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# Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant

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**Nepenthes pitcher plants are typically carnivorous, producing pitchers with varying combinations of epicuticular wax crystals, viscoelastic fluids and slippery peristomes to trap arthropod prey, especially ants. However, ant densities are low in tropical montane habitats, thereby limiting the potential benefits of the carnivorous syndrome. *Nepenthes lowii*, a montane species from Borneo, produces two types of pitchers that differ greatly in form and function. Pitchers produced by immature plants conform to the 'typical' *Nepenthes* pattern, catching arthropod prey. However, pitchers produced by mature *N. lowii* plants lack the features associated with carnivory and are instead visited by tree shrews, which defaecate into them after feeding on exudates that accumulate on the pitcher lid. We tested the hypothesis that tree shrew faeces represent a significant nitrogen (N) source for *N. lowii*, finding that it accounts for between 57 and 100 per cent of foliar N in mature *N. lowii* plants. Thus, *N. lowii* employs a diversified N sequestration strategy, gaining access to a N source that is not available to sympatric congeners. The interaction between *N. lowii* and tree shrews appears to be a mutualism based on the exchange of food sources that are scarce in their montane habitat.**

**Keywords:** *Nepenthes*; carnivory; mutualism; tree shrew; supplementary nitrogen sources

## 1. INTRODUCTION

*Nepenthes lowii* is unusual among carnivorous plants in that it produces pitchers lacking the features normally associated with arthropod prey capture, the normal mode of nutrition in pitcher plants (Juniper *et al.* 1989). The pitchers of most *Nepenthes* species studied

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to date employ combinations of epicuticular wax crystals, viscoelastic fluids and slippery peristomes to capture and retain prey, most notably Formicidae (Moran 1996; Gaume *et al.* 2002; Bohn & Federle 2004; Gorb *et al.* 2004; Gaume & Forterre 2007; Bauer *et al.* 2008; Di Giusto *et al.* 2008). Owing to its arrangement of overlapping epidermal cells arranged in radial ridges, the peristome (a collar-like structure that lines the pitcher rim) provides an anisotropic, highly wettable surface that in species such as *Nepenthes rafflesiana* and *Nepenthes bicalcarata* is slippery for arthropods when wet (Bohn & Federle 2004; Bauer *et al.* 2008) and is fundamental to those species' prey-trapping mechanisms.

*Nepenthes* produce dimorphic pitchers: 'terrestrial' pitchers rest on the ground and are usually ovoid in shape, whereas 'aerial' pitchers are funnel-shaped and produced above ground level, supported by attachment of the pitcher tendril to adjacent vegetation (Clarke 1997). Terrestrial *N. lowii* pitchers are produced only on immature plants, are 'typical' in structure and apparently function as arthropod traps (figure 1a), but the aerial pitchers are heavily lignified and the lid is held away from the mouth, which is very broad (figures 1d and 2). The inner surfaces are rough, lack epicuticular wax crystals and are not steeply inclined, while the rim is narrow and the peristome is reduced to a series of small bumps. The lower surface of the lid in both pitcher types is covered with coarse bristles and specialized nectar glands that secrete a buttery, white exudate. Clarke (1997) reported that aerial pitchers of *N. lowii* from Mount Pagon in Brunei contained large amounts of vertebrate faeces, but no invertebrate prey. This combination of aerial pitcher characteristics and contents indicated a possible interaction with vertebrates and a reduction in the features normally associated with arthropod capture. We conducted field observations and experiments on *N. lowii* and the animals that visit its pitchers to determine the primary source of foliar nitrogen (N), testing the hypothesis that *N. lowii* derives a measurable amount of the nutrient from vertebrate faeces.

