THE PITCHER PLANTS (*NEPENTHES* SPECIES) OF SINGAPORE

Weng Ngai Lam and **Hugh T. W. Tan (Editors)**

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Cover photograph of *Nepenthes rafflesiana* © Weng Ngai Lam

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CHAPTER 1: INTRODUCTION

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***Carnivorous plants*. —** Carnivorous plants are plants that are capable of attracting, capturing and deriving benefit from animal prey to supplement their nutrient requirements (Ellison & Adamec, 2018). Carnivorous plants may be conveniently divided into five main groups according to the mechanisms they employ for prey capture (Table 1.1). Although collectively termed ‘pitcher plants’, members of the pitfall trap group are taxonomically unrelated and are almost completely geographically separate (Brewer & Schlauer, 2018). Tropical pitcher plants, or *Nepenthes* species, represent the largest taxon among these plants, and constitute the only genus of the family Nepenthaceae (Krol et al., 2012).

Table 1.1. A simplified classification of the known carnivorous plant taxa by trapping mechanism. Adapted from Krol et al. (2012); the list of taxa is not exhaustive, and does not include taxa for which carnivory is still uncertain and/or poorly studied.

Trapping mechanism	Carnivorous plant taxa
Snap-traps	*Dionaea muscipula*; *Aldrovanda vesiculosa*
Fly-paper traps	*Drosera* species; *Pinguicula* species; *Byblis* species; *Roridula* species; *Drosophyllum lusitanicum*; *Triphyophyllum peltatum*; *Ibicella lutea*; *Philcoxia* species
Pitfall traps	*Nepenthes* species; *Heliamphora* species; *Sarracenia* species; *Darlingtonia californica*; *Cephalotus follicularis*; several Bromeliaceae species (*Brocchinia reducta* and *Catopsis berteroniana*)
Bladder traps	*Utricularia* species
Eel traps	*Genlisea* species

***The genus* Nepenthes. —** Tropical pitcher plants are represented by the monogeneric family Nepenthaceae. While the other ‘pitcher plants’ (Table 1.1) are generally small, low-growing herbs, most *Nepenthes* species are semi-woody vines which often exceed several metres in length.

Krol et al. (2012) recognised >110 *Nepenthes* species, but the many new species discoveries in recent times are likely to have taken this number to >160 species (Clarke et al., 2018). Many of the recent *Nepenthes* species discoveries have been made in the Philippines, where military conflict had hindered earlier explorations (McPherson, 2011; Clarke et al., 2018).

***Distribution*. —** *Nepenthes* species diversity is concentrated in Southeast Asia, with Borneo, the Philippines and Sumatra being home to the largest numbers of species (Clarke et al., 2018). Many *Nepenthes* species are highland species with highly confined geographical ranges (McPherson, 2009, 2010, 2011; van der Ent et al., 2015).

Three *Nepenthes* species are native to Singapore, namely *Nepenthes ampullaria* Jack, *Nepenthes gracilis* Korth and *Nepenthes rafflesiana* Jack. Unlike the majority of highland species, all three of these species have large geographical ranges and are relatively abundant across their distributions (Clarke, 1997; McPherson, 2009). They are found in Borneo, Sumatra, Peninsular Malaysia and Singapore. *Nepenthes ampullaria* is also found in New Guinea, the Maluku Islands and southern Thailand, and *Nepenthes gracilis* is found in southern Thailand as well (McPherson, 2009).

Plant morphology**.** — The *Nepenthes* plant is a semi-woody vine or scrambler that is usually incapable of standing erect without external support (Fig. 1.1). The three native *Nepenthes* species are all light-demanding species that thrive in conditions of high illumination and thus depend heavily on their climbing habit to gain access to above canopy light conditions when growing in forests. This is done with the help of prehensile tendrils that are produced when the plant transitions into the climbing ontogenic stage (see section below on Pitcher morphology; Fig. 1.1).

Nepenthes plants may reproduce asexually through the development of basal side shoots that emerge from axillary buds along stems sprawling across the soil surface (Fig. 1.2). Although the three native species do not produce stolons (modified lateral shoots for vegetative reproduction) as some specialised lithophytic species do (e.g., *Nepenthes campanulata* [Clarke et al., 2014]), the development of basal side shoots from mature, unsupported vines is an important form of vegetative growth in native species populations and allows *Nepenthes* populations to spread quickly into canopy gaps created by forest disturbances (e.g., treefall gaps or embankments).

Pitcher morphology**.** — The pitcher is the highly modified tip of a tendril that is the extension of the midrib of an expanded leaf stalk or petiole known as a phyllode (Schulze et al., 1997). For convenience in this book, we refer to this phyllode as the leaf's leaf blade or lamina, since it performs all the functions of a typical lamina (Fig. 1.1). The leaf origin of the pitcher is evident in *Nepenthes* seedlings, whose leaves lack tendrils and remain largely undifferentiated in pitcher form (Fig. 1.3). Functional diversification between pitcher and lamina portions of the *Nepenthes* leaf is clearly defined, with the laminae mainly responsible for photosynthesis, while pitchers are mainly for prey capture (Pavlovič & Saganová, 2015). Such specialisation of parts does not occur in pitcher plant

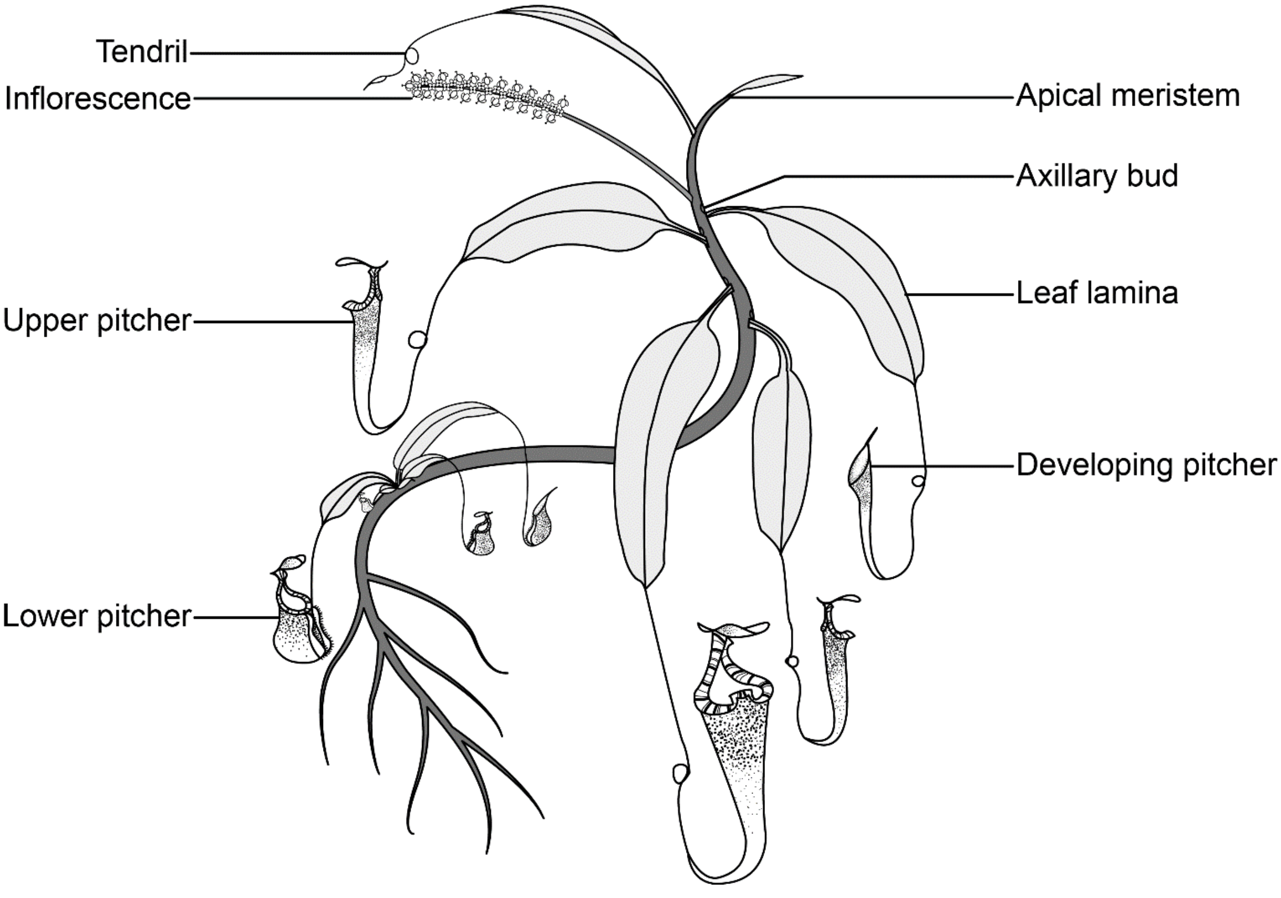

Fig. 1.1. Morphology of a typical *Nepenthes* plant. (Illustration by: Felicia Leong Wei Shan).

Fig. 1.2. *Nepenthes* × *hookeriana* basal sprouts from dormant axillary buds in an old stem. (All photographs in this chapter by: Weng Ngai Lam).

Fig. 1.3. Typical *Nepenthes* seedlings in cultivation, in which pitcher and leaf blade portions of the leaf remain poorly differentiated.

Fig. 1.4. Typical lower (left) and upper (right) pitchers of *Nepenthes rafflesiana*. Scale bars represent 5 cm.

species of the Sarraceniaceae or trumpet pitcher family, where the leaf is a simple structure that consists of a pitcher and lid only, and where the whole organ performs both photosynthetic and carnivorous functions (Pavlovič & Saganová, 2015).

Most *Nepenthes* species produce two pitcher types which reflect the developmental phase of the plant (Fig. 1.4). The lower or rosette pitchers are typically produced by immature and as yet non-climbing plants or basal side-shoots, on stems with comparatively short internodes. The upper or 'aerial' pitchers are produced by mature, vining plants, and are often borne on prehensile tendrils which both position the pitchers in more exposed microenvironments as well as facilitate the climbing habit of the plant (Fig. 1.1). *Nepenthes ampullaria* in particular also produces "ground" pitchers which are clusters of lower pitchers that emerge from or close to the ground from greatly reduced rosettes of leaves called nanophylls.

Generalisable morphological differences between the two pitcher types are summarised in Table 1.2. Intermediate pitchers are typically produced as a plant transitions from one ontogenic stage to the

Table 1.2. Typical morphological differences between upper and lower pitchers.

Character	**Lower pitcher**	**Upper pitcher**
Fringed wings	Present	Absent
Size	Variable, advancing with increasing age of plant or stem	Somewhat constant
Orientation with respect to tendril	Facing tendril attachment	Facing away from tendril attachment
Tendril	Simple	Often prehensile
Pitcher colouration	Often more intensely coloured	Typically drably coloured (more often green)

Fig. 1.5. Lower (left most) and upper (right most) pitchers of *N. gracilis*, and three intermediate pitchers between them. The gradual transition from lower-pitcher type traits (towards the left) to upper-pitcher type traits (towards the right) is evident in this example. In particular, fringed wings are increasingly lost (black arrows), and pitchers increasingly orientate to face away from, rather than towards, the tendril, at the point of attachment to the pitcher (white arrows). Notice also the gradual transition in pitcher colour, as well as the presence of prehensile tendrils in the upper (right most) and most advanced intermediate (second from the right) pitchers. Pitchers from this photograph were collected from Sembawang Avenue, and did not originate from the same plant. Scale bar represents 5 cm.

next (Tan, 1997). Unlike upper or lower pitchers, however, these seldom retain consistent morphological forms, but share varying degrees of overlapping characters with both upper and lower pitcher forms, metamorphosing with the production of each new (and thus more ontogenically-advanced) leaf (Fig. 1.5).

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CHAPTER 2: SINGAPORE SPECIES AND HYBRIDS

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Introduction. — Three *Nepenthes* species occur naturally in Singapore, viz., *Nepenthes ampullaria*, *Nepenthes gracilis* and *Nepenthes rafflesiana* (Tan et al., 1997). The three species are easily distinguished by the clear differences in pitcher morphology and size (Fig. 2.1), and also differ significantly in other vegetative traits. Three *Nepenthes* hybrids, resulting from pairwise combinations of the three native species, are also found naturally in Singapore (Tan et al., 1997). One of these hybrids, *Nepenthes gracilis* × *Nepenthes rafflesiana*, may presently be locally extinct, but information regarding this hybrid is still presented here since spontaneous hybridisation can still result in the natural recreation of the hybrid, and it is impossible to confirm that this hybrid is not still present in unexplored areas of Singapore forests. Although hybrids are not often named in the scientific literature, two of the local hybrids, *Nepenthes* × *hookeriana* (= *Nepenthes ampullaria* ×

Fig. 2.1. Size and morphological differences between typical mature pitchers of the three Singapore *Nepenthes* species. From left to right: *Nepenthes gracilis* lower and upper pitchers, *Nepenthes ampullaria* lower pitcher and *Nepenthes rafflesiana* upper and lower pitchers. (All photographs in this chapter by: Weng Ngai Lam).

Nepenthes rafflesiana) and *Nepenthes* × *trichocarpa* (= *Nepenthes ampullaria* × *Nepenthes gracilis*), retain the names with which they had been originally described as species (Cheek et al., 2019), and will be referred to in this book by these given names.

In the following sections, brief notes concerning the natural history and ecology of the three species are presented. Detailed taxonomic descriptions are not included, since these are readily available in the literature. National conservation status categories of the species were obtained from Chong et al. (2009).

Nepenthes ampullaria

National conservation status: Vulnerable.

Distribution: Central Catchment Nature Reserve, Kent Ridge Park, Western Catchment Area and resam-dominated scrubland (areas mostly overgrown by *Dicranopteris linearis* and/or *Dicranopteris curranii* ferns) within the urban landscape.

Description: *Nepenthes ampullaria* is one of the most unique species within the genus. The lower pitchers are globose and have a large, gaping mouth which is fully exposed to rain and falling canopy debris because the small, reflexed pitcher lid affords little cover. Lower pitchers are often produced by axillary shoots in clumps at the base of mature plants, and may often be completely buried in leaf litter (Fig. 2.2). The leaves in such basal clumps are termed 'nanophylls' and are often greatly reduced, such that sprawling 'carpets' of pitchers appear to casual observers to grow out of the ground (Fig. 2.3). Such a unique growth form is believed to facilitate the semi-detritivorous habit of this species by allowing pitchers to capture more debris from the canopy (see Chapter 5: Prey and Carnivory). Basal clumps are sometimes also produced along the entire length of climbing stems (Fig. 2.4).

Fig. 2.2. Basal clumps of *Nepenthes ampullaria* buried beneath the leaf litter of resam-dominated scrubland. A hole had to be cleared in the vegetation (left) in order for these pitchers to be exposed for photography (right). Location: Holland Woods.

Fig. 2.3. Typical basal clumps with lower pitchers of two colour variants of *Nepenthes ampullaria*. Location: Nee Soon Swamp Forest.

Fig. 2.4. Basal clumps of climbing *Nepenthes ampullaria* stems. These stems may stretch many metres in length, ascending to the canopy. The basal clumps are likewise produced across most of their lengths. Locations: Kent Ridge Park (left); Nee Soon Swamp Forest (right).

Significant intraspecific variation in pitcher colouration exists in *Nepenthes ampullaria*, but that variation is not well represented in local populations of the species. In Singapore, *Nepenthes ampullaria* pitchers may be pure green (Fig. 2.5) or with varying combinations of green-, red- or bronze-speckled patterns. However, more extreme colour forms are known from Borneo, which have not been observed to date in Singapore. For example, pure red or 'tricolour' (Fig. 2.6) varieties with striped peristomes are known from Borneo and New Guinea, and are highly coveted in the ornamental plant trade.

Fig. 2.5. A basal clump of pure green *Nepenthes ampullaria* pitchers from an old mature vine. The 55 mm-wide lens cap is for scale. Location: Nee Soon Swamp Forest.

Fig. 2.6. Bornean *Nepenthes ampullaria* colour forms in cultivation. The colour form on the left is termed 'tricolour' amongst horticulturists because of the presence of three different colours on a single pitcher (maroon and green flecks on a red base).

Fig. 2.7. The rarely-encountered upper pitcher of *Nepenthes ampullaria*. Location: Nee Soon Swamp Forest.

Upper pitchers are produced only very rarely in *Nepenthes ampullaria* plants. Tan et al. (1997) had only observed upper pitchers in one individual plant locally, and suggested that *Nepenthes ampullaria* upper pitchers are non-functional because the observed specimen did not retain any fluids owing to the angle at which the pitcher was held. However, personal observations suggest that upper pitchers are more common than previously thought (Fig. 2.7). Observed specimens also contained fluids as well as small amounts of prey and detritus, showing that the pitchers are functional to a limited extent.

Fig. 2.8. A young *Nepenthes gracilis* plant sprawling across the hot, clayey ground in early successional, resam-dominated scrubland. Location: Holland Woods.

Nepenthes gracilis

National conservation status: Common.

Distribution: Central Catchment Nature Reserve, Kent Ridge Park, Western Catchment Area, resam-dominated scrubland within the urban landscape, Pulau Tekong, Pulau Ubin and the Southern Islands.

Description: *Nepenthes gracilis* is one of the most commonly occurring *Nepenthes* species in Southeast Asia, being a fast-growing, early successional species that can exploit areas of disturbance with relative ease (Fig. 2.8).

Nepenthes gracilis pitchers are typically uniformly coloured, with lower pitchers being green, orange, red or maroon while upper pitchers are almost always green (Fig. 2.9). The tiny pitchers are produced in abundance (Fig. 2.10), and are considered short-lived in comparison to pitchers of other *Nepenthes* species (Table 5.1). *Nepenthes gracilis* also invests little in adaptations that enhance prey capture, such as abundant nectar secretions, viscoelastic fluids or large, flared peristomes. Unsurprisingly, its prey are primarily small ants and other small crawling species (Moran et al., 1999; Chin et al., 2014; Gaume et al., 2016; Lam et al., 2018).

Fig. 2.9. Typical lower (left) and upper (right) pitchers of *Nepenthes gracilis*. Location: Holland Woods.

Fig. 2.10. A “carpet” of *Nepenthes gracilis* lower pitchers. Location: Holland Woods.

Fig. 2.11. Leaf and stem morphology of a typical *Nepenthes gracilis* shoot. Location: Sembawang Avenue.

The leaf and stem morphology of *Nepenthes gracilis* are distinct amongst *Nepenthes* species, as this species' leaves are sessile (stalkless) and decurrent (i.e., leaf blades extend down along the stem), and its stem is angular (Fig. 2.11). These traits are usually evident in the hybrids of *Nepenthes gracilis* as well, albeit to a lesser degree, and are key to their identification in the field on plants that lack pitchers.

Fig. 2.12. Typical habit and upper pitchers of *Nepenthes gracilis*. Location: Holland Woods.

Nepenthes rafflesiana

National conservation status: Vulnerable.

Distribution: Central Catchment Nature Reserve, Kent Ridge Park, Western Catchment Area, resam-dominated scrubland within the urban landscape, coastal cliffs of the Southern Islands.

Description: *Nepenthes rafflesiana* is the largest native *Nepenthes* species, being capable of producing magnificent pitchers regularly exceeding 20 cm tall (Fig. 2.13). Its shoots are easily recognisable by a silvery sheen caused by the fine, white hairs on stems and young leaves (Fig. 2.14). Its leaves are distinctly stalked (petiolate) and its leaf blades have a leathery texture. Together, these traits easily distinguish *Nepenthes rafflesiana* from other *Nepenthes* species growing in the same area, even in the absence of pitchers.

The upper and lower pitchers of *Nepenthes rafflesiana* differ significantly in morphology (Figs. 2.13, 2.14), and trap distinctly different prey taxa (Moran, 1996). This phenomenon is known as pitcher dimorphism, and is likely to reduce competition for prey between juvenile and adult plants in a population, while maximising prey capture in the microhabitats in which such pitchers are borne.

Fig. 2.13. Large *Nepenthes rafflesiana* lower (left) and upper (right) pitchers. Note that the leaves in the right image are decurrent and therefore belong to *Nepenthes gracilis*. The handheld global positioning system (GPS) receiver measures 16 cm long, and is included for scale in the first image. Locations: MacRitchie Reservoir forest (left); Holland Woods (right).

Fig. 2.14. *Nepenthes rafflesiana* upper pitcher in a treefall gap. The distinctive shoot of this species with its silvery sheen and petiolate leaves is also clearly visible in the photograph. Upper pitchers of *Nepenthes rafflesiana* are usually drably coloured (left) but the first upper pitchers produced immediately after a growing stem transitions into an upper pitcher-bearing life stage usually retain the vibrant colours of their lower pitchers (right). Locations: MacRitchie (left); Nee Soon Swamp Forest (right).

In addition to the commonly inhabited vegetation types described in Chapter 3: Habitats, substantial populations of *Nepenthes rafflesiana* have also been found growing on coastal cliffs of the south of Singapore Island and the Southern Islands. These include Labrador Nature Reserve (now extinct), Sentosa and Pulau Tekukor (Fig. 2.15). It is unclear if *Nepenthes rafflesiana* is able to succeed in these environments because of the species' intrinsic adaptions (e.g., tolerance to salt spray) or if these aggregated distributions are merely the result of historical contingencies.

An immense amount of intraspecific variation in pitcher colour and morphology exists in *Nepenthes rafflesiana*, although this is significantly less for the Sumatra, Peninsular Malaysia and Singapore populations as compared to their counterparts from Borneo (Clarke, 2001). The giant form of this species, sometimes termed *Nepenthes rafflesiana* var. *gigantea*, produces pitchers which regularly exceed 30 cm in height and may contain up to one litre of fluid (Clarke, 1997; McPherson, 2009). Another form, also known as *Nepenthes rafflesiana* var. *alata*, has fringed wings which run up the length of its tendrils (Clarke, 1997; McPherson, 2009) (Fig. 2.16). These forms are sometimes circulated in horticultural trade, but are not native to Singapore. Nevertheless, local wild plants of *Nepenthes rafflesiana* still vary considerably, most notably in pitcher colouration.

Furthermore, the typical Bornean variety of *Nepenthes rafflesiana* (sometimes referred to as *Nepenthes rafflesiana* var. *typica* in the literature) differs significantly from the variety native to Singapore and Peninsular Malaysia. Both the upper and lower pitchers of typical Bornean *Nepenthes rafflesiana* are stouter and more round in overall form than typical *Nepenthes rafflesiana* pitchers in Singapore, Peninsular Malaysia and Sumatra. Additionally, native *Nepenthes rafflesiana*

Fig. 2.15. *Nepenthes rafflesiana* thrives on coastal cliffs. Location: Pulau Tekukor.

Fig. 2.16. Two uncommon *Nepenthes rafflesiana* varieties: *Nepenthes rafflesiana* var. *alata* from Borneo (left), grown in a private garden—the fringed wings of this form develop along the tendrils of lower pitchers; and the upper pitcher of a native, all green form (right).

lack viscoelastic fluids in both upper and lower pitchers, and have a more persistent inner waxy layer in lower pitchers (though this as well is variable; the waxy inner layer is lost very early in juvenile plants in typical Bornean *Nepenthes rafflesiana*, but may persist even in mature lower pitchers in some Singapore varieties of the species [Fig. 5.11]). These morphological differences may have resulted from character displacement caused by prey competition between sympatric *Nepenthes* species of the more species-rich *Nepenthes* communities in Borneo. Coincidentally, a Borneo-endemic species, *Nepenthes hemsleyana*, which is sympatric and closely related to *Nepenthes rafflesiana*, has similar but more elongated pitchers than *Nepenthes rafflesiana*, lacks viscoelastic fluids and retains a waxy inner layer (Bonhomme et al., 2011). It is possible, though speculative, that the absence of *Nepenthes hemsleyana* and several other sympatric competitors from Singapore may relax competitive pressures on Singaporean *Nepenthes rafflesiana* populations (Lam et al., 2018), resulting in the loss of some pitcher traits otherwise important for prey resource partitioning in Bornean *Nepenthes* communities. Although viscoelastic fluids are important for the capture of flying prey, Singapore *Nepenthes rafflesiana* pitchers still catch large numbers of flying prey without possessing such an attribute (see Chapter 5: Prey and Carnivory).

Nepenthes × *trichocarpa* (= *Nepenthes ampullaria* × *Nepenthes gracilis*)

National conservation status: None, because the status is provided only for species and not hybrids.

Distribution: Central Catchment Nature Reserve, Kent Ridge Park, Western Catchment Area, resam-dominated scrubland within the urban landscape.

Description: *Nepenthes* × *trichocarpa* is another commonly occurring natural hybrid that thrives in open resam-dominated scrubland. Pitchers of *Nepenthes* × *trichocarpa* share traits from both *Nepenthes ampullaria* and *Nepenthes gracilis*, and, unsurprisingly, also has a prey spectrum that overlaps with both of its parent species (Peng & Clarke, 2015). Spotted or speckled (Fig. 2.17) and green (Fig. 2.18) varieties of the hybrid are common in Singapore, and casual observation suggests that pitcher colouration of *Nepenthes* × *trichocarpa* individuals is determined by the *Nepenthes ampullaria* parent. Whereas *Nepenthes ampullaria* in Singapore seldom bears upper pitchers, *Nepenthes* × *trichocarpa* readily produces them (Figs. 2.17, 2.18).

The stem and leaves of *Nepenthes* × *trichocarpa* are in all respects intermediate in appearance between its parent species. Like *Nepenthes ampullaria*, a fine brown covering of hairs coats its young stems and leaf blade margins (Fig. 2.19), but unlike the leaves of *Nepenthes ampullaria*, those of *Nepenthes* × *trichocarpa* lack petioles and may have more waxy leaf blades—both traits inherited from *Nepenthes gracilis* (Figs. 2.18, 2.19). These distinct vegetative traits allow easy identification of the hybrid in the absence of pitchers.

Fig. 2.17. Speckled upper pitcher of *Nepenthes* × *trichocarpa*. Location: Holland Woods.

Fig. 2.18. Male *Nepenthes* × *trichocarpa*. Pure green upper pitcher (left). Male inflorescence (right). Location: Holland Woods.

Fig. 2.19. The characteristic appearance of the shoots of *Nepenthes ampullaria* (left) and *Nepenthes* × *trichocarpa* (right). Under conditions of high illumination, fine brown hairs densely cover the surfaces of *Nepenthes ampullaria* shoots, and this characteristic can be used to easily identify its hybrids.

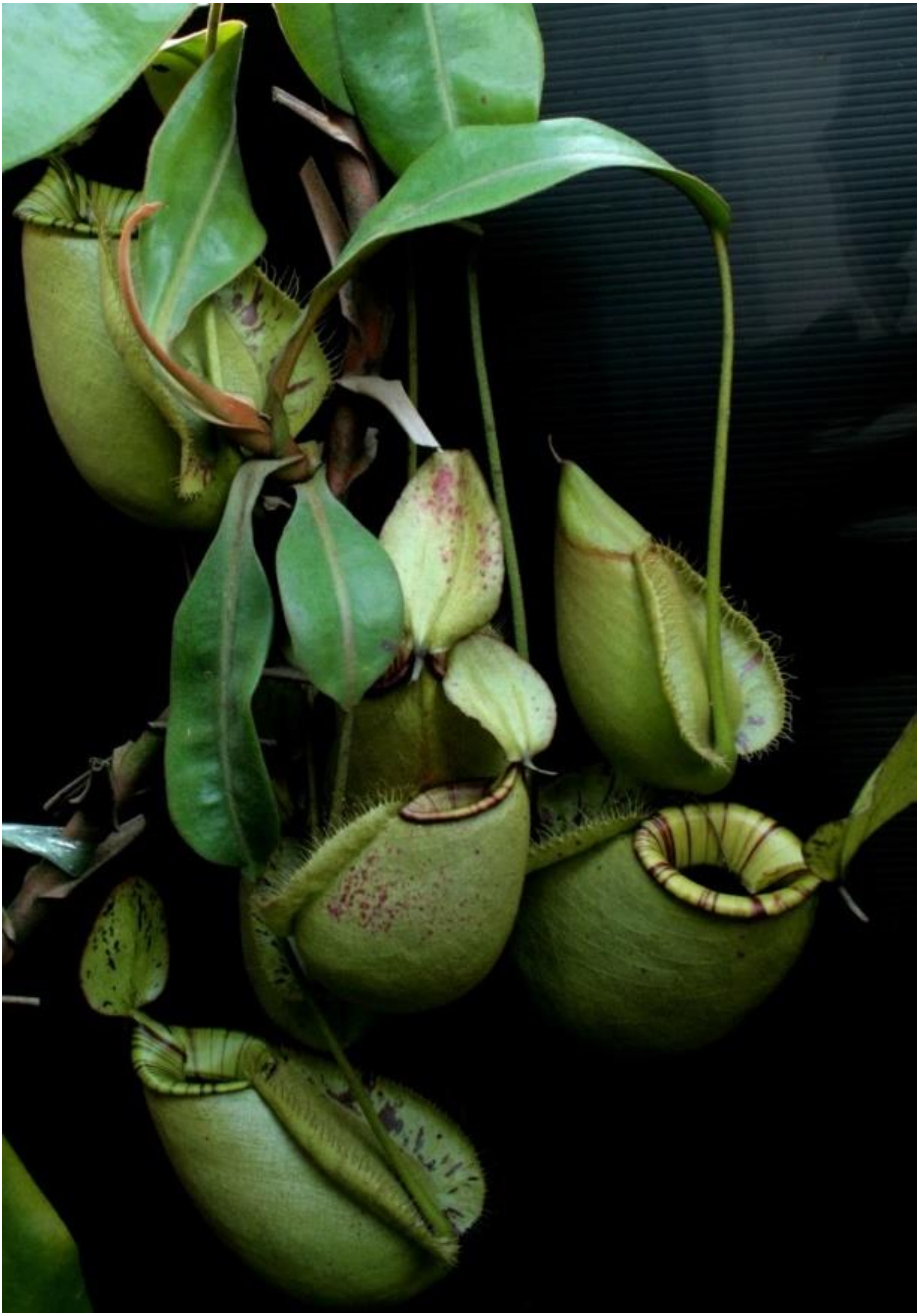

Fig. 2.20. Lower pitchers on a basal shoot of a *Nepenthes* × *hookeriana* plant in a private collection.

Nepenthes × *hookeriana* (= *Nepenthes ampullaria* × *Nepenthes rafflesiana*)

National conservation status: None, because the status is provided only for species and not hybrids.

Distribution: Central Catchment Nature Reserve, Kent Ridge Park, Western Catchment Area.

Description: Once mistaken as a natural species, *Nepenthes* × *hookeriana* occurs commonly in adinandra belukar (secondary forest on degraded soil from poor agricultural practices) and resam-dominated scrubland where populations of its parent species are found growing together. However, *Nepenthes* × *hookeriana* is typically more shade-tolerant than *Nepenthes gracilis* or its hybrids (*Nepenthes* × *trichocarpa* and *Nepenthes gracilis* × *Nepenthes rafflesiana*), and is thus often the only hybrid that persists in the undergrowth of older secondary forests and/or adinandra belukar.

Fig. 2.21. A *Nepenthes* × *hookeriana* lower pitcher in adinandra belukar undergrowth. Location: Kent Ridge Park.

Nepenthes ampullaria is highly variable in pitcher colouration, while *Nepenthes rafflesiana* is highly variable in both pitcher form and pitcher colouration. It is thus of no surprise that hybrids between the two are similarly variable. *Nepenthes* × *hookeriana* is a popular ornamental plant, and a vast diversity of artificially produced hybrids are also readily available from local nurseries or private collectors. WNL's own experience breeding this hybrid suggests that pitcher colouration of the hybrid is often inherited from the *Nepenthes ampullaria* parent, while pitcher form is often strongly influenced by that of the *Nepenthes rafflesiana* parent (WNL, pers. obs.). Natural populations of *Nepenthes* × *hookeriana* have been found to have a prey spectrum intermediate to that of both of its parent species (Peng & Clarke, 2015).

In the field, *Nepenthes* × *hookeriana* may be distinguished from *Nepenthes ampullaria* by its thick, leathery leaves which are sometimes strongly petiolate (all characteristics of *Nepenthes rafflesiana*; Fig. 2.23); and from *Nepenthes rafflesiana* by the presence of a fine brown indumentum on growing tips and young stems (which are characteristics of *Nepenthes ampullaria*; Figs. 2.20, 2.23).

Fig. 2.22. Upper pitchers of two *Nepenthes* × *hookeriana* plants. Location: Kent Ridge Park.

Fig. 2.23. Leaves (left) and shoot (right) of a typical *Nepenthes* × *hookeriana* plant. Notice the thick leathery leaf blades (left), which are characteristic of *Nepenthes rafflesiana*, and the fine brown hairs covering the apical meristem (right), which are characteristic of *Nepenthes ampullaria*. Location: Kent Ridge Park.

Nepenthes gracilis × *Nepenthes rafflesiana*

National conservation status: None, because the status is provided only for species and not hybrids.

Distribution: Presumed locally extinct but more extensive field work may reveal plants of this hybrid growing in the wild in Singapore.

Description: This hybrid is relatively uncommon, perhaps because the vastly differing flower morphologies of *Nepenthes gracilis* and *Nepenthes rafflesiana* greatly limit the probability of shared pollinators between the two (see Chapter 7: Flowering and Reproduction—Hybridisation between *Nepenthes* species). The hybrid has been recorded from Singapore before (Tan et al., 1997), and HTWT had previously observed one plant in Kent Ridge Park, but that individual is now no longer alive.

Nevertheless, *Nepenthes gracilis* × *Nepenthes rafflesiana* is still found in parts of southern Peninsular Malaysia where populations of the two species co-occur (Tan et al., 1997). Pitchers of *Nepenthes gracilis* × *Nepenthes rafflesiana* are intermediate in appearance between both parent species.

The possible extinction of* Nepenthes mirabilis *in Singapore. — *Nepenthes mirabilis* is a widespread species with a large geographic range extending from Northern Australia to Southern China (Clarke, 1997; McPherson, 2009). Although this species is highly adaptable to a wide variety of habitats, it is most often associated with the waterlogged, low-nutrient soils of peat swamp forests (Adam et al., 1992). It is commonly found in the state of Johore in Peninsular Malaysia and is thus believed by some to have occurred naturally in primeval Singapore (Tan et al., 1997; Cheek & Jebb, 2012). However, the freshwater swamp forests in Singapore differ significantly in nutrient levels and ecology from peat swamp forests in Johore or Borneo (Clews et al., 2018), and no herbarium or written records show the presence of *Nepenthes mirabilis* in Singapore. The occurrence of this species in primeval Singapore is thus plausible, albeit unlikely.

Poaching. — Poaching is the biggest threat to many rare *Nepenthes* species worldwide (Lee, 2009; McPherson, 2009, 2011). Many *Nepenthes* species, particularly mountain-growing species, are confined to very small geographic ranges, making them vulnerable to extinction (Clarke, 1997, 2001; Gronemeyer et al., 2014). Fortunately, a significant number of *Nepenthes* habitats are located within designated protected areas, which makes logging and habitat destruction an unlikely cause of extinction (McPherson, 2011). Instead, poaching has been and continues to be the greatest threat to these plants, because of the high value that rare *Nepenthes* species (or colour forms) fetch in the ornamental plant market (Robinson et al., 2009; Cheek et al., 2018; Devi et al., 2019).

Notwithstanding the threat that their horticultural appeal could present, as well as the concomitant issue of habitat clearance eliminating still extant populations, the three *Nepenthes* species native to Singapore are in no significant risk of extinction. All three species and their hybrids have a large geographic range, and are relatively fast-growing species that can establish new populations in novel, disturbed habitats. Furthermore, the value of the three native *Nepenthes* species on the global ornamental plant market is relatively low, in comparison to other, rarer *Nepenthes* species, giving little incentive for poachers to collect and sell wild plants from Singapore's nature reserves. Nevertheless, opportunistic poaching by uninformed park visitors continues to be a significant threat to local *Nepenthes* populations. *Nepenthes ampullaria* and *Nepenthes rafflesiana* are

Fig. 2.24. A *Nepenthes ampullaria* vine from which a stem cutting appears to have been taken. The angle, location (just above the axillary bud) and cleanness of the cut edge suggest that the stem had been removed intentionally for propagation, rather than pruned by maintenance workers or snapped by natural disturbance events (left); the vine was located along a footpath (right), and was thus more susceptible to poaching because of its accessibility to park visitors. Location: Kent Ridge Park.

especially vulnerable to poaching because of their more attractive pitchers (WNL, pers. obs.). Populations of *Nepenthes ampullaria* plants have been completely exterminated from Lower Peirce Reservoir Park, and severely depleted along the trails of Kent Ridge Park by poaching over the last two decades (HTWT, pers. obs.; Fig. 2.24). *Nepenthes* hybrids are also regularly poached from their natural habitats (WNL, pers. obs.). Although hybrids cannot be assessed for a conservation status category because they do not form independent, self-perpetuating populations, the loss of many such plants from parks and nature reserves is a significant loss of biodiversity in Singapore.

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CHAPTER 3: HABITATS

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Vegetation type association. — Plant carnivory is a metabolically costly investment, owing to the morphological and physiological adaptations associated with prey capture and digestion (Givnish et al., 1984; Osunkoya et al., 2007, 2008; Pavlovič & Saganová, 2015). Givnish et al. (1984) observed that carnivorous plants are largely restricted to habitats with high light intensities, high moisture and low nutrient levels. This observation led to the development of the cost-benefit model of plant carnivory (Givnish et al., 1984; Pavlovič & Saganová, 2015), which states that the carnivorous syndrome is only beneficial when the environment allows the benefits of carnivorous nutrient acquisition to outweigh the costs associated with the production of carnivorous organs. Because rainfall is generally high across Southeast Asia, where *Nepenthes* species mostly occur, moisture availability may not be as strong a factor in determining *Nepenthes* species occurrence as compared to light availability and soil-nutrient levels.

