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A comparative exploration of the inquiline and prey species of *Nepenthes rafflesiana* pitchers in contiguous and fragmented habitat patches in Singapore

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Abstract. The Raffles' pitcher plant (*Nepenthes rafflesiana* Jack, Nepenthaceae) is a carnivorous plant found naturally across multiple habitat types both within and outside of the Central Catchment Nature Reserve (CCNR)—the largest contiguous forested habitat in Singapore. *Nepenthes rafflesiana* produces lower and upper pitchers during distinct ontogenetic stages, and both of these pitcher types trap highly diverse but compositionally differentiated spectra of invertebrate prey. The fluid-filled pitchers of *N. rafflesiana* are also habitats for many specialised aquatic invertebrate species, known as inquilines. However, neither the prey spectra nor inquiline communities of *N. rafflesiana* pitchers are well characterised to a high taxonomic resolution. In this study, we surveyed pitcher-prey assemblages and inquiline communities in 78 *N. rafflesiana* pitchers within and outside of the CCNR in Singapore, as well as the plant communities in the 10 × 10 m plots in which the *N. rafflesiana* plants were documented. Plant communities of *N. rafflesiana* plots within the CCNR were found to be much richer in species and differed compositionally from those outside of the CCNR. Inquiline communities, too, were observed to differ compositionally between pitchers within and outside of the CCNR, as well as between pitcher types. However, asymptotic inquiline species richness was not significantly lower in pitchers outside of the CCNR, as compared to those within it. Lower pitchers generally contained more inquiline species than upper pitchers. Finally, prey assemblages differ in composition only marginally between pitchers within and outside of the CCNR, but were strongly differentiated between pitcher types.

Key words. pitcher plant, phytotelmata, Culicidae, astigmatic mite, biodiversity, habitat fragmentation

INTRODUCTION

Nepenthes pitcher plants utilise fluid-filled leaves to trap and digest invertebrate prey from which they obtain nutrients to survive in nutrient-deficient habitats. Most *Nepenthes* species produce two types of pitchers, which may differ significantly in morphology and in the prey that they trap—a phenomenon known as pitcher dimorphism (Moran, 1996). Lower pitchers are produced by immature plants in the rosette stage of growth, and are often borne on long tendrils that allow them to rest in the undergrowth (Clarke, 1997). Upper pitchers are produced by mature plants which are in a climbing stage of growth and are often borne on prehensile tendrils which simultaneously facilitate the climbing habit of the main vine (Clarke, 1997). Three *Nepenthes* species are found in Singapore, viz. *Nepenthes ampullaria* Jack, *N. gracilis* Korth, and *N. rafflesiana* Jack (Tan et al., 1997). *Nepenthes ampullaria* is unique among *Nepenthes* species in that it seldom produces upper pitchers (Clarke, 1997; Tan et al., 1997; McPherson, 2009). *Nepenthes gracilis* produces lower and upper pitchers that are morphologically very similar to each other (Tan et al., 1997), whereas *N. rafflesiana*, the largest of the three species, produces lower and upper pitchers which are morphologically distinct from each other (Fig. 1a, b). Prey resource partitioning between lower and upper pitchers in this species is also very pronounced (Moran, 1996; Di Giusto et al., 2008). However, many studies of pitcher prey contents lack the taxonomic resolution necessary to truly characterise pitcher prey spectra, with the vast number of studies only identifying prey to the taxonomic level of order or family (Moran, 1996; Moran et al., 1999; Chin et al., 2014; Peng & Clarke, 2015; Gaume et al., 2016).

Some animal species have adapted to life in the aquatic environment of pitchers, and utilise them as habitats (Thienemann, 1932; Beaver, 1979). These animals are known as inquilines, and are often highly specialised in their use of *Nepenthes* pitchers (Thienemann, 1932). Inquiline species which are completely reliant on *Nepenthes* pitchers in one or more of their life stages are known as nepenthebionts

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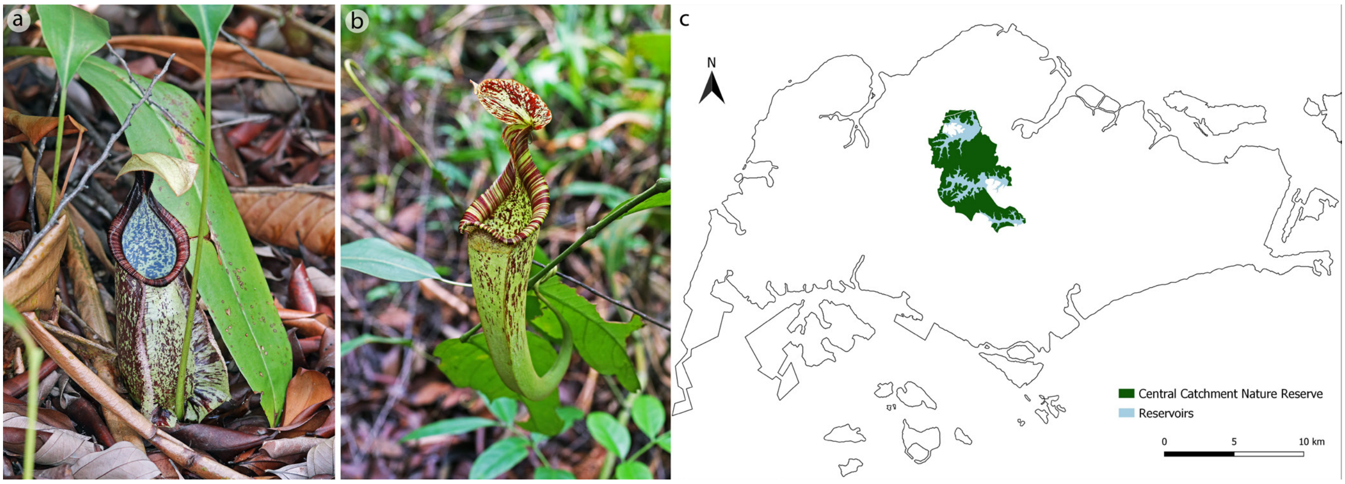

Fig. 1. *Nepenthes rafflesiana* lower (a) and upper (b) pitchers in situ; location of the Central Catchment Nature Reserve (CCNR) in Singapore (c). Data sources for (c): Singapore Public Data (https://data.gov.sg); Global Administrative Areas Database version 3.6 (https://gadm.org/data.html). (Photographs by: Lam Weng Ngai).

(Thienemann, 1932), and many nepenthebiont species are associated with one or only very few *Nepenthes* host species (Clarke & Kitching, 1995; Fashing & Chua, 2002; Das & Haas, 2010; Bittleston, 2018; Lam & Tan, 2019). However, inquiline biology and biodiversity are poorly understood in comparison to that of their host plant. It remains unclear how many inquiline species may be sustained by *Nepenthes* species in Singapore, how inquiline biodiversity is distributed geographically across *Nepenthes* populations, and whether or not inquiline communities differ between upper and lower pitcher types within *Nepenthes* species.

The Central Catchment Nature Reserve (CCNR; Fig. 1c) is the largest contiguous forest fragment of Singapore, and is composed of at least five distinct vegetation types: primary, old secondary and young secondary dryland forests, freshwater swamp forests, and scrubland or non-forests (Corlett, 1997; Yee et al., 2011). Fragments of natural vegetation also exist outside of the CCNR (Yee et al., 2011; 2016), and these may also serve as important habitats for local biodiversity (Koh & Sodhi, 2004; Ng et al., 2011; Tan et al., 2012; Soh & Ngiam, 2013). However, most of such vegetation patches are small and isolated, often highly altered by anthropogenic land-use histories, and thus floristically less diverse than habitats in the CCNR (Sim et al., 1992; Neo et al., 2017). It is generally understood that insect and pitcher inquiline biodiversity in such habitats follows similar patterns (Koh & Sodhi, 2004; Lim et al., 2019), but this does not stop such patches from being important habitats for many invertebrate species (Ng et al., 2011; Tan et al., 2012; Soh & Ngiam, 2013; Sing et al., 2016). *Nepenthes* species occur both within and outside of the CCNR (Fig. 2), and often even exhibit higher fecundity rates in the open habitats outside of it (Lim et al., 2019). For example, *Nepenthes* species often thrive in adinandra belukar—a type of anthropogenic heath forest which forms spontaneously on nutrient leached soils that are characteristic of past intensively-farmed lands in Singapore and Peninsular Malaysia (Sim et al., 1992; Tan et al., 1997; Lim et al., 2019)—occurring outside of the CCNR. However, we still do not know enough about how inquiline diversity varies between contiguous and fragmented habitat patches to confidently assess the distribution of inquiline diversity across sites within and outside of the CCNR and/or the effectiveness of sites outside of the CCNR as reservoirs of inquiline biodiversity.

