2100	9-13	1-4	uS	+	+	+	+	-	/
-	1(5-7)	150-535-1200	700-970-1400	7-12	0-4	Us	±	-	±	+	-	/
-	1(2-6)	200-390-655	250-870-1600	5-10	0-5	US	-	+	-	+	-	/
-	1(2)	170-366-575	190-555-1650	5-12	0-3	Us	±	-	+	±	-	/
+	/	350-812-1700	/	10-15	0	U	+	+	++	+	/	+
+	/	200-460-850	/	8-12	0	Us	-	-	-	+	/	+
+	/	160-260-410	/	11-16	0	uS	-	-	±	+	/	/
+	/	100-1030-2600	/	14-18	0	Usp	±	+	++	+	/	/
-	1(2-14)	120-515-1200	350-1190-2500	11-14	0-2	Us	-	-	±	+	-	/
+	/	ND	ND	10-15	0	U	±	+	+	±	/	/
+	/	100-295-625	/	3-7	0	US	-	-	±	+	/	+
-	1, 2-5	190-425-760	270-950-3400	4-7	3-6	Usp	-	-	±	+	-	+
-	1(2-3)	250-383-750	520-725-900	6-11	0-1	Us	+	-	+	/	-	±

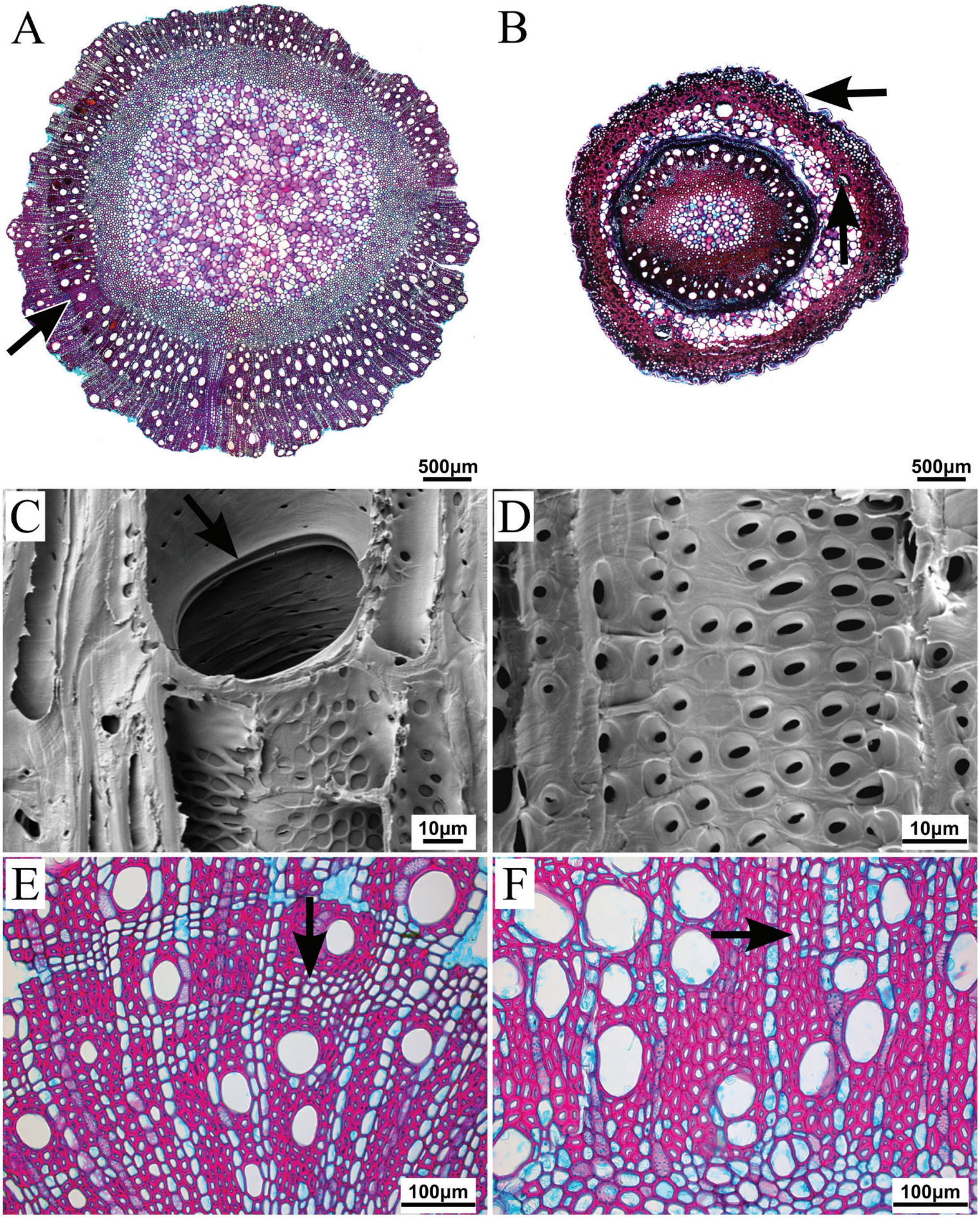

FIGURE 12. Wood anatomical sections of Nepenthaceae. Transverse light microscope sections (A, B, E, F), radial (C) and tangential (D) scanning electron microscopy surfaces of *Nepenthes* wood. (A) *Nepenthes khasiana*, mature stem (bark detached) showing wood with indistinct growth ring (arrow), (B) *Nepenthes muluensis*, entire juvenile stem with pronounced cuticle and lignified areas in both the outer stem area (cortex) (arrow) and the inner stem part (wood and outer pith region), (C) *Nepenthes tobaica*, bordered, simple perforation plate with rim (arrow), (D), *Nepenthes smilessi*, alternate intervessel pits (E), *N. smilessi*, tendency to form banded axial parenchyma (arrow), and (F) *N. edwardsiana*, diffuse-in-aggregates axial parenchyma (arrow).

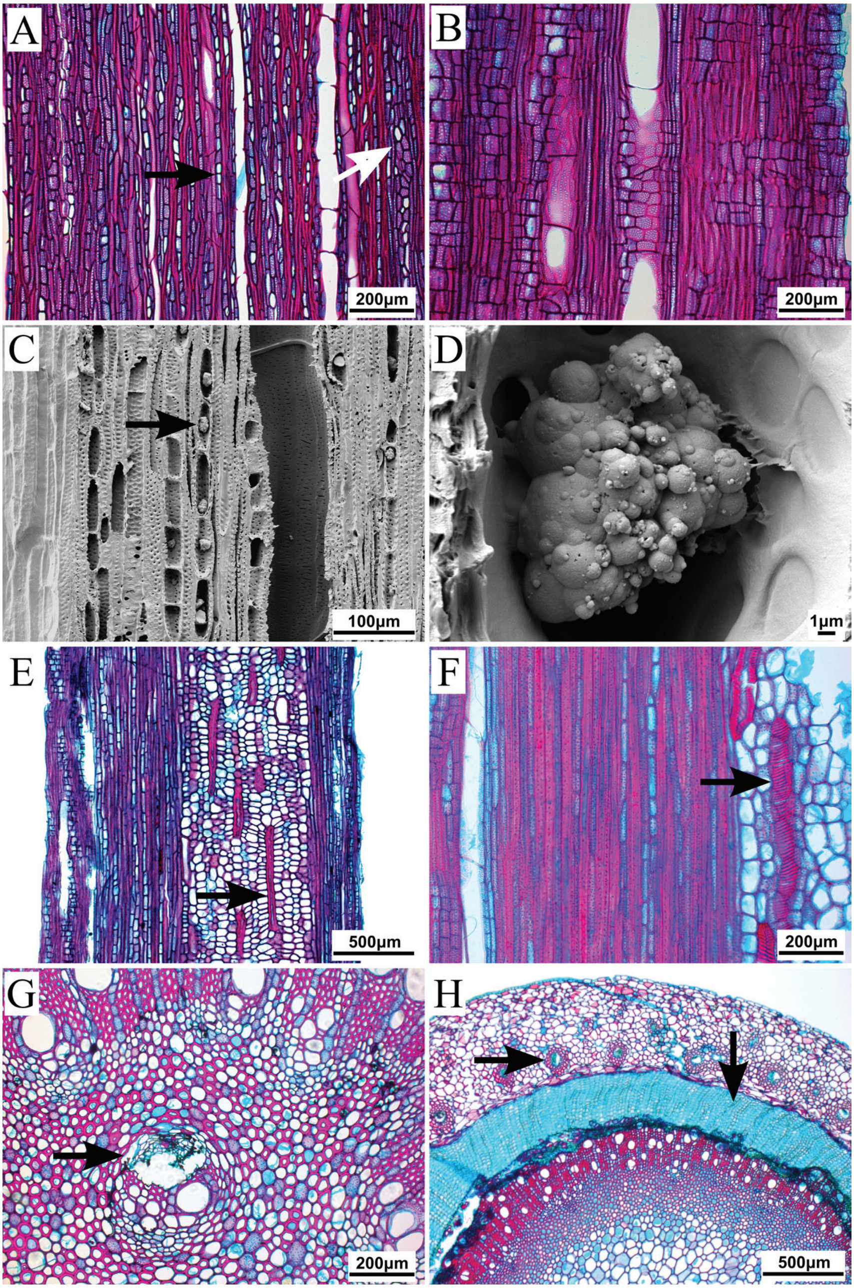

Figure 13. Light microscope sections of tangential (A), radial (B, E) and transverse (F, G, H) views, and scanning electron microscope images (C, D) of tangential surfaces of *Nepenthes* wood. (A) *Nepenthes khasiana*, overview showing dense uniseriate (black arrow) and narrow multiserate rays (white arrow). (B) *Nepenthes gymnamphora*, overview of rays with mainly square to upright ray cells. (C, D) *N. ampullaria*, abundant silica grains in ray cells (arrow), (E) *Nepenthes reinwardtiana*, thick-walled, helically-banded sclereids within the pith (arrow), (F) *N. burbidgeae*, detail of thick-walled, helical idioblast in pith (arrow), (G) *Nepenthes tobaica*, medullary bundle (arrow) and (H) *Nepenthes ventricosa*, cortical vascular bundles inside cortex, deep seated periderm with cork cylinder (arrow).

Discussion

Wood anatomical diversity in *Nepenthes*: We present the most extensive wood anatomical survey of *Nepenthes* to date. The species sampled represent the full diversity in growth habit, ecology and phylogenetic position, providing a better understanding of the wood anatomical diversity in the genus (Table 4). Because of the strict conservation rules and monopodial growth habit for *Nepenthes*, we were forced to incorporate many juvenile specimens, but found that only pith lignification and maximum ray width were correlated with juvenility (Supplementary Data Table S5 online).

Our observations confirm earlier wood descriptions by Metcalfe and Chalk (1950) and Carlquist (1981, 2010), stating that all species have diffuse porous wood with solitary vessels (Fig. 12A, B), simple, bordered perforation plates (Fig. 12C) and alternate intervessel pits of 5–7µm (Fig. 12D). We also found dimorphic vessel elements with an equal number of longer, narrow vessel elements vs. shorter and wider ones in the maceration slides (cf. Carlquist 1981, 2010). Further, fibres have distinctly bordered pits in tangential and radial walls, and the axial parenchyma is diffuse in aggregates (Fig. 12F) with a tendency to form narrow bands (1–4 cells) (Fig. 12E) for most species, with exceptions of much wider bands in the mature wood samples of *N. ampullaria* (up to 8 cells wide) and *N. rafflesiana* (up to 10 cells wide). Rays are typically uniseriate and multiseriate (up to 14 cells wide; Fig. 13A) and consist of a combination of upright and square cells (Fig. 13B), although most juvenile samples only showed uniseriate rays.

More interestingly, we found helical idioblasts (cf. Carlquist 2010) in all but four species investigated. These peculiar cells are mostly either thin-walled or occasionally very thick-walled (Figs. 13E, F), and often occur in the pith, the cortex and rarely in multiseriate rays. Similar-looking ‘spiral tracheids’ were noted previously only in the bark/cortex and tall rays of rhizomes (Heinricher, 1906) and leaves (Kny & Zimmerman, 1885; Carlquist, 1981, 2010). Furthermore, our extended study provides clear evidence for the presence of silica bodies in ray cells (Fig. 12C, D) and in the secondary phloem of most species analyzed, although silica grains were previously only observed in *N. alata* (Carlquist 2010). In addition, most species had some level of lignification in the pith (Figs. 14B and 15B), with marked lignification occurring in a larger portion of the pith in the few remaining species. Medullary bundles (Fig. 13G) were present in the pith of eight species, often associated with the species having more lignified pith (Figs. 14B and 15B). Furthermore, we found cortical vascular bundles in a ring-like arrangement surrounding the periderm producing a large phellem cylinder in *N. ventricosa* (Fig. 13H). In this species, the phellogen is initiated far inside the stem, but we cannot comment whether this is a common feature for *Nepenthes* since the outer stem portions were often missing in our slides. Finally, we observe for the first time that fibres are occasionally septate in a number of species (Figs. 14C and 15C).

Phylogenetic relevance of wood anatomy characters in Nepenthes and Caryophyllales: *Silica bodies.* In the rays of 25 of the 39 *Nepenthes* species studied (Table 4), silica bodies were found; nine of these contained silica in huge quantities (Fig. 13C, D). Silica was not recorded in Carlquist's (1981) initial wood study of *Nepenthes*, although he later reported grains in one species (Carlquist 2010). We found a gain/loss pattern in the trait optimization of silica amongst species of *Nepenthes* (Figs. 14 and 15), which is probably related to the different edaphic conditions that *Nepenthes* species have evolved (see section on abiotic factors). Since silica occurs in only a limited number of flowering plant genera, it is considered of high diagnostic value (Carlquist, 1988). Nevertheless, within our Caryophyllales analysis, the silica-bearing genera are widely scattered within the non-core group (*Ancistrocladus* (Gottwald & Parameswaran, 1968)*, Dioncophyllum* (Gottwald & Parameswaran, 1968) and *Nepenthes* and within the core group (*Limoneum* (Carlquist & Boggs, 1996) and *Rhabdodendrum* (Carlquist, 2010) (Fig. 16A). In addition to these, Carlquist (2003a) records several additional families in the 'non-core' Polygonaceae that include silica in ray cells.

Helical idioblasts. Helical thickenings in the cell walls of various types of idioblastic cells (Carlquist, 2010) appear to be characteristic of the carnivorous clade in Caryophyllales, for which *Nepenthes* is a typical example (Fig. 16B). Helical idioblasts, with either very thin lignified walls in a spiral arrangement or extremely thick lignified walls resembling fibre-sclereids (Figs. 13E, F), occur in the pith and cortex of nearly all *Nepenthes* species observed, and have occasionally been found in multiseriate rays as well. The function of these peculiar cells remains unknown, but has been associated with water storage (Kny & Zimmerman, 1885; Heinricher, 1906; Metcalfe & Chalk, 1950) or protection against insects or other predators (Carlquist 2010). Similar idioblasts (but with 'wide lumina') have only been observed outside *Nepenthes* in the root cortex of the related genus *Drosera* (Oels 1879). *Ancistrocladus* have idioblastic cells so unique that Carlquist (2010) coined them as 'ancistrocladan cells,' which are a grouping of apotracheal parenchyma cells with banded walls that co-occur with normal axial parenchyma cells. In the same non-core clade, *Triphyophyllum* was reported to have helical idioblasts in the axial parenchyma (Gottwald & Parameswaran, 1968), but was later discounted based on the further investigation (Carlquist, 2010). *Anacampseros*, closely related to Portulacaceae and Cactaceae, also have helical idioblasts in the rays (Carlquist, 2010). And although not termed as helical idioblast *per se*, the typical wide-band tracheids in Cactaceae can also be listed here (Mauseth & Landrum, 1997).

FIGURE 14. Wood and pith anatomical characters optimized on the empirical trees of the full *Nepenthes* trnK-matK produced in BEAST. Wood characters include (A) silica presence, (B) axial parenchyma distribution and (C) presence of septate fibres. The pith characters (D), lignification and medullary bundle presence are combined in one map, with black diagonal bands laid over lignification-keyed color. Mature wood specimens indicated with *. Posterior support values generated by BEAST analyses indicated for bpp ≥ 0.90 with † and for bpp threshold 0.80 with ‡. The scale bar is in units of substitutions/site.

FIGURE 15. Wood and pith anatomical characters optimized on the empirical trees of the full *Nepenthes* nrITS produced in BEAST. Wood characters include (A) silica presence, (B) axial parenchyma distribution and (C) presence of septate fibres. The pith characters (D), lignification and medullary bundle presence are combined in one map, with black diagonal bands laid over lignification-keyed color. Mature wood specimens indicated with *. Support values generated by BEAST analyses are indicated for threshold bpp ≥ 0.90 with †. The scale bar is in units of substitutions/site.

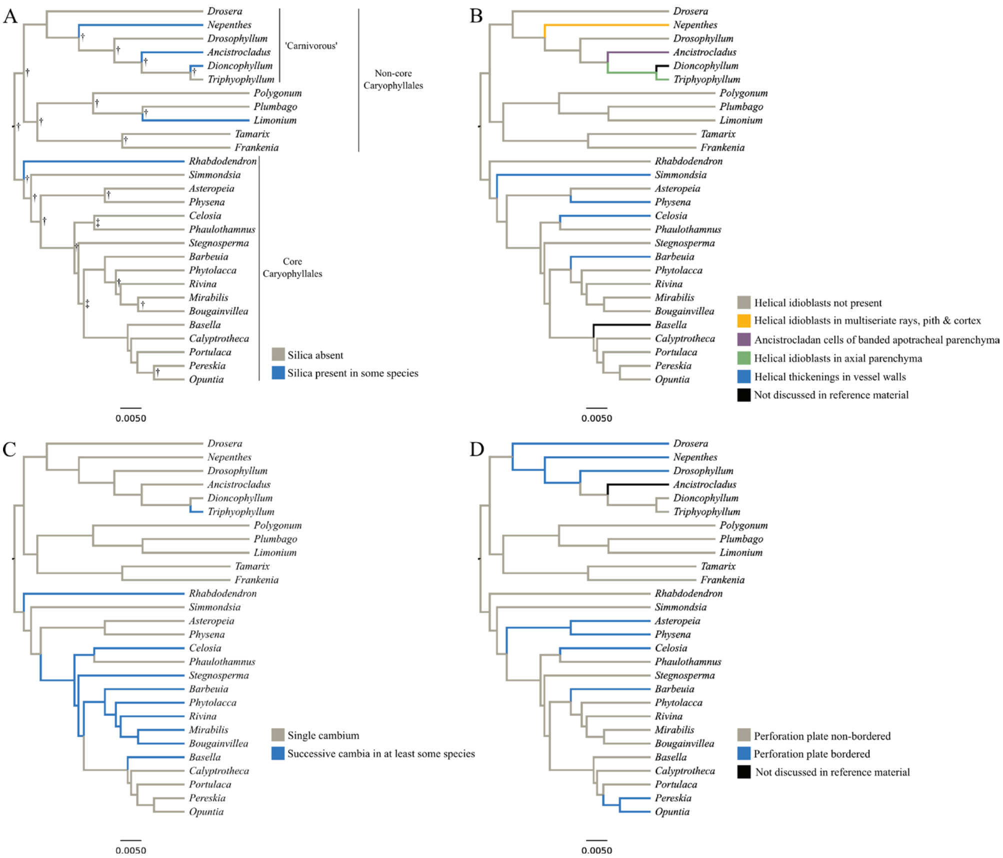

FIGURE 16. Four wood characters mapped on the Caryophyllales order sensu Soltis et al. (2011), with characters optimized on a maximum likelihood tree based on 19 genes from the plastid, nuclear and mitochondrial genomes produced in BEAST. Genera included in the mapping have woody species and referenced anatomical observations. The 'Carnivorous' clade includes the non-carnivorous genera *Ancistrocladus* and *Triphyophyllum*. Support values generated by BEAST analyses indicated for bpp ≥ 0.90 with † and for bpp threshold 0.80 with ‡. The scale bar is in units of substitutions/site.

Single vs. successive cambia. Our results show that single cambia are symplesiomorphic for Caryophyllales, from which acquisition of successive cambia was derived (Fig. 16C). Although this is in line with assumptions made in the past about this wood anatomical character (Rodman, 1994), it should be noted that short-lived plants might not acquire successive cambia because a single cambium provides sufficient support (Carlquist, 2010). Likewise, initiation of multiple cambia may favour the evolution from annual, herbaceous life forms to perennial, woody life forms. Since the shift from herbaceousness towards derived woodiness is characterized by massive convergent evolution (Lens et al. 2013a), it is not surprising that successive cambia have developed multiple times in Caryophyllales (Fig. 16C).

Perforation plates. Like all Caryophyllales species, members of *Nepenthes* have simple perforation plates in their wood (Fig. 12C). Vestigial scalariform perforation plates in the primary xylem were observed by Carlquist (2010), who illustrated gyre tips of the primary xylem fringing the perforation plate. He also occasionally observed multiple perforations plated in *Nepenthes* wood, which we were unable to locate, and in *Dionaea*. The perforation plates of *Nepenthes* and its most closely related genera, *Drosera* and *Drosophyllum,* are clearly bordered (Fig. 16D). Of the families in our analyses, bordered perforation plates only occur in four other families; in Cactaceae (*Pereskia* and *Opuntia;* Carlquist 2010), Amaranthaceae (*Celosia;* Carlquist 2003), Asteropeiaceae (*Asteropeia;* Carlquist 2006), and in Physenaceae (*Physena;* Carlquist 2006). The latter three families have a variable degree of minimally bordered to non-bordered perforation plates as well (Carlquist, 2010). Other Caryophyllales families with bordered perforation plates include Anacampserotaceae, Portulacaceae, Talinaceae, Montiaceae and some genera within Caryphyllaceae and Plumbaginaceae (Carlquist, 2010).

Influence of abiotic factors on wood anatomy: Wood anatomy is fairly conservative at the genus level (van den Oever et al., 1981; Noshiro & Baas, 2000; Lens et al., 2004). Yet minor wood anatomical variation exists in widely dispersed genera covering diverse temperature and precipitation regimes, and these characters are usually associated with vessel adaptations, such as vessel diameter and density, vessel element length, and fine-scale intervessel pit characters (Carlquist 1966, 1975; Baas 1976; Lens et al. 2011, 2013; Scholz et al. 2013). Since *Nepenthes* occupies a variable range of habitats, from coastal mangroves to mountain summits, and inhabits a wide spectrum of soil types, temperatures and precipitation, we investigated the influence of all these environmental factors to variation in stem anatomy.

Soil type. For *Nepenthes*, soil type is one of the main factors in ecological preference (van der Ent et al. 2015; Schwallier et al. 2016). This is not surprising because carnivorous plants, like *Nepenthes,* evolved alternative strategies for nutrient acquisition in an environment where traditional resources from the soil are limiting, giving them an advantage in such ecosystems. Such edaphically stressed environments include acidic kerangas (heath) and peat swamp forests on ultramafic bedrock. Ultramafic soil is extremely rich in iron, magnesium and nickel, but often poor in silica content (Brooks, 1988). Ultramafic soils are especially prevalent in the northern mountains of Malaysian Borneo (van der Ent et al., 2015), the southern Philippines, Sulawesi and other *Nepenthes*-inhabited islands of the Malay Archipelago. Absence of silica in some of the *Nepenthes* species could be explained in two ways. The most straightforward is a simple lack of soluble silica available in the soil where the plants investigated were growing. A second possibility could be mechanisms blocking root uptake of silica (Parry & Kelso, 1977). We found no support for uptake blockage of silica as our trait optimization displays an unlikely gain/loss pattern of such a scenario (Figs. 14 and 15). Interestingly, two ultramafic endemic species of Mount Kinabalu and Mount Tambuyukon, *N. edwardsiana* and *N. villosa,* lack silica in their ray cells. In *N. burbidgeae,* another species native to ultramafic soils, we observed abundant silica in one wood sample from the Sabah Parks Kinabalu Botanical Garden (i.e. not grown on ultramafic soil), while we could only find a small amount of silica grains in another sample collected in the wild on ultramafic soil on Mount Kinabalu. This may suggest that all *Nepenthes* species have the ability to store silica in their wood as long as it is available in the soil. Similarly, silica was also present in seven of the eight greenhouse-grown specimens analyzed (Table 4). Our data, therefore, provides evidence of a possible link between edaphic factors (ultramafic bedrock) and wood anatomical variation (strongly reduced presence or even absence of silica in ray cells).

Precipitation. Vessel maxima were wider when species lived in locations that received more precipitation (Supplementary Data Table S5 online). Also, multiseriate ray height (in both maximum or average measures) increased with increasing annual precipitation. Six of the species studied survive through seasonal drought stress in Cambodia, Sumatra and Thailand: *N. bokorensis, N. kerrii, N. neoguineensis, N. smilesii, N. thorelli* and *N. tobaica* (McPherson, 2009)*. Nepenthes bokoriensis, N. smilessii* and *N. thorellii* occur in exceptionally seasonably dry areas where the driest month average only 20 mm, 5 mm and 6 mm of rain, respectively. We found that all of these species exposed to drought stress had pronounced pith lignification with often thick-walled lignified pith cells (Table 4). *Nepenthes tobaica* for example, which is known to grow in seasonably dry areas of Sumatra (McPherson, 2009) with a threefold average decrease in precipitation from the wettest to the driest month, show marked lignification in the entire pith. Increased stem lignification may help to alleviate drought stress in avoiding water loss through the stems during drier periods (Lens et al. 2013b), which has also been found in grasses (Lens et al. 2016).

Although we were not able to section the outer stem parts for most our samples, we observed that the periderm with a pronounced cork layer was initiated deeply within the stem of *N. ventricosa* (Fig. 13H). Also, *N. muluensis* (Fig. 12B) shows a large lignified pith area, wood with thick fibre walls and a thick lignified layer at the outer part of the cortex and thick cuticle. The features of each of these two species could be alternative strategies to protect the stem during drought. In addition to this, half of the species studied had thick-walled fibres, reflecting a higher wood density. Although there is much noise/inconsistencies in the relationship between wood density and environmental factors (Swenson & Enquist, 2007), several studies have found a link between increased wood density and increased drought stress resistance (Chave et al. 2006, 2009; Lens et al. 2013a; Lens et al. 2013b).

Beyond the stem, leafs and roots likely play a role in drought tolerance in *Nepenthes*. *Nepenthes pervillei,* for example, develops long, pronounced roots (Adlassnig et al., 2005) to obtain water in its rocky cliff habitat (Juniper et al., 1989). In addition, two of our wild harvested Cambodian species, *N. smilessi* and *N. thorelli,* experience such severe drought in the dry season that their aboveground stem parts die off completely, relying on tuberous rootstock for regrowth when rain commences (McPherson, 2009; Mey, 2010). In addition, all of the drought exposed *Nepenthes* species have relatively narrow and leathery leaves to reduce evapotranspiration compared to more moist-living ones (McPherson, 2012).

Most *Nepenthes* species, however, are regularly or even consistently exposed to wet conditions, especially the numerous higher altitude species (McPherson, 2009). Fossil and biogeographic evidence (Krutzsch, 1988; Meimberg et al., 2001) suggests that the genus may have been able to occupy fairly moist ecological habitats for the duration of its evolutionary history, from the humid tropics of what is now France during the Eocene, making its way to Southeast Asia via the Middle East before it underwent aridification. This gives good reason to believe that most *Nepenthes* species are not suited to withstand the stresses imposed from drier or drought conditions, especially if other features like tuberous rootstock, stem lignification or leaf size and texture are not adaptively developed as they are in the Cambodian species. From a conservation perspective, this is especially important given that *Nepenthes* will not likely track tolerable habitat boundaries fast enough to keep up with the sharply changing future climate (Schwallier et al. 2016).

The influence of growth habit on wood anatomy: The basic life forms of *Nepenthes* ranges from self-supporting rosette shrubs, to scramblers and woody climbers with stems dramatically varying from just a few centimetres to over 20 meters long (McPherson, 2009). The mature wood anatomy of the lianoid *Nepenthes* species studied share several characteristics with non-related lianoid lineages (Carlquist, 1989), including vessel dimorphism, simple perforation plates, abundant axial parenchyma, and wide multiseriate rays (Table 4). We found that multiseriate rays were longer in taller lianas (Supplementary Data Table S5), allowing them more flexibility. Another typical lianoid wood character is the presence of wide vessel diameters that can reach over 200 µm in Marcgraviaceae, for example, and even 400 µm in Apocynaceae (Lens et al. 2005; Lens et al. 2008). The mature wood samples representing all the vigorously climbing *Nepenthes* lianas (McPherson, 2009) in our study, however, had an average tangential vessel diameter of only 64 µm. The widest average vessels in our analysis were found in *N. gymnamphora* (104 µm, individuals growing up to 20m) and *N. veichii* (107 µm, individuals reaching up to 10m; Table 1; McPherson 2009). It is known that vessel widening is more pronounced towards the base of stems (Olson et al., 2014), justifying the exclusion of juvenile specimens in this comparison.

Mechanical strength through pith lignification may compensate for the lack of sufficient support in juvenile stems. These younger stems have a broad pith area and narrow wood cylinder that need to carry heavy pitchers with their contents. For instance, *N. rajah* produces one of the most impressive pitcher traps in the genus, recorded to hold over three litres of water (Clarke & Wong, 1997). To accommodate this heavy trap, the plant itself is rather stout and self-supporting, with a coinciding wood anatomy. Our mature sample of *N. rajah* had the greatest wood production and stem diameter of all of the specimens sampled, with the extensive wood cylinder providing extra mechanical support for the plant. The greenhouse-grown specimens investigated, which were artificially supported, had less rigidity and consequently more abundant parenchyma both inside and outside of the wood cylinder, and more thin-walled fibres compared with wild-collected specimens. Underdeveloped fibres and abundant non-lignified parenchyma have previously been reported for greenhouse grown lianas (Lens et al. 2008). For our greenhouse specimens, it appears that the controlled environment (artificial support since seedling stage, lack of wind and other stresses including drought) influenced the wood anatomy.

