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

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Abstract

- **Background and Aims** *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*. We further assess this diversity 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.
- **Methods** 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.
- **Key Results** 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. Precipitation and growth habit (stem length) are linked with vessel density and multiseriate ray height, while soil type correlates with vessel diameter, vessel element length and maximum ray width. For Caryophyllales as a whole, silica grains, successive cambia and bordered perforation plates are the

result of convergent evolution. Peculiar helical sculpturing patterns within various cell types occur uniquely within the insectivorous clade of non-core Caryophyllales.

- **Conclusions** 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*, while other typical *Nepenthes* characters evolved convergently in different Caryophyllales lineages.

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

INTRODUCTION

Nepenthes L. is a genus of carnivorous woody plants including around 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 and Jebb 2001; Meimberg and 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 and 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 and 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 the 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. 2011; 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 and Chalk 1950; Pant and 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 and Chalk 1950), pith, cortex and rhizome rays (Metcalfe and Chalk 1950; Carlquist 2010) and in the stem cortex (Metcalfe and 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; 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, 2008a, 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, one in the field 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) (Table 1).

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 1) 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 because sampling would have killed the plant.

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. In this paper, we focus on wood characters, but comment also on pith or cortex characters. Since the stem samples material had been dried, we were often unable to section the entire stem. In most species, the cortex part in our sections was limited to only a few cell layers at best, making it possible to screen for helical idioblasts but not for the presence of cortical bundles nor the occurrence of deep-seated periderm.

Nepenthes sequences of the nuclear ribosomal marker nrITS and the plastid marker *trn*K-*mat*K 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 with default parameters (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). *Nepenthes* trees have been deposited in TreeBASE (no. 19543; see http://www.treebase.org/) and the Caryophyllales trees of Soltis *et al.* (2011) can also be found in TreeBASE (no. 11267).

For the *Nepenthes* analysis, nrITS and *trn*K-*mat*K were analyzed independently rather than concatenated due to the extensive levels of hybridization between *Nepenthes* species (Clarke and Wong 1997; McPherson 2009). With two separate trees, we were able to include more wood species at the highest possible support than concatenated trees, which require heavy pruning. For the independent analyses of nrITS and *trn*K-*mat*K 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. For the resulting

TN93, equal base frequencies and gamma were selected for nrITS. For HKY, estimated base frequencies and gamma were selected for in *trn*K-*mat*K. 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).

Because BEAST co-estimates tree topology and branch length uncertainties together with the trait model, trees were first produced using all *Nepenthes* species with marker data to maximize topology results and then pruned of species lacking wood data in Mesquite v.2.75 (Maddison and Maddison 2011) to create a set of empirical trees to use for the wood anatomy trait optimization. The main reason for pruning taxa post-analysis rather than prior to the analysis is because the choice of outgroup could be influential on the ingroup topology, resolution and support levels. 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 optimised 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 of 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 1.

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. 1A) and *N. rajah*. Wood diffuse porous. Vessels almost exclusively solitary with simple perforation plates (Fig. 1C); 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. 1D), pits 5–7 μm in horizontal diameter. Gums occasionally present in *N. ampullaria, N. bokorensis, 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. pervillei, 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. thorelii* 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. 1E)*, 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. 2A); multiseriate rays usually 2–(3–4) seriate, occasionally up to 14-seriate in *N. bokorensis* 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. 2C–D) and additionally in the axial parenchyma of *N. rafflesiana*. Helical idioblasts scarcely 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 1). *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. 2E, F) are present in the pith in all species except *N. bokorensis, N. edwardsiana, N. lamii* and *N. maxima.* Medullary collateral bundles are present in the pith of *N. burbidgeae, N. macfarlanei, N. pilosa, N. reinwardtiana, N. sanguinea*, *N. stenophylla, N. tobaica* (Fig. 2G) and *N. veitchii*. Concentric amphivasal cortical bundles were present for *N. ventricosa* (Fig. 2H). Helical idioblasts were present in the cortex of all species for which we could section parts of the cortex (n = 14), and 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.*

For only one species, *N. ventricosa*, we were able to observe the deep-seated origin of the periderm, showing a pronounced cork cylinder (Fig. 2H); the outer part of the other samples that were available to us – except for the juvenile twig of *N. muluensis* (Fig. 1B) – was too destroyed due the drying process, making sectioning impossible. Therefore, we cannot state whether the deep-seated periderm formation is typical of the entire genus. Likewise, the presence of the outer lignified zone in the cortex in *N. muluensis* (Fig. 1B) cannot be generalized for *Nepenthes* as a whole.