The primary aim of this study was thus to compile a comprehensive list of the inquiline species inhabiting *N. rafflesiana* pitchers, and to examine differences in inquiline community composition between lower and upper pitchers, as well as between pitchers found within and outside of the CCNR. The secondary aims were to characterise the vegetation types, both within and outside of the CCNR, in which *N. rafflesiana* is commonly found, and to compile a preliminary list of prey species trapped by *N. rafflesiana* lower and upper pitchers within and outside of the CCNR. This study was done in tandem with that of Lim et al. (2019) which focused on *N. ampullaria*. The sampling methodology utilised in both studies was identical, and described in more detail by Lim et al. (2019). However, topographic slope, canopy height, and presence of senesced inflorescences which were quantified by Lim et al. (2019) were not quantified for this study.

MATERIAL AND METHODS

Study sites. Pitcher samples were collected from all four areas of the CCNR (termed "sites" in this study; Table 1): Mandai (North), Nee Soon (East), MacRitchie (South), and Chestnut (West). Additionally, samples were also collected at three sites outside the CCNR, which are not disclosed to prevent poaching since they are not protected areas. Two of the three sites are found on state land awaiting development, while the last is a park. Two of the three sites are located on mainland Singapore (one located centrally, the other in the south), while the last site was located on an offshore island. Collections were made between July 2017 and February 2019.

Plot establishment and sampling procedure. Thirteen 10 × 10 m plots (nine within and four outside of the CCNR) were established as described in Lim et al. (2019) around

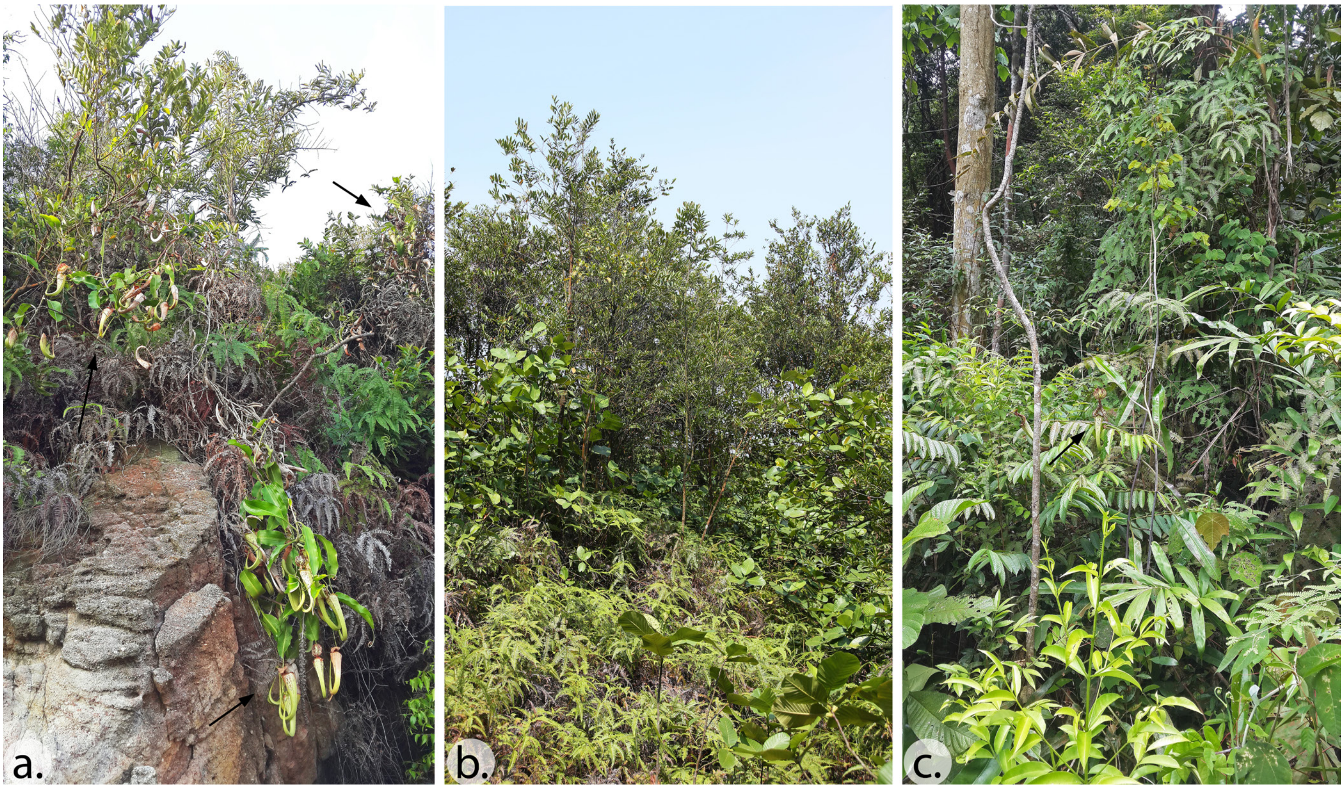

Fig. 2. Habitat types in which *Nepenthes rafflesiana* plants were found in this study: (a) coastal cliffs; (b) adinandra belukar; (c) old secondary forests (in this case, a tree fall gap within an old secondary forest). *Nepenthes rafflesiana* plants are indicated by an arrow in panels a and c. Coastal cliffs (a) and adinandra belukar (b) type habitats were mainly found outside the Central Catchment Nature Reserve (CCNR), while old secondary forests type habitats were only found within the CCNR. Despite their different locations, plant communities in which *N. rafflesiana* were found in coastal cliff habitats (a) and typical adinandra belukar habitats (b) were compositionally highly similar and may both be classified as adinandra belukar type plant communities. (Photographs by: Lam Weng Ngai).

Table 1. Summary of the number of sites, plots, and pitchers sampled within and outside the Central Catchment Nature Reserve (CCNR), in this study.

Location	**No. of sites**	**No. of plots**	**No. of lower pitchers**	**No. of upper pitchers**
Within CCNR	4	9	41	10
Outside CCNR	3	4	12	15
Totals	**7**	**13**	**53**	**25**

patches of *N. rafflesiana* that had been identified in earlier ground surveys. All vascular plants within each plot were identified to as fine a taxonomic resolution as possible. Seven to 10 *N. rafflesiana* pitchers were randomly sampled in each plot, unless the total number of pitchers in a plot was less than seven, in which case all pitchers were sampled. This resulted in a mean of 6.5 ± 1.4 (S. D.) pitchers per plot and a total of 53 lower and 25 upper pitchers sampled across all plots and locations (Table 1). Pitcher dimensions, pitcher fluid pH and volumes, pitcher prey/sediment volumes, and the canopy cover above pitchers were also measured as described in Lim et al. (2019).

After transportation back to the laboratory, all prey items found in pitchers were sorted using a stereozoom microscope and identified to as fine a taxonomic resolution as possible with the help of experts and guides (Bolton, 1994; Marshall, 2012; Tan, 2012). Inquiline dipteran larvae were reared to the fourth instar stage or until the emergence of adults for morphological identification. These were identified morphologically under stereozoom and light microscopes using published keys and descriptions (Edwards & Given, 1928; Colless, 1965; Peyton, 1977; Sirivanakarn, 1977; Mattingly, 1981; Choo et al., 1997). Since mites are often found in very large quantities (up to several thousand) in pitchers, these had to be subsampled prior to identification. Five hundred μL of pitcher fluids containing histiostomatid mites were collected haphazardly using a micropipette and stored in 70% ethanol solution. Mites from these samples were cleared in Nesbitt's solution, mounted in Hoyer's medium on microscope slides, and identified using phase contrast and interference microscopy. Only the presence or absence of mite species was recorded. All specimen sorting and identification methodologies used in this study followed those described in Lim et al. (2019).