## 2. MATERIAL AND METHODS

The study was conducted in montane cloud forest on Gunung Mulu, Sarawak, Malaysia, in June 2008 (4.045835 N, 114.924967 E, 1800–2300 metres above sea level). Observations were made to determine the appropriate experimental methods and revealed that terrestrial *N. lowii* pitchers trap arthropod prey, including Hymenoptera (Formicidae), Diptera, Orthoptera, Coleoptera and Arachnida. In contrast, aerial pitchers do not trap measurable quantities of arthropod prey (although occasional catches are likely), but are visited by mountain tree shrews (*Tupaia montana*), which feed on the exudate on the pitcher lid and defaecate into the pitchers (see the electronic supplementary material, methods). We then performed observations to document the nature of the interaction between *N. lowii* and *T. montana*, focusing on the value of *T. montana* faeces to *N. lowii*. Descriptive observations that are relevant to the design and underlying assumptions in our experiments are provided in the electronic supplementary material.

Video cameras (model DCR-HC 46E, Sony Corporation, Tokyo, Japan) in waterproof casings were set up on tripods, each focusing on one aerial pitcher of *N. lowii*. Recordings were made on three aerial pitchers from different plants. A total of 35 h of recordings were made, at an approximate recording rate of 5 h d<sup>-1</sup>, between noon and sunset over a period of 7 days.

Stable isotope analyses were performed to determine the origin of foliar N in *N. lowii*. For full details of the procedures and experimental design, see the electronic supplementary material, methods. The following samples were collected, all from separate plants: laminar (leaf blade) material from the leaf above the youngest functional

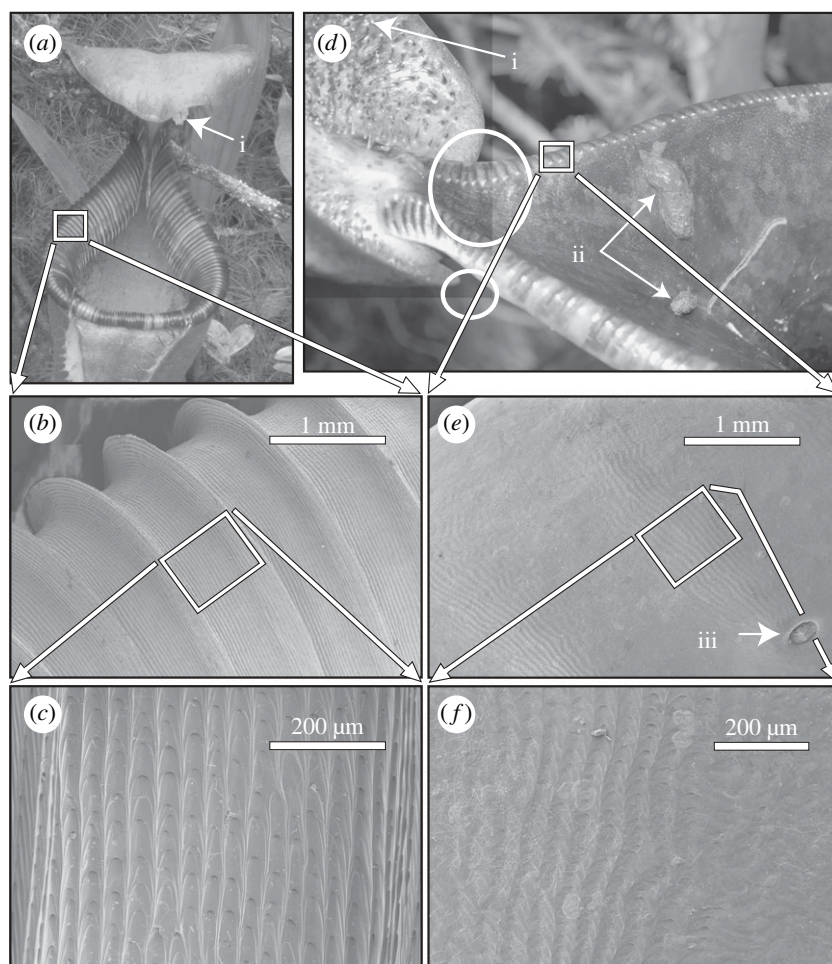


Figure 1. (a) Close-up of a terrestrial pitcher of *Nepenthes lowii*, showing the ridged peristome and waxy bloom on the inner rear surface (epicuticular wax crystals) typical of insect-trapping *Nepenthes* species. (b and c) Microstructure of terrestrial pitcher peristome, showing large- (b) and small-scale (c) radial ridges, the latter formed by overlapping epidermal cells. (d) Aerial *N. lowii* pitcher. (e and f) Microstructure of aerial pitcher peristome. Note flattening of the large-scale ridges (e) and partial or complete reduction in small-scale ridges (f), resulting in loss of wettability and trapping capacity. Scale bars, (b and e), 1 mm; (c and f), 200  $\mu\text{m}$ . i, accumulated exudates; ii, freshly deposited *Tupaia montana* faeces; iii, nectar gland.