Other species display a marked intraspecific difference, illustrating nicely the impact of the environment on the habit. In *N. maxima,* for example, distinct ecotypes have evolved in response to different environments. The most common form is a vigorous climbing stem up to 19 meters long growing in heath or dipterocarp forests, which is very different from the reduced, diminutive form occurring in the seasonal dry savannahs of Central Sulawesi. There, the stems have a maximum self-supporting length of only 35 cm (McPherson, 2009). This shorter form additionally evolved waxy-edged leaves, which was also likely in response to the heated arid environment. In addition, the species *N. lowii* forms a compact rosette or short stem only of 1-2 meters above the ground in exposed areas, because there is no need to produce a climbing stem to reach sunlight. In contrast, the forest ecotype of *N. lowii* is a vigorous climber of up to 10 meters. In other words, collecting wood samples of *Nepenthes* in the field enables establishment of a more accurate link of the impact of growth habit and environment on the wood anatomy, which may significantly vary within *Nepenthes*, even at the species level.

Conclusions

With the pace of anthropogenic climate change necessitating urgent attention, focus on the links between ecology and the anatomical restrictions or pliability of plants that have deep-seated cultural, traditional and economic importance, such as *Nepenthes*, call for our attention. The wood anatomy of *Nepenthes* is generally rather uniform, but several stem anatomical adaptations in the species facing drought stress or growing in ultramaphic soils have been found. The omnipresence of helical idioblasts in the pith and cortex of *Nepenthes* represents a synapomorphy for the genus, and supports its phylogenetic position within the carnivorous clade of Caryophyllales. Other typical *Nepenthes* characters, such as silica grains and bordered perforation plates, evolved convergently in different Caryophyllales lineages. Given our evidence on the conservative nature of most characters in our study, it is unlikely that the progressively changing environmental future predicted by the Intergovernmental Panel on Climate Change (IPCC, 2014) will result in a rapid shift towards more pronounced lignification in the stems, or deep root systems, characters that have been associated with drought stress resistance within *Nepenthes*. In the *Nepenthes* habitat of Southeast Asia, predictions include an increase in monsoon duration and intensity and conversely more drought exposure during the months of July-October (IPCC, 2013). Further investigation on drought stress resistance in the genus could include water transport measures in the xylem to estimate the pressure inducing 50% loss of hydraulic conductivity (P50). In addition, minimum midday water potential measures (Psi min) can be performed to estimate levels of native embolism formation throughout the year in order to have an idea about the hydraulic safety margin (Psi min – P50; Choat et al 2012). This is especially important for the high altitude species that normally thrive in very wet environments throughout the year, offering important conservation information for this iconic plant family.

Supplementary Table 1. Specimen information for wood anatomical investigations of *Nepenthes*. Vouchers are deposited in the Netherlands (L) or Sabah (SNP).

Species	**Voucher**	**Collector**	**Sample source**
N. ampullaria Jack	HBL 20130439	Robert, NA	Hortus botanicus Leiden
N. bicalcarata Hook.f.	L 0578253	Meijer, W	Naturalis
N. bokorensis 1 Mey	HBL 20130443	Mey, F	Hortus botanicus Leiden
N. burbidgeae 1 Mey	SNP 3034	unknown	Sabah National Parks Herbarium
N. burbidgeae 2 Mey	None available	unknown	Kinabalu Parks greenhouse
N. chaniana C.Clarke, C.Lee & S.McPherson	HBL 20130453	Gert, H	Hortus botanicus Leiden
N. distillatoria L.	L.1852283	Schiffner, VF	Naturalis
N. edwardsiana H.Low ex Hook.f.	L.4149074-77	Schwallier, R	Author fieldwork collection
N. gracilis Korth.	L.4149080	Schwallier, R	Author fieldwork collection
N. gracillima Ridl.	L.0885964	Shah, M	Naturalis
N. gymnamphora Reinw. ex Nees	L 0841088	Ploem, JC	Naturalis
N. hemsleyana Macfarl.	L.1852302	Vogel, EF de	Naturalis
N. hirsuta Hook.f.	L.1852307	Kessler PJA	Naturalis
N. kerrii M.Catal. & Kruetr.	HBL 20130464	Smith, A	Hortus botanicus Leiden
N. khasiana Hook.f.	L.1852586	Chand, TR	Naturalis
N. lamii Jebb and Cheek	L.1852590	Willis, FR	Naturalis
N. lowii Hook.f.	L.0577375	Fuchs, HP	Naturalis
N. macfarlanei Hemsl.	L.0885965	Corner, EJH	Naturalis
N. madagascariensis Schmid-Holl.	L.2055350	Sider, A, Knirsch, W, & Andriantiana, JL	Author fieldwork collection
N. maxima Reinw.	HBL 20130476	Mey, F	Hortus botanicus Leiden
N. mirabilis (Lour.) Druce	L.4149383	Schwallier, R	Author fieldwork collection
N. muluensis M.Hotta	L.0577349	Julaihi, LCJ & Jemree, S	Naturalis
N. neoguineensis Macfarl.	L.1856618	Takeuchi, WN	Naturalis
N. pervillei Blume	L.1856547	Bernardi, L	Naturalis
N. pilosa	KNPA15666	Molidin, D.	Sabah National Parks Herbarium
N. rafflesiana Jack	HBL 817241	van Heeringen	Hortus botanicus Leiden
N. rajah Hook.f.	SNP 2895	Phillipps A. & Gunsalam, A	Sabah National Parks Herbarium
N. reinwardtiana Miq.	L.0885508	Burck, W	Naturalis
N. rhombicaulis Sh. Kurata	HBL 20130492	Hoogenstrijd, G	Hortus botanicus Leiden
N. sanguinea Lindl.	L.0885489	Cockburn, PF	Naturalis
N. smilesii Hemsl.	L.4149133	Chayamarit, K, Pooma, R & Cheek, MD	Naturalis
N. stenophylla	KNPA15594	Liming Haur	Sabah National Parks Herbarium
N. tentaculata Hook f.	L.4149110	Schwallier, R	Author fieldwork collection
N. thorelii Lecomte	L.0885456	Smitinand, T & Sleumer, HO	Naturalis
N. tobiaca Danser	L.0885442	Alston, AHG	Naturalis
N. tomoriana Danser	L.0885414	Vogel, EF de	Naturalis
N. ventricosa Blanco	HBL 20130507	Hoogenstrijd, G	Hortus botanicus Leiden
N. veitchii	SNP 08123	Repin, R & Geoffary, G	Sabah National Parks Herbarium
N. villosa Hook.f.	L.4149389	Schwallier, R	Author fieldwork collection
N. spec.nov.	L.4149135	Chayamarit, K, Pooma, R & Cheek, MD	Naturalis

Locality	Specimen diameter (mm)
Unknown	4.46
Malaysia, Borneo, Sabah, Beaufort, Seratok	6.5
Cambodia, Bokor Hill	3.37
Mount Kinabalu, Sabah	10
unknown	7
Borneo	5.62
Sri Lanka,Kalutara	2.74
Malaysia, Borneo, Sabah	4.22
Malaysia, Borneo, Sabah, Poring	2.92
Malaysia, Paking, Gunung Pahang	1.9
Indonesia, Java	5.6
Brunei, Borneo, Belait, Bukit Teraja Path	3.3
Indonesia, Borneo, Kalimantan Timur	3.39
Thailand	2.25
India, Assam, Khasi Hills, Cherrapunj	4.52
Indonesia, New Guinea, Mount Jaya	2.18
Malaysia, Borneo, Sabah, Mt. Kinabalu	9
Malaysia, Pahang, Sungai Tahan	2.55
Madagascar, Fianarantsoa, Mahavelona	3.76
Indonesia, Sulawesi, Lake Poso	5.09
Malaysia, Borneo, Sabah, Poring	6.47
Borneo, Gunung Murud Summit	1.6
Papua New Guinea, East Sepik, Sitipa River	2.27
Seychelles, Silhouette	3.4
Ranau, Kinabalu Park, Mesilau Golf Course, Sabah	6
Borneo, Mt. Kinabalu area	5.41
Ranau, Pig Hill, Sabah	13
Indonesia, Sumatra, Sumatera Barat	3.69
Indonesia, Pangulubao, Lake Toba	2.37
Malaysia, Pahang, Gunung Tapis	6.03
Thailand, Yubon Ratchathani	3.34
Ranau, Mamut Ridge, Sabah	8
Malaysia, Borneo, Sabah, Mt. Kinabalu	1.86
Thailand, Loei, Phu Kradung.	4.41
Indonesia, Sumatra, Sumatera	2.35
Indonesia, Sulawesi, Lake Matano	2.47
Unknown	5.31
Long Pasia, Sabah	7
Malaysia, Borneo, Sabah, Mt. Kinabalu	6.84
Thailand, Trat-Klong Yai, Saphan Hin	1.84

Supplementary Table 2. Accessions for DNA sequence data mined from GenBank.

***Nepenthes* species**	**trnK-matK**	**nrITS**
N. adnata Tamin and M.Hotta ex Schlauer	AF315866	AB675864
N. alata Blanco		HM204891
N. alba Ridl.		JX042564
N. albomarginata T.Lobb *ex* Lindl.	DQ991358	HM204892
N. ampullaria Jack*	KP978671	KP978762
N. andamana M.Catal.		KR698380
N. aristolochioides Jebb & Cheek	AF315900	
N. bellii K. Kondo	AF315926	AB675868
N. bicalcarata Hook.f.*	DQ007089	
N. bokorensis Mey*		KR698372
N. bongso Korth.	AF315865	
N. boschaniana Korth.		HM204893
N. burbidgeae Hook.f. ex. Burb.*	AF315921	AB675869
N. burkei Mast.	DQ840247	AB675870
N. campanulata Sh.Kurata		KR698373
N. chaniana C.Clarke, C.Lee & S.McPherson*	KP978703	KP978823
N. clipeata Danser	AF315877	AB675873
N. diatas Jebb & Cheek	AF315915	AB675876
N. distillatoria L.*	AF315886	
N. dubia Danser	AF315869	
N. edwardsiana H.Low ex Hook.f.*	KP978694	KP978811
N. ephippiata Danser	AF315906	
N. eustachya Miq.	AF315867	
N. eymae Sh.Kurata	AF315930	
N. faizaliana Adam & Wilcock	AF315917	AB675879
N. fusca Danser	KP978728	KP978867
N. glabrata J.R.Turnbull & A.T.Middleton	AF315928	AB675881
N. glandulifera C.Lee		HM204895
N. gracilis Korth.	KP978679	JX042555
N. gracillima Ridl.*	DQ007066	HM204896
N. gymnamphora Reinw. ex Nees*	AF315864	HM204897
N. hamata J.R.Turnbull & A.T.Middleton	AF315914	
N. hemsleyana Macfarl.*		JX042557
N. hirsuta Hook.f.*	AF315889	AB675916
N. hurrelliana Cheek & A.L.Lamb		KR698374
N. inermis Danser	AF315870	
N. insignis Danser	AF315881	HM204898
N. kampotiana Lecomte		KR698376
N. kerrii M.Catal. & Kruetr.*		
N. khasiana Hook.f.*		KT354296
N. kongkandana M.Catal. & Kruetr.		KR698377
N. lavicola Wistuba & Rischer	AF315935	
N. lamii Jebb and Cheek*	AF315905	
N. longifolia Nerz & Wistuba	AF315871	AB675885
N. lowii Hook.f.*	KP978722	KP978857
N. macfarlanei Hemsl.*	AF315894	HM204900
N. macrovulgaris J.R.Turnbull & A.T.Middleton	AF315934	AB675886
N. madagascariensis Schmid-Holl.*	AF315883	
N. maxima Reinw.*	AF315913	HM204901
N. merrilliana Macfarl.	AF315912	AB675887
N. mikei B.R.Salmon & Maulder	AF315911	
N. mira Jebb & Cheek	DQ007085	
N. mirabilis (Lour.) Druce*	KP978702	AB675889
N. muluensis M.Hotta*	AF315933	
N. murudensis Culham ex Jebb & Cheek	DQ007084	
N. neoguineensis Macfarl.*	AF315896	AB675917

N. northiana Hook.f.		HM204903
N. ovata Nerz & Wistuba	AF315873	AB675892
N. peltata Sh.Kurata		KR698378
N. pervillei Blume*	AF315885	AB675893
N. petiolata Danser	AF315902	
N. pilosa Danser*	AF315919	
N. rafflesiana Jack*	AF315910	HM204904
N. rajah Hook.f.*	KP978690	KP978801
N. ramispina Ridl.	DQ007083	JX042563
N. reinwardtiana Miq.*	AF315907	HM204905
N. rhombicaulis Sh. Kurata*	AF315874	AB675897
N. sanguinea Lindl.*	AF315923	
N. sibuyanensis Nerz	DQ840246	
N. smilesii Hemsl.*		AB675899
N. spathulata Danser	DQ007081	AB675900
N. spectabilis Danser	AF315868	HM204908
N. stenophylla Mast.*	AF315922	AB675903
N. sumatrana (Miq.) Beck	AF315872	AB675904
N. talangensis Nerz & Wistuba	AF315924	AB675905
N. tentaculata Hook f.*	KP978674	KP978767
N. thorelii Lecomte*	AF315890	
N. tobaica Danser*	AF315899	AB675907
N. tomoriana Danser*	AF315898	
N. truncata Macfarl.	AF315904	HM204910
N. veitchii Hook.f.*	AF315895	AB675909
N. ventricosa Blanco*	AF315892	HM204911
N. vieillardii Hook.f.		HM204912
N. villosa Hook.f.*	KP978726	KP978864
N. vogelii Schuit. & de Vogel		KR698379

Supplementary Table 3. References used for construction of character optimizations for silica presence, helical thickenings, successive cambia and perforation plate border in the Caryophyllales order presented in Fig. 16.

Family	**Genus**	**Character reference**
Dioncophyllaceae	*Triphyophyllum*	Gottwald and Parameswaran 1968; Carlquist 1988, 1999, 2010
	Dioncophyllum	Gottwald and Parameswaran 1968; Carlquist, 2010
Ancistrocladaceae	*Ancistrocladus*	Gottwald and Parameswaran 1968; Carlquist, 2010
Drosophyllaceae	*Drosophyllum*	Metcalfe and Chalk 1950; Carlquist 1988, 1995, 2010
Droseraceae	*Drosera*	Metcalfe and Chalk 1950; Carlquist 1988, 1995, 2010
Nepenthaceae	*Nepenthes*	this study
Frankeniaceae	*Frankenia*	Metcalfe and Chalk 1950; Carlquist 1988, 2010; Olson et al. 2003
Tamaricaceae	*Tamarix*	Metcalfe and Chalk 1950; Carlquist 1988, 1995, 2010
Polygonaceae	*Polygonum*	Metcalfe and Chalk 1950; Carlquist 1988, 2003, 2010
Plumbaginaceae	*Plumbago*	Metcalfe and Chalk 1950; Carlquist and Boggs 1996; Carlquist 2010
	Limonium	Metcalfe and Chalk 1950; Carlquist and Boggs 1996; Carlquist 2010
Rhabdodendraceae	*Rhabdodendron*	Carlquist 2001
Simmondsiaceae	*Simmondsia*	Carlquist 2002, 2010
Asteropeiaceae	*Asteropeia*	Carlquist 2006, 2010
Physenaceae	*Physena*	Carlquist 2006, 2010
Achatocarpaceae	*Phaulothamnus*	Carlquist 2000a, 2010
Amaranthaceae	*Celosia*	Carlquist 2003b
Stegospermataceae	*Stegnosperma*	Carlquist 1999
Barbeuiaceae	*Barbeuia*	Carlquist 1999
Phytolaccaceae	*Phytolacca*	Carlquist 2000b
	Rivina	Carlquist 2000b
Nyctaginaceae	*Mirabilis*	Carlquist 2004
	Bougainvillea	Carlquist 2004
Basellaceae	*Basella*	Carlquist 1999a; b
Didieraceae	*Calyptrotheca*	Carlquist 1999a; Carlquist 2010
Portulaceae	*Portulaca*	Carlquist 1999a; Carlquist 2010
Cactaceae	*Pereskia*	Mauseth and Landrum 1997; Carlquist 1999b; Carlquist 2010
	Opuntia	Carlquist 1999b, 2010

Supplementary Table 4. Data used in pairwise comparisons of our measured *Nepenthes* wood anatomical characters. Precipitation data averaged from extracted from species' localities. Soil and plant length referenced from the descriptive texts of McPherson (2009) and the IUCN (2015).

Nepenthes species	Juvenile specimen	Growing on ultramafic soil	Maximum plant length (m)	Precipitation driest month (mm)	Annual precipitation (mm)
N. ampullaria	-	-	20	169	3216
N. bicalcarata	+	-	25	175	3153
N. bokorensis	+	-	7	20	2458
N. burbidgeae1	+	+	20	106	2369
N. burbidgeae2	+	+	20	106	2369
N. chaniana	+	-	8	109	2075
N. distillatoria	+	-	9	128	3797
N. edwardsiana	+	+	15	105	2411
N. gracilis	-	-	2	150	2944
N. gracillima	+	-	3	130	2618
N. gymnamphora	-	-	20	96	2916
N. hemsleyana	+	-	6	173	2953
N. hirsuta	+	-	7	181	3223
N. kerrii	+	-	4	38	2442
N. khasiana	-	-	12	6	1875
N. lamii	+	-	4	226	3107
N. lowii	-	-	13	166	2624
N. macfarlanei	+	-	4	125	2646
N. madagascariensis	-	-	1.5	32	1798
N. maxima	+	-	19	185	3064
N. mirabilis	-	-	15	101	2478
N. muluensis	+	-	4	209	3117
N. neoguineensis	+	-	10	173	2866
N. pervillei	+	-	8	66	2134
N. pilosa	+	-	4	111	1887
N. rafflesiana	-	-	15	156	2921
N. rajah	-	+	1.5	111	2243
N. reinwardtiana	+	-	13	152	2747
N. rhombicaulis	-	-	35	NA	NA
N. sanguinea	-	-	10	135	2770
N. smilesii	-	-	5	6	1281
N. stenophylla	+	-	12	176	2683
N. tentaculata	+	-	8	156	2811
N. thorelii	-	-	4	5	1589
N. tobiaca	-	-	10	100	2307
N. tomoriana	-	+	8	73	1983
N. veitchii	+	-	10	186	3026
N. ventricosa	-	-	10	69	2411
N. villosa	-	+	8	117	2340

Supplementary Table 5. Pairwise comparisons of *Nepenthes* wood anatomical characters together with specimen and species character and abiotic measures. (p < 0.05 marked with *).

	Juvenile specimen	Grows on ultramaphic soil	Maximum plant length	Precipitation driest month	Annual precipitation
Pith lignification	0.27	-0.08	0.23	0.18	0.12
Vessel min.	0.03*	0.22	0.12	-0.02	-0.09
Vessel average	0.24	0.05*	0.25	0.17	0.12
Vessel max.	0.25	0.00*	0.23	0.27	0.18
Vessel density min	-0.03*	-0.18	0.10	0.17	0.13
Vessel density avg.	-0.13	-0.19	0.03*	0.05*	0.04*
Vessel density max	-0.17	-0.23	0.02*	-0.06	-0.05*
Vessel element length min.	0.13	-0.16	-0.08	-0.09	-0.10
Vessel element length average	0.00*	-0.11	-0.08	-0.03*	-0.07
Vessel element length max	-0.06	-0.04*	0.00*	-0.01*	-0.12
Multiseriate ray height min.	0.12	-0.12	-0.11	-0.21	0.01*
Multiseriate ray height avg.	0.10	-0.31	0.12	0.02*	0.28
Multiseriate ray height max.	0.11	-0.26	0.27	0.06	0.30
Rays exclusively uniseriate	0.23	-0.17	-0.05*	0.17	0.06
Max ray width (nr. cells)	-0.29	0.04*	-0.13	-0.26	-0.07
Silica bodies in rays	0.16	-0.10	0.02*	0.13	0.06
Fibre-tracheids thick-walled	0.09	-0.25	-0.15	0.19	0.19

Chapter Four

Ontogeny and anatomy of the dimorphic pitchers of *Nepenthes rafflesiana*

Rachel Schwallier, Valeri van Wely, Mirna Baak, Rutger Vos, Bertie Joan van Heuven, Erik Smets, Rogier R. van Vugt and Barbara Gravendeel

An enigmatic feature of tropical pitcher plants belonging to the genus *Nepenthes* involves their dimorphic prey-capturing pitfall traps. The conspicuously shaped upper and lower pitchers grow from a swollen leaf tendril tip until finally opening as insect-alluring devises. Upper pitchers are usually more slender and have their openings facing away from the tendril. They evolve higher up on the plant near the canopy and are adapted to catch flying prey species. Lower pitchers have their opening facing towards the tendril. They evolve lower on the plant near the forest floor and are adapted for luring crawling prey species and plant debris. Few have studied the ontogeny of these traps from an anatomical and quantitative morphological perspective. Whether one pitcher type in *N. rafflesiana* might be a modified form of the other was assessed by tracking the development of microstructures and shape using microscopic investigations and three-dimensional morphometrics with landmark analysis. Four important morphological changes occurring during pitcher ontogeny were identified and defined as curvation, elongation, inflation and maturation phases, respectively. Pitcher length indicated progress through developmental phases, and we propose to use it as a tool for indication of developmental stage. Microstructure development coincided with the developmental phases defined. Ontogenetic shape analysis indicated that upper and lower pitcher types develop with similar phase progression, but have no directly overlapping morphology. This means that upper pitchers are not a derived state from lower pitchers. Independent developmental programs might have evolved to produce distinctly shaped upper and lower pitchers to exploit different food sources.

Keywords: 3D geometric morphometrics (3D GM), carnivorous plants, development, landmark analysis, microstructure, pitcher plants

Introduction

The tropical carnivorous plant genus *Nepenthes* is characterized by one of the most striking morphological features of plants: an insect-luring pitfall trap. These traps, modified leaf tips called pitchers, evolved when traditional nutrient resources were scarce and facilitate the capture of an abundant nitrogen alternative (Juniper et al., 1989; Schulze et al., 1997). Due to high resource costs of pitcher production (Osunkoya et al., 2007, 2008; Ellison & Gotelli, 2009), the genus is most competitive in areas of low nutrient availability throughout the Malay Archipelago, Australia, India and Madagascar (Cheek et al., 2001; Meimberg & Heubl, 2006). Throughout their region, the 140+ recorded species (Cheek & Jebb, 2013) attract foraging, flying or crawling insects such as flies, ants or termites through visual lures (Moran et al., 1999; Moran, Clarke, & Gowen, 2012), extrafloral nectar bribes (Jan, 2007; Bauer et al., 2009a; Chin et al., 2010) and olfactory cues (Di Giusto et al., 2010). Food sources that are captured and retained via mechanisms such as slippery 'aquaplaning' surfaces near the trap opening (Bohn & Federle, 2004; Bauer et al., 2008), viscous pitcher fluid (Gaume & Forterre, 2007; Di Giusto et al., 2008; Bonhomme et al., 2011b) and waxy inner surfaces (Gaume & Di Giusto, 2009; Scholz et al., 2010; Gorb et al., 2013) are broken down through enzyme rich digestive fluids (Owen & Lennon, 1999; Owen et al., 1999; Thornhill et al., 2008). Shape (Chin et al., 2010; Moran et al., 2013) and the pitchers various microstructures (i.e. digestive glands, extra-floral nectaries, cells that release wax etc.) play a direct role in the capture and retention of prey (Bauer & Federle, 2009; Di Giusto et al., 2010; Bonhomme et al., 2011b; Ulrike Bauer, 2012; Gorb et al., 2013). Although the majority of species are believed to be prey generalists, capturing a wide variety of insects for their diet (Moran & Clarke, ; Juniper et al., 1989; Bauer et al., 2009a), several recent studies show that many species are quite specialized in their pursuits. Pitchers of *N. lowii* and *N. rajah*, for example, are distinctively shaped (Clarke et al., 2009; Chin et al., 2010) for their diet specialization: they receive nutrient-rich feces from small mammals that sit over the trap opening while they feed on nectar produced in the lid. The single detritivore described, *Nepenthes ampullaria,* has an open-mouth and minimized lid to capture leaf-litter (Moran et al., 2003; Pavlovič et al., 2011) and the remarkable *N. gracilis* flicks large ants into its trap that are perched under the slippery waxed lid surface when a mere drop of rain falls from above (Bauer et al., 2012).

Beyond the amazing architectural diversity of pitchers and microstructures within the genus (Moran, Booth, & Charles, 1999; Bonhomme et al., 2011; Bauer et al., 2012), pitcher dimorphism of an individual plant is also quite striking (Rembold et al., 2010; Bauer et al., 2011). The first mature pitcher type, or lower pitcher, is produced on or just above the forest floor during the earlier stages of mature plant growth, when plants are still self-supporting shrubs. With maturity, the majority of the species of *Nepenthes* become sprawling and climbing lianas that, in addition to lower pitchers, also produce morphologically distinct upper pitchers, possibly due to exposure to different conditions of humidity, light and/or temperature. These upper pitchers are produced from leaf tips that occur higher up in the vegetation. Inventories of upper and lower pitchers show variation in diet (Jebb, 1991; Moran, 1996; Rembold et al., 2010), which suggests that having two functionally different pitchers could be an evolutionarily advantage to capture a broader prey diversity. In a comparative study of many data sources, Rembold et al. (2010) found that the dimorphic pitchers of *N. gracilis, mirabilis and N. madagascariensis* captured ground dwelling ants and that the upper pitchers additionally attracted flying, flower-visiting insects. Moran (1996) found similar results in *N. rafflesiana*.

Despite the large differences in the two mature pitcher types, little is known about them from an ontogenetic and morphometric perspective. With the increasing interest and number of publications involving this genus, a solid framework of pitcher development and its associated microstructures is a much-needed base. Although botanical morphology has historically been an important tool to taxonomists and ecologists, very few botanical studies have employed the more recent technologies of 3D geometric-morphometric analysis yet (Viscosi et al., 2009; van der Niet et al., 2010).

In this work, we study the ontogeny of both upper and lower pitcher types of *N. rafflesiana* based on 3D geometric-morphometrics and microscopic microstructure analysis for the following research questions; (i) Are their overlapping forms throughout the progression of dimorphic pitcher development or are the pitchers distinct morphologically throughout development? (ii) Does development of microstructures correspond with progression in pitcher development? (iii) Is microstructure development different in the two pitcher types?

Materials and Methods

Study organism: *Nepenthes rafflesiana* Jack occurs abundantly in the heath forest of Borneo, the southern half of peninsular Malaysia and more sparsely in Sumatra (Meimberg et al., 2001) on nutrient-poor white sands (Clarke & Wong, 1997). Mature plants produce a relatively high number of strongly dimorphic pitchers (Fig. 17) each growing season, making this species an ideal candidate for our ontogenetic study. Pitchers were monitored over a two-year period from seven mature plants grown in the Hortus botanicus of Leiden University in a south-facing window, at approximately 21°C, 65% humidity.

Traditional morphometrics: In year one, we employed traditional morphometric analysis on seven lower pitchers and the sole upper pitcher growing in the botanical garden twice a week. The starting point of development in our study began at the moment of 90° pitcher-tendril curvature as this was the point for which the various morphological features scored were distinguishable for measurement. Lower pitchers were identified as those with a tendril attachment born from the front, or opening side, of the pitcher, facing towards the tendril. They are ellipsoid-shaped and bear ladder-like structures (Fig. 17). The distinguishable upper pitchers have a rear tendril-attachment, are

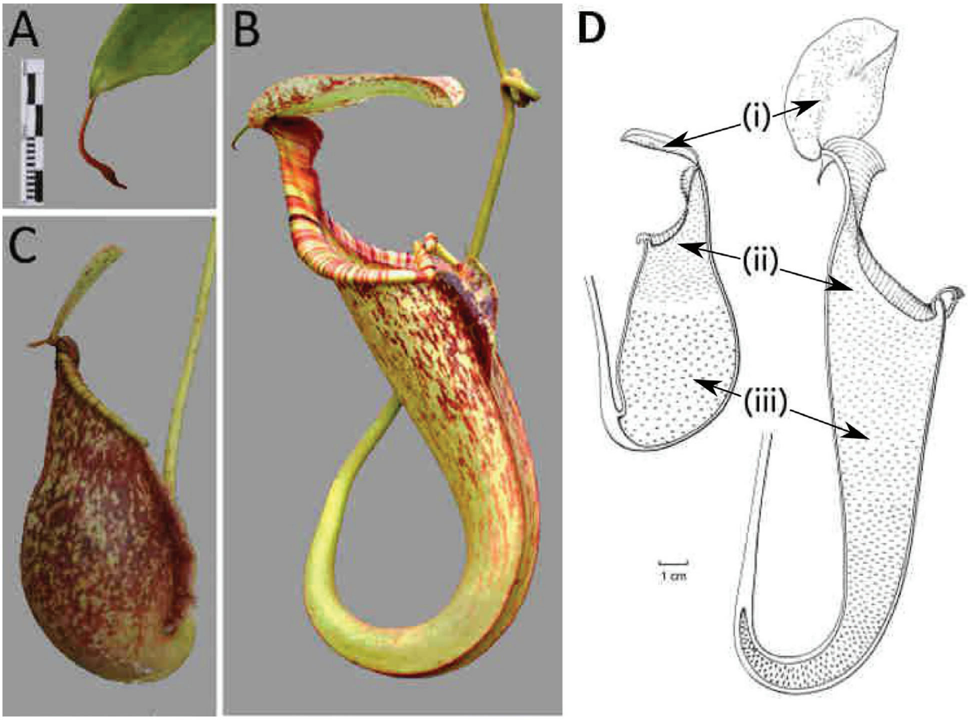

FIGURE 17. Pitcher dimorphism in *Nepenthes rafflesiana*: (A) The swollen tendril tip, (B) mature lower pitcher and (C) upper pitcher. Scale bar = 40 mm. Schematic longitudinal sections of *Nepenthes* (D) lower (left) and upper (right) pitcher indicating (i) peristome, (ii) waxy glands and (iii) digestive glands.

funnel-shaped and have a prominent, sculpted protrusion on the front side of the pitcher at maturity (Fig. 17). They have their opening facing away from the tendril. We measured pitcher length with an electronic digital caliper (0-150mm) from the hinge of the lid to the lowest point of the curved pitcher base as previously described by (Owen & Lennon, 1999).