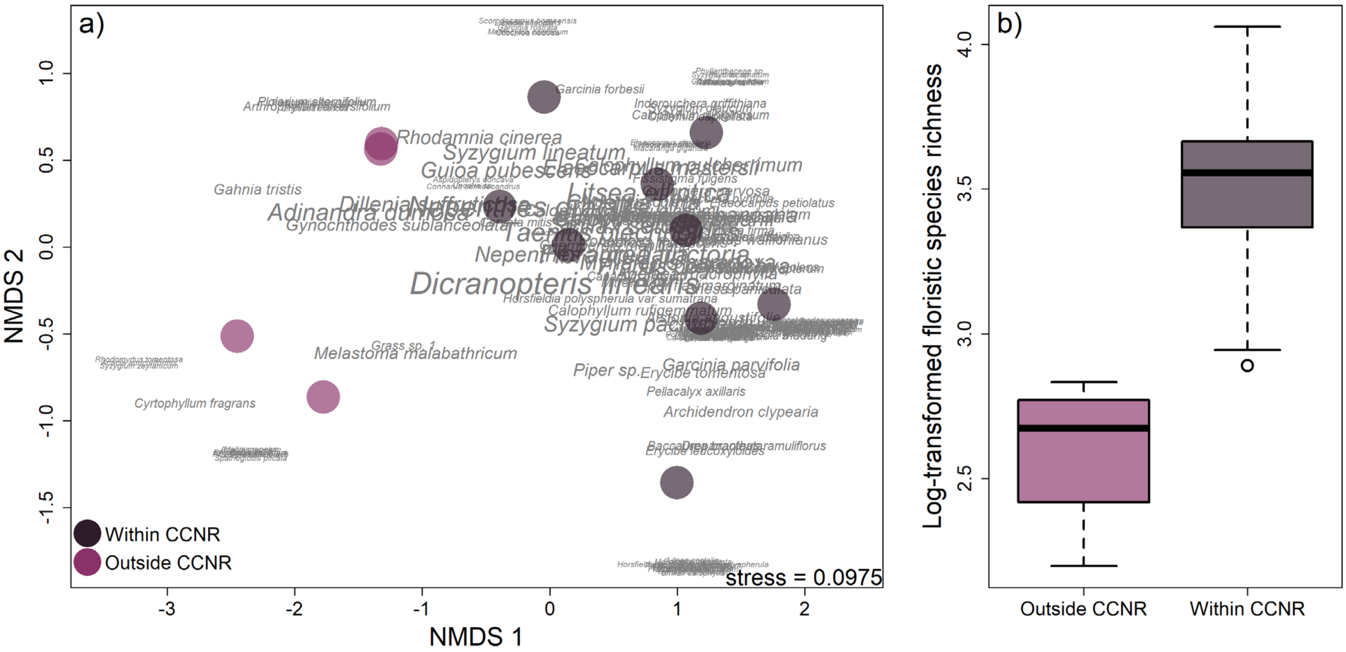

Fig. 3. Two-dimensional NMDS plot of the plant communities co-occurring with *Nepenthes rafflesiana* in plots located within (brown points) and outside (pink points) of the CCNR (a), and box and whisker plot comparing the log-transformed floristic species richness of these locations (b). Plant communities differed significantly in composition (a; pseudo-$F_{1,11} = 3.80$, p-value < 0.001) and species richness (b; $T_{11} = 4.74$, p-value $= 0.001$). Each point in the NMDS plot (a) represents the plant communities of a single plot. Colours are translucent, so that overlapping points may be distinguished. Points which are located closer to each other in the NMDS plot share more similar plant communities. Texts represent plant species centroids, with font sizes proportional to the number of plots in which each was 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. Bold lines in the box and whisker plot represent median log-transformed species richness, boxes represent interquartile ranges, whiskers represent maxima/minima and points represent outliers.

Statistical analyses. Plant community, inquiline community, and prey assemblage differences were examined using distance-based multivariate techniques. Prey taxa were first grouped by intermediate taxonomic ranks prior to analysis: ants (Hymenoptera: Formicidae) were grouped by subfamilies or tribes; spiders (Arachnida: Araneae), crickets or katydids (Orthoptera), flies (Diptera), true bugs (Hemiptera), and beetles (Coleoptera) were grouped by families; and all other taxa were grouped by orders or suborders. Pairwise distances between samples were computed using the Jaccard distance for plant communities (for which only presence–absence data were recorded), and the Bray-Curtis dissimilarity index for inquiline communities and prey assemblages (for which data of the abundance of each taxon was considered). Data were visualised using two- or three-dimensional nonmetric multidimensional scaling (NMDS), and differences between pitcher types (upper or lower pitchers) and location (within or outside of the CCNR) were analysed using permutational (non-parametric) multivariate analysis of variance (PERMANOVA; Anderson, 2001) using 9,999 permutations. Both the NMDS and PERMANOVA routines were performed with the 'vegan' R package (Oksanen et al., 2016). Asymptotic inquiline species richness was estimated, and sample-size- and coverage-based species-richness rarefaction was performed with the 'iNEXT' R package (Hsieh et al., 2016). Prey taxa were not grouped in estimations of asymptotic species richness.

Finally, the inquiline species richness of each *N. rafflesiana* pitcher was also modelled against all possible combinations of the variables: *location* (outside of or within the CCNR), *pitcher type* (lower or upper), *pitcher size* (the standardised first principle component of log-transformed pitcher height, pitcher mouth diameter, and pitcher fluid depth), *canopy cover* experienced by individual pitchers (transformed by raising to the power of five and standardised). This was done using generalised linear models with Poisson error structures and the log link function, with sampling plot and site as nested random effects. Models were fitted with the 'lme4' R package (Bates et al., 2015). Model selection was performed using Akaike's Information Criterion with correction for small sample sizes (AICc) according to Burnham and Anderson (2002). All statistical analyses were performed in the R statistical computing environment v3.4.0 (R Core Team, 2018).

Inquiline specimens were deposited in 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 in the online digital repository Figshare (Lam et al., 2020b).

RESULTS

Plot structural and floristic characteristics. A total of 173 vascular plant species from 115 genera and 65 families were identified from the 13 plots surveyed in this study.

Table 2. Inquiline species found in all 78 sampled *Nepenthes rafflesiana* lower and upper pitchers outside and within the Central Catchment Nature Reserve (CCNR). Numbers indicate the number of pitchers in which each inquiline taxon was present, while the corresponding percentages (in parentheses) convert that number into a proportion of the total pitchers sampled within lower/upper pitchers outside of or within the CCNR.

Inquiline Species	**Family (Higher Classification)**	**Putative Feeding Habit**	**Outside CCNR**		**Within CCNR**		**Total Obs**
			Lower (n = 12)	**Upper (n = 15)**	**Lower (n = 41)**	**Upper (n = 10)**	
Dasyhelea nepenthicola	Ceratopogonidae (Diptera)	Saprotrophs (and/ or detritivores)	6 (50.0%)	2 (13.3%)	2 (4.9%)	–	10
Endonepenthia schuitemakeri	Phoridae (Diptera)		9 (75.0%)	8 (53.3%)	25 (61.0%)	3 (30.0%)	45
***Phorid* sp.**							
Armigeres giveni	Culicidae (Diptera)	Detritivores (filter feeders)	–	–	2 (4.9%)	6 (60.0%)	8
Ar.* cf. *kuchingensis			–	–	1 (2.4%)	1 (10.0%)	2
***Culex brevipalpus* complex**			–	–	29 (70.7%)	2 (20.0%)	31
Cx. curtipalpis			–	1 (6.7%)	8 (19.5%)	1 (10.0%)	10
Cx. navalis			–	–	1 (2.4%)	–	1
Tripteroides nepenthis			–	–	1 (2.4%)	–	1
Tp. tenax			10 (83.3%)	10 (66.7%)	27 (65.9%)	2 (20.0%)	49
***Lestodiplosis* sp.**	Cecidomyiidae (Diptera)	Facultatively-saprotrophic predators	1 (8.3%)	–	3 (7.3%)	–	4
***Nepenthosyrphus* sp.**	Syrphidae (Diptera)		–	–	4 (9.8%)	2 (20.0%)	6
***Creutzeria* spp.**	Histiostomatidae (Acari)	Microscopic detritivores (and/ or saprotrophs)	1 (8.3%)	–	4 (9.8%)	–	5
Undescribed genus 1 sp. 1			3 (25%)	–	2 (4.9%)	–	5
Undescribed genus 1 sp. 2			–	–	1 (2.4%)	–	1
***Nepenthacarus* sp.**			–	1 (6.7%)	1 (2.4%)	–	2
***Zwickia* spp.**			1 (8.3%)	3 (20.0%)	–	–	4
Unidentified Nematoda sp./spp.			4 (33.3%)	2 (13.3%)	9 (22.0%)	–	15

Plant communities from plots within the CCNR differed significantly in composition (pseudo-$F_{1,11}$ = 3.80, *p*-value < 0.001; Fig. 3a) and species richness (T_{11} = 4.74, *p*-value < 0.001; Fig. 3b) from those outside of the CCNR. Despite significant differences in location (Fig. 2a, b) and land use histories of the three sites outside of the CCNR, the floristic composition of these sites were very similar, and clearly represented adinandra belukar (Sim et al., 1992). Across all sites, *N. rafflesiana* most often co-occurred with common secondary forest and long lived-pioneer trees (*Adinandra dumosa*, *Dillenia suffruticosa*, *Guoia pubescens*, *Litsea elliptica*, *Rhodamnia cinerea* etc.), climbers (e.g., *Nepenthes gracilis*, *Smilax setosa*, and *Fibraurea tinctoria*) and ferns (e.g., *Dicranopteris linearis* and *Taenitis blechnoides*), many of which are key components of adinandra belukar (Sim et al., 1992).

