A new way to identify living species of *Nepenthes* (Nepenthaceae): More data needed!

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Summary

We present a possibly simple and effective way to identify living specimens of the various species of *Nepenthes*. The volume and height of the pitchers are closely correlated variables of which the mathematical relationship between them can be expressed in a simple formula. These formulas, preferably of the upper pitchers when available, are slightly different for most species investigated, especially for the largest pitchers on specimens. This method is easily applicable in Botanical Gardens, which generally have a small, highly similar set of species. However, the results are based on few data (few specimens per species, only from two botanical gardens). Thus, please help, by providing more data, especially of hybrids, to further test the method.

Like to Help?

a. Note of a specimen its identification (species name), name and number of collector, origin (of course when known). Please, indicate how reliable you consider the identification to be.
b. empty all **upper** pitchers per specimen (avoid lower pitchers, unless upper pitchers are lacking). Note how many pitchers you measure and, if only lower pitchers are available, indicate this.
c. fill the emptied pitchers with water (keep mouth horizontal, Fig. 1c) and poor the content into a measuring cylinder and note for each pitcher the volume in preferably cubic centimeters.
d. measure the height of the upper pitchers (Fig. 1a & b) and note the heights per pitcher in centimeters.
e. send the data to the Dr. Paul Kessler, Hortus botanicus in Leiden (address under authors), do not forget your own name and address.
f. Many thanks!

Introduction

One of the attractive plant genera in Asia is *Nepenthes* L., a genus of *c.* 87 species (Jebb & Cheek 1997). The genus is famous for its leaf tips, which are transformed into pitchers. The pitchers apply "slippery wax crystals on the inner pitcher wall and 'insect aquaplaning' on the wet upper rim (peristome)" (citation from Bauer *et al.* 2011) to trap and digest mainly arthropods. The arthropods are lured to the pitchers by extrafloral nectaries (Merbach *et al.* 1999). The additional nitrogen and

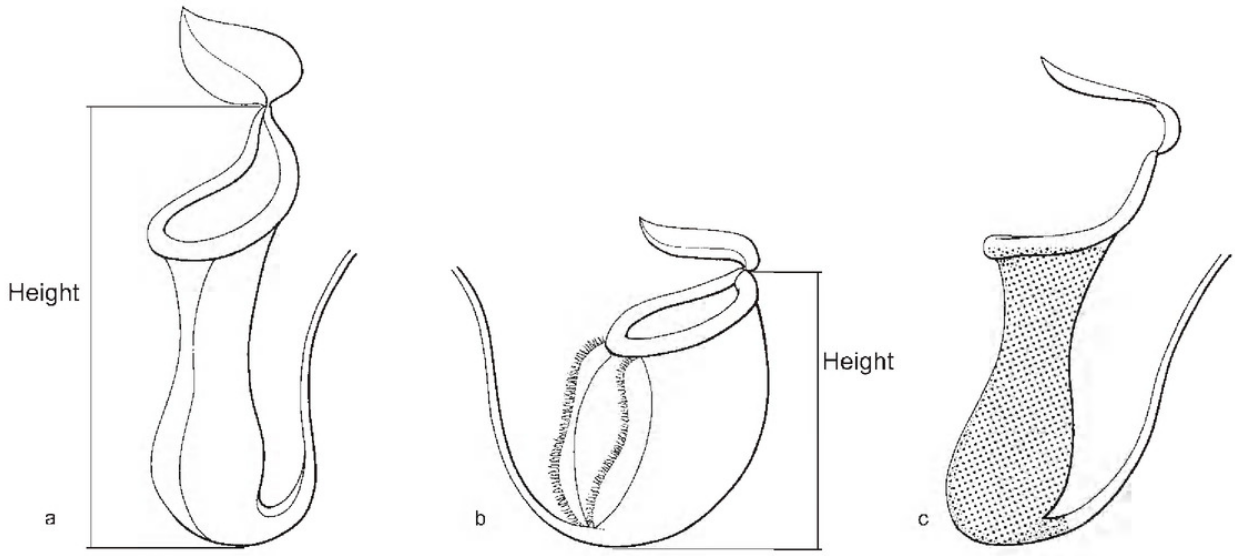

Figure 1: Showing how to measure height of the pitcher (a & b) and the content (c). a. upper pitcher (petiole attachment and lid at same side of pitcher); b. lower pitcher (petiole attachment and lid at opposite sides of pitcher).

phosphate thus obtained allows the pitcher plants to grow in nutrient poor surroundings. However, not all pitcher plants trap arthropods for extra nutrients, *N. ampullaria* Jack (Fig. 2a) is reported to grow in dense woods and to trap leaf litter (Moran *et al.* 2003), while *N. rajah* Hook.f., the species with the largest pitchers, is occasionally reported to catch small mammals (Phillipps & Lamb 1996: 131), but this is probably accidental as the species is reported to live mutualistically with small mammals that defecate in the pitchers (Greenwood *et al.* 2011; Wells *et al.* 2011).