We additionally measured width directly under the peristome and at half and one quarter of the total pitcher length.

Scanning Electron Microscopy (SEM): In year one, seven lower pitchers were scanned along with the sole upper pitcher growing in the botanical garden. Year two allowed us to increase the number of replicates for each phase (n = 5, for all but the mature upper pitcher n = 3, which was more limited in material). Freshly collected pitchers were fixed in formaldehyde-acetic acid-alcohol (FAA) (925ml Ethanol 50%, 50ml Formalin 37%, 25ml Acetic Acid 100%) for seven days and then stored in a 50% ethanol solution before SEM preparation. Dissections were made from the microstructure-containing zones (Juniper et al., 1989; Adlassnig et al., 2011) and dehydrated via a series of Ethanol solutions: 50%-70%-80%-96%-100%-100%, with ten-minute incubation steps. Dehydrated samples were critical point dried using the Leica EM CPD300. Samples were mounted on SEM-stubs and sputter-coated with a 10nm layer of Platinum/Palladium-alloy using the Quorum Q150TS. SEM imaging of microstructures was performed at 5.0 kV using the JEOL JSM-7600F SEM.

Light Microscopy (LM): FAA-fixed pitchers were dehydrated through a series of ethanol solutions (50%-70%-90%-96%-100%-100%) for a minimum of eight hours per step. HistoClear replaced ethanol via a gradual increase in HistoClear (25%-50%-75%-100%-100%). HistoClear was subsequently replaced by paraplast at 60°C (33%-50%-67%-100%-100%). Hardened paraplast samples were sectioned at 8 μm with an E. Leitz Wetzlar microtome, applied on object-glasses and stained with Etzolds staining solution (stock: 10 mg Basic Fuchsin, 40 mg safranin, 150 mg Astra Blue, 2 ml Acetic Acid filled up to 100 ml with demi-water) for two hours and washed with demi-water. Paraplast was removed by washing three times for five minutes with HistoClear. DPX was applied between object-glasses and cover-glasses and left to dry overnight. Microscopy slides were observed with an upright Zeiss Axio Imager, M2 Zeiss light microscope and plan Apochromatic 5x (0.16 correction), 10x (0.18 corr.), 20x (0.8 corr.), 40x (0.95 corr.) and 63x (0.95 corr.) objectives. Digital images were obtained with a five megapixel AxioCam MRc 5 and associated Axio Vision SE64 Rel. 4.8 software.

Detection of sugars in peristomal fluid: Peristomal fluid (10 μl) was mixed with 2 μl Fehling's solution A (stock: 3.45 grams hydrated copper sulfate and 50 ml demi-water) and 2 μl Fehling's solution B (stock: 8.25 grams sodium potassium, 3.34 grams sodium hydroxide and 23.8 ml demi-water). The mixture was heated for 5 minutes in a water bath (90ºC). A blue to red color shift indicated the presence of monosaccharaides in the peristomal fluid.

Freshly collected pitchers were fixed in liquid nitrogen and ground with an electric blender into powder. A total of 50 mg of this powder was ultrasonicated in 0.75 ml of CH_3OH-d_4 and 0.75 ml of KH_2PO_4 buffer in D_2O (pH 6.0) containing 0.1% (w/w) TMSP for 15 min followed by centrifugation for 13 min at 13,000 rpm. An aliquot of 0.8 ml of the supernatant was transferred into a NMR tube for NMR measurements at IBL in Leiden.

^{1} H NMR spectra were recorded at 25°C on a Bruker 600 MHz AVANCE II NMR spectrometer operating at a proton NMR frequency of 600.13 MHz equipped with TCI cryoprobe and Z-gradient system. CD3OD was used for internal lock purposes. For 1D-1H NMR spectra, a total of 32,768 data points were recorded covering a spectral window of 9615 Hz. A total of 128 scans of standard one-pulse sequence with 30 degrees flip angle for excitation and

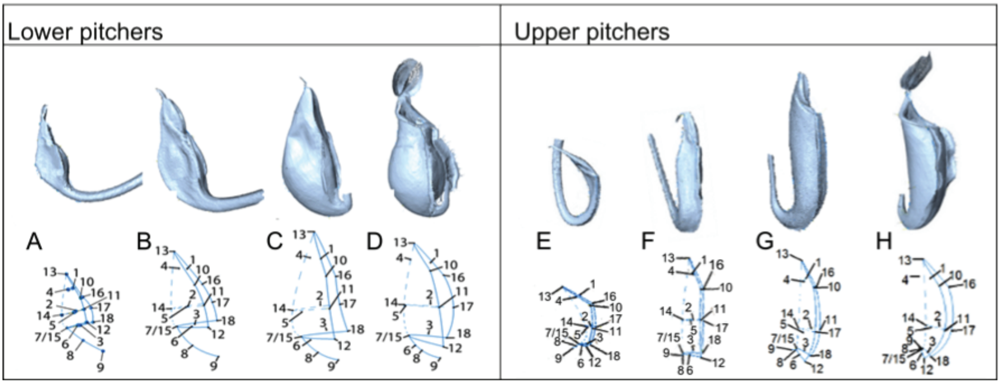

FIGURE 18. (A) Three-dimensional models of *Nepenthes rafflesiana* pitchers from all developmental phases and their landmarked coordinates used for morphometric analysis. Lower pitchers of (A) Curvation phase, (B) Elongation phase, (C) Inflation phase and (D) Maturation phase. Upper pitchers (E-H) are presented with corresponding phases.

presaturation during 2 s relaxation delay were employed with an effective field of cBl=50 Hz for suppression of the residual H2O signal.

3D surface laser scanning and landmark-based geometric morphometrics: Upper and lower pitchers from all developmental stages were three-dimensionally (3D) scanned with a NextEngine 3D scanner HD 2020i. Pitchers were scanned at a maximum resolution of 40.000 points per inch, using the laser triangulation method with multiple laser-stripes sweeping over the pitcher surface. Raw 3D data were auto-aligned, trimmed and refined using ScanStudio HD software version 1.3.2. Eighteen landmarks, which describe the overall pitcher shape (Fig. 18 and S5), were applied to the 3D models using Landmark 3.0.0.6.**Pipeline created in Galaxy for statistical analysis of pitchers:** We wrote several tools in R to analyze the 3D *Nepenthes* pitcher scans, although the tools could be used for any 3D file that is landmarked. The tools created can be downloaded through the Galaxy platform (https://zenodo.ord/record/9981) or installed via the Galaxy Tool Shed from repository name 'nepenthes_3dpca' (Goecks et al., 2010). Several separate programs together form a pipeline, and begins with the 'Get Data' tool, which prompts importation of the Landmark software file (.dta file). To use the coordinates in the .dta file, we created the 'DtaConverter' tool, which converts the file to a comma-separated file (.csv). A procrustes analysis was used to minimize differences between pitchers through rotation and scaling (Kendall, 1989) before we performed a principal component analysis (PCA). In the PCA, the number of variables is reduced to a new set of variables – a combination of variables called components; the first of which explains the greatest variance of the set (Wold et al., 1987). Regression analysis and permutation tests (10,000 resampling events) were performed on PC1, PC2 and centroid size. Our PCA results were imported into the 'PCA plot' tool to create a graph of the results, into the ‘Variance plot’ tool to produce a bar plot with the variance calculated from the standard deviation and the ‘Csize plot’ tool to produce a graph of the centroid size of the scanned pitchers was plotted against a chosen Principal Component. A workflow was made of these tools in Galaxy to streamline processing.

Results

Four developmental phases describe pitcher ontogeny: Four distinct stadia based on clear morphological changes paralleled in upper and lower pitcher ontogeny were identified. These were accordingly defined as the curvation phase, elongation phase, inflation phase and maturation phase. Pitchers are first distinguishable as a swollen tendril tip (Fig. 17A). In concert with our method of morphometric analysis, the first developmental phase is defined as the curvation phase, characterized by a strong curvature at the junction of tendril attachment to the pitcher. Pitchers in the elongation phase increased considerably in length and depth (Figs. S3 & S4). The flattened pitcher appearance changes in the inflation phase (Figs. S1 & S2), when width increases and length growth continues (Figs. S3 & S4). The coloration pattern of the pitcher, which is known to play a role in insect attraction (Moran et al., 2012), also becomes more apparent in this phase (Figs. S1 & S2). The maturation phase is characterized by the lid opening. Phases identified during traditional morphometric analysis led our investigation of microstructure development through Scanning Electronic Microscopy (SEM) and Light Microscopy (LM).

Extra-floral nectary and peristomal teeth: Progressive pitting of the peristomal nectaries characterizes the development of upper and lower pitchers of *N. rafflesiana*. Peristomal teeth begin as lateral ridges in the curvation phase and elongate through the remaining phases, eventually engulfing the peristomal glands in deep pockets between the mature curved teeth (Figs. 19 & 20). Light microscopy reveals glands of mature lower pitchers to have hollow cavities, surrounded by xylem tissue (Fig. 21). Peristomal fluid from on top of the peristome contained high amounts of sugars, indicated by a quick (within 1 minute) color shift when mixed with Fehling’s solutions. NMR analysis of exudates from this gland further showed the presence of sugars (Fig. S11). HNMR spectra of secretions from glands found on the underside of lid also showed sugar presence (Fig. S11). An additional stalked gland on the underside of the peristome was also visible with LM and was occasionally covered with structures reminiscent of left over fragments of a collapsed balloon-like structure (Fig. 21).

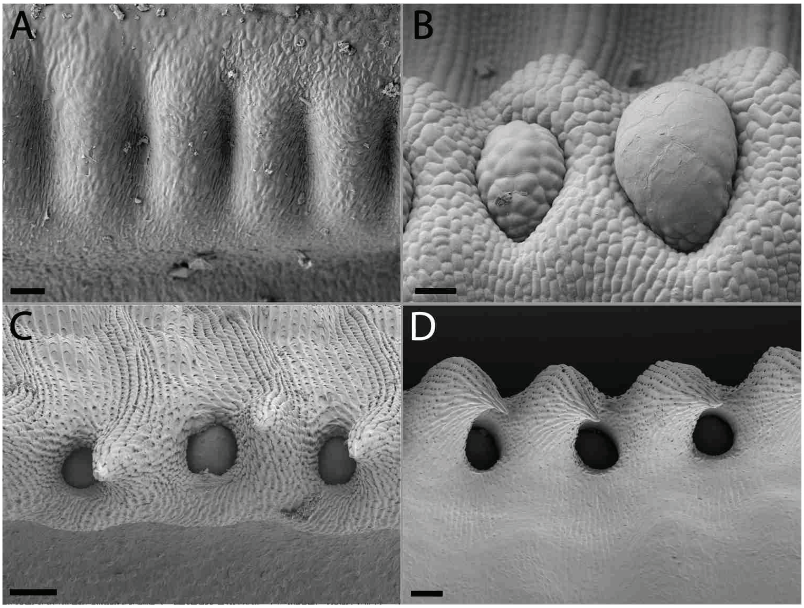

FIGURE 19. Peristomal gland development of lower *Nepenthes rafflesiana* pitchers. (A) Curvation phase - formation of peristome starts, glands absent. (B) Elongation phase - ridges clear, glands start developing at inner peristome. (C) Inflation phase - peristomal gland depressed in pits as peristomal teeth develop around them. (D) Maturation phase - Peristomal glands completely sunken into pits, flanked by fully developed peristomal teeth. (A, B) Scale bar = 20 μm & (C, D) scale bar = 100 μm.

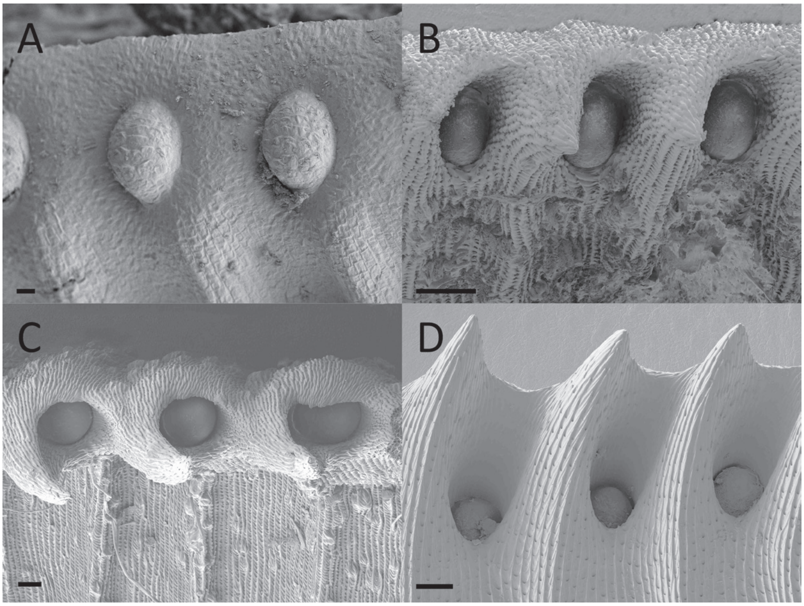

FIGURE 20. Peristomal gland development of upper *Nepenthes rafflesiana* pitchers. (A) Curvation phase - formation of peristome and glands. (B) Elongation phase - ridges and peristomal teeth clear, glands pitted in peristome. (C) Inflation phase - peristomal gland depressed in pits. (D) Maturation phase. Scale bar = 100 μm.

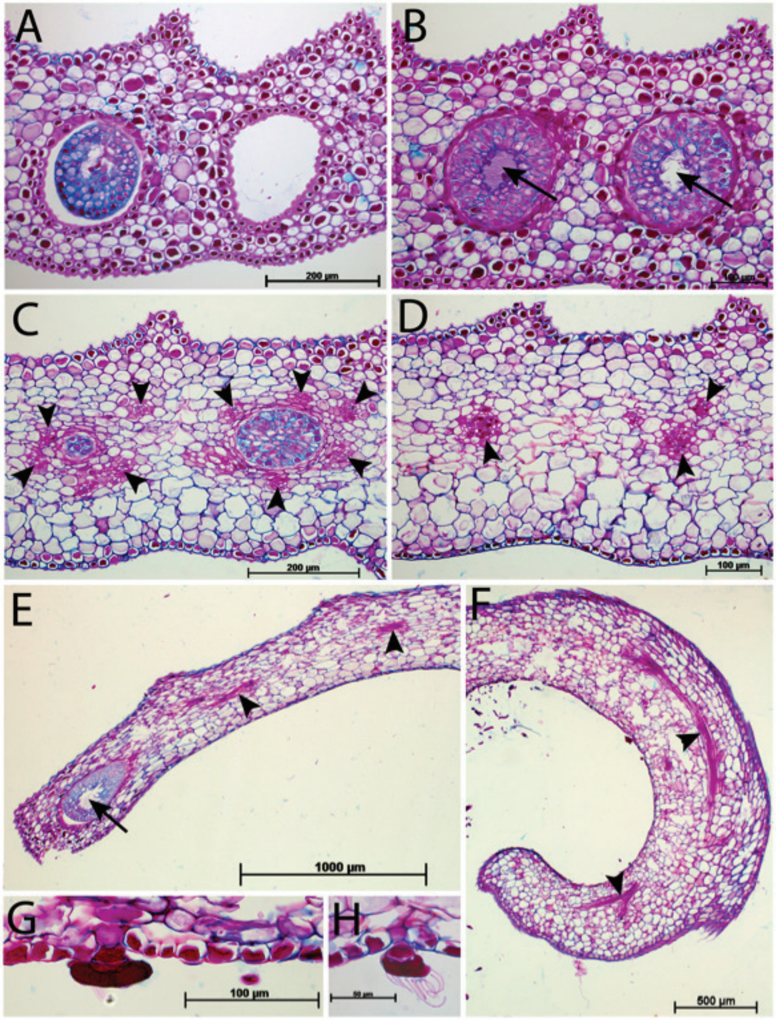

FIGURE 21. Light microscopy images of peristomal glands and vascular tissue from a mature *Nepenthes rafflesiana* pitcher. (A) Glands are present at the bottom of the pits between the peristomal teeth. (B) Cross sections through these glands show a hollow cavity (arrows) within peristomal glands. (C) Vascular tissue (arrowheads) in proximity to the deepest point of the peristomal glands. (D) Directly behind the glands, vascular tissue is also present. (E) Vascular tissue in the inner arm of the peristome near the peristomal gland (longitudinal section). (F) Vascular tissue present in the outer arm of the peristome. (G) Second type of gland, from the underside of the outer arm of the peristome. (H) Fragments of a structure disrupted during fixing were observed multiple times on these glands. A-D: Cross sections, E-H: Longitudinal sections.

Functional digestive glands formed early in pitcher development: Digestive glands of the dimorphic pitchers of *N. rafflesiana* mature early in development. Gland development begins in the curvation phase and final size is quickly reached in the elongation phase (Figs 22 & 23). Digestive glands actively secrete substance in the elongation phase of the lower pitcher as SEM showed substances on these glands that were completely absent from the surrounding tissue (Fig. 22). Upper pitchers are at least active during the inflation phase as there is notable fluid inside the still unopened pitchers. Although gland size remains unchanged during inflation and maturation, the distance between the glands increases (Figs. S7 & S8). Epidermal cells surrounding the glands differentiate to form an envelope structure over the top of the glands. These structures, or epidermal ridges (Owen et al., 1999), were found to be most prominent in the uppermost regions of the digestive zone. Upper pitcher ridging of digestive glands develops earlier and more progressively than in lower pitchers.

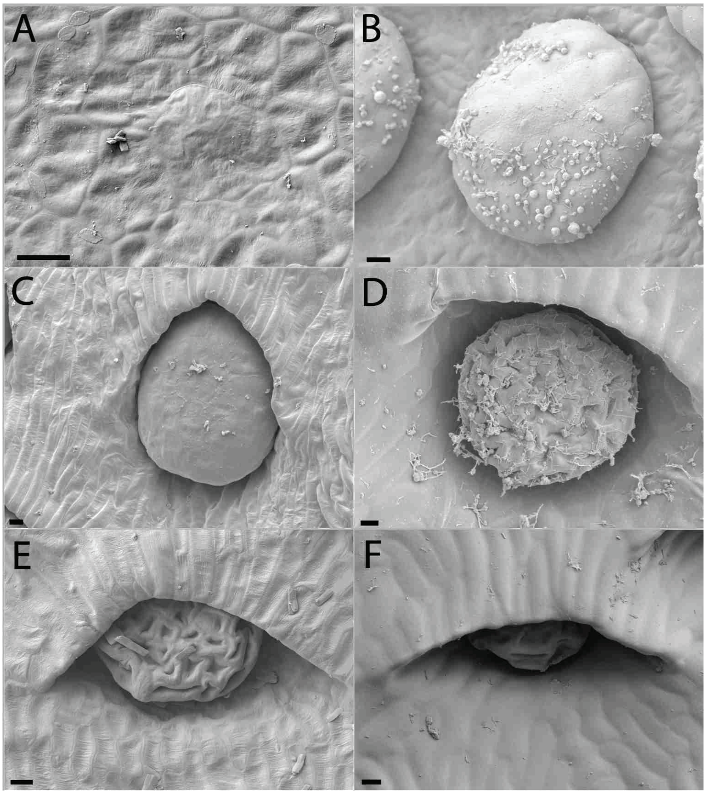

FIGURE 22. Digestive gland development of lower *Nepenthes rafflesiana* pitchers. (A) Curvation phase - small hump of cells visible. (B) Elongation phase - final size of gland reached and actively secreting. (C) Inflation phase - surrounding tissue changes, differentiates and envelops gland, which is even further exaggerated (D) higher on the pitcher. (E) Maturation phase – glands sunken into depression, and even further enveloped (F) higher up the pitcher. Scale bar = 10 μm.

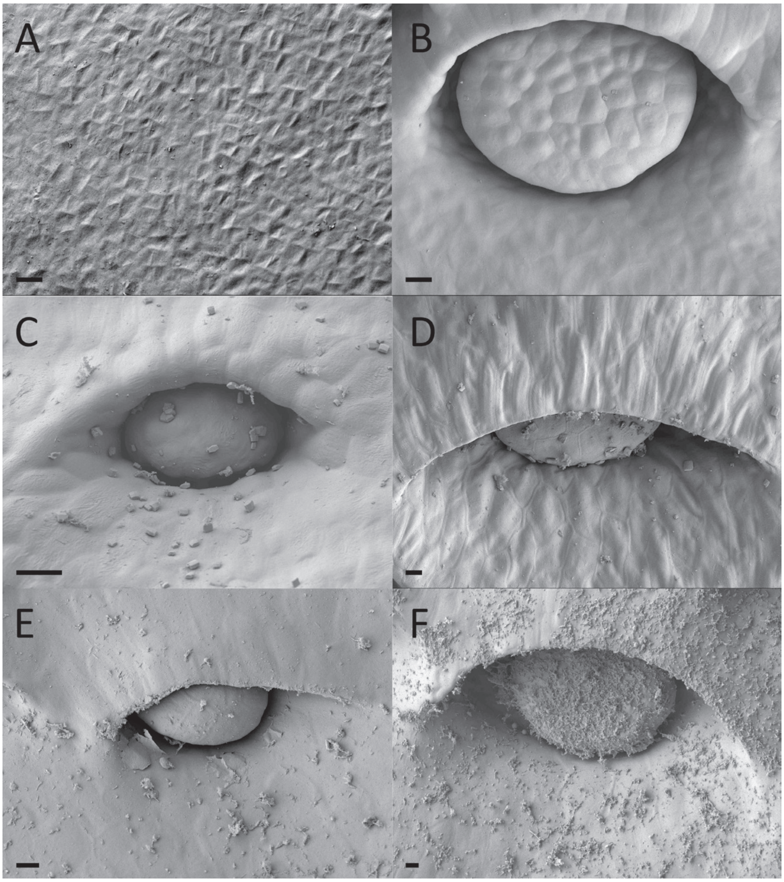

FIGURE 23. Digestive gland development of upper *Nepenthes rafflesiana* pitchers. (A) Curvation phase – faint cell formation visible. (B) Elongation phase - gland maximum size reached and slightly enveloped. (C) Inflation phase – gland more depressed into pit and enveloped considerably (D) higher up the pitcher. (E) Maturation phase – gland enveloped and even more so (F) higher up the pitcher. Scale bar = 10 μm.

Waxy scales and lunate cells appear during pitcher inflation: Lunate cells and the waxy layer that covers this area are typical features of the waxy zone of upper pitchers. Both structures are absent during the first two developmental phases (Figs. 24 & 25). Lunate cells can be observed during the Inflation phases, along with a sparse amount of wax. It was only in the mature upper pitcher that large amounts of wax were found to cover the entire waxy zone.

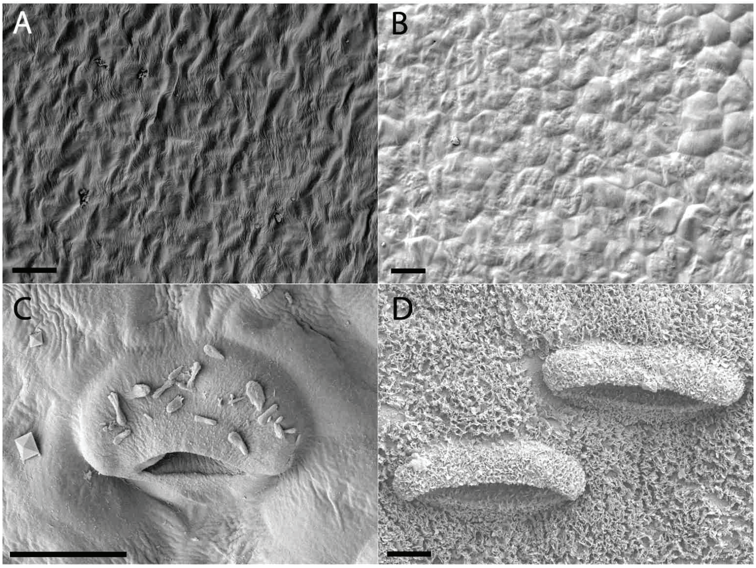

FIGURE 24. Waxy layer progression and lunate cell development of lower *Nepenthes rafflesiana* pitchers. (A) Curvation phase and (B) Elongation phase - lunate cells and waxy layer completely absent. (C) Inflation phase - lunate cells present and wax crystals on surface (D) Mature phase – significantly waxed surface completely covering the now wider lunate cells and surrounding tissue. Scale bar = 10 μm.

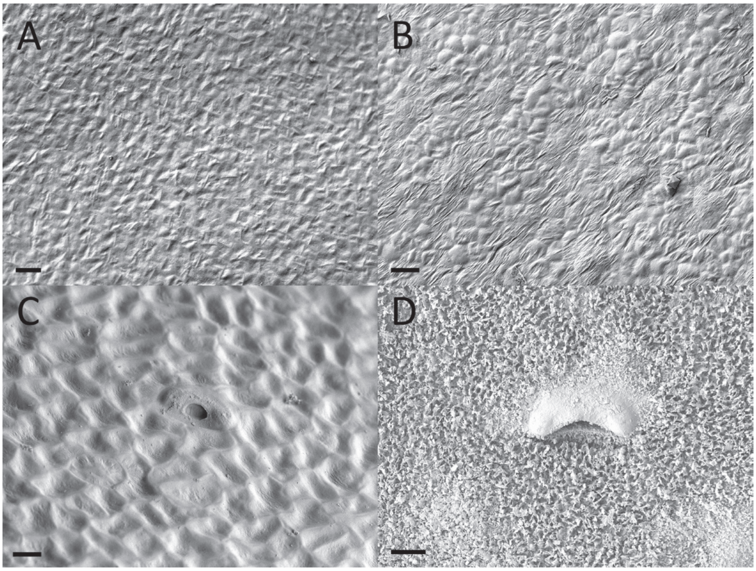

FIGURE 25. Waxy layer progression and lunate cell development of upper *Nepenthes rafflesiana* pitchers. (A) Curvation phase and (B) Elongation phase - lunate cells and waxy layer absent. (C) Inflation phase - lunate cell begins to develop, no wax yet present (D) Maturation phase - defined lunate cells and wax present. Scale bar = 10 μm.

Ontogeny of upper and lower pitchers: Laser scanning contributed large and accurate measurement quantifications. Our results show that upper and lower pitcher types develop into completely separate shapes from the onset of development in *N. rafflesiana.*

Discussion

Four different developmental stages are present in pitcher development: In this study, we were able to define four stages of pitcher development based on distinct morphological characteristics. The growth curves of all measured pitchers were found to follow a very similar pattern. Pitcher development was found to be rather constant even under quite variable climate conditions (i.e. light levels experienced in the greenhouses in Leiden during spring, summer, autumn and winter). However, we found the initiation of new pitchers to be slower during winter months. This suggests that day/night-light hours might be one of the largest contributing factors in pitcher development. Also, the moment of tendril curvation and the duration of this first phase are variable, ranging between ten and forty-nine days. From the moment of pitcher elongation, pitcher growth was found to be very similar (Fig. 20). A growth curve of *N. alata* obtained by Owen & Lennon (1999) only showed pitcher size development starting at 40mm, thereby presumably missing the curvation phase in their graph. This was possibly emitted or avoided on purpose (although not stated explicitly in the methodology), because of the variable length of the curvation phase. We found that after the curvation phase, pitcher size increases gradually during the last three phases of development. We therefore propose to use total pitcher size as an indicator of the developmental phase of a pitcher.

Based on the principal component coefficients, we found three main variables that affect PC1: wing length, tendril curvation and pitcher depth. PC1 was also found to be dependent on the pitcher size, further strengthening our suggestion that pitcher size can be used as an indicator for developmental phase. PC2 on the other hand is mainly composed of landmarks describing pitcher width. Since pitcher width increases drastically during inflation, PC2 accurately separates pitchers from the elongation phase and the inflation phase, as well as mature pitchers (Fig. S6). The mature upper pitcher (n = 3) was clearly separated from all the lower pitchers (n=6) when PC1 was plotted against PC2 (Fig. S6). Taken together, the four variables described are important features when separating *Nepenthes* pitchers of different developmental stages using geometric morphometrics.

Peristomal glands are surrounded by vascular tissue: Peristomal glands developed early during lower pitcher ontogeny, as these glands might be so complex that their development requires more time, hence their early development. Also, developing such glands late in ontogeny would be troublesome, because the surrounding tissue would already have differentiated and dedifferentiation of this tissue would in such case be required in order to enable the formation of peristomal glands.