*N. lowii* aerial pitcher of mature plants ( $n = 20$ ); lamina from the leaf above the youngest functional aerial pitcher of *Nepenthes muluensis* plants (a sympatric 'typical' non-faeces-using species,  $n = 20$ ) and fresh *T. montana* faeces from aerial *N. lowii* pitchers ( $n = 10$ ).  $\delta^{15}\text{N}$  values were determined using a PDZ Europa ANCA-GSL elemental analyser and PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK). Using the ISOERROR1\_03 two end-member mixing model as a basis (Shearer & Kohl 1989; Phillips & Gregg 2001), we estimated the 95 per cent confidence interval (CI) for N input from tree shrew faeces to mature *N. lowii* plants based on the following statistic:

$$\%N_{\text{faeces}} = \frac{\delta^{15}\text{N}_{\text{lowii aerial}} - \delta^{15}\text{N}_{\text{muluensis}}}{\delta^{15}\text{N}_{\text{faeces}} - \delta^{15}\text{N}_{\text{muluensis}}} \times 100,$$

where  $\delta^{15}\text{N}_{\text{lowii aerial}}$ ,  $\delta^{15}\text{N}_{\text{muluensis}}$  and  $\delta^{15}\text{N}_{\text{faeces}}$  represent the mean values for lamina material taken from above the newest fully functional aerial pitcher of *N. lowii*, lamina material taken from above the newest fully functional aerial pitcher of *N. muluensis* and *T. montana* faeces, respectively. See methods in the electronic supplementary material for an explanation of the model. All data were tested for homoscedasticity and normality; datasets not satisfying these assumptions were transformed and then re-tested prior to running parametric tests.

For s.e.m., pitchers were excised from pitchers in the field and transferred to glutaraldehyde (4% in 0.1 M sodium phosphate buffer) for fixation. They were then stained with osmium tetroxide, dehydrated via an ethanol dilution series (25, 50, 75 and 96%, in 1 h steps) and critical point dried. The samples were sputter coated with gold and examined in a FEI XL30-FEG scanning electron microscope (FEI Co., Hillsboro, OR, USA) at 10 kV.

### 3. RESULTS

In a typical interaction, *T. montana* jumps onto an aerial pitcher to feed on the exudate produced by the glands on the underside of the lid (figure 2, electronic supplementary material, video). The animal then defaecates into the pitcher (figure 1d) and scent-marks the lid using its anogenital glands. Individual *T. montana* exhibit highly regulated foraging behaviour, moving from one pitcher to another to feed. The scent marking of pitchers identifies them as a valuable resource (Kawamichi & Kawamichi 1979). *Tupaia montana* routinely visits the same 'circuit' of pitchers, spending much of its foraging time on pitcher visits (from analysis of video recordings, mean interval ( $\pm$ s.e.) between pitcher visits =  $96 \pm 7$  min,  $n = 2100$  min ( $5 \text{ h d}^{-1}$ , 7 days)). Although *T. montana* is frugivorous, it is unable to process fibrous parts of fruits and can only extract and swallow the sugar-rich juices (Emmons 1991). We observed nectar sources to be scarce above 1800 m on Gunung Mulu, but this apparent deficit is offset by *N. lowii*, the lid nectaries of which produce the most copious quantities of exudate observed thus far among the Nepenthaceae (Clarke 1997).





Figure 2. *Tupaia montana* feeding at an aerial pitcher of *Nepenthes lowii*.