Until now, species were always identified morphologically (Clark 1997; Jebb & Cheek 1997; Cheek & Jebb 2001). This is often difficult because differences might be small or characters may be difficult to find. A second way of identification, presently being developed, is DNA-barcoding whereby short sequences of two or three regions in the chloroplast DNA should be typical for a species. This is still a rather expensive and time consuming method, which still has to be performed in a laboratory.

Three-dimensional shapes that are reiterated on an object are usually constant in proportions like the pitchers, young and older, on the same individual. The iterations may show variation in sizes, but the overall shape remains constant. Consequently, the mathematical relations between the various components that make up for the three-dimensional shape can be expressed in relatively simple formulas.

Here we like to investigate whether the pitchers of *Nepenthes* possess two or more variables that show a constant, mathematical relationship per species, but differ slightly to strongly among species. If these are found, then pitcher variables can be used to identify the species even outside a laboratory.

Material and Methods

The volume of the (preferably) upper pitchers and several simple measurements like height and width of the pitcher, *etc*. were measured. It quickly became evident that especially the height of the pitchers in combination with the volume provided sufficiently different results between the species to ignore the other variables.

The volume of the pitchers was measured by first emptying and cleaning the pitcher from all fluids and debris, then filling it with water till the rim of the mouth, which generally meant that the

Figure 2a: *Nepenthes ampullaria* Jack showing detritus in pitchers (photo: Rogier van Vugt, Malaysia, Gunung Jerai); b: *N. albomarginata* T.Lobb ex Lindl., green form (photo: Rogier van Vugt, Hortus botanicus University of Leiden, originally from Malaysia, Sarawak, Bako National Park); c: *N. albomarginata* T.Lobb ex Lindl., purple form from Malaysia (photo: Rogier van Vugt, Malaysia, Bukit Bendera).

mouth of the pitcher needed to be held horizontally (normally the mouth is somewhat diagonal; Fig. 1a & b). The content was then collected in a cylinder with a scale. Depending on the size of the pitchers, cylinders of 10, 50, 100, and 250 ml were used. The height of the pitchers was measured with a ruler from the base of the cup (where the tendril ends and the actual pitcher begins) to where the lid is attached to the pitcher (highest point of the diagonal mouth; Fig. 1c). The ruler must be held parallel to the pitcher in such a way that the ruler is in line with the two measuring points.

The species and specimens studied (Table 1) are all measured from living collections in the Hortus botanicus Leiden, Leiden University, Leiden, The Netherlands and the Royal Botanic Gardens Melbourne, Melbourne, Australia. Care was taken to measure all pitchers per plant, and also, when possible, more than one specimen per species, whereby the geographical origin of the sample was noted.

The mathematical relationship between volume and height was analyzed per species with Microsoft Office Excel 2007, whereby various types of functions were examined and the highest R^2-values were used to select the best fitting function.

Results

The measurements, volumes and heights, are shown in Fig. 3a. The best fitting functions are plotted through the data. The formulas of the functions and their R^2-values are shown in Table 2. For most species it appeared that simple exponential functions described the mathematical relation between volume and height best, the two exceptions are *N. albomarginata* T.Lobb ex Lindl. (Borneo) with a linear function and *N. mirabilis* (Lour.) Druce var. *mirabilis* with a polynomial function.

Discussion

The measurements per species were obtained from a number of genetically different individuals and from a number of cuttings of these individuals (Table 1); the only two exceptions were *N. mirabilis* var. *globosa* M.Catal. and *N. alata* Blanco of which only one plant was available in the Hortus botanicus Leiden and the Royal Botanic Gardens Melbourne, respectively. Measuring various speci-

Table 1. The measurements of species and specimens.

