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Preliminary checklist of the inquiline and prey species of *Nepenthes ampullaria* pitchers across vegetation types in Singapore

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Abstract. The contents of 147 pitchers from the pitcher plant *Nepenthes ampullaria* were sampled from three sites across Singapore. The primary aim of the study was to compile a comprehensive checklist of the inquiline species inhabiting *Nepenthes ampullaria* pitchers across the key *Nepenthes* habitat types in Singapore, with the secondary aims of compiling a preliminary checklist of *Nepenthes ampullaria* prey species, and identifying structural or floristic habitat characteristics associated with reproducing populations of *Nepenthes ampullaria* plants. The data suggested that *Nepenthes ampullaria* flowering occurs more regularly in habitats with little canopy cover—such as in urban, resam-dominated scrub, or in large treefall gaps. A total of 23 inquiline morphospecies and 72 prey morphospecies were observed, and 20 of these inquiline morphospecies could be confidently identified at least to the genus level. This also included an undescribed genus of histiostomatid mite, and one possibly undescribed taxon from the hoverfly genus *Nepenthosyrphus*. Among the prey species, the ant *Meranoplus malaysianus* represents a first record in Singapore. Inquiline diversity was not equally distributed across all samples, but was much higher in the Nee Soon Swamp Forest than in Kent Ridge Park and the urban sites, with 12 inquiline species being found only in the Nee Soon Swamp Forest. However, abiotic conditions of pitchers (fluid pH, volume and depth, pitcher dimensions and sediment volumes in pitchers) differed little between pitchers from the three different sites, thus suggesting that abiotic conditions of the larger environment, such as the absence of adult food resources in the surrounding vegetation, and/or dispersal limitations, such as the anthropogenic barriers separating inquiline source populations in the Nee Soon Swamp Forest from Kent Ridge Park and urban vegetation patches, are more important in determining inquiline community composition in *Nepenthes ampullaria* phytotelmata.

Key words. pitcher plant, phytotelmata, diptera, inquiline, biodiversity, habitat fragmentation

INTRODUCTION

The genus *Nepenthes* comprises carnivorous plants which capture and digest animal prey using modified-leaf pitfall traps known as pitchers. The genus has its centre of diversity in Southeast Asia, and three species, namely *Nepenthes ampullaria*, *Nepenthes gracilis*, and *Nepenthes rafflesiana*, are native to Singapore (Tan et al., 1997). Despite their carnivorous function, *Nepenthes* pitchers are important plant-held aquatic habitats, also known as phytotelmata (singular, phytotelma; Maguire, 1971). These are often inhabited by specialised aquatic organisms known as inquilines (Beaver, 1979; Clarke & Kitching, 1993; Mogi & Chan, 1997).

Phytotelmata are useful model systems for the study of ecology and biodiversity because of their small size, short generation times, contained structure and hierarchical spatial arrangement (Srivastava et al., 2004; Gaume et al., 2019). For example, pitchers of the North American purple pitcher plant, *Sarracenia purpurea*, have been used extensively for the study of bottom-up (Gray et al., 2006), top-down (Kneitel & Miller, 2002; Canter et al., 2018), temperature-structuring (Hoekman, 2010) and dispersal (Kneitel & Miller, 2003) processes on community structures. *Nepenthes* species have been used similarly for the study of pairwise-interspecific interactions (Schöner et al., 2015; 2017; Leong et al., 2018; Lim et al., 2018; Lam & Tan, 2019), and the taxonomy and biology of many individual inquiline species have been well documented in recent years (Mogi & Chan, 1996; Borkent & Grafe, 2012; Fashing, 2002; 2004; 2008; Das & Haas, 2010; Rotheray et al., 2012; Lam et al., 2018; Gaume et al., 2019). However, the use of whole *Nepenthes* inquiline communities as models for the study of community or ecosystem processes or biodiversity phenomena remains minimal, owing largely to the poor characterisation of inquiline species (although see Gaume et al., 2019). For example, a comprehensive study of inquiline food webs in six *Nepenthes* species could only identify inquilines to generic level (Clarke & Kitching, 1993), and the use of metabarcoding for the investigation of host-inquiline specificity in another study (Bittleston et al., 2015) could not achieve species-level identification either. In both of these examples, low taxonomic resolution hindered the process of ecological inference.

In addition, the distribution of inquiline species across *Nepenthes* pitcher phytotelmata is an aspect of inquiline communities that has generally been overlooked. The occurrence of species in a given habitat is constrained by dispersal limitations, abiotic filters or biotic interactions (Kraft & Ackerly, 2014). Dispersal limitations refer to spatial or geographical barriers that prevent a species from dispersing into a given habitat. For example, large distances between source populations and a focal community may prevent the colonisation of the focal community despite its suitability for a species. Abiotic filters are environmental factors that limit the survival or establishment of a species at a site. Although the abiotic environments in pitchers are comparable across habitats and plant populations, the occurrence and persistence of inquilines may still be influenced by factors on a more macro scale, such as the absence of suitable food plants for terrestrial inquiline adults. Negative interspecific biotic interactions such as predation or competition may also reduce the abundance of a species. For example, predation by the predatory mosquito *Toxorhynchites acaudatus* may drive certain other mosquito species to local extinction. Thus, although *Nepenthes* species are found across multiple habitat types across Singapore and Southeast Asia, the occurrence of inquiline species in *Nepenthes* pitchers across these habitats is unlikely to be uniform.

Understanding the distribution of inquiline biodiversity across *Nepenthes ampullaria* pitchers locally is important also because inquiline communities in novel habitats may diverge from those that are present in less disturbed forests. Chou et al. (2015) suggested that *Aedes albopictus* mosquitoes, which are vectors of dengue hemorrhagic fever, may breed in *Nepenthes ampullaria* pitchers that are not colonised by predatory *Toxorhynchites acaudatus* mosquito larvae. This may mean that *Toxorhynchites acaudatus* range restriction can allow pitchers to become breeding habits for *Aedes* spp. However, this speculation remains to be tested, and we know of no study as yet which has examined the possibility of this in Singapore.

The primary aim of this study was thus to compile a comprehensive checklist of the inquiline species inhabiting *Nepenthes ampullaria* pitchers across the key *Nepenthes* habitat types in Singapore. Furthermore, aside from being model communities for inquiline species, pitchers are also natural pitfall traps that passively sample the invertebrate communities in their immediate vicinity. Also, populations of *Nepenthes ampullaria* in Singapore are small and categorised as nationally vulnerable by the 2nd Edition of the Singapore Red Data Book (Davison et al., 2008), and not all populations of these plants in the secondary forests of the Central Catchment Nature Reserve (CCNR) appear to be reproducing populations, because *Nepenthes* spp. are early-successional climbers that lose competitive dominance in later-successional forests (WNL, pers. obs.). The secondary aims of this study were thus to compile a preliminary checklist of the prey species of *Nepenthes ampullaria* pitchers in Singapore, and to identify structural or floristic habitat characteristics associated with reproducing populations of *Nepenthes ampullaria* plants.

MATERIAL & METHODS

Study sites. Sample collection was performed at four sites across Singapore (data from the last two urban scrub sites are combined, and these are hereafter referred to as a single site): (1) Kent Ridge Park (Fig. 1a; 1°17′08.09″N; 103°47′16.73″E), (2) Nee Soon Swamp Forest (Fig. 1b; 1°22′29.93″N, 103°48′40.63″E), and (3) two urban scrub sites in central and northern Singapore Island (Fig. 1c; precise locations of sites are withheld to prevent poaching) between August 2017 to February 2018. Forests in Kent Ridge Park are known as adinandra belukar (Sim et al., 1992) and are early-successional, native-dominated forests (Yee et al., 2016), while those of the Nee Soon Swamp Forest are native-dominated forests that occur along a continuum of early- to late-succession (Yee et al., 2016; Clews et al., 2018). These three sites differ significantly in their respective area, location, land use history and protection status (Table 1).

Table 1. Comparison of general characteristics between the three sites surveyed in this study.

Site	No. of Plots	Contiguous habitat area (ha)	Location	Land-use History	Protection Status
Nee Soon Swamp Forest	10	1,400	Central Catchment Nature Reserve	Part forest reserve, part low density fruit plantations (Clews et al., 2018)	Nature reserve
Kent Ridge Park	2	40	Southern ridges	Intensive gambier and rubber agriculture, subsequently abandoned (Sim et al., 1992)	Public park
Urban scrub	5	8	Two residential districts	Cleared land awaiting development	None

Fig. 1. Representative habitat photographs of (a) Kent Ridge Park—the uniform age and heights of the canopy trees (mostly *Adinandra dumosa*) is characteristic of the adinandra belukar forest type of the southern ridges. A large *Nepenthes rafflesiana* population dominates the undergrowth in this photograph; (b) treefall gap in Nee Soon Swamp Forest—the fallen tree in this photograph is *Prunus polystachya*, a common tree species in native-dominated secondary forests of the Central Catchment Nature Reserve. *Nepenthes ampullaria* plants are shown growing vigorously in the undergrowth; (c) Resam-dominated scrubland with open canopy in the urban sites—small tree saplings can be seen amidst the resam scrub, and *Nepenthes gracilis* plants can be seen in the foreground (arrowed). (Photographs by: Lam Weng Ngai).

