Convergent and divergent evolution in carnivorous pitcher plant traps

Chris J Thorogood[1*], Ulrike Bauer[2], Simon J Hiscock[1]

[1]Botanic Garden, University of Oxford, Rose Lane, Oxford OX1 4AZ; [2]School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ.

*Correspondence to Chris Thorogood: chris.thorogood@obg.ox.ac.uk, 01865 610301

Contents List:

Twitter accounts (not to appear on publication):

@thorogoodchris1 (personal account but fine to promote to this)

@OBGHA (University of Oxford Botanic Garden)

@OxfordPlants (University of Oxford Dept. of Plant Sciences)

Summary

The pitcher trap is a striking example of convergent evolution across unrelated carnivorous plant lineages. Convergent traits that have evolved across pitcher plant lineages are essential for trap function, suggesting key selective pressures are in action. Recent studies have also revealed patterns of divergent evolution in functional pitcher morphology within genera. Adaptations to differences in local prey assemblages may drive such divergence, and ultimately speciation. Here we review recent research on convergent and divergent evolution in pitcher plant traps, with a focus on the genus *Nepenthes,* which we propose as a new model for research into adaptive radiation and speciation.

Key Words: functional morphology, adaptive radiation, speciation, *Nepenthes*, Sarraceniaceae, *Cephalotus*

Introduction

Carnivorous plants have inspired generations of scientists since Darwin's early experiments (Darwin, 1875). Strikingly similar trap morphologies, attractive features, and trapping mechanisms have evolved convergently across unrelated carnivorous plant lineages, which caused confusion among early taxonomists (Ellison and Gotelli, 2001). A prime example is the pitcher trap which has evolved independently at least six times (Givnish*,* 2015). Here we focus on the three best known pitcher plant genera: the paleotropical *Nepenthes* (c. 150 species), the neotropical *Sarracenia, Darlingtonia* and *Heliamphora* (ca. 30 species) and the monotypic Australian *Cephalotus follicularis* (Juniper et al., 1989). Pitfall traps have evolved a further three times independently in the Bromeliaceae and Eriocaulaceae in the form of tightly bound leaf 'tanks' that capture water and nutrients (Schulte et al., 2009).

Nepenthes, Cephalotus and the Sarraceniaceae show a remarkable convergence in trap morphology (Fig. 1). All three groups produce highly specialised leaf-derived pitfall traps to attract, capture, retain, kill and digest animal prey. Nectar is widely used as a primary attractant, often aided by volatiles and contrasting colour patterns in the visible or UV spectrum. Specialised slippery surfaces, often with strikingly similar micromorphology, lead arthropods to slip and fall into a pool of digestive liquid at the base of the pitcher. Notwithstanding this, recent research highlights multiple examples of divergent trap morphology, especially in the genus *Nepenthes* (Moran & Clarke, 2010, Bauer et al., 2012). This diversity of pitcher morphologies is mirrored by a diversification in utilized nutrient sources which may include arthropods, other animal prey, faeces and organic matter such as leaf litter. Adaptation to any of these nutrient sources may lead to divergent selective pressures which drive a diversification in pitcher morphology (Pavlovic, 2012). Here we highlight recent research into the convergent and divergent evolution in trap morphology and nutrient acquisition strategies across the pitfall-trapping carnivorous plants, with a focus on the genus *Nepenthes,* which has received significant attention in recent years.

Evolution of the pitcher

Many of the structures and compounds used by carnivorous plants to trap and digest prey are shared with non-carnivorous plants in association with defence against pathogens (Renner and Specht, 2013). Phylogenetic data indicates that *Nepenthes* evolved from a *Drosera*-like progenitor (Meimberg et al., 2000; Thorogood, 2010). Fusion of the leaf margins led to the tubular, so-called *epiascidiate* leaf in which the upper leaf surface became the inner surface of the tube. Spontaneous mutations can lead to epiascidiate leaves in non-carnivorous plants such as *Codiaeum variegatum* (Juniper et al., 1989; Fig. 2A). Heubl et al. (2006) suggest that the spontaneous occurrence of tubular leaves could have conveyed a selective advantage by facilitating water and nutrient storage, analogous to the evolution of tight leaf rosettes in bromeliads (Givnish, 2015). Digestive glands secrete digestive enzymes and absorb nutrients in pitcher plants (Juniper et al., 1989). Glands are anatomically similar across carnivorous sundews *Drosera* (Fig 2B) and *Drosophyllum* (Fig 2C), *Nepenthes* pitcher plants (Fig 2D), and their non-carnivorous relatives, such as *Plumbago* (Fig.