Competitive interactions between co-occurring *Nepenthes* species can further limit their occupancies in shared habitats and favour the development of habitat specialisation between sympatric species. This phenomenon is known as resource partitioning, which is a stabilising niche mechanism that allows the coexistence of competing species (HilleRisLambers et al., 2012). Habitat resource partitioning was recently demonstrated in ultramafic montane *Nepenthes* species in Borneo (van der Ent et al., 2015). However, such differentiation has not yet been tested formally among lowland *Nepenthes* species, which tend to be more generalised in habitat use (McPherson, 2009; Schwallier et al., 2016).

Although the three species native to Singapore, viz., *Nepenthes ampullaria*, *Nepenthes gracilis* and *Nepenthes rafflesiana*, are largely sympatric where their geographical ranges overlap (Clarke, 2001), they nonetheless appear to differ slightly in their habitat preferences (Table 3.1). Tropical heath forests (locally known as kerangas; Fig. 3.1) and peat swamp forests, two of the most important habitat types occupied by lowland *Nepenthes* species in Borneo and Sumatra, are not found in Singapore. Instead, local *Nepenthes* populations today tend to be associated with nutrient-poor habitats formed through anthropogenic activities (Tables 3.2, 3.3). For example, one of the most important *Nepenthes* habitats in Singapore is adinandra belukar, a type of secondary forest in Singapore and southern Peninsular Malaysia formed after over-exhaustive agricultural exploitation, which is physiognomically and floristically very similar to tropical heath forests (Sim et al., 1992). *Nepenthes* populations in primeval Singapore may have occurred along steep slopes which were prone to landslides and/or retained insufficient soil for the establishment of large trees (Fig. 3.4).

In the following section, three important *Nepenthes* habitats in Singapore are discussed, viz., resam-dominated vegetation, native species-dominated secondary forests and reservoir or quarry edges.

(i) Resam-dominated vegetation: Vegetation regenerating on clear-felled wasteland within the urban matrix of modern Singapore appears to develop along two stable-state trajectories. Acidic, nitrogen- and phosphorus-limited clayey soils with high aluminium toxicity appear to favour the establishment of resam (*Dicranopteris linearis*)-dominated scrubland (Figs. 3.1, 3.2) and this can subsequently develop into long-lived, pioneer-dominated, albeit species-poor and successionally stagnant adinandra belukar (Sim et al., 1992; Chua et al., 2016) or other forms of native-dominated forests (Chua et al., 2016). On the other hand, nutrient-rich soils resulting from an agricultural legacy permit the establishment of invasive grass and legume species that eventually develop into Moluccan albizia (*Falcataria moluccana*)-dominated waste woodlands (Yee et al., 2016, 2019; Neo et al., 2017).

The former vegetation type, resam-dominated scrubland, is one of the most important habitats for *Nepenthes* species in Singapore.

Table 3.1. Summary of reported habitat preferences (i.e., frequent association) between *Nepenthes ampullaria*, *Nepenthes gracilis* and *Nepenthes rafflesiana* from the literature. Table references: [1]Adam et al. (1992); [2]Clarke (1997); [3]Clarke (2001); [4]McPherson (2009); [5]Tan et al. (1997); [6]Hung et al. (2017).

	Nepenthes ampullaria	***Nepenthes gracilis***	***Nepenthes rafflesiana***
Habitat associations			
Peat swamp forests	✓[1, 2, 3]	✓[2, 3, 4]	
Kerangas (heath) forests	✓[1, 2, 3, 4]	✓[1, 2, 3, 4]	✓[1, 3, 4]
Forest margins		✓[4]	✓[4]
Gaps in primary forests	✓[1]		
Coastal forests		✓[4]	✓[6]
Adinandra belukar	✓[5]	✓[5]	✓[5]
Open areas	✓[1]	✓[1, 3, 4]	✓[1, 2, 3, 4]
Other human-modified landscapes	✓[1, 3]	✓[1, 2, 3, 4]	✓[1]
Soil or vegetation characteristics			
Wet soils	✓[1, 2, 3, 4]	✓[4]	✓[2, 4]
Sandy soils	✓[2, 3]	✓[4]	✓[2, 3, 4]
Clay soils			✓[2, 4]
High vegetation cover	✓[1, 4]		✓[4]

Fig. 3.1. Typical kerangas (heath forest) in Bako National Park, Sarawak, Malaysia. (All photographs in this chapter, unless otherwise stated, by: Weng Ngai Lam).

Table 3.2. Distribution of *Nepenthes* species across the major vegetation types in Singapore.

Vegetation type	**Classification**	**Sub-classification**	**Dominant species**	**Presence of *Nepenthes* species**
Closed-canopy swamp forests	Mangrove forests	-	Rhizophoraceae (*Rhizophora* species, *Bruguiera* species), *Avicennia* species, *Sonneratia* species	Absent
	Freshwater swamp forests	-	Myristicaceae, *Gynotroches axillaris*, *Baccaurea bracteata* (Chong et al., 2018)	*Nepenthes ampullaria* common; *Nepenthes gracilis* and *Nepenthes rafflesiana* sometimes
Closed-canopy dryland forests	Primary lowland or coastal hill Dipterocarp forests	-	Dipterocarpaceae (*Anisoptera* species, *Dipterocarpus* species, *Hopea* species, *Shorea* species)	Absent
	Native species-dominated secondary forests (Yee et al., 2016, 2019)	Late successional forests	-	Present in low densities (often *Nepenthes ampullaria*)—mainly in treefall gaps and patches of disturbance
		Early successional forests—*Trema*-*Mallotus*-*Macaranga* belukar (Tan et al., 2010)	*Trema* species, *Mallotus paniculatus*, *Macaranga* species	Largely absent
		Early successional forests—adinandra belukar (Sim et al., 1992)	*Adinandra dumosa*, *Rhodamnia cinerea*, *Ploiarium alternifolium*, *Cyrtophyllum fragrans*, etc.	Common
	Waste woodland secondary forests (Yee et al., 2016)	-	*Falcataria moluccana*, *Leucaena leucocephala*, *Acacia auriculiformis*, *Cecropia pachystachya*, etc.	Absent
	Reclaimed land forests	-	*Casuarina equisetifolia*	Absent
	Abandoned-land secondary forests (Yee et al., 2016)	Abandoned plantations	*Hevea brasiliensis*	Absent
		Abandoned kampungs or orchards	*Durio zibethinus*, *Nephelium lappaceum*, *Spathodea campanulata*, etc.	Absent

Vegetation type	**Classification**	**Sub-classification**	**Dominant species**	**Presence of *Nepenthes* species**
Open canopy vegetation	Grass-dominated scrubland	Lalang-dominated	*Imperata cylindrica*, *Acacia* species	Largely absent; *Nepenthes gracilis* sometimes
		Mown-lawns	*Axonopus compressus*	Absent
	Resam-dominated vegetation	Resam-dominated scrubland: low-growing, early successional resam scrubland	*Dicranopteris* species (resam), *Lycopodiella cernua*, *Dillenia suffruticosa*	Common
		Resam-dominated patches: tall, arrested successional patches within closed-canopy forests (Chua et al., 2016)	*Dicranopteris* species	Only along margins

Fig. 3.2. Panoramic view of typical resam-dominated scrubland which developed over cleared land at Sembawang Avenue, Singapore.

Resam-dominated vegetation is a habitat in which sunlight is abundant, but where nutrient limitations (Goldsmith et al., 2011; Chua et al., 2016) or allelopathic effects from the resam ferns (Kato-Noguchi et al., 2012) are thought to hinder other fast-growing plants such as grasses or short-lived pioneer tree species from dominating. It is under such conditions that plant carnivory is both possible and worthwhile, given the high metabolic costs incurred by carnivorous investments (Givnish et al., 1984; Pavlovič & Saganová, 2015). Accordingly, in the absence of anthropogenic interventions (e.g., poaching or repeated disturbance events; see section below on Climate change and other human impacts on the *Nepenthes* species of Singapore), resam-dominated scrubland is the single most important habitat type for all three native *Nepenthes* species in Singapore, which grow robustly and flower regularly in such habitats.

Of the three species, *Nepenthes gracilis* is the most dominant in this vegetation type. This fast-growing species is able to recruit quickly on bare ground to exploit open areas and is usually among the pioneer plant species that colonise a recently cleared patch of land (Fig. 3.5). *Nepenthes ampullaria* and *Nepenthes rafflesiana* may also recruit in young resam-dominated scrubland, but they typically take longer to establish sexually reproducing populations. In resam-dominated scrubland, *Nepenthes* species typically grow within the resam fern matrix or scramble over young tree saplings in order to gain access to more sunlight (Fig. 3.6).

Resam-dominated vegetation can also occur as scattered patches within otherwise closed-canopy forests, e.g., persisting in areas which may have been previously cleared or farmed within the native-dominated secondary forests of the Central Catchment Nature Reserve (Fig. 3.7; Chua et al., 2016). These resam-dominated patches appear to be distinguishable from those which develop over the nutrient-poor soils of clear-felled wasteland in the urban environment. Unlike the resam-dominated scrubland which is often populated by saplings of slow-growing pioneer tree species, *Lycopodiella cernua* and *Nepenthes* species, resam-dominated patches within closed-canopy forest often grow much taller and more densely so as to completely exclude other plant species from their matrices (Fig. 3.7; WNL, pers. obs.). The reason for this is still unclear. *Nepenthes* species are occasionally found along the edges of such patches, but never within them (WNL, pers. obs.).

Fig. 3.3. Resam-dominated vegetation along the Mersing highway in southeastern Peninsular Malaysia, an ideal *Nepenthes* habitat created by road construction. Top: flowering *Nepenthes* × *hookeriana* (indicated by arrow) along the vegetation edge. Bottom: *Macaranga heynei* (indicated by arrows), a short-lived pioneer tree species, can sometimes also be found in this type of vegetation. (Photographs by: Chen Liang Fang).

Fig. 3.4. Steep slopes such as those of the Gunung Pulai recreational forest in Johore, Peninsular Malaysia, are ideal habitats for *Nepenthes* species and may have been the main habitat type occupied by these species in primeval Singapore.

Fig. 3.5. *Nepenthes gracilis* plants sprawling across the hot, sandy ground in early successional, resam-dominated scrubland. Location: Sembawang Avenue, Singapore.

Fig. 3.6. *Nepenthes gracilis* vines (arrows indicate the tips of two shoots) climbing over an *Adinandra dumosa* treelet. Location: Holland Woods, Singapore.

Fig. 3.7. A patch of resam-dominated vegetation within the Nee Soon Swamp Forest. *Nepenthes* species are seldom found within the matrices of such patches, although they are occasionally found along the edges.

(ii) Native species-dominated secondary forests: Ecological succession is the natural, predictable change in the composition of an ecological community over time. Resam-dominated scrubland is one of several vegetation types that represents the earliest stages of succession in Singapore (Corlett, 1991). As maturing tree species gradually form a closed canopy, some herbaceous and understorey species may gradually die out when deprived of direct sunlight (Corlett, 1991). Typically, fast-growing pioneer tree species such as *Trema* species, *Mallotus paniculatus* and *Macaranga* species are the first to gain dominance within the canopy layer in early successional forests. Forests in this transitory state of succession are sometimes termed *Trema-Mallotus-Macaranga* belukar (belukar means ‘bush’ or ‘secondary forest’ in Malay) (Tan et al., 2010). In the typical successional trajectory, fast-growing pioneer tree species are eventually replaced by late successional (or ‘tall secondary forest’) tree species and finally by primary forest (or ‘climax’) tree species (Tan et al., 2010). However, in highly degraded sites in which soil nutrients have been exhausted and dispersal limitations prevent the recruitment of late successional tree species, long-lived pioneer tree species may gain dominance in the early successional stages instead, leading to the development of a state of arrested succession (Chua et al., 2016). This is thought to have been the case for the development of the unique forest type known as adinandra belukar (Fig. 3.8), which is named after its most dominant tree species, *Adinandra dumosa*, a long-lived pioneer species (Fig. 3.9) (Sim et al., 1992).

Early successional forests: As semi-woody vines, *Nepenthes* species persist with varying degrees of success in native species-dominated secondary forests. They are typically absent from *Trema-Mallotus-Macaranga* belukar, but may be found in abundance in adinandra belukar (Table 3.2), where *Nepenthes* plants which succeed in reaching the canopy flower and pitcher profusely (Fig. 3.10). Alternatively, they may persist in areas where tree development is hindered, for example, along steep slopes. Leaf litter in adinandra belukar is often very resistant to decomposition and accumulates in thick layers beneath tree canopies (Fig. 3.11; Chua et al., 2016). *Nepenthes* seedlings are unable to recruit under such conditions and young plants which do not succeed in climbing a host tree during the transitional phase from open- to closed-canopy adinandra belukar often perish or enter a languid, pitcherless state in the undergrowth.

Fig. 3.8. Typical adinandra belukar vegetation. Other common adinandra belukar species, such as *Dillenia suffruticosa* (black arrow), *Polyscias diversifolia* (white arrow on the right side of the figure) and *Gahnia tristis* (white arrow on the left side of the figure), are also clearly visible in the photograph. Location: Kent Ridge park.

Late successional forests: *Nepenthes* species are also present in late successional, native species-dominated secondary forests across the Central Catchment Nature Reserve. Such forests may have substrates that are richer in nutrients than in adinandra belukar, and are also more floristically diverse. *Nepenthes* species generally do not fare well in such forests, as strong competition for light greatly suppresses the growth of these weak climbers. Nevertheless, late successional native species-dominated secondary forests tend to be highly heterogeneous environments, and forest gaps associated with uneven terrain, resam-dominated patches or disturbance events such as treefalls are habitats in which *Nepenthes* species can survive.

Fig. 3.9. A flowering branch of *Adinandra dumosa*, also known as tiup-tiup—a species often found growing alongside *Nepenthes* species in resam-dominated scrubland and adinandra belukar. Location: Holland Woods, Singapore.

Fig. 3.10. *Nepenthes gracilis* (two arrows on left side of the figure) and *Nepenthes rafflesiana* (arrow on right side of the figure) pitchering and flowering in the canopy of adinandra belukar. Location: Kent Ridge Park, Singapore.

Fig. 3.11. *Nepenthes ampullaria* and *Nepenthes gracilis* in the undergrowth of old adinandra belukar in Kent Ridge Park, Singapore. Note the thick layer of leaf litter.

Treefall gaps occur naturally when natural senescence, lightning or wind bring down large canopy trees, resulting in canopy openings that permit the establishment of tree saplings and other opportunistic light-demanding species (Fig. 3.12). Treefall gaps are optimal, ephemeral habitats for *Nepenthes* species (Fig. 3.13). However, the establishment of *Nepenthes* species populations in such gaps is often contingent on their being able to persist in a state of suspended, languid growth in the undergrowth in the intervening periods between population establishment and gap creation. This is because *Nepenthes* species more often disperse into treefall gaps through vegetative growth, rather than through seedling recruitment. Existing *Nepenthes* vines in the undergrowth of adjacent vegetation may detect increases in light intensity resulting from a canopy opening and grow in the direction of this light influx. These plants quickly establish themselves in the opening and may multiply vegetatively when their vines come into contact with the bare substrate in the gap (Fig. 3.14). In this regard, *Nepenthes* species do not represent forest gap specialists that persist indefinitely in forests by following treefall gaps in a shifting mosaic landscape (Clark, 1991). Instead, they may be better described as early-successional species which can persist in late successional forests by reliance upon periodic disturbance events. Of the three native species, *Nepenthes ampullaria* appears to be the most tolerant of shade conditions and is thus the species most commonly found in late successional forests of the Central Catchment Nature Reserve (Fig. 3.15).

Fig. 3.12. Tree saplings burst to life in a recent treefall gap. Location: Macritchie Reservoir forest, Singapore.

Fig. 3.13. A large population of *Nepenthes ampullaria* thrives in a gap created by a fallen *Prunus polystachya* tree (left). This treefall created a large canopy opening (right), which evidently led to the accelerated growth of the *Nepenthes ampullaria* in the undergrowth. Location: Nee Soon Swamp Forest, Singapore.

(iii) Reservoir or quarry edges: A final habitat type often inhabited by *Nepenthes* species is reservoir or quarry edges. This habitat type has not been included in Table 3.2 because it does not constitute a distinct vegetation type. This novel habitat type is the result of human modification of the landscape and presents a unique set of habitat factors that are not often naturally available to plants in Singapore.

Many of Singapore's 17 reservoirs, particularly those in the Central Catchment Nature Reserve, were constructed by impoundment—that is, the damming of tributaries to prevent natural outflow of water from the reservoir (Ng et al., 2011). This resulted in the formation of large, open freshwater bodies that had not been present in primeval Singapore. The abandonment of quarries led to the formation of similar, novel freshwater habitats.

The edges of reservoirs or quarries are high-light and -water environments with relatively stable, albeit limited, substrate. This contrasts with natural freshwater swamps or forest streams, which may provide moist substrates, but are typically dominated by tall trees and have acidic, unstable, anoxic substrates. Freshwater swamp tree species are typically unable to exploit the outermost margins of reservoirs or quarries because of the steep drop in the amount of soil leading into these artificial water bodies and the vastly differing abiotic attributes of these edge habitats. The sharp boundary between water and land is thus often exploited by weedy herbaceous and/or climbing species such as *Nepenthes*. *Nepenthes gracilis* (Fig. 3.16) is most commonly found to inhabit this unique habitat and so are *Nepenthes rafflesiana* and *Nepenthes ampullaria*, although to lesser extents (Fig. 3.17).

Fig. 3.14. *Nepenthes ampullaria* basal side shoot in a treefall gap. The main vine (arrows) can be seen running along the soil surface. Location: MacRitchie Reservoir forest, Singapore.

Fig. 3.15. *Nepenthes ampullaria* vines in the undergrowth of a native species-dominated forest. Note the abundance of woody debris surrounding the plants and the dark green colouration of the foliage caused by the low ambient light conditions. Location: Nee Soon Swamp Forest, Singapore.

Fig. 3.16. *Nepenthes gracilis* (indicated by the arrow) with *Dillenia suffruticosa* and *Acacia auriculiformis* along the edge of Little Guilin, an abandoned quarry in Bukit Batok, Singapore.

Fig. 3.17. A *Nepenthes rafflesiana* plant growing along the edge of Upper Pierce Reservoir, Singapore.

***Climate change and other human impacts on the* Nepenthes *species of Singapore*.** — Climate change is often associated with increasing frequencies and intensities of weather pattern extremes (Rosenzweig et al., 2001; Coumou & Rahmstorf, 2012). Dry spells, characterised by increased temperatures and prolonged periods of reduced or no rain, may significantly alter vegetation dynamics in tropical countries like Singapore (Phillips et al., 2009). *Nepenthes* plants are known to abort pitchers and cease pitcher production during dry spells (WNL, pers. obs.; Fig. 3.17). As pitchers may lose a large amount of moisture through the exposed surface of pitcher fluids, pitcher abortion (accompanied by the reabsorption of pitcher fluids in existing pitchers) may be a water conservation response that is activated when plants are water-stressed.

While dry spells are seldom fatal to mature *Nepenthes* plants, their impacts on seedlings and inquiline communities can be severe. A prolonged dry spell in early 2014 resulted in the loss of whole cohorts of *Nepenthes* seedlings and juvenile plants and the complete cessation of pitcher production in all three *Nepenthes* species for almost half a year in all the Kent Ridge Park *Nepenthes* populations (WNL, pers. obs.). Although comparative studies of inquiline communities before and after the dry spell were not performed, the complete loss of suitable breeding habitats is likely to have resulted in the local extinction of many nepenthebiont inquilines. Studies of *Nepenthes ampullaria* and *Nepenthes rafflesiana* inquiline communities across Singapore revealed that those of Kent Ridge Park are depauperate in comparison to those of more drought-buffered populations in Nee Soon Swamp Forest (Lim et al., 2019; Lam et al., in press). Although many other factors may explain the low species richness of Kent Ridge Park's inquiline communities, it is likely that periodic re-occurrences of dry spells such as the 2014 event contribute significantly to this.

Many forested habitats in which *Nepenthes* species are found are located within parks and nature reserves which are protected by Singapore law. Forested habitats are thus expected to remain as refugia for *Nepenthes* populations for decades to come, but this is not the case with resam-dominated

Fig. 3.17. A *Nepenthes rafflesiana* plant aborts its pitchers during a dry spell. Note the rapid browning of the young pitcher in the centre of the photograph; pitchers often dry up more gradually during natural leaf senescence. Location: Mandai Reservoir Forest, Singapore.

Fig. 3.18. A large, sprawling *Nepenthes gracilis* plant on bare ground in a patch of young resam-dominated scrubland. Unfortunately, this plant was destroyed several months after the photo was taken, when the entire site was mown to the ground. Location: Rosewood Drive, Singapore.

scrubland found within the urban matrix. Many such sites have been devastated by poachers (see section on poaching in Chapter 2) and overenthusiastic nature-lovers, or completely eradicated by development. Furthermore, resam-dominated scrubland is highly susceptible to damage by trampling and mowing. Paths made through the resam-dominated scrubland often take months to close up and can significantly modify the microhabitat in ways that are not yet fully understood. Mowing is sometimes done on sites in early successional stages to prevent the development of resam-dominated scrubland, often to the detriment of such vegetation and its plant species (Fig. 3.18).

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CHAPTER 4: HERBIVORY

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Introduction. — The vegetative organs of *Nepenthes* are relatively well defended chemically, by a plethora of secondary metabolites (Aung et al., 2002; Rischer et al., 2002; Shin et al., 2007; Gwee et al., 2014; Van Thanh et al., 2015), and physically, through high densities of fibrous tissue in plant leaves and stems (Choo et al., 1997). Accordingly, herbivory occurs at relatively low rates in the three native *Nepenthes* species, and may be summarised into four categories:

1. Pitcher herbivory by leaf-mining species
2. Leaf herbivory by generalist lepidopterans
3. Seed herbivory by the pitcher blue butterfly
4. Seed herbivory by the Florida pink scavenger

Pitcher herbivory by leaf-mining species. — The larvae of several insect taxa are known to feed by tunnelling through the mesophyll of leaves—a habit known as leaf-mining. Leaf-mining allows insect larvae to feed on plant tissues without exposing themselves to predators present on plant surfaces. Leaf-mining trails are occasionally observed in the pitcher walls of all three *Nepenthes* species (WNL, pers. obs.; Fig. 4.1), and are likely to be caused by the pitcher-miner moth *Phyllocnistis nepenthae* (Lepidoptera: Gracillariidae) (Hering, 1930; Choo et al., 1997). *Phyllocnistis nepenthae* appears to be the only known pitcher-mining species in Singapore, and is reported to lay its eggs on the under surface of the pitcher lid (Choo et al., 1997). The damage caused by leaf-mining organisms is typically minimal (Choo et al., 1997)—pitchers affected by leaf-mining herbivores appear capable of attracting, trapping and digesting prey, albeit at a presumably lower efficiency than pitchers free from herbivory.

Leaf herbivory by generalist lepidopterans. — No leaf herbivores are known to specialise on the foliage of *Nepenthes* plants, and our personal observations have been of generalist lepidopterans such as tussock (Erebidae) and bagworm (Psychidae; Fig. 4.2) moth caterpillars. The damage caused by such species is typically localised and minimal.

Fig. 4.1. Leaf-mining damage caused by the larvae of the *Phyllocnistis nepenthae* moth, in an upper pitcher of *Nepenthes gracilis*. Location: Holland Woods. (Photograph by: Weng Ngai Lam).

Fig. 4.2. The 'bag' of a bagworm moth caterpillar on the leaf of a *Nepenthes ampullaria* pitcher. Bagworm moth caterpillars use silk and plant material to construct a case in which they reside. Location: Holland Woods. (Photograph by: Weng Ngai Lam).

Seed herbivory by the pitcher blue butterfly. — (Text and photographs in this section are adapted from Tan [2010]). The capsules (= fruits) and seeds of *Nepenthes* are often fed upon by a specialist lepidopteran species, the pitcher blue (*Virachola kessuma deliochus*; Fig. 4.3; Chen & Chan, 1997; Clarke, 1997). The pitcher blue is a small butterfly belonging to the Lycaenidae or gossamer-winged butterfly family (Corbet & Pendlebury, 1993; Ek-Amnuay, 2012). It is rarely seen in Singapore and sightings are restricted to the few locations where pitcher plants are found in abundance. The adults are fast fliers. They usually stay high up in the crowns of trees and only make occasional visits to flowers in the vicinity. Larval stages of the pitcher blue feed exclusively on the developing seeds of pitcher plants. All three local *Nepenthes* species, namely, *Nepenthes gracilis*, *Nepenthes ampullaria* and *Nepenthes rafflesiana*, have been found to be larval food plants for the pitcher blue.

Physical description of the adult butterfly: On the upper side, the forewings of the male are mostly black except for a basal patch of shiny blue at the dorsum, while its hindwings are shining blue with the exception of a black costal border and a pale brown dorsum. In contrast, the female is pale blue with broad brown borders on both fore- and hindwings (Figs. 4.3–4.5). On the underside, both fore- and hindwings are pale brown and marked with white striae in similar arrangement as for the four-line blues (*pavana* group of *Nacaduba* species) but without basal striae in the hindwing. Each hindwing has a small tornal lobe, a prominent subtornal orange-crowned black spot in space 2, blue and green metallic scales in space 1b and a white-tipped filamentous tail at the end of vein 2. Each hindwing has a prominent subtornal orange-crowned black spot in space 2, blue metallic scales in space 1b and a white-tipped filamentous tail at the end of vein 2. There is also a small black tornal lobe and a small black marking surrounded by orange and blue metallic scales in space 1a. Like many other lycaenid butterflies, the female has proportionately longer labial palpi (sensory appendages on the sides of the mouth) than the male (Fig. 4.5).

Fig. 4.3. An adult female pitcher blue perching on a capsule of *Nepenthes gracilis*. (This and all subsequent photographs in this chapter by: Horace Tan).

Fig. 4.4. The upper side view of an adult female pitcher blue.

Fig. 4.5. Comparison of adult male (left) and female (right) pitcher blue butterflies. Note that the labial palpi of the female (right) are proportionately longer than those of the male (left).

Eggs of the pitcher blue: Eggs of the pitcher blue are typically laid singly, and rarely in pairs or triplets, at the tip of a capsule (Figs. 4.6–4.8). Occasionally, eggs are laid at the lower portion of the capsule near the calyx. It is not uncommon to see multiple egg-bearing capsules on one cluster of fruits (Figs. 4.6, 4.7). The dome-shaped egg is small (about 0.8–0.9 mm in diameter) with a depressed micropyle. The surface is covered with a reticulated pattern of intersecting raised ridges (Fig. 4.9). When freshly laid, the egg is whitish with a yellowish green undertone (Figs. 4.9, 4.10). Each egg takes about three and a half to four days to hatch.

Fig. 4.6. Eggs (arrowed) at the tip of capsules of *Nepenthes gracilis*.

Fig. 4.7. Eggs (arrowed) at the tip of capsules of *Nepenthes rafflesiana*.

Fig. 4.8. Close-up views of egg-bearing capsules of (a) *Nepenthes gracilis*, (b) *Nepenthes rafflesiana* and (c) *Nepenthes ampullaria*.

Fig. 4.9. Upper view (left) and side view (right) of an egg of the pitcher blue on the stigma of the capsule.

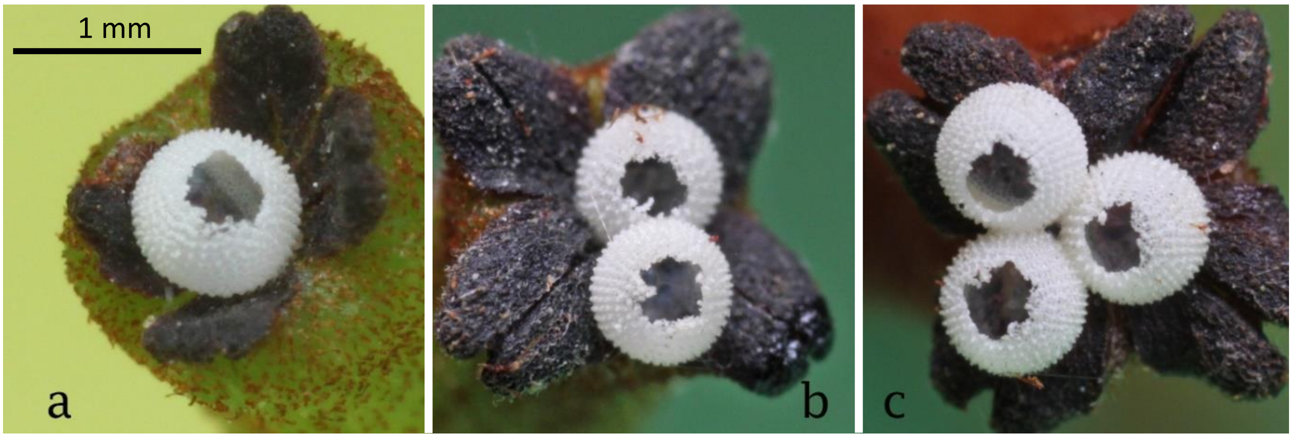

Fig. 4.10. Empty egg shells of the pitcher blue showing varying numbers of eggs laid at the stigmata of capsules.

Larval stages of pitcher blue: The larval stages of the pitcher blue comprise four instars. In each instar, the caterpillar bores through the wall of the capsule and feeds on the developing seeds contained within. Once the entry is made, the caterpillar stays within the capsule to feed and rest until the seeds are depleted. When this happens, the caterpillar moves on to the next capsule. This feeding sequence is only interrupted by moulting events which also take place within the confined space of the capsule. From the 2nd instar onwards, the caterpillar also has a dorsal nectary organ on the dorsum of the 7th abdominal segment. As a result, ants have been observed to attend to the caterpillar when they are outside the capsule (Fig. 4.23).

First instar: The young caterpillar emerges after nibbling away a sufficiently large portion of the egg shell. Measuring 1.1–1.2 mm in length, its pale yellowish-brown body sports long fine setae, a dark brown head capsule and a large and oval shaped prothoracic shield. A dark brown anal plate is also featured with a prominent black spot (Fig. 4.11).

The newly hatched caterpillar makes its way to the side of the egg-bearing capsule and starts to bore into it (Figs. 4.12, 413). After about two days of feeding and growth, it reaches about 3.5 mm in length. It then stays dormant within the capsule for its moult to the 2nd instar.

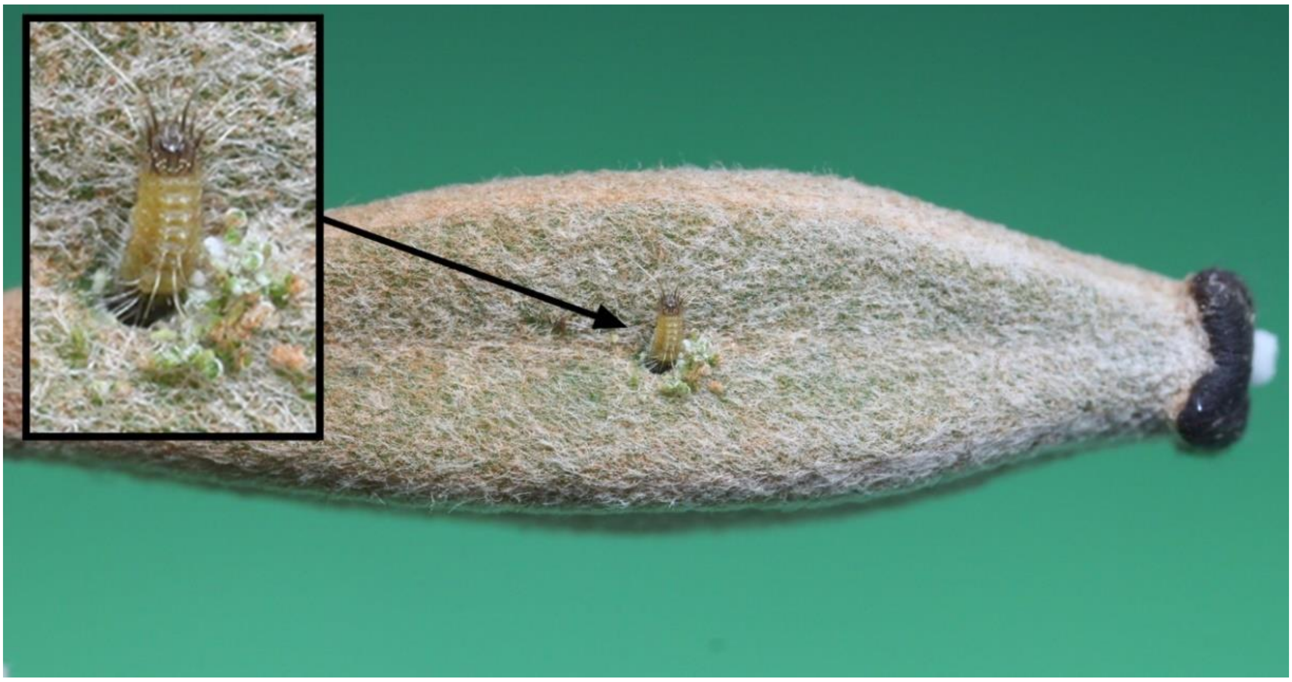

Fig. 4.11. A newly hatched pitcher blue caterpillar boring into a capsule of *Nepenthes rafflesiana*.

Fig. 4.12. A newly hatched pitcher blue caterpillar boring into a capsule of *Nepenthes gracilis* (left). The caterpillar’s body is entirely inside the capsule, leaving only the anal plate visible at the entrance (right).

Fig. 4.13. Side (top photograph) and top (bottom photograph) views of a 1st instar caterpillar of the pitcher blue.

Second instar: Covered with numerous setae, the body of the 2nd instar caterpillar is brown to reddish brown (Figs. 4.14–4.16). The head capsule is yellowish brown in colour and the dark brown prothoracic shield is roughly triangular in shape. The caterpillar grows up to a length of about 5.5–6.0 mm, and after about two days in this instar, it moults again.

Third instar: The prothoracic shield is still dark brown and triangular but now features a short tooth midway along its base (Fig. 4.17). The basal body colour is reddish brown but with lighter colouration on the dorsum of the middle body segments (Figs. 4.18, 4.19). This instar takes about three days to complete, with a body length reaching about 9 mm.

Fourth instar: As in the previous two instars, the 4th (and final) instar caterpillar has numerous body setae and long lateral setae (Fig. 4.20). The body colour is reddish brown to wine red (Figs. 4.20, 4.21). One prominent change from the 3rd instar is seen in the prothoracic shield which has now become pale yellowish, and is bearing large black spots in its four corners. The dorsal nectary organ is rather prominent at this stage (Figs. 4.22, 4.23).

Fig. 4.14. Side (top photograph) and top view (bottom photograph) of a 2nd instar caterpillar of the pitcher blue.

Fig. 4.15. A sequence of photographs showing the boring effort of a 2nd instar caterpillar.

Fig. 4.16. A late 2nd instar caterpillar found inside a capsule, with nearly all developing seeds devoured.

Fig. 4.17. Views of anterior segments of pitcher blue caterpillars in 3rd (a) and 4th instar (b), showing the dark brown prothoracic shields.

Fig. 4.18. Side (top photograph) and top (bottom photograph) views of an early 3rd instar caterpillar of the pitcher blue.

Fig. 4.19. A capsule of *Nepenthes gracilis* opened to reveal a late 3rd instar caterpillar of the pitcher blue.

On the last feeding day of the 4th instar, the first two thoracic segments and 3rd to 5th abdominal segments become much paler in colouration (shades of yellow, orange and even beige), giving the caterpillar a distinctly banded appearance (Fig. 4.24). After four to five days of growth and reaching a maximum length of around 15–16 mm in the final instar, the body of the caterpillar gradually shrinks. The caterpillar ceases eating and wanders around for a pupation site. Its preference is for a previously eaten capsule with sufficient room within to accommodate it. The pupation site could also be a spot on the surface of a leaf. The caterpillar readies itself for pupation by spinning a silk girdle and a silk pad (Figs. 4.25, 4.26).

Fig. 4.20. Two views of an early 4th instar caterpillar of the pitcher blue.

Fig. 4.21. A 4th instar caterpillar feeding through a hole it made on a capsule of *Nepenthes gracilis*.

Fig. 4.22. Anterior segments of a 4th instar caterpillar showing its prothoracic shield (left). Posterior segments showing the dorsal nectary organ (DNO, right).

Fig. 4.23. A 4th instar caterpillar being attended to by an ant, which is likely to be feeding on nectar secreted by the caterpillar's dorsal nectary organ.

Fig. 4.24. A late 4th instar caterpillar. Note the banded appearance.

Fig. 4.25. Side (top photograph) and top (bottom photograph) views of a pre-pupatory caterpillar on a leaf.

Pupal stage of pitcher blue: Pupation takes place after one day of the pre-pupal stage. The slightly hairy pupa has the typical lycaenid shape, yellowish brown in basal colour with variable number of black to dark brown patches on the pupal surface (Figs. 4.26–4.28). The pupa has a length of about 9–10 mm. Eight days later, the pupa becomes darkened, signalling the imminent emergence of the adult (Fig. 4.29). Patches of green and blue can be seen in the wing pads through the pupal skin (Fig. 4.29). The next day, the adult butterfly emerges from the mature pupa (Fig. 4.30).