Correlations with developmental stem stages, growth habit and abiotic preferences

Complete pairwise comparison data and results are presented in Tables S4 and S5 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. 3 and 4. Posterior support values generated by the BEAST analyses are indicated on Figs. 3 and 4 as icons when Bayesian posterior probabilities (bpp) ≥ 0.80 and ≥ 0.90. Although the major bifurcations of Figs. 3 and 4 are well supported, it should be noted that polytomies exist in *Nepenthes,* and the resulting low phylogenetic resolution might affect interpretation of the evolution of particular character states. There is no single wood character that defines one entire subclade. Silica grains (Figs. 3A and 4A), for example, are lost seven times throughout the *trn*K-*mat*K phylogeny. Markedly lignified pith (Figs. 3B and 4B) is present in a number of independent clades in both *trn*K-*mat*K and ITS. Likewise, presence of occasional septate fibres (Figs. 3C and 4C) is scattered throughout the phylogeny. Seven of the eight species with medullary bundles also have a marked lignification of the pith (Figs. 3C and 4C). Clear axial parenchyma bands (Fig. 3D and 4D) and medullary bundle presence in the pith (Fig. 3B and 4B) are derived features that evolved multiple times independently.

Character optimizations for a selection of woody genera in Caryophyllales are presented in Fig. 5. Posterior support values generated by the BEAST analyses are indicated on Fig. 5 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. 5B). Other typical *Nepenthes* features, such as the presence of silica grains, have evolved convergently within the order (Fig. 5A). Successive cambia (Fig. 5C) and non-bordered vessel perforation plates (Fig. 5D) have evolved in numerous Caryophyllales families independently as well.

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 1). 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).

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. 1A, B), simple, bordered perforation plates (Fig. 1C) and alternate intervessel pits of 5–7µm (Fig. 1D). 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. 1F) with a tendency to form narrow bands (1–4 cells) (Fig. 1E) 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. 2A) and consist of a combination of upright and square cells (Fig. 2B), 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 thin-walled or occasionally very thick-walled (Figs. 2E, 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 and Zimmerman 1885; Carlquist 1981, 2010). Furthermore, our extended study provides clear evidence for the presence of silica bodies in ray cells (Fig. 1C, D) and in the secondary phloem of most species analysed, 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. 3B and 4B), with marked lignification occurring in a larger portion of the pith in the few remaining species. Medullary bundles (Fig. 2G) were present in the pith of eight species, often associated with the species having more lignified pith (Figs. 3B and 4B). Furthermore, we found cortical vascular bundles in a ring-like arrangement surrounding the periderm producing a large phellem cylinder in *N. ventricosa* (Fig. 2H). 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. 3C and 4C).

Phylogenetic relevance of wood anatomy characters in Nepenthes *and Caryophyllales*