Species [origin] (no. genetically unique plants)	Specimen number*	No. pitchers measured	Pitcher type
N. alata Blanco [unknown] (1)	020224 (RBGM)	16	upper
N. albomarginata T.Lobb ex Lindl. [Borneo, Sarawak] (1)	20050998 (HB)	6	upper
N. albomarginata T.Lobb ex Lindl. [Malay Peninsula] (6)	960378 (cutting) (HB)	6	upper
	960378 (cutting) (HB)	4	upper
	950470 (HB)	2	upper
	960382 (HB)	3	upper
	950467 (HB)	1	upper
	950466 (HB)	1	upper
	960375 (HB)	4	upper
N. ampullaria Jack [cultivated] (2)	20051574 (cutting) (HB)	6	lower
	20051574 (cutting) (HB)	7	lower
	20040012 (HB)	3	lower
N. maxima Reinw. ex Nees [cultivated] (1)	HBL31076 (cutting) (HB)	9	upper
	HBL31076 (cutting) (HB)	3	upper
N. merrilliana Macfarl. [cultivated] (2)	930070 (cutting) (HB)	6	upper
	930070 (cutting) (HB)	6	upper
	930070 (cutting) (HB)	5	upper
	20060048 (HB)	1	upper
N. mirabilis (Lour.) Druce var. *globosa* M.Catal. [cultivated] (1)	20090052 (HB)	7	lower
N. mirabilis (Lour.) Druce var. *mirabilis* [various] (3)	930053 (HB) [Sulawesi]	6	upper
	960329 (cutting) (HB) [Malay Peninsula]	9	upper
	960329 (cutting) (HB) [Malay Peninsula]	3	upper
	960329 (cutting) (HB) [Malay Peninsula]	3	upper
	20050997 (HB) [Australia]	1	upper
N. smilesii Hemsl. [cultivated] (1)	792749-19755 (cutting) (HB)	6	upper
	792749-19755 (cutting) (HB)	3	upper
	792749-19755 (cutting) (HB)	5	upper
N. ventricosa Blanco [cultivated] (3)	910137 (HB)	15	upper
	930733 (HB)	3	upper
	020245 (RBGM)	6	upper

* HB = Hortus botanicus Leiden; RBGM = Royal Botanical Gardens Melbourne.

Table 2. The formulas of the best fitting functions and their R^2-values.			
Species (number of genetically unique plants)	Formula of best fitting functions	R^2-values	Type of function
N. alata Blanco (1)	$1.2359e^{0.2882x}$	0.9578	exponential
N. albomarginata T.Lobb ex Lindl. (Borneo) (1)	$2.3005x - 13.089$	0.9392	linear
N. albomarginata T.Lobb ex Lindl. (Malay Peninsula) (6)	$1.2602e^{0.306x}$	0.9583	exponential
N. ampullaria Jack (2)	$2.2767e^{0.3982x}$	0.7242	exponential
N. maxima Reinw. ex Nees (1)	$8.4462e^{0.1046x}$	0.9379	exponential
N. merrilliana Macfarl. (2)	$5.3004e^{0.1773x}$	0.9019	exponential
N. mirabilis (Lour.) Druce var. *globosa* M. Catal (1)	$0.5727e^{0.5277x}$	0.958	exponential
N. mirabilis (Lour.) Druce var. *mirabilis* (3)	$0.1082x^2 + 1.7353x - 8.1235$	0.8899	polynomial
N. smilesii Hemsl. (1)	$0.5987e^{0.3953x}$	0.9378	exponential
N. ventricosa Blanco (3)	$1.1037e^{0.3941x}$	0.922	exponential

mens was done to check if the functions were indeed species-specific or individual-specific. The sample is still small and needs further elaboration with more species and more individuals per species.

A second problem is that only specimens from two botanical gardens (mainly Leiden) were used. Usually, the conditions per garden are controlled in the same way for all specimens present, which may decrease the variability among genetically different specimens. Thus, additional measurements from other gardens or private collections are in high demand.

The extra data may also help to solve a third problem. The genetically different individuals often still came from the same area of origin (*e.g.*, 6 specimens of *N. albomarginata* T.Lobb ex Lindl. from the Malay Peninsula) and, therefore, the full variability per species has not yet been explored completely.

Botanical gardens prefer to have true species in their collections and no hybrids. However, the trade in pitcher plants is generally a trade in hybrids. Adding measurements on hybrids will be a great addition and allows us to see whether the measurements will be intermediate or not with those of the parent species.

When we consider the results so far, then we found little or no variation between the individuals of a species or for the different pitchers on the same plant specimen, thus volume and height of pitchers are specific for the species. Small and large pitchers within the same species show the same shape ratios. This is demonstrated by the high R^2 values (Table 2), which are generally above 0.9 (with *N. ampullaria* – Fig. 2a – and *N. mirabilis* var. *mirabilis* as exceptions). Mind you, our R^2 values are probably inflated as we had to treat all measurements, often of the same plant, as independent in order to find a mathematical function. The artificially higher R^2 values are not a problem as they were all only used to select the best fitting functions.