Fig. 4.26. Side (top photograph) and top (bottom photograph) views of a pupa of the pitcher blue on a leaf.

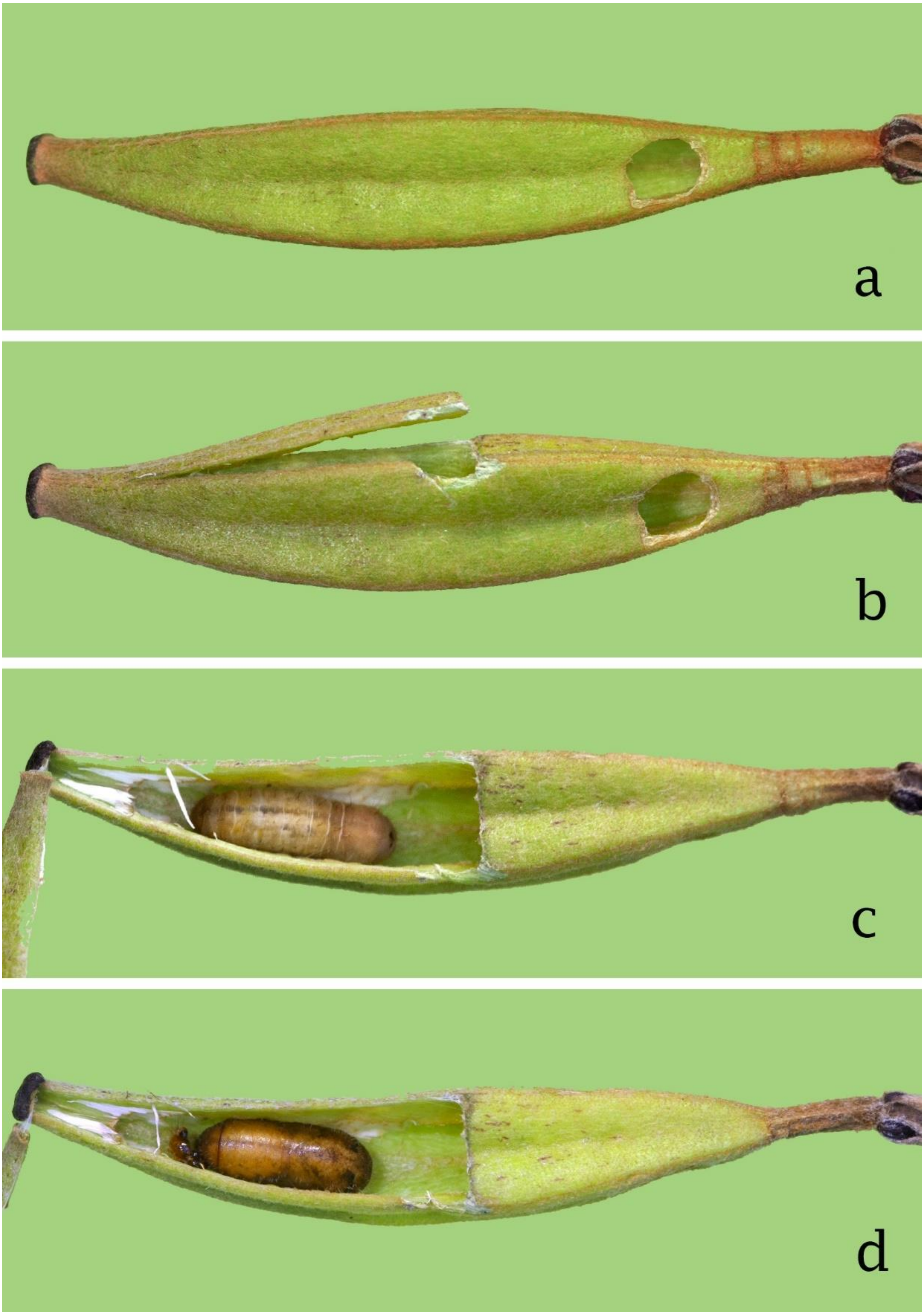

Fig. 4.27. A sequence of photographs revealing the pupation site of a pitcher blue caterpillar within an empty capsule of *Nepenthes rafflesiana*. a, Intact but empty capsule; b, a cut made to lift off a section of the capsule skin; c, a late pre-pupatory caterpillar revealed; d, the pupa a day later.

Fig. 4.28. A pupa-bearing capsule of *Nepenthes ampullaria*. A glimpse of the anterior portion of the pupa through the hole (top). The full view of the pupa after the capsule is split open (bottom).

Fig. 4.29. Two views of a mature pupa of the pitcher blue.

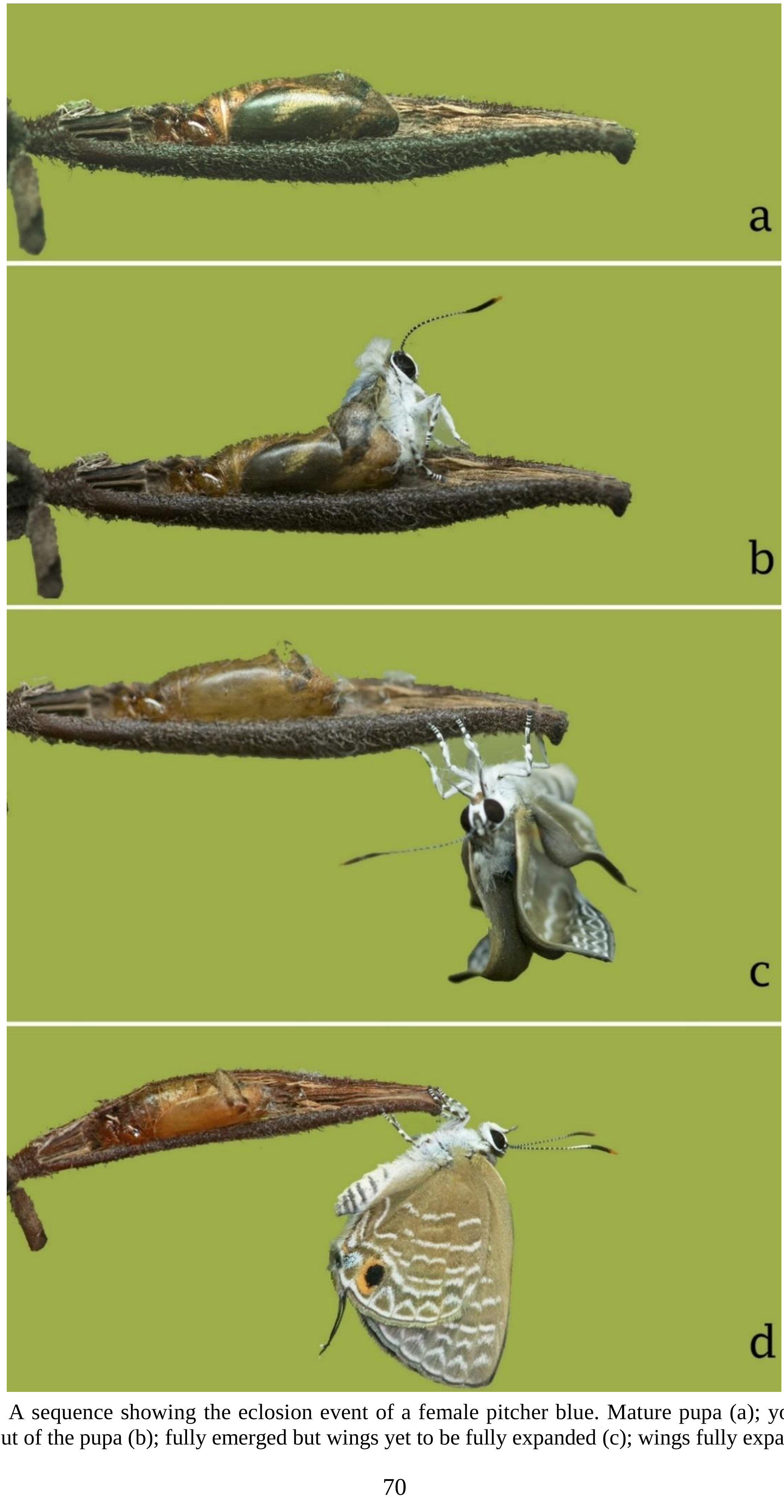

Fig. 4.30. A sequence showing the eclosion event of a female pitcher blue. Mature pupa (a); young adult halfway out of the pupa (b); fully emerged but wings yet to be fully expanded (c); wings fully expanded (d).

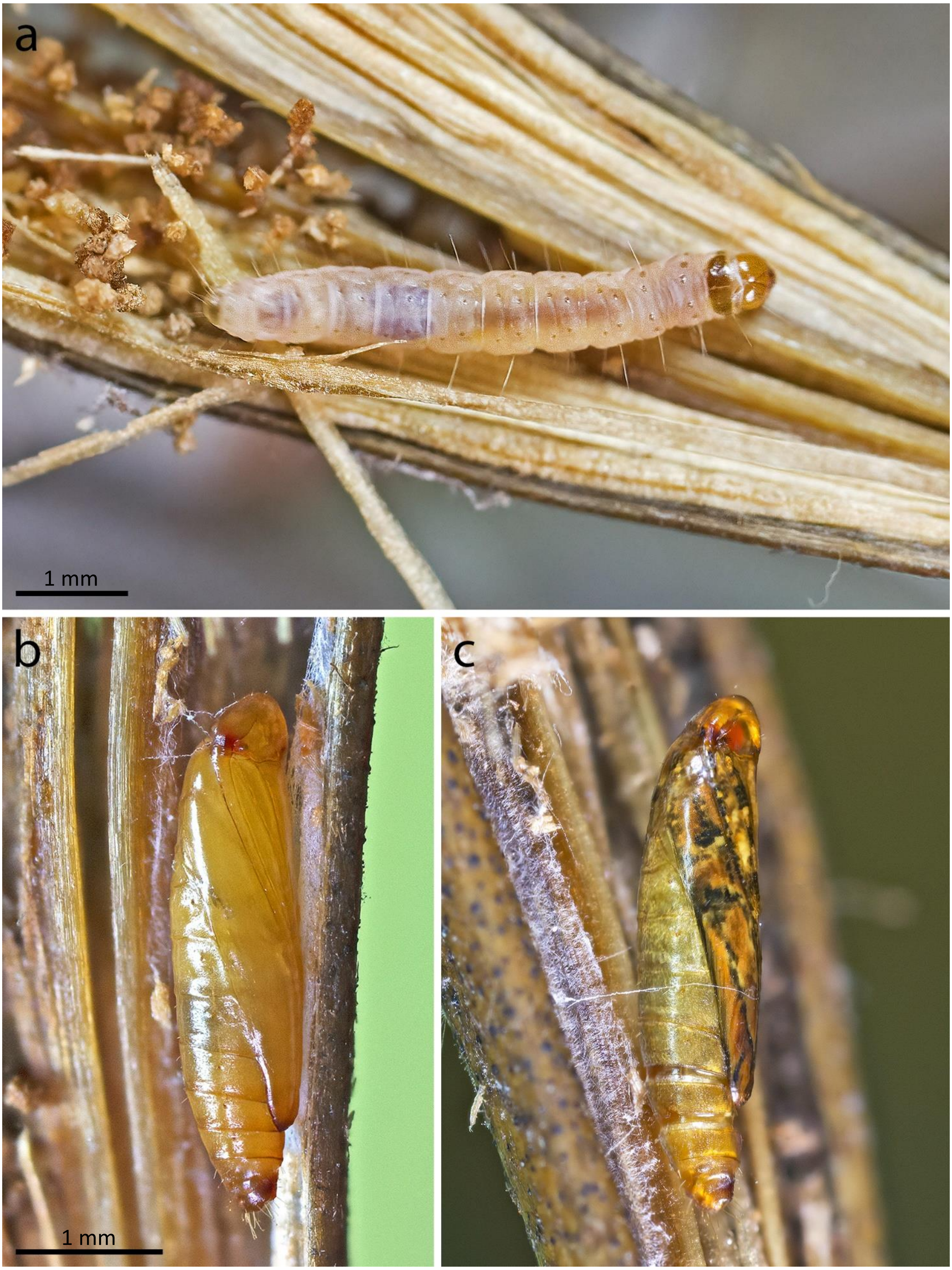

Fig. 4.31. Larva (a) and two stages of pupal development (b, c) of the Florida pink scavenger in a fruit of *Nepenthes gracilis*. Panels b and c share the same scale bar.

Fig. 4.32. Top (a, b), ventral (c) and side (d) views of the eclosed Florida pink scavenger adult. Scale bars represent 1 mm, with panels a and c, and panels b and d, sharing the same scale bars.

Seed herbivory by the Florida pink scavenger. — The Florida pink scavenger (*Pyroderces badia*; Lepidoptera: Cosmopterigidae) is a small moth species known from the southern USA, whose larvae are scavengers on a wide range of commercial fruits and vegetables (Hodges, 1962; Adamski et al., 2006). We observed larvae of this species consuming the seeds of *Nepenthes gracilis* (Fig. 4.31), and the species was identified after these larvae were reared to adulthood (Fig. 4.32). The Florida pink scavenger is likely to be a generalist herbivore which feeds opportunistically on the fruits and/or seeds of *Nepenthes* species. However, the incidence of such herbivory is likely to be low, as we have only observed this on one instance. Furthermore, the Florida pink scavenger may be an introduced species since we know of no records of this species from the region.

The effect of specialist seed predators on **Nepenthes** ***phenology.*** — Despite their negative impact on *Nepenthes* seed survival, specialist seed predators such as the pitcher blue butterfly may play key roles in the maintenance of *Nepenthes* phenology. Like many plant species, *Nepenthes* species demonstrate clear phenological patterns in flowering and fruiting (see Chapter 7: Flowering and Reproduction). Synchronous fruiting is likely to reduce the relative numbers of seeds lost to seed predators because seeds are produced in numbers far greater than predator populations can consume in a given time (a phenomenon known as predator satiation; Rathcke & Lacey, 1985). Furthermore, the prolonged absence of seed resources during the intervening months between flowering/fruiting episodes greatly suppresses specialist predator populations and prevents them from growing to a level where seed predation may exceed seed production. Thus, the presence of specialist seed predators such as the pitcher blue butterfly acts as a selection pressure that may maintain well-synchronised flowering periods within *Nepenthes* species, as individuals flowering before or after peak periods suffer high levels of pre-dispersal seed mortality (Rathcke & Lacey, 1985; Elzinga et al., 2007; Valdés & Ehrlén, 2017).

Indirectly, this may also lead to the reduction of hybridisation success between co-occurring *Nepenthes* species. *Nepenthes* species usually flower at different times of the year (see Chapter 7: Flowering and Reproduction), and hybridisation thus happens only when individual plants flower sooner or later than others of their species, thus coinciding with the flowering time of an individual of a different species. Seed predators such as the pitcher blue ensure that the probability of survival of such seeds are always lower than those produced during the temporally separated peaks in each species' flowering time. The loss or reduction of specialist seed predators like the pitcher blue, presumably due to the loss of nectar sources and/or suitable habitats, is thus one of several possible explanations why *Nepenthes* hybrids are more often found in human-modified landscapes where disturbance events are much higher (Peng & Clarke, 2015).

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CHAPTER 5: PREY AND CARNIVORY

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Introduction. — Carnivorous nitrogen sequestration in pitcher plants occurs as a result of four successional processes: prey attraction, capture, retention and digestion. Each of these processes are facilitated by morphological, physiological and even ecological adaptations of *Nepenthes* pitchers, and are reviewed in the following sections.

Prey attraction. — The first process in carnivorous nitrogen sequestration is the attraction of prey to pitchers, and more specifically, to the prey trapping surfaces of pitchers (Fig. 5.1).

Fig. 5.1. *Iridomyrmex* cf. *anceps* feeding on nectar which has accumulated together with the early morning dew on the underside of a *Nepenthes gracilis* lower pitcher lid. Location: Holland Woods, Singapore. (All photographs in this chapter, unless otherwise stated, by: Weng Ngai Lam).

(i) Visual attractants: The vibrant colouration of pitcher bodies, lids (Fig. 5.2) and peristomes (Fig. 5.3) in many *Nepenthes* species suggest strongly that these organs aid in the visual attraction of certain prey types, but this has never been shown conclusively before. Moran et al. (1999) demonstrated that colours of *Nepenthes rafflesiana* upper pitcher bodies contrasted strongly against colours of their peristomes in three spectral regions (ultraviolet [UV], green and blue regions) corresponding to the trichromatic sensitivity maxima in insects. Contrast patterns of *Nepenthes gracilis* upper pitchers were much less pronounced than those in *Nepenthes rafflesiana*. The authors then compared prey spectra of the two species, and, finding that *Nepenthes rafflesiana* pitchers trapped more moths and flies than *Nepenthes gracilis*, concluded that pitcher peristome–body contrast patterns facilitate the visual attraction of flying prey to pitchers. Moran (1996) did similarly using upper pitchers of the closely-related *Nepenthes rafflesiana* (in which UV contrast patterns were strong) and *Nepenthes hemsleyana* (in which UV contrast patterns were weak) to infer that UV contrast patterns aided in prey capture in *Nepenthes rafflesiana*. While the conclusions of these authors are plausible, the use of pitcher prey contents for the comparison of mechanisms of attraction is clearly inappropriate, since only a fraction of attracted pitcher visitors are successfully trapped and retained by pitchers. Later studies also proved that traits such as pitcher fragrance and fluid viscoelasticity—which *Nepenthes gracilis* and *Nepenthes hemsleyana* lack—contribute to the attraction, capture and retention of large numbers of both crawling and flying prey in *Nepenthes rafflesiana* upper pitchers (Di Giusto et al., 2008, 2010), thus showing that observations of pitcher prey spectra alone cannot be used to determine the effect of visual or UV contrast patterns on prey attraction.

Fig. 5.2. The lid of a *Nepenthes rafflesiana* lower pitcher, as viewed from below (left) and from above (right). Nectaries are clearly visible as small black dots (a few of these arrowed) on the underside of the lid (left).

Fig. 5.3. The peristome of *Nepenthes rafflesiana* is often brilliantly coloured, with peristome teeth that extend several millimetres beyond its inner margin, and present a formidable barrier to prey attempting to escape from pitchers. Visible also in this image is the nectar that has accumulated between the peristome ridges.

Nevertheless, it is still possible that *Nepenthes* pitchers rely on visual attractants to increase the rates of pitcher visitation by potential prey species. One of the strongest pieces of evidence for this is the phenomenon of pitcher phototropism or light orientation (Fig. 5.4). Many *Nepenthes* pitchers orientate to face the direction of a directional light source (e.g., an opening in the undergrowth). Of the three *Nepenthes* species native to Singapore, the lower pitchers of *Nepenthes gracilis* and *Nepenthes rafflesiana*, which are often found in low-light environments of the undergrowth, are known to display this phenomenon (WNL, unpublished data). Upper pitchers of these two species, which often occur in high-light environments, do not display this phenomenon, and neither do the semi-detritivorous pitchers of *Nepenthes ampullaria*. It is thus possible that there is an adaptive function for the orientation of pitchers towards a light source and, since pitchers do not fulfil a photosynthetic function (Pavlovič & Saganová, 2015), the most likely explanation for this is that pitchers are more visually attractive to prey or ovipositing inquiline mutualists when thus oriented.

Fig. 5.4. *Nepenthes rafflesiana* lower pitchers on a dark forest floor naturally orientate to face the direction of the strongest light source—in this case a small opening in the canopy above. This is suggestive of an adaptive visual attraction mechanism in pitchers, which is most effective when light falls on pitchers from their fronts. Location: Kent Ridge Park.

The peristomes and pitcher fluids of *Nepenthes* have also been shown recently to emit a blue fluorescence under UV excitation (Kurup et al., 2013). Fluorescence is the emission of light—in this case blue light with 430–460 nm wavelength—by a substance upon absorption of light of a different wavelength—in this case near-UV radiation with 366 nm wavelength. Kurup et al. (2013) found that the substance responsible for the fluorescence could be removed by dichloromethane and methanol, but not water or *n*-hexane. The authors attempted to eliminate peristome fluorescence in field tests with *Nepenthes khasiana* pitchers by masking peristomes with an acetone extract of pitcher inner walls, or by completely trimming off the peristome, and found this to drastically reduce prey capture. While it is likely that peristome fluorescence contributes in some ways to prey attraction, the highly significant results of the field study experiments of Kurup et al. (2013) must be interpreted with caution, since peristome removal vastly alters many other prey attraction and capture attributes of pitchers, while acetone masking eliminates the scent and nectar of pitchers (WNL, pers. obs.), in addition to masking their fluorescent emissions. In our experience, the application of acetone to *Nepenthes* pitcher peristomes also leads to rapid pitcher senescence.

Some authors consider the circular mouths of pitchers similar to the circular appearance of most flowers, particularly the capitula of Asteraceae species. Nevertheless, Batesian mimicry is an unlikely explanation for such a similarity, since such flowers are not frequently found in the habitat of *Nepenthes* species (Joel, 1988; Moran, 1996). Instead, Biesmeijer et al. (2005) suggested that circular, 'dark centre' contrast patterns formed by floral guides, stingless bee nest entrances and pitcher plants represent convergent evolution of visual stimuli meant to exploit the innate attraction of certain Hymenoptera towards such visual cues. Although an attractive suggestion, such an argument vastly oversimplifies the visual appearance of pitchers. Our own observations suggest that the waxy inner surfaces of pitchers, which have been overlooked in all the studies cited above, are actually the most UV-reflective surfaces in pitchers (Fig. 5.5), and since these surfaces are clearly visible from the front view of a pitcher, insects may see pitchers as a 'bright centre' rather than a 'dark centre' as supposed by Biesmeijer et al. (2005) (Fig. 5.5b, d). It is possible that the visual appearance of pitchers simply accentuates their visibility and recognisability, without constituting any form of mimicry or sensory exploitation. Furthermore, the nectar of some pitcher plants has been shown to be UV absorptive (Joel et al., 1985), and this may well constitute an honest signal of food availability to potential prey (Bennett & Ellison, 2009). Although pitchers invest in adaptations that seem to maximise their visual appeal to potential prey taxa, the exact mechanisms of prey visual attraction to pitchers remain poorly understood and should be an area of future research.

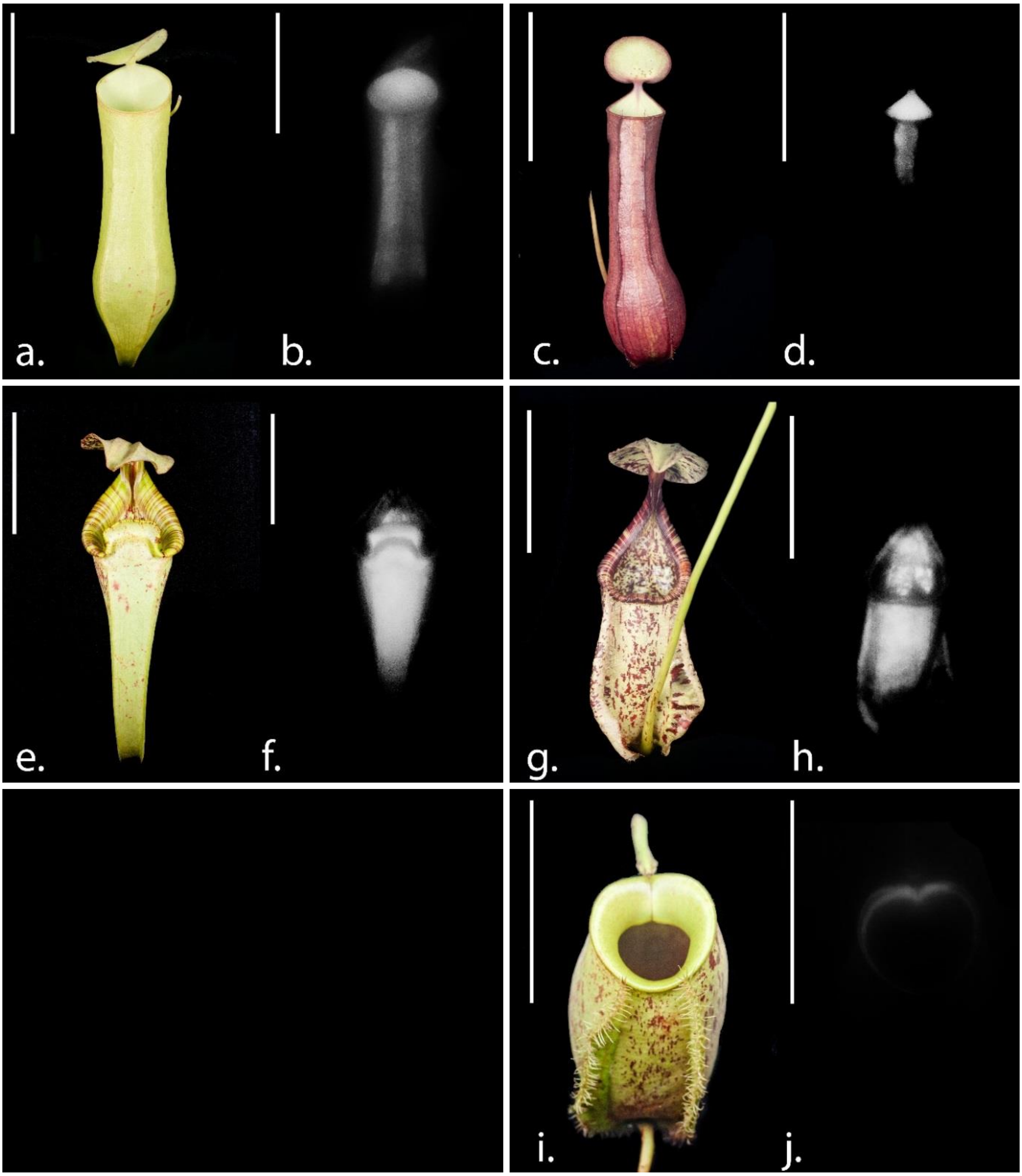

Fig. 5.5. Front views of upper (left) and lower (right) pitchers of the three native *Nepenthes* species, *Nepenthes gracilis* (a–d), *Nepenthes rafflesiana* (e–h) and *Nepenthes ampullaria* (i, j). Pitchers were first photographed with visible light (supplied by an on-camera flash; a, c, e, g, i), then with UV light only (b, d, f, h, j). Note that *Nepenthes ampullaria* (i, j) and *Nepenthes rafflesiana* (e–h) pitchers pictured do not possess wax crystals on their inner surfaces. Scale bar represents 5 cm. (Photographs by: Chui Shao Xiong and Joshua Sim).

(ii) Olfactory attractants: Pitcher scents have been shown to play an important role in attracting potential prey species to pitchers (Di Giusto et al., 2008; Di Giusto et al., 2010). Among the three native *Nepenthes* species, *Nepenthes rafflesiana* upper pitchers are the most strongly scented, while the scent of their lower pitchers is often weaker, and those of *Nepenthes gracilis* and *Nepenthes ampullaria* are often too weak to be detected (WNL, pers. obs.). Di Giusto et al. (2010) showed using gas chromatography-mass spectrometry that the peristomes of *Nepenthes rafflesiana* pitchers from Borneo were the main sources of olfactory attractants. These volatile compounds are most likely found in the pitcher nectar, which is produced most abundantly at the peristomes (Di Giusto et al., 2008; Bauer et al., 2009, 2016; Di Giusto et al., 2010). Both upper and lower pitchers emit a large spectrum of volatile compounds, many of which are also found in floral scents. However, upper pitchers emitted volatile compounds in 20-fold greater quantities, and with significantly greater diversity, than lower pitchers. The authors also demonstrated that the scent of *Nepenthes rafflesiana* upper pitchers facilitates the attraction of a diverse spectrum of potential prey taxa, although only a fraction of these were successfully trapped by pitchers. Previous researchers (Moran, 1996; Di Giusto et al., 2008) had suggested that pitcher fragrance increases the attractiveness of pitchers to flying prey taxa, but many ant taxa also respond to floral scents (Rostás & Tautz, 2011), and may be similarly attracted to pitchers by their fragrance. Some researchers have even observed that *Nepenthes rafflesiana* in Brunei emit significantly different scents during the day and during the night (U. Bauer, pers. comm.), suggesting that olfactory attraction may be somewhat targeted towards prey which forage at different times of the day.

Additionally, recent studies (Baby et al., 2017) showed that *Nepenthes* pitchers constantly emit carbon dioxide (CO_2) gas, which may act as a gaseous chemical attractant for flying insect prey, such as lepidopterans, which oviposit selectively on fruits (which respire rather than photosynthesise) (Sage, 2002). CO_2 may be emitted as a by-product of trap respiration (Baby et al., 2017), prey decomposition and/or inquiline respiration.

(iii) Nectar: Extrafloral nectaries are found on many above ground parts of the *Nepenthes* plant (Merbach et al., 2001; Fig. 5.6), and the nectar reward supplied at these sites is a key attractant in pitchers (Bennett & Ellison, 2009). Nectar is often produced most abundantly on the inner edge of the peristomes of pitchers (Merbach et al., 2001; Bauer & Federle, 2009; Bauer et al., 2016; Horner et al. 2018), but in *Nepenthes gracilis*, a large amount of that nectar is produced on the underside of pitcher lids (Bauer et al., 2012; Fig. 5.1). Bauer et al. (2009) observed that newly opened upper pitchers of *Nepenthes rafflesiana* produced very little nectar, but that nectar secretions

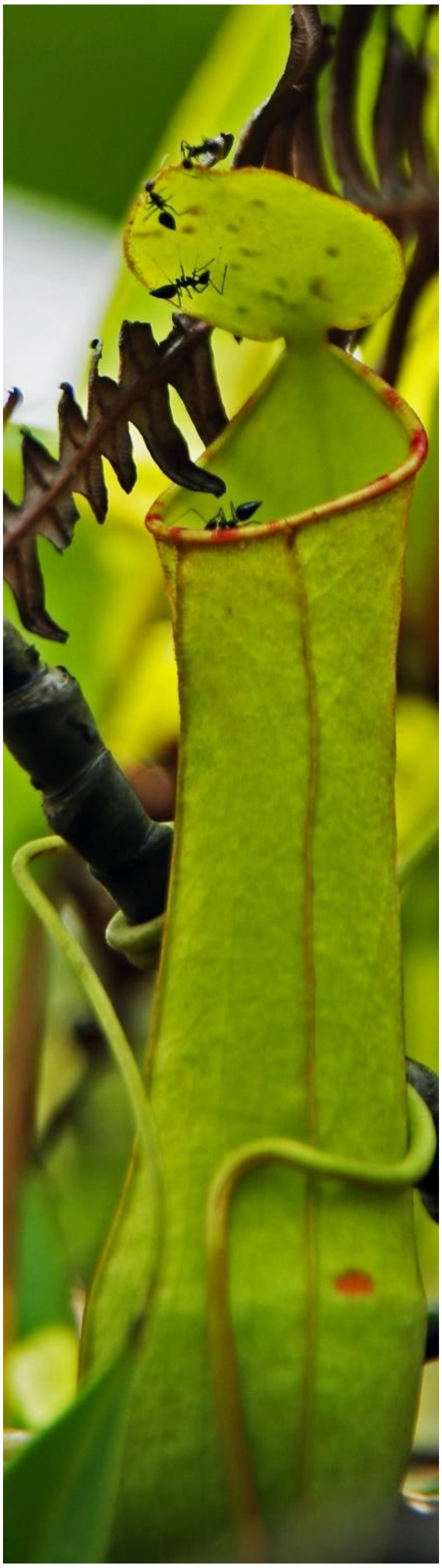

Fig. 5.6. *Prenolepis* cf. *jerdoni* ants feeding on nectar secreted by a *Nepenthes gracilis* upper pitcher. The large black extrafloral nectaries are clearly visible on the underside of the pitcher's lid. Location: Kent Ridge Park, Singapore.

Fig. 5.7. Only a small fraction of pitcher visitors is successfully trapped by *Nepenthes* pitchers. Many, like this *Philidris* species worker feeding from the rear edge of the peristome of a *Nepenthes rafflesiana* upper pitcher, successfully consume pitcher nectar without even endangering their lives. Unexpectedly, studies have shown that low but stochastic prey trapping efficiencies can actually lead to higher eventual total prey-capture numbers.

increased steadily over the first two weeks of the pitchers' lives. As only a fraction of pitcher visitors becomes trapped by them, it is likely that prey species may learn to associate *Nepenthes* pitchers with the nectar reward they offer.

Prey capture. — Although a significant amount of nectar can be harvested without endangering the safety of pitcher visitors (Fig. 5.7), pitcher visitors are often forced to position themselves precariously in order to obtain the richest nectar collections. Prey, which slip off these surfaces, fall directly into pitcher fluids below. Prey capture encompasses the process by which prey individuals fall into pitcher fluids, and may occur through four groups of mechanisms, as described below.

(i) Peristome-assisted prey capture: The primary trapping surface of most *Nepenthes* species is the peristome. When dry, the peristome is actually a relatively secure foothold for pitcher-visiting arthropods (Bohn & Federle, 2004; Bauer et al., 2008). However, this changes completely when the peristome is wet, as the microstructure of its hydrophilic, micro-rough surface causes water to spread evenly across the grooves between peristome ridges, forming thin, homogeneous films of water across the peristome surfaces that prevent the adhesive pads of arthropod tarsi from making the necessary contact with peristome surfaces (Bohn & Federle, 2004; Bauer & Federle, 2009). This wetness-dependent characteristic of pitcher peristomes means that peristomes are sometimes extremely slippery, and sometimes hardly so. Condensation (e.g., dew), rain or extrafloral nectar secretions may wet and thus 'activate' pitcher peristomes, while dry weather and windy conditions may result in their deactivation (Bohn & Federle, 2004; Bauer et al., 2008). The presence of sugar-rich, hygroscopic nectar on peristomes also enhances surface wetting by condensation (Bauer & Federle, 2009). Importantly, intermittent activation and deactivation of peristomes has been shown to help pitchers better exploit the foraging-recruiting behaviours of ants, which are primary prey sources for many lowland *Nepenthes* species (Bauer et al., 2015a). This is because most foraging ants do not actively seek out novel food sources, but instead follow pheromone trails laid down by scout ants which had earlier discovered a new food source. Peristomes that are 100%-effective would capture all visiting ant scouts, but would never succeed in trapping the bulk of pheromone-recruited foraging ant workers. In contrast, dry, non-slippery peristomes allow scout ants to survive and thus lay pheromone trails from the pitcher to the ant colony, resulting in the mass recruitment of large numbers and continuous streams of foraging ants. Pitchers have been shown to trap ants in 'batch-capture' events when their peristomes are intermittently activated by rain, condensation or nectar secretions (Bauer et al., 2015a). This unpredictable, batch-capture phenomenon has been shown to result in higher overall capture rates in the long run (Bauer et al., 2015a), and may also allow the sustenance of a mutualism between the ant colony and pitcher plant, as workers succeed in bringing large volumes of pitcher nectar back to the ant colony (Joel, 1988; Lam & Tan, 2018).

(ii) Lid-assisted prey capture: It has been shown that some species, such as *Nepenthes gracilis*, may also catch prey by means of a lid that is transformed into a 'torsional spring system' when hit by rain drops (Bauer et al., 2012; Bauer et al., 2015b). Prey species often feed on the underside of *Nepenthes gracilis* pitchers' lids (Fig. 5.8). Rain is common in the tropical rainforest and tropical monsoon climate zones across *Nepenthes gracilis*' native range, and the fortuitous impact of a rain droplet on the lid, which is stiff yet flexible at its neck, propels

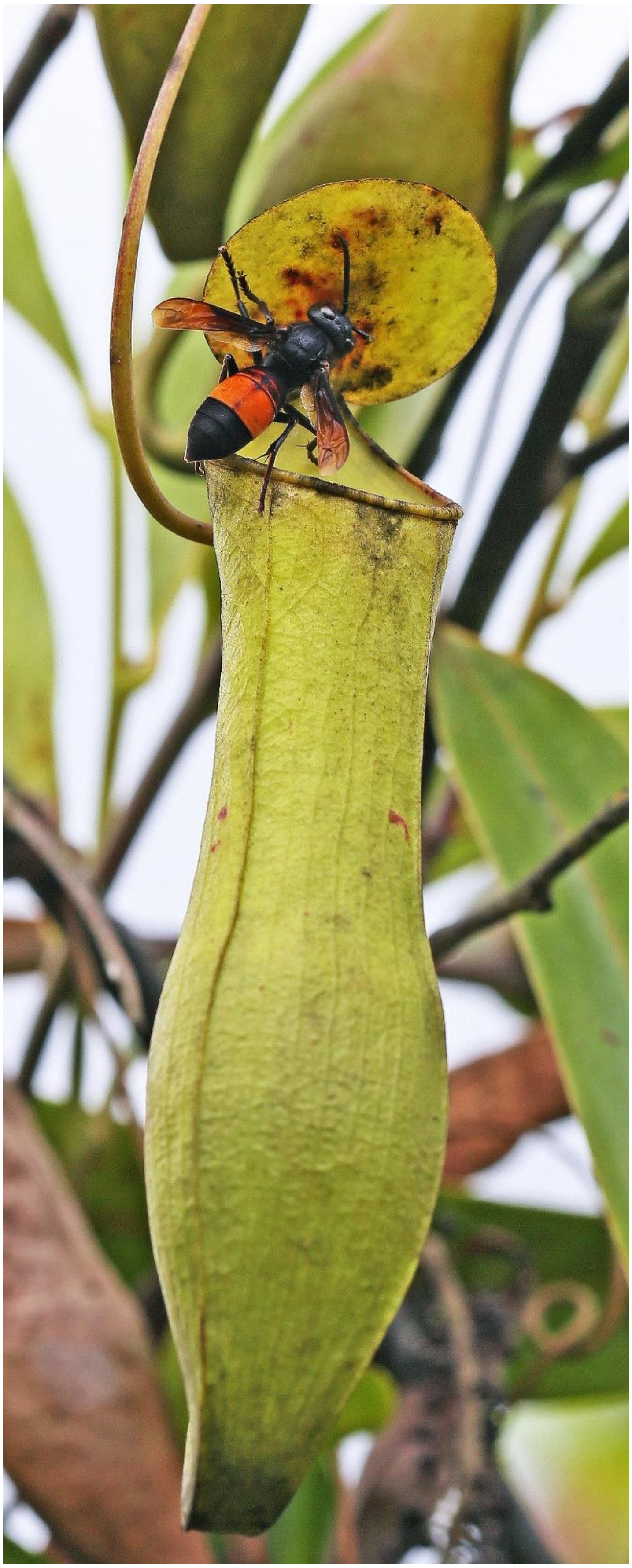

Fig. 5.8. A *Vespa affinis* hornet feeding on the underside of the lid of a *Nepenthes gracilis* upper pitcher. Location: Kent Ridge park, Singapore.

prey directly into pitcher fluids below. The researchers had observed a beetle being trapped in such a manner while hiding under its lid when it began raining, and were thus led to examine the potential trapping function of this pitcher organ. Evidence for such a function is found in (i) the presence of friction-reducing wax crystals on the underside of the lid; (ii) a relatively higher number of extrafloral nectaries found on the underside of the lid; and (iii) a relatively stiff lid in comparison to that of other *Nepenthes* pitchers. It remains unclear if this trapping mechanism is functional in other species of *Nepenthes*, although this is unlikely in the case of *Nepenthes rafflesiana* (whose lid does not possess the physical properties necessary for such a mechanism), or *Nepenthes ampullaria* (whose lids are reflexed backwards).