The presence of vascular tissue in the proximity of the peristomal glands at the inner peristome indicates that these glands are indeed secretory glands, as was previously assumed. Hooker (1859) described these peristomal glands as nectaries. Despite reference to the existence of peristomal glands in *Nepenthes* (Pant & Bhatnagar, 1977; Juniper et al., 1989; Owen & Lennon, 1999), the glands that we observed in *N. rafflesiana* lower pitchers were shaped unlike traditional nectaries (Smets & Cresens, 1988). We found sugar-containing nectar on top of the peristome produced by the peristomal glands at the inside of the peristome-rim. NMR comparisons of secretions in the lid (with known nectar production (Di Giusto et al., 2010) and the peristome tissue show that they both contain extra-floral nectaries used to attract animals (Jan, 2007; Bauer et al., 2008, 2009b; Bauer & Federle, 2009; Bennett & Ellison, 2009). It is possible that capillary forces might be involved in transporting this nectar between the peristomal ridges and onto the peristome. Another remarkable finding was that the peristomal glands were hollow. Multiple cells seem to have disintegrated at the site of the cavity, indicating that this intracellular space is formed lysigenously (Fahn, 1990).

Based on their morphology, the second type of peristomal gland (found at the underside of the outer peristome) (Fig. 21) shows similarities to volatile-producing osmophores (Vogel et al., 1990) and oil glands (Fahn, 1979). The presence of scent-producing osmophores is plausible since it has been shown that the peristome is the main source of scent production (Di Giusto et al., 2010). Balloon-like structures as found in Lamiaceae might facilitate volatile evaporation. The fragments still visible might have been part of such a balloon-like structure on the gland. Based on the findings of Di Giusto et al. (2010) that upper pitchers emit a larger quantity of odours, we expected these glands to be more abundantly present in upper pitchers.

Digestive glands appear early in development: Digestive glands were found to mature early during development of upper pitchers, as was expected based on their importance for insect digestion (Thornhill et al., 2008) and apparent complex structure requiring a long developmental period. The digestive glands seem to be fully functional at the second developmental phase, since secretion in these closed pitchers can exclusively be observed on the surface of the digestive glands (Figure 22 and 23). This is in accordance with Thornhill et al. (2008), who observed digestive glands early in development of *N. tobaica* and *N. ventricosa* as well as enzyme activity in the digestive fluid, prior to lid opening.

SEM images from the inflation phase showing a bulging peristome and collapsed lunate cells in the waxy zone. The latter are presumably a consequence of the air trapped inside the inflated pitcher during fixation, thereby decreasing the fixation efficacy of FAA. When we cut the pitcher open prior to fixation this artifact disappeared.

Waxy scales and lunate cells formed later during development or not at all: Waxy scales and lunate cells were not observed in lower pitchers from the first two developmental phases, indicating that lunate cells and wax are formed later in pitcher development. However, the absence of these structures in these specific pitchers might also have to do with the age of the specific plant at the moment of pitcher formation. Gaume & Di Giusto (2009) have shown an ontogenetic loss of wax in juvenile *N. rafflesiana typica* plants; the waxy layer was found to be reduced in successively produced pitchers on juvenile plants. Ultimately, this results in the formation of pitchers (both lower as well as upper pitchers) without a waxy layer. An investment tradeoff has been suggested as a possible explanation for a reduced waxy layer in pitchers. Highly viscoelastic digestive fluid can be produced as an alternative trapping mechanism in pitchers and was found to be more effective in trapping flying insects than the waxy layer (Bonhomme et al., 2011b). These insects can only be caught higher up in the vegetation, though, so it might be that the plant has to wait until it has reached sufficient height first before it pays off to produce more digestive fluid and less waxy layers in the pitchers.

Gaume and Di Giusto (2009) found that the presence of a waxy layer in *N. rafflesiana typica* had no effect on the number of prey insects found in the lower pitchers. Furthermore, they found that the upper pitchers were completely devoid of such a waxy layer, and that lower pitchers only have a waxy layer when they are formed on juvenile plants. Lower pitchers on mature plants are devoid of a waxy layer.

Changes in 3D morphology of upper and lower pitchers show different trends: Three-dimensional models were obtained for the developmental phases of upper and lower pitchers. Landmarks were applied on the obtained 3D models (Fig. 18) and used for geometric morphometric analysis. PC1 explained 70.9% of the variance of these 3D models and PC 2 explained 14.9%. Lower pitchers in the early developmental phases have the highest PC1 and PC2 scores (Fig. S6) with both scores decreasing during pitcher development. Based on these two components, the upper pitcher morphology seems to be entirely separated from lower pitcher morphology.

A negative linear correlation was observed when PC1 was plotted against pitcher size (as centroid size). Based on regression analysis for PC1 and pitcher size, we could indeed reject H_0 and conclude that PC1 is dependent on pitcher size ($p<0.001$). PC2 seems to show a similar negative correlation with centroid size but this is contradicted by regression analysis ($p>0.05$).

PC1 mainly describes changes in height (x) and depth (z). The most important single landmark in this respect is landmark 9, which describes the position of the lowest point of the curved tendril at the back of the pitcher. Both height (x) and depth (z) coordinates of landmark 9 are major contributors to PC1. Secondly, the length of the wing (x coordinates of landmark 10, 12, 16 and 18) is important when separating the pitchers along with pitcher depth (described by z coordinates of landmarks 7, 15 and 14). In turn, PC2 mainly describes y-coordinates and thereby the width of the pitcher (y coordinates of landmarks 2, 3, 5 and 6).

Pitcher dimorphism is often described as being an ontogenetic process (Di Giusto et al., 2008; Moran et al., 2013) because lower pitchers initiate and develop on the plants first before upper pitchers appear. As we found that paedomorphy is not a likely driver of the major phenotypic difference between upper and lower pitcher forms, alternative pathways such as developmental plasticity within genes caused by epigenetic factors or completely separate genes controlling shape/development could be at play. If environmental conditions were mainly controlling pitcher type, though, you expect to see lower pitchers in the place of upper ones (and vice versa) at times, but this is never observed to our knowledge either in the field or in cultivation. Also, upper and lower pitcher types are often produced simultaneously, further indicating that more must be at play than the environment alone.

The few studies investigating leaf development in *Nepenthes* from a molecular perspective (Pinthong et al., 2009; Zakaria et al., 2016) report an increase in proteins during development progresses, but genes responsible for pitcher development are not yet identified.

Conclusions

In this research, we defined four important morphological ontogenetic stages in upper and lower pitchers of *Nepenthes rafflesiana*. These stages are of great importance for separating pitchers using Geometric Morphometrics. Pitcher 3D models of different developmental phases were separated with PCA based on increases in wing length, tendril curvation, pitcher depth (PC1) and pitcher width (PC2). Functional digestive glands were found to develop early during lower pitcher development. Extra-floral nectariferous peristomal glands also developed early during upper pitcher ontogeny in the elongation phase. Lunate cells and a waxy layer were not observed in the first two developmental phases in upper pitchers which might be caused by ontogenetic loss of the waxy layer in upper pitchers. Although microstructures and generalized changes occurred in parallel in upper and lower pitcher types, morphometric shapes of upper and lower pitchers are independent ontogenetic processes, with no overlapping quantitative shape. Based on these findings, we conclude that upper pitchers are not a derived state from lower pitchers. Independent developmental programs must have evolved to produce distinctly shaped upper and lower pitchers to exploit different food sources available near the canopy and forest floor, respectively. It would be very interesting to study differences in pitcher morphology between *N. rafflesiana* and other species of *Nepenthes* and possible correlations with diet composition. In addition to this, more knowledge about the genes involved in pitcher initiation and development would move the understanding of pitcher ontogeny forward considerably.

Supplementary material Figures S1-S11

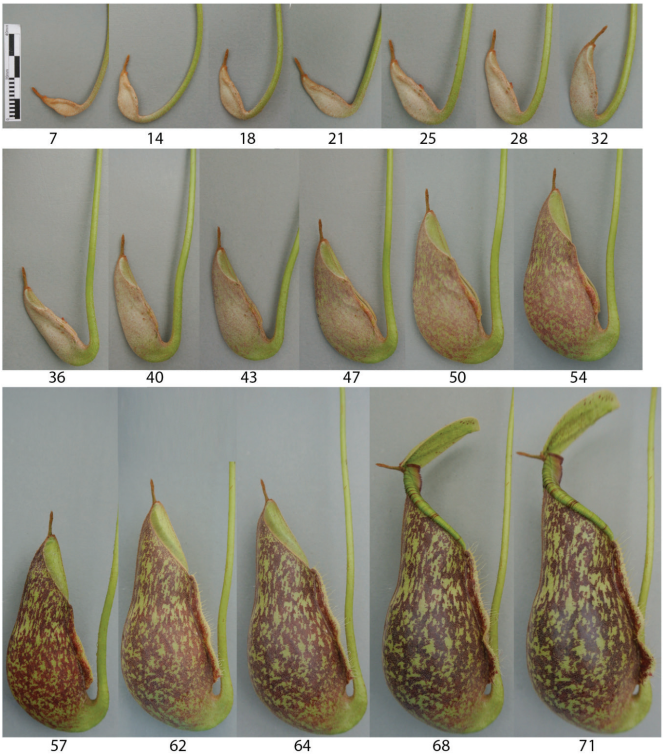

FIGURE S1. *Nepenthes rafflesiana* lower pitcher ontogeny. Numbers below pictures indicate days after tendril begins to curve. Scale bar = 40 mm.

FIGURE S2. *Nepenthes rafflesiana* upper pitcher phases. (A) Curvation phase, (B) Elongation phase, (C) Inflation phase and (D) Maturation phase. Scale bar = 40 mm.

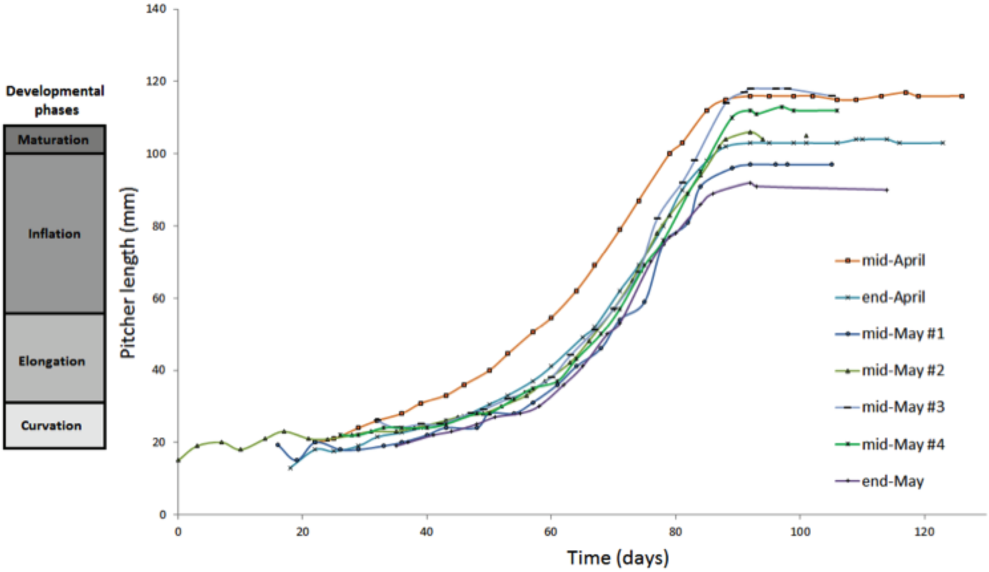

FIGURE S3. Pitcher length of seven *Nepenthes rafflesiana* lower pitchers throughout ontogeny. Pitchers named according to maturation date.

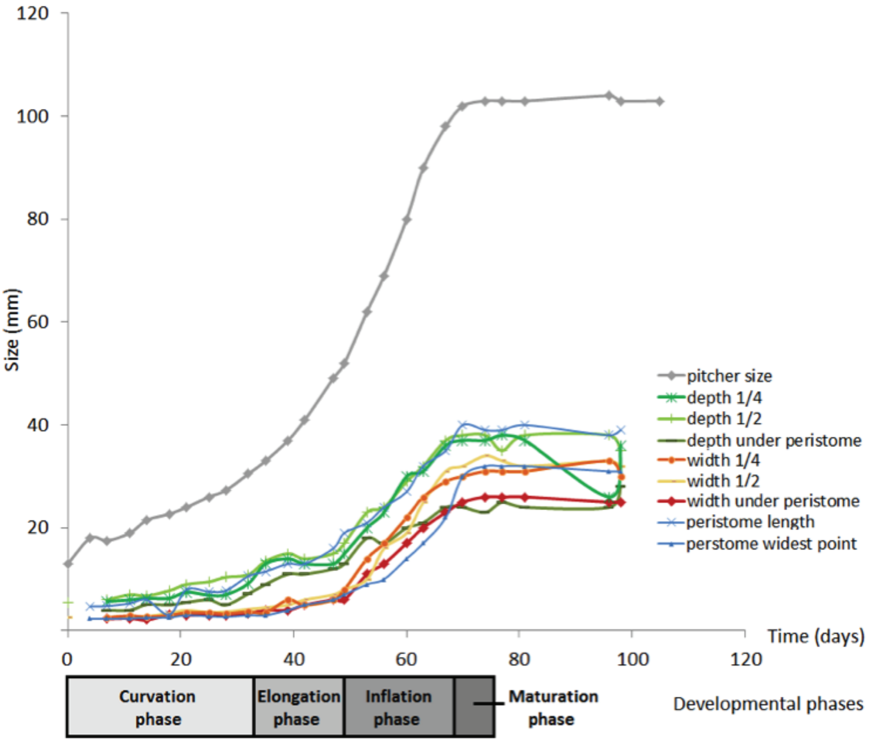

FIGURE S4. Width and depth measurements of developing *Nepenthes rafflesiana* lower pitcher.

Landmark	Type	Description
1	III	Under peristome betw 13 & 16, indicating width (right)
2	III	Halfway between 17 & 14, indicating width (right)
3	III	Halfway between 18 & 15, indicating width (right)
4	III	Under peristome between 13 & 10 indicating width (left)
5	III	Halfway between 11 & 14, indicating width (left)
6	III	Halfway between 12 & 15, indicating width (left)
7	III	Back of pitcher, opposite to lowest point of wing
8	III	Between lowest landmarks (7/15 & 9), on pitcher back
9	II	Lowest point of tendril curvation
10	II	Highest point of pitcher wing (right)
11	III	Middle point of pitcher wing (right)
12	II	Lowest point of pitcher wing (right)
13	II	Directly behind the spur
14	III	Back of pitcher, opposite to middle point of wing
15	III	Back of pitcher, opposite to lowest point of wing
16	II	Highest point of pitcher wing (left)
17	III	Middle point of pitcher wing (left)
18	II	Lowest point of pitcher wing (left)

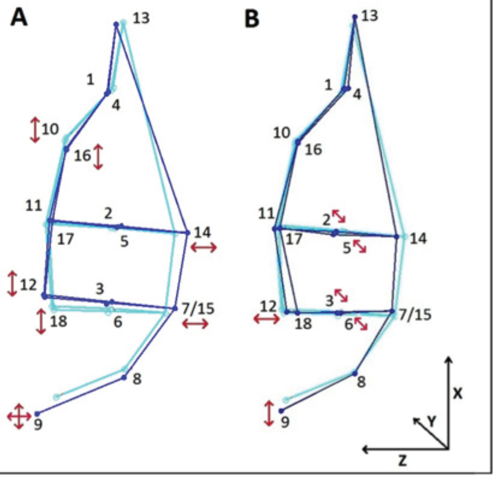

FIGURE S5. Landmark category and placement description on *Nepenthes rafflesiana* pitchers. Type I landmarks involve the juxtaposition of two types of tissue, type II is a maximum or minimum (top of pitcher, for example) and type III uses type II landmarks as a reference (between top and bottom of pitcher, for example). The right side of the figure shows a side view of pitcher wireframe with landmarks contributing to shape changes. Numbers correspond to landmarks in adjacent table. Red arrows indicate principal component coefficients above 0.200, and the orientation of the arrow indicates the axis (x, y or z). (A) Based on the Principal component coefficients, PC1 encompasses three main shape variables: wing length (landmarks 10,12,16 and 18), tendril curvation (landmark 9) and pitcher depth (landmarks 7/15 and 14). (B) Wireframe of PC2 shows that variance in PC2 is largely described by an increasing pitcher width during development (landmark 2, 3, 5 and 6).

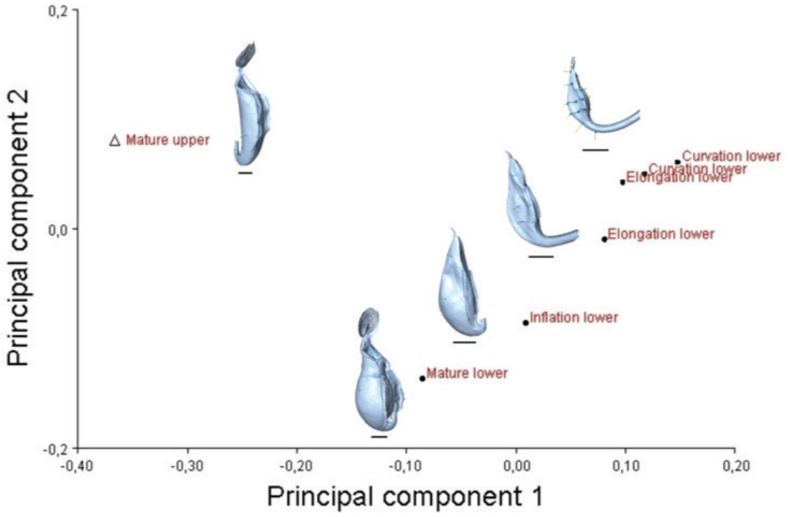

FIGURE S6. Principal component results for the developmental series of *Nepenthes rafflesiana* upper (left side series) and lower (right side series) pitchers. Principal component 1 and 2 separate pitchers from different developmental phases. PC1 accounts for 70.9% of the variance, mainly based on wing length, tendril curvation and pitcher depth. PC2 describes 14.9% of the variance, mainly based on pitcher width.

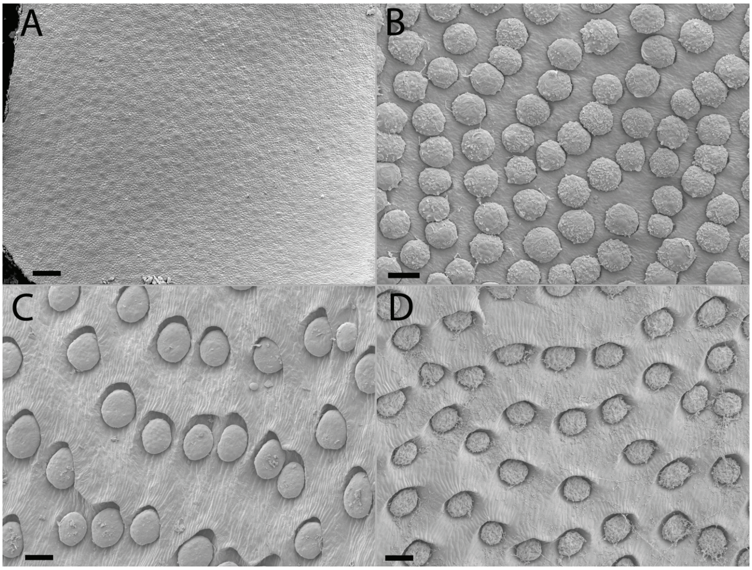

FIGURE S7. Overview of digestive gland development of lower *Nepenthes rafflesiana* pitchers. Developmental phases include the (A) Curvation phase, (B) Elongation phase, (C) Inflation phase and (D)Maturation phase. Scale bar = 100 μm.

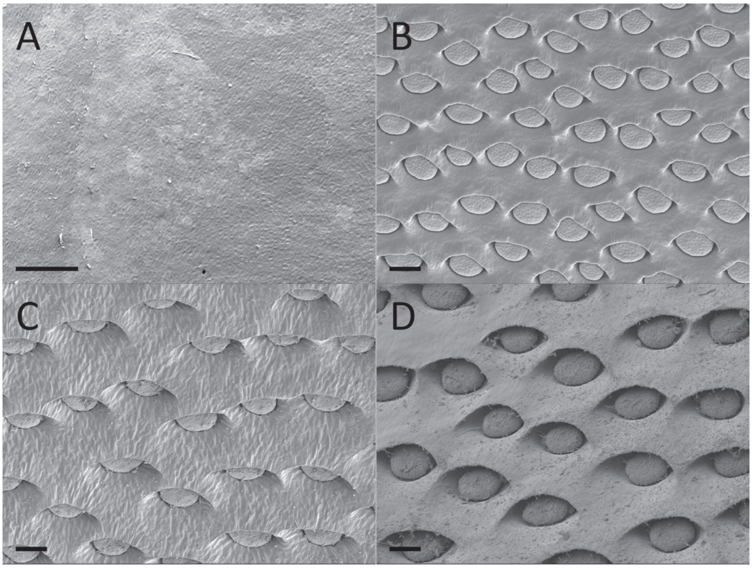

FIGURE S8. Overview of digestive gland development of upper *Nepenthes rafflesiana* pitchers. Developmental phases include the (A) Curvation phase, (B) Elongation phase, (C) Inflation phase and (D)Maturation phase. Scale bar = 100 μm.

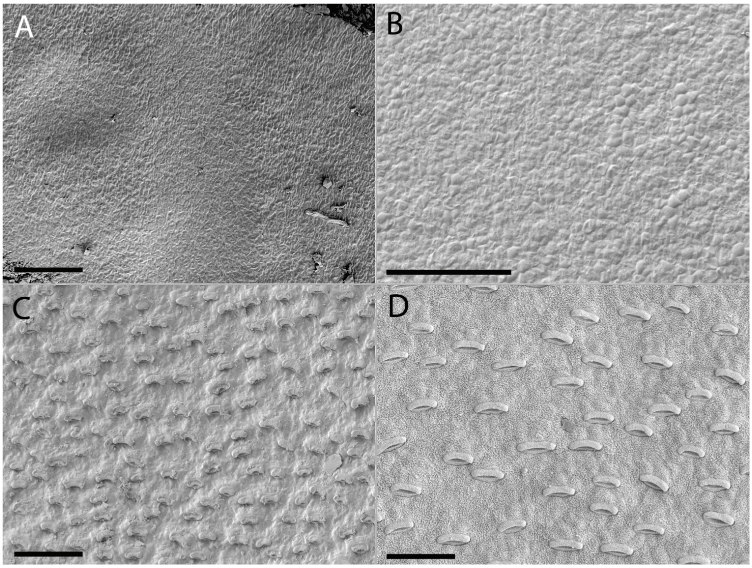

FIGURE S9. Overview of waxy layer progression and lunate cell development of lower *Nepenthes rafflesiana* pitchers. Developmental phases include the (A) Curvation phase, (B) Elongation phase, (C) Inflation phase and (D)Maturation phase. Scale bar = 100 μm.

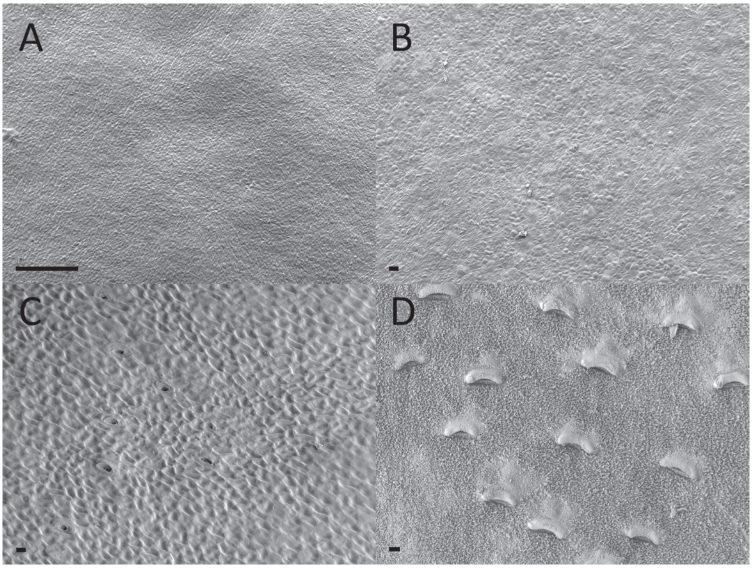

FIGURE S10. Overview of waxy layer progression and lunate cell development of upper *Nepenthes rafflesiana* pitchers. Developmental phases include the (A) Curvation phase, (B) Elongation phase, (C) Inflation phase and (D)Maturation phase. Scale bar = 100 μm.

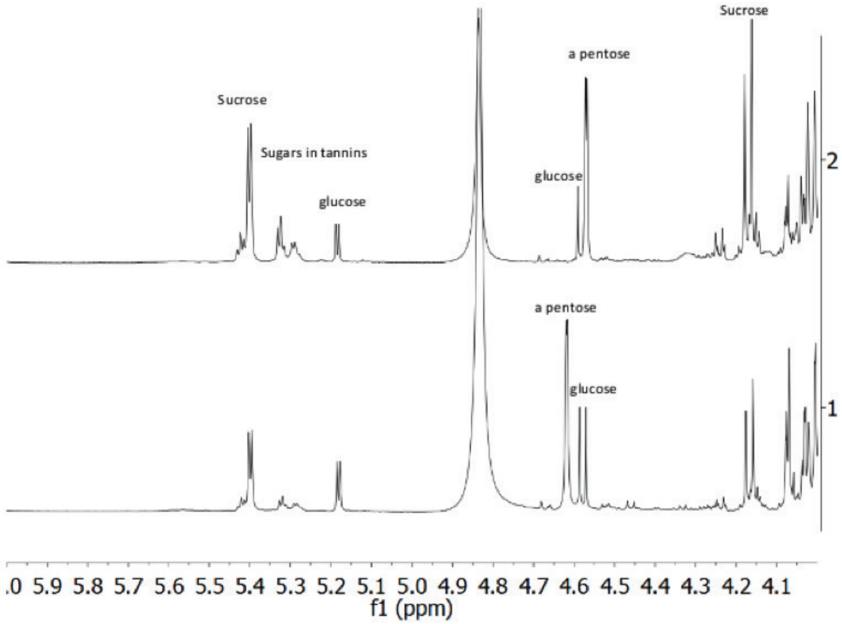

FIGURE S11. Sugar region of HNMR spectra of lid (1) and peristome (2) secretion.

Chapter Five

Journal of Ethnobiology and Ethnomedicine (2015) 11:24

Traps as treats: a traditional sticky rice snack persisting in rapidly changing Asian kitchens

Rachel Schwallier, Hugo J de Boer, Natasja Visser, Rogier R van Vugt and Barbara Gravendeel

An accessory to modern developing economies includes a shift from traditional, laborious lifestyles and cuisine to more sedentary careers, recreation and convenience-based foodstuffs. Similar changes in the developed western world have led to harmful health consequences. Minimization of this effect in current transitional cultures could be met by placing value on the maintenance of heritage-rich food. Vitally important to this is the preservation and dissemination of knowledge of these traditional foods. Here, we investigate the history and functionality of a traditional rice snack cooked in *Nepenthes* pitchers, one of the most iconic and recognizable plants in the rapidly growing economic environment of Southeast Asia. Social media was combined with traditional ethnobotanical surveys to conduct investigations throughout Malaysian Borneo. Interviews were conducted with 25 market customers, vendors and participants from various ethnical groups with an in-depth knowledge of glutinous rice cooked in pitcher plants. The acidity of pitcher fluid was measured during experimental cooking to analyze possible chemical avenues that might contribute to rice stickiness. Participants identifying the snack were almost all (96%) from indigenous Bidayuh or Kadazandusun tribal decent. They prepare glutinous rice inside pitcher traps for tradition, vessel functionality and because they thought it added fragrance and taste to the rice. The pH and chemical activity of traps analyzed suggest there is no corresponding effect on rice consistency. Harvest of pitchers does not appear to decrease the number of plants in local populations. The tradition of cooking glutinous rice snacks in pitcher plants, or *peruik kera* in Malay, likely carries from a time when cooking vessels were more limited, and persists only faintly in tribal culture today because of value placed on maintaining cultural heritage. Social media proved a valuable tool in our research for locating research areas and in interviewing respondents, and we endorse its further use in ethnobotanical investigations. Our gathered data urges for the preservation of sustainable, tribal plant use for the prosperity of both health and culture.

Keywords: Borneo, carnivorous plants, ethnobotany, glutinous rice, *Nepenthes*, Malaysian tribes, traditional food

Introduction

The culture and preparation of food triggers memories, shapes our everyday life and reflects the priorities and progression of cultures. The developed western world's current revival of eating local, 'slow' food and its trending diet named after our Paleolithic ancestors is an imperative reaction to the rampant crises of obesity, food allergies, cardiovascular disease, type II diabetes and other habit-induced health incursions (Popkin, 2006). These are owned from a long-endured transition in habits acquired during rapid economic growth (Davey et al., 2013). Present day countries with freshly advancing economic environments undergo a similar shift from traditional foodstuffs to convenience foods and from laborious jobs and lifestyles to more sedentary careers and recreation (Popkin et al., 2012). The implications of these changes are apparent at very early stages in these shifting economies (Davey et al., 2013).

Maintenance of tradition in food preparation is one solution to minimize the gap experienced in the western world. Vitally important to the continued benefits traditional foodstuffs offer, is simply preserving the knowledge involved in its preparation and history (Pilgrim et al., 2008). Here, we offer a contribution to this knowledge bank by uncovering the history and culture of a luring glutinous rice snack cooked inside a carnivorous plant trap.