Plot establishment and sampling procedure. *Nepenthes* pitcher plants often occur in aggregations across landscapes. This may be caused by historical disturbance events which alter microsite soil and vegetation characteristics, random dispersal of propagules and/or simply through the vegetative propagation of individual plants over time (WNL, pers. obs.). *Nepenthes ampullaria* populations were identified at each site during site reconnaissance and earlier site visits. Square plots of 10 × 10 m dimensions were established around identified *Nepenthes ampullaria* patches. All vascular plants within each plot were identified to as low a taxonomic level as possible. Twig cuttings were made of plants which could not be reliably identified in the field. These were pressed and dried in a 60°C oven, and subsequently identified using published keys (Chong et al., 2016; Lim et al., 2016; Neo et al., 2016; Tan et al., 2016) and consultation with experts. The heights of three canopy trees within the plot were also measured using an electronic clinometer (Haglöf, Sweden), but this was assigned a value of zero in exposed plots where adult trees (with a diameter at breast height [DBH] of > 5 cm) were absent. Slope was determined using the “Angle” application on an iPhone VII smartphone (Apple Inc., Cupertino, CA) laid flat against the soil surface at three corners of the plot. The presence of water bodies (forest streams) within or close (< 10 m) to the plot was also noted. Proximity to water bodies is associated with periodic flooding, soft, unstable substrates, and plant and animal communities associated with freshwater swamp forests. The presence or absence of *Nepenthes ampullaria* inflorescences, both fresh and dry, on *Nepenthes ampullaria* vines within each site was also noted as an indicator of whether or not the observed population had been flowering regularly. *Nepenthes ampullaria* flowers seasonally during April–May and September–October (WNL, unpublished data), but old, dried inflorescences or infructescences often remain on plants for a long time (1–2 years; WNL, pers. obs.) after flowering has ceased. The presence of dried inflorescences on stems is thus a robust method for determining if plants have flowered recently.

Five to 10 *Nepenthes ampullaria* pitchers were randomly sampled in each plot, resulting in 147 pitchers sampled across all plots and sites. Pitchers that were damaged or did not contain fluids were excluded from the sample. Sampled pitchers were excised at their tendrils, and had their complete contents (inquilines, prey and pitcher fluids) emptied into 50-mL Falcon™ tubes. Pitcher dimensions (i.e., pitcher mouth inner diameter and pitcher height) and phytotelma geometry (maximum pitcher width at fluid level, fluid depth and fluid volume) were also measured. Pitcher age was estimated using the leaf-count method as done in Lam et al. (2019). In this method, the youngest pitcher on an actively growing shoot is assigned an age of ‘1’, and pitchers on each subsequent (older) leaf are assigned ages of increasing integer values.

Specimen sorting and identification. Pitcher prey were sorted using a stereomicroscope and identified to as low a taxonomic level as possible with the help of experts and guides (Bolton, 1994; Marshall, 2012; Tan, 2012). As many prey specimens were highly degraded by inquiline and microbial activity and/or enzymatic action, the majority could only be classified at the familial or ordinal levels. Where generic or specific identifications were possible, these were listed under their lowest taxonomic levels in the final checklist.

Dipteran larva inquilines were identified using published keys and descriptions (Edwards & Given, 1928; Colless, 1965; Peyton, 1977; Sirivanakarn, 1977; Mattingly, 1981; Choo et al., 1997). To increase the accuracy of identification, a portion of the dipteran inquiline species were also reared to adults to obtain associative adult morphological characters. The rest of the samples were reared to the fourth instar stage and stored in 80% ethanol (vol./vol.) or mounted on slides.

Microscopic inquiline taxa (viz., rotifers, nematodes and mites) could not be individually counted and identified. The abundance of rotifers and nematodes were estimated using an index scale (0–3, with 0 indicating absence, and 3 indicating a very high density of individuals). Histiostomatid mites could not be identified using the stereozoom microscope and typically occur in very high numbers (>100 individuals per pitcher). These mites were thus collected haphazardly using a micropipette and stored in 70% ethanol solution (vol./vol.). Approximately 500 μL of pitcher fluids containing mites were thus collected in such a manner from each pitcher sample and these were subsequently identified by NJF. Mite specimens were cleared in Nesbitt's solution and mounted in Hoyer's medium on microscope slides (Krantz & Walter, 2009), then observed and identified using phase contrast and interference microscopy. The abundance of histiostomatid mites was not recorded and instead, only the presence or absence of each species was recorded.

Statistical analyses. Pairwise Jaccard distances were computed between the plant communities in the 10 × 10 m plots, and between the prey assemblages of the individual *Nepenthes ampullaria* pitchers. Nine of the 147 pitchers sampled did not contain prey, and these were thus excluded from the analysis. The Jaccard distance (D_J) between two samples may be represented by Equation (1):

$$D_J = \frac{b + c}{a + b + c} \qquad (1)$$

where a is the number of species shared between the two samples, b is the number of species from the first sample that do not occur in the second, and c is the number of species in the second sample that do not occur in the first. The Jaccard distance is the complement to the original similarity index of Jaccard (1900), and is commonly used in ecology for the quantification of community compositional differences (Legendre & Legendre, 2012). The Jaccard distance, which utilises presence–absence rather than abundance data, was selected for the analysis of prey assemblages, for which individual prey counts were measured, because of the very high amounts of variation between the abundances of the different prey groups. This high variation meant that (i) prey assemblages could not be resolved using non-metric multidimensional scaling (NMDS) when abundance-based dissimilarity indices such as Bray-Curtis dissimilarity were used, and (ii) prey groups with high abundances were likely to bias the results of analyses, even if appropriate data transformations were performed (Warton et al., 2012).

The plant communities of plots and prey assemblages of pitchers were then visualised using two- and three-dimensional NMDS plots, respectively. Between-site differences were analysed using permutational (non-parametric) multivariate analysis of variance (PERMANOVA; Anderson, 2001) for both the plant communities of plots and the prey assemblages of pitchers. Both the NMDS and PERMANOVA were performed with the 'vegan' R package (Oksanen et al., 2018). Site inquiline species richness was compared using sample-size- and coverage-based species-richness rarefaction (interpolation) and extrapolation with the 'iNEXT' R package (Chao et al., 2014; Hsieh et al., 2016). All statistical analyses were performed in the R statistical computing environment v3.4.0 (R Core Team, 2017).

The presence or absence of inflorescences on *Nepenthes ampullaria* vines was modelled against the fixed effects of first (NMDS1) and second (NMDS2) NMDS axis scores of the plant communities of each plot, average canopy height within a plot, average canopy cover within a plot, and average incline within a plot. This was done using logistic mixed-effects models with the three sites (viz., urban, Kent Ridge Park and Nee Soon Swamp Forest) as random intercepts, using the *lme4* R package (Bates et al., 2015). Six models, viz., five models containing one each of the five fixed effects, and a null model containing only random effects, were fitted to the data, and compared using Akaike's information criterion with correction for small sample sizes (AICc) according to Burnham & Anderson (2002).

Inquiline specimens were deposited at the Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum (LKCNHM), National University of Singapore, and the Reference Collection, Environmental Health Institute, National Environmental Agency of Singapore. All data were deposited on the online digital repository Figshare (Lim et al., 2019).

RESULTS

Plot structural and floristic characteristics. One hundred and eighty-nine vascular plant species from 127 genera and 68 families were identified from the 17 plots surveyed across the three sites. Plant communities clearly differed between the sites, and resolved clearly in ordination space (Fig. 2). The Nee Soon Swamp Forest is known to contain patches of abandoned-land forests (Clews et al., 2018), which were used previously as plantations or settlements and are characterised by the presence of commercially important tree species such as rubber (*Hevea brasiliensis*) or fruit trees (Yee et al., 2016). However, none of the 10 plots surveyed within the Nee Soon Swamp Forest had plant species compositions resembling such abandoned-land forests, suggesting that such forest types are generally unsuitable for *Nepenthes ampullaria*.

Inflorescences (both fresh and dried) of *Nepenthes ampullaria* were observed in seven of these 17 plots. Models of flowering probability (Table 2) suggested that *Nepenthes ampullaria* flowering probability was higher in plots with low canopy coverage (Fig. 3b) and which had floristic compositions that represented earlier successional forests (i.e., low NMDS1 score; Fig. 2), although the latter model was only 1.2 AICc units better than the null model (Table 2). Correlations between flowering probability and NMDS2 (Fig. 2), canopy height (Fig. 3a), and incline (Fig. 3c) were generally poor. Flowering probability also differed across sites, with inflorescences observed in four out of five urban plots, one out of two plots in Kent Ridge Park, and only two out of 10 plots in the Nee Soon Swamp Forest.

Inquiline diversity. A total of 23 inquiline morphospecies, of which 20 could be confidently identified at least to genus level, from 14 genera and nine families were identified in our surveys (Table 3). The vast majority of these (16 species) were from the order Diptera, and among these, Culicidae (10 species) was the most well represented family. Many inquiline species, viz., *Armigeres giveni*, *Tripteroides nepenthis*, *Tp. nepenthisimilis*, *Culex brevipalpus* complex, *Culex curtipalpis*, *Culex hewitii*, *Culex navalis*, *Uranotaenia moultoni*, and *Toxorhynchites acaudatus*, and *Xenoplatyura beaveri* (Mycetophilidae), *Nepenthosyrphus* sp. (Syrphidae), and Phorid sp.1, were only found in pitchers in the Nee Soon Swamp Forest (Table 3; Fig. 2). Accordingly, rarefaction predicted that inquiline species richness in the Nee Soon Swamp Forest (estimate = 19.0; 95% confidence interval [CI] = [19.0, 20.2]) was approximately twice that of urban sites (estimate = 9.2; 95% CI = [19.0, 13.7]) and Kent Ridge Park (estimate = 10.0 species; 95% CI = [9.1, 22.9]) (Fig. 4).