(iii) Inquiline-assisted prey capture: Finally, prey capture can also be facilitated by pitcher inquilines. Inquilines are specialised organisms which live within pitchers (see Chapter 6: Inquilines). Two crab spider species (Thomisidae), *Thomisus nepenthiphilus* and *Misumenops nepenthicola*[1], inhabit the pitchers of *Nepenthes gracilis* locally (Fig. 5.9). Most crab spiders are ambush predators which capture prey with their characteristically enlarged forelegs and potent venom (Morse, 1981), and *Thomisus nepenthiphilus* and *Misumenops nepenthicola* are no exception. Both species ambush potential pitcher prey as these forage at the mouths of pitchers (Fig. 5.10), just as flower crab spiders ambush flower-visiting insects as they forage at flowers. In doing so, crab spiders significantly increase the probability that pitcher visitors are caught (Lim et al., 2018; Lam & Tan, 2019). The spiders typically drag captured prey items into pitchers to feed, eventually dropping these carcasses into the pitcher fluids below (Lim et al., 2018). The feeding behaviour of crab spiders thus increases pitcher prey-capture rates, but lowers nutrient values of individual prey (Lim et al., 2018). Such a trade-off is beneficial for the *Nepenthes gracilis* host when levels of prey in the environment are low (Lim et al., 2018) and when the prey caught by spiders are large, and infrequently trapped by pitchers (Lam & Tan, 2019). Both of these criteria are likely to be fulfilled in most natural contexts, suggesting that the *Nepenthes gracilis*–crab spider relationship is a generally mutualistic one (Lam & Tan, 2019). The biology of *Nepenthes* inquiline crab spiders is discussed in greater detail in Chapter 6: Inquilines.

Crab spiders are not the only inquiline taxa to assist *Nepenthes* pitchers in prey capture. In Borneo, the predatory inquiline ant, *Colobopsis schmitzi*, is known to do the same for pitchers of *Nepenthes bicalcarata*, which it inhabits (Bazile et al., 2012). However, *Colobopsis schmitzi* individuals are likely to have a greater impact on prey retention in their attacking of prey that have already fallen into pitchers (Bonhomme et al., 2011a), than on prey capture per se (see the section below on Inquiline-assisted prey retention). It is likely that many more cases of prey capture mutualisms remain to be discovered between *Nepenthes* and its inquilines, as inquiline species are generally poorly studied in comparison to their plant hosts.

(iv) Other prey capture mechanisms: Other structures may also contribute directly or indirectly to prey capture in pitchers. Most notably, the waxy inner wall and viscoelastic property of fluids may also play minor roles in prey capture, even if the fluids' key function may be in the retention rather than direct capture of prey (see the section below on Prey retention). For example, ants sometimes wander from the peristome onto the inner surfaces of pitchers, from which they lose their foothold and fall into the pitcher fluids.

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[1] *Misumenops nepenthicola* is also known as *Henriksenia labuanica* (Striffler & Rembold, 2009), although this name has not been accepted in the World Spider Catalog (http://www.wsc.nmbe.ch/).

Fig. 5.9. Female *Thomisus nepenthiphilus* (left) and *Misumenops nepenthicola* (right) waiting in typical ambush posture for visiting prey under the peristomes of a *Nepenthes gracilis* upper (left) and lower (right) pitcher. Location: Holland Woods, Singapore.

Fig. 5.10. *Thomisus nepenthiphilus* (arrowed) in a *Nepenthes gracilis* upper pitcher with a freshly killed flesh fly (Sarcophagidae) in its grasp. Location: Holland Woods, Singapore.

Prey retention. — Captured prey may take several minutes to several hours to drown in the fluids of *Nepenthes* pitchers. Prey appear to perish more quickly in more acidic fluids (Bazile et al., 2015). But while they are still alive, prey typically make numerous attempts to escape from pitchers. Several pitcher adaptations hinder such an escape, and these may be divided into three different categories.

(i) Viscoelastic pitcher fluids: The fluids of several *Nepenthes* species are highly viscoelastic (Gaume & Forterre, 2007; Di Giusto et al., 2008; Bonhomme et al., 2011b; Bazile et al., 2015). It is likely that pitcher fluids in such species are composed of a polysaccharide-based hydrogel (Huang et al. 2015)—the same composition that gives rise to flypaper-trap stickiness in the distantly related sundews (*Drosera* species) (Gaume & Forterre, 2007; Adlassnig et al., 2010). Viscoelastic pitcher fluids stick to and thus exhaust drowning prey as they struggle to escape (Gaume & Forterre, 2007; Di Giusto et al., 2008), and is surprisingly effective even when diluted by rainwater (Gaume & Forterre, 2007).

Although many *Nepenthes* species have been shown to possess viscoelastic pitcher fluids (Bauer et al., 2011; Bonhomme et al., 2011b), fluids of the three native *Nepenthes* species lack viscoelasticity (WNL, pers. obs.). This is surprising, since studies conducted in Borneo have repeatedly demonstrated the viscoelastic properties of *Nepenthes rafflesiana* fluids. Even when grown under identical (ex situ common garden) conditions, pitcher fluids of Singapore *Nepenthes rafflesiana* and Borneo *Nepenthes rafflesiana* contrast starkly in viscoelasticity, suggesting that the cause for this difference is genetic, and not environmental. One possible reason for this is that the absence of ecologically similar competitor species (e.g., *Nepenthes hemsleyana* and *Nepenthes mirabilis*) from Singapore may relax competitive pressures on Singaporean *Nepenthes rafflesiana* populations (Lam et al., 2018), resulting in the loss of pitcher traits otherwise important for prey resource partitioning in Bornean *Nepenthes* communities.

(ii) Waxy inner surfaces of pitcher walls: The inner surfaces of most pitchers may be divided into two sections, glandular and waxy surfaces (Fig. 5.11), although waxy surfaces are not present in all species (they are absent from *Nepenthes rafflesiana* upper pitchers and from *Nepenthes ampullaria* pitchers; Fig. 5.12). The glandular surfaces typically occupy the lower portions of the pitcher body, and are studded with black glands which are visible to the naked eye (Figs. 5.11, 5.12). Numerous microscopic epicuticular wax crystals, which are composed primarily of long-chain aldehydes and primary alcohols, cover the surface of the waxy zone, being arranged with their sharp edges facing outwards (i.e., towards the pitcher cavity) (Gaume et al., 2002; Riedel et al., 2007). At the subcellular level, these wax crystals create a surface that is microscopically rough, reducing the real contact area that can be gained between arthropod adhesive pads and pitcher-wall surfaces, and thus greatly hinder pad adhesion of arthropod prey attempting to haul themselves out of pitcher fluids (Gorb et al., 2005; Scholz et al., 2010). At the cellular level, downward-pointing idioblasts (also known as ‘lunate cells’, owing to their crescent shape) offer poor contact edges for arthropod claws to gain traction against (Gaume et al., 2002; Poppinga et al., 2010). Another proposed mechanism for wax crystal-mediated slipperiness is that wax crystals detach easily from the inner wall, contaminating insect adhesive pads and thus rendering them ineffective (Gaume et al., 2002; Gaume et al., 2004; Gorb et al., 2005). However, this proposal has been challenged by more recent investigations (Scholz et al., 2010).

It has been observed (Bonhomme et al., 2011b) that most *Nepenthes* species possess either viscoelastic fluids or waxy inner surfaces, but rarely both. This suggests that these two adaptations are functionally redundant, as both facilitate prey retention in pitchers. Incidentally, the lower pitchers of Singapore *Nepenthes rafflesiana* lack viscoelastic pitcher fluids which their Bornean counterparts possess, but Bornean *Nepenthes rafflesiana* lower pitchers lose their waxy inner surfaces quickly as they mature (most juvenile plants produce wax-less pitchers by the time they are 30 cm in height [Di Giusto et al., 2009]), while those in Singapore do so at a significantly slower rate (many large plants still possess narrow zones of wax; WNL, pers. obs.; M. Scharmann, pers. comm.; Fig. 5.11).

Fig. 5.11. Waxy (W) and glandular (G) inner surfaces of dissected *Nepenthes gracilis* upper (left) and *Nepenthes rafflesiana* lower (right) pitchers, with scale bars for size reference. Notice how the waxy inner surface is reduced with increasing pitcher size in *Nepenthes rafflesiana* lower pitchers.

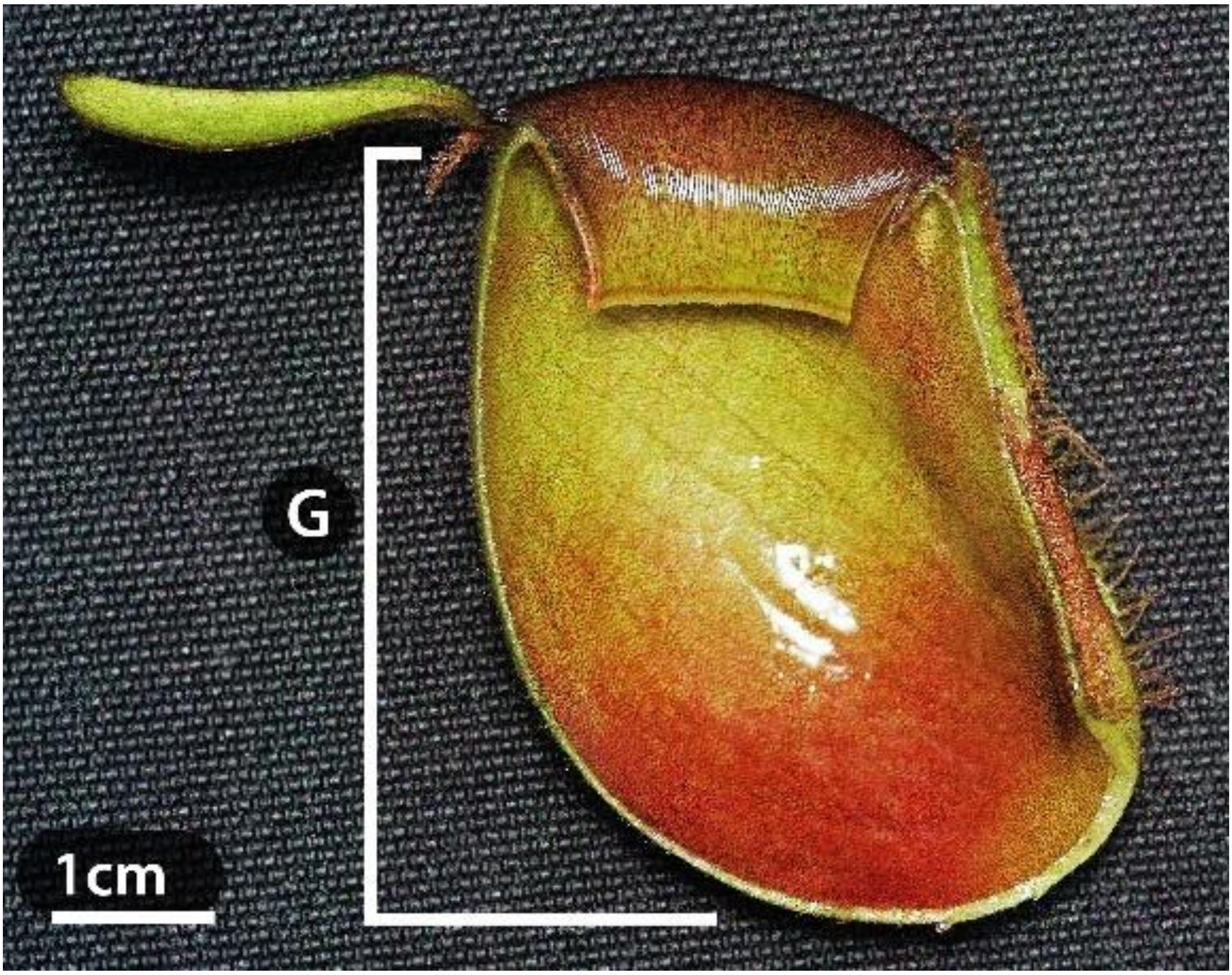

Fig. 5.12. Glandular (G) inner surface of dissected *Nepenthes ampullaria* lower pitcher.

Fig. 5.13. The sticky web of *Xenoplatyura beaveri* in a *Nepenthes ampullaria* lower pitcher (left) and a *Xenoplatyura beaveri* larva in a dissected *Nepenthes ampullaria* lower pitcher (right). The carcass of a large ant (black arrow; possibly *Camponotus* species) and leaf debris (white arrows) can be seen ensnared in the web (left), suggesting that webs of this species may facilitate prey retention in *Nepenthes ampullaria*. Location: Nee Soon Swamp Forest, Singapore.

(iii) Inquiline-assisted prey retention: Pitcher inquilines may also facilitate prey retention in pitchers. Crab spiders in *Nepenthes gracilis* pitchers may, in addition to ambushing pitcher-visitors at the peristome, attack struggling prey as they are floating on the surface of the pitcher fluids (Karl & Bauer, 2020). Such a behaviour is not well documented for crab spiders, but has been observed in the predatory inquiline ant, *Colobopsis schmitzi*, which attacks large *Polyrhachis pruinosa* ants as they try to escape from *Nepenthes bicalcarata* pitchers in Borneo (Bonhomme et al., 2011a). A predatory, web-building fungus gnat, *Xenoplatyura beaveri*, may also facilitate prey retention in *Nepenthes ampullaria*. *Xenoplatyura beaveri* inhabits pitchers of *Nepenthes ampullaria* in the forests of the Central Catchment Nature Reserve in Singapore, and builds sticky webs over the fluid surface of its pitchers to ensnare emerging inquiline adults (more information about this species is found in Chapter 6: Inquilines) (Fig. 5.13). Lam et al. (2018b) suggested that *Xenoplatyura beaveri*'s sticky webs may also serve to ensnare fallen prey (Fig. 5.13), which would otherwise escape *Nepenthes ampullaria* pitchers with a reasonably high success rate since this pitcher lacks wax crystals on its inner walls (Fig. 5.12).

Prey digestion. — After repeated, unsuccessful attempts at escape, prey become exhausted and drown in pitcher fluids. Prey digestion is the next phase in the carnivorous–nutrient sequestration process, and comprises the conversion of prey tissue into forms that can be taken up by pitchers. Two pathways are responsible for this digestion process.

(i) Enzyme-assisted digestion: *Nepenthes* pitchers secrete a wide array of hydrolytic enzymes which break down macromolecules into smaller molecules which can be absorbed by pitchers (Hatano & Hamada, 2008, 2012; Lee et al., 2016). Some of these enzymes are secreted constitutively, while the secretion of others is elicited by the detection of prey or chitin—the primary constituent in arthropod exoskeletons and fungal cell walls—in pitcher fluids (Eilenberg et al., 2010; Hatano & Hamada, 2012; Rey et al., 2013; Buch et al., 2015; Yilamujiang et al., 2016; Saganová et al., 2018).

The two most important nutrients that pitchers obtain from their prey are nitrogen and phosphorus (Schulze et al., 1997; Moran & Moran, 1998; Moran et al., 2010), although nitrogen is likely to be more limiting to the growth of *Nepenthes* in situ (Osunkoya et al., 2007). Prey protein is a rich source of nitrogen, while phosphorus is likely to be obtained primarily from prey nucleic acids. The aspartic protease nepenthesin is the enzyme responsible for protein degradation in the fluids of most *Nepenthes* species examined to date (Amagase et al., 1969; Takahashi et al., 2005; Hatano & Hamada, 2008; Rey et al., 2013; Kadek et al., 2014; Buch et al., 2015; Yilamujiang et al., 2016; Saganová et al., 2018), and the protein species that is found in greatest quantities in the fluids of most pitchers (Fig. 5.14). Additionally, cysteine (Stephenson & Hogan, 2006) and serine (Lee et al., 2016) proteases have also been identified, with the latter being named 'neprosin'. Phosphatases and nucleases have been identified in *Nepenthes* fluids or expressed in pitcher wall cells (Stephenson & Hogan, 2006; Lee et al., 2016). However, their presence in the fluids of *Nepenthes* pitchers has not been as well documented as would have been expected if these were as abundant or ubiquitous as their protease counterparts (Hatano & Hamada, 2008, 2012).

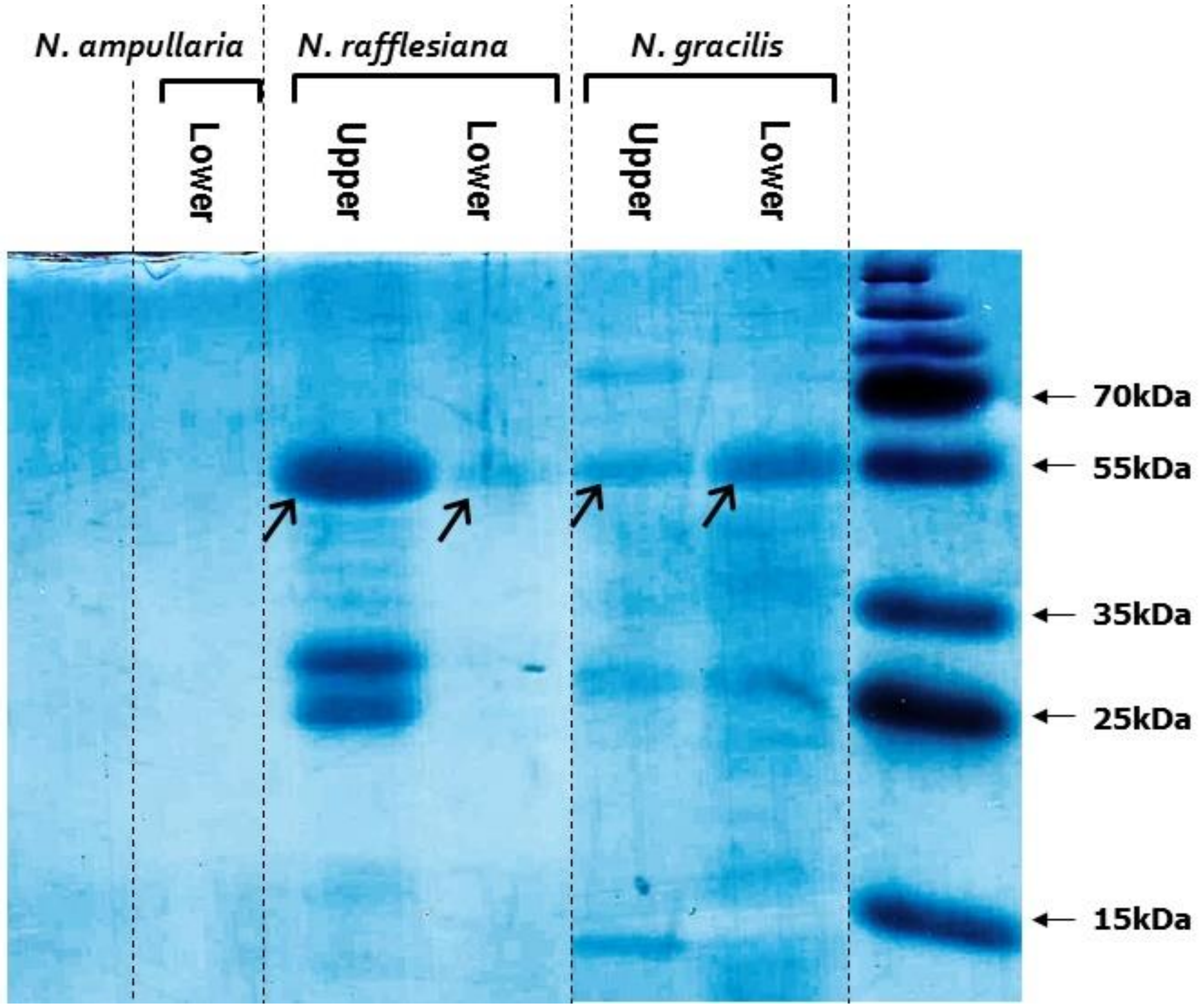

Fig. 5.14. The protease nepenthesin (black arrows) is the most abundant protein species in the fluids of native *Nepenthes* species. The figure shows Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pitcher fluid proteome bands of the three native *Nepenthes* species, and their pitcher types. Pitcher fluids were filtered through a 0.20-µm membrane, and then concentrated approximately 40× using 10 kDa Vivaspin ultrafiltration centrifugal devices (Sartorius Stedim, Gottingen, Germany) before SDS-PAGE. SDS-PAGE is a commonly employed technique used to separate a protein mixture by the molecular weights of its components. This image also shows that enzymes are expressed in different quantities and proportions in the fluids of different *Nepenthes* species and pitcher types. (Data from: W. N. Lam & G. S. Anand, unpublished data).

Fluid protease activities differ greatly between species and pitcher types in the three native *Nepenthes* species (Figs. 5.14, 5.15). Fluids of *Nepenthes gracilis* and *Nepenthes rafflesiana* lower pitchers contain low protease levels, while those of *Nepenthes rafflesiana* upper pitchers typically secrete large amounts of enzymes (Figs. 5.14, 5.15). The semi-detritivorous *Nepenthes ampullaria*, which seldom produces upper pitchers, appears to secrete negligible amounts of proteases into its pitcher fluids (Figs. 5.14, 5.15), even when secretion is elicited using prey, ammonium chloride or chitin (Saganová et al., 2018).

(ii) Inquiline assisted digestion: *Nepenthes* pitchers are important habitats to many specialist organisms, known as inquilines (Beaver, 1979). Inquilines are generally regarded as nutritional mutualists of pitchers which facilitate the breakdown of prey protein via the ammonium pathway (Moran et al., 2010; Adlassnig et al., 2011; Lam et al., 2017)—detritivorous inquilines consume prey protein, assimilate some of the nutrients, and excrete the rest in the form of pitcher-absorbable ammonium and phosphates. Most of the metazoan inquiline species inhabiting pitchers are detritivores which consume prey carcasses or the microorganisms which feed on them (Clarke & Kitching, 1993).

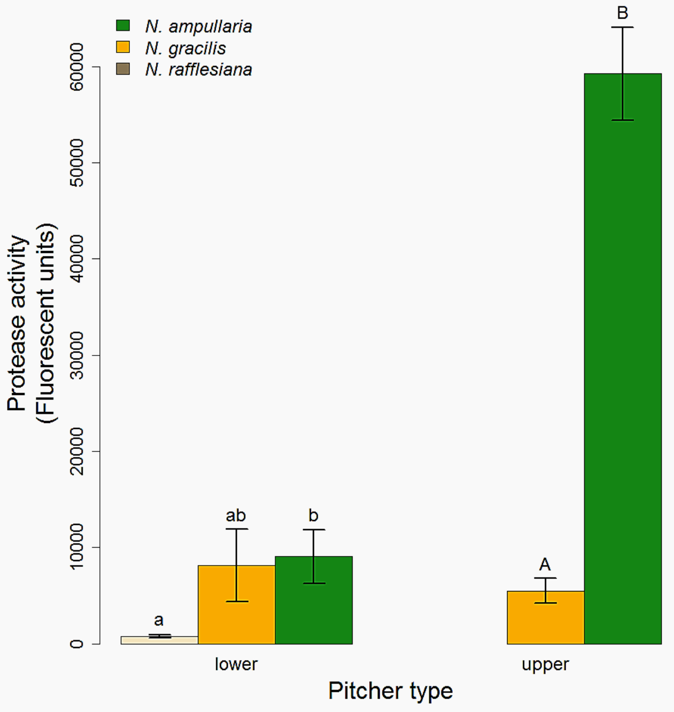

Fig. 5.15. Fluid protease activities in the three native *Nepenthes* species and their pitcher types, showing stark differences in levels of proteases between pitcher species and types. Data are represented as means ± SE; $n = 10–13$. Protease activity was measured using a fluorescein isothiocyanate-labelled casein (FITC-casein) protease detection kit (Sigma-Aldrich, St. Louis, MO) with a 24-hour incubation duration and 100 mM Gly-HCl, pH 2.5 reaction buffer. Bars sharing the same letter represent non-significant differences under Tukey's post-hoc *T*-test (data were log-transformed for normality prior to statistical testing). (Data from: W. N. Lam & G. S. Anand, unpublished data).

However, inquilines may not always benefit their host nutritionally. Nutritional mutualisms, like all positive species interactions, often have very context-dependent outcomes (Bronstein, 1994, 2009; Chamberlain et al., 2014). That is, the net outcome of the inquiline–pitcher plant interaction may shift from positive (mutualistic) to neutral (commensal) to negative (parasitic) depending on environmental factors such as resource abundance, resource types, temperature, inquiline abundances, etc. Most metazoan inquilines eventually pupate and emerge from pitchers as winged adult insects, exporting a significant amount of nutrients from pitchers when they leave (Scharmann et al., 2013). Detritivorous inquilines can thus benefit their *Nepenthes* host nutritionally only if they facilitate the release of more nutrients from recalcitrant substrates than that which they assimilate. For example, it was found that phorid larvae increased nitrogen availability in *Nepenthes gracilis* pitchers when these trapped large *Polyrhachis pruinosa* ant prey, which have a high surface area to volume ratio and hard exoskeletons (Leong et al., 2018). However, this benefit was lost and even reversed when *Nepenthes gracilis* pitchers trapped medium-sized *Dolichoderus thoracicus* and small-sized *Philidris* species ant prey, respectively (Leong et al., 2018). This was likely because phorid larvae increase nitrogen cycling by fragmenting prey carcasses and converting prey tissue into ammonia—services which are only beneficial when the prey species cannot be processed efficiently by proteolytic enzymes in pitcher fluids (Leong et al., 2018). It has also been shown that inquiline-phorid larvae tend to increase nitrogen availability to pitchers when small amounts of prey are trapped, but that these benefits are lost when prey volumes are high (Leong et al., 2019).

Compared to detritivorous metazoan inquilines, the role of inquiline predators and microbes in prey digestion are relatively poorly studied. Studies to date suggest that microbes, on the whole, contribute greatly to the degradation of prey tissue in pitchers (Lam et al., 2017, 2019). *Nepenthes* pitcher microbes exhibit a range of enzymatic activities including those that are proteolytic, chitinolytic, cellulolytic, amylolytic and xylanolytic (Chan et al., 2016), which may contribute substantially to the total enzyme activity present in pitcher fluids (Takeuchi et al., 2011). It has also been suggested that microbe- and plant-derived chitinase activities are complementary and act jointly for the complete digestion of chitin from insect exoskeletons (Chan et al., 2016). Nutrients released from prey digestion are partly assimilated by microbes, but these can eventually become available to the host plant when the microbes die (Adlassnig et al., 2011) or when they are consumed by other inquilines such as filter-feeding dipteran larvae, which excrete nutrients back into the pitcher fluid (Lam et al., 2017). Nitrogen-fixing bacterial genera such as *Bradyrhizobium*, *Methylosinus* and *Burkholderia* from the orders Rhizobales and Burkholderiales, respectively, have also been observed to occur abundantly in *Nepenthes rafflesiana* from Borneo (Sickel et al., 2016). These bacteria are commonly found in the rhizosphere, where they promote plant growth by converting atmospheric nitrogen into ammonia, an accessible form of nitrogen for plants. However, whether these bacteria can contribute significantly to nitrogen absorbed by *Nepenthes* pitchers has yet to be determined.

Similarly, inquiline predators are known to have far-reaching effects on the inquiline community structure (Kitching, 2000, 2001), but their role(s) in pitcher-nutrient sequestration have not been well studied. Preliminary research suggests that inquiline predators may increase the nutrient-cycling efficiency of inquiline communities, and in doing so increase the overall yield of nutrients from trapped prey (Lam et al., 2018a; WNL, unpublished data). Given the ubiquity and complexity of inquilines and inquiline communities, a better understanding of their role in nutrient processing should be a key focus of future research.

Prey resource partitioning between* Nepenthes *species. — Resource partitioning is the process by which coexisting species reduce competition between them by altering their relative dependencies upon limiting resources available to them in the environment (Chase & Leibold, 2003; Silvertown, 2004). Competition for common, limiting resources can lead to competitive exclusion, character displacement or adaptive radiation, all of which lead to higher functional diversity between sympatric species and greater divergences in traits associated with resource acquisition. The three *Nepenthes* species native to Singapore exhibit significant differences in many pitcher morphological traits (Table 5.1), suggesting that each of them adopts distinct strategies in the attraction, retention, capture and digestion of different prey groups. Indeed, evidence for resource partitioning between sympatric *Nepenthes rafflesiana* and *Nepenthes gracilis* upper pitchers has been found in resam-dominated scrubland in Singapore (Lam et al., 2018b; Fig. 5.16) and Brunei (Gaume et al., 2016), with the former trapping flower-visiting prey taxa with much greater frequencies than *Nepenthes gracilis* (Fig. 5.17). The semi-detritivorous habit of *Nepenthes ampullaria* (see the section below on Detritivory in *Nepenthes ampullaria*) further reinforces the notion that resource partitioning occurs between the three species (Gaume et al., 2016). Across the whole genus, taking geographical barriers and intrinsic species fitness into consideration, differences in pitcher traits associated with prey attraction, retention and capture are positively correlated with the likelihood of co-occurrence between species pairs, suggesting that prey resource partitioning is an important mechanism that promotes coexistence between sympatric *Nepenthes* species (Lam et al., 2018a).

***Detritivory in* Nepenthes ampullaria.** — *Nepenthes ampullaria* is unique amongst *Nepenthes* species in its habit of developing extensive rosettes of pitchers with reflexed lids and greatly reduced laminae, from lateral shoots produced along prostrate stems (Fig. 2.2). This often results in the formation of extensive 'carpets' of pitchers, which cover considerably large areas of the forest floor. Such a growth habit allows *Nepenthes ampullaria* to collect leaves, flowers and plant debris falling from the canopy, and supports a semi-detritivorous nutrient acquisition strategy in this species (Moran et al., 2003;

Pavlovič et al., 2011). The apparent replacement of traits associated with carnivory by those associated with detritivory may be interpreted as evidence for prey-resource partitioning, since *Nepenthes ampullaria* reduces competition with sympatric carnivorous *Nepenthes* species by specialising on alternative 'prey' resources and thus reducing nutritional niche overlap with sympatric congeners (Pavlovič, 2012; Gaume et al., 2016).

Table 5.1. Summary of key pitcher attributes associated with prey attraction capture, retention and digestion in the native *Nepenthes* species. The data show that species differ significantly in morphology and ecology, suggesting divergences in strategies of attraction, retention, capture and digestion. Quantities are represented as mean ± standard error.

[1] Lim et al. (2019)—*n* = 147 *Nepenthes ampullaria* lower pitchers

[2] Lam et al. (2019)—*N* = 23 upper and 28 lower pitchers of *Nepenthes gracilis*

[3] Lam & Tan (2019)—*n* = 109 *Nepenthes gracilis* upper pitchers

[4] WNL, unpublished data—*N* = 25 upper and 53 lower pitchers of *Nepenthes rafflesiana*

[5] Osunkoya et al. (2008)—*N* = 80–120 pitchers per species, of six species found in Brunei; upper/lower pitcher distinctions were not noted

† values for *Nepenthes rafflesiana* var. *gigantea* are reported, as these are likely to be the closest to those of Singaporean *Nepenthes rafflesiana*, in the authors' opinions

Species	***Nepenthes ampullaria***	***Nepenthes gracilis***		***Nepenthes rafflesiana***	
Pitcher type	**Lower**	**Lower**	**Upper**	**Lower**	**Upper**
Height of pitchers (cm)	4.8 ± 0.1[1]	12.0 ± 0.7[2]	11.2 ± 0.2[3]	12.3 ± 0.4[4]	17.9 ± 0.6[4]
Diameter of pitcher mouths (cm)	1.3 ± 0.1[1]	–	2.5 ± 0.1[3]	2.4 ± 0.1[4]	3.2 ± 0.1[4]
pH of pitcher fluids	5.2 ± 0.1[1]	3.8 ± 0.4[2]	3.0 ± 0.3[2]	4.5 ± 0.2[4]	3.7 ± 0.4[4]
Volume of pitcher fluids (mL)	10.6 ± 1.0[1]	–	–	16.5 ± 3.2[4]	12.6 ± 1.7[4]
Proteolytic activity of fluids (Fig. 5.15)	Low	Medium	Medium	Medium	High
Pitcher half lifetime[5]	2.40 ± 0.99	2.96 ± 0.99	2.96 ± 0.99	1.10 ± 0.01†	1.10 ± 0.01†
Waxy inner surface	No	Yes	Yes	Developmentally lost	No
Viscoelastic fluids	No	No	No	No	No
Prey/detritus volume	Low to medium	Medium	Medium	High	High
Prey/detritus type diversity	Medium	Low	Low	High	Medium to high
Inquiline community diversity	High	Medium	Medium	Medium	Low
Inquiline density	Medium to high	Low	Low	High	Low

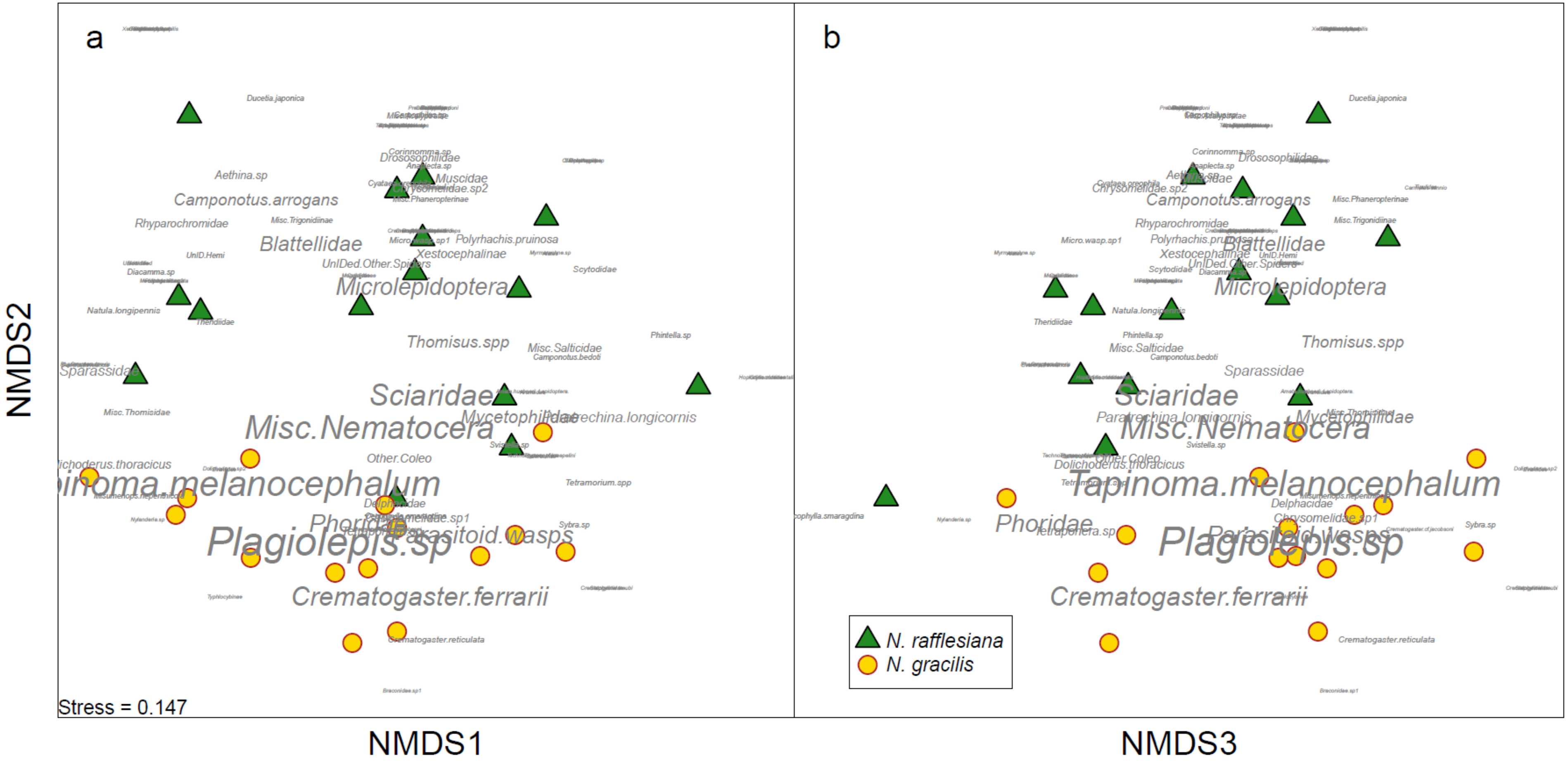

Fig. 5.16. Comparison of the prey assemblages of 15 *Nepenthes rafflesiana* and 15 *Nepenthes gracilis* upper pitchers showed clear resource partitioning between the species (PERMANOVA pseudo-*F* = 2.17, *p*-value = 0.003). (a) First (horizontal) and second (vertical) axes, and (b) second (vertical) and third (horizontal) axes of the three-dimensional non-metric multidimensional scaling (NMDS) ordination plots of pitcher prey assemblages. Points represent the assemblages of individual pitchers; the green triangles represent those of *Nepenthes rafflesiana* and the yellow circles those of *Nepenthes gracilis*. Points that are located closer to each other in the figure share more similar prey assemblages. Text represents prey taxon centroids, with text size scaled proportionally to the number of pitchers each taxon was found in. Figure and data from Lam et al. (2018c).