2E). The glue secreted by *Drosera* glands contains viscoelastic polysaccharides as does the pitcher fluid of many *Nepenthes* species, corroborating a plesiomorphic origin of this characteristic (Bonhomme et al., 2011).

Pitcher evolution in the Sarraceniaceae remains largely unstudied but recent work has established that tissue-specific changes in the orientation of cell divisions establish the pitcher shape in *Sarracenia purpurea* (Fukushima et al., 2015). *Cephalotus* produces pitchers and non-carnivorous flat leaves in synchrony. Recently, Fukushima et al., (2017) sequenced the transcriptome of both leaf types and showed that genes involved in adaxial-abaxial polarity are upregulated in pitcher-bearing shoots. The authors also identified an upregulation of genes involved in sucrose, wax and cutin biosynthesis. The co-existence of carnivorous and non-carnivorous leaves on the same plant in *Cephalotus* promises to yield more mechanistic insights into the evolution of the pitcher plant trap (Fukushima et al., 2017).

Convergent evolution

Convergent traits associated with carnivory, can be seen in both trap morphology and physiology. In *Nepenthes,* a hierarchical micro-topography of the rim (peristome) renders its surface slippery when wet, causing prey to 'aquaplane' on a lubricating fluid film (Bohn and Federle, 2004). In many species, epicuticular wax crystals reduce insects' adhesive pads contact area and performance (Gaume et al., 2004; Scholz et al., 2010). Directional features on the peristome and on the inner pitcher wall provide a grip for insects entering the pitcher, but do not facilitate their exit. Finally, the digestive fluid of many *Nepenthes* species is viscoelastic, facilitating the retention of arthropod prey (Gaume and Forterre, 2007).

Pitchers in the Sarraceniaceae also produce nectar to attract insects, have slippery surfaces for prey capture, and digestive fluids. The functional morphology of the slippery surfaces resembles that of *Nepenthes*, with contact area-reducing microtopography and directional features contributing to the anti-adhesive properties. Inward-pointing trichomes and imbricate cells are common across the family while epicuticular wax layers have only been observed in a few species. Remarkably, the hairy inner pitcher wall of *Heliamphora nutans* is wettable and traps insects via an 'aquaplaning' mechanism, similar to that of the *Nepenthes* peristome (Bauer et al., 2013). Interestingly, the trichomes have a parallel ridge structure resembling the microscopic peristome ridges of *Nepenthes*, which may be crucial for the wettability of both surfaces (D. Labonte, unpublished data). The evolution of two morphologically different superhydrophilic trap surfaces in unrelated plant orders is a striking example of functional convergence (Bauer et al., 2012) which suggests that biomechanical constraints may have driven the evolution of trap microstructures. Studies on the trapping mechanism of *Cephalotus* are scarce, but the similarities in functional morphology suggest that it may be similar to that of *Nepenthes*.

Strong patterns of convergence are also emerging for both attractive and digestive syndromes. Transparent fenestrations are another striking convergent feature in the attractive syndromes of some Sarraceniaceae (Fig. 3A), *Nepenthes* (Fig. 3B), and the pitcher lid of *Cephalotus* (Fig. 3C). Recent work however indicates that fenestrations in *Saraccenia minor* play a more important role in prey attraction (Schaefer and Ruxton, 2014). Studies of the digestive syndromes of *Nepenthes*, *Cephalotus*, *Drosera* and *Dionaea* suggest that orthologous pathogen-defence proteins have been repeatedly co-opted for digestion and as antimicrobial agents in the digestive fluid of carnivorous plants (Bemm et al., 2016). Fukushima et al. (2017) recently showed that *Arabidopsis* genes related to the genes coding for digestive fluid proteins in carnivorous plants are upregulated under biotic and abiotic stresses. The authors suggest that co-option of stress-response proteins may be a widespread pattern in the evolution of carnivorous plant enzymes. Functional convergence is also observed among the predators, filter feeders and detritivores that inhabit digestive pitcher fluids (Bittleston et al., 2016).