The structure of *N. lowii* aerial pitchers facilitates both the feeding of *T. montana* on the lid exudates and defaecation into the pitchers. The thickened tendrils and robust pitchers can support an animal the size of *T. montana* (ca 150 g; Emmons 1991) without breaking. The highly reduced peristome enables *T. montana* to grip the pitcher rim without slipping, while the shape of the mouth and orientation of the lid impede feeding from the side or rear, thereby manoeuvring the animal to sit astride the pitcher orifice, maximizing the likelihood that the animal's hindquarters are positioned over the orifice while it feeds (figure 2).

Using a two end-member isotopic mixing model for  $^{15}\text{N}$ , we estimate that between 57 and 100 per cent of foliar N in *N. lowii* plants possessing aerial pitchers is derived from faeces (95% CI;  $n = 20$ ). The remainder is derived from other sources, including the soil (via the roots), prey capture and leaf litter, which accumulates in pitchers, particularly when they are old and no longer visited by *T. montana* (detritus that falls into pitchers that are visited by *T. montana* is usually knocked out of the pitchers when the animals climb on and off the rim). Foliar N concentrations were significantly higher for *N. lowii* plants producing aerial pitchers than for *N. muluensis*, which had no access to faecal material (mean  $\pm$  1 s.e.  $1.58 \pm 0.10$  and  $1.21 \pm 0.07\%$ , respectively;  $t = 2.90$ ,  $p < 0.01$ , d.f. = 38, *t*-test).

*Nepenthes lowii* aerial pitchers appear to have lost their slipperiness (electronic supplementary material, table). This change is because of the extensive reduction in the microstructure, resulting in flattening of both the larger radial ridges and small-scale ridges

and the relatively gentle incline of the pitcher walls (figure 1d–f). In contrast, these features are retained in terrestrial pitchers (figure 1a–c).

#### 4. DISCUSSION

Immature *N. lowii* plants are carnivorous, producing terrestrial pitchers that trap arthropod prey in the manner typical of the genus, but the initiation of aerial pitcher production signals the switch to *T. montana* faeces as the primary N source in this species. The interaction with *T. montana* enables *N. lowii* to exploit a N source that is unavailable to the sympatric *N. muluensis*. A possible explanation for this strategy is that montane *Nepenthes* are subject to an evolutionary pressure that is absent in lowland habitats: scarcity of ants as potential prey (Collins 1980; Samson *et al.* 1997). For example, on Gunung Mulu, Formicidae are 28 times more abundant per unit area at the foot of the mountain than at the summit (509 and 18 individuals  $\text{m}^{-2}$  at 130 and 2376 metres above sea level, respectively; Collins 1980). Givnish *et al.* (1984) proposed a cost–benefit model for carnivory in plants, stating that the syndrome would be of most benefit to plants in sunny, moist, nutrient-deficient habitats, such as acid bogs or tropical mountain tops. In habitats that would normally favour plant carnivory (e.g. montane cloud forests, where *N. lowii* grows), a scarcity of potential prey represents a limitation on the benefits of the syndrome. *Nepenthes lowii* has responded to this by targeting an alternative N source (*T. montana* faeces) as soon as the plant produces aerial pitchers.

The association between *N. lowii* and *T. montana* appears to be mutualistic, based on the exchange of nutritional resources that are scarce on Gunung Mulu. The importance of the interaction to each species is not yet known, but the geographical range of *N. lowii* falls entirely within that of *T. montana* (Payne *et al.* 1985; Clarke 1997). As faecal inputs by *T. montana* account for 57–100% of foliar N in mature *N. lowii*, the association is likely to be of considerable value to the plant. However, *T. montana* has a wide geographical range in north-western Borneo and is known from sites where *N. lowii* is absent (Payne *et al.* 1985), so some populations do not interact with *N. lowii*.

These results contribute to a growing body of evidence that *Nepenthes* exploit a range of N sources, including arthropods (Moran 1996; Merbach *et al.* 2002), leaf litter (Moran *et al.* 2003) and vertebrate faeces. *Nepenthes lowii* is the only plant species that is currently known to sequester N from both vertebrate and invertebrate sources.

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