Silica bodies. In the rays of 25 of the 39 *Nepenthes* species studied (Table 1), silica bodies were found; nine of these contained silica in huge quantities (Fig. 2C, 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. 3 and 4), 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 to have high diagnostic value (Carlquist 1988). Nevertheless, within our Caryophyllales analysis, the silica-bearing genera are widely scattered within the non-core group (*Ancistrocladus* (Gottwald and Parameswaran 1968)*, Dioncophyllum* (Gottwald and Parameswaran 1968) and *Nepenthes)* and within the core group (*Limonium* (Carlquist and Boggs 1996) and *Rhabdodendrum* (Carlquist 2010)) (Fig. 5A). 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. 5B). Helical idioblasts, with either very thin lignified walls in a spiral arrangement or extremely thick lignified walls resembling fibre-sclereids (Figs. 2E, F), occur in the pith and cortex of nearly all *Nepenthes* species observed, and have occasionally been found in multiseriate rays as well, although their presence is extremely scarce in the rays and in only a few species. The function of these peculiar cells remains unknown, but has been associated with water storage (Kny and Zimmerman 1885; Heinricher 1906; Metcalfe and 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'. These cells 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 and Parameswaran 1968), but was later discounted based upon further investigation (Carlquist 2010). *Anacampseros*, closely related to Portulacaceae and Cactaceae, also have helical idioblasts in the rays (Carlquist 2010). In summary, different types of helical idioblasts characterize the insectivorous clade of non-core Caryophyllales, but it must be stressed that these idioblasts have different ontogenetic pathways, and thereby questioning their homology. They are either derived from the vascular cambium (rays and axial parenchyma) or from the primary ground tissue (pith/cortex).

Single vs. successive cambia. Our results show that single cambia are symplesiomorphic for Caryophyllales, from which acquisition of successive cambia was derived (Fig. 5C). 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. 5C).

Perforation plates. Like all Caryophyllales species, members of *Nepenthes* have simple perforation plates in their wood (Fig. 1C). 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. 5D). 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 Caryophyllaceae 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 and 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, 2013b; 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 involved 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 environments where traditional resources from the soil were limited, 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 and 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. 3 and 4). 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 the six greenhouse-grown specimens for which perlite was a component of the substrate (Table 1). Since perlite is largely made up of silicon dioxide, this would explain the availability of silica for uptake. Unfortunately, we could not trace whether the two other greenhouse grown specimens that lack silica in their ray cells, *N. kerrii* and *N. ventricosa*, had perlite added to the soil medium. 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). 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. thorelii* and *N. tobaica* (McPherson 2009). *Nepenthes bokorensis, N. smilesii* and *N. thorelii* 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 1). *Nepenthes tobaica* for example, grows 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. in press).

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. 2H). Also, *N. muluensis* (Fig. 1B) 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 and 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, b).

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. smilesii* and *N. thorelii,* 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 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) suggest that *Nepenthes* 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, to when it made 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 as 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) (Supplementary Table 4). 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 1). 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. 2008a). 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 20 m) and *N. veitchii* (107 µm, individuals reaching up to 10 m; 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 and 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. 2008a). 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. 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 species facing drought stress or growing in ultramaphic substrate 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 a rapid shift towards characters that have been associated with drought stress resistance within *Nepenthes* such as more pronounced lignification in the stems, or deep root systems will keep the pace needed in the progressively changing environmental future predicted by the Intergovernmental Panel on Climate Change (IPCC 2014). In the *Nepenthes* habitat of Southeast Asia, climate 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 give 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.

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Supplementary Data

Table S1 & S2 presents *Nepenthes* specimen and NCBI GenBank accessions. Table S3 includes references assembled for Caryophyllales genera character optimizations. Table S4 presents referenced growth habit and ecology data used for pairwise comparisons, and the subsequent calculations shown in Table S5.

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Figure legends

Figure 1. 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 (horizontal arrow) and lignified areas in both the outer stem area (cortex) and the inner stem part (wood and outer pith region), the vertical arrow points to the vascular bundle in the cortex, (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 2. 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 (horizontal arrow), deep-seated periderm with cork cylinder (vertical arrow).

Figure 3. Wood and pith anatomical characters optimized on the empirical trees of the full *Nepenthes trn*K-*mat*K 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 4. Wood and pith anatomical characters optimized on the empirical trees of the full *Nepenthes nr*ITS 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 5. 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* (which has

‘Ancistrocladan cells’ (Carlquist 2010)) 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.

Table 1. 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). 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 ^.