Divergent evolution

Morphological trap characteristics, such as size, shape, peristome geometry, and the presence and location of wax crystal layers vary in *Nepenthes* (Bauer et al., 2012). Physiological traits such as attractive volatiles, nectar, and pitcher fluid composition are also variable across the genus. Recent studies show that this diversity is mirrored by a range of nutrient acquisition strategies linked to habitat characteristics (Pavlovic, 2012). *Nepenthes* occur from sea level to 3,000 m elevation across the paleotropics, and prey availability varies between habitats. Ants comprise the bulk of prey in many lowland species (Moran et al., 2001) while flying insects predominate in montane environments. Viscoelastic pitcher fluids are more effective for the retention of flying prey (Di Giusto et al., 2008) and are more common in montane species (Bonhomme et al., 2011).

Epicuticular wax crystals are effective for the retention of ants but are absent or reduced in approximately one third of examined species. Bauer et al. (2012) performed a comparative study of trap morphology which identified wax crystal presence as the ancestral state and showed that wax layers have been lost several times independently. Pitchers without wax crystals frequently have larger and more inward-sloping peristomes than those with wax blooms on the inner wall. Bonhomme et al. (2011) demonstrated that wax loss is associated with montane habitats and with the occurrence of viscoelastic fluids. Moran et al. (2013) linked divergent trapping syndromes to climate, in which viscoelastic fluids are common in perhumid (ever-wet) regions and epicuticular wax crystals are common in both perhumid and seasonal areas (Moran et al., 2013). Climatic conditions as well as the faunal composition of the plants' habitats may exert selective pressures that favour one trapping strategy at the expense of another.

Carnivorous plants typically rely on prey for optimal growth and reproduction (Moran and Moran, 1998), and strong selection pressures should act on traps to maximise their prey intake. Modifications in trap geometry may enable the utilisation of novel nutrient sources, analogous to well-known examples in animals such as the diverse beak shapes of Darwin's finches and the various adaptations of cichlid fish in the African Great Lakes. For example Bauer et al. (2015a) demonstrated that the pitcher lid of *N. gracilis* is adapted to exploit the impact of rain drops for capturing insect prey. The lid of this species functions as a rain-driven torsion spring, flicking insects into the pitcher during heavy rain. The pitchers of *N. albomarginata* (Fig. 2F; 4A) produce a white ring of lichen-mimicking tissue that is specifically attractive to termites (*Hospitalitermes*) which account for >50% of its foliar nitrogen (N) (Moran et al., 2001). *Nepenthes ampullaria* (Fig. 2G) grows in closed-canopy forests and derives up to 40% of its foliar N from leaf litter (Moran et al., 2003; Pavlovic, 2012). The formation of dense pitcher 'carpets' with reflexed lids maximises the intake of leaf litter fall (Moran et al., 2003).

Four Bornean species (Fig. 2H-K, 4C-F) produce pitchers which trap the faeces of mammals (Chin et al., 2010), which can contribute 57-100% of their foliar N (Clarke et al., 2009). Chin et al., (2010) demonstrated that the size and geometry of the pitcher orifices in *N. lowii, N. rajah* and *N. macrophylla* are closely correlated with the body size of tree shrews. All three species have pitchers with large orifices and concave, reflexed lids oriented to optimally position the animal for faeces capture. *Nepenthes rajah* and *N. macrophylla* are closely related to each other, but not to *N. lowii*, according to phylogenetic data (Meimberg et al., 2001) suggesting that tree shrew associations evolved independently at least twice. Records of birds and tree shrews feeding on the nectar of *Nepenthes* that are not associated with mammals (Bauer et al., 2016) suggest that pitchers may be preadapted to attract vertebrates. Broad pitcher orifices could initially have been an adaptation for enhanced water or leaf litter capture. Faeces capture may have conferred a strong selective advantage, facilitating and consolidating further changes in pitcher geometry. *Nepenthes hemsleyana* (Fig. 2K; 4C) produces slender pitchers which capture few insects, but provide a roosting site for bats (*Kerivoula hardwickii*) which provide the plants with a third of their foliar N (Grafe et al., 2011). The pale-coloured tubular pitchers resemble whitish, suspended, tubular flowers that are typically bat-pollinated. Bat-adapted pitcher morphology may be analogous to shifts in floral spur length as an adaptation to pollinators with greater tongue length (Whittall and Hodges, 2007).