Prey taxa. At least 72 morphospecies from 28 families and 10 orders were identified (Table 4). Most of the animal taxa encountered were ground-dwelling, crawling taxa which are common in leaf litter and undergrowth. Prey assemblages were found to differ significantly between sites (pseudo-$F_{2,117}$ = 12.37, *p*-value = <0.00001). Ants were a major component of the prey assemblages of pitchers from all three sites, but a large proportion of pitchers in the Nee Soon Swamp Forest contained as much if not more *Hospitalitermes* spp. termites as well (Nasutitermitinae, Termitidae; Table 4; Fig. 6), which were not encountered at the other two sites. Ground-crawling beetles (several Coleoptera families) and large, predatory hemipterans (*Lisarda* spp. [Reduviidae] and *Metochus* sp. [Rhyparochromidae]) were also found in pitchers from the forested sites (Nee Soon Swamp Forest and Kent Ridge Park), but not in the resam-dominated scrub of urban sites (Table 4; Fig. 6). Instead, plant material, particularly the leaflets of the resam fern (*Dicranopteris linearis*), were found in more than 80% of pitchers in urban sites—a significantly higher proportion than in pitchers in the forested sites (Table 4; Fig. 6). This was not surprising, since *Nepenthes ampullaria* pitchers in urban sites were often found growing under dense layers of resam ferns in the urban sites.

Table 2. Model selection table comparing the six flowering probability models fitted to the data. *df* = degrees of freedom.

Model Rank	Formula	df	AICc	ΔAICc	Weights
1	*~ canopy cover*	3	21.188	0.000	0.810
2	*~ NMDS1*	3	25.846	4.658	0.079
3	*~ null*	2	26.999	5.812	0.044
4	*~ canopy height*	3	27.837	6.649	0.029
5	*~ incline*	3	28.096	6.908	0.026
6	*~ NMDS2*	3	29.650	8.462	0.012

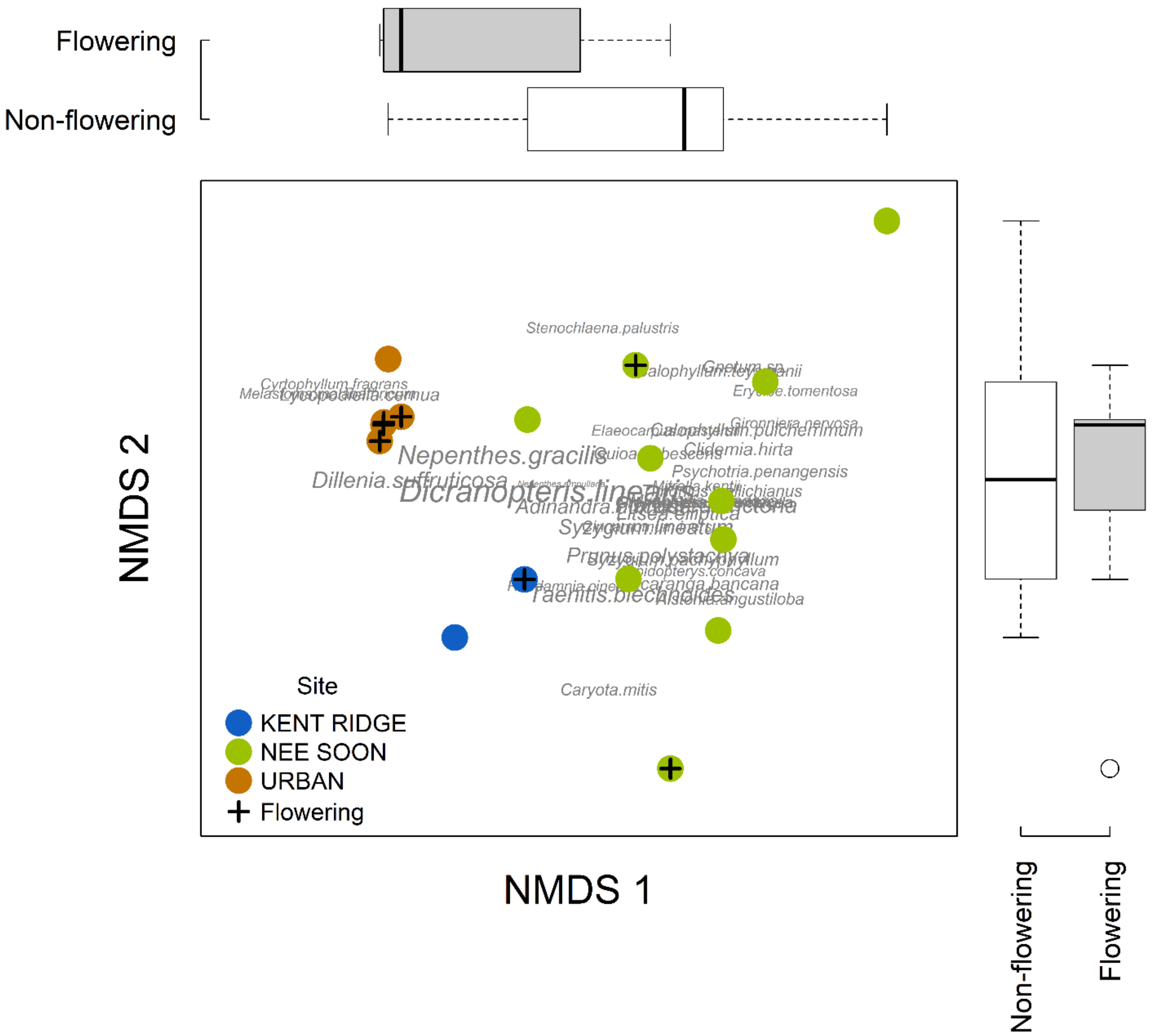

Fig. 2. Plant communities differed significantly between sites (pseudo-$F_{2,14}$ = 3.77, *p*-value = <0.00001), as revealed in this two-dimensional non-metric multidimensional scaling (NMDS) plot of the plant communities of the 15 sampled plots (computed using the Jaccard distance). Points represent plots, which are coloured according to the sites to which they belong; points which are located closer to each other in the diagram share more similar plant communities; a cross denotes that *Nepenthes ampullaria* plants within the plot were flowering or had recently flowered. Texts represent species centroids, with font size proportional to the number of plots in which a species is found (species which were found in two or less plots are not displayed). A species is more likely to occur in a plot if the plot's point is located close to the species' centroid. Stress = 0.0931. Boxplots on the margin show the distribution of the respective NMDS scores between non-flowering (clear) and flowering (grey) plots. Boxes represent interquartile ranges; bold horizontal lines within them represent group medians; whiskers represent maxima and minima; and points represent outliers.

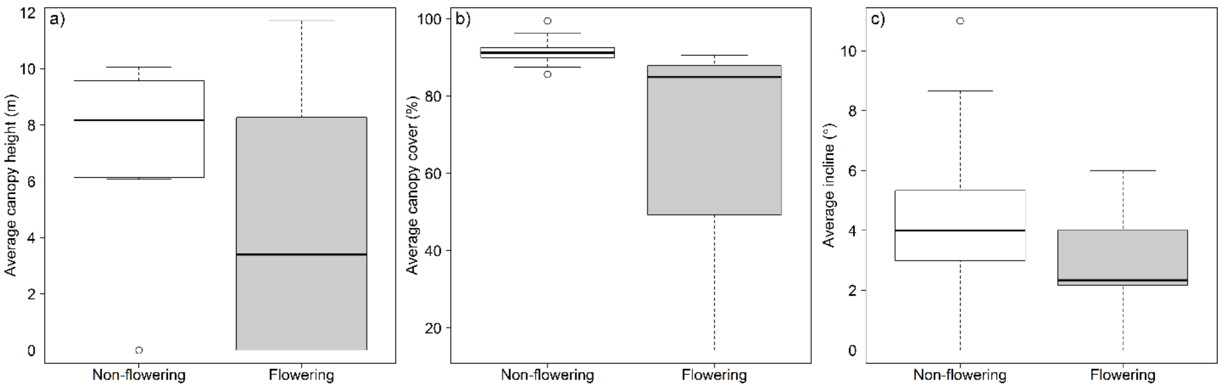

Fig. 3. Plots which contained flowering *Nepenthes ampullaria* plants tended to have (a) slightly lower canopy heights; (b) significantly lower canopy coverage; and (c) similar ground inclines as compared to plots in which no inflorescences (fresh or dried) were found on the *Nepenthes ampullaria* plants. In the diagrams, boxes represent interquartile ranges; bold horizontal lines within them represent group medians; whiskers represent maxima and minima and empty circles represent outliers.

Table 3. Checklist of the inquiline species found in the 147 sampled *Nepenthes ampullaria* pitchers. Ticks or crosses represent the presence or absence, respectively, of the species at the corresponding site.

Species	Family (Higher Classification)	Putative Feeding Habit	Kent Ridge Park	Nee Soon Swamp Forest	Urban Sites	Total Observations
Dasyhelea ampullariae	Ceratopogonidae (Diptera)	Medium saprotrophs (and/or detritivores)	✓	✓	✓	80
Dasyhelea biseriatus						
Dasyhelea nepenthicola						
Endonepenthia schuitemakeri	Phoridae (Diptera)	Large saprotrophs (and/or detritivores)	✓	✓	✓	14
Phorid sp. 1			×	✓	×	
Armigeres giveni	Culicidae (Diptera)	Detritivores (filter feeders)	×	✓	×	1
Tripteroides nepenthis			×	✓	×	43
Tripteroides nepenthisimilis			×	✓	×	32
Tripteroides tenax			✓	✓	✓	48
***Culex brevipalpus* complex**			×	✓	×	10
Culex curtipalpis			×	✓	×	2
Culex hewitii			×	✓	×	6
Culex navalis			×	✓	×	21
Uranotaenia moultoni			×	✓	×	45
Toxorhynchites acaudatus		Large aquatic predator	×	✓	×	36
Corethrella calathicola	Corethrellidae (Diptera)	Small aquatic predator	✓	✓	×	54
Xenoplatyura beaveri	Mycetophilidae (Diptera)	Terrestrial predator	×	✓	×	4
***Nepenthosyrphus* sp. 1**	Syrphidae (Diptera)	Large aquatic predator	×	✓	×	3
***Creutzeria* sp. 1**	Histiostomatidae (Acari)	Microscopic detritivores (and/or saprotrophs)	×	✓	✓	8
Undescribed genus 1 sp. 1 (previously *Zwickia nepenthesiana* [Hirst, 1928], but see text)			✓	✓	✓	49
Undescribed genus 1 sp. 2			✓	×	✓	3
Unidentified Rotifera sp./spp.			✓	✓	✓	11
Unidentified Nematoda sp./spp.			✓	✓	✓	21

