Evolutionary diversification of Nepenthes (Nepenthaceae)

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Chapter Two

Evolution of endemism on a young tropical mountain

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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.

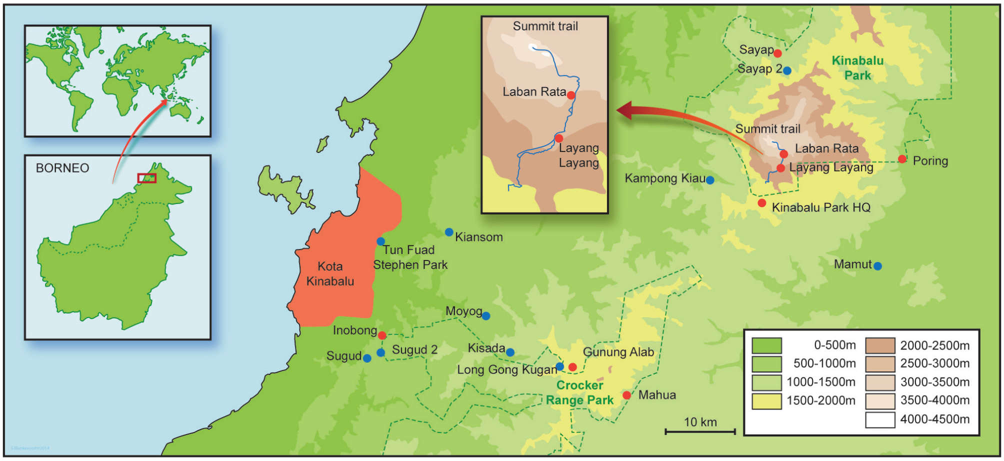

FIGURE 7. Map of the study area. Inset left, location of the study area in the World and in Borneo. Inset middle, detail of the summit trail in Kinabalu Park. The eight expedition stations in Kinabalu Park and Crocker Range Park are indicated with red markers, ten additional sampling sites with blue 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).

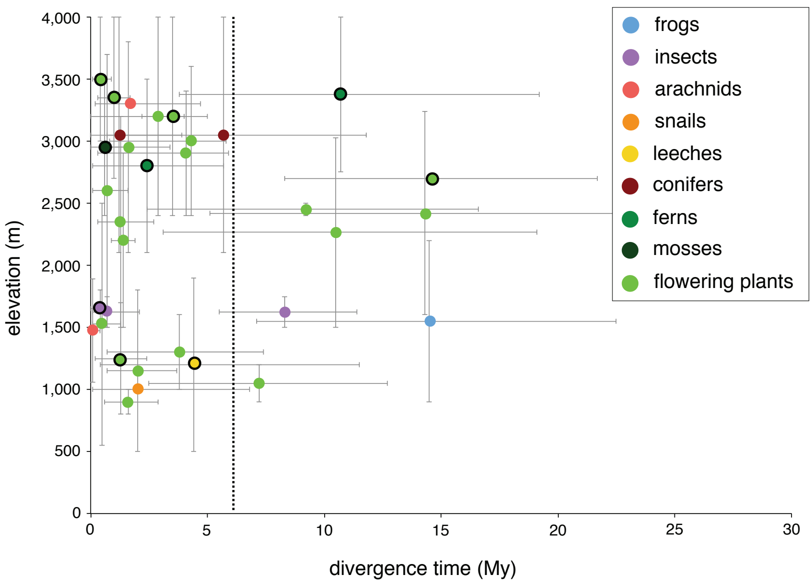

FIGURE 8. Elevations and ages for endemic species. Elevations (mid-points, minima and maxima) and dates of origination derived from molecular dating (averages and 95% credible intervals) for endemic species; eccentric species (see main text) are indicated with a black circle. The vertical dashed line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

