Evolutionary diversification of Nepenthes (Nepenthaceae)

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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.