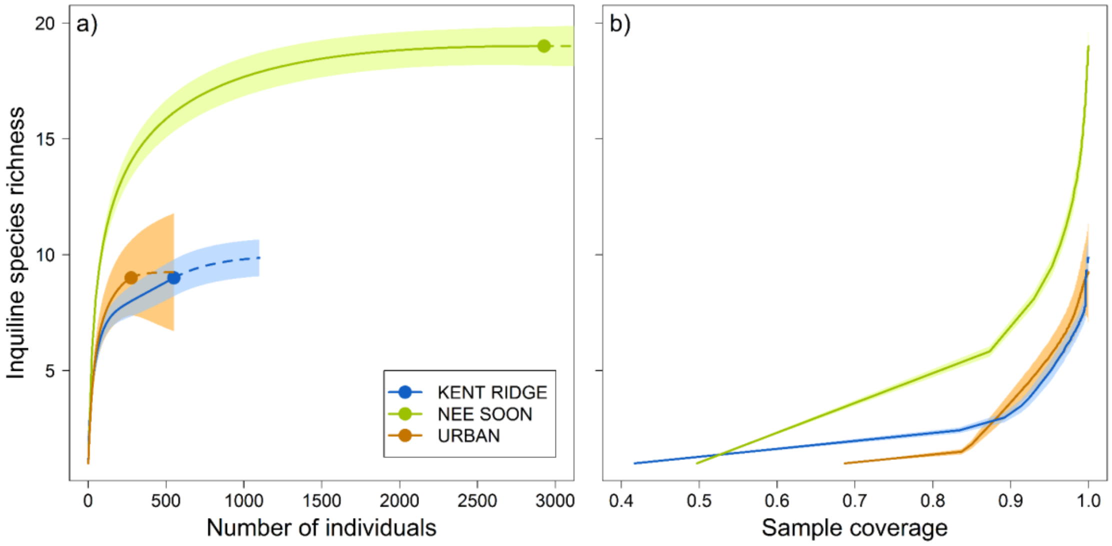

Fig. 4. Sample-size- (a) and coverage-based (b) rarefaction curves show that inquiline species richness was significantly higher in old secondary forests of Nee Soon Swamp Forest than in adinandra belukar and resam-dominated scrub in Kent Ridge Park and the urban sites, respectively. Lines represent the interpolated (continuous) and extrapolated (dashed) species richness of each forest type, as a function of the number of individuals sampled within it (a) and the estimated sample coverage (b); shaded regions represent the 95% confidence intervals of these estimates; points represent the observed species richness (these are omitted from panel b to prevent the obscuring of other details in the figure).

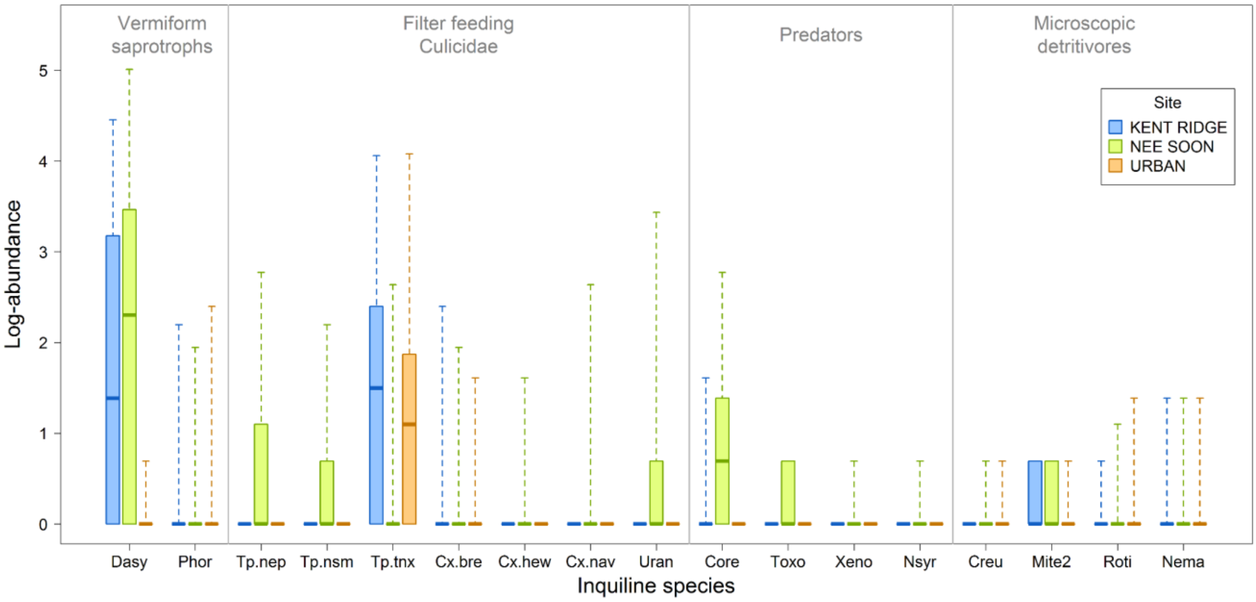

Fig. 5. Box and whisker plots show that inquiline taxa were found in different abundances across the different forest types, with several species being confined to the old secondary forests of Nee Soon Swamp Forest. Bold horizontal lines represent median log-transformed number of each inquiline taxa in pitchers from each forest type (denoted by colours); boxes represent interquartile range; whiskers represent maximum values. *Dasy* = *Dasyhelea* spp.; *Phor* = Phoridae; *Tp.nep* = *Tripteroides nepenthis*; *Tp.nsm* = *Tp. nepenthisimilis*; *Tp.tnx* = *Tp. tenax*; *Cx.bre* = *Culex brevipalpus* complex; *Cx.hew* = *Cx. hewitii*; *Cx.nav* = *Cx. navalis*; *Uran* = *Uranotaenia moultoni*; *Core* = *Corethrella calathicola*; *Toxo* = *Toxorhynchites acaudatus*; *Xeno* = *Xenoplatyura beaveri*; *Nsyr* = *Nepenthosyrphus* sp. raff; *Creu* = *Creutzeria* spp.; *Mite2* = Histiostomatidae genus 1; *Roti* = rotifers; *Nema* = nematodes. Rare inquiline taxa which were present in fewer than three samples are not displayed.

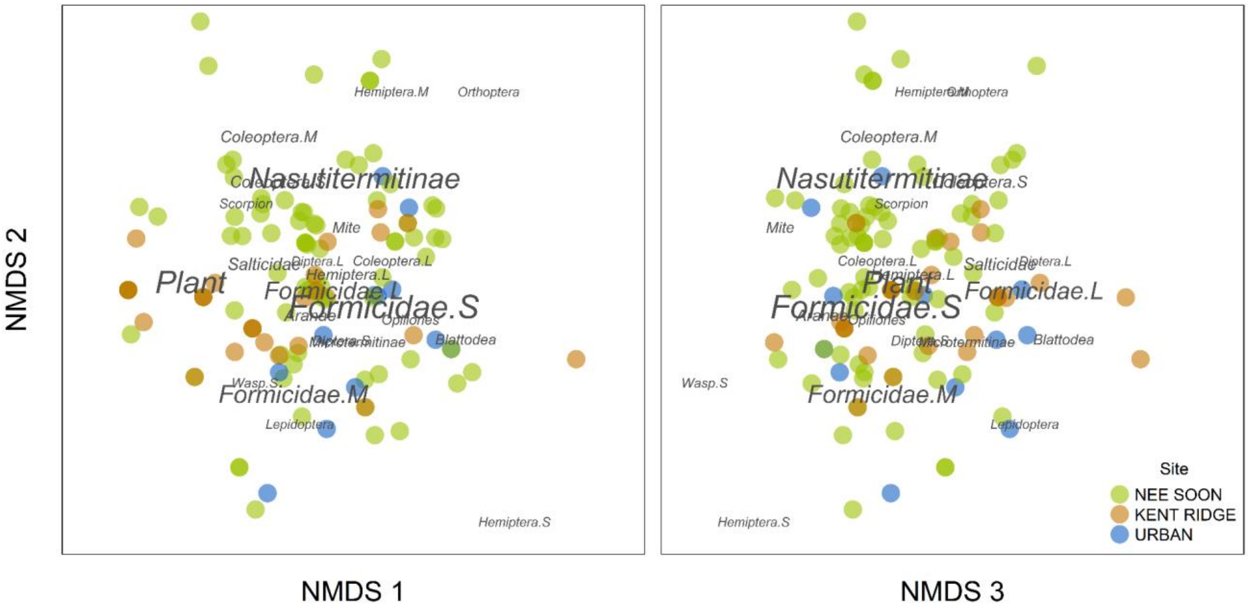

Fig. 6. Prey assemblages differed significantly between sites (pseudo-$F_{2,117}$ = 12.37, *p*-value = <0.00001), as revealed in this three-dimensional non-metric multidimensional scaling (NMDS) plot of the prey assemblages of the 15 sampled plots (computed using the Jaccard distance). Points represent pitchers, which are coloured according to the sites to which they belong; points which are located closer to each other in the diagram share more similar prey assemblages. Texts represent prey group centroids, with font size proportional to the number of pitchers in which a prey group is found (prey groups which were found in only one pitcher are not displayed). A prey group is more likely to occur in a pitcher if the pitcher's point is located close to the prey group's centroid. Stress = 0.1233. The letters S, M, or L appended to taxon names represent the size class the prey taxa were sorted into (for example, *Formicidae.S* = small ants; see Table 4).