Nepenthes bicalcarata (Fig. 2L, 4B) has evolved a unique mutualistic association with ants (*Colobopsis schmitzi*) which defend the plant against herbivores in exchange for nectar and nesting sites (Merbach et al., 2007; Bonhomme et al., 2010) similar to other myrmecophytes. Analogies can be drawn with sea anemones providing anemonefish with shelter from predators in return for defence against cnidarian predators (Godwin and Fautin 1992). *Nepenthes bicalcarata* may benefit from colony waste for nutrition (Bazile et al., 2012), prevention of nutrient export by pitcher-dwelling Diptera (Scharmann et al., 2013) and maintaining the slipperiness of the trap by cleaning the peristome (Thornham et al., 2012).

Divergent trap forms have also evolved across the Sarraceniaceae and may be linked to ecological niche in *Sarracenia*. Most species produce trumpet-shaped traps (Fig. 4I), but those of *S. minor* (Fig. 4G) and *S. psitticina* (Fig. 3D) are hooded with concealed entrances. Forms of *Sarracenia minor* show specificity either towards flying insects (tall pitchers) or ground-dwelling prey (shorter pitchers with dense trichomes) depending on habitat differences (Stephens et al., 2015).

Adaptive radiation and speciation

Adaptive radiations are characterised by rapid speciation, recent common ancestry, and a strong link between phenotype and environment (Schluter, 2000). The genus *Nepenthes* meets these criteria because it has undergone rapid speciation (Meimberg and Heubl, 2006), and functional pitcher morphology is linked to prey availability and climate (Clarke and Moran, 2016). Many *Nepenthes* species have overlapping distributions and produce hybrids. *Nepenthes rajah* and *N. villosa*, both confined to the Mount Kinabalu National Park in Borneo, overlap and hybridise. *Nepenthes rajah* is established to capture faeces, but nothing is known about the nutrient sources of *N. villosa* or the hybrid. The more tubular pitcher, smaller orifice and horizontal lid suggest that faecal capture is unlikely in *N. villosa*. Divergence, despite ongoing hybridisation at intermediate altitudes, is also seen in ragworts on Mount Etna (*Senecio*, Asteraceae) (Filatov et al., 2016) where strong ecological and phenotypic differences between the high and low altitude species evolved due to strong selection at just a few key genes (Chapman et al., 2016). Selection at a few key genes may also feature in *Nepenthes* but this is yet to be explored.

Co-occurring *Nepenthes* species are established to capture different combinations of prey in a given location (Chin et al., 2014). Hybridisation between co-occurring *Nepenthes* species is widespread (M. Scharmann et al., unpublished data), so if hybrids arise from parents with different pitcher morphologies, novel pitcher traits may arise and facilitate the utilisation of novel nutrient sources. On the other hand, Pavlovic (2012) hypothesised that intermediate hybrid phenotypes of insect-trapping *N. rafflesiana* and detritivorous *N. ampullaria* may perform poorly and be outcompeted. Indeed, preliminary data suggest that the quantity and variety of prey caught by lowland hybrids may be less than that of their parent taxa in some cases. This could be due to a loss of specialized structures in hybrids of parent species that target specific types of prey (Peng and Clarke, 2015). Further work should explore this hypothesis by investigating the fitness of hybrids of species with divergent nutrient acquisition strategies, for example *Nepenthes hemsleyana* and *N. rafflesiana* which rely on mutualistic bats (Grafe et al., 2011), and insects (Bauer et al., 2015b), respectively. *Nepenthes rafflesiana, N. hemsleyana, N. ampullaria, N. bicalcarata* and *N. albomarginata* all have overlapping distributional ranges and have evolved distinct specialisations linked to nutrient sources. Given that sympatric *Nepenthes* species appear to target different prey taxa (Chin et al., 2014), interspecific competition for prey may have been a driver of disruptive selection and speciation in the genus (Thorogood 2010).