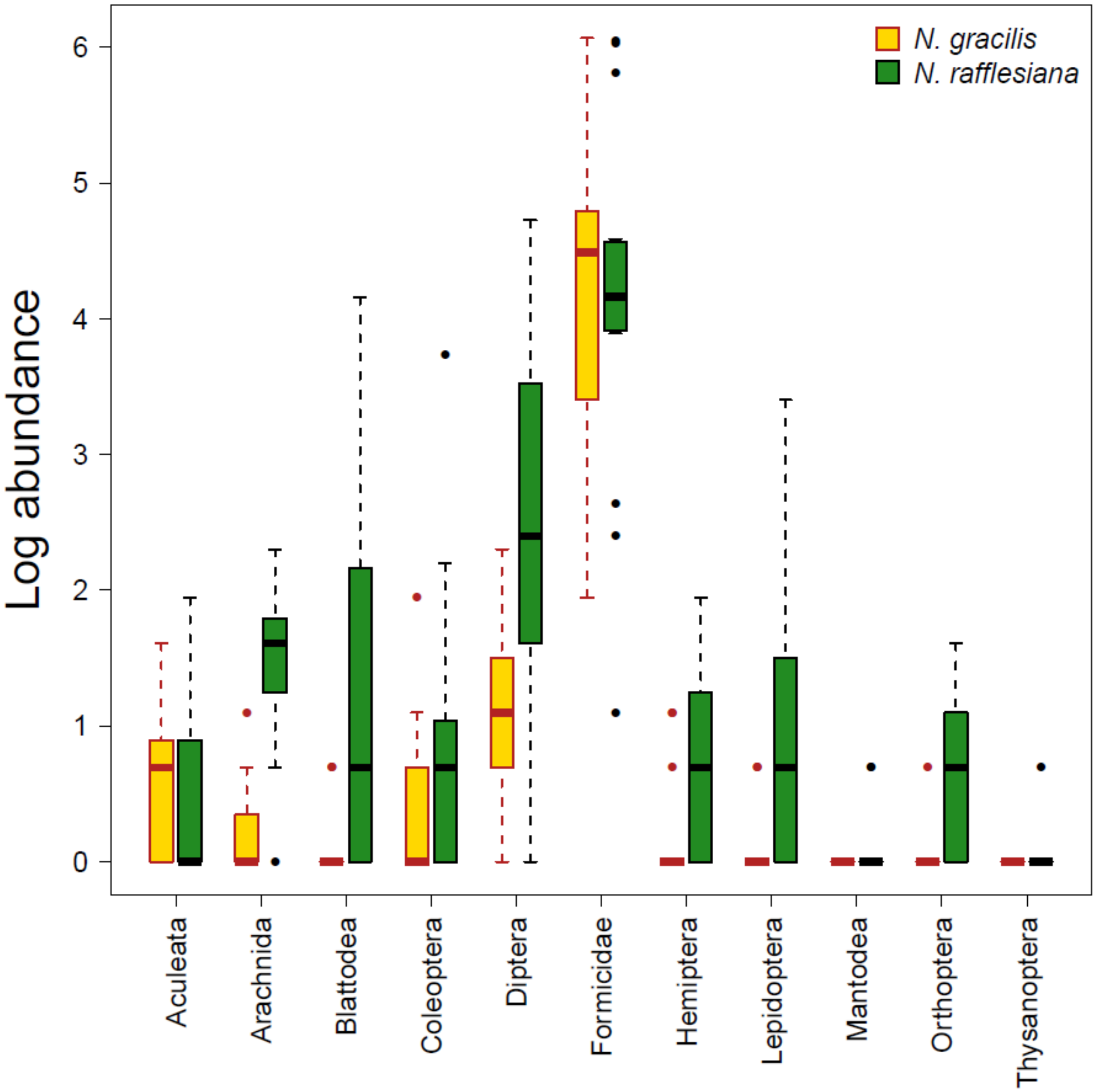

Fig. 5.17. Amounts of prey from each arthropod order trapped by the 15 *Nepenthes rafflesiana* and 15 *Nepenthes gracilis* upper pitchers represented in Fig. 5.16. *Nepenthes rafflesiana* clearly traps greater numbers of Blattodea (cockroaches), Diptera (flies), Hemiptera (true bugs—mainly leaf- and planthoppers), Lepidoptera (moths and butterflies) and Orthoptera (crickets, katydids and grasshoppers) than *Nepenthes gracilis*. Prey abundances are log-transformed; boxes represent interquartile ranges; bold lines within boxes represent median values; whiskers extend to maximum and minimum values; and points represent outliers. Figure and data from Lam et al. (2018c).

Fig. 5.18. Pitchers of *Nepenthes ampullaria* in the undergrowth. Pitchers of this species accumulate large amounts of leaf litter, and have been shown to derive a significant amount of nitrogen from this.

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CHAPTER 6: INQUILINES

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Introduction. — The aquatic environment of *Nepenthes* pitchers is an important habitat for many specialised organisms known as inquilines (Beaver, 1979; Clarke & Kitching, 1993; Fig. 6.1). Pitchers are often described in the scientific literature as 'phytotelmata' (singular, phytotelma), meaning water bodies held by or within terrestrial plant organs (Maguire, 1971; Kitching, 2001). *Nepenthes* inquilines may be classified into three categories based on the degree of their dependence upon *Nepenthes* pitcher phytotelmata for survival (Thienemann, 1932). Nepenthexenes are species which may colonise *Nepenthes* pitchers opportunistically, although they are not typically a part of *Nepenthes* phytotelma communities. Nepenthephiles are frequently found in pitcher phytotelmata, but are not completely dependent upon these for survival. Nepenthebionts are obligately associated with *Nepenthes* pitchers, and are completely dependent on pitcher phytotelmata during at least one stage of their life cycle. The vast majority of metazoan inquiline species found in pitchers of the three native *Nepenthes* species appear to be nepenthebionts (Choo et al., 1997; WNL, pers. obs.).

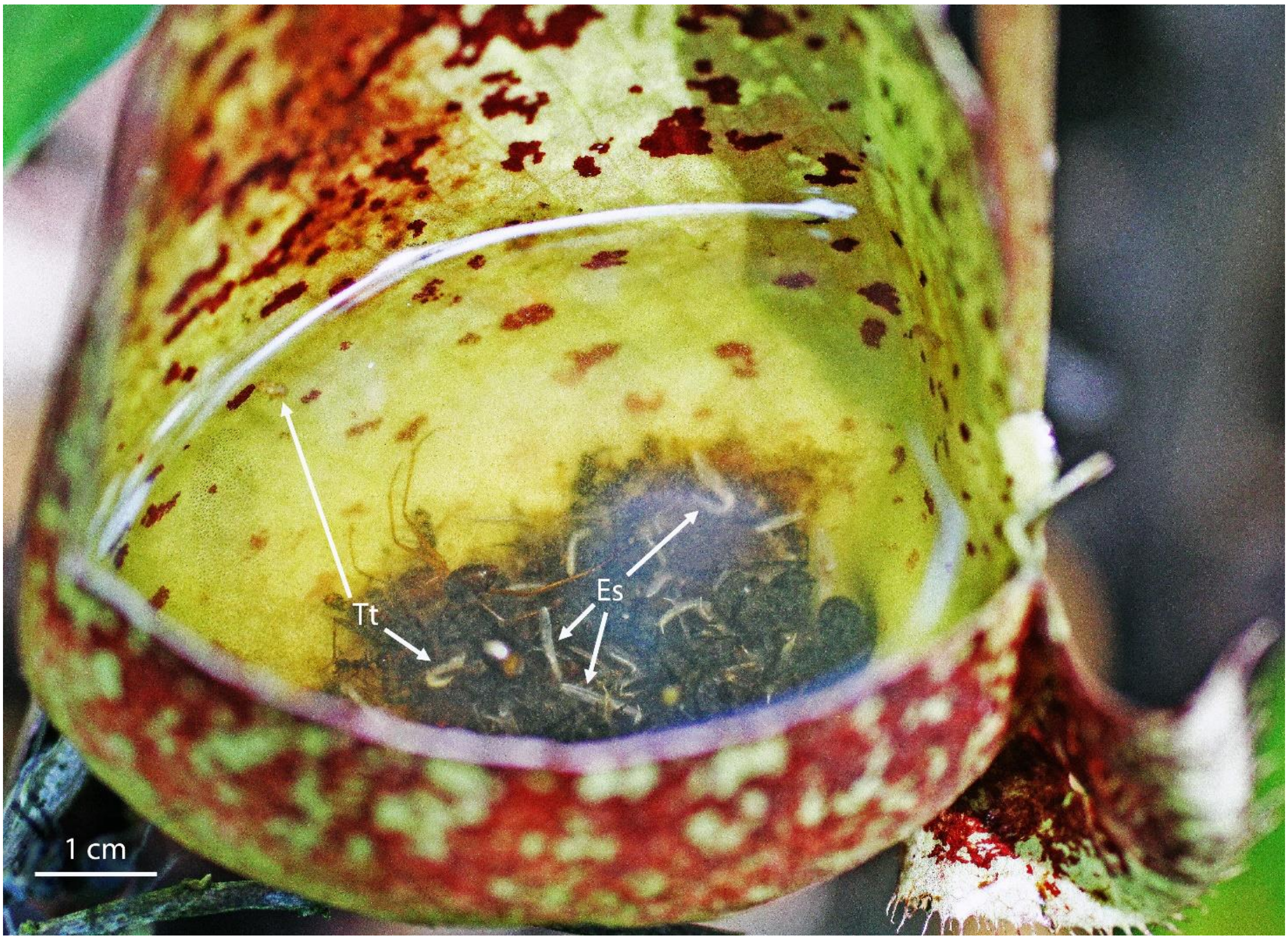

Fig. 6.1. *Endonepenthia schuitemakeri* (arrows labelled Es) and *Tripteroides tenax* (arrows labelled Tt) are common inhabitants of *Nepenthes* pitchers in Singapore and are shown here to be feeding on prey carcasses at the bottom of this dissected *Nepenthes rafflesiana* pitcher. Location: Kent Ridge Park. (Photograph by: Weng Ngai Lam).

Diversity and distribution. — *Nepenthes* inquiline species are not distributed uniformly across landscapes and between *Nepenthes* species and pitcher types. Although many inquiline species can inhabit the pitchers of different *Nepenthes* species, most exhibit a certain degree of host specificity (Table 6.1), and inquiline communities thus differ significantly between *Nepenthes* species (Clarke & Kitching, 1993; Gaume et al., 2019; Gilbert et al., 2020). Furthermore, many *Nepenthes* inquiline species appear to be highly sensitive to forest fragmentation or disturbance, and are not found in compatible host pitchers in younger, more disturbed or fragmented landscapes (Lim et al., 2019; Fig. 6.2). This may have arisen owing to the possible dependency of adult forms on food sources that are only available in forests (e.g., the nectar of certain plant species), and/or the dependency of species on large populations of both pitchers and prey (predatory inquiline species appear to be more sensitive than the other inquiline species of *Nepenthes ampullaria*; Table 6.1).

Inquiline diversity at the geographical scale is known to correlate positively with biogeographical factors such as land mass and proximity to the centre of *Nepenthes* diversity (Borneo and the Philippines), as well as the number of co-occurring *Nepenthes* species in the geographical region (Beaver, 1985; Kitching, 2000). Unsurprisingly therefore, the species richness of *Nepenthes* inquilines in Borneo appears to be significantly greater than that recorded from Singapore. This is evident from the number of top predator species—which is often correlated to overall taxon diversity (Sergio et al., 2006)—recorded from *Nepenthes ampullaria* pitchers in the two geographical regions. Up to three *Toxorhynchites* (Diptera: Culicidae; Clarke & Kitching, 1993) and two *Nepenthomyia* (Diptera: Calliphoridae; Mogi & Chan, 1996) species are known from the pitchers of *Nepenthes ampullaria* in Borneo, but only one of each have been recorded from *Nepenthes ampullaria* pitchers in Singapore (Mogi & Chan, 1997; Lim et al., 2019). Furthermore, it is possible that the continued reduction in *Nepenthes* population sizes over the years has resulted in the extinction of several inquiline species previously present in Singapore. Recent comprehensive surveys conducted across Singapore failed to detect a few species that had previously been reported from *Nepenthes* species locally (Lim et al., 2019; Lam et al., in press). These include *Nepenthomyia* species (Calliphoridae) and *Pierretia* species (Sarcophagidae), two large predatory fly larvae from *Nepenthes ampullaria* and *Nepenthes rafflesiana*, respectively, as well as *Culex coerulescens* from *Nepenthes ampullaria* and *Nepenthes gracilis*, and *Uranotaenia xanthomelaena* from *Nepenthes rafflesiana* (Mogi & Chan, 1997).

The microbes. — Mature *Nepenthes* pitchers harbour high densities of microbes including prokaryotic bacteria and eukaryotic fungi, protozoa and algae (Adlassnig et al., 2011; Bittleston, 2018). Microbes may be introduced to the phytotelmata of open *Nepenthes* pitchers directly from the air or transferred from raindrops, prey carcasses, other diet components (e.g., leaf litter), and possibly the bodies of inquilines. Some bacteria have also been found in unopened *Nepenthes* pitchers (Chou et al., 2014; Takeuchi et al., 2015), and these could originate from endogenous bacteria that live on or within plant tissues.

Within the *Nepenthes* microbial community, prokaryotic bacteria are likely to be the most abundant and diverse (Bittleston, 2018). Bacterial phyla which occur most commonly across various species of *Nepenthes* pitchers from different parts of the world are the Proteobacteria, Bacteroidetes and Actinobacteria while the orders commonly represented include the Rhodospirallales, Actinomycetales and Rhizobiales (Chou et al., 2014; Takeuchi et al., 2015; Chan et al., 2016; Sickel et al., 2016). However, the specific bacteria taxa present in individually sampled pitchers tends to be highly variable.

Table 6.1. Known associations between nepenthebiont and nepenthiphile inquiline species and their *Nepenthes* pitcher host in Singapore (Mogi & Chan, 1997; Lim et al., 2019; Lam et al., in press; pers. obs.).

Family	**Species**	***Nepenthes ampullaria***	***Nepenthes gracilis* (both)**	***Nepenthes rafflesiana* (upper)**	***Nepenthes rafflesiana* (lower)**
Biting midges (Ceratopogonidae)	*Dasyhelea ampullariae*	✓	✓	Occasionally?	Occasionally?
	Dasyhelea biseriata	✓	✓	Occasionally?	Occasionally?
	Dasyhelea nepenthicola	✓	Occasionally	✓	✓
	Lestodiplosis species	–	✓	–	Occasionally
Scuttle flies (Phoridae)	*Endonepenthia schuitemakeri*	✓	✓	✓	✓
	Endonepenthia species 1	✓	–	–	✓
	Megaselia species 1	✓	–	–	–
	Megaselia species 2	–	✓	–	–
Mosquitoes (Culicidae)	*Armigeres giveni*	–	–	✓	✓
	Armigeres cf. *kuchingensis*	–	–	Occasionally	Occasionally
	Culex brevipalpus complex	✓	✓	✓	✓
	Culex curtipalpis	Occasionally	✓	✓	✓
	Culex hewitii	✓	–	–	–
	Culex navalis	✓	✓	–	Occasionally
	Toxorhynchites acaudatus	✓	–	–	Occasionally
	Tripteroides nepenthis	✓	–	–	Occasionally
	Tripteroides nepenthisimilis	✓	–	–	–
	Tripteroides tenax	✓	✓	✓	✓
	Uranotaenia moultoni	✓	Occasionally	–	–
Hover flies (Syrphidae)	*Nepenthosyrphus* species 1	✓	–	✓	✓
	Nepenthosyrphus species 2	–	✓	–	–
Miscellaneous dipteran genera	*Corethrella calathicola* (Corethrellidae)	✓	–	–	–
	Xenoplatyura beaveri (Mycetophilidae)	✓	–	–	–

Family	**Species**	***Nepenthes ampullaria***	***Nepenthes gracilis* (both)**	***Nepenthes rafflesiana* (upper)**	***Nepenthes rafflesiana* (lower)**
Astigmatid mites (Histiostomatidae, Acari)	*Creutzeria* species	✓	✓	✓	✓
	Nepenthacarus species	✓	✓	✓	✓
	New Genus 1 species 1 (previously *Zwickia nepenthesiana* [Hirst, 1928])	✓	✓	–	✓
	New Genus 1 species 2	✓	✓	–	Occasionally
	New Genus 2	?	✓	–	–
	Zwickia species	–	✓	✓	✓
Crab spiders (Thomisidae, Aranae)	*Thomisus nepenthiphilus*	–	✓	–	–
	Misumenops nepenthicola	–	✓	–	–

Eukaryotic microbes also reside in *Nepenthes* pitchers but have received lesser attention. In a study utilising pitchers of *Nepenthes ampullaria*, *Nepenthes gracilis* and *Nepenthes rafflesiana* from Singapore, Bittleston et al. (2018) found that the green algae *Pseudomuriella* (Chlorophyceae) was the most abundant eukaryotic microbe, while other algae such as *Goniomonas* and *Microthamnion* (Trebouxiophyceae) were also relatively abundant. The second most abundant eukaryotic microbes were the Gregarines (Apicomplexa) (Bittleston et al., 2018), which are parasitic protists living in the guts of invertebrates, presumably introduced through arthropod prey. Other common protozoa include ciliates (Ciliophora), flagellates (e.g., Euglenophyta and Cercozoa) and various amoeba (e.g., *Acanthamoeba*). The most abundant fungus and fourth most abundant eukaryotic microbes were *Saccharomycetes* yeasts (Bittleston et al., 2018).

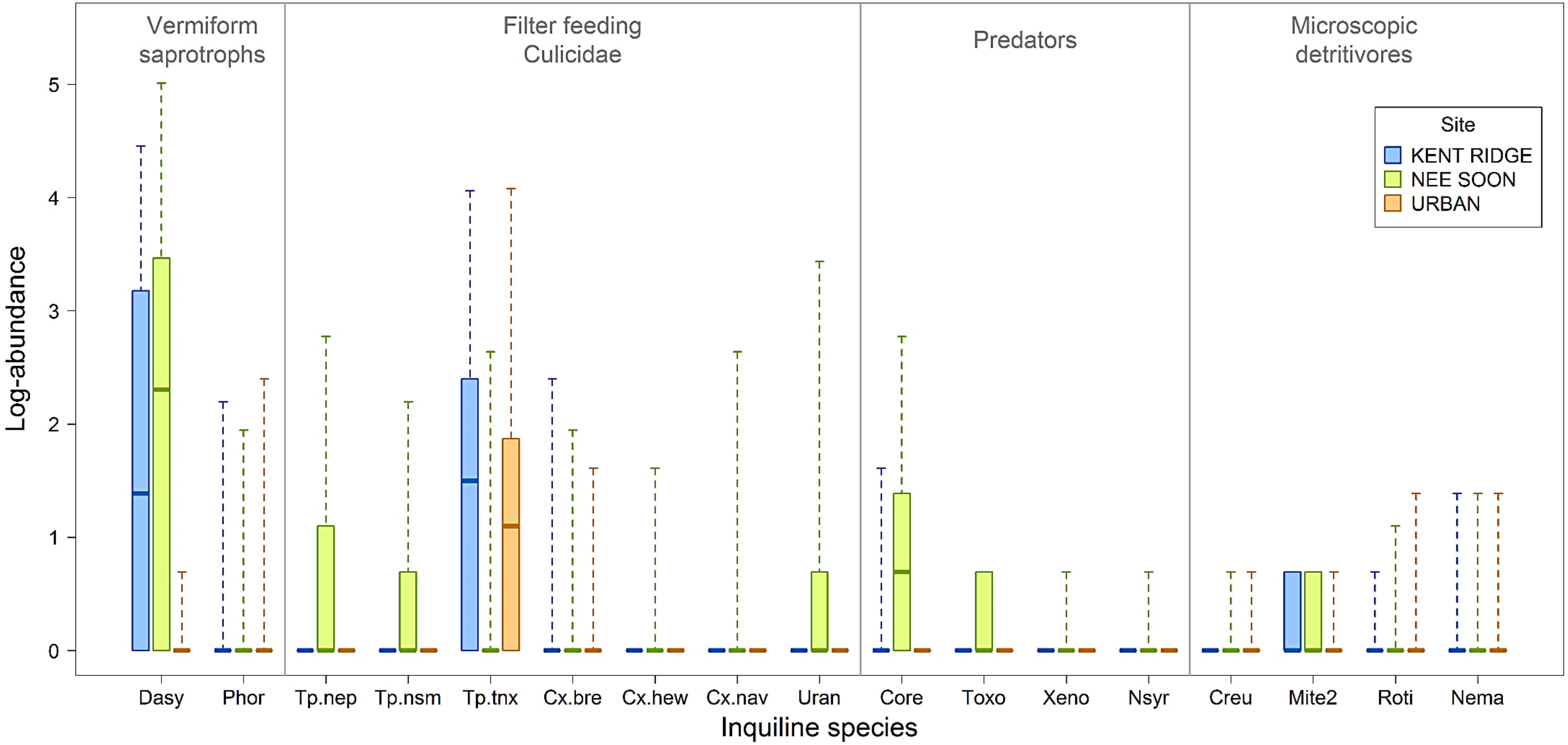

Fig. 6.2. Log abundances of 17 inquiline taxa found in the pitchers of *Nepenthes ampullaria* across three habitat types in Singapore: Nee Soon Swamp Forest, an intact freshwater swamp forest; Kent Ridge Park, an isolated adinandra belukar forest fragment in the south of Singapore; and urban resam-dominated scrubland. It is clear from the figure that many inquiline taxa found in Nee Soon Swamp Forest are absent from the other two, human-modified habitats. Bold horizontal lines represent median log-transformed numbers of each inquiline taxon in pitchers from each forest type (denoted by colours); boxes represent interquartile ranges; whiskers represent maximum values. *Dasy* = *Dasyhelea* species; *Phor* = Phoridae; *Tp.nep* = *Tripteroides nepenthis*; *Tp.nsm* = *Tripteroides nepenthisimilis*; *Tp.tnx* = *Tripteroides tenax*; *Cx.bre* = *Culex brevipalpus* complex; *Cx.hew* = *Culex hewitii*; *Cx.nav* = *Culex navalis*; *Uran* = *Uranotaenia moultoni*; *Core* = *Corethrella calathicola*; *Toxo* = *Toxorhynchites acaudatus*; *Xeno* = *Xenoplatyura beaveri*; *Nsyr* = *Nepenthosyrphus* species 1; *Creu* = *Creutzeria* species; *Mite2* = Histiostomatidae genus 1; *Roti* = rotifers; *Nema* = nematodes. Rare inquiline taxa which were present in fewer than three samples are not displayed. (*n* = 147; Figure reproduced from Lim et al. [2019]).

The colonisation of *Nepenthes* phytotelmata by introduced microbes is determined by various pitcher fluid properties. Pitcher fluids of unopened *Nepenthes* pitchers contain antimicrobial compounds whose production is induced by the presence of bacteria and fungi (Eilenberg et al., 2010; Buch et al., 2013). However, the main determinant of *Nepenthes* bacterial communities appears to be pitcher fluid pH, which may have an even stronger influence than the pitcher's geographical location (Kanokratana et al., 2016; Bittleston, 2018; Bittleston et al., 2018). The acidity of pitcher fluids can inhibit certain microbes while selecting for others, contributing to environmental filtering of bacterial diversity (Gilbert et al., 2020). For instance, *Acidocella*, a genus of acidophilic bacteria, is abundant only in *Nepenthes* pitchers with highly acidic fluids, regardless of host species (Kanokratana et al., 2016; Sickel et al., 2016). Since the pH of pitcher fluids changes with pitcher age (Higashi et al., 1993) and prey captured (Hua & Li, 2005; Bittleston et al., 2018), this could also suggest temporal shifts in the composition of *Nepenthes* microbe communities.

Nepenthes microbe communities are also affected by host species (Bittleston et al., 2018). This might be partly attributed to interspecific variations in trapping strategies, prey spectra and diets (Bazile et al., 2015; Gaume et al., 2016), which can result in different introduced microbes, or favour the establishment of microbes which feed on specific resources. For instance, the pitcher fluids of *Nepenthes hemsleyana*, a Bornean species which acquires a significant portion of its nutrients from bat faeces, were found to be enriched with vertebrate gut symbionts as well as saprophytic bacteria, which can derive nutrients from bat faeces and urine (Sickel et al., 2016). Similarly, the presence of bacteria taxa in *Nepenthes ampullaria* which cannot be found in other *Nepenthes* species examined in Borneo (Kanokratana et al., 2016) could be linked to its unique diet of leaf litter (Moran et al., 2003; Pavlovič et al., 2011).

Although *Nepenthes* microbes potentially compete with their host plants for prey-derived nutrients, they might also be capable of facilitating nutrient sequestration in pitchers (Adlassnig et al., 2011; Lam et al., 2017, 2020). This topic is discussed in detail in Chapter 5: Prey and Carnivory: Prey digestion.

The mites (Histiostomatidae, Acari). — Mites in the astigmatic family Histiostomatidae are perhaps the most ubiquitous invertebrate inquilines in *Nepenthes* pitchers, as well as one of the most diverse taxa among the metazoan (i.e., non-microbial) inquilines (Bittleston et al., 2016). They are, however, also the least investigated and consistently overlooked taxon. Despite their large numbers—occasionally even constituting a larger inquiline biomass than all other metazoan inquilines combined—their presence is often simply acknowledged or completely ignored. For example, mites were not even considered to be part of the metazoan inquiline community in Gaume et al. (2019), even though a new mite species (*Naiadacarus nepenthicola*; Fashing & Chua, 2002) had earlier been described from *Nepenthes bicalcarata* in the same study area in Brunei. Relegating mites to an unimportant status in *Nepenthes* community studies is undoubtedly in part due to their small size (body lengths of adults typically less than 0.5 mm), as well as the lack of knowledge concerning their taxonomy and biology. However, an understanding of the role mites play in *Nepenthes* pitcher communities is essential for a complete understanding of food webs and community dynamics (Fashing, 2002).

Nepenthes pitchers typically contain multiple mite species which are polymorphic, making identification, even to the generic level, difficult. Males often display precopulatory mate guarding and have appendages which are adapted for grasping and clinging to the penultimate female instar (see Fashing, 2004b, 2008). They therefore can be morphologically quite different from females (Fig. 6.3a, b). In addition, the life cycle includes a deutonympal instar (also known as hypopus; Fig. 6.3c) that is morphologically quite different from both males and females, being highly modified for the rigours of dispersal as well as for attachment to phoretic host(s) (i.e., animals onto which the mites attach for dispersal facilitation; see Fashing, 2010). Knowledge of the morphological features of adults as well as deutonymphs of the various species is therefore necessary for accurate identification of the species present in a pitcher.

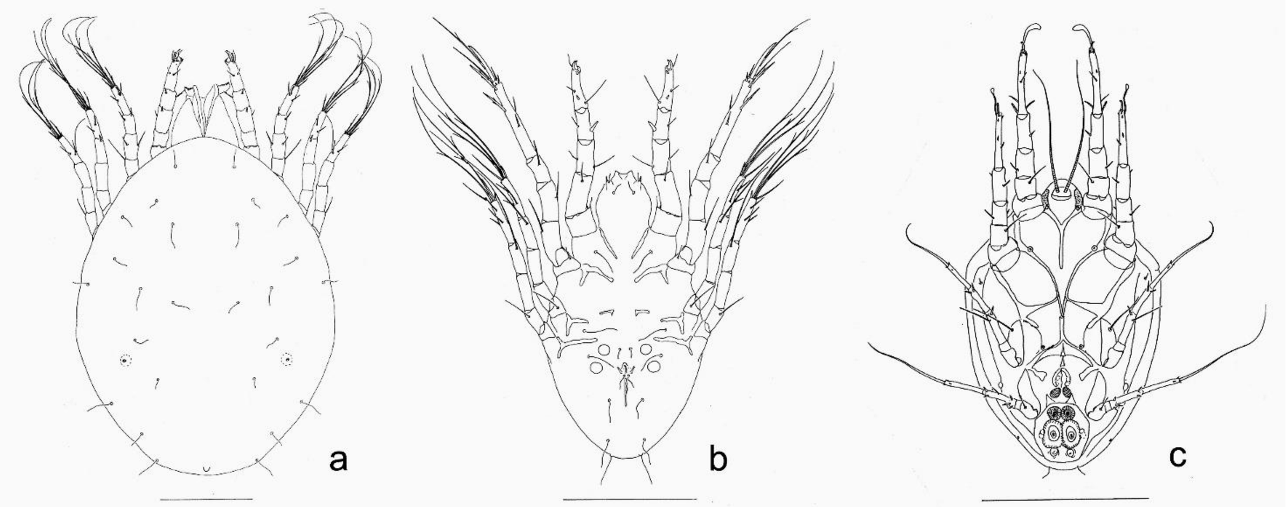

Fig. 6.3. Illustrations demonstrating morphological differences between adults and deutonymphs of *Creutzeria* species collected from *Nepenthes mirabilis* pitchers in Australia. a, female; b, male; c, deutonymph. Scale bars = 100 µm. Figure reproduced from Lim et al. (2019).

(i) Current status concerning the taxonomy of nepenthebiont mites: Oudemans (1915) described *Anoetus guntheri* collected in Sri Lanka from the pitchers of *Nepenthes distillatoria*, but later (1924) established the genus *Zwickia* with *Anoetus guentheri* as its type species. Presumably assuming mite inquilines of *Nepenthes* pitchers all belong to the genus *Zwickia*, Hirst (1928) described a second species from the pitchers of *Nepenthes ampullaria* in Singapore as *Zwickia nepenthesiana*. In 1932, Oudemans described a second genus, *Creutzeria* (*Creutzeria tobaica*), from samples collected from *Nepenthes tobaica* in Java. In 1979, Nesbitt described *Creutzeria seychellensis* collected from the pitchers of *Nepenthes pervillei* in the Seychelles. More recently, Fashing (2002) established a third genus, *Nepenthacarus*, from *Nepenthes mirabilis* in Far North Queensland, Australia, and Fashing & Chua (2002) described *Naiadacarus nepenthicola* (family Acaridae) from the pitchers of *Nepenthes bicalcarata* in Brunei Darussalam. Much remains to be done concerning the taxonomy and systematics of nepenthebiont mites. For example, a survey of the mites collected from six species of *Nepenthes* in Borneo revealed not only the presence of the inquiline genera *Zwickia* (two species), *Creutzeria* (one species), *Nepenthacarus* (one species) and *Naiadacarus* (one species), but also two undescribed genera, one containing three species and the other two species. With more than 160 *Nepenthes* species described to date (Clarke et al., 2018) and a species discovery rate that does not appear to be slowing down (Robinson et al., 2019a, 2019b), it is clear that many nepenthebiont mite taxa still await discovery.

(ii) Taxonomy of Singaporean nepenthebiont mites: Observations by NJF on slide mounted specimens from collections made by K. L. Yeo, Raffles Museum of Biodiversity Research (currently the Lee Kong Chian Natural History Museum) in 2001 revealed five genera inhabiting the three Singapore *Nepenthes* species: *Creutzeria* (Fig. 6.3), *Zwickia* (Fig. 6.4a), *Nepenthacarus* (Fig. 6.4b) and two genera new to science (Fig. 6.4c, d). The presence of the five genera has been corroborated by slide-mounted specimens from recent collections (Lim et al., 2019; Lam et al., in press). One of the undescribed genera (hereafter referred to as New Genus 1) is in all probability the species described by Hirst (1928) as *Zwickia nepenthesiana*. Although Hirst's written description is very brief and his illustrations incomplete, it is obvious from the illustrations that the species does not belong in the genus *Zwickia* nor in a described genus. It is probable that the other four genera are represented by undescribed species. Using metabarcoding of fluid samples rather than microscopic observations of actual specimens, Bittleston et al. (2016) and Bittleston (2018) reported two additional genera present in all three of the Singapore species of *Nepenthes*: *Hormosianoetus* and *Rostrozetes*. It is interesting that neither genus has been reported from studies utilising slide-mounted specimens, and that barcoding did not reveal the presence of the five genera listed above. While *Hormosianoetus* is in the family Histiostomatidae and does contain a species that inhabits phytotelmata, it is found in water-filled tree holes (Fashing, 2010). As far as can be determined, the genus *Rostrozetes* has not been recorded from any type of phytotelma. It is possible that the database used for the metabarcoding did not contain the sequences necessary for the identification of the relevant mite taxa, and it is therefore quite probable that neither the genus *Hormosianoetus* nor the genus *Rostrozetes* inhabits *Nepenthes* pitchers in Singapore.

(iii) Association of nepenthebiont mite genera with Singaporean Nepenthes species: All three species of *Nepenthes* in Singapore harbour mites in their pitchers (Table 6.1). *Nepenthacarus* and *Creutzeria* appear to be generalists and were found in all three species, whereas New Genus 2 appears to be a specialist, inhabiting the pitchers of *Nepenthes gracilis*; one specimen was found in *Nepenthes ampullaria* and could easily have been from a mislabelled sample. *Creutzeria* species are commonly found in *Nepenthes gracilis* pitchers across the island, and are especially abundant in pitchers with turbid fluids and/or high prey contents (J. Ling & WNL, pers. obs.).

(iv) Notes on the biology of nepenthebiont mite genera: Nepenthebiont mite genera may be classified based on their locomotory modes. Most aquatic histiostomatid mites are ambulatory, crawling about on the substrate, and this is also the case for species in New Genus 1 and New Genus 2.

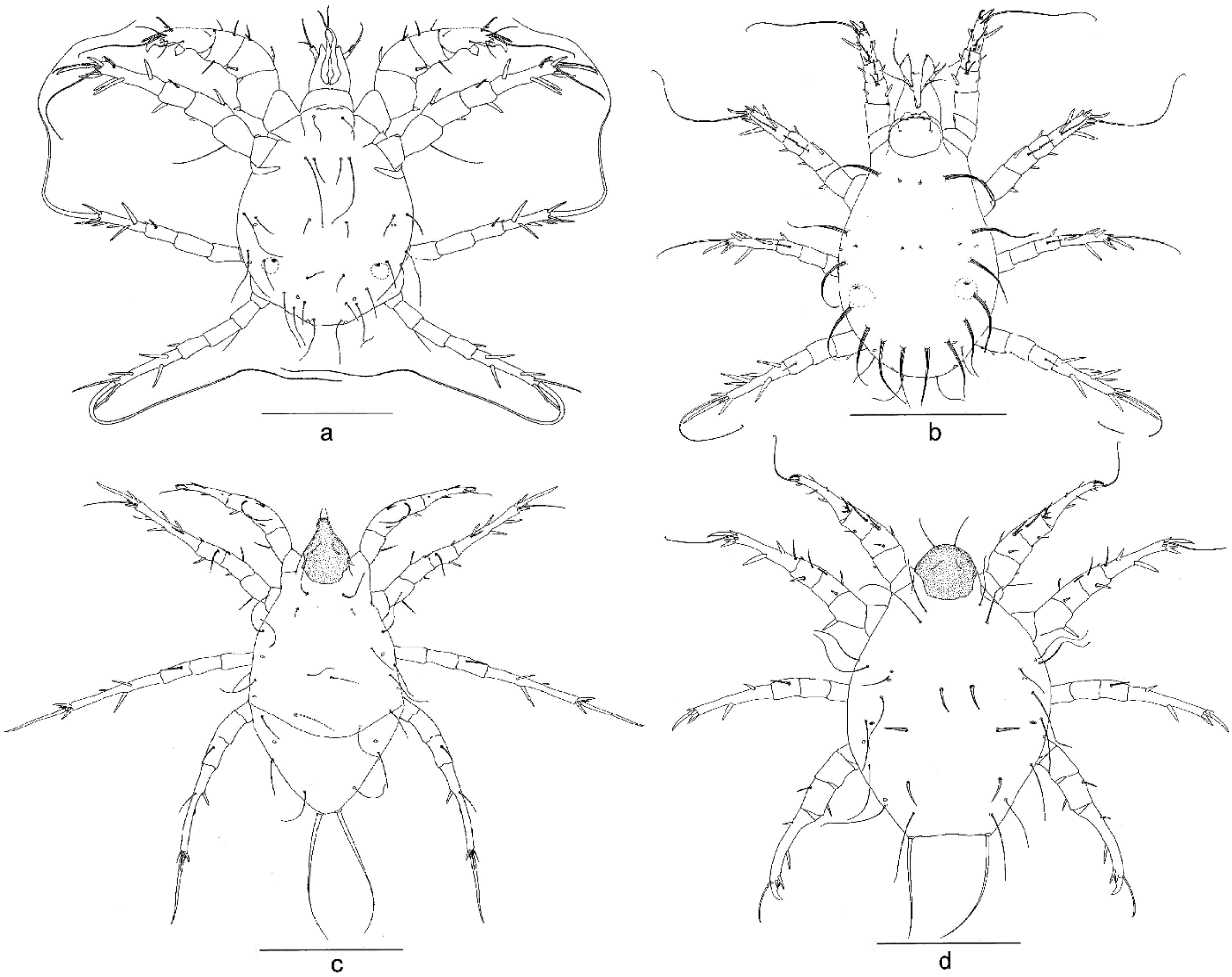

Fig. 6.4. Illustrations of the dorsum of males of mite genera inhabiting *Nepenthes* pitchers in Singapore. a, *Zwickia*; b, *Nepenthacarus*; c, New Genus 1 ("*Zwickia nepenthesiana*" of Hirst [1928]); d, New Genus 2. Scale bars = 150 μm. (Illustration by: Norman J. Fashing).