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	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
*N. ampullaria^*	25-60-105	14-27-40	250-360-470	+	300-500-700	±	+	±	1-8	+	-	1(2-4)	170-760-2100	310-1090-1900	12-14	0-2	Usp	+	-	+	+	-	/
*N. bicalcarata**	40-98-160	8/14/25	300-460-590	-	450-620-780	-	+	-	/	-	+	/	400-800-1800	/	6-10	0	US	-	-	+	+	/	/
N. bokorensis^*	40-68-100	14-24-44	200-325-500	+	410-590-820	-	-	+	1-2	+	-	1(2-4,10)	170-330-550	400-1140-3300	3-7	2-5	US	±	-	+	-	-	/
*N. burbidgeae1**	50-78-100	22-25-30	200-320-500	-	500-650-750	-	-	+	1	-	+	/	250-620-1000	/	12-14	0	U	±	+	+	+	/	+
*N. burbidgeae2**	50-76-120	13-19-24	250-380-525	-	625-770-900	-	-	+	1	-	-	1(2-3)	300-690-1500	700-1070-1700	11-14	0-1	Us	+	+	+	+	-	/
N. chaniana^*	35-47-70	16-24-36	250-340-540	+	415-545-670	-	+	+	2	+	-	1(2)	250-390-750	560-935-1210	7-16	0-2	US	±	-	-	+	-	+
*N. distillatoria**	20-46-75	28-36-40	200-285-375	-	350-525-710	-	+	-	/	-	-	1(2,10)	170-490-1250	750-1350-1950	8-12	0-1	US	±	-	±	+	-	/
*N. edwardsiana**	30-51-70	22-43-52	270-380-550	-	300-515-710	-	-	-	/	-	-	1(2)	260-580-1250	405-810-2250	8-13	0-3	Us	-	-	±	-	-	/
N. gracilis	40-94-170	9-16-23	250-332-550	-	400-549-750	+	-	+	1-4	+	-	1,2-5(6-10)	ND	ND	ND	ND	Usp	+	-	+	+	/	+
*N. gracillima**	25-37-60	20-31-40	240-315-405	-	250-430-600	-	+	±	1	-	-	1(2-3,10)	150-290-600	/	12-16	0	Us	±	-	±	+	/	/
N. gymnamphora	50-104-150	28-33-39	250-370-500	+	400-565-750	-	-	±	1-4	+	-	1 (2)	400-755-1400	600-1365-2100	9-13	1-2	Usp	-	-	±	±	+	/
*N. hemsleyana**	25-37-50	20-24-28	210-360-450	-	310-445-600	±	+	+	1-2	+	-	1(2)	210-690-1200	600-940-1250	10-13	0-2	Us	±	-	±	+	-	/
*N. hirsuta**	15-38-50	32-45-60	280-390-500	-	300-850-625	-	+	±	1	-	+	/	150-370-700	/	9-12	0	uS	+	-	+	+	/	+
N. kerrii^*	30-53-75	16-31-44	200-295-400	-	300-465-800	-	-	-	/	±	+	/	245-710-1210	/	8-14	0	US	-	-	+	+	/	+
N. khasiana	20-61-90	45-52-64	200-260-340	+	350-515-700	±	-	±	1	-	-	1(2-3,14)	170-485-710	230-765-1800	11-14	3-5	Us	-	-	±	+	+	/
*N. lamii**	35-60-105	16-37-48	200-420-710	-	300-505-740	+	-	-	/	±	-	1(5-8)	200-580-1350	450-640-900	7-15	0-1	Us	±	-	±	-	-	/
N. lowii	45-80-115	21-22-30	250-450-730	-	270-465-600	±	-	+	1-7	+	+	1(2-5)	120-470-950	250-660-1800	4-10	0-3	US	-	-	+	+	+	+
*N. macfarlanei**	35-57-90	24-34-48	260-490-720	-	300-605-950	-	+	-	/	-	-	1(2-3)	400-805-1950	100-1500-2200	14-20	0-1	Us	-	+	++	+	-	/
N. madagascariensis	25-54-120	16-29-40	200-265-360	+	300-450-710	-	-	±	4-6	+	-	1(6-12)	180-520-1600	300-1365-3900	3-10	1-6	Usp	±	-	-	+	-	/
N. maxima^*	25-43-95	18-33-46	205-340-570	-	360-565-750	-	-	±	3-4	-	-	1(2-3)	175-470-1150	550-1270-3400	7-12	2-6	uS	±	-	±	-	-	/
N. mirabilis	30-78-150	20-23-34	210-370-710	-	350-520-700	±	-	±	1-4	+	-	1(2)	300-1090-2200	450-1390-3200	9-13	0-2	Usp	±	-	±	+	-	+
*N. muluensis**	25-54-100	20-28-36	250-390-500	-	360-460-610	-	+	-	/	-	+	/	150-400-900	/	6-14	0	Us	-	-	++	+	/	+