Nepenthes L. is an iconic genus from Southeast Asia with modified leaf traps shaped as pitchers that capture and digest their prey. Throughout its growing regions, the pitchers have a functional role in traditional culture (Perry & Metzger, 1980; Jaiswal, 2010; Mey, 2010; Balangcod & Balangcod, 2011). *Nepenthes* are medicinally used to relieve gastro-intestinal discomfort, including dysentery, stomachache and bed-wetting (Wiart, 2006). They are also used to prevent malaria, and the roots contain plumbagin (Likhitwitayawuid et al., 1998), which shows promising *in vitro* and *in vivo* antiplasmodial efficacy (Sumsakul et al., 2014). The plant provides material in housing construction and serves as a protective male sheath in West Papua (Milliken, 1992). Despite their intrigue, the culture of *Nepenthes* in traditional food is not extensively known. Previous accounts of the use of *Nepenthes* in traditional food are framed within larger works of carnivorous plants, food packaging or ethnobotany, which allowed only brief identification of *Nepenthes* in food culture (Pietropaolo & Pietropaolo, 1986; Laistrooglai et al., 2000; Christensen, 2002).

With this study we analyze and present the preparation, culture and significance of the sticky rice snack made inside *Nepenthes* pitchers. Our research was based on the following questions: How and when is the *Nepenthes* sticky rice snack prepared? What is the main motivation for using *Nepenthes* in food preparation? Is there a contribution from the pitcher to chemically induce rice stickiness in the snack? Which species of *Nepenthes* were used in history and now? How does their distribution reflect their usage?

Materials and methods

Area of investigation: Documented areas of cooking rice inside *Nepenthes* pitchers included Thailand (Laistrooglai et al., 2000), the Philippines (Pietropaolo & Pietropaolo, 1986) and Malaysia (Christensen, 2002). These accounts gave us a general base, but social media allowed a more detailed current glimpse of the present day culture and whereabouts of this snack. We scoured personal travel blogs, the photo sharing website ©Flickr and the visual board tool ©Pinterest to uncover that production and consumption of this traditional snack was alive and thriving, especially in the southern parts of Sarawak, Malaysia. We therefore targeted our efforts in Malaysian Borneo, where many indigenous tribes are known to continue their rich traditions (Deavin et al., 2012). Malaysian Borneo sits in the north of this third largest island in the world. It has one of the oldest and richest rainforests and is considered the main center of biodiversity for *Nepenthes* (Clarke & Wong, 1997). Malaysia is a rapidly developing country and, like many 'aspiring' economies, is experiencing a shift towards a more sedentary lifestyle and increased consumption of high caloric processed foods (Davey et al., 2013).

Data collection: In October and November 2014, we worked throughout the two states of Malaysian Borneo, Sabah and Sarawak, with three different questionnaires made for those that had eaten the snack, those that were vendors selling the snack on a market and those who offered more advanced knowledge of the preparation and tradition. We started our research at nine outdoor markets (Figure 1), where we surveyed market customers and vendors by first asking if they recognized a laminated photograph of the sticky rice dish cooked in *Nepenthes,* and then utilized snowball sampling (Martin, 1995) to identify additional areas, markets or informants. On the markets where no pitcher plant snacks were sold, we presented the photograph of the snack to a target of half of the vendors and customers (total n = 299) with the aim of encompassing diversity in tribe, gender and age. Upon recognition, informants were asked to answer our short 'Market' questionnaire designed for customers and vendors who were not selling the snack themselves (n = 11). This included questions about the frequency of their consumption of the snack, the reason they thought rice was cooked inside a *Nepenthes* pitcher, information about the ingredients used to make the dish and the species of *Nepenthes* used in cooking. To identify the species of *Nepenthes* used to make the snack, we showed a laminated photo series of the five regionally growing species to all informants, which offered a portable and convenient *ex situ* method of plant identification (Martin, 1995) with high rates of consistent plant recognition (Thomas et al., 2007). All vendors found selling the sticky rice snack were asked to complete a 'Vendor' focused questionnaire, which additionally asked about the harvesting of the pitchers and market sales.

Further, we prepared the snack along with an indigenous Bidayuh family from Sarawak, Malaysian Borneo by participant observation of the cooking process. With this, we also conducted a more detailed 'Expert' ethnobotanical questionnaire with more depth in the history, culture and preparation of the glutinous rice snack cooked in *Nepenthes* pitchers. The cook-along occurred in the informant's homes in Bau in English. Two informants met whilst conducting

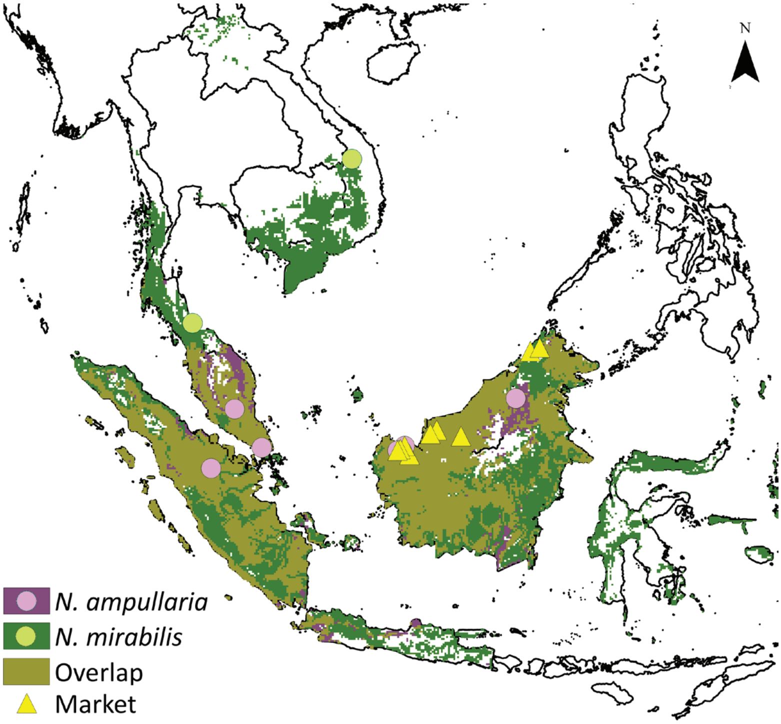

Figure 26. Glutinous rice snack localities and predicted distribution of pitcher plant species used in its preparation. Modeled distribution of the two most widely prepared species of pitcher plants, *Nepenthes ampullaria* and *Nepenthes mirabilis*, based on verified herbarium specimen localities. Beige shading indicates co-occurrence of species. Yellow triangles indicate visited marketplaces. Circles denote areas where the consumption or sale of the glutinous rice snack was identified through social media.

our field study in Malaysia and friended via Facebook also completed 'Expert' questionnaires. One questionnaire was mailed and returned via Facebook in English. The second questionnaire was conducted in Malay in Kuching by a local interpreter who then translated the information to English.

This study complies with the International Society of Ethnobiology (ISE) Code of Ethics, and all participating market vendors and customers were informed of the objectives of our research and questionnaires prior to beginning the interviews. Only those that agreed to participate were interviewed.

Predicting distribution of species uncovered through social media: We modeled the potential distribution of the two prominent species used in preparation that were uncovered in our social media investigation, *Nepenthes ampullaria* Jack and *Nepenthes mirabilis* (Lour.) Druce. These areas of predicted distribution are based on the realized ecological niche preferences of known localities.

To build the ecological niche models (ENMs), we combined localities with soil data derived from the International Soil Reference and Information Centre (ISRIC) (Batjes, 2012) and climate data from WorldClim (http://www.worldclim.org/) at a spatial resolution of 5 arc-minute Environmental Systems Research Institute (ESRI)-grids. We selected a set of soil and climate variables that strongly indicated distribution by using presence localities of all botanical collections from Southeast Asia within our data records. Selection of 8 bioclimate and 10 soil variables was based on the ecology of *Nepenthes* and highest vector loading using Principal Component Analysis (PCA) in R. Variables correlated (|Spearman rho| > 0.7) to these highest predicting variables were excluded. In the R environment (Matloff, 2008), we used MaxEnt version 3.3.3 k (Elith et al., 2006, 2011) to model the projections of the potential distributions. MaxEnt uses presence-only data to predict approximate species distribution based on a probability distribution of maximum entropy, an approach that has been shown to outperform other modeling algorithms (Elith et al., 2011; Aguirre-Gutiérrez et al., 2013). We trained the models using a background sample of all plant material collections from our data sources within Southeast Asia (92°E-165°E, 15°S-22°N), and projected the distributions onto phytogeographical subareas (Van Welzen et al., 2011). Geographic delimitation minimizes over-estimating distribution to islands with suitable abiotic conditions but no recorded species presence, as the sea is a likely dispersal barrier (Schupp et al., 2002; Holt et al., 2005). We present the two species using the 10% training presence threshold in MaxEnt for visual ease. This feature presents a binary visualization of areas encompassing 90% of the predicted distribution area.

Pitcher fluid measurements: In order to assess the possibility of a chemical contribution of the pitcher to the stickiness of the glutinous rice, we measured pH and presence of amylase, an enzyme that catalyzes the hydrolysis of starch into sugars, which is known to break down the starch present in glutinous rice (Limpisut & Jindal, 2002). Amylase activity was measured in fluid of well-developed but still unopened pitchers in order to control for variation due to rainwater dilution or insect capture contamination. The pH measurements were made with CholorpHast® pH-indicator strips, and amylase starch hydrolysis was tested with iodine. *Nepenthes* pitchers have glands that secrete digestive fluid, which breaks down their captured prey (Tökés et al., 1974). Through the in depth questionnaires, we found that pitcher fluid was not used in the cooking process, however, there was still potential that glands release substance while they were being steamed during preparation. We therefore measured the pH of distilled water steamed in three *N. ampullaria* pitchers in ten-minute increments for the standard one-hour steam identified in our cook-along. To ascertain the pH needed to alter rice viscosity, we cooked white rice in acidic acid-amended cooking liquid with a pH of 7, 6, 5 or 4. We blended the rice after cooking and measured viscosity with a Brookfield High Shear cap2000+ (United Nations, 2008).

Results and Discussion

Throughout Malaysian Borneo, over 300 people were approached about the glutinous rice snack made with *Nepenthes* pitchers, making this the most extensive ethnobotanical study of pitcher plants conducted so far. *Nepenthes* were identified throughout Malaysian Borneo as *peruik kera,* and the snack as *nasi pulut* (white rice) in *peruik kera.* Two alternative names for the snack were given by two Bidayuh informants; *klimuoh* from one informant on the Serian Central market and *tramuo* from a family in Bau.

Consumers of carnivorous plants are of indigenous tribal decent: In our investigations, all participants that recognized this snack via our photographs were of indigenous tribal decent save for one Chinese immigrant who had purchased the snack on a Bidayuh market, suggesting that this preparation of *Nepenthes* pitchers has roots in the indigenous culture of plant use. We showed our identification card to over 300 customers and vendors on nine market locations throughout Malaysian Borneo (Figure 26), and either vendors or customers on all but two markets recognized the snack. On the markets where the snack was not sold, participants who had eaten the snack identified themselves as belonging to the Bidayuh, (n = 2) Dyak, (n = 4) or Kadazandusun (n = 4) tribes. Those of Iban and Orang Ulu tribes did not identify with the photographs of the snack in our survey. In the two markets with vendors selling the snack, pitchers were being sold and prepared by

Bidayuh families (n = 11). Bidayuh (n = 3) and Kadazandusun (n = 1) tribal families that were not vendors on the market identified themselves as making the snack in their homes. There was also a Javanese/Malay family (n = 1) that was taught how to make the snack from Bidayuh friends.

The three Bidayuh families answered our most detailed questionnaire. Both the Bidayuh and Kadazandusun tribes are non-nomadic lowland tribes of Sarawak and Sabah, respectively (Deavin et al., 2012). The Bidayuh, previously identified as Land Dyak, and the Kadazandusun are both egalitarian societies, which distinguishes them from the more highland tribes of both states. Tribes not positively identifying the sticky rice snack also lack documentation of such use in social media or descriptive ethnobotanical analyses (Thomas et al., 2007), suggesting that it is at the least not as widely or currently used in their present day culture. Save for the Kelabit tribe of the Northern Bario Highlands, all tribes identified as having ties with the snack through social media were approached in our market surveys. We acknowledge that some people may not have shared knowledge with us due to language barrier or fear, as it was expressed to us in the markets of Sabah that these plants are protected and cannot be legally harvested for such use. No such fear was voiced in any markets of Sarawak, which in general had more open communication. Indeed the United Nations Declaration of the Rights of Indigenous Peoples (United Nations, 2008) and the more regional Sarawak Law and Forest Ordinance of 1958 (Human Resource Management Unit, 1958) protects the preservation of the cultural heritage in using CITES protected material, such as *Nepenthes,* as long as it is not traded internationally without permits.

The preparation of carnivorous plants also reaches into other traditional cultures. Norwegians douse *Pinguicula vulgaris* L., appropriately common-named 'butterwort', leaves in cow's milk to make thick sour milk called *tjukkmjølk* (Nilsson & Nilsson, 1958). Southwestern Australian aborigines consumed *Drosera* spp. roots (Hammond, 1933), and Europeans distilled large quantities of *Drosera* spp. in a drink called *Rosa Solis*, which was promoted to 'stir up lust' in those who drank it (Plat, 1609). Even the aquatic *Utricularia vulgaris* L. has edible turions and leaves (Chiej, 1984).

Species used are common and sustainably harvested: The species used for glutinous rice preparation revealed in social media coincided with those found in traditional ethnobotanical analysis. *Nepenthes ampullaria* and *N. mirabilis* were the two species most identified through social media and are widely distributed (Figure 26). For these two species, our ecological niche models predicted potential distribution with significant confidence ($p < 0.05$). Amazingly, the distribution predictions combined with the information

Figure 27. Modern preparation of glutinous rice snack prepared in *Nepenthes ampullaria* pitchers. Pitchers placed in steamer by indigenous Bidayuh family of Bau, Malaysia

uncovered through social media reveals that pitchers of *N. mirabilis* are only prepared in areas where *N. ampullaria* is not distributed (Figure 26), indicating that *N. ampullaria* is the preferred species for preparation. Participant interviews identified *N. ampullaria* (n = 24), *N. bicalcarata* (n = 2), *N. gracilis* (n = 2), *N. mirabilis* (n =1) or *N. veitchii* (n = 1) as species used for preparation, and *N. ampullaria* was the only species prepared for sale on the market. All market and cook-along preparations filled only *N. ampullaria* pitchers and selectively chose pitchers of this species that had developed sturdy trap walls, not yet too brittle from age. Interviews indicate that the identified species of use were the same as those prepared by the mothers and grandmothers that taught them how to cook the dish.

The most prevalently used *Nepenthes* species are located within lowland areas, where they are easily accessible to tribal villages and harvested in such a way that this does not threaten the persistence of local populations, i.e. by cutting off selected pitchers only and leaving the remainder of the plant intact so that it is sustained by the remaining and newly produced pitchers. Wild collection in this way ensures the future of the food-source for the people for which it provides. This system of sustainable harvesting is a mainstay of indigenous natural resource consumption as indigenous tribes have long relied on wild collections from their environment (Bharucha & Pretty, 2010).

Preparation of the dish changed when modern kitchen utensils became available: Another remarkable interview suggests a more historical preparation of the sticky rice snack. One informant took a highland trek with tribal guides who harvested *Nepenthes* pitchers, coated them in a thick mud and then placed them directly on the coals of the fire to cook rice. The sterility of unopened pitchers (Buch et al., 2013) might have initially made *Nepenthes* an attractive vessel option for serving food in times when kitchen hygiene was more cumbersome. Modernized cooking methods and supplies allow for deviations to this more rudimentary approach, as all of our 'Experts' steamed the rice snack in large batches, sometimes upwards of hundreds of pitchers at a time, in aluminum pots over an electric cooktop (Figure 27). The basic recipe consists of glutinous hill rice or Thai white glutinous rice cooked in coconut milk. Additions of *sambal udang* (prawns cooked in chili peppers), *rousong* (a dried meat product), chicken, peanut or pandan leaf were found on the Bau Wet market and the Kampung Duyoh Roadside market (Figure 28).

Figure 28. Glutinous rice snack prepared in *Nepenthes ampullaria* pitchers for sale in Sarawak, Malaysia. This photo shows two variations of the snack made with chicken (left) and prawn chili paste (sambal) (right) with peanuts on top. Each pitcher sells for one Malaysian Ringgit on this Kampung Duyoh roadside market.

Figure 29. Presentation of glutinous rice snacks prepared inside traditional packaging in Sarawak, Malaysia. At the Kampung Duyoh roadsite market, sticky rice was prepared inside *Nepenthes ampullaria* pitchers (right) and within the leaf and culm of bamboo (*Bambusa* spp.) (left).

Chemical attributes of the pitchers do not seem to influence rice preparation: Glutinous rice has inherent properties that make it sticky. The dominant starch, amylopectin, has a low gelatinization temperature, giving the moist, sticky texture so highly sought after in many dishes across Asia (Schiller et al., 2006). Although some food preparations, like the addition of vinegar to sushi rice in Japan (Odahara et al., 2004), contribute to rice texture and stickiness, we found no indication that the *Nepenthes* pitcher might be adding to the stickiness of the rice. No amylase activity was detected in our test; the pH remained unchanged throughout the cooking process and the pH needed to actually increase viscosity was measured to be a pH of at least 3, which was a far deviation of the pH of 7 recorded in our cooking experiment.

Pitchers are convenient, biodegradable rice containers: Throughout Southeast Asia, packaging food in natural materials was the default before the introduction of waxed papers, plastics and aluminum containers. Plants used as food containers like the world-spread calabash (*Lagenaria siceraria* (Molina) Standl.) (Price, 1982; United Nations, 2013) and cornhusks (*Zea mays* L.), which wrap Mexican tamales, are especially well known as they are still commonly used. For glutinous rice, bamboo (*Bambusa* spp.) and banana leaf (*Musa* spp.) are the plants most frequently used as packaging, often with elaborate and beautiful design (Laistrooglai et al., 2000). In Thailand and Laos it is common to cook sticky rice in green bamboo directly on an open fire. The charred bamboo package is served and then peeled off to eat the glutinous rice inside (Schiller et al., 2006).

Like these, *Nepenthes* pitchers offer a charming folk packaging that protects the food and has an appeal unmatched by synthetic vessels (Figure 29). In line with their attractive presentation, participants and social media identified that the glutinous rice snack filled inside pitchers were often served to celebrate Ramadan, election parties and harvest festivals. When limitation of serving vessels might otherwise prohibit larger gatherings, pitchers offer an option to feed many people at one time. *Nepenthes* pitchers are convenient, biodegradable containers. In an age in desperate need of waste reduction and resource conservation (Buzz Wikipearl), containers like these are a great solution to a growing environmental global problem. Edible packaging trends as one of the top innovations that will change our lives (Rousseau, 2013), and the buzz around Wikipearl™ shows that consumers desire options that are more eco- and health-friendly.

Added value of social media for carrying out interview-based ethnobotany research: Social media provided a valuable tool in our research, allowing us a present day glimpse of the accessibility of the *Nepenthes* snack and assisting in our post-fieldwork data collection. Flickr, Pinterest and YouTube unearthed present day localities of where we might find the snack and revealed a modern excitement about this heritage-rich food. The revival of food culture through social media pulses around the globe, where blogs, Tweets and Instagrams of your favorite food, recipe and restaurant have created a virtual food community accessible by all linked to technology (Pilgrim & Pretty, 2010). The cronut craze of 2013 brought so much attention via social media that fans flocked to its croissant/donut New York birth-site and even camped outside its doors in hopes of a deep-fried reward. Because of this, we suggest that social media might be used as a promotional weapon for heritage-rich snacks like the *Nepenthes* glutinous rice dish. One ©YouTube video, which originally aired as a popular Malaysian children's cartoon highlighting the traditional *Nepenthes* snack, already has over 4.2 million views (http://youtu.be/9Y2ZisNsOjE). The presence of this dish in social media is a modern version of story telling and passing on of traditional knowledge.

Benefits of a modern culture keeping traditional ways: Carrying on the tradition of food promotes cultural, personal and even biodiversity health. This interplay between nature and culture has become even more apparent as we feverishly attempt to hold on to both before they are lost (United Nations, 2001). Recognizing the 'fast disappearing' traditional knowledge of indigenous communities, the Sarawak Biodiversity Council in Malaysian Borneo focuses their efforts on preserving their valuable heritage. Malaysian Borneo boosts a quickly growing economy and global vibrancy (Institute for Public Health Ministry of Malaysia, 2011). There is a culture of eating out in modern society, with MacDonald's restaurants, KFCs and Burger Kings lining high traffic streets and shopping malls. Echoing this is a 300% increase in the number of obese persons in Malaysia, up from just 4.4% in 1996 to 15% in 2011 (Ravussin et al., 1994). If Malaysia grips tightly onto their food heritage, they might save themselves from the health consequences indebted now in the developed western world. A study with the Pima Indians of Mexico indeed showed that populations with diets more reflective of their traditional ways had lower incidence of obesity and non-insulin dependent diabetes (Alexiades et al., 2013).

In addition to the benefits of human health, preservation of traditional food enhances the link that people have with their natural environment and sustainable forest practices (Miettinen et al., 2011). This need harkens radically in Borneo, where an average of 5% of the total forested area is lost to deforestation each year (Pretty et al., 2009). Maintaining the connection between nature and local people strengthens the value placed on local forests and increases the potential that communities will cry for preservation of these sites despite drives for more immediate, but temporary economic growth (United Nations, 2001).

Conclusion

The human drive to protect heritage resounds in the numerous museums throughout the world, in the detailed stories of histories and through the food served at our tables. Market surveys and social media resources revealed that the glutinous rice snack is still produced and consumed today among traditional communities throughout Southeast Asia. Small-scale commercial sale of glutinous rice snacks in local markets in Sarawak is less widespread, but vendors report easy access to pitchers and consistent sales of their cooked product. The tradition of cooking glutinous rice in *Nepenthes* pitcher plants in Malaysia likely carries from a time when cooking vessels were more limited and persists in tribal culture today because of value placed on maintaining cultural heritage, appeal and the inherent intrigue of these plants. Social media proved a valuable tool in our research, and we endorse its further use in ethnobotanical investigations and spread of knowledge. The documentation and gathered data weigh important for the ethnobotanical preservation of Bornean tribal culture and traditions.

General Discussion and Summaries

The work presented in this thesis includes molecular phylogenetic analyses of *Nepenthes* species for the reconstruction of the evolution of ecological niche diversity, endemism, anatomical and morphological diversity of the genus. The results obtained provide more insight in how best to conserve species of *Nepenthes* to prevent them from going extinct due to global warming and other human induced habitat destruction. The final chapter focuses on traditional use of *Nepenthes* and moves from 'how to conserve' to 'why to conserve'. Explanation of the major findings and conclusions of each chapter are outlined below.

Contributions towards understanding ecological niche diversity in *Nepenthes*: *Chapter 1* presents an expanded multi-locus molecular phylogeny of *Nepenthes* with increased resolution compared to previously published phylogenies. Ecological niche modeling of 15 selected species provided insight in the evolution of ecological preferences. When combined, the quantified genetic distance and ecological divergence revealed two distinct phylogenetic signals, one among the higher altitude species and another with lower altitude species. The higher altitude species were more genetically similar and shared more ecological niche space with each other than the lower altitude species that differed genetically to a larger extent and overlapped much less in ecological niche space. This offers evidence that the higher altitude species may have differing rates and mechanisms of diversification than the lower altitude species. Higher altitude species underwent rapid, recent radiations, catapulted by the opening up of new niche space when the Kinabalu mountain range formed. Lower altitude species are more ancestrally derived, undergoing sympatric speciation and evolving via adaptive processes in response to disruptive natural selection

Not only does this study unravel differing evolutionary histories, but it also shows that the implications of species' tolerances to future changing climate are distinguishable. The ecological niche models presented in this chapter show that climate has and will continue to play an important role in the distribution of *Nepenthes* species. Future climate forecasts show that in just half of a century from now (2070), higher altitude species on average face an overall loss of suitable habitat loss, whereas lowland species show a gain in potential habitat. With changing climate, some areas suitable now, will no longer be suitable in the future for each species. Because preferred habitat occurring in both the present and the future scenarios are likely to be the only areas supporting populations in the future, 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 in botanical gardens and *ex situ* conservation programs such as Ark of Life.

Revealing the evolutionary mechanisms that created endemic species of *Nepenthes* on Mt. Kinabalu: My contribution to the large collaborative work in *Chapter 2* involved untangling the evolutionary origins of the *Nepenthes* species of Mt. Kinabalu. The chapter presents a modification of the publication including detailed phylogenies, ancestral reconstructions and molecular clock analyses of *Nepenthes.* This is the first application of multi-taxon molecular phylogenetics for an entire tropical montane biodiversity hotspot, and our results considerably deepened the understanding of the evolution of endemism in general and the origin of Borneo's biodiversity in particular. Mount Kinabalu proved to be both a cradle of speciation on its top and a museum of ancient relics on its flanks. The plant and animal species investigated both arrived by long distance dispersal (most notably the ferns and mosses) and *in situ* speciation (*Nepenthes edwardsiana, N. x kinabaluensis, N. lowii, N. rajah, N. villosa*).

Expanding the knowledge of the evolution of *Nepenthes* wood anatomical diversity: In *Chapter 4*, wood characters were visually mapped on a molecular phylogeny of the Caryophyllales order for the first time. In addition to this, complete anatomical descriptions were made for *Nepenthes* and ancestral states were reconstructed to illuminate phylogenetically informative characters.

Wood anatomy of *Nepenthes* is diffuse porous, with mainly solitary vessels showing simple, bordered perforation plates and alternate intervessel pits, fibres with distinctly bordered pits (occasionally septate), apotracheal axial parenchyma, and co-occurring uni- and multiseriate rays often including silica bodies. Abiotic conditions (soil type and precipitation) and growth habit (stem length) correlate with multiseriate ray height and width, vessel diameter and presence of silica grains. For Caryophyllales as a whole, silica grains, successive cambia, bordered perforation plates and helically banded idioblasts seem to be the result of convergent evolution. Peculiar helical sculpturing patterns within various cell types occur uniquely within the insectivorous clade of non-core Caryophyllales.

The wood anatomical variation in *Nepenthes* displays variation for some characters dependent on soil type, precipitation and stem length, but is largely conservative. The helical-banded fibre-sclereids that mainly occur idioblastically in the pith and cortex are synapomorphic for *Nepenthes*. Other typical *Nepenthes* characters, such as the presence of silica grains and bordered perforation plates, evolved convergently in different Caryophyllales lineages. This study revealed important patterns of the conservative nature of wood, addressing the inflexibility of wood to rapidly adjust to upcoming drought stress. We report for the first time a possible link between wood anatomy and ultramafic soil, uncovered the omnipresence of peculiar helically-banded idioblasts within the genus and presented support for a hypothesis on convergent evolution of the presence of silica bodies and non-bordered perforation plates for the order Caryophyllales.

Contributions in understanding the development of the uniquely dimorphic carnivorous traps of Nepenthaceae: The previous chapters showed that the current patterns in ecological niche diversity, endemism and wood anatomy in *Nepenthes* are the result of millions of year of evolution. Another morphological adaptation that many species of *Nepenthes* evolved during this timespan are dimorphic carnivorous leaf traps. The ones at the top of the plant, called upper pitchers, are shaped very differently than the ones lower on the plant, called the lower pitchers. Upper pitchers are generally more suitable for capturing flying insects whereas lower pitchers catch more crawling insects and plant debris. I investigated whether lower pitchers were the ancestral state from which higher pitchers evolved in a later stage during the evolution of *Nepenthes*. Landmark analysis of pitcher morphology and investigation of microstructure development within the dimorphic pitchers of *Nepenthes rafflesiana* revealed four parallel states of pitcher ontogeny. They are identified in *Chapter 6* as distinguishable curvation, elongation, inflation and maturation phases.

The curvation phase is characterized by a strong curvature at the junction of tendril attachment to the pitcher. Pitchers in the elongation phase increased considerably in length and depth. The flattened pitcher appearance changes in the inflation phase when width increases and length growth continues. The maturation phase is characterized by the opening of the pitcher by disconnection of the lid.

Pitcher length indicates progress through developmental phases, and its use as a tool for indication of specific developmental stages is proposed. Microstructure development coincides with the developmental phases defined. Ontogenetic shape analysis indicates that upper and lower pitcher types develop with similar phase progression, but have no directly overlapping morphology in any of the four distinct phases, discarding the hypothesis that upper pitchers are a derived state from lower pitchers. This means that independent developmental programs must have evolved to produce distinctly shaped upper and lower pitchers to exploit different food sources.

Calling attention to the value of preserving *Nepenthes* for its cultural tradition: The final chapter focuses on traditional use of *Nepenthes* and moves from 'how to conserve' to 'why to conserve'. In *Chapter 5,* ethnobotanical investigation of a heritage rich snack made with *Nepenthes* pitchers highlights the cultural value of this charismatic group of plants. More than 300 people on markets across Sabah and Sarawak, Malaysia, were questioned, making this the most extensive ethnobotanical study of pitcher plants ever conducted. Unearthed via social media and market surveys, this glutinous rice snack, or *peruik kera* in Malay, is alive in small pockets of indigenous tribal culture. The most common species for snack preparation are *N. ampullaria* and *N. mirabilis*. According to modeled distribution and social media surveys, *N. mirabillis* are only used when *N. ampullaria* does not natively grown in the region. The indigenous tribes identifying the snack as being part of their heritage-rich tradition in Malaysia included those of Bidayuh or Kadazandusun decent. The traps used in preparation were identified as convenient packaging material, especially when large groups gather for celebration or political parties.

Food traditions promote cultural, personal and even biodiversity health. The interplay between nature and culture offer a foothold as we feverishly attempt to hold on to both before they are lost. The compilation of diversification and conservation studies in this thesis are strengthened by the recognition that maintaining the connection between nature and people echoes the value placed on local forests and increases the potential that communities will personally commit to their preservation.