Table 4. Preliminary checklist of all prey taxa found in the 147 *Nepenthes ampullaria* pitchers sampled from the three different sites (Nee Soon, Kent Ridge, and Urban) in this study. Taxa are grouped into intermediate (prey group) and higher order (higher classification) taxa. Numbers indicate the number of pitchers in which each prey group was present, while the corresponding percentages (in parentheses) convert that number into a proportion of the total pitchers sampled within that site (indicated in the last row). Ant taxa were arbitrarily divided into three size classes based on head length (HL), which is a robust estimator of ant body sizes (Kaspari & Weiser, 1999) and which could be assigned even with fragmented prey carcasses.

Higher Classification	Prey Group	Taxa Included	Kent Ridge Park	Nee Soon Swamp Forest	Urban Sites
Plant material—Plantae	Plant material	Leaves/leaflets of *Dicranopteris linearis* (Gleicheniaceae), *Falcataria moluccana* (Fabaceae), *Lycopodiella cernua* (Lycopodiaceae), and other unidentified plants Flowers of *Adinandra dumosa* (Pentaphyllaceae) and other unidentified plants Seed of *Nepenthes* spp. (Nepenthaceae) Tree bark	4 (33.3%)	42 (46.2%)	21 (80.8%)
Cockroaches and Termites—Blattodea	Termites	*Hospitalitermes* sp. (Nasutitermitinae) Unidentified Nasutitermitinae Unidentified Microtermitinae	2 (16.7%)	65 (71.4%)	0 (0%)
	Cockroaches	*Perisphaerus* sp. (Blaberidae) Unidentified Blattodea	2 (16.7%)	4 (4.4%)	1 (3.8%)
Ants—Formicidae (Hymenoptera)	Small ants (HL < 1.0 mm)	*Carebara* spp. (Myrmicinae) *Crematogaster* cf. *ferarri* (Myrmicinae) *Crematogaster* spp. (Myrmicinae) *Meranoplus malaysianus* (Myrmicinae) *Nylanderia* spp. (Formicinae) *Paratrechina longicornis* (Formicinae) *Pheidole affinis* (Myrmicinae) *Philidris* sp. (Dolichoderinae) *Plagiolepis* sp. (Formicinae) *Proceratium deelemani* (Proceratiinae) *Strumigenys* sp. (Myrmicinae) *Tetramorium* cf. *smithi* (Myrmicinae) *Tetramorium* spp. (Myrmicinae) Unidentified small Formicidae	8 (66.7%)	57 (62.6%)	7 (26.9%)
	Medium ants (HL 1.0–2.0 mm)	*Anoplolepis gracilipes* (Formicinae) *Aphaenogaster/Pheidole* sp. (Myrmicinae) *Cataulacus* sp. (Myrmicinae) *Dolichoderus thoracicus* (Dolichoderinae) *Dolichoderus* spp. (Dolichoderinae) *Leptogenys processionalis* group (Ponerinae) Unidentified medium Formicidae	6 (50%)	34 (37.4%)	4 (15.4%)
	Large ants (HL > 2.0 mm)	*Camponotus* cf. *arrogans* (Formicinae) *Diacamma* spp. (Ponerinae) *Odontomachus rixosus* (Ponerinae) *Odontoponera* sp. (Ponerinae) *Polyrhachis pruinosa* (Formicinae) *Polyrhachis* spp. (Formicinae) Unidentified Ponerinae	6 (50%)	34 (37.4%)	4 (15.4%)
True bugs—Hemiptera	Plant hoppers (Delphacidae)	Unidentified Delphacidae	0 (0%)	1 (1.1%)	0 (0%)
	Small bugs	Unidentified small Hemiptera	0 (0%)	2 (2.2%)	0 (0%)
	Medium bugs	*Penthimia* sp. (Cicadellidae)	0 (0%)	2 (2.2%)	0 (0%)
	Large bugs	*Lisarda* spp. (Reduviidae) *Metochus* sp. (Rhyparochromidae)	2 (16.7%)	9 (9.9%)	0 (0%)
Wasps—Aculeata (Hymenoptera)	Small wasps	Unidentified small Hymenoptera	1 (8.3%)	1 (1.1%)	1 (3.8%)
	Large wasps	Unidentified large Hymenoptera	1 (8.3%)	0 (0%)	0 (0%)
True Flies—Diptera	Small flies	Unidentified Drosophilidae Unidentified Mycetophilidae Unidentified Phoridae	0 (0%)	5 (5.5%)	0 (0%)
	Large flies	Unidentified Calyptratae	1 (8.3%)	1 (1.1%)	0 (0%)
Moths & Butterflies—Lepidoptera	Caterpillars	Unidentified Lepidoptera	0 (0%)	1 (1.1%)	1 (3.8%)
Beetles—	Small beetles	Unidentified Chrysomelidae	1 (8.3%)	10 (11%)	3 (11.5%)

Higher Classification	**Prey Group**	**Taxa Included**	**Kent Ridge Park**	**Nee Soon Swamp Forest**	**Urban Sites**
Coleoptera		cf. *Synommatoides* sp. (Dryophthorinae, Curculionidae) Unidentified Curculionidae Unidentified Scyrtidae *Uncifer* sp (Anthribidae)			
	Medium beetles	Unidentified Tenebrionidae	1 (8.3%)	11 (12.1%)	1 (3.8%)
	Large beetles	Unidentified Elateridae *Eulichas* sp. (Eulichadidae)	1 (8.3%)	4 (4.4%)	0 (0%)
Grasshoppers—Orthoptera, Crickets	Crickets (Gryllidae)	*Cardiodactylus singapura* Unidentified Gryllidae	0 (0%)	2 (2.2%)	0 (0%)
Arachnida	Jumping spiders (Salticidae)	Unidentified Salticidae	2 (16.7%)	13 (14.3%)	1 (3.8%)
	Ant spiders (Zodariidae)	*Asceua* sp. (Zodariidae) Unidentified Zodariidae	0 (0%)	2 (2.2%)	0 (0%)
	Other spiders	*Pardosa* sp. (Lycosidae) Unidentified Ctenidae Unidentified Segestriidae	1 (8.3%)	8 (8.9%)	3 (11.5%)
	Mites (Acari)	Unidentified Acari	0 (0%)	9 (9.9%)	0 (0%)
	Harvestmen (Opiliones)	Cf. *Hoplodino continentalis* (Podoctidae)	1 (8.3%)	4 (4.4%)	0 (0%)
	Scorpions (Scorpiones)	Unidentified Scorpiones	0 (0%)	4 (4.4%)	0 (0%)
Centipedes and millipedes—Myriapoda	Centipedes (Chilopoda)	Unidentified Chilopoda	0 (0%)	1 (1.1%)	0 (0%)
Unknown	Unknown	–	0 (0%)	0 (0%)	1 (3.8%)
Total number of pitchers sampled			12	91	26

DISCUSSION

In Singapore, *Nepenthes ampullaria* occurs in at least three habitat types, viz. early-successional, adinandra belukar-type, native-dominated forests, early- to late-successional native-dominated forests, and resam-dominated scrub (Figs. 1, 2). A total of 147 pitchers were surveyed across these three habitat types. However, *Nepenthes ampullaria* does not appear to flower with the same regularity across all habitats in which it is found. Our observations suggest that flowering occurs more regularly in habitats with little canopy cover (Fig. 3b; Table 2)—such as in urban, resam-dominated scrub, or in large treefall gaps. A high diversity of both inquiline (23 morphospecies) and invertebrate prey (72 morphospecies) diversity was also observed in the sampled *Nepenthes ampullaria* pitchers. However, this diversity was not equally distributed across all samples, but was instead highly correlated with the site from which samples were collected. Inquiline species richness was much higher in the Nee Soon Swamp Forest than in Kent Ridge Park and the urban sites (Fig. 4), with 12 inquiline species being found only in the Nee Soon Swamp Forest (Fig. 5; Table 3). However, abiotic conditions of pitchers (fluid pH, volume and depth, pitcher dimensions and sediment volumes in pitchers) differed little between pitchers from the three different sites, thus suggesting that abiotic conditions of the larger environment, such as the absence of adult food resources in the surrounding vegetation, and/or dispersal limitations, such as the anthropogenic barriers separating inquiline source populations in the CCNR from Kent Ridge Park and urban vegetation patches, are more important in determining inquiline community composition in *Nepenthes ampullaria* phytotelmata.

Thienemann (1932) defined three groups of *Nepenthes* pitcher inquilines: nepenthebionts are inquiline species which are obligately dependent on *Nepenthes* pitchers for the completion of at least one stage of their life cycles; nepenthephiles are those which are frequently found in association with *Nepenthes* pitchers, but which may survive and reproduce in other habitats as well; while nepenthexenes are species which only utilise pitchers opportunistically, but are otherwise not reliant on *Nepenthes* pitchers for their survival. All inquiline taxa identified in this study (not including rotifers and nematodes) are nepenthebiont organisms (Fig. 7). Importantly, we did not detect *Aedes* spp., which is classified by some as a nepenthexene species (Mogi & Yong, 1992; Chou et al., 2015), or the larvae of any disease vector species in any of the 147 sampled pitchers. This was so even for pitchers in habitats where predatory *Toxorhynchites acaudatus* were clearly absent, such as Kent Ridge Park, or urban sites nested within residential estates. Chou et al. (2015) had previously shown that larvae of the dengue-transmitting *Aedes albopictus* can survive in pitchers of *Nepenthes ampullaria* in Peninsular Malaysia, although they were rarely encountered in them. The authors hypothesised that this was because of the risk of predation by *Toxorhynchites acaudatus*, which is commonly present in pitchers. A follow up study showed that gravid female *Aedes albopictus* adults often avoided ovipositing in pitcher mimics for reasons that were unclear to the authors (Chou et al., 2016). Our study further confirms that *Aedes* spp. do not occur in *Nepenthes ampullaria* pitchers in Singapore, even in highly-disturbed sites occurring within the urban matrix of residential estates where predatory *Toxorhynchites acaudatus* are absent.