*N. pilosa**	50-94-140	22-26-32	250-400-600	-	600-680-850	-	-	-	2-3	-	+	/	350-770-1100	/	10-15	0	U	+	+	+	+	/	+
*N. rafflesiana^*	25-78-125	18-22-29	275-390-540	+	400-555-740	-	+	+	1-10	+	-	1(2-4)	200-500-1100	1000-1475-2300	7-10	2-3	Usp	+	-	±	+	+	/
N. rajah	50-65-90	8/13/20	150-256-400	+	450-580-700	±	-	+	2-6	-	-	1(2)	150-183-350	150-192-250	10-13	0-1	Usp	±	-	±	+	+	+
*N. reinwardtiana**	40-52-70	28-36-44	210-350-540	-	320-525-700	-	+	-	/	-	-	1(2-4)	150-510-790	400-955-2100	9-13	1-4	uS	+	+	+	+	-	/
*N. rhombicaulis^*	30-45-75	32-38-50	260-375-490	-	400-515-850	±	-	+	1-5	+	-	1(5-7)	150-535-1200	700-970-1400	7-12	0-4	Us	±	-	±	+	-	/
N. sanguinea	15-34-50	20-32-48	240-340-480	+	375-520-710	-	+	±	1-2	-	-	1(2-6)	200-390-655	250-870-1600	5-10	0-5	US	-	+	-	+	-	/
N. smilesii	20-38-50	40-55-64	155-215-325	-	275-420-530	-	±	+	1-4	-	-	1(2)	170-366-575	190-555-1650	5-12	0-3	Us	±	-	+	±	-	/
*N. stenophylla**	60-84-100	14-23-32	200-340-450	-	650-740-850	±	-	-	2-3	-	+	/	350-812-1700	/	10-15	0	U	+	+	++	+	/	+
*N. tentaculata**	25-48-100	16-30-36	270-355-500	-	300-460-700	-	+	-	/	-	+	/	200-460-850	/	8-12	0	Us	-	-	-	+	/	+
N. thorelii	20-37-55	40-48-56	200-305-460	-	300-425-550	-	±	±	1-2	-	+	/	160-260-410	/	11-16	0	uS	-	-	±	+	/	/
N. tobaica	25-54-75	32-41-54	245-360-500	-	450-600-750	-	+	-	/	-	+	/	100-1030-2600	/	14-18	0	Usp	±	+	++	+	/	/
N. tomoriana	25-40-65	36-46-64	260-355-500	-	380-585-850	-	+	±	1-2	±	-	1(2-14)	120-515-1200	350-1190-2500	11-14	0-2	Us	-	-	±	+	-	/
*N. veitchii**	75-107-130	28-35-48	200-304-450	-	500-640-700	ND	-	+	1-2	-	+	/	ND	ND	10-15	0	U	±	+	+	±	/	/
*N. ventricosa^*	40-65-105	15-20-30	200-315-500	-	355-490-605	-	-	+	1-2	+	+	/	100-295-625	/	3-7	0	US	-	-	±	+	/	+
N. villosa	30-53-75	36-49-61	290-380-490	+	400-625-900	-	-	+	1-2	+	-	1, 2-5	190-425-760	270-950-3400	4-7	3-6	Usp	-	-	±	+	-	+
N. sp. (Thai origin)	30-57-90	32-46-58	250-330-460	-	275-435-550	±	+	+	1-2	+	-	1(2-3)	250-383-750	520-725-900	6-11	0-1	Us	+	-	+	/	-	±