Inquiline diversity. Our surveys of *N. rafflesiana* pitcher contents identified 18 inquiline morphospecies, of which 15 could be confidently identified minimally to the generic level. Among these were 11 genera from six families (Table 2). Inquiline communities differed significantly between *location* (i.e., within or outside of the CCNR; pseudo-$F_{1,71}$ = 7.85; *p*-value < 0.001) and *pitcher type* (i.e., upper or lower pitchers; pseudo-$F_{1,71}$ = 5.72; *p*-value < 0.001; Fig. 4). Contrary to expectations however, asymptotic species richness within the CCNR (18.2; 95% CI = [16.3, 35.0]) was not significantly higher than that outside of it (16.0; 95% CI = [11.0, 47.8]; Fig. 5). Several key inquiline taxa, viz. the culicid species *Armigeres giveni*, *Ar.* cf. *kuchingensis* and *Culex brevipalpus* complex, and *Nepenthosyrphus* sp. (Syrphidae) were only found in pitchers in the CCNR (Table 2; Fig. 6). The reverse was true for one taxon, the astigmatic

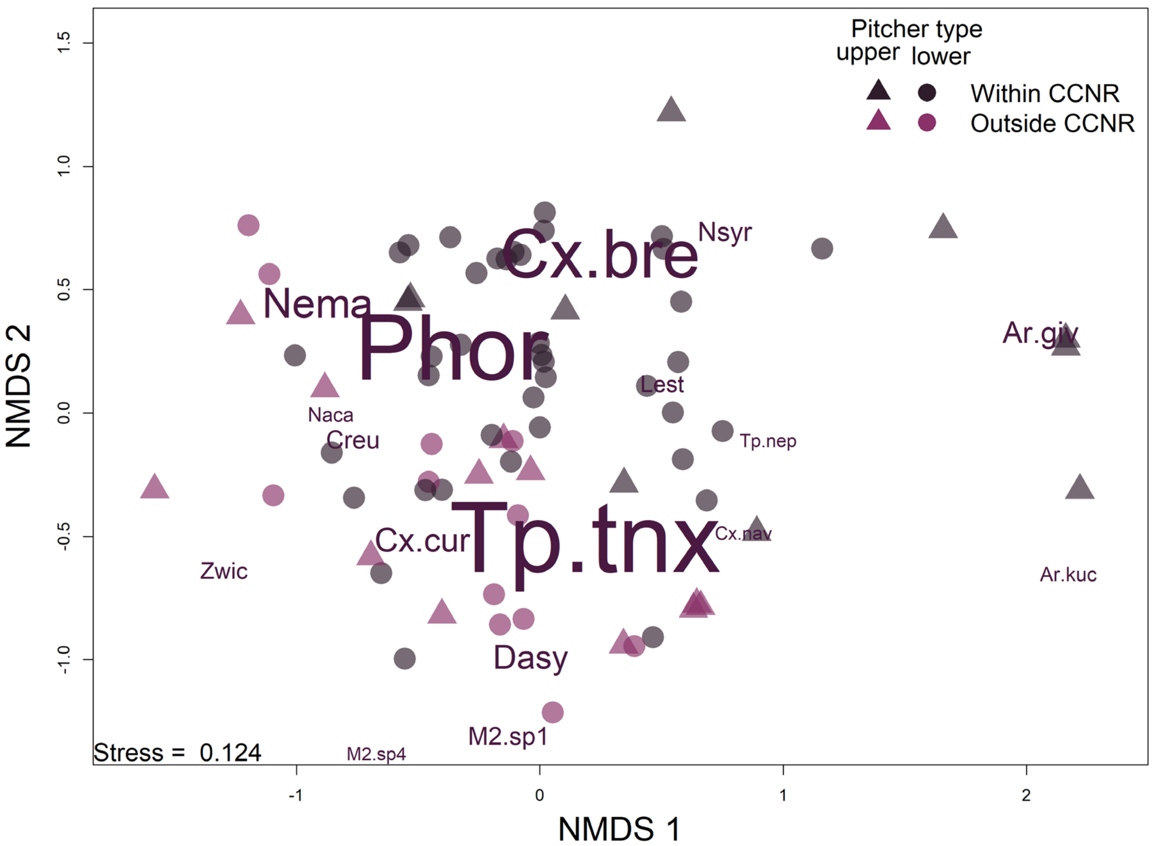

Fig. 4. Two-dimensional NMDS plot of the pitcher inquiline communities of the sampled *Nepenthes rafflesiana* pitchers. Each point represents an inquiline community from an individual pitcher, with its colour denoting the location in which it was found (within [brown] or outside [pink] the Central Catchment Nature Reserve [CCNR]), and its shape denoting its pitcher type (triangles denote upper, and circles, lower, pitchers). Texts represent inquiline species centroids, with font sizes proportional to the number of pitchers in which each was found. A species is more likely to occur in a pitcher if the pitcher's point is located close to the species' centroid. Taxon name abbreviations: Dasy = *Dasyhelea* spp.; Phor = Phoridae; Ar.giv = *Armigeres giveni*; Ar.kuc = *Ar.* cf. *kuchingensis*; Cx.bre = *Culex brevipalpus* complex; Cx.cur = *Cx. curtipalpis*; Tp.tnx = *Tripteroides tenax*; Lest = *Lestodiplosis* sp.; Nsyr = *Nepenthosyrphus* sp. raff; Creu = *Creutzeria* spp.; M2.sp1 = Histiostomatidae genus 1 sp. 1; Naca = *Nepenthacarus* spp.; Zwic = *Zwickia* spp.; Nema = nematodes.

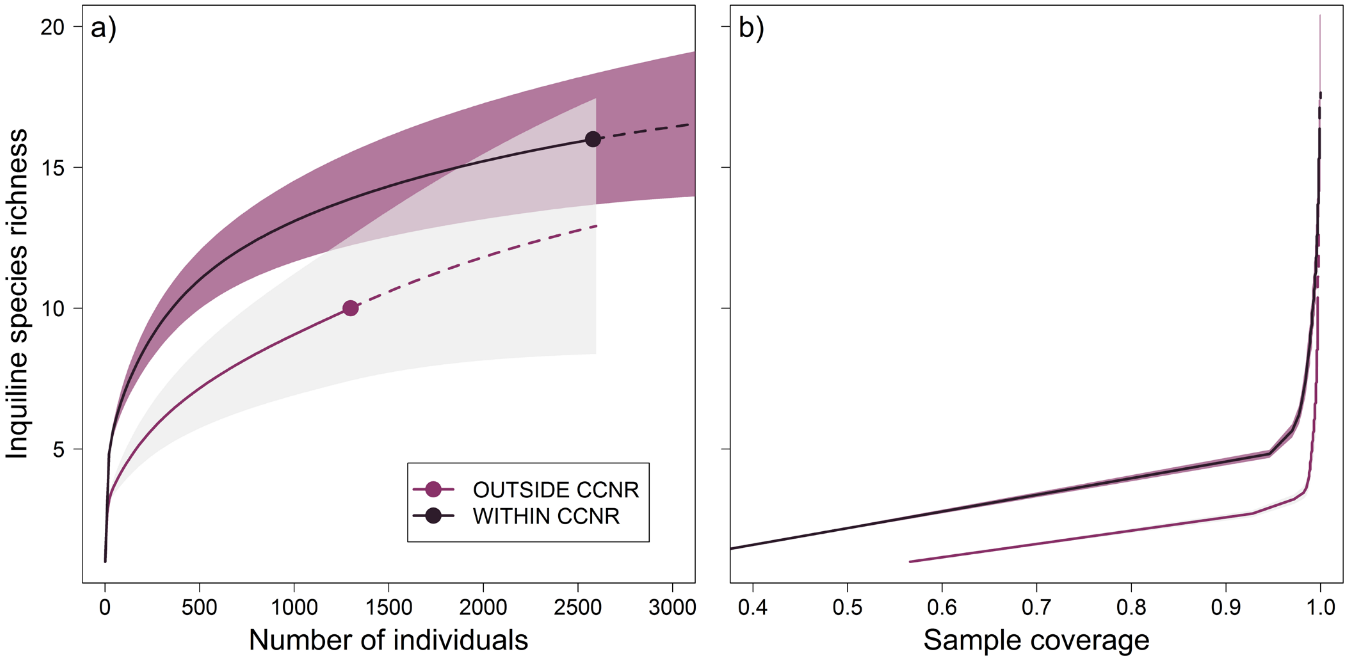

Fig. 5. Sample-size- (a) and coverage-based (b) rarefaction curves of inquiline species richness from pitchers collected outside of (pink lines) and within (brown lines) the Central Catchment Nature Reserve (CCNR). Lines represent the interpolated (continuous) and extrapolated (dashed) species richness 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).

Table 3. Inquiline species richness model coefficients and their corresponding standard errors in the top models (ΔAICc < 2), as well as the best model containing the term **Location**. In the categorical variables **Pitcher type** and **Location**, values represent the estimated differences in intercepts between the two variable levels (upper/lower, and within/outside of the Central Catchment Nature Reserve [CCNR], respectively). R^2 is the marginal R^2 of models—that is, the R^2 of fixed effect components of the model.