Future research directions: To deepen the current insights in the main drivers of diversification of the genus *Nepenthes,* concentrated focus on the production of a multigene based molecular phylogeny including all species would be of great benefit. New developments in Next Generation sequencing and genomics will speed up this progress. Because hybridization played a role in the origin of multiple species, additional network analyses not employed in this study should be carried out as well.

For ecological niche modeling and future climate predictions as presented in *Chapter 1*, taking biotic variables into account would put an enhanced perspective on the requirements needed for survival of *Nepenthes* species. These would include prey species of which carnivorous plants such as *Nepenthes* rely on for survival, but also other organisms such as pollinators, associated symbiotic fauna living such as insect larvae, spiders, mites ants, mammals and birds along with endophytic and mycorrhizal fungi and other microbes.

Molecular dating of multiple clades as presented in *Chapter 2* should be applied to larger communities of other tropical montane biodiversity hotspots. For *Nepenthes*, similar studies carried out on mountains such as the Banjaran Titiwangsa mountain range, The Central Cordillera of New Guinea, the mountains of the Philippines and the Bukit Barisan chain in Sumatra can provide more insight on the major role that mountains play as the evolutionary drivers of speciation in the tropics.

Also valuable for gaging future climate competences of species of *Nepenthes* would be experimental research focusing on the physiological thresholds and responses to predicted future climate extremes such as drought. Additional information to the anatomical survey as presented in *Chapter 3* should include tests on drought stress resistance including water transport measures in the xylem and minimum midday water potential pressure to establish hydraulic safety margins. These measures could be combined with predicted current and future predictions to estimate timescales of when thresholds would be surpassed.

The diversity in tolerances and pliability might be a key to the future success of *Nepenthes*. The framework established in *Chapter 4* of quantitatively defining pitcher shape for *N. rafflesiana* could be expanded with additional species. Associations with different diets or other associations could be explored as well. In addition to this, more knowledge about the genes involved in pitcher initiation and development would move the understanding of the molecular basis of pitcher ontogeny forward considerably.

Finally, we highlight the cultural importance of *Nepenthes* in traditional food culture in *Chapter 5*, but more investigation of the use of *Nepenthes* in other capacities deserves attention especially in additional tribally influenced areas of SE Asia and Madagascar. Social media proved a valuable tool in our research, and we endorse its further use in ethnobotanical investigations and spread of knowledge. Also of interest could be studies on the link of local and national government promotion of cultural heritage and environmentally-linked public opinion. Such a holistic approach to conservation would ensure the preservation of culture, forests, plants and people.

Samenvatting

Voor dit proefschrift zijn moleculair fylogenetische analyses uitgevoerd met soorten uit het tropische bekerplantengenus *Nepenthes.* Deze planten kunnen op arme bodems leven door extra nutriënten op te nemen met behulp van een gemodificeerde bladtop in de vorm van een beker gevuld met verteringssappen. Het merendeel van de soorten is vleesetend, een klein deel leeft van het verteren van mest of van plantendelen.

Met de geproduceerde moleculaire stambomen is een reconstructie gemaakt van de evolutie van de diversiteit aan ecologische niches, endemisme, de anatomie en morfologie. Dankzij deze analyses is nu duidelijker gemaakt waar soorten van *Nepenthes,* die in het wild met uitsterven bedreigd worden, het best beschermd kunnen worden tegen opwarming en andere door de mens veroorzaakte vormen van habitatdestructie. Naast stamboomonderzoek is ook een ethnobotanische analyse uitgevoerd. Hiermee kon de vraag *hoe* deze groep bekerplanten te beschermen tegen uitsterven, nog wat uitgebreider worden beantwoord, namelijk door ook te kijken *waarom* ze een betere bescherming verdienen. De belangrijkste resultaten van dit promotie-onderzoek worden hieronder kort samengevat.

Een beter begrip van de diversiteit aan ecologische niches binnen *Nepenthes*: In *Hoofdstuk 1* wordt een moleculair fylogenetische analyse gepresenteerd op basis van verschillende genen uit het nucleair en chloroplastgenoom. De resolutie van deze stamboom is hoger dan die van voorafgaande publicaties. Voor 15 soorten zijn vervolgens de ecologische niches gemodelleerd om de evolutie daarvan binnen deze groep bekerplanten te kunnen reconstrueren. Onder een niche wordt een afgebakende ecologische ruimte in een ecosysteem verstaan. Door de stamboom- en nichereconstructies te combineren, kon geconcludeerd worden dat in *Nepenthes* twee verschillende fylogenetische signalen aanwezig zijn, één binnen hooggebergtesoorten, en een ander binnen laaglandsoorten. De hooggebergesoorten lijken genetisch gezien meer op elkaar en delen ook meer ecologische niches, terwijl de laaglandsoorten genetisch gezien onderling veel heterogener zijn en minder overlappen qua niches. Verder blijken de hooggebergesoorten voortgekomen te zijn uit een snelle radiatie, die relatief recent plaatsvond op de Kinabalu berg toen deze door vulkanisme gevormd werd. Laaglandsoorten zijn ouder en ontstaan door sympatrische specatie, waarbij reproductieve isolatie ontstond door een veel langduriger proces van natuurlijke selectie.

Dit onderzoek toonde verder aan dat klimaatverandering een belangrijke rol speelde en zal blijven spelen in de verspreiding van de soorten van *Nepenthes*. Scenario's berekend voor de toekomst laten zien dat in slechts

50 jaar tijd (2070) de hoogtegebergtesoorten er sterk in verspreiding op achteruit zullen gaan terwijl de laaglandsoorten er op vooruitgaan. Als de huidige opwarming door blijft gaan, zullen veel gebieden die nu nog geschikt zijn voor hooggebergtesoorten ongeschikt worden voor *Nepenthes*. Ik adviseer dan ook gebieden die nog wel geschikt blijven prioriteit te geven bij natuurbeschermingprojecten. Ook is het van belang dat de genetische diversiteit van hooggebergtesoorten uit deze gebieden bewaard blijft in botanische tuinen en *ex situ* programma's zoals bijvoorbeeld Ark of Life.

Het ontstaan van endemische *Nepenthes* soorten op de Kinabalu: Mijn bijdrage aan het grote internationale project beschreven in *Hoofdstuk 2* betrof het onderzoek naar de oorsprong van de endemische *Nepenthes* soorten op deze tropische berg. In dit hoofdstuk worden gedetailleerde stamboomreconstructies en moleculaire klokanalyses gepresenteerd van diverse organismen waaronder *Nepenthes.* Zo'n exercitie was nog niet eerder gedaan voor een tropische berg vanuit een dermate breed taxonomische perspectief. De restulaten lieten ondubbelzinnig zien dat op de top van de Kinabalu berg vele nieuwe soorten zijn ontstaan, terwijl op de flanken veel voorouders van nieuwe soorten voorkomen. De op de berg onderzochte soorten kwamen daar aangewaaid vanuit verre oorden elders (dit gold vooral voor hele oude plantenfamilies binnen de varens, mossen en de schimmels) maar onstonden ook ter plekke. Dit laatste bleek het geval te zijn voor endemen als *Nepenthes edwardsiana, N. x kinabaluensis, N. lowii, N. rajah,* en *N. villosa*.

Meer inzicht in de evolutie van het hout in *Nepenthes*: In *Hoofdstuk 4* heb ik de evolutie van houtanatomische kenmerken in de Caryophyllales onderzocht. Daarnaast heb ik de variatie in houtanatomie binnen *Nepenthes* beschreven en ontdekt welke kenmerken in de voorouders aanwezig waren en welke kenmerken daar evolutionair van zijn afgeleid.

Het hout van *Nepenthes* is diffuus-porig. De vaten zijn veelal alleenstaand met enkelvoudige doorboringen die een gegroefde wand hebben. Verder heeft *Nepenthes* alternerende vat-stippels, vezels met duidelijke hofstippels, die soms gesepteerd zijn, apotracheaal axiaal parenchym, en samen voorkomende één- tot meerrijige stralen die vaak silicalichamen bevatten. Abiotische omstandigheden (bodemtype en hoeveelheid neerslag) en habitus (liaan of kruidachtige plant) correleren met de lengte en breedte van de mergstralen, de diameter van de vaten en aanwezigheid van silicalichamen. Binnen de Caryophyllales lijken silicalichamen, succesieve cambia, vaten met enkelvoudige doorboringen met een gegroefde wand en helicaal-verdikte idioblasten het resultaat te zijn van convergente evolutie. Afwijkende patronen in diverse celvormen komen alleen voor in de insecteneters binnen de Caryophyllales.

Variatie in houtanatomie van *Nepenthes* correleert met het bodemtype, de hoeveelheid neerslag en de gemiddelde lengte van de stengel, maar de significantie van die correlatie is niet erg uitgesproken. Steencellen en idioblasten in het merg en de cortex zijn synapomorfieën voor *Nepenthes*. Andere kenmerken, zoals de aanwezigheid van silicalichamen en gegroefde vatwanden, zijn meerdere keren gedurende de evolutie van de Caryophyllales ontstaan.

Dit onderzoek liet zien dat het hout van *Nepenthes* relatief éénvormig is en dat soorten niet snel reageren op verdroging van hun leefomgeving door evolutie van nieuwe houtanatomische kenmerken. Op bodems met veel zware metalen blijkt het hout van *Nepenthes* vol te zitten met helicaal-verdikte idioblasten. Deze lijken, samen met silicalichamen en gegroefde vatwanden, het restultaat te zijn van convergente evolutie binnen de Caryophyllales en zijn mogelijk van belang om te kunnen overleven op bodems met een afwijkende mineralensamentstelling.

Ontwikkeling van bodem- en luchtbekers in *Nepenthes*: In de voorafgaande hoofdstukken heb ik laten zien dat de huidige diversiteit aan ecologische niches, endemisme, en houtanatomie binnen *Nepenthes* het resultaat is van miljoenen jaren van evolutie. Een andere morfologische aanpassing aan het leven op arme bodems zijn de bekervallen van *Nepenthes*. De vorm van de bekers bovenin de vegetatie is anders dan die van de bekers vlak boven of op de bosbodem. Luchtbekers zijn aangepast aan het vangen van door de lucht vliegende insecten, terwijl bodembekers meer geschikt zijn voor het opvangen van bladmateriaal en over de bodem rondkruipende insecten. Ik heb onderzocht of luchtbekers een afgeleide vorm zijn van bodembekers. Dat heb ik gedaan door de groei en ontwikkeling van beide type bekervallen van *N. rafflesiana* door de tijd heen te volgen. Tijdens de ontwikkeling kunnen vier verschillende fases worden onderscheiden en ik beschrijf deze in *Hoofdstuk 6*. In de eerste fase zijn de eerste krommingen zichtbaar op de plek waar de beker zich uit het blad ontwikkelt. In de volgende fase vindt verlenging en verbreding plaats. Daarop volgt een fase van opzwellen en uiteindelijk een fase waarin de bekerval opengaat en zijn definitieve vorm aanneemt.

De lengte van de bekerval blijkt een goede indicator te zijn voor de verschillende ontwikkelingsfases. De vorming van microstructuren als klieren vindt plaats gedurende verschillende ontwikkelingsfasen. In geen enkele van de vier verschillende ontwikkelingsfasen blijken de bodem- en luchtbekers overlap te vertonen. De luchtbekers kunnen daarom geen afgeleide vorm zijn van de bodembekers en moeten dus onafhankelijk daarvan ontstaan zijn.

***Nepenthes* als cultureel erfgoed:** Het laatste hoofdstuk gaat over het traditioneel gebruik van *Nepenthes*. In *Hoofdstuk 5* wordt ethnobotanisch onderzoek beschreven waarin het gebruik van bekerplanten in traditionele recepten is onderzocht. Meer dan 300 bezoekers van markten in Sabah en Sarawak in Maleisië zijn hiervoor ondervraagd. Bij het interviewen van consumenten en producenten van deze gerechten is ook gebruik gemaakt van sociale media. Hieruit bleek dat het gerecht *peruik kera*, een kleverige snack van rijst, gekookt en geserveerd in *Nepenthes* bekervallen, ondanks de uitvinding van plastic bakjes, nog onverminderd populair is. De meest algemene soorten die hiervoor gebruikt worden zijn *N. ampullaria* en *N. mirabilis*, maar de laatste soort wordt alleen gebruikt als *N. ampullaria* lokaal niet beschikbaar is. Het gerecht wordt vooral bereid door twee bevolkinsgroepen in Maleisië, de Bidayuh en Kadazandusun, en geserveerd tijdens feestelijke bijeenkomsten met veel deelnemers.

Traditionele gerechten bereid met lokale flora zijn niet alleen het resultaat van een plaatselijke cultuur maar ook van de daar aanwezige biodiversiteit. Als het niet goed gaat met de lokale natuur gaat het ook niet goed met de tradities die daarop gebaseerd zijn en *vice versa*. Zowel de natuur als de lokale tradities beschermen lijkt in dit geval dan ook een uitstekende strategie om *Nepenthes* soorten plaatselijk voor uitsterven te behoeden.

Suggesties voor toekomstig onderzoek: Om meer inzicht te krijgen in de processen die soortvorming binnen *Nepenthes* bevorderen, is het belangrijk dat er een moleculaire stamboom beschikbaar wordt van alle momenteel bekende soorten. Dankzij nieuwe ontwikkelingen op het gebied van tweede en derde generatie DNA sequensers en genoomonderzoek zal een dergelijke stamboom waarschijnlijk niet lang meer op zich laten wachten. Omdat hybridisatie een belangrijke rol speelt in het soortvormingsproces van *Nepenthes* zijn bovendien aanvullende netwerkanalyses nodig.

Voor toekomstige studies naar het behoud van soorten in het wild onder omstandigheden van klimaatopwarming zoals gepresenteerd in *Hoofdstuk 1* zou het waardevol zijn om ook soorten die samenleven met bekerplanten bij het onderzoek te betrekken. Je kunt dan denken aan prooien als vliegende en kruipende insecten, maar ook aan soorten die mest in de bekers achterlaten, zoals kleine zoogdieren. Daarnaast zijn bekerplant afhankelijk van bestuivers en andere biota waarmee ze samenleven, zoals bijvoorbeeld schimmels, en kikker- en muggenlarven, die zich deels in de bekers ontwikkelen.

Moleculaire klokanalyses zoals gepresenteerd in *Hoofdstuk 2* voor de Kinabalu berg in Borneo zouden ook gedaan moeten worden voor andere tropische hotspots van *Nepenthes*. Je kunt dan denken aan het Banjaran Titiwangsa gebergte op het Maleis schiereiland, Puncak Jaya in Papua Nieuw Guinea, diverse gebergtes op de Filippijnen en de Bukit Barisan keten in Sumatra in Indonesië. Door de evolutie van endemen in meerdere gebieden te onderzoeken, kan de rol van tropische bergen in het proces van soortvorming binnen *Nepenthes* verder in kaart gebracht worden.

Aanvullend experimenteel fysiologisch onderzoek aan de droogteresistentie van verschillende soorten *Nepenthes* kan beter helpen voorspellen welke daarvan in het wild zullen uitsterven als de huidige trends aan opwarming doorzetten. Het anatomisch onderzoek zoals gepresenteerd in *Hoofdstuk 3* dient dan voortgezet worden met metingen van watertransport in het xyleem in het laboratorium om de reacties op droogtestress van verschillende soorten *Nepenthes* te kwantificeren.

Ecologische flexibiliteit binnen *Nepenthes* is belangrijk voor het overleven van soorten. Ik heb in *Hoofdstuk 4* de veranderingen in bekervorm gekwantificeerd voor *N. rafflesiana*. Dit onderzoek zou voortgezet moeten worden met andere soorten, niet alleen met insecteneters, maar ook met mestverteerders en vegetarische soorten. Meer kennis over de genen die betrokken zijn bij bekervorming zou het onderzoek naar de vorming en ontwikkeling van deze organen verder kunnen helpen verdiepen.

Tot slot wil ik graag het culturele belang van *Nepenthes* benadrukken. Van diverse soorten zijn de bekers een belangrijk onderdeel van plaatselijke traditionele rijstgerechten. In hoofdstuk 5 is dit gebruik onderzocht voor Maleisië, maar vergelijkbare gerechten worden ook geserveerd en geconsumeerd in andere delen van Zuidoost Azië en Madagascar. Sociale media blijken bij uitstek geschikt om zowel de makers als de consumenten van traditionele recepten te interviewen over het gebruik van bekerplanten in hun gerechten. Ik ben ervan overtuigd dat het gebruik van sociale media een zeer belangrijk instrument zal zijn binnen ethnobotanisch onderzoek maar ook binnen natuurbeleid, zowel nationaal als internationaal. Op deze manier kan immers meer draagvlak gecreeërd worden voor een samenleving waarin we zorgvuldig gebruik maken van zowel de lokale natuurlijke hulpbronnen aanwezig binnen natuurgebieden, als de traditionele kennis van de mensen die in en rond die gebieden leven.

References

Addo-Bediako A., Chown S., & Gaston K. (2000) Thermal tolerance, climatic variability and latitude. *PNAS*, **267**, 739–475.

Adlassnig W., Peroutka M., Lambers H., & Lichtscheidl I.K. (2005) The roots of carnivorous plants. *Plant and Soil*, **274**, 127–140.

Adlassnig W., Peroutka M., & Lendl T. (2011) Traps of carnivorous pitcher plants as a habitat: composition of the fluid, biodiversity and mutualistic activities. *Annals of Botany*, **107**, 181–94.

Aguirre-Gutiérrez J., Carvalheiro L.G., Polce C., van Loon E.E., Raes N., Reemer M., Biesmeijer J.C., & Chapman M.G. (2013) Fit-for-purpose: species distribution model performance depends on evaluation criteria – Dutch hoverflies as a case study. *PLOS ONE*, **8**, e63708.

Alamgir M., Mukul S.A., & Turton S.M. (2015) Modelling spatial distribution of critically endangered Asian elephant and Hoolock gibbon in Bangladesh forest ecosystems under a changing climate. *Applied Geography*, **60**, 10–19.

Alamsyah F. & Ito M. (2013) Phylogenetic analysis of Nepenthaceae, based on interal transcribed spacer nuclear ribosomal DNA sequences. *Acta Phytotaxonomica Geobotanica*, **64**, 113–126.

Albert V.A., Williams S.E., & Chase M.W. (1992) Carnivorous plants: phylogeny and structural evolution. *Science (New York, N.Y.)*, **257**, 1491–1495.

Alexiades M.N., Peters C.M., Laird S.A., Binnqüist C.L., & Castillo P.N. (2013) The missing skill set in community management of tropical forests. *Conservation biology: the journal of the Society for Conservation Biology*, **27**, 635–7.

Araújo M.B., Alagador D., Cabeza M., Nogués-Bravo D., & Thuiller W. (2011) Climate change threatens European conservation areas. *Ecology Letters*, **14**, 484–92.

Araújo M.B., Ferri-Yáñez F., Bozinovic F., Marquet P.A., Valladares F., & Chown S.L. (2013) Heat freezes niche evolution. *Ecology Letters*, **16**, 1206–1219.

Baas P. (1976) Some functional and adaptive aspects of vessel member morphology. *Wood structure in biological and technological research* (ed. by P. Baas, A. Bolton, and D. Catling), pp. 157–81. Leiden Univeristy Press, Leiden.

Baas P., Werker E., & Fahn A. (1983) Some ecological trends in vessel characters. *IAWA Bulletin*, **4**, 141–59.

Baek H.J., Lee J., Lee H.S., Hyun Y.K., Cho C., Kwon W.T., Marzin C., Gan S.Y., Kim M.J., Choi D.H., Lee J., Lee J., Boo K.O., Kang H.S., & Byun Y.H. (2013) Climate change in the 21st century simulated by HadGEM2-AO under representative concentration pathways. *Asia-Pacific Journal of Atmospheric Sciences*, **49**, 603–618.

Baker H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution*, **9**, 347–349.

Balangcod T.D. & Balangcod A.K.D. (2011) Ethnomedical knowledge of plants and healthcare practices among the Kalanguya tribe in Tinoc, Ifugao, Luzon, Philippines. *Indian Journal of Traditional Knowledge*, **10**, 227–238.

Baldwin B., Kyhos D., Dvorak J., & Carr G. (1991) Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). *PNAS*, **88**, 1840–3.

Baldwin B.G., Crawford D.J., Francisco-Ortega J., Kim S.-C., Sang T., & Stuessy T.F. (1998) Molecular phylogenetic insights on the origin and evolution of oceanic island plants. *Molecular Systmatics of Plants II* (ed. by D.E. Soltis, P.S. Soltis, and J.J. Doyle), pp. 410–441. Springer US, Boston.

Barkman T.J. & Simpson B.B. (2001) Origin of high-elevation Dendrochilum species (Orchidaceae) endemic to Mount Kinabalu, Sabah, Malaysia. *Systematic Botany*, **26**, 658–669.

Batjes N.H. (2012) ISRIC-WISE derived soil properties on a 5 by 5 arc-minutes global grid (ver. 1.2).

Bauer U., Bohn H.F., & Federle W. (2008) Harmless nectar source or deadly trap: *Nepenthes* pitchers are activated by rain, condensation and nectar. *Proceedings. Biological sciences / The Royal Society*, **275**, 259–65.

Bauer U., Clemente C.J., Renner T., & Federle W. (2012) Form follows function: morphological diversification and alternative trapping strategies in carnivorous *Nepenthes* pitcher plants. *Journal of Evolutionary Biology*, **25**, 90–102.

Bauer U. & Federle W. (2009) The insect-trapping rim of *Nepenthes* pitchers: surface structure and function. *Plant Signaling & Behavior*, **4**, 1019–23.

Bauer U., Grafe T.U., & Federle W. (2011) Evidence for alternative trapping strategies in two forms of the pitcher plant, *Nepenthes rafflesiana*. *Journal of Experimental Botany*, **62**, 3683–92.

Bauer U., Willmes C., & Federle W. (2009a) Effect of pitcher age on trapping efficiency and natural prey capture in carnivorous *Nepenthes rafflesiana* plants. *Annals of Botany*, **103**, 1219–26.

Bauer U., Willmes C., & Federle W. (2009b) Effect of pitcher age on trapping efficiency and natural prey capture in carnivorous *Nepenthes rafflesiana* plants. *Annals of Botany*, **103**, 1219–26.

Beaman J.H., Friis I., & Balslev H. (2005) Mount Kinabalu: hotspot of plant diversity in Borneo. *Plant diversity and complex patters: local, regional and global dimensions.* (ed. by I. Friis and H. Balslev), pp. 103–127. Det Kongelige Danske Videnskabernes Selskab, Copenhagen.

Bennett K.F. & Ellison A.M. (2009) Nectar, not colour, may lure insects to their death. *Biology Letters*, **5**, 469–72.

Bertrand R., Lenoir J., Piedallu C., Riofrío-Dillon G., de Ruffray P., Vidal C., Pierrat J.-C., & Gégout J.-C. (2011) Changes in plant community composition lag behind climate warming in lowland forests. *Nature*, **479**, 517–520.

Best R., Caulk N., & Stachowicz J. (2013) Trait vs. phylogenetic diversity as predictors of competition and community composition in herbivorous marine amphipods. *Ecology Letters*, **16**, 72–80.

Bharucha Z. & Pretty J. (2010) The roles and values of wild foods in agricultural systems. *Philosophical transactions of the Royal Society of London*, **365**, 2913–26.

Blanchet S., Helmus M.R., Brosse S., & Grenouillet G. (2014) Regional vs local drivers of phylogenetic and species diversity in stream fish communities. *Freshwater Biology*, **59**, 450–462.

Blomberg S.P., Garland T., & Ives A.R. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 717–45.

Bohn H.F. & Federle W. (2004) Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. *PNAS*, **101**, 14138–43.

Bonhomme V., Gounand I., Alaux C., Jousselin E., Barth D., & Gaume L. (2011a) The plant-ant *Camponotus schmitzi* helps its carnivorous host-plant *Nepenthes bicalcarata* to catch its prey. *Journal of Tropical Ecology*, **27**, 15–24.

Bonhomme V., Pelloux-Prayer H., Jousselin E., Forterre Y., Labat J.-J., & Gaume L. (2011b) Slippery or sticky? Functional diversity in the trapping strategy of *Nepenthes* carnivorous plants. *New Phytologist*, **191**, 545–54.

Brandt A.J., Seabloom E.W., & Hosseini P.R. (2009) Phylogeny and provenance affect plant-soil feedbacks in invaded California grasslands. *Ecology*, **90**, 1063–1072.

Brockington S.F., Alexandre R., Ramdial J., Moore M.J., Crawley S., Dhingra A., Hilu K., Soltis D.E., & Soltis P.S. (2009) Phylogeny of the Caryophyllales *sensu lato*: revisiting hypotheses on pollination biology and perianth differentiation in the core Caryophyllales. *International Journal of Plant Sciences*, **170**, 627–643.

Brooks R. (1988) *Serpentine and its vegetation. A multidisciplinary approach.* Dioscorides Press, Inc., Portland.

Buch F., Rott M., Rottloff S., Paetz C., Hilke I., Raessler M., & Mithöfer A. (2013) Secreted pitfall-trap fluid of carnivorous *Nepenthes* plants is unsuitable for microbial growth. *Annals of Botany*, **111**, 375–83.

Buel, J.W. (1887) *Sea and Land*. J. S. Robertson. Toronto.

Burns J. & Strauss S.Y. (2011) More closely related species are more ecologically similar in an experimental test. *PNAS*, **108**, 5302–5307.

Cadena C.D., Kozak K.H., Gomez J.P., Parra J.L., McCain C.M., Bowie R.C.K., Carnaval A.C., Moritz C., Rahbek C., Roberts T.E., Sanders N.J., Schneider C.J., VanDerWal J., Zamudio K.R., & Graham C.H. (2011) Latitude, elevational climatic zonation and speciation in New World vertebrates. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 194–201.

Carlquist S. (1966) Wood anatomy of compositae: a summary, with comments on factors controlling wood evolution. *Aliso*, **6**, 25–44.

Carlquist S. (1975) Wood anatomy and relationships of Lactoridaceae. *American Journal of Botany*, **102**, 128–134.

Carlquist S. (1981) Wood anatomy of Nepenthaceae. *Bulletin of the Torrey Botanical Club*, **108**, 324–330.

Carlquist S. (1984) Vessel grouping in dicotyledon wood: significance and relationship to imperforate trachery elements. *Aliso*, **10**, 505–525.

Carlquist S. (1988) *Comparative wood anatomy: systematic, ecological and evolutionary aspects of dicotyledon wood.* Springer-Verlag, Berlin.

Carlquist S. (1989) Anatomy of vine and liana stems: a review and synthesis. *The Biology of Vines* (ed. by F. Putz and H. Mooney), pp. 53–71. Cambridge University Press, Cambridge.

Carlquist S. (1995) Wood anatomy of Caryophyllaceae: ecological, habital, systematic, and phylogenetic implications. *Aliso* **14**, 1–17.

Carlquist S. (1999a) Wood, stem, and root anatomy of Basellaceae with relation to habit, systematics, and cambial variants. *Flora* **194**, 1–12.

Carlquist S. (1999b) Wood anatomy, stem anatomy, and cambial activity of *Barbeuia* (Caryophyllales). *IAWA Journal* **20**, 431–440.

Carlquist S. (2001) Wood and stem anatomy of Rhabdodendraceae is consistent with placement in Caryophyllales sensu lato. *IAWA Journal* **22**, 171–181.

Carlquist S. (2002) Wood anatomy and successive cambia in *Simmodsia* (Simmodsiaceae): evidence for inclusion in Caryophyllales S.L. *Madorno* **49**, 158–164.

Carlquist S. (2003a) Wood anatomy of Polygonaceae: analysis of a family with exceptional wood diversity. *Botanical Journal of the Linnean Society*, **141**, 25–51.

Carlquist S. (2003b) Wood and stem anatomy of woody Amaranthaceae s.s.: ecology, systematics and the problems of defining rays in dicotyledons. *Botanical Journal of the Linnean Society*, **143**, 1–19.

Carlquist S. (2004) Lateral meristems, successive cambia and their products: a reinterpretation based on roots and stems of Nyctaginaceae. *Botanical Journal Of The Linnean Society* **146**: 129–143.

Carlquist S. (2006) Asteropeia and Physena (Caryophyllales): A case study in comparative wood anatomy. *Brittonia*, **58**, 301–313.

Carlquist S. (2010) Caryophyllales: a key group for understanding wood anatomy character states and their evolution. *Botanical Journal of the Linnean Society*, **164**, 342–393.

Carlquist S. & Boggs C. (1996) Wood Anatomy of Plumbaginaceae. *Bulletin of The Torrey Botanical Club*, **123**, 135–147.

Carroll C. (2010) Role of climatic niche models in focal-species-based conservation planning: assessing potential effects of climate change on Northern Spotted Owl in the Pacific Northwest, USA. *Biological Conservation*, **143**, 1432–1437.

Cavender-Bares J., Ackerly D.D., Baum D.A., & Bazzaz F.A. (2004) Phylogenetic overdispersion in Floridian oak communities. *The American Naturalist*, **163**, 823–843.

Chase M.W., Christenhusz M.J.M., Sanders D., & Fay M.F. (2009) Murderous plants: Victorian Gothic, Darwin and modern insights into vegetable carnivory. *Botanical Journal of the Linnean Society*, **161**, 329–356.

Chave J., Coomes D., Jansen S., Lewis S.L., Swenson N.G., & Zanne A.E. (2009) Towards a worldwide wood economics spectrum. *Ecology Letters*, **12**, 351–366.