Both genera are found in the detrital layer at the base of pitchers. *Creutzeria* species, on the other hand, are natatory, and are active, adept swimmers. In fact, they are unable to walk and must swim to move about (see Fashing et al., 1996). Legs I are used primarily for grasping the substrate while resting, or in the case of males, also for clasping females. Legs II to IV, with their long terminal setae, are used for propelling themselves through the water column. *Nepenthacarus* species are adapted to life in and on the water surface film (see Fashing, 2002). They appear to move about either on the water surface or while partially submerged in the surface film utilising legs I to III. Members of the genus have occasionally been observed crawling about on debris or arthropod parts floating on the water surface. *Zwickia* species are interesting in that they are natatory as well as ambulatory, although swimming appears to be a much more laborious task than walking (see Fashing, 2004b). Individuals are usually found moving about on the substrate at the bottom of the pitchers using legs I and II, but are occasionally observed propelling themselves forward using backward movements of legs III with their extremely long setae. When swimming, legs IV appear to be dragged behind, perhaps being used as rudders.

Inquiline mite species are likely to coexist by specialising in different habitat strata (free-swimming ones in the pelagic stratum and crawling ones in the benthic stratum) and on different food resources. The small size of nepenthebiont mites makes it difficult to determine their feeding behavior and diet. However, mouthpart morphology suggests that resource partitioning does occur between the genera (Fig. 6.5). Lam et al. (2020) found that fluid microbes appear to compete with *Creutzeria* mites, and do not appear to be consumed by them. The chelicerae of *Creutzeria* (Fig. 6.5a) resemble brushes and are flexible, not ridged. Individuals are often observed swimming up or down in the water column while brushing the surface of decomposing insect cuticle, presumably feeding on the microbial film that grows on prey carcasses. The chelicerae of *Zwickia* (Fig. 6.5b) also appear to be brush-like, but are more ridged and narrow distally to a point. It is possible they are used to extract the microbial film growing in crevices as well as brushing from the surface. The chelicerae of *Nepenthacarus* (Fig. 6.5c)

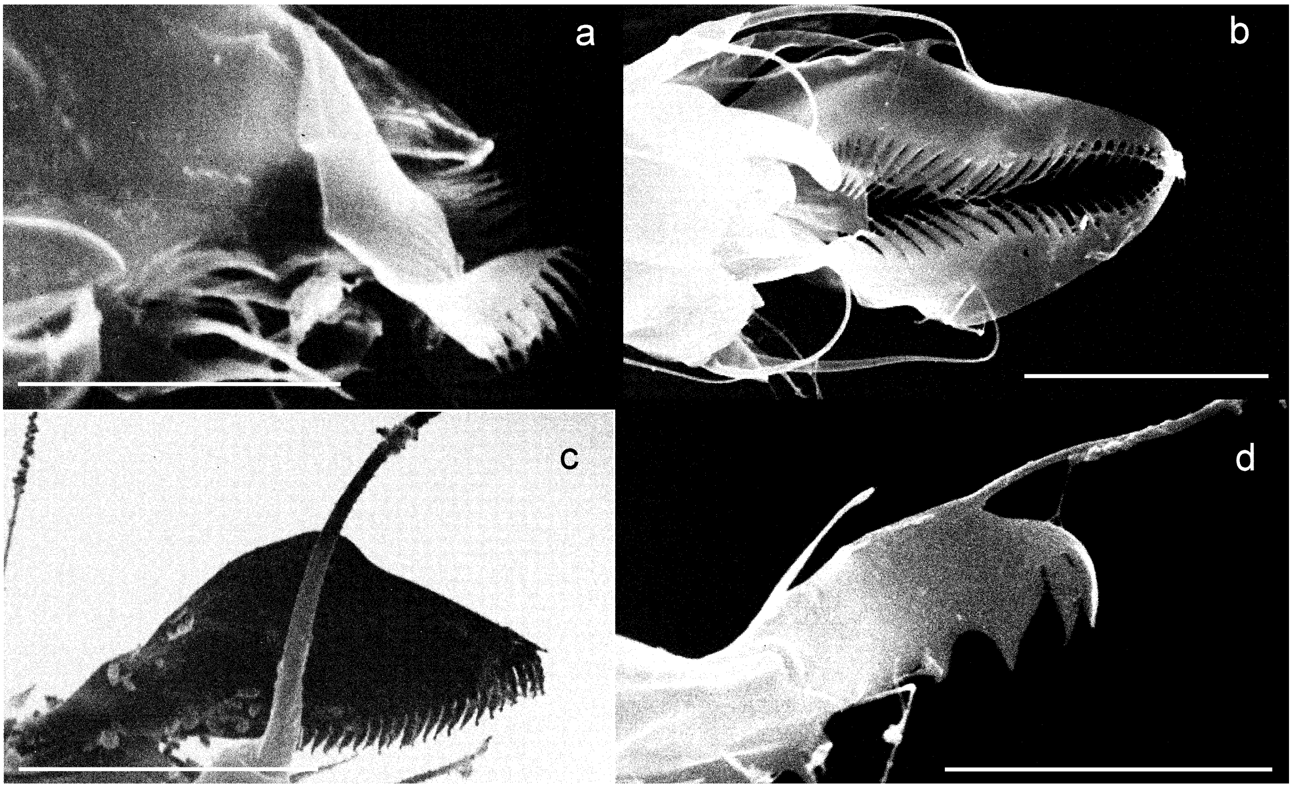

Fig. 6.5. Scanning electron microscope (SEM) photographs exemplifying morphological differences in the chelicerae of *Nepenthes* histiostomatid mites. a, *Creutzeria* species; b, *Zwickia* species; c, *Nepenthacarus* species; d, New Genus 2. Scale bars = 10 µm. (Photographs by: Norman J. Fashing).

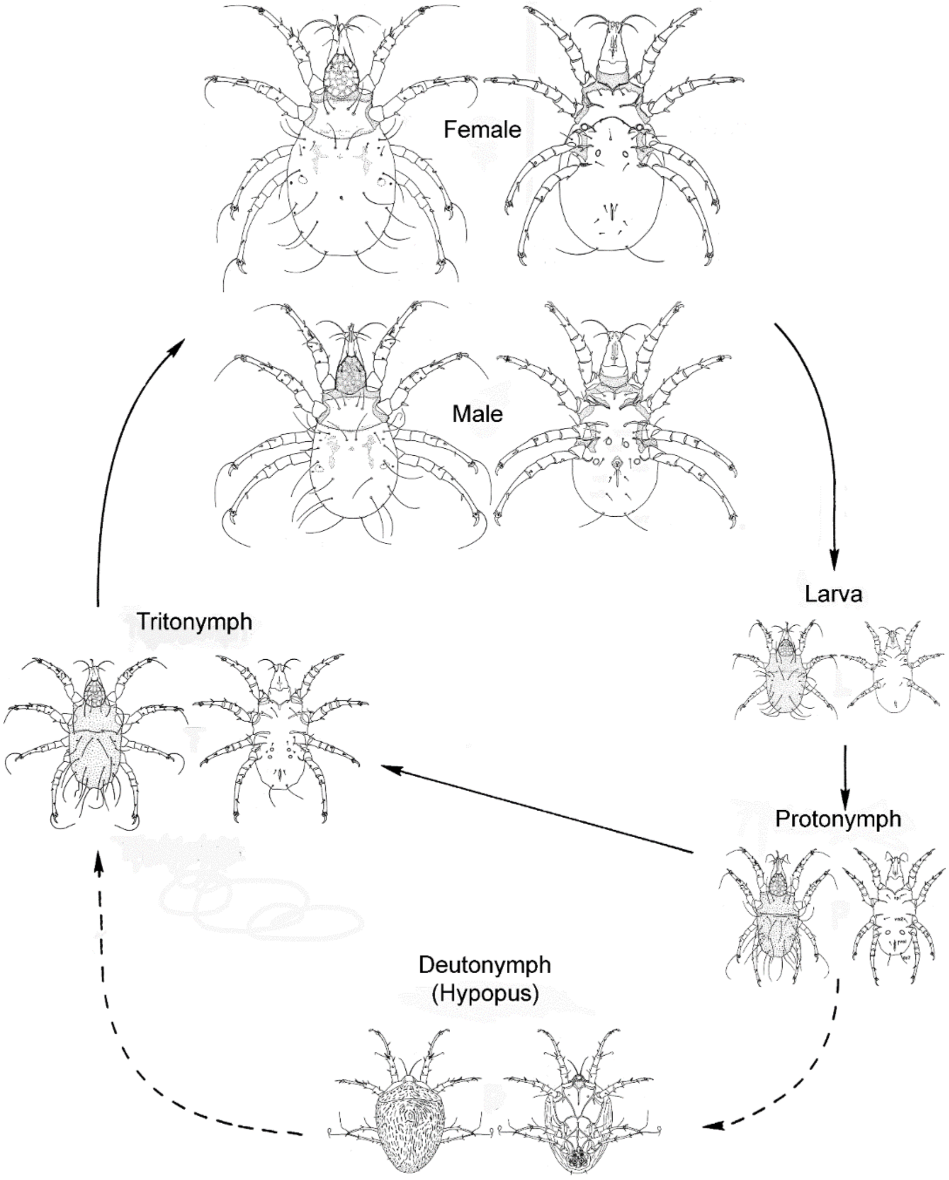

Fig. 6.6. Life cycle of *Hormosianoetus mallotae*, a histiostomatid inhabitant of water-filled tree-holes. Broken lines reflect the facultative deutonymphal stage. Modified from Fashing (2010).

appear to be quite ridged with short, rake-like teeth. It is possible they are used to push microbes and small debris from the surface film toward the oral cavity. The chelicerae of New Genus 2 (Fig. 6.5d) are perhaps the most interesting since they are atypical for histiostomatid species. They appear to be quite ridged, with knife-like serrations rather than brush or rake-like teeth. It is possible members of the genus feed on small particles cut from decomposing pitcher prey.

Little is known concerning the dispersal of inquiline mites between individual *Nepenthes* pitchers. The life cycle of most free-living astigmatic mites contains a heteromorphic deutonympal stage (Fig. 6.6), and nepenthebionts are no exception. The deutonymph is highly adapted for dispersal, lacking functional mouthparts and possessing a terminal sucker plate on the venter for attaching to a host that utilises the same habitat (a phenomenon known as ‘phoresy’). It is also more heavily sclerotised and resistant to desiccation. Deutonymphs are typically facultative, forming under adverse conditions, and often in low numbers. The host for only one species of nepenthebiont mite is known. *Creutzeria* species inhabiting *Nepenthes madagascariensis* have been found to disperse via emerging inquiline chloropid frit flies (Diptera: Chloropidae; Ratsirarson & Silander, 1996); however, the hosts for other nepenthebionts remain unknown. Both inter- and intraplant dispersal remain a mystery. Since *Nepenthes* species possess multiple pitchers in close proximity on a given plant, it is possible that deutonymphs walk from one pitcher to another for intraplant dispersal. Such behaviour has been observed for deutonymphs of the histiostomatid genus *Sarraceniopus*, which inhabit the pitchers of *Sarracenia* species in North America (Fashing, 2004a). Interplant dispersal, however, would require phoresy on a host inhabiting the same *Nepenthes* species. The dispersal ecology of inquiline mites remains a promising area for future research.

The mosquitoes (Culicidae). — Mosquito larvae are the most diverse and commonly encountered metazoan inquiline taxon in most *Nepenthes* species (Bittleston, 2018). Eleven species from five genera of mosquitoes are known from pitchers of the three Singapore *Nepenthes* species (Table 6.1). These mosquitoes are for the most part nepenthebionts that are well adapted to living in the acidic pitcher fluids. They may also be recorded from other habitats (e.g., *Tripteroides tenax* from nipah palm [*Nypa fruticans*] leaf or inflorescence axils), but these are rare occurrences (Mattingly, 1981).

Tripteroides tenax (Figs. 6.7, 6.8c) is the most ubiquitous inquiline mosquito species, and can be found in all three species of *Nepenthes* pitchers in large numbers, even in habitats which are open and highly disturbed. In contrast, *Armigeres giveni* and *Armigeres* cf. *kuchingensis* (Fig. 6.8a) are specialised in *Nepenthes rafflesiana*, which has the most acidic pitcher fluids among the three native *Nepenthes* species. *Nepenthes ampullaria* harbours the largest number of mosquito species with nine species recorded (Table 6.1). This may be because of the larger amount of nutrients obtained from leaf litter utilisation, and a weak acidic fluid environment (~pH 5; Table 5.1; Gaume et al., 2019). Species such as *Culex hewitti*, *Culex navalis* and *Tripteroides nepenthisimilis* are largely confined to pitchers of Singapore *Nepenthes ampullaria*, suggesting that their breeding habits are highly specific.

Given that the pitchers are readily available water sources, there is concern that they are suitable breeding habitats for mosquito vectors of human diseases. It is important to note that the mosquito nepenthebionts in Table 6.1 are not vector species in Singapore and do not transmit disease-causing pathogens. *Aedes albopictus* has been reported to occur naturally in *Nepenthes ampullaria* pitchers in a zoo in Malacca, Peninsular Malaysia, although the number of larvae found was considerably low and occurred mainly in older pitchers (Mogi & Yong, 1992), which may not be as acidic. This suggests that while *Aedes albopictus* is capable of breeding in *Nepenthes* pitchers, it is generally still an unattractive habitat for these vectors as they are rarely found within the pitchers (Chou et al., 2015a, 2015b). No *Aedes* species larvae were found breeding naturally in recent surveys of 147 *Nepenthes ampullaria* (Lim et al., 2019) and 78 *Nepenthes rafflesiana* (Lam et al., in press) pitchers across

Fig. 6.7. A newly emerged *Tripteroides tenax* mosquito adult rests on the inner walls of *Nepenthes gracilis*. Location: Kent Ridge Park. (Photograph by: Weng Ngai Lam).

multiple habitat types in Singapore, despite the adults being abundant in the environments where these were sampled. The *Nepenthes* pitchers in Singapore are thus not a significant habitat for the larvae of dengue vectors (Chou et al., 2015b).

Other than the generalised host-biting behaviour of each mosquito genus, the specific host preferences of the nepenthebionts are largely unknown. Generally, females of *Armigeres* species tend to bite humans and other mammals, while *Uranotaenia* (Fig. 6.8d) are known to take blood meals mostly from amphibians and reptiles (Harbach, 2013). *Culex* species (Fig. 6.8b) tend to be ornithophilic (feeding on birds), although they may exhibit a preference for mammals as well (Harbach, 2013). Not much is known about the blood-feeding habits of *Tripteroides* species except that only a few species occasionally bite humans (Mattingly, 1981).

Toxorhynchites acaudatus (Fig. 6.8e) are large, reddish, predatory larvae commonly found in the pitchers of *Nepenthes ampullaria* and occasionally also in *Nepenthes rafflesiana* (Table 6.1). *Toxorhynchites* species are non-haematophagus and do not need to take blood meals to lay eggs. In fact, they are voracious predators when they are in the larval stage, and readily attack mosquito and other dipteran larvae. The proteins obtained from this lifestyle are stored for oogenesis (egg production) when they become adults (Steffan & Evenhuis, 1981). Owing to its cannibalistic nature, only one *Toxorhynchites acaudatus* larva is usually found in each pitcher.

Several species of larvae (e.g., *Culex* species [Fig. 6.8b] and *Uranotaenia moultoni* [Fig. 6.8d]), have been observed to adopt behaviours to evade detection by the *Toxorhynchites acaudatus* predator. Except for the continuous movement of the mouthbrushes for filter feeding of detritus, they are usually motionless and positioned upside down along the walls of the pitchers (Mogi & Chan, 1996). In contrast, *Tripteroides tenax* are frequently found on the detritus upside down or on the water surface, and are generally more vulnerable to predation by *Toxorhynchites acaudatus* (Mogi & Chan, 1996).

The scuttle flies (Phoridae). — Scuttle flies are a diverse family of small, hump-backed flies which are so named because of their habit of scuttling across surfaces instead of taking flight (Marshall, 2012). The larvae of many scuttle fly species are saprotrophic and one species, *Megaselia scalaris*, is commonly used in the estimation of post-mortem intervals in homicide investigations (Reibe & Madea, 2010). Unsurprisingly, scuttle flies are also saprotrophs in the pitchers of many *Nepenthes* pitchers (Clarke & Kitching, 1993; Disney, 2004). Phorid larvae are often the largest saprotrophic inquilines in *Nepenthes* pitchers and fragment large prey carcasses which smaller inquilines like *Dasyhelea* species and filter-feeding culicids are unable to consume directly (WNL, pers. obs.). Their messy feeding habits thus increase the surface areas of prey carcasses for enzymatic degradation and/or access to other inquiline species, facilitating nutrient release to pitchers (Lam et al., 2017; Leong et al., 2018) and potentially also the feeding activities of other inquiline species (Lam et al., 2017).

Although a considerable number of phorids have been described from *Nepenthes* phytotelmata (Disney, 2004), species identification continues to be a significant barrier to the study of these, owing in part to similarities in larval morphology between most inquiline phorid species. Differences in pupal and adult morphologies suggest that at least four species are still extant in Singapore (Table 6.1), with *Endonepenthia schuitemakeri* being the most common among these (Figs. 6.9, 6.10). *Endonepenthia schuitemakeri* appears to be a generalist species that is able to utilise the pitchers of many different *Nepenthes* species, and is commonly found in the pitchers of exotic ornamental *Nepenthes* species cultivated in local nurseries and private collections (WNL, pers. obs.). It is naturally found in all three native *Nepenthes* species, but usually occurs in lower numbers in the pitchers of *Nepenthes ampullaria* (Beaver, 1983), where supplies of prey carcasses are lower and less regular owing to the semi-detritivorous habit of this species (see Chapter 5: Prey and Carnivory: Detritivory in *Nepenthes ampullaria*).

Fig. 6.8. Mosquito larvae. a, *Amigeres* cf. *kuchingensis;* b, *Culex brevipalpus* complex; c, *Tripteroides tenax*; d, *Uranotaenia moultoni*; e, *Toxorhynchites acaudatus*. Scale bars = 1 mm. (Photographs by: Yeo Huiqing).

Another common phorid species is *Megaselia* species 2 (Figs. 6.11, 6.12), which, though only found in the pitchers of *Nepenthes gracilis* (Table 6.1), is widely distributed across Singapore. This species appears to preferentially colonise pitchers which contain large amounts of prey, and are often associated with foul-smelling, putrefied pitcher fluids. The pupae of *Megaselia* species 2 attach themselves around the peristomes of pitchers (Fig. 6.12). Larvae, pupae and adults of *Megaselia* species 2 are all significantly smaller than those of *Endonepenthia schuitemakeri* in corresponding life stages.

In Singapore, *Megaselia* species 1 (Fig. 6.13) and *Endonepenthia* species 1 are found only in the Central Catchment Nature Reserve (CCNR), but never in scrubland or adinandra belukar fragments outside of it (Lim et al., 2019; Lam et al., in press; note that *Megaselia* species 1 is referred to as Phorid species 1 in Lim et al., 2019, and *Endonepenthia* species 1 as Phorid species in Lam et al., in press). *Megaselia* species 1 appears to be an early coloniser of *Nepenthes ampullaria* pitchers; it completes its larval life cycle quickly and often pupates before the arrival of predatory *Toxorhynchites acaudatus* larvae (Fig. 6.8e). *Toxorhynchites acaudatus* larvae appear to feed preferentially on the larvae of *Endonepenthia* species rather than on the larvae of other culicids in the laboratory (WNL, pers. obs.). Little is known about *Endonepenthia* species 1, which is similar to, albeit smaller than, *Endonepenthia schuitemakeri* in all respects. Its pupae do not appear to congregate in the manner that *Endonepenthia schuitemakeri* do and are coloured in a distinctly different manner.

Fig. 6.9. *Endonepenthia schuitemakeri* larva (a) and pupal casing (b). Both photographs share the same scale bar, which is shown in panel a. (Photographs by: Tiffany Q. H. Lum).

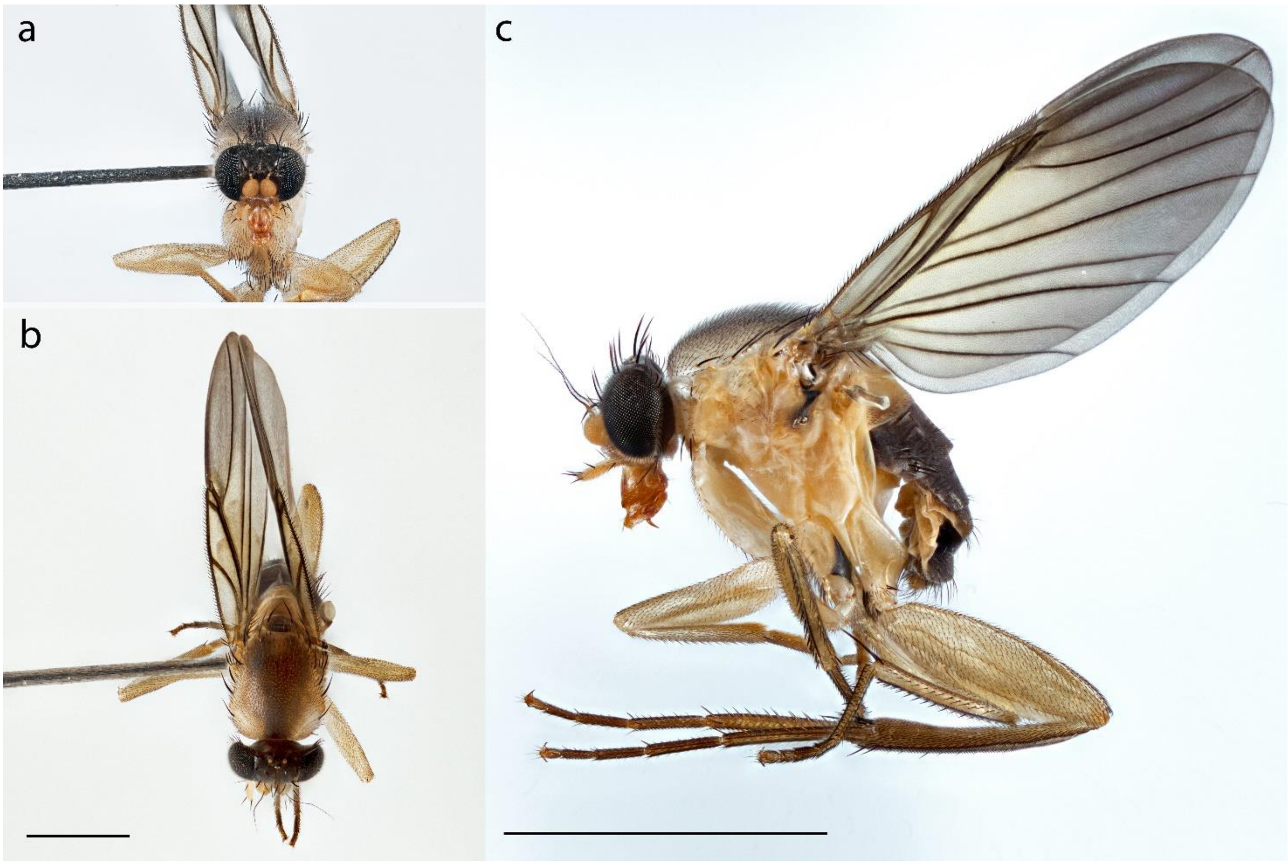

Fig. 6.10. Frontal (a), dorsal (b) and lateral (c) views of an adult female *Endonepenthia schuitemakeri*. Scale bars represent 1 mm, with panels a and b sharing the scale bar in panel b. (Photographs by: Robyn J. Y. Lim).

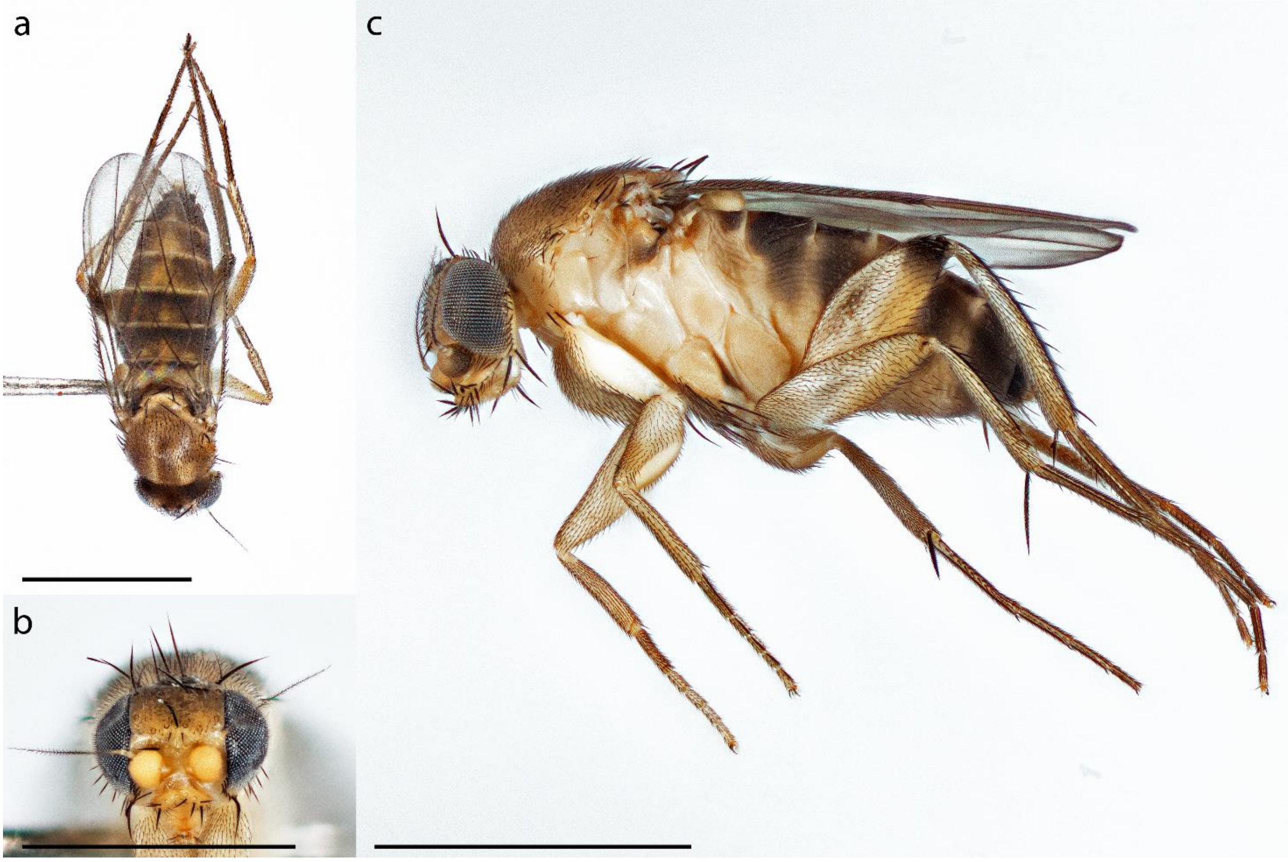

Fig. 6.11. Dorsal (a), frontal (b) and lateral (c) views of an adult *Megaselia* species 2. Scale bars = 1 mm. (Photographs by: Robyn J. Y. Lim).

Fig. 6.12. Pupae of *Megaselia* species 2 in an upper pitcher of *Nepenthes gracilis*. This species typically colonises *Nepenthes gracilis* pitchers in which large amounts of rotting prey carcasses have accumulated. Location: Kent Ridge Park. (Photograph by: Weng Ngai Lam).

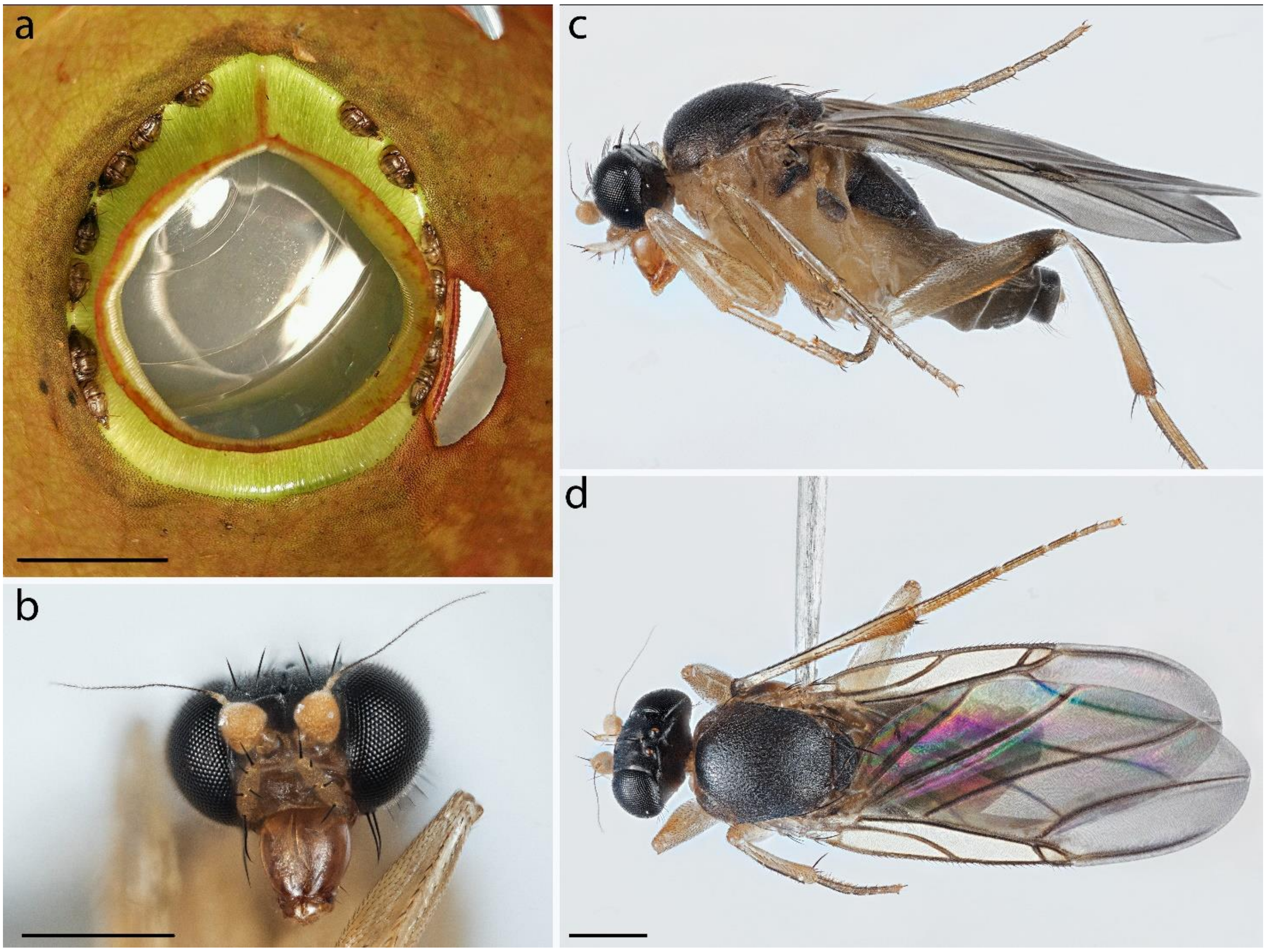

Fig. 6.13. Pupae of *Megaselia* species 1 attach themselves to the under surface of the peristome in a *Nepenthes ampullaria* pitcher (a; photograph is taken from a bottom-up angle of a transversely dissected pitcher). Frontal (b), lateral (c) and dorsal (d) views of an adult *Megaselia* species 1. Scale bars = 1 cm (a); 0.5 mm (b, d). Panels c and d share the same scale bar. (Photographs by: Tiffany Q. H. Lum).

The biting midges (*Dasyhelea *species, Ceratopogonidae). — The larvae of *Dasyhelea* are small and slender (Fig. 6.14), and are often found amongst the detritus in the pitchers of all three Singapore *Nepenthes* species (Dover et al., 1928; Choo et al., 1997). Differentiating the *Dasyhelea* species can be difficult, and many studies (e.g., Clarke & Kitching, 1993; Bittleston et al., 2016; Gaume et al., 2019; Lim et al., 2019) considered the abundance of the whole genus without noting the associations of each species with specific *Nepenthes* hosts. This taxon is most commonly recorded from the pitchers of *Nepenthes ampullaria,* but also occur, albeit less frequently, in the pitchers of *Nepenthes gracilis* and *Nepenthes rafflesiana* (Beaver, 1979; Mogi & Chan, 1997; Chua & Lim, 2012; Lim et al., 2019). However, the degree to which the different *Dasyhelea* species specialise in one or more *Nepenthes* host species is unclear, as various published studies have reported different associations and association strengths (Wirth & Beaver, 1979; Mogi & Chan, 1997; Lam et al., in press). All three *Dasyhelea* species have been collected at least once from each of the three *Nepenthes* species (Beaver, 1979, 1983; Wirth & Beaver, 1979; Choo et al., 1997; Mogi & Chan, 1997; Lim et al., 2019; Lam et al., unpublished data). Species from another ceratopogonid genus, *Forcipomyia*, also inhabit *Nepenthes* pitchers in Southeast Asia. However, this genus is not known from Singapore pitchers.

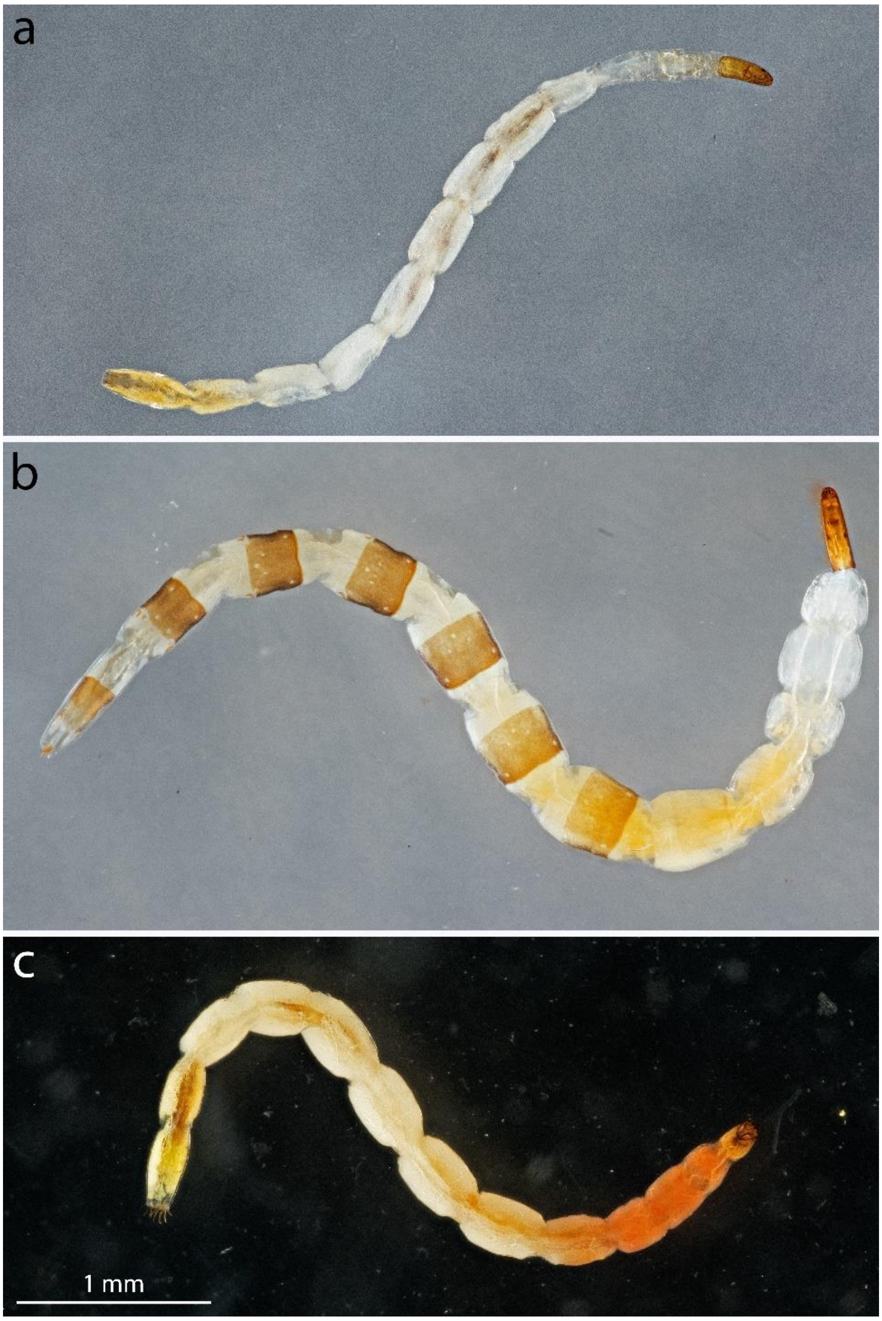

Fig. 6.14. Photographs of *Dasyhelea biseriata* (a), *Dasyhelea ampullariae* (b) and *Dasyhelea nepenthicola* (c) larvae. Images share the same scale bar, which is shown in panel c, and are all oriented so that anterior ends of the larvae are to the right side of the image. (Photographs by: Tiffany Q. H. Lum).