Model rank	Pitcher type	Pitcher size	Canopy cover	Location	ΔAICc	Model weight	R^2
1	0.546 (± 0.171)	–	–	–	0.00	0.28	0.149
2	0.477 (± 0.207)	0.034 (± 0.058)	–	–	1.94	0.11	0.152
3	0.519 (± 0.178)		0.043 (± 0.078)	–	1.99	0.10	0.152
…							
5	0.538 (±0.181)	–	–	0.023 (± 0.162)	2.27	0.09	0.148

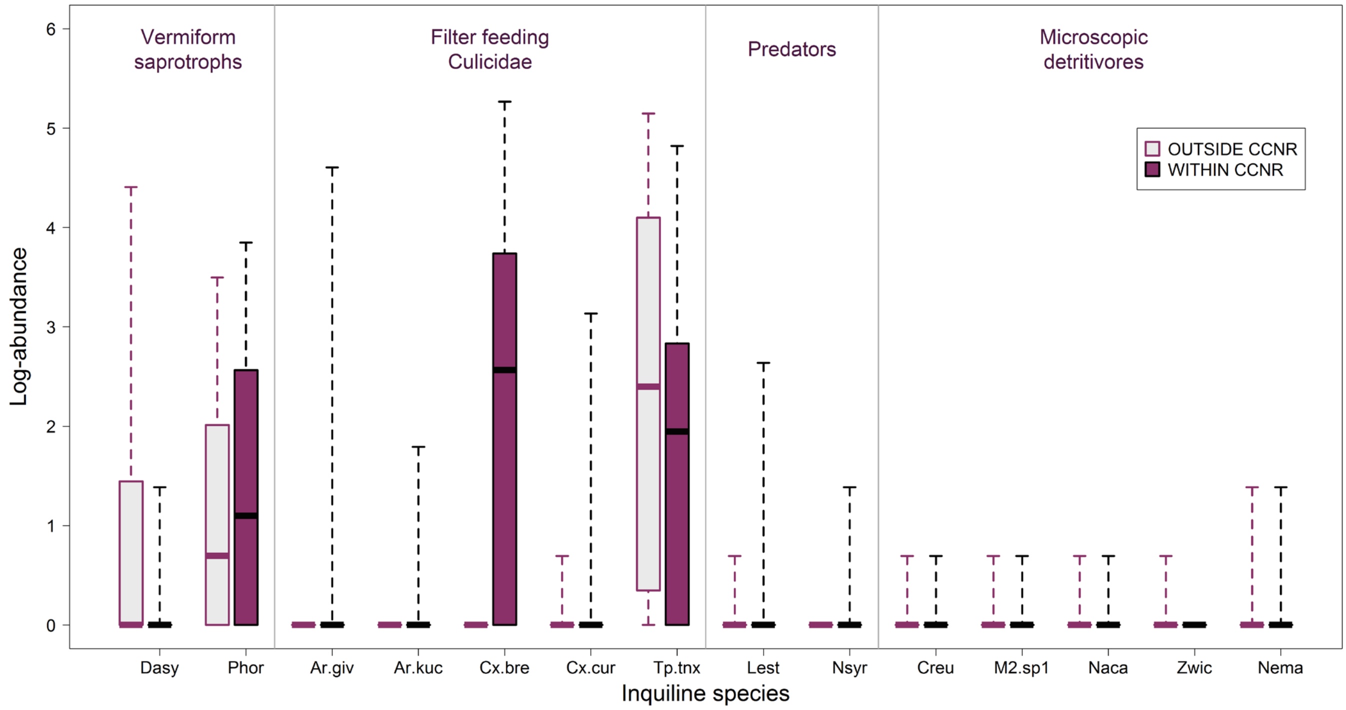

Fig. 6. Box and whisker plots show that inquiline taxa were found in different abundances across the different forest types, with several species being confined to the old secondary forests within the Central Catchment Nature Reserve (CCNR). Bold horizontal lines represent median log-transformed number of each inquiline taxon in pitchers from each forest type (denoted by colours); boxes represent interquartile range; whiskers represent maximum values. Taxon names are abbreviated as done in the previous figure; rare inquiline taxa which were present in only one sample are not displayed.

mite *Zwickia* spp., which was found only in pitchers outside, but not within the CCNR (Table 2; Fig. 6).

The best model of inquiline species richness contained only the term *pitcher type*. This model estimated a 0.55 (95% CI = [0.21, 0.88]; Table 3) difference in log-transformed species richness between upper and lower pitchers, meaning that, on average, upper pitchers contain 0.7 more inquiline species than lower ones (Fig. 7a). All three models within the top model set (ΔAICc < 2) also contained the term *pitcher type* with an effect size estimate which was similar to that of the top model, albeit with weak effects of *pitcher size* (second best model with ΔAICc = 1.94; 95% CI of standardised effect size = [−0.08, 0.15]; Table 3; Fig. 7b) and *canopy cover* (third best model with ΔAICc = 1.99; 95% CI of standardised effect size = [−0.11, 0.20]; Table 3; Fig. 7c). However, *location* (outside of or within the CCNR) had a negligible effect on inquiline species richness of individual pitchers, and the best model containing this term had a ΔAICc value of 2.27 (Table 3; Fig. 7d).

Prey taxa. Approximately 160 morphospecies from ≥ 60 families and 16 orders were identified from the 78 *N. rafflesiana* pitchers surveyed in this study (Table 4). Prey assemblages were found to differ significantly between upper and lower pitchers (pseudo-$F_{1,65}$ = 3.25, *p*-value < 0.001; Fig. 8), but only weakly between pitchers found within and outside of the CCNR (pseudo-$F_{1,65}$ = 1.54, *p*-value = 0.066; Fig. 8). Lower pitchers trapped significantly more spiders (Arachnida: Araneae) or harvestmen (Arachnida: Opiliones) species, and many times more small myrmicine (*Pheidole* spp., *Carebara* spp., *Crematogaster* spp., *Tetramorium* spp.) and *Nylanderia*

Table 4. Preliminary list of all prey taxa found in the 78 *Nepenthes rafflesiana* pitchers sampled in this study. Taxa are grouped into intermediate (**Intermediate Classification**) 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 lower/upper pitchers outside of or within the Central Catchment Nature Reserve (CCNR).

Higher classification	**Intermediate classification**	**Taxa included**	**Outside CCNR**		**Within CCNR**	
			Lower (n = 12)	**Upper (n = 15)**	**Lower (n = 41)**	**Upper (n = 10)**
Ants—Formicidae (Hymenoptera)	Dolichoderinae	*Dolichoderus thoracicus* *Dolichoderus* sp. *Iridomyrmex anceps* *Philidris* sp. *Tapinoma melanocephalum* *Tapinoma* sp. *Technomyrmex schimmeri* *Technomyrmex* sp.	9 (75%)	8 (53.3%)	18 (46.2%)	3 (30.0%)
	Formicinae (Camponotini)	*Camponotus arrogans* *Camponotus* cf. *moeschi lygaeus* *Dinomyrmex gigas* *Polyrhachis pruinosa* *Polyrhachis saevissima* complex *Polyrhachis proxima* *Polyrhachis abdominalis* *Polyrhachis illaudata* *Polyrhachis* spp.	8 (66.7%)	7 (46.7%)	10 (25.6%)	5 (50.0%)
	Formicinae (Lasiini)	*Nylanderia* cf. *bourbonica* *Nylanderia* spp.	5 (41.7%)	3 (20.0%)	7 (17.9%)	1 (10.0%)
	Formicinae (Oecophyllini)	*Oecophylla smaragdina*	0 (0.0%)	2 (13.3%)	0 (0.0%)	0 (0.0%)
	Formicinae (Plagiolepidini)	*Plagiolepis* spp.	2 (16.7%)	5 (33.3%)	2 (5.1%)	1 (10.0%)
	Myrmicinae (Attini)	*Pheidole aglae* *Pheidole* cf. *cariniceps* *Pheidole plagiaria* *Pheidole singaporensis* *Pheidole* spp.	2 (16.7%)	2 (13.3%)	18 (46.2%)	0 (0.0%)
	Myrmicinae (Crematogastrini)	*Carebara diversa* *Carebara* cf. *affinis* *Carebara* spp. *Catalaulacus* sp. *Crematogaster reticulata* *Crematogaster treubi* *Crematogaster jacobsoni* *Crematogaster cylindriceps* *Crematogaster* spp. *Meranoplus malaysianus* *Tetramorium* cf. *floricola* *Tetramorium* cf. *smithi* *Tetramorium* cf. *tonganum* *Tetramorium* spp.	9 (75%)	5 (33.3%)	16 (41%)	2 (20.0%)
	Myrmicinae (Solenopsidini)	*Monomorium floricola*	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Ponerinae	*Diacamma pallidum* *Diacamma* spp. *Platythyrea parallela* *Leptogenys chinensis* group spp. *Odontomachus rixosus* Other unidentified Ponerinae spp.	8 (66.7%)	7 (46.7%)	6 (15.4%)	1 (10.0%)