Conclusions and perspectives

Pitcher plants are one of the most striking examples of convergent evolution in the plant kingdom. Repeated patterns of adaptation for prey capture across unrelated groups include similar surface micro-morphology and gland anatomy. A recent surge in research is now providing growing evidence that adaptive radiations may be driven by nutrient competition. New *Nepenthes* species are described every year and little or nothing is known about their trapping strategies. Mammalian faeces-capture in pitcher plants was discovered only recently from extensive field observations on well-known species in North Borneo. Ecological studies are now required across the genus, including the many poorly-known species described recently from the Philippines. This will build a platform for exploring adaptive radiation, for example through examining competitive exclusion and niche partitioning *in situ*. The increasing availability of genomic data also offers new and exciting opportunities for studying the molecular basis of carnivorous plant evolution. In particular, the quantification of genetic divergence and positive selection in relation to phenotypic divergence. A combined approach exploring these ecological and molecular aspects will greatly enhance our understanding of adaptive radiations in carnivorous pitcher plants in the future.

Acknowledgements: we thank Hugh Dickinson for the SEM preparations, Kate Pritchard for supplying plant material, and Bruno Nevado for his constructive comments on the manuscript.

References

1. **Bauer U, Bohn HF, Federle W. 2008.** Harmless nectar source or deadly trap: *Nepenthes* pitchers are activated by rain, condensation and nectar. *Proc. R. Soc. B*. 275: **259**–265.
2. **Bauer U, Paulin M, Robert D, Sutton GP. 2015a.** Mechanism for rapid passive-dynamic prey capture in a pitcher plant. *PNAS.* **112**: 13384–13389.
3. **Bauer U, Clemente CJ, Renner T, Federle W. 2012**. Form follows function: morphological diversification and alternative trapping strategies in carnivorous *Nepenthes* pitcher plants. *Journal of Evolutionary Biology*. **25:** 90-102. doi: 10.1111/j.1420-9101.2011.02406.x.
4. **Bauer U, Federle W, Seidel H, Grafe TU, Ioannou CC. 2015b.** How to catch more prey with less effective traps: explaining the evolution of temporarily inactive traps in carnivorous pitcher plants. *Proceedings of the Royal Society B.* **282**: 20142675.
5. **Bauer U, Rembold K, Grafe TU. 2016.** Carnivorous *Nepenthes* pitcher plants are a rich food source for a diverse vertebrate community. *Journal of Natural History.* **50:** 483-495.
6. **Bauer U, Scharmann M, Skepper J, Federle W. 2013**. 'Insect aquaplaning' on a superhydrophilic hairy surface: how *Heliamphora nutans* Benth. pitcher plants capture prey. *Proceedings of the Royal Society B***. 280**: 20122569.http://dx.doi.org/10.1098/rspb.2012.2569
7. **Bazile V, Moran JA, Le Moguédec G, Marshall DJ, Gaume L. 2012.** A carnivorous plant fed by its ant symbiont: a unique multi-faceted nutritional mutualism. *PLoS ONE***. 7**(5): e36179. doi:10.1371/journal.pone.0036179.
8. **Bemm F, Becker D, Larisch C, Kreuzer I, Escalante-Perez M, Schulze WX, Ankenbrand M, Van de Weyer A-L, Krol E, Al-Rasheid KA et al., 2016**. Venus flytrap carnivorous lifestyle builds on herbivore defense strategies. *Genome Research*. **26**: 1–14.
9. **Bittleston LS, Pierce NE, Ellison AM, Pringle A. 2016.** Convergence in multispecies interactions. *Trends in Ecology & Evolution*. **31**: 269-280.
10. **Bohn HF, Federle W. 2004.** Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. *PNAS*. **101**: 14138–14143.
11. **Bonhomme V, Gounand I, Alaux C, Jousselin E, Barthelemy D, Gaume L. 2010.** The plant-ant *Camponotus schmitzi* helps its carnivorous host-plant *Nepenthes bicalcarata* to catch its prey. *Journal of Tropical Ecology*. **27**: 15–24.
12. **Bonhomme V, Pelloux-Prayer H, Jousselin E, Forterre Y, Labat JJ, Gaume L. 2011.** Slippery or sticky? Functional diversity in the trapping strategy of *Nepenthes* carnivorous plants. *New Phytologist*. **191**: 545–554.