Noteworthy inquiline taxa.

Mosquitoes (Insecta: Diptera: Culicidae)

Tripteroides tenax (Fig. 7) was the most commonly encountered mosquito species, and the only one which occurred in all three sites (Table 3; Fig. 5). This species appears to be a generalist species which also inhabits the pitchers of *Nepenthes rafflesiana* and *Nepenthes gracilis* (HY & SGL-P, pers. obs.). *Tripteroides tenax* was found in much greater numbers in pitchers outside of the Nee Soon Swamp Forest (Fig. 5), suggesting that this species performed poorly under competition and predation pressures. Indeed, Mogi & Chan (1996) have shown previously that this species is far less resistant to *Toxorhynchites acaudatus* predation than *Uranotaenia moultoni* and *Tripteroides nepenthis*, which occur only in the Nee Soon Swamp Forest.

Previous studies of *Nepenthes* inquiline communities in Singapore identified *Culex eminentia* as a common inquiline species (Mogi & Chan, 1996; Mogi & Chan, 1997). However, this is more likely to be a complex of several closely-related species, viz. *Culex eminentia*, *Culex lucaris*, *Culex acutipalus*, and *Culex brevipalpus* (Colless, 1965; Sirivanakarn, 1977). Briefly, the complex is defined by a few larval characters such as weak seta 1-A (4–6 branches) on the antenna, and seta 5-C (2–4 branches) and 6-C markedly flattened and short on the head. The four species are morphologically very similar, and it is difficult to identify them accurately. The larvae of *Culex brevipalpis*, *Culex eminentia*, and *Culex acutipalus* have been recorded from *Nepenthes ampullaria* in Singapore previously (Colless 1965; Sirivanakarn, 1977), with observations of at least two species co-occurring in the same pitcher (Colless, 1965; Sirivanakarn, 1977). *Culex lucaris* was also recorded from Singapore, although it was not specified if the collection was from *Nepenthes ampullaria*.

Fig. 7. Habitus of a *Tripteroides tenax* fourth instar larva. Photograph by: HY.

Mites (Arachnida: Sarcoptiformes: Histiostomatidae)

Although astigmatic mites, especially inquiline genera of the family Histiostomatidae, are common in *Nepenthes* pitchers, they are generally overlooked in community studies or their presence acknowledged but relegated to minor roles (Fashing, 2002). This is undoubtedly due to their small size and the lack of knowledge concerning their taxonomy and biology. For example, when mite presence was acknowledged, mites were commonly referred to by family name or lumped under the genus *Zwickia* or the genus *Creutzeria*, the two nepenthebiont mite genera known at the time (e.g., see Beaver, 1979, Clarke & Kitching 1993, Clarke, 1998). When Clarke & Kitching (1993) published their work on food webs of six *Nepenthes* species in Borneo, mites were lumped under the genus *Zwickia* and placed in a single functional group which was isolated from the rest of the food web. A later analysis of those same six Bornean *Nepenthes* species (NJF, unpublished data) revealed not only the presence of the inquiline genus *Zwickia* (2 species), but also the inquiline genera *Creutzeria* (1 species), *Nepenthacarus* (Fashing, 2002) (1 species), *Naiadacarus* (Fashing & Chua, 2002) (1 species), and two undescribed genera, one containing three species and the other two species. Although their small size makes it difficult to observe the feeding behavior of astigmatic mites, mouthpart morphology can be used as an aid in such studies (e.g., see Fashing, 1998). The morphological differences observed in the mouthparts of *Nepenthes* mite inquilines (Fig. 8a–c) implies that further study will reveal a more accurate placement in food webs (NJF, pers. obs). Furthermore, preliminary experiments suggest that mites are the preferred prey of *Corethrella calathicola* (WNL, unpublished data), showing that these organisms are by no means isolated from pitcher phytotelma food webs. In addition, inquiline mites are ubiquitous in *Nepenthes* pitchers, and are occasionally so abundant that they constitute a larger inquiline biomass than all other inquilines combined (WNL & NJF pers. obs.).

However, both the biology and taxonomy of inquiline mites remain poorly understood. Identification of genera and species requires not only the association of males with females (Fig. 9a, b), but also with the deutonymphal instar (a.k.a., hypopus; Fig. 9c). Males of histiostomatid species often display precopulatory mate guarding and are thus morphologically adapted for grasping and clinging to the penultimate female instar (see Fashing, 2004, 2008). The deutonymphal instar is nonfeeding and morphologically quite different from both males and females, being modified for the rigours of dispersal as well as for attachment to a respective phoretic host(s) (see Fashing, 2010). All three are therefore necessary for accurate identification and the description of new species.

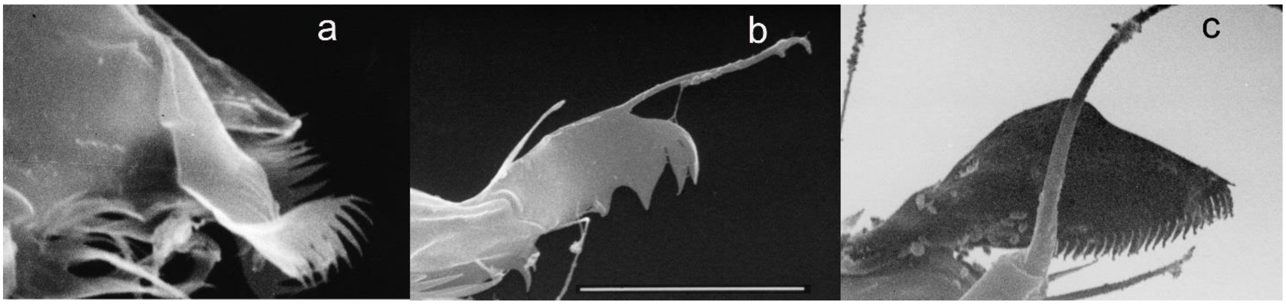

Fig. 8. Scanning electron microscope (SEM) photographs exemplifying morphological differences in the chelicerae of *Nepenthes* histiostomatid mites: (a) *Creutzeria* sp., (b) Undescribed genus 1, (c) *Nepenthacarus*. Scale bar = 10 micrometers. Photograph by: NJF.

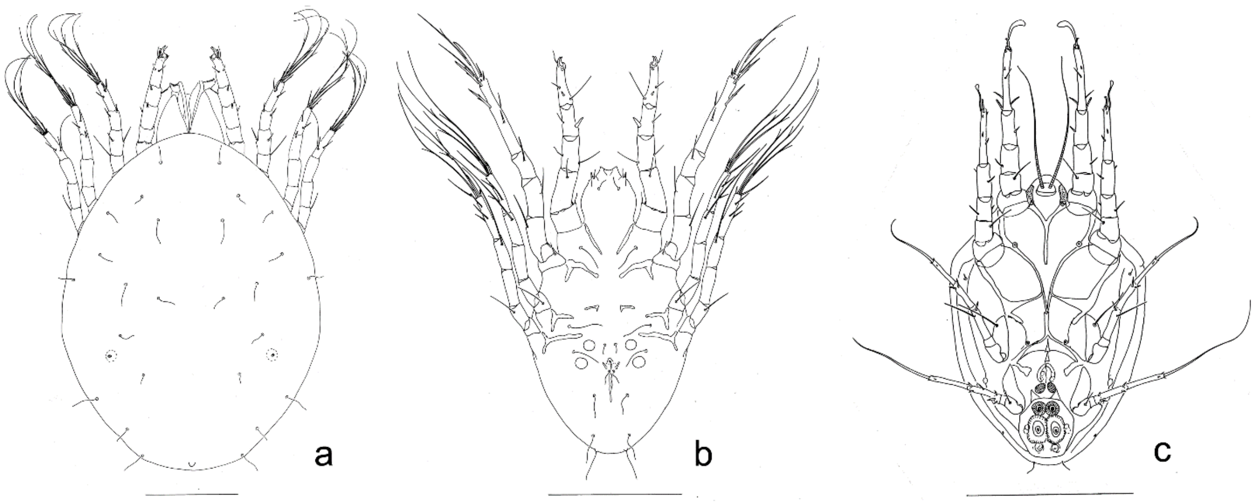

Fig. 9. Illustrations demonstrating morphological differences between adults and deutonymphs of *Creutzeria* sp*:* (a) female, (b) male, (c) deutonymph. Scale bars = 100 micrometers. Illustration by: NJF.

Using a sub-sampling procedure, we identified two histiostomatid genera in the 147 sampled pitchers in this study, viz. *Creutzeria* and an undescribed genus that has been observed in other *Nepenthes* species as well (Table 2; Fig. 10; NJF, pers. obs.). The undescribed genus is most likely the species described by Hirst (1928) as *Zwickia nepenthesiana* collected from *N. ampullaria* pitchers in Singapore. Hirst's written description is brief and his illustrations incomplete; however, it is obvious from the illustrations that the species does not belong in the genus *Zwickia* nor in a described genus, and we thus refrain from referring to this species as *Zwickia nepenthesiana*. *Creutzeria* and the undescribed genus evidently occupy divergent niches, with the former being a more pelagic genus that actively swims about in pitchers using fast beating movements of legs II-IV (Fashing et al., 1996; Fashing, 2008), and the undescribed genus being an ambulatory, benthic genus that always remains in the detrital layer (J. Ling, WNL & NJF, pers. obs.). It is possible that the undescribed genus contains two species (Table 3), although this differentiation is putative. Leg and body setation do not seem to vary greatly between the two potential species; however, some male specimens have a prominent projection of the anterior dorsal shield and other male specimens lack the projection (NJF unpublished data). It is possible that this could merely be morphological variation within a single species. Further study is necessary to determine if there are two species.