Higher classification	**Intermediate classification**	**Taxa included**	**Outside CCNR**		**Within CCNR**	
			Lower (n = 12)	**Upper (n = 15)**	**Lower (n = 41)**	**Upper (n = 10)**
	Other unidentified Formicidae spp.		1 (8.3%)	0 (0.0%)	18 (46.2%)	2 (20.0%)
Bees and wasps—Aculeata (Hymenoptera)	Aculeata	*Ceratina* sp. (Apidae) Unidentified wasp spp.	1 (8.3%)	2 (13.3%)	1 (2.6%)	1 (10.0%)
Cockroaches and termites—Blattodea	Cockroaches	*Anaplecta calosoma* *Blatella* spp. *Periplaneta americana* Other unidentified Blattodea spp.	0 (0.0%)	7 (46.7%)	10 (25.6%)	5 (50.0%)
	Isoptera (Termites)	Nasutitermitinae spp. Unidentified termite spp.	1 (8.3%)	1 (6.7%)	13 (33.3%)	2 (20.0%)
Flies—Diptera	Phoridae	Unidentified spp.	2 (16.7%)	5 (33.3%)	1 (2.6%)	2 (20.0%)
	Sciaridae	Unidentified spp.	1 (8.3%)	8 (53.3%)	0 (0.0%)	0 (0.0%)
	Drosophilidae	Unidentified spp.	0 (0.0%)	6 (40.0%)	1 (2.6%)	1 (10.0%)
	Syrphidae	Unidentified sp.	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Muscidae	Unidentified spp.	0 (0.0%)	3 (20.0%)	0 (0.0%)	1 (10.0%)
	Calliphoridae	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Mycetophilidae	Unidentified sp.	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Unidentified small diptera spp.		0 (0.0%)	4 (26.7%)	0 (0.0%)	0 (0.0%)
True bugs—Hemiptera	Cicadellidae	Xestocephalinae sp. Other unidentified Cicadellidae spp.	0 (0.0%)	1 (6.7%)	0 (0.0%)	1 (10.0%)
	Delphacidae	Delphacidae	2 (16.7%)	2 (13.3%)	0 (0.0%)	1 (10.0%)
	Membracidae	*Gargara flavocarinata*	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
	Reduviidae	*Lisarda* spp.	0 (0.0%)	0 (0.0%)	7 (17.9%)	0 (0.0%)
	Rhyparochromidae	*Metochus* cf. *uniguttatus*	4 (33.3%)	3 (20.0%)	1 (2.6%)	0 (0.0%)
	Unidentified hemiptera		0 (0.0%)	2 (13.3%)	1 (2.6%)	0 (0.0%)

Higher classification	**Intermediate classification**	**Taxa included**	**Outside CCNR**		**Within CCNR**	
			Lower (n = 12)	**Upper (n = 15)**	**Lower (n = 41)**	**Upper (n = 10)**
Beetles—Coleoptera	Anthribidae	*Basitropis* sp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
	Chrysomelidae	*Cryptocephaus* sp. *Dercetisoma* sp. (Galerucinae) *Arcastes biplagiata* (Galerucinae) Other spp.	0 (0.0%)	2 (13.3%)	2 (5.1%)	2 (20.0%)
	Curculioniae	*Trochorhopalus sumatranus* (Dryophthoridae)	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Elateridae	Unidentified spp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (20.0%)
	Eucnemidae	Unidentified spp.	0 (0.0%)	2 (13.3%)	0 (0.0%)	0 (0.0%)
	Nitidulidae	*Epuraea* sp. *Carpophilus* sp.	0 (0.0%)	3 (20.0%)	0 (0.0%)	0 (0.0%)
	Scarabidae	Unidentified Sericini (Melolonthinae) sp. Other unidentified spp.	2 (16.7%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Scirtidae	Unidentified sp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
	Staphylinidae	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Other unidentified Coleoptera spp.		0 (0.0%)	0 (0.0%)	6 (15.4%)	0 (0.0%)
Moths & butterflies—Lepidoptera	Lepidoptera	Psychidae larva Other unidentified Lepidoptera larvae Unidentified moth and butterfly spp.	0 (0.0%)	6 (40.0%)	1 (2.6%)	3 (30.0%)
Grasshoppers, crickets, and katydids—Orthoptera	Acrididae	*Traulia azureipennis* *Xenocatantops humilis* *Oxya* sp.	0 (0.0%)	1 (6.7%)	1 (2.6%)	0 (0.0%)
	Gryllacrinidae	Unidentified sp.	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Gryllidae	*Svistella* sp. *Odontogryllodes latus* Unidentified Trigonidiinae sp.	0 (0.0%)	3 (20.0%)	0 (0.0%)	1 (10.0%)
	Mogoplistidae	*Cycloptiloides* cf. *timah* Other unidentified sp.	0 (0.0%)	1 (6.7%)	2 (5.1%)	0 (0.0%)
	Tettigoniidae	*Conocephalus melaenus* *Euconocephalus picteti* Unidentified Phaneropterinae sp.	0 (0.0%)	4 (26.7%)	0 (0.0%)	0 (0.0%)
	Other unidentified Orthoptera spp.		0 (0.0%)	0 (0.0%)	1 (2.6%)	1 (10.0%)

Higher classification	**Intermediate classification**	**Taxa included**	**Outside CCNR**		**Within CCNR**	
			Lower (n = 12)	**Upper (n = 15)**	**Lower (n = 41)**	**Upper (n = 10)**
Spiders, scorpions, mites, and harvestmen—Arachnida	Barychelidae	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Clubionidae	*Nusatidia camouflata*	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Corinnidae	*Corinnomma* sp.	1 (8.3%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Ctenidae	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Liocranidae	*Oedignatha scrobiculata*	0 (0.0%)	0 (0.0%)	2 (5.1%)	0 (0.0%)
	Oonopidae	*Ischnothyreus* sp. Unidentified sp.	1 (8.3%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Oxyopidae	*Oxyopes* sp.	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Salticidae	*Ptocasius weyersi* *Harmochirus brachiatus* *Myrmarachne* sp. *Bavia* sp. *Phintella* sp. Other unidentified spp.	3 (25%)	3 (20.0%)	6 (15.4%)	1 (10.0%)
	Thomisidae	*Thomisus* sp.	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)
	Zodariidae	*Asceua* sp. *Cryptothele sundaica* *Mallinella annulipes*	0 (0.0%)	0 (0.0%)	3 (7.7%)	0 (0.0%)
	Other Unidentified Aranae spp.		1 (8.3%)	0 (0.0%)	2 (5.1%)	0 (0.0%)
	Acari	Unidentified spp.	0 (0.0%)	0 (0.0%)	5 (12.8%)	0 (0.0%)
	Opiliones	Unidentified spp.	1 (8.3%)	0 (0.0%)	7 (17.9%)	0 (0.0%)
	Scorpiones	Unidentified spp.	0 (0.0%)	0 (0.0%)	2 (5.1%)	1 (10.0%)
Miscellaneous animal taxa	Dermaptera	Unidentified spp.	1 (8.3%)	1 (6.7%)	1 (2.6%)	0 (0.0%)
	Isopoda	Unidentified spp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
	Mantodea	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Mollusca	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Myriapoda	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Psocoptera	Unidentified sp.	0 (0.0%)	0 (0.0%)	1 (2.6%)	0 (0.0%)
	Thysanoptera	Unidentified spp.	0 (0.0%)	1 (6.7%)	1 (2.6%)	0 (0.0%)
Plant material	Plantae	*Dicranopteris linearis* (Gleicheniaceae) leaflets Other unidentified flowers Other unidentified leaves/leaflets	4 (33.3%)	2 (13.3%)	4 (10.3%)	1 (10.0%)