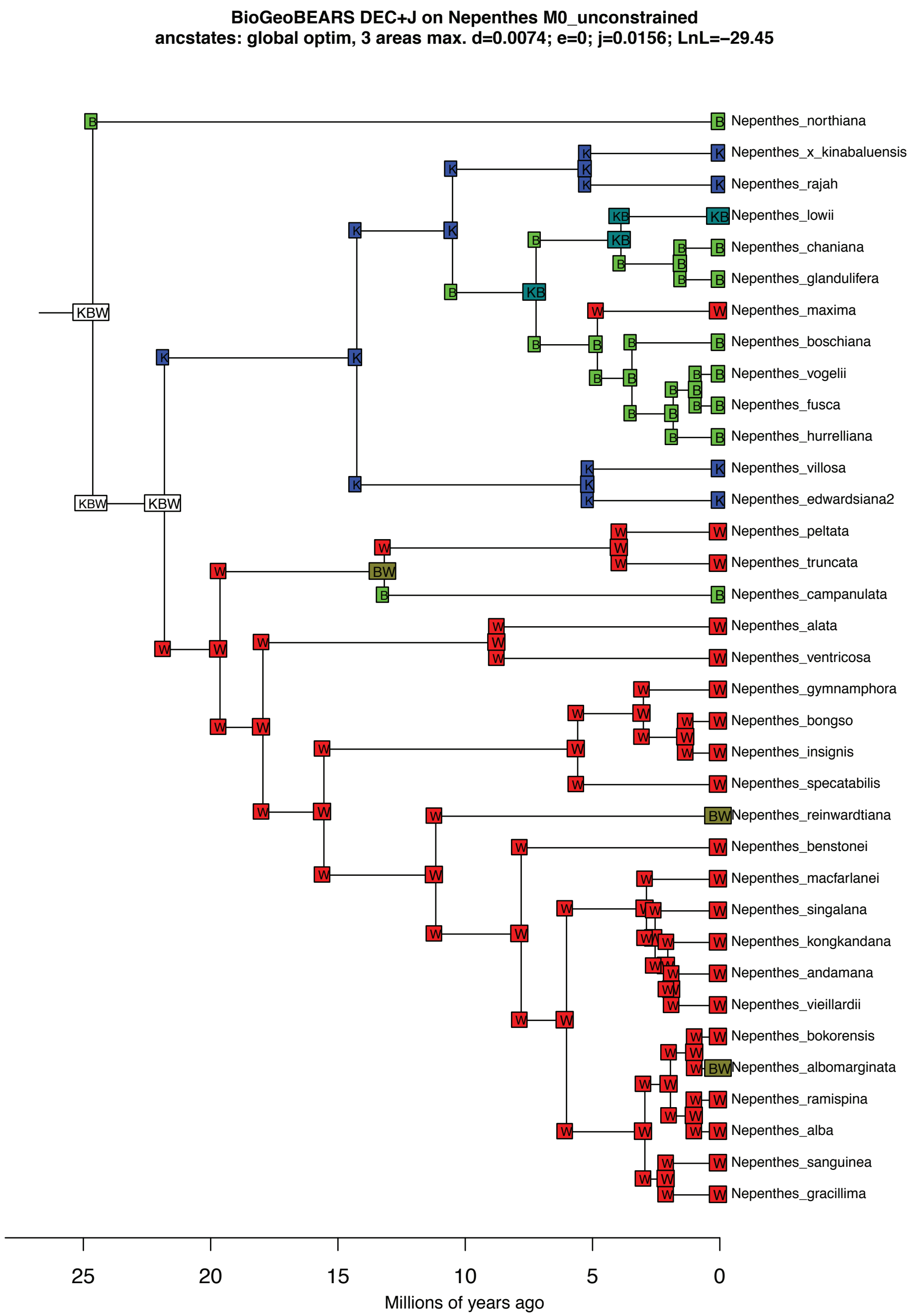

FIGURE 9. Ancestral range estimations for *Nepenthes*.