Notwithstanding the sampling intensity and broad coverage of habitat type accomplished in this study, it is unlikely that the inquiline mite diversity of *N. ampullaria* pitchers in Singapore was comprehensively captured. Earlier observations by NJF on collections made by K. L. Yeo, Raffles Museum of Biodiversity Research, revealed at least one other mite genus, *Nepenthacarus,* is often present in *N. ampullaria* pitchers as well as the pitchers of *N. gracillis* and *N. rafflesiana*. A second possibly undescribed genus commonly found in *N. gracillis* pitchers but rarely in pitchers of *N. ampullaria* was also observed. It is thus likely that the sub-sampling procedure employed in this study led to significant compromises in the detection probability of rare or less abundant mite genera.

Hoverflies (Insecta: Diptera: Syrphidae)

Another poorly studied taxon encountered in this study was the hoverfly genus *Nepenthosyrphus*. *Nepenthosyrphus* sp. (Fig. 11) were found singly in three out of the 94 sampled *Nepenthes ampullaria* pitchers in the Nee Soon Swamp Forest. This species is also known to occur in *Nepenthes rafflesiana* pitchers from the same site (RJYL, SHW & WNL, pers. obs.). Another, distinct species is also known to inhabit pitchers of *Nepenthes gracilis* locally (Mogi & Chan, 1996; Y. Y. Chou, RJYL & WNL, pers. obs.). The larvae of all known *Nepenthosyrphus* spp. are large aquatic predators which feed voraciously on *Dasyhelea* spp., culicid and/or phorid larvae (Mogi & Chan, 1996; WNL, pers. obs.), but may also be facultatively saprotrophic (Clarke & Kitching, 1993). *Nepenthosyrphus* spp. are found in the pitchers of many *Nepenthes* species, including *Nepenthes albomarginata* (Gaume et al. 2019), *Nepenthes ampullaria* (Mogi & Chan, 1996; 1997; Cresswell, 1998), *Nepenthes bicalcarata* (Clarke & Kitching, 1993; Gaume et al., 2019), *Nepenthes gracilis* (Beaver, 1979; Clarke & Kitching, 1993; Mogi & Chan, 1996), *Nepenthes mirabilis* (Clarke & Kitching, 1993), and *Nepenthes reinwardtiana* (Mogi & Chan, 1996). However, identification of *Nepenthosyrphus* species appears to be challenging, and was not achieved in any of the seven studies cited above. Five *Nepenthosyrphus* species have been described to date (Thompson, 1971; Rotheray et al., 2012), but the species collected in this study does not appear to match descriptions of any of these five. However, it could not be ascertained if the *Nepenthosyrphus* sp. collected in this study represents an undescribed taxon. Members of the hoverfly (Syrphidae) family are important pollinators of plants, and the biology of this obscure genus is thus a promising area for future studies.

Undetected inquiline taxa. Several inquiline species that had previously been recorded in *Nepenthes ampullaria* pitchers in Singapore were not encountered in our surveys (Table 5). Incidentally, three of the six species in this list are nepenthexenous ones, which only utilise pitchers opportunistically. The frogs *Microhyla borneensis* (a nepenthephile) and *Microhyla nepenthicola* (a nepenthebiont) are known to breed in *Nepenthes ampullaria* pitchers in Borneo (Das & Haas, 2010), and it is certain that many other *Nepenthes* species are utilised by frogs, especially in montane habitats (Rembold, 2009; C. C. Lee pers. comms.), but nepenthebiont frog species are not known to occur in Singapore. The absence of the toad *Bufo melanostictus*, the frog *Kalophrynus pleurostigma* and the crab *Geosesarma perracae* from samples cannot be used to infer the persistence of these species in the sampled habitats, since pitcher utilisation by these species is infrequent to begin with.

The absence of nepenthebiont species from samples, however, is a greater cause of concern. The most recently published local *Nepenthes* inquiline survey was done by Mogi & Chan (1997), more than 20 years ago. In that study, a fly *Nepenthomyia* sp. was found at three of the six sampled locations (Mogi & Chan, 1997), and thus seems to have been relatively widespread. Indeed, we have observed this species before in the Nee Soon swamp forest (WNL, pers. obs.), but did not encounter it in the 147 *N. ampullaria* pitchers sampled in this study. It is remotely possible that only young individuals (first or second instar) of *Nepenthomyia* sp. were mistakenly identified as phorid larvae, with which they bear superficial similarity, but that these were not successfully reared to adult as the success rate of phorid rearing was only ~50%. Without more substantial observational data we can only speculate that *Nepenthomyia* sp. is likely to be present still in Singapore, albeit in small or low density populations. On the other hand, the mosquito *Culex coerulescens* was observed by Mogi & Chan (1997) in only ~1% of *Nepenthes ampullaria* pitchers at just one of six sampled locations, suggesting either a very low population density that may have already been approaching extinction, or that this species breeds in habitats other than pitchers of *Nepenthes ampullaria*. However, the latter proposition is unlikely because *Culex*

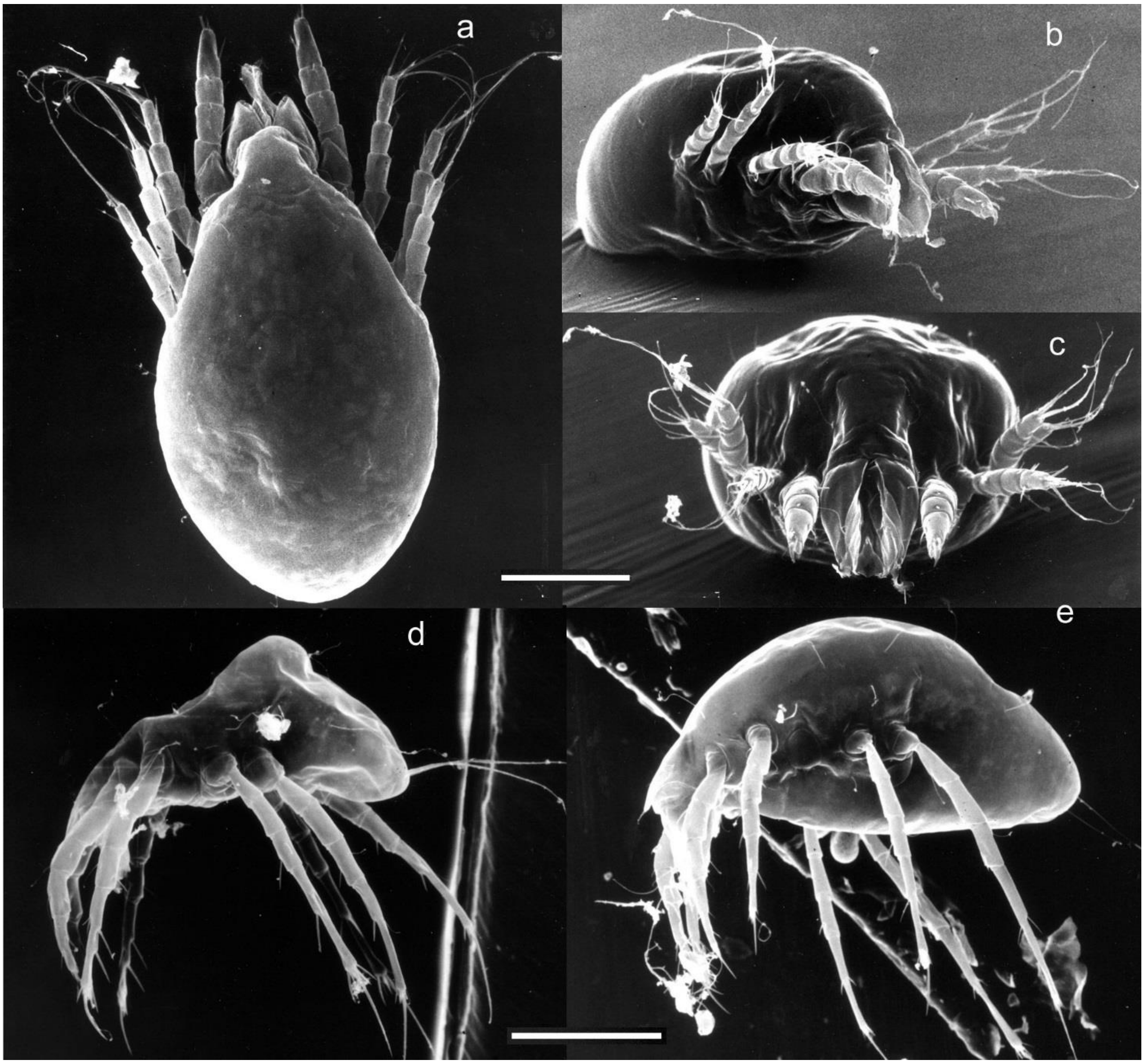

Fig. 10. SEM photographs of the inquiline mites of *N. ampularia* encountered in this study: *Creutzeria* sp. (a) female dorsal, (b) lateral, and (c) frontal views; undescribed genus I, sp. 1 (d) male lateral, and (e) female lateral views. Scale bars = 100 micrometers. Photograph by: NJF.