The hover flies (**Nepenthosyrphus** ***species, Syrphidae).*** — Hover flies are a large family of true flies (Diptera). Members of the taxon are often flower-visiting, and many possess striking colouration that allows them to mimic other hymenopteran flower visitors (Marshall, 2012). *Nepenthosyrphus* is a small and poorly studied genus of hover flies containing only six described species (Thompson, 1971; Rotheray et al., 2012). *Nepenthosyrphus* species are described as "yellow pollinose flies with carinate face and massive hind femora", and are also unique in having eyes that are covered with a layer of fine, soft hairs (Thompson, 1971). *Nepenthosyrphus* species have been observed in the pitchers of many different *Nepenthes* species, but the vast majority were not identified to species (Clarke & Kitching, 1993; Mogi & Chan, 1996; Cresswell, 2000; Gaume et al., 2019). Given the range over which the genus is found (Thompson, 1971), the diversity of *Nepenthes* species hosts in which they occur (Thompson, 1971; Clarke & Kitching, 1993; Cresswell, 2000; Rotheray et al., 2012), and the degree to which these predators specialise behaviourally in prey capture (Mogi & Chan, 1996), it is very likely that the genus contains many times more species than the six which are currently described. Two morphospecies of *Nepenthosyrphus* species have been observed in the pitchers of local *Nepenthes* species (Figs. 6.15–6.17).

Nepenthosyrphus species 1 larvae (Fig. 6.15) are found primarily in the lower pitchers of *Nepenthes rafflesiana*, but also occur occasionally in *Nepenthes rafflesiana* upper pitchers and *Nepenthes ampullaria* lower pitchers. *Nepenthosyrphus* species 1 has been observed to feed on large prey items and inquiline phorid larvae (WNL, pers. obs.), and is thus more commonly encountered in pitchers that have accumulated large amounts of prey, since phorids are also attracted to the smell of foul, decomposing prey. *Nepenthosyrphus* species forage by coiling their bodies around their meal, before ingesting it apparently whole. Mogi & Chan (1996) reported that *Nepenthosyrphus* species from Borneo and Singapore fed upon mosquito larvae (*Aedes aegyptii*, *Culex eminentia* and *Tripteroides tenax*). Our observations suggest that *Nepenthosyrphus* species 1 greatly prefers phorid (*Endonepenthia schuitemakeri*, in our experiments) to culicid (*Tripteroides tenax*) prey, although the latter is still consumed at low frequencies (WNL et al., unpublished data). Cannibalistic behaviour has not been observed among *Nepenthosyrphus* species individuals. *Nepenthosyrphus* species 1 adults (Fig. 6.16) took approximately two weeks to emerge from pupae reared in the laboratory (Y. Y. Chou & WNL, unpublished data), but adult behaviour has never been observed in the field.

Fig. 6.15. *Nepenthosyrphus* species 1 larva (left) and pupae, attached to the cut out inner wall of a *Nepenthes rafflesiana* lower pitcher (right). Scale bars represent 5 mm. (Photographs by: Weng Ngai Lam).

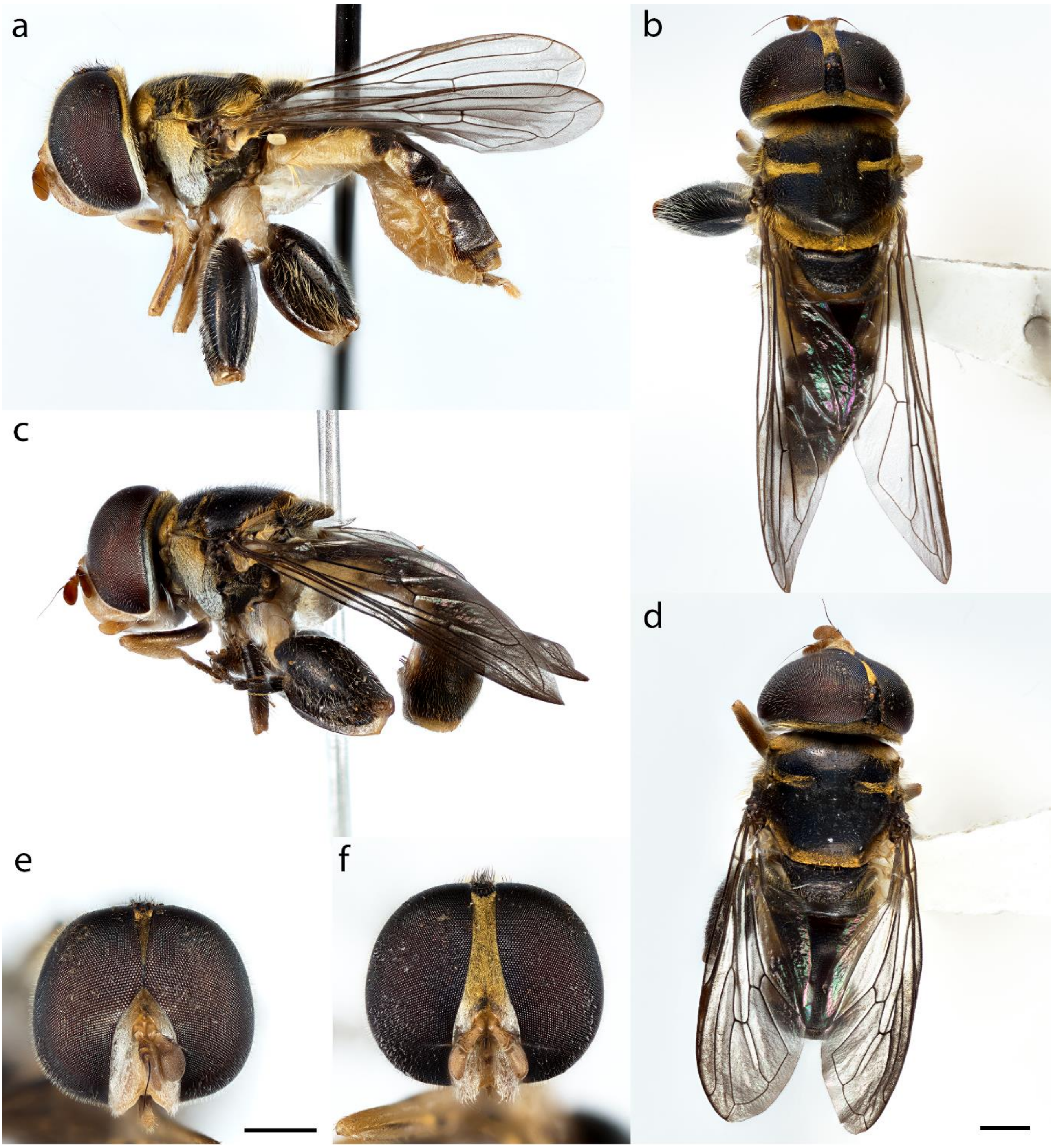

Fig. 6.16. Adult female (a, b, f) and male (c, d, e) *Nepenthosyrphus* species 1 from lower pitchers of *Nepenthes rafflesiana*. Scale bars represent 1 mm, with panels a–d, and panels e–f, sharing the same scale bars respectively. (Photographs by: Chou Ying Yi).

Nepenthosyrphus species 2 (Fig. 6.17) has not been observed extensively. This species is found in very low frequencies in *Nepenthes gracilis* pitchers in the CCNR. Both larvae and adults are significantly smaller in size than *Nepenthosyrphus* species 1, and *Nepenthosyrphus* species 2 appears to prefer feeding on culicid rather than phorid prey (WNL et al., unpublished data).

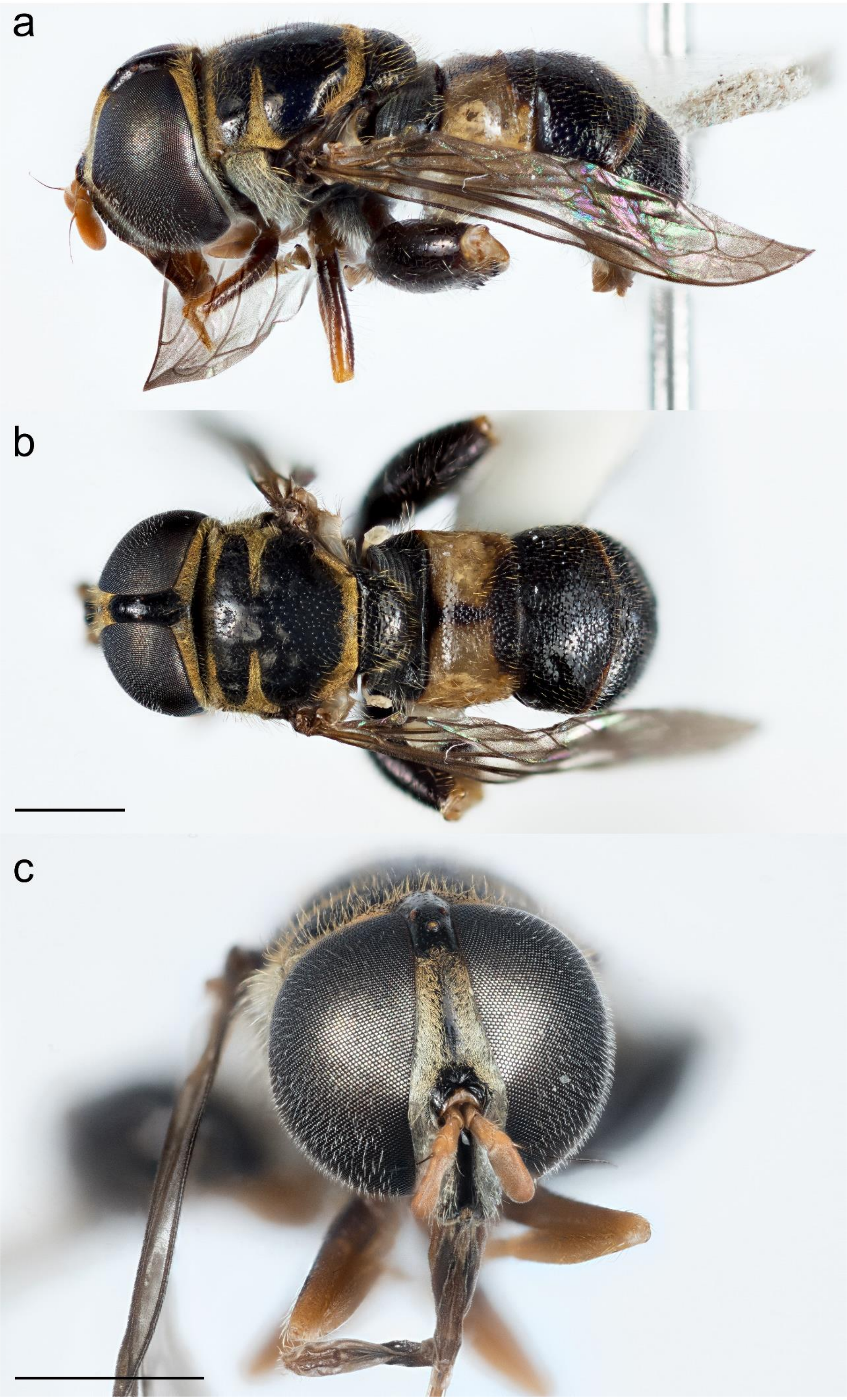

Fig. 6.17. Adult female *Nepenthosyrphus* species 2 from an upper pitcher of *Nepenthes gracilis*. Scale bars = 1 mm, with panels a and b sharing the same scale bar. (Photographs by: Chou Ying Yi).

The small predators (*Lestodiplosis *species and* Corethrella calathicola*). — *Lestodiplosis* is a genus of gall midges (family: Cecidomyiidae) while *Corethrella calathicola* belongs to the small, monotypic family Corethrellidae, sometimes known as frog-biting or parasitic midges (Borkent & Grafe, 2012). Adults of *Corethrella* species feed on the blood of frogs, to whose mating calls they are attracted (Borkent & Grafe, 2012), but little is known about the adult biology of *Lestodiplosis* species.

Lestodiplosis species larvae are common inhabitants of *Nepenthes gracilis* pitchers (Choo et al., 1997; Chua & Lim, 2012; Gaume et al., 2019), where multiple individuals are known to collectively attack and feed on inquiline *Dasyhelea* species larvae (Mogi & Chan, 1996). However, it is unlikely that *Dasyhelea* species are the only food resource of *Lestodiplosis* species, since both taxa do not appear to co-occur in sufficient frequencies in situ for one to be completely reliant on the other as a prey resource (Gaume et al., 2019; WNL, unpublished data). Beaver (1983) had proposed that *Lestodiplosis* species feed on the larvae of *Endonepenthia* spp. as well, but we have never observed this in controlled experiments (K. Fonseka & W. N. Lam, unpublished data). It is possible that *Lestodiplosis* species are facultatively bacterivorous and/or saprotrophic, allowing them to feed on alternative food sources when *Dasyhelea* prey are absent.

Corethrella calathicola is commonly found in *Nepenthes ampullaria* pitchers throughout the region (Beaver, 1979; Mogi & Yong, 1992; Clarke & Kitching, 1993; Mogi & Chan, 1996), and is also present in pitchers of *Nepenthes bicalcarata* and *Nepenthes veitchii* in Borneo (Borkent & Grafe, 2012; Gaume et al., 2019). It is the only species of its genus to inhabit *Nepenthes* pitchers (Borkent & Grafe, 2012). Furthermore, it is also the only member of the *appendiculata* species group to be found in the old world, with all other members of this lineage being neotropical tree hole-inhabiting species (Borkent & Grafe, 2012).

The larvae of *Corethrella calathicola* (Fig. 6.18) have been reported to feed on *Dasyhelea* species and young *Tripteroides tenax* mosquito larvae in the laboratory (Mogi & Chan, 1996). However, mosquito larvae are unlikely to be a major component of their natural diet, since the largest *Corethrella calathicola* larvae are still significantly smaller than the third or fourth instar larvae of

Fig. 6.18. The larva of *Corethrella calathicola*. (Photograph by: Robyn J. Y. Lim).

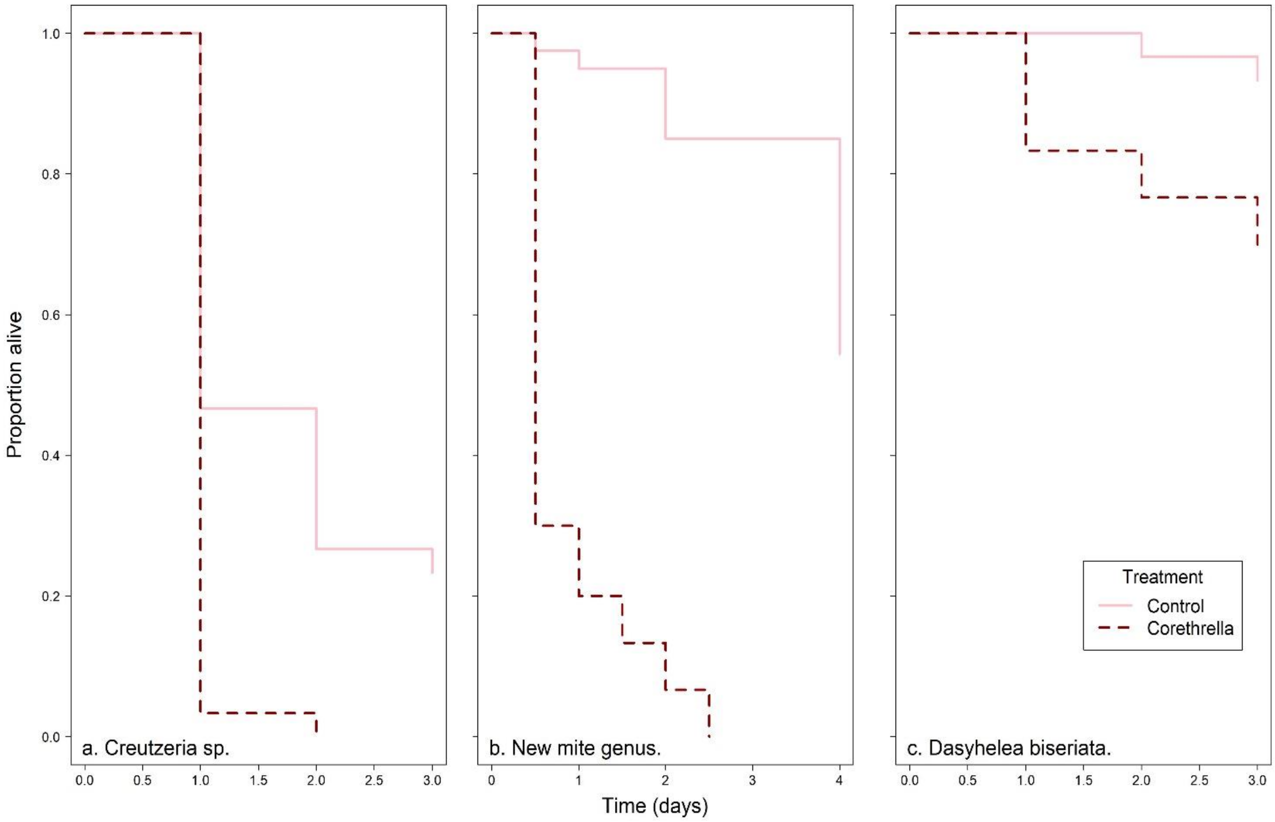

Fig. 6.19. Kaplan-Meier survival curves of 10 (a) *Creutzeria*, (b) undescribed mite genus 1 mites, and (c) *Dasyhelea biserata* in the presence (dashed red lines) and absence (continuous pink lines) of one third to fourth instar *Corethrella calathicola* individual. (*n* = 3; data from K. Fonseka & W. N. Lam, unpublished data).

most coexisting mosquito species. We have found that *Corethrella calathicola* feed on both *Creutzeria* and undescribed mite genus 1 mites at a far higher rate than they do on *Dasyhelea biseriata* (Fig. 6.19).

Unlike the predatory mosquito *Toxorhynchites acaudatus*, cannibalism is not common among the small predators, and multiple individuals of *Lestodiplosis* species (in *Nepenthes gracilis*; Gaume et al., 2019) and *Corethrella calathicola* (in *Nepenthes ampullaria*; Lim et al., 2019) are often found together in individual pitchers. Nevertheless, cannibalism does occur between individuals of *Corethrella calathicola* when these are artificially starved in the laboratory (Mogi & Chan, 1996).

The web-spinning mycetophilid (**Xenoplatyura beaveri*****, Mycetophilidae).*** — This predatory web-forming mycetophilid species was previously thought to be found only in Peninsular Malaysia and Singapore (Matile, 1979; Beaver, 1983; Clarke & Kitching, 1993), but was recently shown to be present in *Nepenthes ampullaria* in Borneo as well (Gaume et al., 2019). It occurs mainly in the pitchers of *Nepenthes ampullaria*, but has also been observed in pitchers of *Nepenthes hirsuta* and *Nepenthes rafflesiana* var. *gigantea* (WNL, pers. obs.). Unlike the other inquiline dipteran larvae, *Xenoplatyura beaveri* does not inhabit the pitcher fluids, but instead constructs and lives on sticky, mucilaginous webs in the upper regions of pitchers (Fig. 6.20). *Xenoplatyura beaveri* larvae often construct their webs within the cavity which exists between the inward fold of the peristome and the pitcher wall, and/or across the lumen of the entire pitcher (Fig. 6.20). Emerging dipteran inquiline adults are snared in these sticky webs when they attempt to leave pitchers, and *Xenoplatyura beaveri* appears to kill them by smothering them with more mucus secretions, before consuming their carcasses whole (Beaver, 1983; Mogi & Chan, 1996; Lam et al., 2018a). *Xenoplatyura beaveri* defecates into pitcher fluids after consuming the prey. Laboratory experiments have found that the feeding, excretion and even metamorphosis of *Xenoplatyura beaveri* individuals increase the

Fig. 6.20. The sticky web of *Xenoplatyura beaveri* enclosing the mouth of a *Nepenthes ampullaria* pitcher (left), and the underside of the peristome of a dissected *Nepenthes ampullaria* pitcher revealing a *Xenoplatyura beaveri* larva (arrow) and its web (right). Notice also the pupa of an unidentified phorid behind the larva—the phorid adult is likely to be ensnared in this web at the moment when it emerges from its pupal case. *Xenoplatyura beaveri* larvae often retreat to the narrow cavity underneath the overhanging peristome of *Nepenthes ampullaria* when threatened. Location: Nee Soon Swamp Forest. Scale bars = 1 cm. (Photographs by: Weng Ngai Lam).

Fig. 6.21. Pupa (left) and adult (right) of a *Xenoplatyura beaveri* individual reared to adulthood in the laboratory. (Photographs by: Weng Ngai Lam).

concentration of ammonium in pitcher fluids, thus returning nitrogen from emerging dipteran inquilines to pitcher phytotelmata (Lam et al., 2018a). Little is known concerning the biology of adults.

The crab spiders (Thomisidae, Araneae). — Two crab spider species (Thomisidae) are known to inhabit the pitchers of *Nepenthes gracilis* locally—*Thomisus nepenthiphilus* (Fig. 6.22) and *Misumenops nepenthicola* (Fig. 6.23) (Choo et al., 1997; Mogi & Chan, 1997; Chua & Lim, 2012). Both species are also regularly found in pitchers of *Nepenthes rafflesiana* in Borneo (Clarke, 1997, 2001; Karl & Bauer, 2020), although this is only very rarely observed in Singapore (Choo et al., 1997; WNL, pers. obs.). *Thomisus nepenthiphilus* carcasses have even been found in *Nepenthes rafflesiana* upper pitchers (Lam et al., 2018b), suggesting that *Nepenthes rafflesiana* in Singapore may possess adaptations that prevent or deter crab spider occupation (e.g., large downward-pointing peristome spines). Neither species inhabits the pitchers of *Nepenthes ampullaria* (Beaver, 1983), which are often filled with too much fluid to allow crab spiders sufficient nest-building space on pitcher walls. Curiously, *Thomisus nepenthiphilus* have also been found to be regular occupants of *Nepenthes ventricosa* × *Nepenthes alata* pitchers at a site in Sembawang in Singapore (Fig. 6.24). These plants are common in the ornamental plant trade, and were presumably ‘released’ into the habitat by plant owners who no longer desired to maintain them. Pitchers of *Nepenthes gracilis* and *Nepenthes ventricosa* × *Nepenthes alata* are morphologically and functionally similar, and it is likely that pitcher-inhabiting crab spiders may be able to adapt to living in pitchers of the latter taxon without difficulty.

Fig. 6.22. *Thomisus nepenthiphilus* adult male (left) and female (right), on upper and lower pitchers of *Nepenthes gracilis*, respectively. Scale bars = 1 cm. (Photographs by: Weng Ngai Lam).

Fig. 6.23. Adult female *Misumenops nepenthicola* in an upper pitcher of *Nepenthes gracilis*. (Photographs by: Weng Ngai Lam). Scale bars = 1 cm.

Fig. 6.24. Female *Thomisus nepenthiphilus* waiting in typical ambush posture for visiting prey under the peristomes of a *Nepenthes gracilis* upper pitcher (left) and a *Nepenthes ventricosa* × *Nepenthes alata* upper pitcher (right). Location: Sembawang Avenue. Scale bars = 1 cm. (Photographs by: Weng Ngai Lam).

Behaviour: Mature individuals of *Thomisus nepenthiphilus* and *Misumenops nepenthicola* dwell solitarily (Clarke, 2001; Lim et al., 2018; Karl & Bauer, 2020), and are both known to ambush and feed on pitcher-visiting arthropods (Fig. 6.24). This behaviour is discussed in detail in Chapter 5: Prey and Carnivory, and is likely to be beneficial towards their *Nepenthes gracilis* host (Lam & Tan, 2019). *Misumenops nepenthicola* is also known to consume dipteran larvae inhabiting the fluids of pitchers in Singapore (Chua & Lim, 2012), although Karl & Bauer (2020) observed this behaviour only very rarely in Borneo. This feeding behaviour was captured on film in the famous BBC documentary ‘Planet Earth’ (Episode 8: ‘Jungles’), which was narrated by Sir David Attenborough. Both *Misumenops nepenthicola* and *Thomisus nepenthiphilus* are also frequently cannibalistic. Female adults of both species are often observed consuming male adults, and less often, one another (Fig. 6.26). Lam & Tan (2019) found that pitchers inhabited by adult female *Thomisus nepenthiphilus* often contained carcasses of *Misumenops nepenthicola*, confirming observations in the laboratory that *Thomisus nepenthiphilus* often attacks and consumes its smaller competitor (Fig. 6.26).

Thomisus nepenthiphilus and *Misumenops nepenthicola* are nepenthebionts and are obligately dependent upon their pitcher plant hosts for survival. Both species live, feed and breed in pitchers, and are seldom found anywhere else except in or near pitchers. In fact, both spider species are so at home in pitchers that they always retreat into pitchers when disturbed. It is commonly observed that these spiders dive into pitcher fluids and hide among prey carcasses when frightened, emerging only after the perceived threat is past (Clarke, 1997; Karl & Bauer, 2020; Fig. 6.25).

Rembold et al., (2012) observed that another crab spider species in *Nepenthes madagascariensis*, *Synema obscuripes*, often creates an additional web to seal off pitchers in which they build their nests. This is not observed in either of the Singapore species, whose silk nests are often pressed and well camouflaged against the white inner walls of pitchers (Fig. 6.27; WNL, pers. obs.). Nests of *Misumenops nepenthicola* appear to consist of a single layer of silk over the nest cavity, with eggs and larvae held in direct contact with pitcher walls (Fig. 6.27; Karl & Bauer, 2020). Those of *Thomisus nepenthiphilus* appear significantly more robust, with eggs/larvae completely encircled within a spherical silk nest which is in turn attached by its sides against the pitcher wall (WNL, pers. obs.). The female spiders often guard their nests fiercely, attacking objects many times their size which threaten their eggs or larvae. This aggression is especially evident in *Thomisus nepenthiphilus*. However, adult crab spiders appear to abandon their young shortly after the young emerge from their nests, and newly emerged spiderlings are often observed leaving the pitcher in which they were born at a very young age (Fig. 6.28; Karl & Bauer, 2020).

Fig. 6.25. When threatened, *Misumenops nepenthicola* instinctively retreats into the narrow bases of *Nepenthes gracilis* pitchers, where it is often submersed in pitcher fluids and hidden from view. The figure shows top (left) and side (right) views of an adult female *Misumenops nepenthicola* hiding in a dissected *Nepenthes gracilis* pitcher. Scale bars represent 1 cm. (Photographs by: Weng Ngai Lam).

Fig. 6.26. From left to right: female *Thomisus nepenthiphilus* consuming a female *Misumenops nepenthicola*; female *Misumenops nepenthicola* consuming a smaller *Misumenops nepenthicola* (presumably a male); female *Thomisus nepenthiphilus* consuming a male congener. Scale bars = 1 cm. (Photographs by: Weng Ngai Lam).

Fig. 6.27. The nest of *Misumenops nepenthicola*, guarded by the mother (left). The nest is torn open, revealing approximately 30 juvenile spiders beneath its single silk layer (right). Both photographs share the same scale bar. Location: Holland Woods. (Photographs by: Weng Ngai Lam).

Fig. 6.28. Newly emerged *Thomisus nepenthiphilus* on the lid of a *Nepenthes gracilis* pitcher—perhaps waiting to be carried away by a gust of wind. Location: Holland Woods. (Photograph by: Weng Ngai Lam).

Other inquiline spider species: *Misumenops nepenthicola* and *Thomisus nepenthiphilus* are both nepenthebionts, and are thus found only in or around *Nepenthes* pitchers. But several nepenthexenous spider taxa have also been observed to utilise *Nepenthes* pitchers to varying degrees. The masked crab spider, *Thomisus guangxicus*, has been observed on *Nepenthes gracilis* pitchers on multiple occasions, and may utilise pitchers the way they utilise flowers—as hunting sites on which to ambush foraging insects (Fig. 6.29; WNL, pers. obs.). Unlike the nepenthebiont crab spider species, however, *Thomisus guangxicus* does not retreat into pitchers when threatened, nor has it been observed to nest in pitchers. Additionally, wandering spiders (*Heteropoda jaegerorum*, family: Sparassidae) and orb-weaver spiders (*Parawixia dehaani*, family: Araneidae) have also been observed on several occasions within pitchers (WNL, pers. obs.). It is unclear if these simply hide in pitchers during the day or if these spider taxa also utilise pitchers as foraging sites.

Fig. 6.29. The masked crab spider, *Thomisus guangxicus*, on a *Nepenthes gracilis* upper pitcher. This species is not typically associated with pitchers, but has been observed to use pitchers in a way comparable to other nepenthebiont Thomisids. Location: Sembawang Avenue. (Photograph by: Weng Ngai Lam).

CHAPTER 6 LITERATURE CITED

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CHAPTER 7: FLOWERING AND REPRODUCTION

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***Flower and inflorescence morphology.* —** *Nepenthes* are dioecious plants—that is, a single individual is only capable of bearing flowers of a single sex (Fig. 7.2), i.e., the plants are unisexual. *Nepenthes* flowers are racemes or panicles (Figs. 7.1–7.3). Inflorescences of *Nepenthes gracilis* and *Nepenthes rafflesiana* are raceme-like panicles (Fig. 7.3a), while those of *Nepenthes ampullaria* are typical panicles (Fig. 7.3b; Clarke, 1997). Each *Nepenthes* flower has four tepals whose upper surfaces often secrete nectar (Fig. 7.4). Tepals are similar looking perianth segments that occur in one whorl, unlike the sepals and petals of a typical flower which occur in at least two separate whorls.

Fig. 7.1. A large male *Nepenthes rafflesiana* plant flowers profusely after successfully reaching the top of an *Adinandra dumosa* tree. Location: Holland Woods. (All photographs in this chapter by: Weng Ngai Lam).

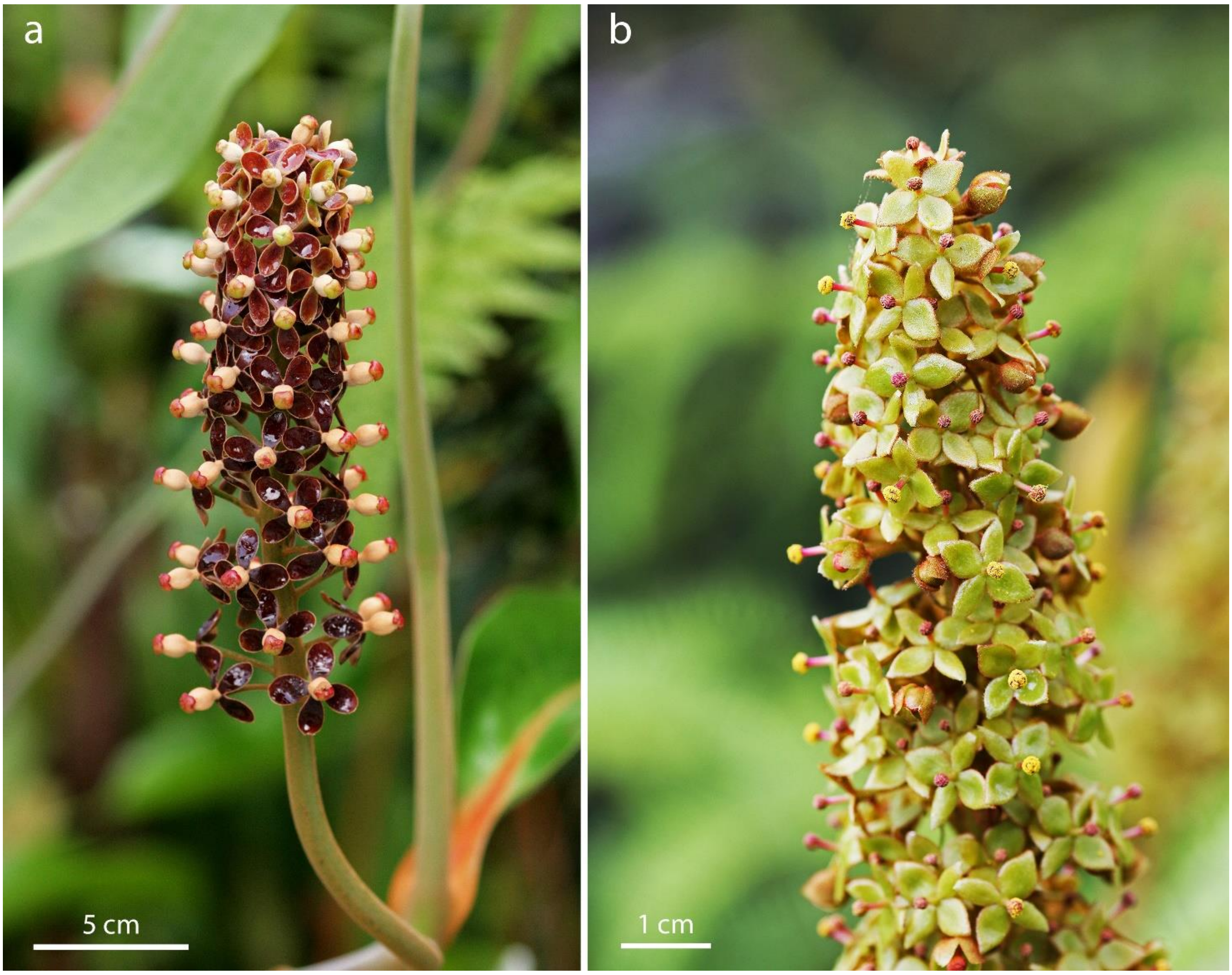

Fig. 7.2. Female *Nepenthes rafflesiana* (a) and male *Nepenthes ampullaria* (b) inflorescences. Locations: private collection (a) and Holland Woods (b).

Female flowers of a single inflorescence typically all open within a few days of each other (Fig. 7.2a), while the opening of individual flowers in male inflorescences (Fig. 7.2b) progresses slowly—often over the course of several weeks. In both sexes this progression is always in an acropetal direction (i.e., from bottom to apex) within individual inflorescences (Figs. 7.1, 7.3a) or sub-inflorescences (Figs. 7.2b, 7.3b). A female inflorescence may thus only be receptive to pollination over a short period of one to two weeks, while a male inflorescence may produce pollen continuously over the course of several weeks. It is generally regarded amongst *Nepenthes* horticulturists that female *Nepenthes* flowers are receptive to male pollen for only a period of no more than a week.

Flowering phenology. — Most flowering plants do not flower continuously, but instead do so in a synchronised manner seasonally (Schaik, 1993). The synchronisation of such flowering episodes between spatially separated populations of plants is usually accomplished by the means of joint responses to environmental cues. For example, temperate plants typically flower in response to the increases in day length and/or temperature which accompany the arrival of spring or summer (Smith-Ramirez et al., 1994), while tropical dipterocarps do so supra-annually after periods of exceptionally low rainfall (Schaik, 1993).