13. **Chapman MA, Hiscock SJ, Filatov DA. 2016**. The genomic bases of morphological divergence and reproductive isolation driven by ecological speciation in *Senecio* (Asteraceae). *Journal of Evolutionary Biology*. **29**: 98-113.
14. **Chin L, Moran JA, Clarke CM. 2010.** Trap geometry in three giant montane pitcher plant species from Borneo is a function of tree shrew body size. *New Phytologist*. **186:** 461-470.
15. **Chin L, Chung AY, Clarke CM. 2014.** Interspecific variation in prey capture behavior by co-occurring *Nepenthes* pitcher plants – evidence for resource partitioning or sampling-scheme artefacts? *Plant signaling and Behavior,* **9**: e27930.
16. **Clarke CM, Bauer U, Lee CC, Tuen AA, Rembold K, Moran JA. 2009.** Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant. *Biology Letters*. **5**: 632-635.
17. **Clarke CM, Moran JA. 2016.** Climate, soils and vicariance - their roles in shaping the diversity and distribution of *Nepenthes* in Southeast Asia. *Plant and Soil*. **403**: 37-51.
18. **Darwin, 1875.** Insectivorous plants. London: John Murray.
19. **Giusto BD, Grosbois V, Fargeas E, Marshall DJ, Gaume L. 2008.** Contribution of pitcher fragrance and fluid viscosity to high prey diversity in a *Nepenthes* carnivorous plant from Borneo. *Journal of Biosciences*. **33**: 121-36.
20. **Ellison AM, Gotelli NJ. 2001.** Evolutionary ecology of carnivorous plants. *Trends in Ecology and Evolution*. **16**: 623-629.
21. **Filatov DA, Osborne OG, Papadopulos AS. 2016.** Demographic history of ecological speciation in *Senecio* altitudinal hybrid zone on Mt. Etna. *Molecular Ecology*. **25**: 2467-2481.
22. **Fukushima K, Fang X, Alvarez-Ponce D, Cai H, Carretero-Paulet L, Chen C, Chang TH, Farr KM, Fujita T, Hiwatashi Y et al., 2017.** Genome of the pitcher plant *Cephalotus* reveals genetic changes associated with carnivory. *Nature Ecology & Evolution* **1**: 59. doi:10.1038/s41559-016-0059.
23. **Fukushima K, Fujita H, Yamaguchi T, Kawaguchi M, Tsukaya H, Hasebe M. 2015**. Oriented cell division shapes carnivorous pitcher leaves of *Sarracenia purpurea*. *Nature Communications.* **6**: 6450. doi: 10.1038/ncomms7450.
24. **Gaume L, Perret P, Gorb E, Gorb S, Labat JJ, Rowe N. 2004**. How do plant waxes cause flies to slide? Experimental tests of wax-based trapping mechanisms in three pitfall carnivorous plants. *Arthropod structure & development.* **33**: 103–11.
25. **Gaume L, Forterre Y. 2007.** A Viscoelastic Deadly Fluid in Carnivorous Pitcher Plants. *PLoS ONE* **2(11)**: e1185. doi:10.1371/journal.pone.0001185.
26. **Givnish TJ. 2015.** New evidence on the origin of carnivorous plants. *PNAS*. 112: 10–11.
27. **Godwin J, Fautin DG. 1992**. Defense of host actinians by anemonefishes. *Copeia*. **3**: 902–908.
28. **Grafe TU, Schöner CR, Kerth G, Junaidi A, Schöner MG. 2011.** A novel resource-service mutualism between bats and pitcher plants. *Biology Letters*: **7**: 436-9.
29. **Heubl G, Bringmann G, Meimberg H. 2006.** Molecular phylogeny and character evolution of carnivorous plant families in caryophyllales – Revisited. *Plant Biology*. **8**: 821–830.
30. **Juniper BE, Robins RJ, Joel DM. 1989.** The carnivorous plants. London, UK. Academic Press.