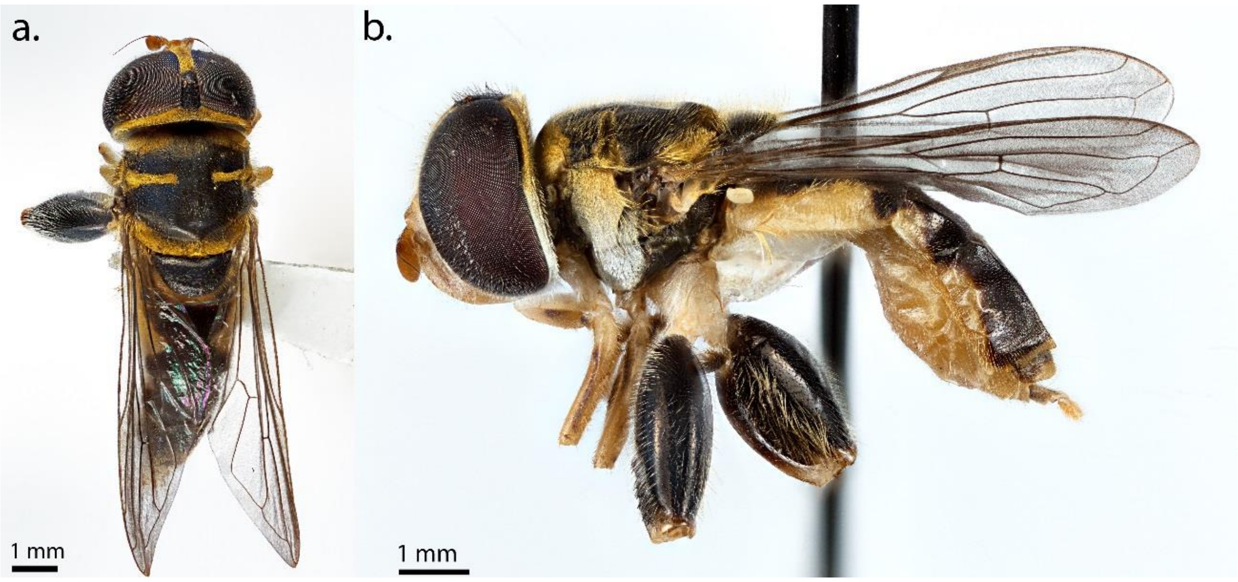

Fig. 11. Adult female *Nepenthosyrphus* sp. (a) dorsal and (b) lateral views. Photograph by: RJYL.

Table 5. Previously recorded inquiline species from *Nepenthes ampullaria* pitchers in Singapore that were not detected in this study.

Higher taxonomic rank	Species	Pitcher obligacy	Reference	Remarks
Flies (Diptera)	*Culex coerulescens* (Culicidae)	Nepenthebiont	Mogi & Yong, 1992; Mogi & Chan, 1997	Filter feeder. Found in greater frequency and densities in *Nepenthes bicalcarata* in Borneo (Cresswell, 2000; Gaume et al., 2019)
	Nepenthomyia sp. (Calliphoridae)	Nepenthebiont	Mogi & Chan, 1996; 1997	Predator
Mites (Acari)	*Nepenthacarus* sp. (Histiostomatidae)	Nepenthebiont	NJF pers. obs. (see text)	May have been missed due to sub-sampling procedure employed for mite identification (see text)
	Undescribed genus 2 (Histiostomatidae)	Nepenthebiont	NJF pers. obs. (see text)	
Frogs (Anura)	*Cf. Bufo melanostictus* (Bufonidae)	Nepenthexene	Dover et al., 1928	Identification uncertain
	Kalophrynus pleurostigma (Microhylidae)	Nepenthexene	Lim & Ng, 1991; Malkmus & Dehling, 2008	
Crabs (Decapoda)	*Geosesarma perracae* (Sesarmidae)	Nepenthexene	Tan & Ng, 2008	Only a single reported sighting locally

coerulescens is known only from *Nepenthes* pitchers, and is not found in the shorter-lived pitchers of *Nepenthes gracilis* or *Nepenthes rafflesiana* (Mogi & Yong, 1992; Cresswell, 2000; Gaume et al., 2019). It is thus possible that *Culex coerulescens* has become locally extinct within the last 20 years. Future research should examine the possible cause(s) of this, so as to develop effective mitigating measures.

Preliminary checklist of pitcher prey taxa. Prey identification was generally difficult owing to the extensive degradation in many samples. As enzymatic activity alone seldom causes fragmentation of prey carcasses (WNL, pers. obs.), this degradation was strong evidence that inquiline communities efficiently fragmented and consumed prey carcasses in *Nepenthes ampullaria* pitchers. Nevertheless, we were still able to consolidate a preliminary list of prey taxa (Table 4). *Nepenthes ampullaria*'s prey assemblages were composed largely of ground-dwelling, crawling invertebrate taxa—this is especially evident from the ant (Formicidae), spider (Aranae), and large bug (*Lisarda* spp. and *Metochus* sp. [Hemiptera]) taxa, as well as the abundance of open-air processional column termite prey (Nasutitermitinae) in samples from the Nee Soon Swamp Forest. This is unsurprising since *Nepenthes ampullaria* pitchers are often found on the ground, sometimes completely buried by leaf litter. Additionally, our data corroborates earlier observations in showing that a considerable proportion of *Nepenthes ampullaria*'s "prey" spectrum is composed of plant material (Moran et al., 2003; Pavlovič et al., 2011).

The occurrence of *Meranoplus malaysianus* ants (Fig. 12) in pitchers in this study constitutes the first record of the species in Singapore. This minute species is relatively uncommon, and usually found in leaf litter, albeit not gregariously. Ants of the genus *Meranoplus* are known for their unusually large Dufour's gland, which has been suggested to serve a defensive function in addition to a smaller abdominal poison gland (Hölldobler, 1988). Little is known about the feeding ecology of the species, or even the entire genus in the Oriental region. African species of *Meranoplus* have been observed to mainly forage on the ground or in leaf litter (Bolton, 1981); Australian species have furthermore been observed to be either omnivores, opportunistic seed feeders, or specialised granivores (Andersen, 2006). One species—*Meranoplus mucronatus*—has been documented to tap on and consume honeydew secreted by larvae of plataspid bugs in an apparently trophobiotic relationship (Maschwitz et al., 1987). Extrapolating from this, *M. malaysianus* may possibly be drawn to feed on the nutrient-rich secretions provided by extrafloral nectaries on *Nepenthes* pitchers.

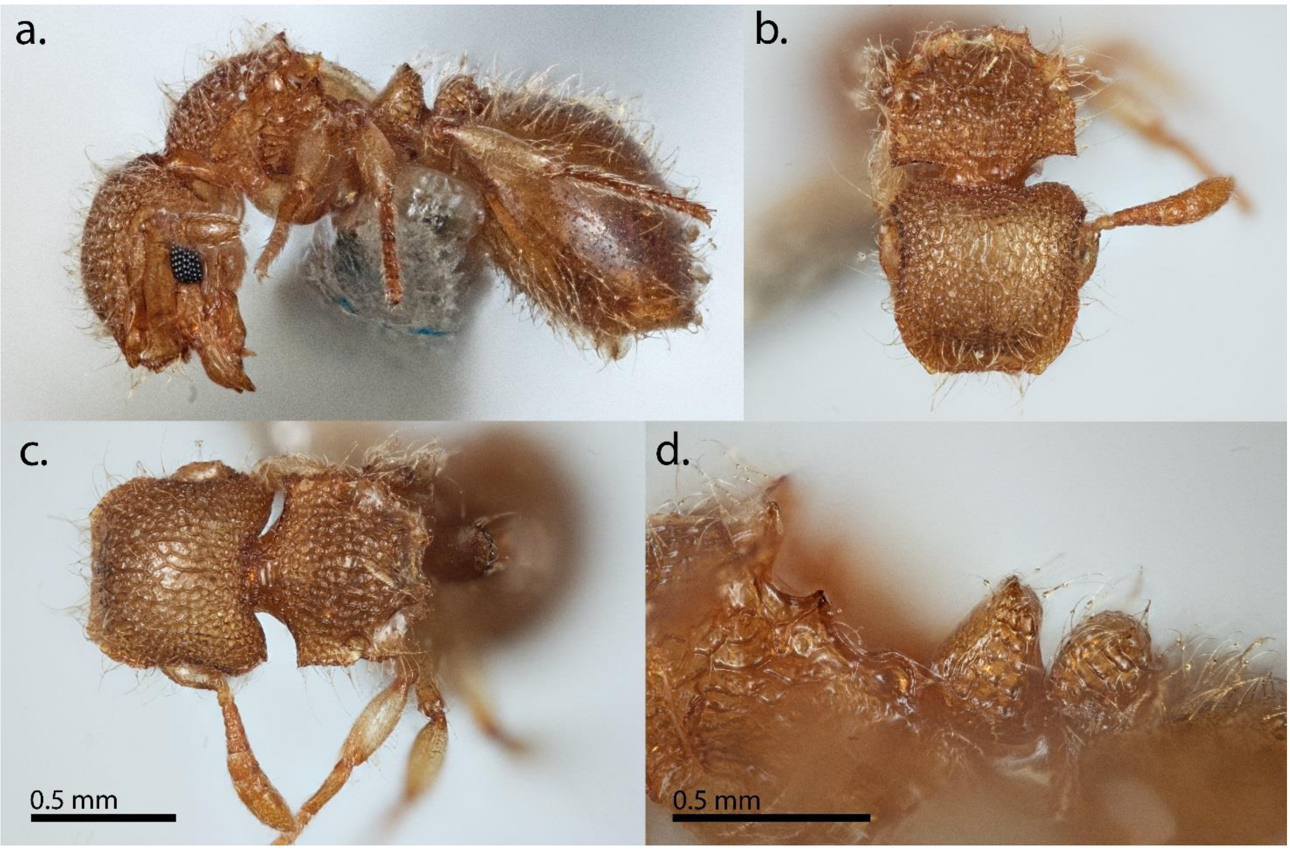

Fig. 12. *Meranoplus malaysianus* (a) lateral, (b) frontal, and (c) dorsal views, as well as a close up of pronotum and petiole (d). Panels a–c share the same scale bar, which is shown in panel c. Photograph by: Chui Shao Xiong.

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