Synchronisation of flowering evidently occurs in *Nepenthes* species (Figs. 7.1, 7.5), and may serve to increase the chance of cross-pollination among individuals (female inflorescences which mature before or after a flowering episode typically experience low fertilisation rates [WNL, pers. obs.]), as

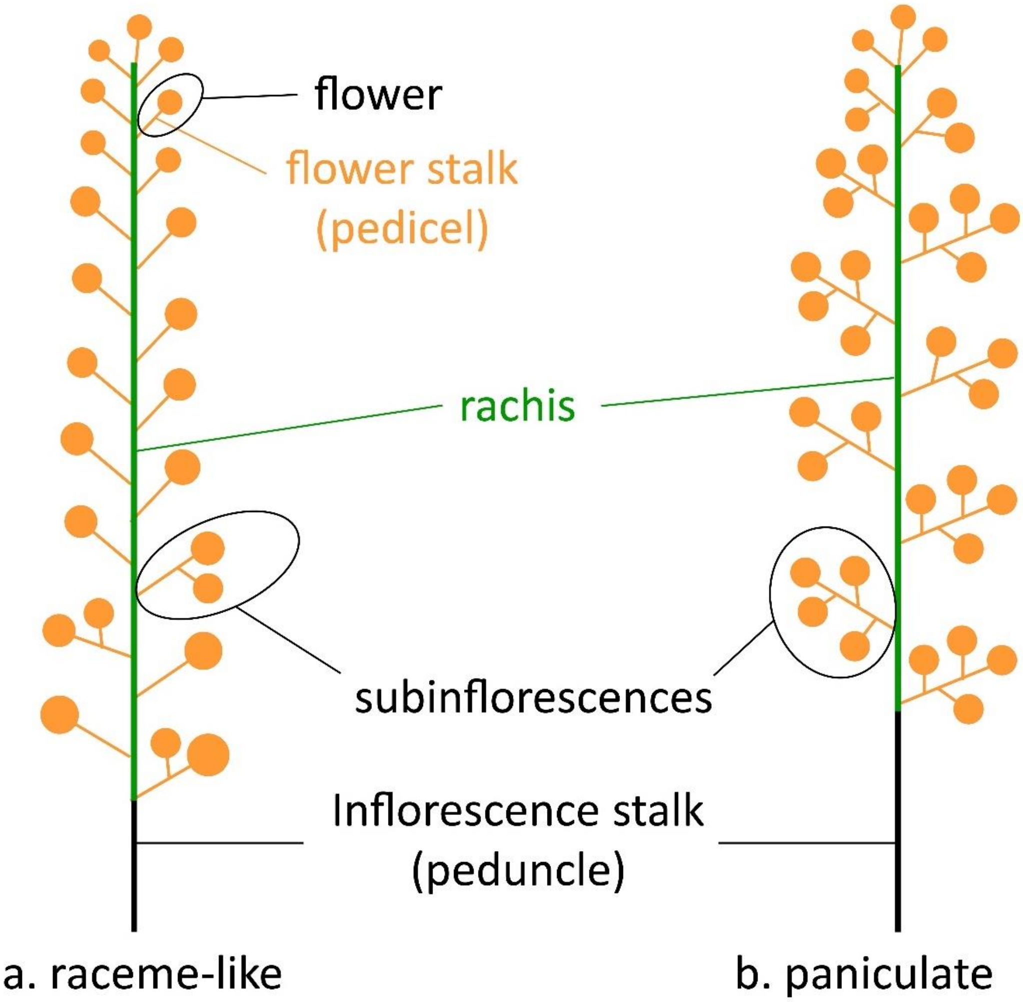

Fig. 7.3. Raceme-like panicle (a) and more typical paniculate (b) inflorescences in *Nepenthes* species.

Fig. 7.4. *Nepenthes rafflesiana* female flowers with nectar copiously secreted from surface of the tepals. Location: private collection.

well as reduce the population sizes of specialist seed predators such as the pitcher blue butterfly (*Virachola kessuma deliochus*; see Chapter 4: Herbivory). However, the environmental cues that are utilised by plants for the synchronisation of flowering are not well studied. Personal observations suggest that flowering episodes among local *Nepenthes* species occur at least twice yearly (Fig. 7.6). Those of *Nepenthes ampullaria* and *Nepenthes rafflesiana* appear to coincide with prolonged periods of little rainfall or to immediately follow them (Fig. 7.6). But flowering episodes of *Nepenthes gracilis* do not seem to follow the same pattern. Significant amounts of overlap occur between flowering episodes of all three species (Fig. 7.6), although hybridisation between *Nepenthes* species does not occur frequently, and may be hindered by pollinator partitioning between species (see section below on Pollination and pollinators).

Fig. 7.5. *Nepenthes ampullaria* female inflorescences in synchronised flowering in separate individuals. Location: Holland Woods.

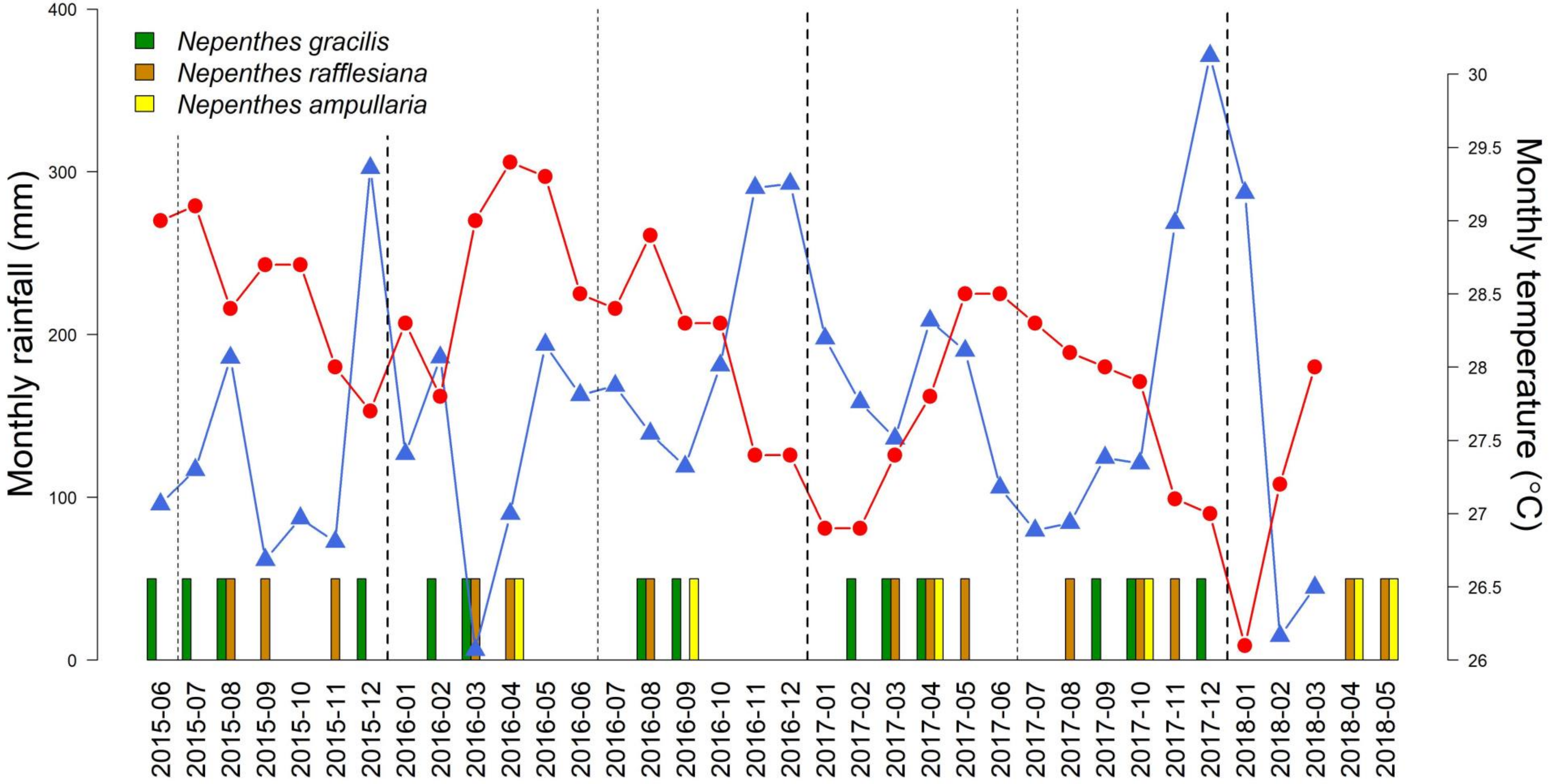

Fig. 7.6. Consolidated observations of in situ flowering episodes in the three native *Nepenthes* species over a period of three years. Bars indicate the observation of a flowering episode in a species (denoted by bar colour) during that month. Data of total monthly rainfall (blue triangles; in mm) and mean monthly surface air temperature (red circles; °C) as recorded from the Changi climate station (data obtained from data.gov.sg) are included for the inference of climate-induced flowering trends. A flowering episode is defined as the synchronised flowering of multiple individuals within a population, or across multiple populations, and was recorded by WNL through haphazard field observations made across multiple sites in Singapore. The regularity of *Nepenthes ampullaria*'s flowering episodes (every April and September or October, after a period of low rainfall) suggests that a flowering episode in October 2015 (2015-10) may have been missed.

Pollination and pollinators. — The pollination biology of *Nepenthes* species is a topic that has received relatively little attention in the literature. Pollen morphology and scented flowers suggest that *Nepenthes* species are insect-pollinated (Clarke, 2001). Furthermore, personal observations suggest that pollinators may be highly efficient at transferring pollen between *Nepenthes* flowers. The authors have repeatedly observed fertilisation rates of more than 90% on female inflorescences of a *Nepenthes rafflesiana* plant whose nearest conspecific neighbour was a male plant that was 96 metres away (Fig. 7.7).

Fig. 7.7. A *Nepenthes rafflesiana* infructescence. The nearest conspecific neighbour to this photographed specimen was a male plant that was located 96 m away, suggesting that pollen was efficiently transported across that distance or greater, at a level of efficiency which resulted in a more than 90% fertilisation rate for this inflorescence. Location: Holland Woods.

Fig. 7.8. A *Polyrhachis pruinosa* worker visits a female *Nepenthes ampullaria* inflorescence. Location: Sembawang Avenue.

Large *Polyrhachis* species ants are one of the most common visitors of *Nepenthes* flowers in the day (Fig. 7.8; Kato, 1993; WNL, pers. obs.), and have been hypothesised to be pollinators of *Nepenthes* species (Macfarlane, 1893). However, using inter-nest aggression experiments (as done in Maeyama & Matsumoto, 2000), the home range of *Polyrhachis pruinosa* ants in Singapore *Nepenthes* habitats was found to be less than 5 m on average (WNL, unpublished data). Ant pollination of *Nepenthes* flowers is thus very unlikely because the range over which ants may transport pollen is far too small for the effective pollination of most *Nepenthes* plants. Such an argument has also been made by other authors (Kato, 1993; Clarke, 2001), who observed that night-visiting moths and calyptrate flies are the more likely pollinators of *Nepenthes*. Nocturnal pollination is also supported by the higher nectar secretions on *Nepenthes* flowers in the night (Kato, 1993).

***Hybridisation between* Nepenthes *species.* —** Hybridisation (also known as ‘crossing’) is the process by which two plants of different species form offspring. Artificial hybridisation of *Nepenthes* species is also done regularly in horticulture to create novel *Nepenthes* hybrids or cultivated varieties for the ornamental plant market. Often, hybridisation produces plants that are more vigorous than both parent species—a phenomenon known as heterosis. Furthermore, few, if any, limitations exist in the ways in which *Nepenthes* species or hybrids may be crossed, which has led to the development of many complex hybrids involving numerous species and/or multiple back-crosses to parent species.

Three *Nepenthes* hybrids, formed by the pairwise combinations between the three native *Nepenthes* species, have been recorded in Singapore (Chapter 2: Singapore Species and Hybrids). However, despite significant overlaps in flowering periods between co-occurring *Nepenthes* species populations (Fig. 7.6), *Nepenthes* hybrid plants are often found in densities far lower than that of their parent species (Peng & Clarke, 2015), and seldom, if ever, occur in mono-hybrid stands (Clarke, 1997; WNL, pers. obs.). This suggests the existence of one or more ecological barriers preventing the self-replication of hybrid populations. Peng & Clarke (2015) also observed that hybrids tend to occur in disturbed sites, and argued that anthropogenic habitat modification disrupts flowering phenology, promoting overlaps in flowering periods that are conducive for hybridisation. However, data to support such a hypothesis were not available (Peng & Clarke, 2015).

We argue that the three native *Nepenthes* species are likely to utilise different pollinator species, and that this interspecific partitioning of pollinators is likely to explain why relatively low rates of hybridisation are observed, as well as why hybrid *Nepenthes* plants do not develop into populations. Firstly, flowers of the three native *Nepenthes* species differ significantly in size and overall morphology. Secondly, if *Nepenthes* hybrids are not limited in fitness during their sterile life stages (i.e., high mortality rates during the period from germination to maturation) as demonstrated in horticulture, then population limitation is most likely the result of propagule limitation. Yet if *Nepenthes* species pollen is interspecifically compatible, as horticulture has likewise demonstrated, and co-occurring and concurrently flowering populations of parent species are frequent, as has been shown earlier (Fig. 7.3), then propagule limitation can only occur as a result of pollen vector limitation. However, the relatively high rates of pollination success observed in the inflorescences of *Nepenthes* species (Fig. 7.7) shows that pollinators are neither limited nor poor dispersal agents. The only logical consequence of these observations is that *Nepenthes* hybrids are limited by their abilities to pollinate or be pollinated, perhaps because they are ineffective at attracting specialist pollinators.

Pollinator–prey conflict. — Pollinator–prey conflict occurs in carnivorous plants when arthropod species can be both prey to its traps and pollinators to its flowers. In the absence of mechanisms preventing pollinator–prey overlap, a carnivorous plant may, hypothetically, depress arthropod populations through their consumption to the extent that these can no longer provide effective pollination services to it.

Jürgens et al. (2012) identified three conditions that must all be met for pollinator–prey conflict to emerge:

1. the carnivorous plant must depend on pollinators for reproduction;
2. the carnivorous plant must be pollen- or pollinator-limited; and
3. an overlap in prey and pollinator species must exist.

Nepenthes species meet the first condition because they are dioecious, insect-pollinated plants that can neither persist indefinitely through asexual reproduction nor self-pollinate. The second condition of pollinator limitation has never been tested in *Nepenthes* communities before, but that of prey limitation has been clearly demonstrated, and prey limitation means pollinator limitation as well if pollinator and prey species overlap (the third condition, which is discussed next). Lam et al. (2018) showed that intraspecific competition between *Nepenthes gracilis* plants results in reduced prey capture in individual pitchers, and the repeated demonstration of resource partitioning between coexisting *Nepenthes* species suggests that prey competition (which necessitates prey limitation) is strong enough to cause niche segregation between sympatric species (Gaume et al., 2016; Lam et al., 2018a, 2018b).

Because the first two conditions of pollinator–prey conflict are likely to be true for most *Nepenthes* species, the occurrence of pollinator–prey conflict can be predicted by the magnitude of the third condition of pollinator–prey overlap. Pollinator–prey overlap can be reduced by temporal or spatial separation of traps and flowers (Jürgens et al., 2012). Temporal separation of traps and flowers occurs in some temperate carnivorous plant species such as the Venus fly trap (*Dionaea muscipula*) and various *Sarracenia* species, which flower in early spring before the production of traps. Spatial separation of traps and flowers is evident in aquatic carnivorous plants (*Utricularia* species, *Aldrovanda vesiculosa*, *Genlisea* species) and many low-growing butterwort (*Pinguicula*) and sundew (*Drosera*) species. These carnivorous plants produce long flower stalks which position their flowers far from their trapping organs, and thus reduce or eliminate (in the case of the aquatic carnivorous plants) the possibility of pollinator–prey overlap.

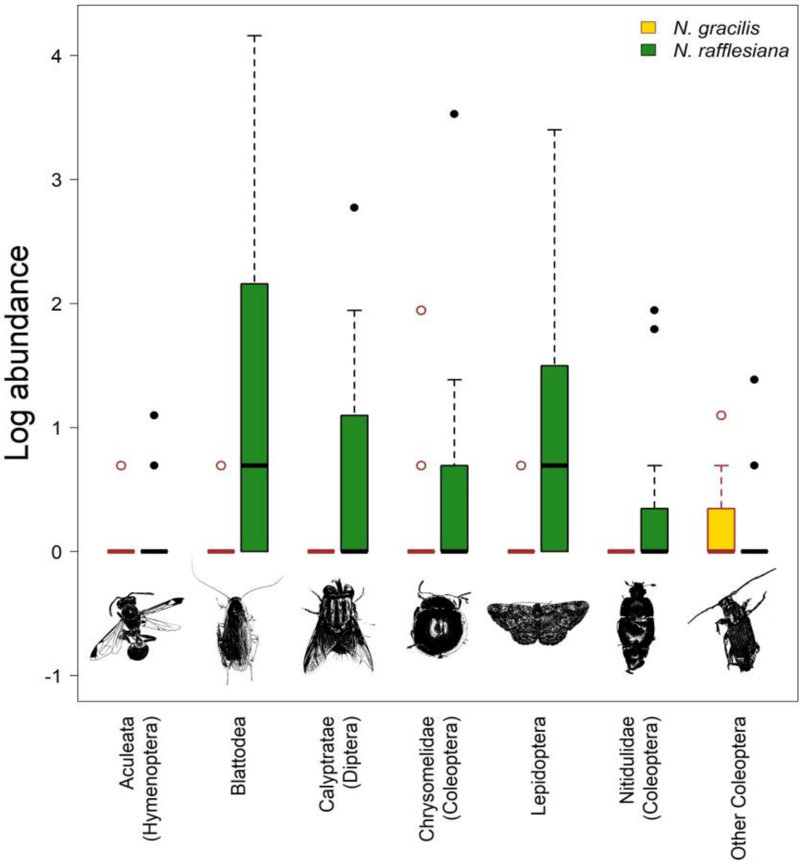

Fig. 7.9. Capture rates of the upper pitchers of *Nepenthes rafflesiana* (green boxes with black borders) and *Nepenthes gracilis* (yellow boxes with brown borders) of seven insect taxa known to pollinate flowers (*n* = 15; data from Lam et al. [2018]). Bold lines represent medians; boxes represent the interquartile range; whiskers represent maxima; points represent outliers.

With the exception of *Nepenthes ampullaria*, whose pitchers are predominantly produced in the undergrowth and are almost always spatially separated from their exposed inflorescences, the other two Singapore *Nepenthes* species do not exhibit discernible temporal and/or spatial separation of traps and flowers. *Nepenthes rafflesiana* and *Nepenthes gracilis* develop pitchers continuously during their flowering periods, and do not elevate their inflorescences significantly beyond the vertical stratum occupied by their upper pitchers. This means that pollinator–prey conflict can potentially occur between *Nepenthes rafflesiana* or *Nepenthes gracilis* and their prey. This situation is exacerbated by the fact that upper pitchers, in particular those of *Nepenthes rafflesiana*, often trap large numbers of flying insect taxa that are known to pollinate other flowering plants (Fig. 7.9). Nevertheless, fertilisation rates in female *Nepenthes rafflesiana* plants are typically high (Fig. 7.7), leading to the conclusion that pollinators of *Nepenthes rafflesiana* must somehow be able to evade capture by its pitchers. Such a proposition, though logically coherent, has never been empirically tested, and should be the focus of future studies in pollinator–prey conflict in *Nepenthes* species. Alternatively, some researchers have observed that pitchers are less strongly scented during the flowering season (U. Bauer & B. Di Giusto, pers. comm.), presumably to reduce the trapping rate of potential pollinators when flowers are receptive to pollination.

Fertilisation and seed development. — In the three Singapore *Nepenthes* species, the male inflorescences senesce shortly after flowering is completed (Fig. 7.10), but female ones persist much longer, and, if successfully fertilised, develop fruits (an inflorescence whose flowers have been fertilised and have developed into fruits is known as an infructescence; Fig. 7.11). Fruits take approximately two months to develop (WNL, pers. obs.). When ripe, the fruits turn yellow or brown and dehisce loculicidally to release many threadlike, wind-dispersed seeds (Figs. 7.12–7.14). Little is known about *Nepenthes* seed dispersal and germination, but observations by us suggest that all three native *Nepenthes* species germinate primarily only on exposed soils in open patches or young, resam-dominated scrub (Fig. 7.15). *Nepenthes* seeds and seedlings appear to have poor tolerance for leaf litter, and thus appear incapable of recruitment in forested habitats where bare ground is scarce.

Fig. 7.10. The male inflorescence of a *Nepenthes gracilis* plant senesces shortly after flowering is completed. Location: Holland Woods.

Fig. 7.11. A cohort of *Nepenthes gracilis* fruits develops together towards maturity. Fertilised (black arrows) and unfertilised (white arrows) fruits are clearly visible in the infructescence on the left. Location: Holland Woods.

Fig. 7.12. *Nepenthes gracilis* with an infructescence of fully dehisced fruits awaiting a gust of wind to disperse its seeds. Location: Holland Woods.

Fig. 7.13. Close-up of the freshly dehisced fruits of a *Nepenthes gracilis* infructescence in Fig. 7.12. Location: Holland Woods.

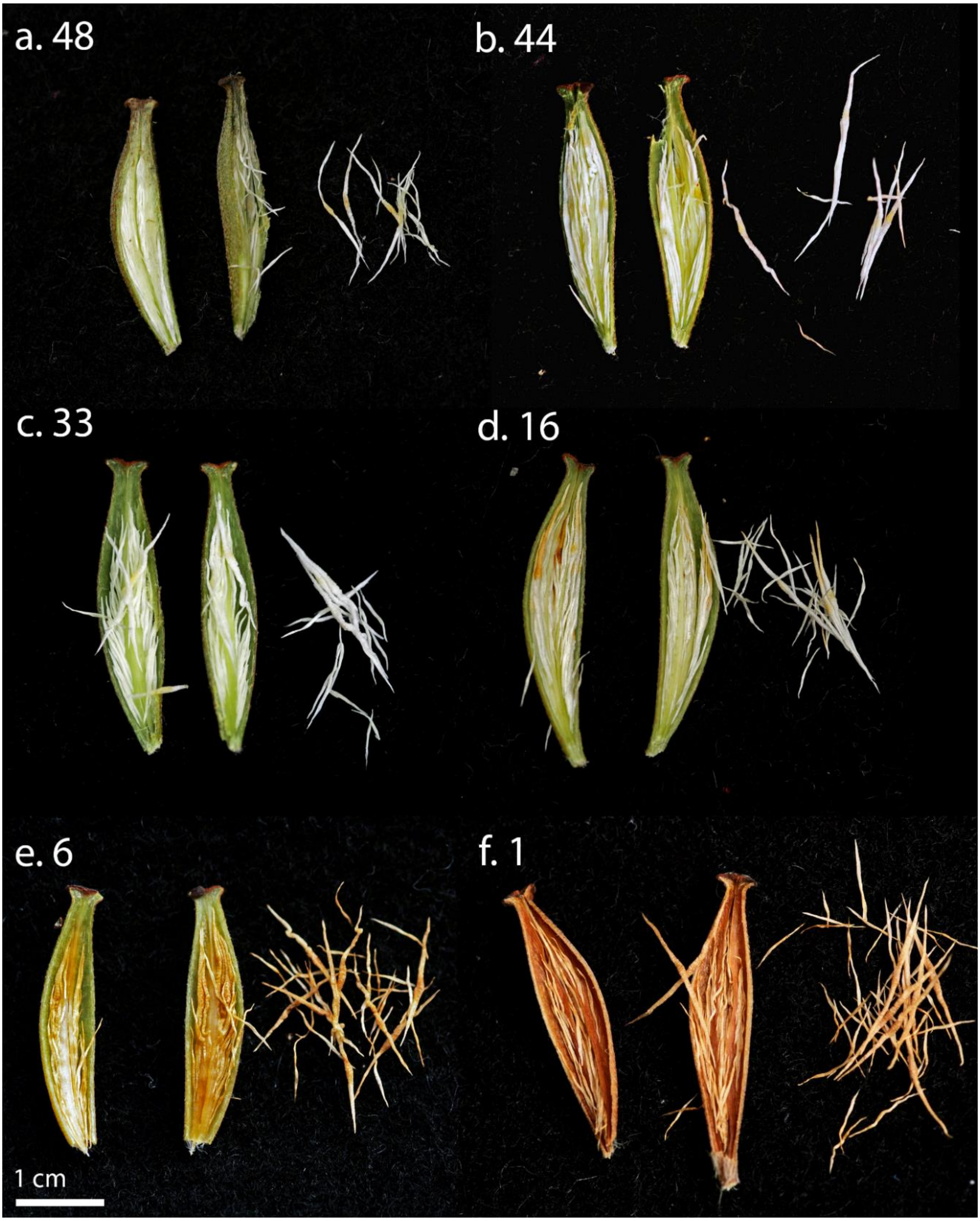

Fig. 7.14. Ripening process in a typical *Nepenthes* fruit that has been dissected. In each of the six panels (a–f), a single fruit of *Nepenthes* × *coccinea* (a horticultural hybrid of *Nepenthes mirabilis* × [*Nepenthes ampullaria* × *Nepenthes rafflesiana*]) was randomly removed from a maturing infructescence and dissected longitudinally. Several unripe seeds were removed and placed beside the dissected fruit in each photograph. Numbers after panel letters indicate the number of days before dehiscence at which a fruit was collected and dissected.

Fig. 7.15. Seedlings of *Nepenthes rafflesiana* (left) and *Nepenthes gracilis* (right) at two exposed sites. *Nepenthes* species typically recruit only on soil surfaces that are relatively free of leaf litter and competing vegetation. Locations: Kent Ridge Park.

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CHAPTER 8: GLOSSARY

To make this book's contents more accessible to non-specialist readers, simplified definitions of technical or scientific jargon are provided below. The definitions here are not universal but are the ones most appropriate to the terms in the contexts in which they are used in this book. Furthermore, definitions provided here are deliberately simplified to be accessible to a broad audience, and are thus not as accurate as definitions provided in scientific dictionaries. Names of species (e.g., *Dolichoderus thoracicus*, an ant species) or higher order taxa (e.g., Asteraceae, a plant family) are not defined here. Additionally, words whose definitions are given at their only occurrence in the book are also not included in this glossary.

adhesive pad — The anatomical structure on arthropod legs that facilitates their adhesion and thus movement on non-horizontal surfaces.
ambulatory — Having the habit of walking. See also *natatory*.
anthropogenic — Having a cause or origin in human activities.
apical meristem — The point (apex) of a shoot or root system from which growth originates via cell division, enlargement and differentiation.
arthropod — The animal phylum containing invertebrate organisms bearing an exoskeleton and jointed legs, such as the insects, spiders, crustaceans, etc.
axillary bud — Embryogenic, as yet undeveloped shoots found at the axils of leaves, which have the potential to develop into leafy branches, leafy and flowering branches, inflorescences (flower clusters) or flowers.
benthic — Describing an aquatic organism that occupies the lowest zone of the water column (i.e., on or just above the substrate). See also *pelagic*.
biogeography — The study of the natural distribution of species through space and time.
cannibalistic — The habit of consuming another member of the same species.
capitulum (plural, **capitula)** — A type of flower cluster where the axis of the shoot is axially compressed, and individual flowers are positioned on a single, lateral plane, as in the capitula of a sunflower, daisy or chrysanthemum.
carnivore (adjective, **carnivorous)** — An organism which feeds primarily on animal tissue. See also *detritivore* and/or *herbivore*.
chelicerae (singular, **chelicera)** — The mouthparts of arthropods in the subphylum chelicerata, which includes animals such as scorpions, spiders, etc.
chitin — A biopolymer that forms the main constituent of the exoskeletons of arthropods and the cell walls of fungi.
community — A group of different, interacting species sharing the same habitat and resource pools at the same point in time.
decurrent — Describing a leaf base which extends down along the stem beyond its point of attachment. See Fig. 2.11.
dehisce — The splitting of a chamber-like plant organ—especially fruits—to release its contents at maturity. *Dehiscence* describes this process of splitting.
detritivore (adjective, **detritivorous)** — An animal that feeds primarily on decomposing organic matter. See also *carnivore* and/or *herbivore*.
deutonymph (also known as **hypopus)** — A developmental stage, in the life cycle of pitcher inquiline mites, adapted for dispersal (via attachment to a suitable phoretic host) between pitchers.
digestion — The breaking down of a large, complex macromolecule (such as protein) into smaller, simpler units (such as amino acids), often to facilitate absorption.
dimorphic (noun, **dimorphism)** — Having two different forms. See also *polymorphic*.
dioecious — Describing a plant species whose flowers are unisexual and borne on different individuals (i.e., the same individual cannot bear bisexual or both male and female flowers), i.e., the species consists of male and female individuals, just like humans.
ecology — The study of how species interact with each other and their environment.

endogenous — Originating within an organism.

enzyme — Biological molecules that accelerate or decelerate specific chemical reactions.

epicuticular wax — A layer of wax deposited upon the outermost surface (cuticle) of plant tissues.

eukaryote — All species whose cell nucleus is enclosed within a nuclear envelope, that is, all species which are not bacteria or archaea.

exoskeleton — The hard outer covering of animals such as arthropods (e.g., insects and crustaceans) and molluscs (e.g., snails).

filamentous —Threadlike in form.

genus (plural, **genera**; adjective, **generic)** — The taxonomic rank above species (see *Taxonomy*). *Monogeneric* describes a family containing only a single genus.

geographic range — The area over which a species naturally occurs.

gland — A biological structure that secretes one or more compounds. *Glandular* refers to a surface containing glands.

habitat — The environment in which a species lives.

haematophagus — The habit of feeding on blood.

herbivore — An organism that feeds primarily on plant material or algae. See also *carnivore* and/or *detritivore*.

hybrid — The offspring of two different species. *Hybridisation* is the process by which a hybrid is produced.

hydrolytic — Describing a chemical reaction in which a larger molecule is broken into smaller molecules as it reacts with a molecule of water. *Hydrolysis* refers to such a chemical reaction.

hygroscopic — Describing a substance which absorbs moisture from the air.

idioblast — A cell that differs in form and function from its immediate neighbours.

inflorescence — A cluster of flowers borne on a single shoot system.

infructescence — An inflorescence whose flowers each have developed into a fruit.

instar — A developmental stage in the life cycle of insects and arthropods. The first instar is the least mature stage of development.

lamina (also known as the **leaf blade)** — The portion of the leaf that is typically expanded, flattened and adapted for light capture for photosynthesis in most plants. See Fig. 1.1 for illustration.

larva (plural, **larvae)** — The juvenile, pre-metamorphosis stage(s) of an insect.

lithophytic — Describing a plant that often grows upon rocks or the cracks in rocks.

lumen — The cavity of a hollow, tubular biological structure.

metabolic — A process associated with an organism’s metabolism, which consists of the basic chemical processes sustaining life.

metazoa (adjective, **metazoan)** — A multicellular animal species.

microbe — A species that is too small to be visible to the naked eye, including archaea, bacteria, fungi, protists, etc.

mimicry — The resemblance of one species to another, often with an adaptive function. *Batesian mimicry* is a specific type of mimicry in which a species lacking the physiological investments of the anti-predator defence or pollinator-attracting reward resembles one that does. For example, a harmless snake species (mimic) resembles a venomous one (model), or a carnivorous plant secreting only small amounts of nectar to lure insects to their death (mimic) resembles a flower that secretes large amounts of nectar to reward insects for their services in effecting pollen transfer (model).

morphology — The study of the physical structure of organisms. See also *physiology*.

mutualism — An interaction between two species from which both benefit. *Mutualists* are species which participate in a mutualism.

nanophyll — The reduced leaf that bears ground pitchers of *Nepenthes ampullaria* and other carpet-forming *Nepenthes* species (see Chapter 2: Singapore Species and Hybrids: *Nepenthes ampullaria*).

natatory — Having the habit of swimming. See also *ambulatory*.

native — Describing a species that is found naturally in an area.

nectary — A gland that secretes nectar. Extrafloral nectaries refer to nectaries found on plant parts other than the flowers.

nepenthebiont, nepenthephile, nepenthexene — Terms that classify *Nepenthes* inquiline species into groups according to their degree of dependence upon *Nepenthes* pitchers as habitats. Nepenthexenes are species that may colonise *Nepenthes* pitchers opportunistically, although they are not typically a part of *Nepenthes* phytotelma communities. Nepenthephiles are frequently found in pitcher phytotelmata, but are not completely dependent upon these for survival. Nepenthebionts are obligately associated with *Nepenthes* pitchers, and are completely dependent on pitcher phytotelmata during at least one stage of their life cycle.

nocturnal — Describing a species that is active mostly at night.

nuclease — An enzyme that catalyses the breaking of long chains of (deoxyribo- [DNA] or ribo- [RNA]) nucleic acids into shorter chains of the same.

nucleic acid — Molecules responsible for the encoding of genetic information in cells.

olfactory — Relating to smell.

ontogeny (adjective, **ontogenic)** — The natural and predictable development of an organism through its lifetime.

panicle — A cluster of flowers with two or more degrees of branching, i.e., a raceme of racemes. See Fig. 7.3 for illustration.

pelagic — Describing an aquatic organism that occupies the middle zone of the water column that is between the surface and the bottom of the aquatic habitat. See also *benthic*.

petiole (also known as the **leaf stalk)** — The stalk attaching a leaf blade to the stem. See Fig. 1.1 for illustration.

perianth — The collection of floral parts in a flower which are non-reproductive (viz., the sepals and/or petals or tepals).

peristome — The structure surrounding the mouth of pitchers.

phenology — The study of cyclic and/or seasonal biological phenomena, such as flowering, fruit production, migration, etc.

pheromone — A chemical secreted by an organism whose function is to stimulate behavioural or physiological changes in other individuals of the same species.

phosphatase — An enzyme that catalyses the removal of a phosphate molecule from a phosphate-containing molecule.

phototropism — The phenomenon in which a plant or plant part grows or moves towards a directional light source.

physiology — The study of the processes associated with the functioning of living systems. See also *morphology*.

physiognomy — The appearance and structure of a plant community.

phytotelma (plural, **phytotelmata)** — A water body held by or within a terrestrial plant organ (e.g., fluid-containing pitchers, tree holes, tank bromeliads etc.).

pollen — The substance, produced by male flowers or floral parts, which produces sperm cells for the sexual reproduction of plants. See also *pollinate*.

pollination — The physical transfer of pollen from male flowers or floral parts to the stigma of a flower of the same species. A *pollinator* is the animal agent that facilitates this process.

polymorphic (noun, **polymorphism)** — Having multiple different forms. See also *dimorphic*.

population — A group of interbreeding individuals of the same species.

predator — An organism that kills and consumes another. A *seed predator* is an organism which consumes seeds or parts of seeds in a way that renders them no longer viable. It is thus different from a herbivore in that the act of consumption kills the individual plant, whereas a herbivore may eat parts or one part of the plant such as its leaves. See also *carnivore* and/or *herbivore*.

prehensile — Describing a structure that is capable of grasping by means of wrapping around an object.

protease — An enzyme that catalyses the breaking of long protein chains into shorter protein chains and/or amino acids. *Aspartic*, *cysteine* and *serine proteases* are three different families of proteases which are distinct in the structures of their active sites.

prothoracic shield — A flat, plate/shield-like structure found on the first segment (prothorax) of a caterpillar.

propagule — Any plant structure that may develop into a new, separate plant individual when detached from the parent plant.

protist/protozoa — Microscopic eukaryotes such as amoeba, dinoflagellates, etc. The terms are not synonymous, but differences are irrelevant to their usage in this book.

pupa (plural, **pupae)** — The intermediate life stage of an insect, which it goes through as it develops (metamorphoses) from larval to adult life stages. *Pupation* describes the process in which a larva transforms into a pupa.

raceme — A kind of flower cluster that consists of a single vertical axis with stalked flowers inserted along the length with the oldest flowers at the base (proximal end) and flowers getting progressively younger towards the tip (distal end). See Fig. 7.3 for illustration.

recruitment — The addition of new individuals to a population; in the context of *Nepenthes* plants, the survival of seedlings past the germination stage.

respiration — The process in which a cell obtains energy from nutrients and oxygen.

reticulated — Having a netlike pattern.

rhizosphere — The soil surrounding a plant's root ball.

rosette — In reference to the *Nepenthes* plant: an immature plant (and/or its leaves and stem) which has not yet begun to climb—i.e., the plant's stem remains compact, with short internodal distances.

sclerotised — Describing a hardened biological substance or structure.

senescence — Deterioration owing to the natural process of ageing.

sequestration — The process by which an organism takes up a substance from the environment.

seta (plural, **setae)** — A hair-like biological structure.

stigma (plural, **stigmata)** — The floral part that receives pollen. See also *pollination* and/or *pollen*.

species (plural, **species)** — The taxonomic rank below genus (see *Taxonomy*).

succession (ecological) — The natural process in which an ecological community changes compositionally over time.

Sympatry (adjective, **sympatric)** — The occurrence of two or more populations of different species in the same geographic area at the same time.

taxon (plural, **taxa)** — Any taxonomic unit, regardless of hierarchy (see *Taxonomy*).

taxonomy (adjective, **taxonomic)** — The study of naming and classifying biological organisms. The seven major taxonomic ranks in such a system, in increasing order of hierarchy, are: species, genus (pl. genera), family, order, class, phylum (pl. phyla), kingdom. For example: the common pitcher inquiline mosquito *Tripteroides tenax* is the species from the genus *Tripteroides* with the specific epithet '*tenax*'; the *Tripteroides* genus belongs to the mosquito family Culicidae, which belongs to the order of the true flies, Diptera, which belongs to the phylum Arthropoda, which belongs to the kingdom Animalia.

tendril — A specialised plant organ, or part of an organ, that is like a coiled spring in structure and used to facilitate climbing by grasping adjacent structures. See Fig. 1.1.

type species — Relating to taxonomy, a species that is permanently associated with a genus name, and thus a species which will remain associated with that genus name even if the genus is subsequently revised.

tornal — Associated with the rearmost region of the wing of a butterfly.

variety — Relating to plant taxonomy, a taxonomic rank below that of species (see *Taxonomy*).

vermiform — Wormlike in form.

viscoelastic — Describing a substance that possesses both viscous and elastic properties.

volatile — Describing a substance that evaporates quickly at room temperature and pressure.

wavelength — The period of a light wave. In the visible spectrum, warm colours have lower wavelengths, while cool colours have higher wavelengths.

whorl — A group of three or more leaves or floral parts which are arranged radially around a single point along the stem or stalk.

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