Chave J., Muller-Landau H.C., Baker T.R., Easdale T.A., ter Steege H., & Webb C.O. (2006) Regional and phylogenetic variation of wood density across 2456 neotropical tree species. *Ecological Applications*, **16**, 2356–2367.

Chazdon R.L., Careaga S., Webb C., & Vargas O. (2003) Community and phylogenetic structure of reproductive traits of woody species in wet tropical forests. *Ecological Monographs*, **73**, 331–348.

Cheek M. & Jebb M. (2001) Nepenthaceae. *Flora Malesiana*, **15**, 1–157.

Cheek M. & Jebb M. (2013) Recircumscription of the *Nepenthes alata* group (Caryophyllales: Nepenthaceae), in the Philippines, with four new species. *European Journal of Taxonomy*, **69**, 1–23.

Cheek M., Jebb M., & Nooteboom H.P. (2001) *Flora Malesiana. Series I: Spermatophyta = Flowering plants. Vol. 15: Nepenthaceae.* Nationaal Herbarium Nederland, Leiden.

Chen I.C., Shiu H.J., Benedick S., Holloway J.D., Cheye V.K., Barlow H.S., Hill J., & Thomas. C.D. (2009) Elevation increases in moth assemblages over 42 years on a tropical mountain. *PNAS*, **106**, 1479–1483.

Chiej R. (1984) *Encyclopedia of Medicinal Plants.* Little, Brown, New York.

Chin L., Moran J.A., & Clarke C. (2010) Trap geometry in three giant montane pitcher plant species from Borneo is a function of tree shrew body size. *New Phytologist*, **186**, 461–70.

Choat B., Jansen S., Brodribb T.J., Cochard H., Delzon S., Bhaskar R., Bucci S.J., Feild T.S., Gleason S.M., Hacke U.G., Jacobsen A.L., Lens F., Maherali H., Martínez-Vilalta J., Mayr S., Mencuccini M., Mitchell P.J., Nardini A., Pittermann J., Pratt R.B., Sperry J.S., Westoby M., Wright I.J., & Zanne A.E. (2012) Global convergence in the vulnerability of forests to drought. *Nature*, **491**, 752–5.

Christensen H. (2002) *Ethnobotany of the Iban & the Kelabit.* Nepcon; University of Aarhus; Forest Department Sarawak, Malaysia,

Clarke C. & Moran J.A. (2011) Incorporating ecological context: a revised protocol for the preservation of *Nepenthes* pitcher plant specimens (Nepenthaceae). *Blumea*, **56**, 225–228.

Clarke C. & Wong K.M. (1997) *Nepenthes of Borneo.* Natural History Publications in association with Science and Technology Unit, Sabah.

Clarke C.M., Bauer U., Lee C.C., Tuen A.A., Rembold K., & Moran J.A. (2009) Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant. *Biology letters*, **5**, 632–5.

Colwell R.K. & Futuyma D.J. (2014) On the measurement of niche breadth and overlap. *Ecological Society of America*, **52**, 567–576.

Corlett R.T. (2011) Impacts of warming on tropical lowland rainforests. *Trends in Ecology and Evolution*, **26**, 606–13.

Cottam M.A, Hall R., Sperber C., Kohn B.P., Forster M.A., Batt G.E. (2013) Neogene rock uplift and erosion in northern Borneo: evidence from the Kinabalu granite, Mount Kinabalu. *Journal Geological Society London*, **170**, 805–816.

Couvreur T.L.P., Porter-Morgan H., Wieringa J.J., & Chatrou L.W. (2011) Little ecological divergence associated with speciation in two African rain forest tree genera. *BMC Evolutionary Biology*, **11**, 296.

Crisp M.D., Arroyo M.T.K., Cook L.G., Gandolfo M.A., Jordan G.J., McGlone M.S., Weston P.H., Westoby M., Wilf P., & Linder H.P. (2009) Phylogenetic biome conservatism on a global scale. *Nature*, **458**, 754–6.

Cuénoud P., Savolainen V., Chatrou L.W., Powell M., Grayer R.J., & Chase M.W. (2002) Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. *American Journal of Botany*, **89**, 132–144.

Culmsee H. & Leuschner C. (2013) Consistent patterns of elevational change in tree taxonomic and phylogenetic diversity across Malesian mountain forests. *Journal of Biogeography*, **40**, 1997–2010.

Darwin C.R. (1875) *Insectivorous Plants.* John Murray, London.

Darwin C.R. (1859) *The Origin of Species.* John Murray, London.

Das I. & Haas A. (2010) New species of Microhyla from Sarawak: Old World's smallest frogs crawl out of miniature pitcher plants on Borneo (Amphibia: Anura: Microhylidae). *Zootaxa*, **2571**, 37–52.

Davey T.M., Allotey P., & Reidpath D.D. (2013) Is obesity an ineluctable consequence of development? A case study of Malaysia. *Public Health*, **127**, 1057–62.

Deavin G., Denis J.I., Djandam A.M., Dols H., Lajumin P., Lanting A.Y., Lasimbang R., Spence J., & Widjojo N. (2012) *The Human Heart of Borneo.* WWF Heart of Borneo Global Initiative.

Dormann C.F., Elith J., Bacher S., Buchmann C., Carl G., Carré G., Marquéz J.R.G., Gruber B., Lafourcade B., Leitão P.J., Münkemüller T., Mcclean C., Osborne P.E., Reineking B., Schröder B., Skidmore A.K., Zurell D., & Lautenbach S. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 027–046.

Doyle J.J. & Doyle J. (1990) Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13–15.

Drummond A.J., Suchard M.A., Xie D., & Rambaut A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Elith J., Graham C.H., Anderson R.P., Dudik M., Ferrier S., Guisan A., Hijmans R.J., Huettmann F., Leathwick J.R., Lehmann A., Li J., Lohmann L.G., Loiselle B.A., Manion G., Moritz C., Nakamura M., Nakazawa Y., Overton J.M.C., Peterson A.T., Phillips S.J., Richardson K., Scachetti-Pereira R., Schapire R.E., Soberon J., Williams S., Wisz M.S., & Zimmermann N.E. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.

Elith J., Phillips S.J., Hastie T., Dudík M., Chee Y.E., & Yates C.J. (2011) A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, **17**, 43–57.

Ellison A.M. & Gotelli N.J. (2001) Evolutionary ecology of carnivorous plants. *Trends in Ecology & Evolution*, **16**, 623–629.

Ellison A.M. & Gotelli N.J. (2009) Energetics and the evolution of carnivorous plants - Darwin's "most wonderful plants in the world". *Journal of Experimental Botany*, **60**, 19–42.

van der Ent A., Sumail S., & Clarke C. (2015) Habitat differentiation of obligate ultramafic *Nepenthes* endemic to Mount Kinabalu and Mount Tambuyukon (Sabah, Malaysia). *Plant Ecology*, **216**, 789–807.

Fahn A. (1979) *Secretory tissues in plants.* Academic Press, London.

Fahn A. (1990) *Plant anatomy.* Pergamon Press, Oxford.

Favre A., Päckert M., Pauls S.U., Jähnig S.C., Uhl D., Michalak I., & Muellner-Riehl A.N. (2014) The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. *Biological Reviews*, **90**, 236-253.

Felsenstein J. (1973) Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Zoology*, **22**, 240–249.

Felsenstein J. (1985) Phylogenies and the comparative method. *The American Naturalist*, **125**, 1–15.

Francisco-Ortega J., Jansen R.K., & Santos-Guerra A. (1996) Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *PNAS*, **93**, 4085–4090.

Gathorne-Hardy F.J., Davies R.G., Eggleton P., & Jones D.T. (2002) Quaternary rainforest refugia in Southeast Asia: using termites (I*soptera*) as indicators. *Biological Journal of the Linnean Society*, **75**, 453–466.

Gaume L. & Forterre Y. (2007) A viscoelastic deadly fluid in carnivorous pitcher plants. *PloS ONE*, **2**, e1185.

Gaume L. & Di Giusto B. (2009) Adaptive significance and ontogenetic variability of the waxy zone in *Nepenthes rafflesiana*. *Annals of Botany*, **104**, 1281–91.

Gawin D.F., Rahman M.A., Ramji M.F.S., Smith B.T., Lim H.C., Moyle R.G., & Sheldon F.H. (2014) Patterns of avian diversification in Borneo: the case of the endemic Mountain Black-eye *Chlorocharis emiliae*. *The Auk*, **131**, 86–99.

Gernhard T. (2008) The conditioned reconstructed process. *Journal of Theoretical Biology*, **253**, 769–778.

Ghalambor C.K., Huey R.B., Martin P.R., Tewksbury J.J., & Wang G. (2006)

Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology*, **46**, 5–17.

Di Giusto B., Bessière J.-M., Guéroult M., Lim L.B.L., Marshall D.J., Hossaert-McKey M., & Gaume L. (2010) Flower-scent mimicry masks a deadly trap in the carnivorous plant *Nepenthes rafflesiana*. *Journal of Ecology*, **98**, 845–856.

Di Giusto B., Grosbois V., Fargeas E., Marshall D.J., & Gaume L. (2008) Contribution of pitcher fragrance and fluid viscosity to high prey diversity in a *Nepenthes* carnivorous plant from Borneo. *Journal of Biosciences*, **33**, 121–36.

Givnish T.J. (1997) Adaptive radiation and molecular systematics: issues and approaches. *Molecular Evolution and Adaptive Radiation* (ed. by T.J. Givnish and K.J. Systma), pp. 1–54. Cambridge University Press, Cambridge.

Godoy O., Kraft N.J.B., & Levine J.M. (2014) Phylogenetic relatedness and the determinants of competitive outcomes. *Ecology Letters*, **17**, 836–844.

Goecks J., Nekrutenko A., & Taylor J. (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible and transparent computational research in the life sciences. *Genome Biology*, **11**, R86.

Goodman M. (1981) Decoding the pattern of protein evolution. *Progress in Biophysics and Molecular Biology*, **38**, 105–64.

Gorb E. V, Baum M.J., & Gorb S.N. (2013) Development and regeneration ability of the wax coverage in *Nepenthes alata* pitchers: a cryo-SEM approach. *Scientific Reports*, **3**, 3078.

Gottwald H. & Parameswaran N. (1968) Das sekundäre xylem und die systematische stellung der Ancistrocladaceae und Dioncophyllaceae. *Stellung and Botanisches Jahrbuch*, **88**, 49–69.

Graham C.H., Carnaval A.C., Cadena C.D., Zamudio K.R., Roberts T.E., Parra J.L., Mccain C.M., Bowie R.C.K., Moritz C., Baines S.B., Schneider C.J., Vanderwal J., Rahbek C., Kozak K.H., & Sanders N.J. (2014) The origin and maintenance of montane diversity: integrating evolutionary and ecological processes. *Ecography*, **37**, 711–719.

Graham C.H., Ron S.R., Santos J.C., Schneider C.J., & Moritz C. (2004) Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, **58**, 1781.

Greenwood M., Clarke C., Lee C.C., Gunsalam A., & Clarke R.H. (2011) A unique resource mutualism between the giant Bornean pitcher plant, *Nepenthes rajah*, and members of a small mammal community. *PLOS ONE*, **6**, e21114.

Grubb P.J. (1971) Interpretation of the "Massenerhebung" Effect on tropical mountains. *Nature*, **229**, 44–45.

Hall R. (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. *Biogeography and Geological Evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 99–131. Backhuys Publishers, Leiden.

Hammond J. (1933) *Winjan's People.* Imperial Printing Co., Perth.

Heibl C. (2011) Package phyloclim: integrating phylogenetics and climatic niche modelling.

Heinricher E. (1906) Biologie von *Nepenthes*: speciell der Javanischen N. melamphora. *Annals du Jardin de Buitenzorg*, **20**, 277–298.

Hellinger E. (1909) Neue Begründung der Theorie quadratischer Formen von unendlichvielen Veränderlichen. *Journal für die reine und angewandte Mathematik*, **136**, 210–271.

Heubl G., Bringmann G., & Meimberg H. (2006) Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales - revisited. *Plant Biology*, **8**, 821–30.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Holt R., Barfield M., & Gomulkiewicz R. (2005) Theories of niche conservatism and evolution: could exotic species be potential tests? *Species Invasions: Insights into Ecology, Evolution, and Biogeography.* (ed. by D. Sax, J. Stachowicz, and S. Gaines), pp. 259–290. Sinauer Associates, Sunderland.

Hooker J.D. (1859) XXXV. On the origin and development of the pitchers of *Nepenthes*, with an account of some new Bornean plants of that genus. *Transactions of the Linnean Society of London*, **22**, 415–424.

Hoorn C., Mosbrugger V., Mulch A., & Antonelli A. (2013) Biodiversity from mountain building. *Nature Geoscience*, **6**, 154–154.

Huey R.B. & Webster T.P. (1976) Thermal biology of anolis lizards in a complex fauna: the Christatellus group on Puerto Rico. *Ecology*, **57**, 985–994.

Human Resource Management Unit, Chief Minister Department of Sarawak in cooperation with State Attorney General. (1958) Laws of Sarawak: Forest Ordinace. Kuching.

Hutchinson G.E. (1957) Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415–427.

IAWA C. (1989) IAWA list of microscopic features for hardwood identification. *International Association of Wood Anatomists Bulletin*, **10**, 221–332.

IPCC (2013) IPCC summary for policymakers in climate change 2013: the physical science basis. Stockholm: Cambridge University Press.

IPCC (2014) *Climate Change 2014: Impacts, adaptation and vulnerability. Contribution of working group II to the fifth assessment report of the Intergovernmental Panal on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York.

IUCN. 2015. The IUCN Red List of Threatened Species. Version 2014.3. http://www.iucnredlist.org/

Institute for Public Health Ministry of Malaysia. (2011) National Health and Morbidity Survey 2011.

Jackson S.T. & Overpeck J.T. (2000) Responses of plant populations and communities to environmental changes of the late Quaternary. *The Paleontological Society*, **26**, 194–220.

Jaiswal V. (2010) Culture and ethnobotany of Jaintia tribal community of Meghalaya, Northeast India - A mini review. *Indian Journal of Traditional Knowledge*, **9**, 38–44.

Jakob S.S., Heibl C., Rödder D., & Blattner F.R. (2010) Population demography influences climatic niche evolution: evidence from diploid American Hordeum species (Poaceae). *Molecular Ecology*, **19**, 1423–38.

Jan B. (2007) Sweet but dangerous: nectaries in carnivorous plants. *Acta agrobotanica*, **60**, 31–37.

Janzen D.H. (1967) Fire, vegetation structure, and the Ant X *Acacia* interaction in Central America. *Ecological Society of America*, **48**, 26–35.

Jebb (1991) An account of *Nepenthes* in New Guinea - Wikipedia, the free encyclopedia. *Science in New Guinea*, **17**, 7–54.

Jennings D.E. & Rohr J.R. (2011) A review of the conservation threats to carnivorous plants. *Biological Conservation*, **144**, 1356–1363.

Jiang L., Tan J., & Pu Z. (2010) An experimental test of Darwin's naturalization hypothesis. *The American Naturalist*, **175**, 415–23.

Juniper B., Robins R., & Joel D.M. (1989) *The carnivorous plants.* Academic Press, London; San Diego.

Katoh K., Misawa K., Kuma K., & Miyata T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.

Kendall D. (1989) A survey of the staistical theory of shape. *Statistical Science*, **4**, 87–120.

Kidner C., Groover A., Thomas D.C., Emelianova K., Soliz-Gamboa C., & Lens F. (2016) First steps in studying the origins of secondary woodiness in Begonia (Begoniaceae): combining anatomy, phylogenetics, and stem transcriptomics. *Biological Journal of the Linnean Society*, **117**, 121–1.

Kitayama K. (1992) An altitudinal transect study of the vegetation on Mount Kinabalu, Borneo. *Vegetatio*, **102**, 149–171.

Knox E.B. & Palmer J.D. (1995) Chloroplast DNA variation and the recent radiation of the giant senecios (Asteraceae) on the tall mountains of eastern Africa. *PNAS*, **92**, 10349–10353.

Kny L. & Zimmerman A. (1885) Die bedeutung der spiralzellen von *Nepenthes*. *Berichte der Deutschen Botanischen Gesellschaft*, **3**, 123–128.

Korner C. & Spehn E. (2002) *Mountain Biodiversity: a Global Assessment.* Parthenon, Boca Raton.

Kozak K.H. & Wiens J.J. (2006) Does niche conservitism promote speciation? a case study in North American salamanders. *Evolution*, **60**, 2604–2621.

Krutzsch W. (1988) Paleogeography and historical phytogeography (paleochorology) in the Neophyticum. *Plant Systematics and Evolution*, **162**, 5–61.

Laistrooglai A., Mosikarat P., & Wigran M. (2000) Packaging design with natural materials: A study for conservation. Silpakorn Univeristy Research & Development, Bangkok.

Larsson A. (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics*, **30**, 3276–3278.

Lens F., Davin N., Smets E., & del Arco M. (2013a) Insular woodiness on the canary islands: a remarkable case of convergent evolution. *International Journal of Plant Sciences*, **174**, 992–1013.

Lens F., Dressler S., Jansen S., van Evelghem L., & Smets E. (2005) Within balsaminoid Ericales: a wood anatomical approach. *American Journal of Botany*, **92**, 941–953.

Lens F., Endress M.E., Baas P., Jansen S., & Smets E. (2008a) Wood anatomy of Rauvolfioideae (Apocynaceae): a search for meaningful non-DNA characters at the tribal level. *American Journal of Botany*, **95**, 1199–1215.

Lens F., Kårehed J., Baas P., Jansen S., Rabaey D., Huysmans S., Hamann T., & Smets E. (2008b) The wood anatomy of the polyphyletic Icacinaceae s.l., and their relationships within asterids. *Taxon*, **57**, 525–552.

Lens F., Luteyn J.L., Smets E., & Jansen S. (2004) Ecological trends in the wood anatomy of Vaccinioideae (Ericaceae s.l.). *Flora*, **199**, 309–319.

Lens F, Picon-Cochard C, Delmas CEL, Signarbieux C, Buttler A, Cochard H, Jansen S, Chauvin T, Doria LC, del Arco M, Delzon S. (In press) Herbaceous angiosperms are not more vulnerable to drought-induced embolism than angiosperm trees. *Plant Physiology* DOI:10.1104/pp.16.00829.

Lens F., Sperry J., Christman M., Choat B., Rabaey D., & Jansen S. (2011) Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus Acer. *New Phytologist*, **190**, 709–23.

Lens F., Tixier A., Cochard H., Sperry J.S., Jansen S., & Herbette S. (2013b) Embolism resistance as a key mechanism to understand adaptive plant strategies. *Current Opinion in Plant Biology*, **16**, 287–292.

Liew T., Schilthuizen M., & Bin Lakim M. (2009) The determinants of land snail diversity along a tropical elevational gradient: insularity, geometry and niches. *Journal of Biogeography*, **37**, 1071–1078.

Likhitwitayawuid K., Kaewamatawong R., Ruangrungsi N., & Krungkrai J. (1998) Antimalarial naphthoquinones from *Nepenthes thorelii*. *Planta Medica*, **64**, 237–41.

Limpisut P. & Jindal V.K. (2002) Comparison of rice flour pasting properties using Brabender Viscoamylograph and Rapid Visco Analyser for evaluating cooked rice texture. *Starch/Stärke*, **54**, 350–357.

Lobo J.M., Jiménez-Valverde A., & Real R. (2008) AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography*, **17**, 145–151.

Lomolino M. (2001) Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography*, **10**, 3–13.

Losos J.B. (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, **11**, 995–1003.

Losos J.B., Leal M., Glor R.E., De Queiroz K., Hertz P.E., Rodríguez Schettino L., Lara A.C., Jackman T.R., & Larson A. (2003) Niche lability in the evolution of a Caribbean lizard community. *Nature*, **424**, 542–5.

Maddison W.P. & Maddison D.R. (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75. http//mesquiteproject.org. .

Madriñán S., Cortés A.J., & Richardson J.E. (2013) Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, **4**, 1–7.

Martin G.J. (1995) *Ethnobotany: A methods manual.* Chapman & Hall, London.

Matloff N. (2008) R for Programmers. 1–104.

Mauseth J.D. & Landrum J. V. (1997) Relictual vegetative anatomical characters in Cactaceae: the genus *Pereskia*. *Journal of Plant Research*, **110**, 55–64.

McPherson S.R. (2012) *The New Nepenthes.* Redfern Natural History Productions Ltd, England.

McPherson S.R. (2009) *Pitcher Plants of the Old World Vol. 1 Redfern Natural History.* Redfern Natural History Productions Ltd, England.

Meimberg H., Dittrich P., Bringmann G., Schlauer J., & Heubl G. (2000) Molecular phylogeny of Caryophyllidae. s.l. based on matK sequences with special emphasis on carnivorous taxa. *Plant Biology*, **2**, 218–228.

Meimberg H. & Heubl G. (2006) Introduction of a nuclear marker for phylogenetic analysis of Nepenthaceae. *Plant Biology*, **8**, 831–40.

Meimberg H., Wistuba A., Dittrich P., & Heubl G. (2001) Molecular phylogeny of Nepenthaceae based on cladistic analysis of plastid trnK intron sequence data. *Plant Biology*, **3**, 164–175.

Merbach M.A., Merbach D.J., Maschwitz U., Booth W.E., Fiala B., & Zizka§ G. (2002) Mass march of termites into the deadly trap. *Nature*, **415**, 36–37.

Merckx V.S.F.T., Hendriks K., Arumugam N., Chung A.Y.C., Geml J., Janssens S.B., Joan, Lens F., Pereira T., Shim P.-S., Sugau J.B., Katja, Tuh F.Y.Y., de Boer H., Dow R., Gravendeel B., Jocqué M., Biun A., Feijen F.A.A., Beentjes K.K., Buang M.M., Feijen H., Geurts R., Hovenkamp P., Majapun R.J., Rahman H., Smit H., Suleiman M., Yahya B.E., Peijnenburg T.C.A., Kappes H., Morgado L.N., Sabran S., Schwallier R., Sol N., Sumail S., Mennes C.B., Khoo E., Neupane S., Sawang A., Spait M., Thomas D.C., Nais J., Repin R., Lakim M., & Menno S. (2015) Evolution of endemism on a young tropical mountain. *Nature*, **524**, 347–350.

Merow C., Smith M.J., & Silander J.A. (2013) A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, **36**, 1058–1069.

Metcalfe C. & Chalk L. (1950) *Anatomy of the dicotyledons, vol. 11.* Claredon Press, Oxford.

Mey F.S. (2010) Introduction to the pitcher plants (*Nepenthes*) of Cambodia.

Cambodian Journal of Natural History, **2**, 106–117.

Miettinen J., Shi C., & Liew S.C. (2011) Deforestation rates in insular Southeast Asia between 2000 and 2010. *Global Change Biology*, **17**, 2261–2270.

Miettinen J., Shi C., Tan W.J., & Liew S.C. (2012) 2010 land cover map of insular Southeast Asia in 250m spatial resolution. *Remote Sensing Letters*, **3**, 11-20.

Miller M., Holder M.T., Vos R., Midford P., Liebowitz T., Chan L., Hoover P., & Warnow T. (2010)

Milliken W. (1992) Ethnobotany of the Yali of West Papua. Edinburgh: Royal Botanic Garden; 1992. 7, 19 & 30.

Moran J.A. (2001) Termite prey specialization in the pitcher plant *Nepenthes albomarginata* - evidence from stable isotope analysis. *Annals of Botany*, **88**, 307–311.

Moran J.A. (1996) Pitcher dimorphism, composition prey and the mechanisms of prey attraction in the pitcher plant *Nepenthes rafflesiana* in Borneo. *Journal of Ecology*, **84**, 515–525.

Moran J.A., Booth W.E., & Charles J.K. (1999) Aspects of pitcher morphology and spectral characteristics of six Bornean *Nepenthes* pitcher plant species: implications for prey capture. *Annals of Botany*, **83**, 521–528.

Moran J.A., Clarke C., & Gowen B.E. (2012) The use of light in prey capture by the tropical pitcher plant *Nepenthes* aristolochioides. *Plant Signaling & Behavior*, **7**, 957–960.

Moran J.A., Clarke C.M., & Hawkins B.J. (2003) From carnivore to detritivore? Isotopic evidence for leaf litter utilization by the tropical pitcher plant *Nepenthes ampullaria*. *International Journal of Plant Sciences*, **164**, 635–639.

Moran J.A., Gray L.K., Clarke C., & Chin L. (2013) Capture mechanism in Palaeotropical pitcher plants (Nepenthaceae) is constrained by climate. *Annals of Botany*, **112**, 1279–91.

Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B., & Kent J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

van der Niet T., Zollikofer C.P.E., León M.S.P. De, Johnson S.D., & Linder H.P. (2010) Three-dimensional geometric morphometrics for studying floral shape variation. *Trends in Plant Science*, **15**, 423–6.

Nilsson R. & Nilsson G. (1958) Studies concerning Swedish ropy milk: the antibiotic qualities of ropy milk. *Archiv für Mikrobiologie*, **31**, 191–197.

Nongrum I., Kumar S., Kumaria S., & Tandon P. (2012) Genetic variation and gene flow estimation of *Nepenthes khasiana* Hook. Threatened insectivorous plant of India as revealed by RAPD markers. *Journal of Crop Science Biotechnology*, **15**, 101–105.

Noshiro S. & Baas P. (2000) Trends in wood anatomy within species and genera: case study in Cornus s.l. (Cornaceae). *American Journal of Botany*, **87**, 1495–1506.

Nürk N.M., Uribe-Convers S., Gehrke B., Tank D.C., & Blattner F.R. (2015) Oligocene niche shift, Miocene diversification – cold tolerance and accelerated speciation rates in the St. John's Worts (Hypericum, Hypericaceae). *BMC Evolutionary Biology*, **15**, 1–13.

Odahara M., Sokooshi H., Takahashi T., Okadome H., & Ohtsubo K. (2004) The effect of sushi vinegar on texture of sushi rice before and after storage under low temperature. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **51**, 620–625.

van den Oever L., Baas P., & Zandee M. (1981) Comparative wood anatomy of Symplocos and latitude and altitude of provenance. *IAWA Bulletin new series*, **2**, 3–24.

Olson ME, Gaskin JF, Ghahremani-Nejad F. (2003) Stem anatomy is congruent with molecular phylogenies placing *Hypericopsis persica* in *Frankenia* (Frankeniaceae): comments on vasicentric tracheids. *Taxon* **52**: 525–532.

Olson M.E., Anfodillo T., Rosell J.A., Petit G., Crivellaro A., Isnard S., León-Gómez C., Alvarado-Cárdenas L.O., & Castorena M. (2014) Universal hydraulics of the flowering plants: vessel diameter scales with stem length across angiosperm lineages, habits and climates. *Ecology Letters*, **17**, 988–997.

Ornelas J.F., Sosa V., Soltis D.E., Daza J.M., Gonzlez C., Soltis P.S., Gutirrez-Rodriguez C., de los Monteros A.E., Castoe T.A., Bell C., & Ruiz-Sanchez E. (2013) Comparative phylogeographic analyses illustrate the complex evolutionary history of threatened cloud forests of Northern Mesoamerica. *PLoS ONE*, **8**, .

Osunkoya O.O., Daud S.D., Di-Giusto B., Wimmer F.L., & Holige T.M. (2007) Construction costs and physico-chemical properties of the assimilatory organs of *Nepenthes* species in Northern Borneo. *Annals of Botany*, **99**, 895–906.

Osunkoya O.O., Daud S.D., & Wimmer F.L. (2008) Longevity, lignin content and construction cost of the assimilatory organs of *Nepenthes* species. *Annals of Botany*, **102**, 845–53.

Owen T.P. & Lennon K.A. (1999) Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (Nepenthaceae). *American Journal of Botany*, **86**, 1382–1390.

Owen T.P., Lennon K.A., Santo M.J., & Anderson A.N. (1999) Pathways for nutrient transport in the pitchers of the carnivorous plant *Nepenthes alata*. *Annals of Botany*, **84**, 459–466.

Pacifici A.M., Foden W.B., Visconti P., Watson J.E.M., Butchart S.H.M., Kovacs K.M., Scheffers B.R., Hole D.G., Martin T.G., Akçakaya H.R., Corlett R.T., Huntley B., Bickford D., Carr J.A., Hoffmann A.A., Midgley G.F., Pearce-Kelly P., Pearson R.G., Williams S.E., Willis S.G., Young B., & Rondinini C. (2015) Assessing species vulnerability to climate change. *Nature Climate Change*, **5**, 215–225.

Pant D.D. & Bhatnagar S. (1977) Morphological studies in *Nepenthes* (Nepenthaceae). *Phytomorphology*, **27**, 13–34.

Parry D. & Kelso M. (1977) The ultrastructure and analytical microscopy of silicon deposits in the roots of *Saccharum officinarum* (L.). *Annals of Botany*, **4**, 855–862.

Pavlovič A., Slováková L., & Šantrůček J. (2011) Nutritional benefit from leaf litter utilization in the pitcher plant *Nepenthes ampullaria*. *Plant, Cell & Environment*, **34**, 1865–73.

Pearman P.B., Guisan A., Broennimann O., & Randin C.F. (2008) Niche dynamics in space and time. *Trends in Ecology & Evolution*, **23**, 149–58.

Pearson R.G., Raxworthy C.J., Nakamura M., & Townsend Peterson A. (2007) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.

Perry L.M. & Metzger J. (1980) *Medicinal plants of East and Southeast Asia: attributed properties and uses*. MIT press, Cambridge, MA.

Phillips S.J., Anderson R.P., & Schapire R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Pietropaolo J. & Pietropaolo P. (1986) *Carnivorous Plants of the World*. Timber Press, Portland.

Pilgrim S. & Pretty J. (2010) *Nature and Culture: Rebuilding Lost Connections*. Earthscan, London.