31. **Lim YS, Schöner CR, Schöner MG, Kerth G, Thornham DG, Scharmann M, Grafe TU. 2015**. How a pitcher plant facilitates roosting of mutualistic woolly bats. *Evolutionary Ecology Research.* **16**: 581-591.
32. **Meimberg H, Dittrich P, Bringmann G, Schlauer J, Heubl G. 2000.** Molecular phylogeny of Caryophyllidae s.l. based on MatK sequences with special emphasis on carnivorous taxa. *Plant Biology.* **2**: 218–228.
33. **Meimberg H, Heubl G. 2006.** Introduction of a nuclear marker for phylogenetic analysis of Nepenthaceae. *Plant Biology*. **8**: 831–840.
34. **Meimberg H, Wistuba A, Dittrich P, Heubl G. 2001.** Molecular phylogeny of Nepenthaceae based on cladistic analysis of plastid trnK Intron sequence data. *Plant Biology*. **3**:164-175.
35. **Merbach MA, Zizka G, Fiala B, Merbach D, Booth WE, Maschwitz U. 2007**. Why a carnivorous plant cooperates with an ant – selective defence against pitcher-destroying weevils in the myrmecophytic pitcher plant *Nepenthes bicalcarata* Hook. F. *Ecotropica* **13**: 45-56.
36. **Moran JA, Clarke CM, Hawkins BJ. 2003.** From carnivore to detritivore? Isotopic evidence for leaf litter utilization by the tropical pitcher plant *Nepenthes ampullaria International Journal of Plant Science*. **164**: 635–639.
37. **Moran JA, Clarke CM. 2010**. The carnivorous syndrome in *Nepenthes* pitcher plants: current state of knowledge and potential future directions. *Plant Signalling and Behaviour*. **5**: 644-648.
38. **Moran JA, Merbach MA, Livingston NJ, Clarke CM, Booth WE. 2001.** Termite prey specialization in the pitcher plant *Nepenthes albomarginata*. Evidence from Stable Isotope Analysis. *Annals of Botany*. **88**: 307-311.
39. **Moran JA, Gray LK, Clarke CM, Chin L. 2013.** Capture mechanism in Palaeotropical pitcher plants (Nepenthaceae) is constrained by climate. *Annals of Botany.* **112:** 1279–1291.
40. **Moran JA, Moran AJ. 1998.** Foliar reflectance and vector analysis reveal nutrient stress in prey-deprived pitcher plants (*Nepenthes rafflesiana*). *International Journal of Plant Sciences*. **159**: 996-1001.
41. **Pavlovic A. 2012**. Adaptive radiation with regard to nutrient sequestering strategies in the carnivorous plants of the genus *Nepenthes*. *Plant Signalling and Behaviour*. **7**: 295-297.
42. **Peng HS, Clarke CM. 2015.** Prey capture patterns in *Nepenthes* species and natural hybrids - are the pitchers of hybrids as effective at trapping prey as those of their parents? *Carnivorous Plant Newsletter.* **44**: 62–79.
43. **Renner T, Specht CD. 2013.** Inside the trap: gland morphologies, digestive enzymes, and the evolution of plant carnivory in the Caryophyllales. *Current Opinion in Plant Biology.* **16**:436-42.
44. **Schaefer HM, Ruxton GD. 2014.** Fenestration: a window of opportunity for carnivorous plants. *Biology Letters*. **10:** 20140134.
45. **Scharmann M, Thornham DG, Grafe TU, Federle W. 2013.** A novel type of nutritional ant-plant interaction: ant partners of carnivorous pitcher plants prevent nutrient export by dipteran pitcher infauna. *PLoS One*. **8**(5): e63556. doi: 10.1371/journal.pone.0063556.
46. **Schluter D. 2000**. The ecology of adaptive radiation. Oxford: Oxford Univ. Press.
47. **Scholz I, Bückins M, Dolge L, Erlinghagen T, Weth A, Hischen F, Mayer J, Hoffmann S, Riederer M, Riedel M, Baumgartner W. 2010.** Slippery surfaces of pitcher plants: *Nepenthes* wax crystals minimize insect attachment via microscopic surface roughness. *Journal of Experimental Biology*. **213**:1115-1125.

48. **Schöner MG, Schöner CR, Simon R, Grafe TU, Puechmaille SJ, Ji LL, Kerth G. 2015.** Bats are acoustically attracted to mutualistic carnivorous plants. *Current Biology*. **25**: 1911–1916.
49. **Stephens JD, Rogers WL, Heyduk K, Cruse-Sanders JM, Determann RO, Glenn TC, Malmberg RL. 2015.** Resolving phylogenetic relationships of the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Molecular Phylogenetics and Evolution*. **85**: 76–87.
50. **Thornham DG, Smith JM, Grafe TU, Federle W. 2012.** Setting the trap: cleaning behaviour of *Camponotus schmitzi* ants increases long-term capture efficiency of their pitcher plant host, *Nepenthes bicalcarata*. *Functional Ecology.* **26**: 11–19.
51. **Thorogood CJ. 2010.** The Malaysian *Nepenthes*: Evolutionary and Taxonomic Perspectives. Nova Publishers, New York.
52. **Whittall JB, Hodges SA. 2007**. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature Letters*. **447**: 706-710.