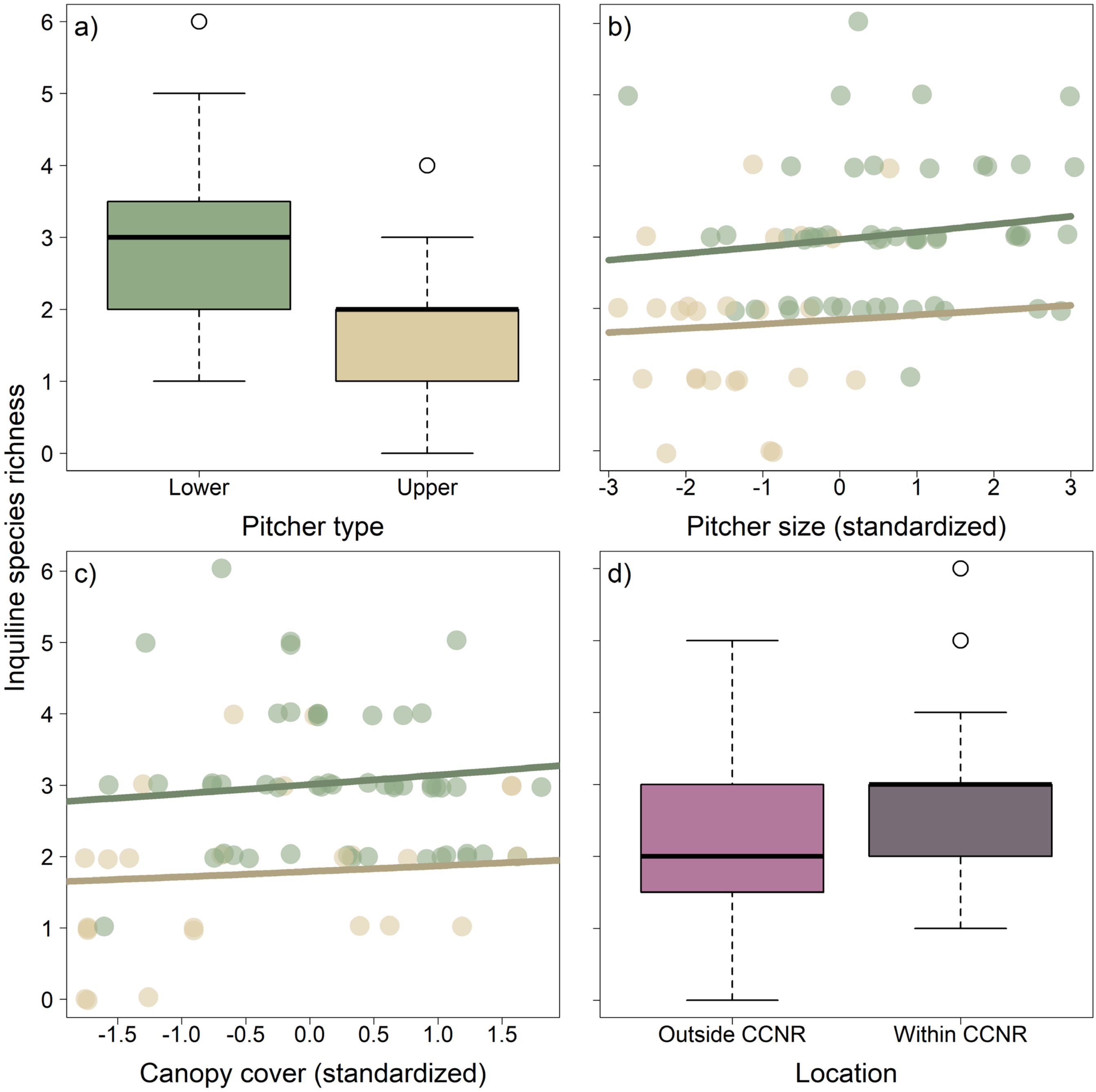

Fig. 7. Box and whisker (a, d) and scatter (b, c) plots showing the relationships between the inquiline species richness of individual pitchers and pitcher type (a), pitcher size (b), canopy cover (c), and location (within or outside of the CCNR; d). The plots show that inquiline species richness was higher in lower than upper pitchers (a), and pitcher size (b) and canopy cover (c) had weak positive effects on inquiline species richness, but there was no significant difference in inquiline species richness between pitchers outside of and within the Central Catchment Nature Reserve (CCNR) (d). In (a) and (d), boxes represent interquartile ranges, whiskers represent maxima and minima, and points represent outliers. In (b) and (c), points (green = lower, beige = upper pitchers) represent the species richness of individual pitchers, and lines represent the model predictions of the second (b; ΔAICc = 1.94) and third (c; ΔAICc = 1.99) best models for lower (green) and upper (beige) pitchers.

spp. (Formicinae: Lasiini) ants than upper pitchers. On the other hand, upper pitchers trapped cockroaches (Blattodea), beetles (Coleoptera), crickets or katydids (Orthoptera), moths or butterflies (Lepidoptera) and large *Polyrhachis* spp. and *Dinomyrmex gigas* ants (Formicinae: Camponotini) more frequently than lower pitchers (Table 4; Fig. 8). In general, prey items were often larger in body size (and thus nutritional content) in upper pitchers.

DISCUSSION

We examined the pitcher contents of 78 *N. rafflesiana* pitchers found in 13 plots within and outside of the CCNR. Differences in plant community composition in plots within and outside of the CCNR were very evident (Fig. 2). Outside of the CCNR, *N. rafflesiana* occurred mostly in adinandra belukar-type habitats (Fig. 2a, b). Primary forest or old secondary forest vegetation types constitute a large component of the forested land area in the CCNR (Yee et

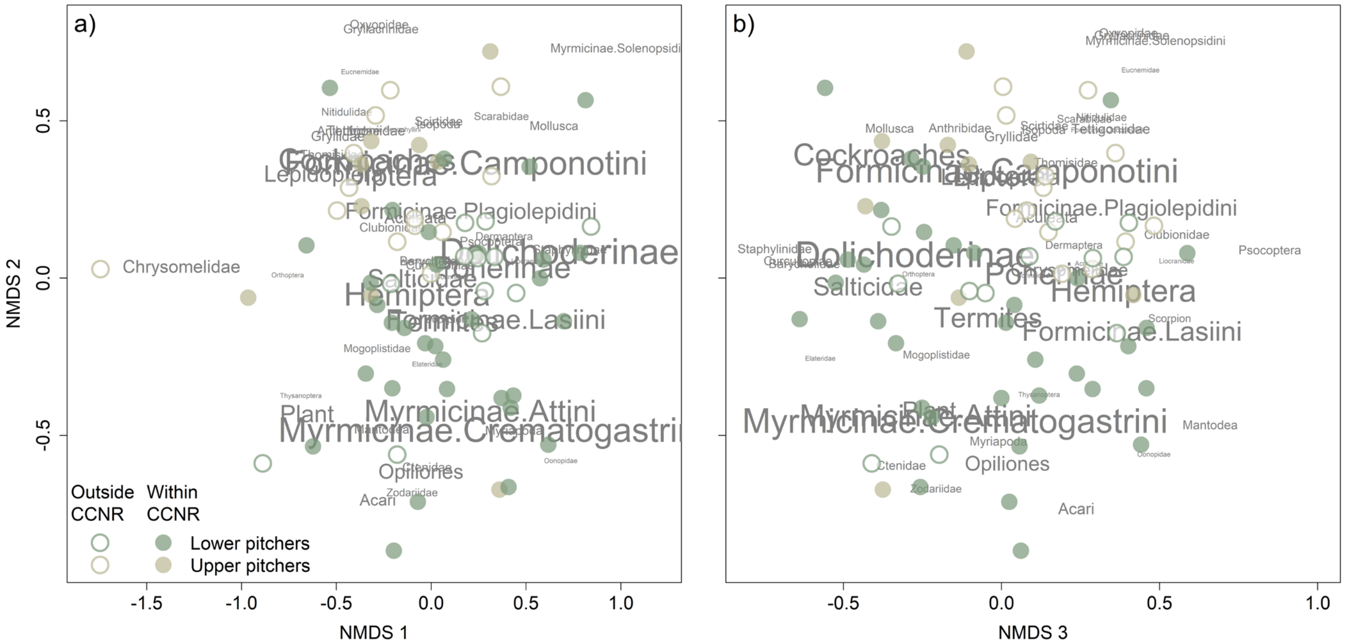

Fig. 8. First and second (a) and second and third (b) dimensions of the three-dimensional NMDS of pitcher prey assemblages. Points represent prey assemblages of individual pitchers, being coloured according to pitcher type (with green points representing lower pitchers and beige ones representing upper ones) and are filled for pitchers found within the Central Catchment Nature Reserve (CCNR) and unfilled for those outside of it. Texts denote prey taxon centroids and are sized proportionally with the frequency of occurrence of the taxa they denote. Points found close to species centroids are more likely to contain the corresponding prey taxon. Stress = 0.174.

al., 2016), but *N. rafflesiana* was not found in these forest types, and occurred mostly in what would be described as resam patches, edges, or treefall gaps (Goldsmith et al., 2011; Chua et al., 2016; Yee et al., 2016, 2019) (Fig. 2c). Inquiline communities within pitchers also differed significantly between pitcher types and the locations of plots in which they were sampled (within or outside of the CCNR; Figs. 4, 6; Table 2). Contrary to expectation, however, asymptotic inquiline species richness of pitchers within and outside of the CCNR were not significantly different (Fig. 5). Finally, prey assemblages of *N. rafflesiana* upper and lower pitchers were significantly different as expected, but differences due to location (within or outside of the CCNR) were relatively weak (Fig. 8), suggesting that differing trapping mechanisms between upper and lower pitchers drive a deterministic prey partitioning between them.