Pilgrim S.E., Cullen L.C., Smith D.J., & Pretty J. (2008) Ecological knowledge is lost in wealthier communities and countries. *Environmental Science & Technology*, **42**, 1004–1009.

Pinthong K., Chaveerach A., Tanee T., Sudmoon R., & Mokkamul P. (2009) Differential expressed protein in developing stages of *Nepenthes gracilis* Korth. pitcher. *Pakistan Journal of Biological Sciences*, **12**, 526–529.

Plat H. (1609) *Delightes for Ladies: to adorn their persons, tables, closets, and distillatories with beauties, banquets, perfumes and waters*. Hymfrey Lownes, London.

Popkin B.M. (2006) Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. *The American Journal of Clinical Nutrition*, **84**, 289–298.

Popkin B.M., Adair L.S., & Ng S.W. (2012) Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition reviews*, **70**, 3–21.

Pretty J., Adams B., Berkes F., de Athayde S.F., Dudley N., Hunn E., Maffi L., Milton K., Rapport D., Robbins P., Sterling E., Stolton S., Tsing A., Vintinnerk E., & Pilgrim S. (2009) The intersections of biological diversity and cultural diversity: towards integration. *Conservation and Society*, **7**, 100–112.

Price S. (1982) When is a calabash not a calabash? *New West Indian Guide / Nieuwe West-Indische Gids*, **56**, 69–82.

Price T.D., Hooper D.M., Buchanan C.D., Johansson U.S., Tietze D.T., Alström P., Olsson U., Ghosh-Harihar M., Ishtiaq F., Gupta S.K., Martens J., Harr B., Singh P., & Mohan D. (2014) Niche filling slows the diversification of

Himalayan songbirds. *Nature*, **509**, 222–5.

Prinzing A., Durka W., Klotz S., & Brandl R. (2001) The niche of higher plants: evidence for phylogenetic conservatism. *PNAS*, **268**, 2383–9.

Raes N. & ter Steege H. (2007) A null-model for significance testing of presence-only species distribution models. *Ecography*, **30**, 727–736.

Rahbek C. (1995) The elevational gradient of species richness: a uniform pattern? *Ecography*, **18**, 200–205.

Rahbek C. (2004) The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters*, **8**, 224–239.

Ravussin E., Valencia M.E., Esparza J., Bennett P.H., & Schulz L.O. (1994) Effects of a traditional lifestyle on obesity in Pima Indians. *Diabetes Care*, **17**, 1067–1074.

Raxworthy C.J., Ingram C.M., Rabibisoa N., & Pearson R.G. (2007) Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (Phelsuma) from Madagascar. *Systematic Biology*, **56**, 907–23.

Rembold K., Fischer E., Striffler B.F., & Barthlott W. (2012) Crab spider association with the Malagasy pitcher plant *Nepenthes madagascariensis*. *African Journal of Ecology*, **51**, 188–191.

Rembold K., Fischer E., Wetzel M. a., & Barthlott W. (2010) Prey composition of the pitcher plant *Nepenthes madagascariensis*. *Journal of Tropical Ecology*, **26**, 365–372.

Robinson A., Nerz J., & Wistuba A. (2011) *Nepenthes epiphytica*, a new pitcher plant from East Kalimantan. *New Nepenthes* (ed. by N. Sanders, M. Lessard, C. Fitzpatrick, R. Dunn, and S. McPherson), pp. 35–51. Redfern Natural History Productions Ltd, Poole.

Robinson A.S., Fleischmann A.S., Mcpherson S.R., Heinrich V.B., Gironella E.P., & Peña C.Q. (2009) A spectacular new species of *Nepenthes* L . (Nepenthaceae) pitcher plant from central Palawan, Philippines. *Botanical Journal of the Linnean Society*, **159**, 195–202.

Rodman J. (1994) Cladistic and phenetic studies. *Caryphyllales* (ed. by H. Behnke and T. Mabry), pp. 279–301. Springer-Verlag, Berlin & Heidelberg.

Rodriguez-Castaneda G., Dyer L.A., Brehm G., Connahs H., Forkner R.E., & Walla T.R. (2010) Tropical forests are not flat: how mountains affect herbivore diversity. *Ecology Letters*, **13**, 1348–1357.

Rokas A. & Carroll S.B. (2005) More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. *Molecular Biology and Evolution*, **22**, 1337–1344.

Rousseau S. (2013) *Food and social media: You are what you tweet.* AltaMira Press, Plymouth.

Le Roux P.C. & McGeoch M.A. (2008) Rapid range expansion and community reorganization in response to warming. *Global Change Biology*, **14**, 2950–2962.

Schäferhoff B., Müller K.F., & Borsch T. (2009) Caryophyllales phylogenetics: disentangling Phytolaccaceae and Molluginaceae and description of Microteaceae as a new isolated family. *Willdenowia - Annals of the Botanic Garden and Botanical Museum Berlin-Dahlem*, **39**, 209–228.

Schiffers K., Borne E., Lavergne S., Thuiller W., & Travis J. (2013) Limited evolutionary rescue of locally adapted populations facing climage change. *Philosophical Transactions of the Royal Society of London*, **368**, 20120083.

Schiller J., Rao S., Inthapanya P., & Hasadong (2006) Glutinous rice in Laos. *Rice in Laos* (ed. by J. Schiller, M. Chanphengxay, B. Linquist, and S. Rao), pp. 197. International Rice Research Institute, Los Banos.

Schoener T.W. & Gorman G.C. (1968) Some niche differences in three lesser antillean lizards of the genus Anolis. *Ecology*, **49**, 819–830.

Scholz A., Rabaey D., Stein A., Cochard H., Smets E., & Jansen S. (2013) The evolution and function of vessel and pit characters with respect to cavitation resistance across 10 *Prunus* species. *Tree Physiology*, **33**, 684–694.

Scholz I., Bückins M., Dolge L., Erlinghagen T., Weth A., Hischen F., Mayer J., Hoffmann S., Riederer M., Riedel M., & Baumgartner W. (2010) Slippery surfaces of pitcher plants: *Nepenthes* wax crystals minimize insect attachment via microscopic surface roughness. *The Journal of Experimental Biology*, **213**, 1115–25.

Schreiber S.G., Hacke U.G., & Hamann A. (2015) Variation of xylem vessel diameters across a climate gradient: insight from a reciprocal transplant experiment with a widespread boreal tree. *Functional Ecology*, **29**, 1392–1401.

Schulze W., Schulze E.D., Pate J.S., & Gillison A.N. (1997) The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californica*. *Oecologia*, **112**, 464–471.

Schupp E., Milleron T., & Russo S. (2002) *Dissemination limitation and the origin and maintenance of species- rich tropical forests. In seed dispersal and frugivory: Ecology, evolution and conservation.* CABI Publishing, Wallingford.

Schwallier R., Raes N., de Boer H., Vos R., van Vugt R., & Gravendeel B. (2016) Phylogenetic analysis of niche divergence reveals distinct evolutionary histories and climate implications for tropical carnivorous plants. *Diversity and Distributions*, **22**, 97–110.

Schwery O., Onstein R.E., Bouchenak-Khelladi Y., Xing Y., Carter R.J., & Linder H.P. (2015) As old as the mountains: the radiations of the Ericaceae. *New Phytologist*, **207**, 355–367.

Scriven S.A., Hodgson J.A., McClean C.J., & Hill J.K. (2015) Protected areas in Borneo may fail to conserve tropical forest biodiversity under climate change. *Biological Conservation*, **184**, 414–423.

Shaw R.G. & Etterson J.R. (2012) Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytologist*, **195**, 752–765.

Silvertown J., Dodd M., Gowing D., Lawson C., & McConway K. (2006) Phylogeny and the hierarchical organization of plant diversity. *Ecology*, **87**, 39–49.

Smets E.F. & Cresens E.M. (1988) Types of floral nectaries and the concepts character and character state - a reconsideration. *Acta Botanica Neerlandica*, **37**, 121–128.

Soberón J. & Nakamura M. (2009) Niches and distributional areas: concepts, methods, and assumptions. *PNAS*, **106**, 19644–19650.

Soberón J. & Peterson A.T. (2005) Interpretation of models of fundamenatal ecological niches and species' distributional areas. *Biodiversity Informatics*, **2**, 1–10.

Sodhi N.S., Posa M.R.C., Lee T.M., Bickford D., Koh L.P., & Brook B.W. (2010) The state and conservation of Southeast Asian biodiversity. *Biodiversity and Conservation*, **19**, 317–328.

Solereder H. (1908) *Systamatic anatomy of the dicotyledons: a handbook for laboratories of pure and applied botany.* Claredon Press, Oxford.

Soltis D.E., Smith S.A., Cellinese N., Wurdack K.J., Tank D.C., Brockington S.F., Refulio-Rodriguez N.F., Walker J.B., Moore M.J., Carlsward B.S., Bell C.D., Latvis M., Crawley S., Black C., Diouf D., Xi Z., Rushworth C.A., Gitzendanner M.A., Sytsma K.J., Qiu Y.L., Hilu K.W., Davis C.C., Sanderson M.J., Beaman R.S., Olmstead R.G., Judd W.S., Donoghue M.J., & Soltis P.S. (2011) Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany*, **98**, 704–730.

La Sorte F.A. & Jetz W. (2010) Projected range contractions of montane biodiversity under global warming. *Proceedings. Biological Sciences/The Royal Society*, **277**, 3401–10.

Stamatakis A., Hoover P., & Rougemont J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, **57**, 758–771.

van Steenis C. (1964) Plant geography of the mountain flora of Mt. Kinabalu. *Proceedings of the Royal Society London*, **161**, 7–38.

Sumsakul W., Plengsuriyakarn T., Chaijaroenkul W., Viyanant V., Karbwang J., & Na-Bangchang K. (2014) Antimalarial activity of plumbagin in vitro and in animal models. *BMC Complementary and Alternative Medicine*, **14**, 15.

Swenson N. & Enquist B. (2007) Ecological and evolutionary determinants of a key plant functional trait: wood density and its community-wide variation across latitude and elevation. *American Journal of Botany*, **94**, 451–459.

Takyu M., Aiba S., & Kitayama K. (2003) Changes in biomass, productivity and decomposition along topographical gradients under different geological conditions in tropical lower montane forests on Mount Kinabalu, Borneo. *Oceologica*, **134**, 397–404.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., & Kumar S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–9.

The HadGEM2 Development Team, Martin G.M., Bellouin N., Collins W.J., Culverwell I.D., Halloran P.R., Hardiman S.C., Hinton T.J., Jones C.D., McDonald R.E., McLaren A.J., O'Connor F.M., Roberts M.J., Rodriguez J.M., Woodward S., Best M.J., Brooks M.E., Brown A.R., Butchart N., Dearden C., Derbyshire S.H., Dharssi I., Doutriaux-Boucher M., Edwards J.M., Falloon P.D., Gedney N., Gray L.J., Hewitt H.T., Hobson M., Huddleston M.R., Hughes J., Ineson S., Ingram W.J., James P.M., Johns T.C., Johnson C.E., Jones A., Jones C.P., Joshi M.M., Keen A.B., Liddicoat S., Lock A.P., Maidens A. V., Manners J.C., Milton S.F., Rae J.G.L., Ridley J.K., Sellar A., Senior C.A., Totterdell I.J., Verhoef A., Vidale P.L., & Wiltshire A. (2011) The HadGEM2 family of Met Office Unified Model climate configurations. *Geoscientific Model Development*, **4**, 723–757.

Thomas E., Vandebroek I., & van Damme P. (2007) What works in the field? A comparision of different interviewing methods in ethnobotany with special reference to use of photographs. *Economic Botany*, **61**, 376–384.

Thornhill A.H., Harper I.S., & Hallam N.D. (2008) The Development of the digestive glands and enzymes in the pitchers of three *Nepenthes* Species: *N. alata*, *N. tobaica*, and *N. ventricosa* (Nepenthaceae). *International Journal of Plant Sciences*, **169**, 615–624.

Tökés Z.A., Woon W.C., & Chambers S.M. (1974) Digestive enzymes secreted by the carnivorous plant *Nepenthes macferlanei* L. *Planta*, **119**, 39–46.

Ulrike Bauer B.D.G. (2012) With a flick of the lid: a novel trapping mechanism in *Nepenthes gracilis* pitcher plants. *PloS ONE*, **7**, e38951.

United Nations. (2008) United Nations Declaration on the Rights of Indigenous Peoples.

United Nations. World Population Prospects: 2000 Revision Population Database. http://esa.un.org/unpd/wpp/index.htm. Accessed Jan 10 2015.

United States Environmental Agency. (2013) Reducing wasted food & packaging, a guide for food services and packaging.

de Vienne D.M., Giraud T., & Martin O.C. (2007) A congruence index for testing topological similarity between trees. *Bioinformatics*, **23**, 3119–3124.

Vijverberg K., Kuperus P., Breeuwer J.A.J., & Bachmann K. (2000) Incipient adaptive radiation of New Zealand and Australian Microseris (Asteraceae): an amplified fragment length polymorphism (AFLP) study. *Journal of Evolutionary Biology*, **13**, 997–1008.

Viscosi V., Fortini P., Slice D.E., Loy A., & Blasi C. (2009) Geometric morphometric analyses of leaf variation in four oak species of the subgenus *Quercus* (Fagaceae). *Plant Biosystems*, **143**, 557–587.

Vogel S., Renner S.S., Bhatti J.S., & Kothekar V.S. (1990) *The role of scent glands in pollination: on the structure and function of osmophores.* Smithsonian Institution Libraries, Wahsington.

Walpole M.J. & Leader-Williams N. (2002) Tourism and flagship species in conservation. *Biodiversity and Conservation*, **11**, 543–547.

Warren B.H., Simberloff D., Ricklefs R.E., Aguilée R., Condamine F.L., Gravel D., Morlon H., Mouquet N., Rosindell J., Casquet J., Conti E., Cornuault J., Fernández-Palacios J.M., Hengl T., Norder S.J., Rijsdijk K.F., Sanmartín I., Strasberg D., Triantis K.A., Valente L.M., Whittaker R.J., Gillespie R.G., Emerson B.C., & Thébaud C. (2015) Islands as model systems in ecology and evolution: prospects fifty years after MacArthur-Wilson. *Ecology Letters*, **18**, 200–217.

Warren D.L., Glor R.E., & Turelli M. (2008) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution*, **62**, 2868–83.

Watson D.M. (2002) A conceptual framework for studying species composition in fragments, islands and other patchy ecosystems. *Journal of Biogeography*, **29**, 823–834.

van Welzen P.C., Ferry Silk J.W., & Alahuhta J. (2005) Plant distribution patterns and plate tectonics in Malesia. *Biologiska Skrifter*, **55**, 199–217.

Van Welzen P.C., Parnell J.A., & Slik J.F. (2011) Wallace's Line and plant distributions: two or three phytogeographical areas and where to group Java? *Biological Journal of the Linnean Society*, **103**, 531–545.

White T., Bruns T., Lee S., & Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* (ed. by A. Inns, D. Gelfand, J. Sninsky, and T. White), pp. 315–324. Academic Press, New York.

Whitehead J. (1893) *Exploration of Mount Binabalue, North Borneo.* Gurney & Jackson, London.

Wiart C. (2006) *Medicinal plants of the Asia-Pacific: Drugs for the future.* World Scientific Publishing Co. Pte. Ltd., Singapore.

Williams J.W., Jackson S.T., & Kutzbach J.E. (2007) Projected distributions of novel and disappearing climates by 2100 AD. *PNAS*, **104**, 5738–42.

Wisz M.S., Hijmans R.J., Li J., Peterson A.T., Graham C.H., & Guisan A. (2008) Effects of sample size on the performance of species distribution models. *Diversity and Distributions*, **14**, 763–773.

Wold S., Esbensen K., & Geladi P. (1987) Principal Component Analysis. *Chemometrics and Intelligen Laboratory Systems*, **2**, 37–52.

Wong K. (1996) *Kinabalu, Summit of Borneo.* Sabah Society & Sabah Parks, Kota Kinabalu.

Yesson, C. & Culham, A. (2006) Phyloclimatic modeling: combining phylogenetics and bioclimatic modeling. *Systematic Biology*, **55**, 785–802.

Zachos J., Pagani M., Sloan L., Thomas E., & Billups K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.

Zakaria W., Adibah W.N., Loke K.-K., Goh H.-H., & Mohd Noor N. (2016) RNA-seq analysis for plant carnivory gene discovery in *Nepenthes* x ventrata. *Genomics Data*, **7**, 18–19.

Zwickl D.J. & Hillis D.M. (2002) Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology*, **51**, 588–598.

Acknowledgments

For mentorship: Barbara Gravendeel's contribution to this thesis extends beyond these pages. She both consciously and unconsciously unleashed a consistent message of motivation, efficiency, engagement and model mentorship. I am truly grateful to have worked under her supervision. Hugo de Boer's power in patience and brevity brought much to this thesis. It is no coincidence that he co-authors several chapters. I thank him for the wittily light to 'beast'ly intricate work together. Erik Smets guided this doctorate with honesty and interest. He made it feel like he was routing for the work and for me to be successful, the mark of a great leader. I feel lucky to have worked with him.

For fieldwork: I am grateful to Menno Schilthuizen, who organized the expedition that made me first feel like a biologist. I hope to glean even a fraction of his poise, vigor and awe in all living things. I also thank Sukaibin Sumail for enthusiastically sharing his home forest with me. His knowledge and calmness make him a most amazing *Nepenthes* field companion.

For plant material: I thank Nicolien Sol for her savvy herbarium help. Paul Keßler and Rogier van Vugt provided living material from the Hortus botanicus Leiden and Anton Sieder shared material from the Botanical Garden of the University of Vienna. My gratitude goes also to Frederic Meijer Gardens and Sculpture Park, especially Steve LaWarre and Mandy Stade, who keenly let me to clip material for research in this thesis.

For permits: We thank the CITES offices of the Netherlands, Malaysia and Sabah Biodiversity Centre, especially Rimi Repin, for collection and research permits. This access was the source of much of our data.

For satellite offices: I am most grateful to Shaily Mennon, who ardently offered her cozy office at Grand Valley State University during summer work in Michigan. I thank Lena Struwe for her kindness and hospitality at Rutgers University. Much of this work was written haunting Grand Rapids coffee shops. Thank you Alexandria, Kate & Richard for providing the essentials needed to complete a doctorate thesis - a vibrant environment and delicious caffeine.

For financial support: I want to thank Amanda Cuevas and the P.E.O. Sisterhood, especially Marcia Haas and Frances Kistler, not only for their financial support, but also their unconditional encouragement and unwavering interest. They played a significant role in the completion of this research. The Alberta Mennega Foundation supported studies with several grants for fieldwork and laboratory work. I am grateful for their contribution. I thank the Treub Foundation for supporting our work in Borneo. My thanks go also to the Endless Forms focus Group, International Carvivorous Plant Society, American Society of Plant Biologists, Vernon I. Cheadle Foundation and, of course, Naturalis Biodiversity Center for supporting conference attendance and lab work.

For science support: The photographic geniuses, Christian Ziegler, Petra Sonius and Joris van Alphen have my whole appreciation for their colorful contributions. My *Nepenthes* enthusiast friends provided gusto and more for this thesis, especially Marcel van den Broek, Ch'ien Lee, Drew Martinez, Shawn Mayes, Stewart McPherson and Alastair Robinson.

For human happiness: Grace, my steadfast friend, thank you. To Adam, my office-mate and science brother who warms a room with his presence, what pleasure to share so many days in happy company with you. To my sweet friends Carlie, Tommy and Peter, who put up the most awesome high-fives in every moment worthy of celebration and gave laughter-full distraction when I would have otherwise been too deep in this thesis, thank you. To my market crew, customers and friends, especially Karolina, Ana, Meghan, David and Todd, I am grateful. To my lovely across-office mate, Alexandra, who shared many cups of thoughtful tea with me. To Katja for many slippery gels, thank you. My time in Leiden was so much sweeter with the friendship of Agnese and Sheila. Floris filled me with the most intricately prepared caffeine and good-hearted discussion. Luis, Nico and Ingrid gave great company. Without Danny, this thesis would have never begun. Without Kries, Watie and Mattea, it would have never been completed. Thank you.

For everything: My family.

About the Author

Rachel Marie Schwallier was born in a blizzard on the 11th of January 1982 in Grand Rapids, Michigan, USA. Curiosity of the natural world brewed early in her life on her parent's orchard, and after her 4th grade teacher brought an awe-driving beehive into the classroom, her interest in the sciences catapulted. She asked for flower presses for her birthday, traded pizza parties for microscopes and blanketed her walls with Zoobook magazine tear-outs.

In 2000, her first steps into college were with a volleyball scholarship at Grand Rapids Community College. She transferred to Grand Valley State University where she earned her BSc in secondary education of biology and chemistry in 2004. During her undergraduate years, she oversaw the university greenhouse, which inspired her to begin a master's thesis entitled, *Studies on the influence of seed maturation on desiccation tolerance for long term ex situ orchid seed storage*. During her time there, Rachel completed an international course on the Great Barrier Reef of Australia and fieldwork research in Nicaragua. She completed her MSc degree in 2007.

After graduating and a lovely stint at Frederik Meijer Gardens and Sculpture Park, Rachel began her PhD research at Naturalis Biodiversity Center and the Hortus botanicus of Leiden University in the Netherlands in 2011. Her fieldwork in Malaysian Borneo hallmarked her thesis studies along with volunteer work for Operation Wallacea in Honduras. The findings in this thesis were presented at international conferences in Seekonk (2012), Austin (2012), New Orleans (2013), Boston (2013), Cairns (2014), Edmonton (2015), Savannah (2016) and many local conferences in the Netherlands and Michigan. With travel and research stamping her passport well, Rachel returned to Michigan in 2016 as a Visiting Associate Professor at Grand Valley State University - a still point in her turning world.

SCI Publications

Schwallier R, Gravendeel B, de Boer H, van Heuven BJ, Sieder A, Sumail S, van Vugt RR, Nylinder S, Lens F. In press. Evolution of wood anatomical characters in *Nepenthes* and close relatives of Caryophyllales. *Annals of Botany*.

Schwallier R, Raes N, de Boer H, Vos R, van Vugt R, Gravendeel B. 2016. Phylogenetic analysis of niche divergence reveals distinct evolutionary histories and climate implications for tropical carnivorous plants. *Diversity and Distributions* 22: 97-110. Merckx VSFT, Hendriks KP, Beentjes KK, Mennes CB, Becking LE, Peijnenburg KTCA, Afendy A, Arumugam N, de Boer H, Biun A, Buang MM, Chen P-P, Chung AYC, Dow R, Feijen FAA, Feijen H, Feijen-van Soest C, Geml J, Geurts R, Gravendeel B, Hovenkamp

P, Imbun P, Ipor I, Janssens SB, Jocqué M, Kappes H, Khoo E, Koomen P, Lens F, Majapun R.J, Morgado LN, Neupane S, Nieser N, Pereira JT, Rahman H, Sabran S, Sawang A, **Schwallier R**, Shim P-S, Smit H, Sol N, Spait M, Stech M, Stokvis F, Sugau JB, Suleiman M, Sumail S, Thomas DC, van Tol J, Tuh FYY, Yahya BE, Nais J, Repin R, Lakim M & Schilthuizen M. 2015. Evolution of endemism on a young tropical mountain. *Nature*. 524: 347–350.

Schwallier R, de Boer H, Visser N, van Vugt RR, Gravendeel B. 2015. Traps as treats: a traditional sticky rice snack persisting in rapidly changing Asian kitchens. *Journal of Ethnobiology and Ethnomedicine* 11: 24.

Schwallier R, Bhoopalan V, Blackman S. 2011. The influence of seed maturation on desiccation tolerance in Phalaenopsis amabilis Hybrids. *Scientia Horticulturae* 128: 136-140.

Schwallier R, van Wely V, Baak M, Vos R, van Heuven BJ, Smets E, van Vugt RR and Gravendeel B. Ontogeny and anatomy of the dimorphic pitchers of *Nepenthes rafflesiana*. In preparation for *American Journal of Botany*.

Conference and Public Media

Schwallier R, Gravendeel B, de Boer H, van Heuven BJ, Sieder A, Sumail S, van Vugt RR, Nylinder S, Lens F. 2016. Botany Conference presentation. Evolution of wood anatomical characters in *Nepenthes* and close relatives of Caryophyllales. Savannah, USA.

Schwallier R. 2016. GVSU Science on Tap invited lecturer. Connecting science and the public in an approachable and welcoming setting. Grand Rapids, Michigan.

Schwallier R *et al.* 2015. GVSU seminar series invited lecture. The evolution, endemism and edibility of tropical carnivorous plants revealed. Allendale, Michigan.

Schwallier R, de Boer H, Visser N, van Vugt RR, Gravendeel B. 2015. Botany Conference presentation. Traps as treats: a traditional sticky rice snack persisting in rapidly changing Asian kitchens. Edmonton, Canada.

Schwallier R, Raes N, de Boer H, Vos R, van Vugt R, Gravendeel B. 2015. Botany Conference presentation. Peril for pitcher plants. Edmonton, Canada.

Schwallier R, Raes N, de Boer H, Vos R, van Vugt R, Gravendeel B. 2014. International Carnivorous Plants Conference presentation. 3D morphometrics and pitcher plants. Cairns, Australia.

Schwallier R, Valeri van Wely, Gravendeel B, van Vucht RR. Botany 2013. Conference Presentation. Pitcher ontogeny of *Nepenthes*: defining developmental stages through morphology and microstructures. New Orleans, USA.

Schwallier R, Raes N, Gravendeel B. 2012. Plant Biology Conference presentation. Ecological niche divergence in tropical pitcher plants. Austin, USA.

Schwallier R, Raes N, Gravendeel B. 2012. International Carnivorous Plant

Society presentation. Are we murdering our murderous plants? Seekonk, USA.

Schwallier R, 2011. Naturalis Biodiversity Center. Diversity in carnivorous pitcher plants. Leiden, The Netherlands.

Schwallier R, Blackman S. 2007. American Society of Plant Biologists Conference. The influence of maturation on seed quality in *Encyclia tampensis*. Chicago, USA.

Schwallier R. Looking for a botanical toilet on Mt. Kinabalu. 2012. Scientific America.

Media for Kinabalu expedition featured in, but not limited to the following: The journal *Science,* Science Daily, Huffington Post, CBS News, Scientific Malaysian Magazine, Wildlife Extra, Daily Express, The Why Files, The Brunei Times and many more.

Media for Ethnobotanical research in Malaysia featured in the following: Science Daily, Beyond Babel, Science Nordic, Forskning, Alpha Galileo.

Grand Valley State University featured my alumni story and research in, 'Finding Fellowships,' July 2015. Grand Valley Magazine.

Joris van Alphen. Photo and research featured in, 'The Mystery of Mount Kinabalu,' February 2013 of National Geographic.

PE&RC Training and Education Statement

The C.T. De Wit Graduate School PE&RC PRODUCTION ECOLOGY & RESOURCE CONSERVATION

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

Niche differentiation, molecular phylogenetics, wood anatomy, ontogeny and traditional use of *Nepenthes* (Nepenthaceae: Caryophyllales) (2012-2016)

Writing of project proposal (4.5 ECTS)

Niche differentiation of tropical pitcher plants (2012)

Post-graduate courses (7.5 ECTS)

Geometric morphometrics training course; Transmitting Science, Barcelona, Spain (2012) Online tools for ecological modelling, visualization and geo-processing; Consortium for Conservation Biologists, Baltimore, USA (2013) Scanning Electron Microscopy (SEM) of *Nepenthes* wood; Leiden University (2013) Anatomical slide preparation and light microscopy; Leiden University (2013)

Laboratory training and working visits (12 ECTS)

Ecological niche modelling of tropical pitcher plants; Grand Valley State University (2012)

Invited review of (unpublished) journal manuscript (1 ECTS)

Blumea: *Nepenthes* taxonomy (2013)

Deficiency, refresh, brush-up courses (4.5 ECTS)

Plant Families of the Tropics; Leiden University (2012)

Competence strengthening / skills courses (4.2 ECTS)

Time management; Leiden University (2012) Far from help; Wilderness Medical Training, Glenmore Lodge, Scotland (2012) Communication in science; Leiden University (2013) Effective communication; Leiden University (2013) On being a scientist; Leiden University (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

6th Workshop plant-insect interaction; UVA (2011) Mini-symposium collections and spatial analysis-introduction of Naturalis Geoportal; Naturalis (2011) PhD Day; PE&RC (2011) Voyager GIS data discovery and spatial analysis with Naturalis Geoportal; Naturalis (2012)

Discussion groups / local seminars / other scientific meetings (9 ECTS)

Tuesday colloquia series of Naturalis (2012-2016) Character evolution focus group meetings (2013-2016)

International symposia, workshops and conferences (12.7 ECTS)

Plant Biology; poster presentation; Austin, USA (2012) International Carnivorous Plant Society conference; oral presentation; Seekonk, USA (2012) Botany conference; poster presentation; New Orleans, USA (2013) International Carnivorous Plant Society conference; poster presentation; Cairns, Australia (2014) Botany conference; oral and poster presentation; Edmonton, Canada (2015) Grand Valley State University fall symposium; oral presentation; Allendale, USA (2015)

Lecturing / supervision of practicals / tutorials (5.4 ECTS)

Plant Families of the Tropics course - carnivorous plants lecture; Leiden University (2012-2013) Geometric morphometric application and analysis with 3D laser scanning; Leiden University (2014) Honduran biodiversity lecture; Muskegon Community College, USA (2016)

Co-supervision of MSc Student (3 ECTS)

Valeri van Wely: *Nepenthes* (Nepenthaceae: Caryophyllales) pitcher ontogeny