FIGURE LEGENDS:

Figure 1. Pitcher plants show a remarkable convergence of traits associated with carnivory. *Nepenthes* pitchers (A) comprise a pitcher chamber containing digestive fluid, a rim (peristome), and roof-like lid. The pitcher lid shows interspecific variation but is believed to have an attractive function and in some species to prevent dilution of the digestive fluid with rainwater. Attractive nectar glands are numerous on the underside of the pitcher lid and along the inner edge of the peristome, which also plays a key role in prey capture. *Cephalotus* pitchers (B) resemble those of *Nepenthes* in their ovoid shape, ridged peristome and lid. The trapping surfaces feature parallel ridges on the peristome teeth and downward-pointing imbricate cells in the funnel-shaped region. *Sarracenia* pitchers (C) have slippery surfaces that resemble those of *Nepenthes*, in which contact area-reducing microtopography and directional features contribute to the anti-adhesive properties of the trap. Inward-pointing trichomes and imbricate cells are common across the family while epicuticular wax layers have only been observed in a few species. L=lid; P=Peristome; PC=Pitcher Chamber; T=Tendril. Images not to scale.

Figure 2 (A) mutant leaf of *Codiaeum variegatum* showing extension of midrib from leaf blade, homologous to the *Nepenthes* tendril (white arrow), and espiascidiate leaf (black arrow); Scanning Electron Microscope (SEM) images of stalked digestive glands of (B) *Drosera* and (C) *Drosophyllum*, which secrete digestive enzymes, absorb nutrients and trap prey via localized tentacle-bending reaction which, together with sticky mucilage, aid retention. Glands homologous with these are embedded on the inner wall of *Nepenthes* pitchers, such as *N. inermis* (D); calyx glands of non-carnivorous *Plumbago* (E) are anatomically similar to the mucilage glands of *Drosera* and *Drosphyllum* pointing to a common ancestral gland structure; (F) pitchers of *N. albomarginata* showing the white band of lichen-mimicking tissue (arrow) which attracts termites; (G) the closely packed pitchers of *N. ampullaria* on the forest floor; (H) tree shrew (*Tupaia montana*) feeding on a pitcher lid of *N. lowi* (from Bauer et al., 2016); (I) pitcher of *N. rajah* showing reflexed lid (arrow); (J) *Tupaia montana* faeces inside a pitcher of *N. macrophylla* (arrow); (K) roosting bat (*Kerivoula hardwickii*) inside a pitcher of *N. hemsleyana;* (L) pitcher of *N. bicalcarata* with nectar-producing thorns (arrow) on which mutualistic ants (*Colobopsis schmitzi*) (inset) feed.

Figure 3. Striking examples of convergence in morphological adaptations to the pitfall trap include (i) domed pitchers with fenestrations which operate as light traps in which 'false exits' disorient flying prey in *Sarracenia psittacina* (A), *Nepenthes aristolochioides* (B) and the lid of *Cephalotus follicularis* (C) as well as (ii) the remarkably similar ridged peristome structures of *C. follicularis* and *N. villosa* (D).

Figure 4. Divergence in the carnivorous syndrome in *Nepenthes* (A-F) and Sarraceniaceae (G-J). Several species of *Nepenthes* have diverged (at least partially) from the carnivorous syndrome and coevolved mutualistic relationships with animal partners: *N. albomarginata* (A) has a white band of hairs which attract termites (*Hospitalitermes bicolor*); (B) *N. bicalcarata* produces swollen tendrils in which mutualistic ants (*Camponotus schmitzi*) rear broods; (C) *N. hemsleyana* lacks traits associated with insect attraction has modified pitcher shape and fluid level to facilitate bat roosting (Lim et al., 2015) and an elongated inner pitcher wall to reflect the ultrasound calls of bats, enabling them to locate and identify plants in dense vegetation (Schöner et al., 2015); montane species which associated mainly with tree shrews (*Tupaia montana*) include *N. macrophylla* (D), *N. lowii* (E) and *N. rajah* (F); examples of divergent of pitcher morphologies across the Sarraceniaceae lineage include hooded pitchers with fenestrations (G) *Sarracenia minor* and (H) *Darlingonia californica*, as well as the open pitfall traps of (I) *Sarracenia* and (J) *Heliamphora*. Images not to scale.