Nepenthes plants show two kinds of pitchers, lower (ground) and upper (aerial) pitchers (Moran 1996). Lower pitchers are attached to the leaf with the site opposite to the lid (Fig. 1b, 2a, somewhat difficult to see) and are generally present in the lower part of the plant. They are usually larger, especially basally, than the upper pitchers. Lower pitchers can even become very large when resting

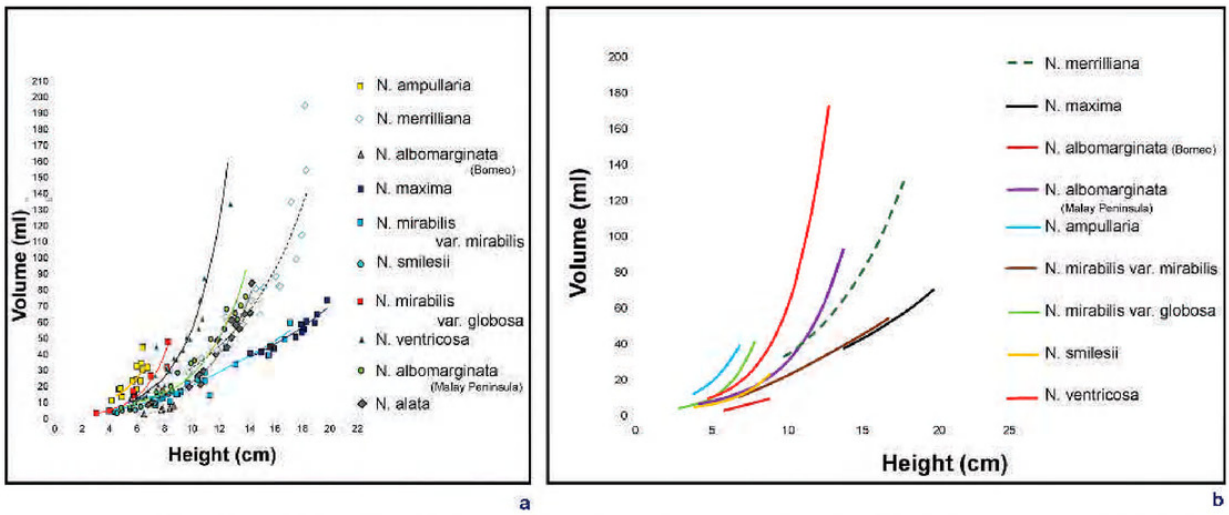

Figure 3a: Species-specific relationships between volume and height for the pitchers of some species of *Nepenthes*. These relationships are the species-specific functions that can be used for identification of the species; b: The representations of the mathematical functions that best fit the relationship between volume and height and which can be used for the identification of the species.

on the soil. Upper pitchers are more slender and attached to the leaf with the side that carries the lid (Fig. 1a, 2b, c); these are always in the upper part of the plant. A species like *N. ampullaria* generally only has basal pitchers, very exceptionally also upper ones (Clarke & Moran 1994; Fig. 2a). The differences between the types of pitcher probably explain the difference between the two varieties of *N. mirabilis*. Of var. *globosa* only one plant was present in the Hortus botanicus Leiden with only lower pitchers, while the other variety showed plants with upper pitchers. On the other hand, the difference may also be due to evolutionary differentiation. The method we developed was mainly applied to upper pitchers (Table 1), with *N. ampullaria* and *N. mirabilis* var. *globosa* as exceptions. Of the latter two, only normal lower pitchers were used and not the exceptionally large ones. Though we like to concentrate on the upper pitchers, as these are usually generally available, a comparison with the lower pitchers will prove interesting. These data are welcome too, but take care to note which measurements were taken from lower pitchers.

Only the data of *N. albomarginata* T.Lobb ex Lindl. showed different results for Borneo (Fig. 1b) and the Malay Peninsula (Fig. 1c). The various plants from these regions also looked morphologically quite different, and, therefore, the localities were separated in the analysis. This means that the identification method may be robust enough to separate geographical variants of widespread species.

By only plotting the best fitting functions of each species (Fig. 3b), the graphs become much clearer and more useful as a determination chart. Several of the functions cross each other. This is mainly the case for the smaller sized pitchers. Thus, it is best practice to always measure the largest pitchers on a specimen, then identification is easiest, *e.g.* check the graphs of *N. albomarginata* from the Malay Peninsula, *N. maxima* Reinw. ex Nees, *N. merrilliana* Macfarl., *N. mirabilis* var. *globosa*, and *N. ventricosa* Blanco. Where species-specific functions more or less completely cross, additional determination keys are needed to identify the species correctly. This means that the original determination keys are still partly needed. This is especially the case with the species with smaller pitchers (Fig. 3a).

Two taxa show a different optimal function than exponential, linear for *N. albomarginata* from Borneo and polynomial for *N. mirabilis* var. *mirabilis*. This may seem very anomalous, but the R^2 values for an exponential function were only slightly lower. It is still uncertain whether the pitcher

length/contents ratio of these species really follows other mathematical functions or that it is due to a limited sample size.

The graphs in Fig. 3b can be used for identification. Measure the height and volume of the largest pitchers of your plants and plot them in the diagram. The nearest line should indicate the correct species.

Conclusion

The method presented here has great potential to help identifying living species of *Nepenthes*. Still, many more species need to be measured and added to the determination graph, whereby geographical variation has to be checked. Also, the influence of hothouse conditions has to be compared with the measurements of wild specimens.

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