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

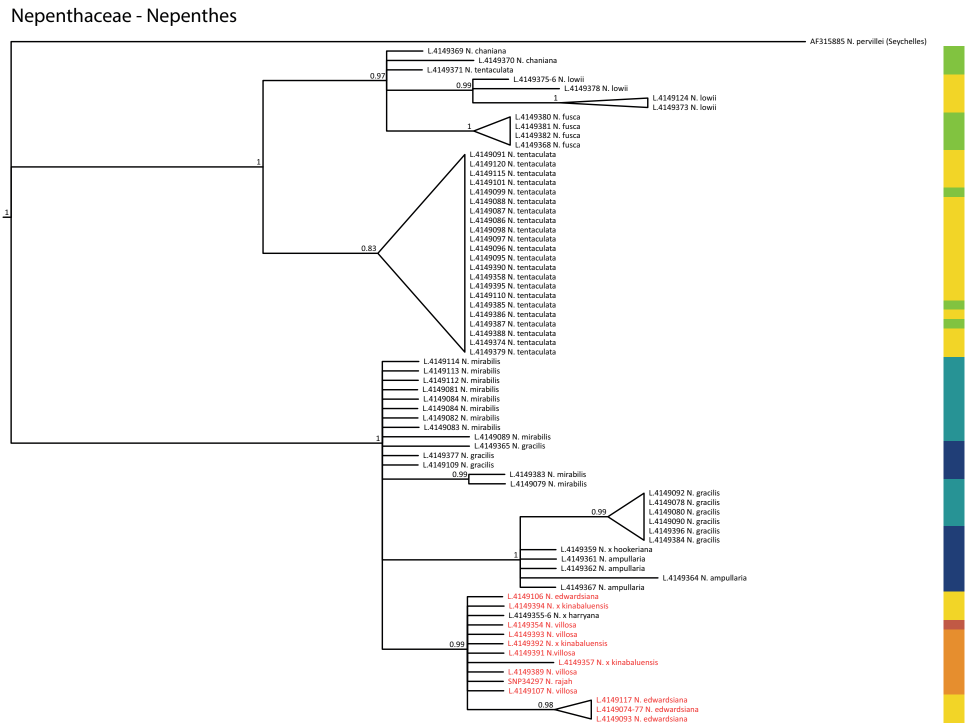

FIGURE 10. Phylogenetic reconstructions for Nepenthaceae.

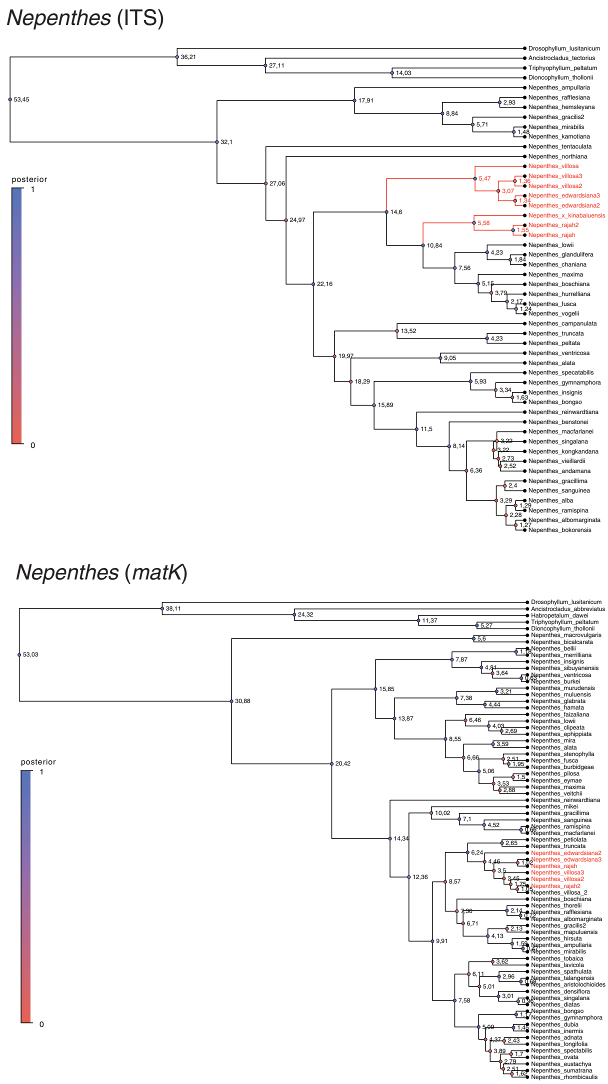

FIGURE 11. Chronograms for *Nepenthes*. Node colors represent posterior probabilities. Endemic species printed in red.

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 neighboryingMount 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).