Our data showed that several inquiline dipteran species, viz. *Armigeres giveni*, *Ar.* cf. *kuchingensis*, *Culex brevipalpus*, *Cx. navalis*, and *Tripteroides nepenthis* (all Culicidae), and *Nepenthosyrphus* sp. (Syrphidae) are clearly restricted to the CCNR (Table 2). Despite this, rarefaction analyses showed that unique inquiline species were not accumulated at a faster rate relative to abundances of sampled inquiline individuals in the CCNR compared to outside of it (Fig. 5), neither were there significant differences in inquiline species richness between pitchers found within the CCNR and those found outside of it (Fig. 7). This was probably because at least one inquiline taxon (*Zwickia* spp. [Histiostomatidae]) was found only in pitchers outside of the CCNR, and these were also more frequently inhabited by some inquiline species (e.g., *Dasyhelea nepenthicola* [Ceratopogonidae]) which otherwise occurred only rarely within the CCNR (Table 2).

Lim et al. (2019) had earlier found that *N. ampullaria* pitchers from the Nee Soon Swamp Forest (a watershed within the CCNR) contained significantly higher numbers of inquiline species than those in adinandra belukar of an urban forest (Kent Ridge Park), and in resam-dominated scrub of urban natural vegetation fragments. In the case of *N. ampullaria*, pitchers occurring outside the CCNR all contained much simpler inquiline communities which often lacked predators and were dominated by a single detritivorous species (*Tripteroides tenax* [Culicidae]) (Lim et al., 2019). In the case of *N. rafflesiana*, however, *Nepenthosyrphus* sp. (Syrphidae) and *Lestodiplosis* sp. (Cecidomyiidae) are the only known predators in these shorter-lived pitchers in Singapore (Choo et al., 1997; Lam et al., 2019), and both of these species are not common and facultatively saprotrophic (Lam et al., 2019; Lam et al., 2020a). Thus, differences in the inquiline communities within and outside of the CCNR were represented mainly by a turnover of detritivorous species (Table 2; Fig. 4), with some being found more outside of the CCNR (e.g., *Tp. tenax* [Culicidae], *Dasyhelea nepenthicola* [Ceratopogoniidae], and *Zwickia* sp. [Histiostomatidae]) and others being found more often within it (e.g., *Armigeres* spp. and *Cx. brevipalpus* complex [both Culicidae]). Our findings thus point indirectly to the role of inquiline predators in maintaining inquiline biodiversity in *Nepenthes* pitcher phytotelmata.

Several non-specific inquiline–host associations were observed in this study. *Culex navalis* (two individuals) and *Tp. nepenthis* (one individual) were found in only one *N. rafflesiana* lower pitcher each in the CCNR. These two species commonly inhabit the pitchers of *N. ampullaria* in the CCNR (Lim et al., 2019). Their presence in *N. rafflesiana* pitchers may have been the result of occasional oviposition

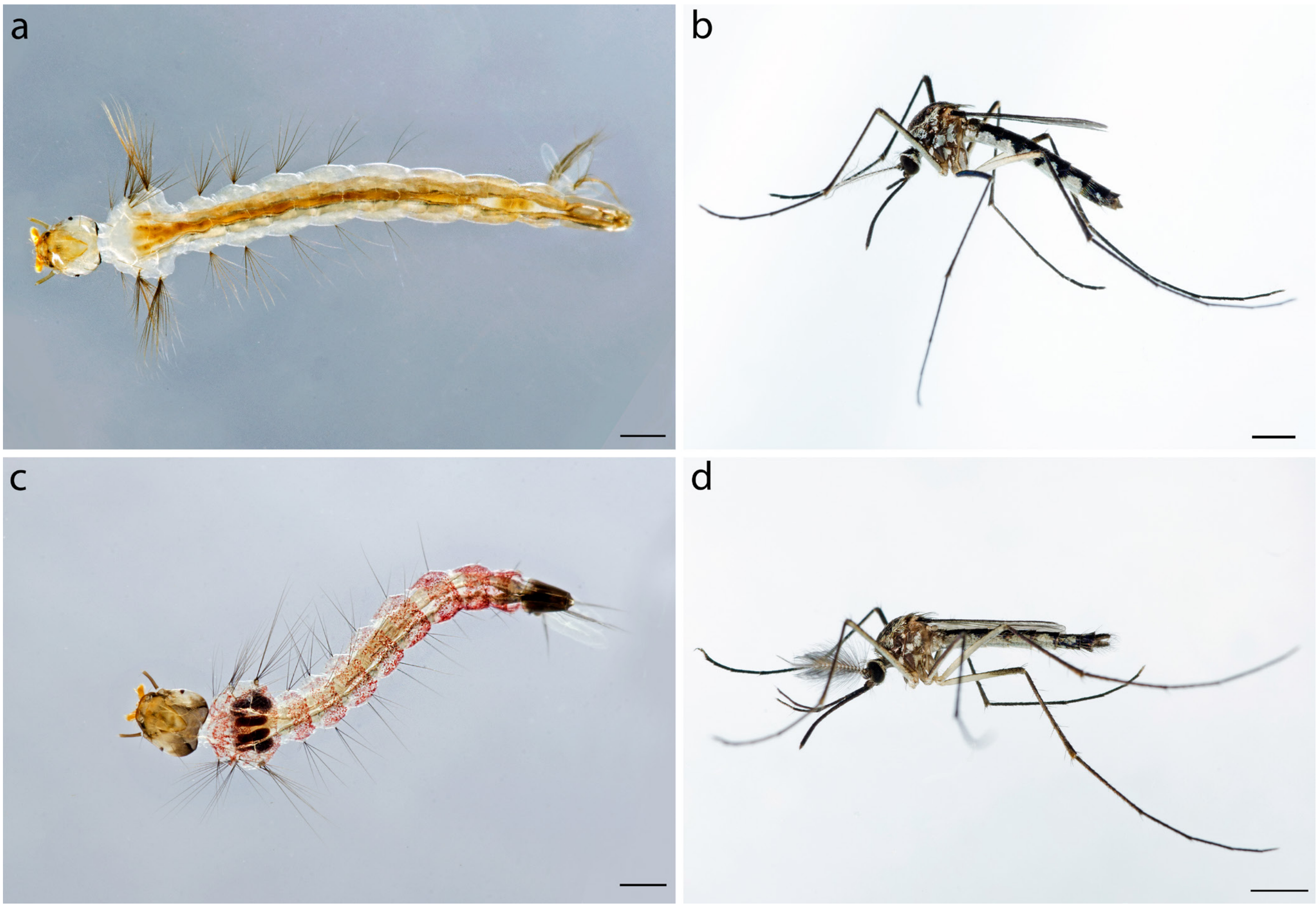

Fig. 9. Habitus of *Armigeres giveni* fourth instar larva (a); *Ar. giveni* female adult (b); *Ar.* cf. *kuchingensis* fourth instar larva (c); *Ar.* cf. *kuchingensis* male adult (d). Scale bars represent 1 mm. (Photographs by: Yeo Huiqing).

by gravid female mosquitoes in non-target hosts, and it is unclear if these individuals would have survived to maturity in nature. On the other hand, despite the low numbers of observations made of *Ar.* cf. *kuchingensis* and *Lestodiplosis* sp., these taxa are less likely to be the result of oviposition in non-target host species. *Lestodiplosis* sp. are not found in great frequencies or abundances even in their preferred host species, *N. gracilis* (WNL, unpublished data), and their presence in numbers of up to 13 in one *N. rafflesiana* lower pitcher suggest that *N. rafflesiana* may be considered a secondary host of *Lestodiplosis* sp.

Culicid inquiline taxa. Mosquito species which are known vectors of disease-causing pathogens, viz. *Aedes aegypti* and *Ae. albopictus* were not found in any of the sampled pitchers. This is unsurprising given that pH of the *N. rafflesiana* pitchers was highly acidic (mean ± SD = 4.2 ± 1.7), and that *Ae. albopictus* and *Ae. aegypti* are only able to tolerate environments which are slightly acidic (pH ≈ 5–7.9) (Sardiñas et al., 2008; Chou et al., 2016; Madzlan et al., 2017). The presence of additional larvicidal agents such as microbes and digestive enzymes in the pitcher fluid can also negatively affect the survivorship of larvae which are not adapted to this harsh environment (Takeuchi et al., 2011; Hatano & Hamada, 2012). Moreover, *Ae. aegypti* is a highly anthropophilic and urban-adapted species, thus the chances of this species ovipositing in the pitchers is very low (Chou et al., 2016).

As with the other *Nepenthes* species, *Tripteroides tenax* and *Culex brevipalpus* complex were the two most common taxa found in the *N. rafflesiana* pitchers. The *Culex brevipalpus* complex consists of six morphologically highly similar species, of which *Culex eminentia* (Barr & Chellapah, 1963; Mogi & Chan, 1997) and *Cx. lucaris* (Sirivanakarn, 1977) have been recorded locally in *N. rafflesiana*. This complex will certainly benefit from a barcoding investigation given their high morphological similarity.

Two species of *Armigeres* seem to be specialists of *N. rafflesiana* pitchers and are only localised in the CCNR. *Armigeres giveni* (Fig. 9a, b) has been recorded in *N. rafflesiana* previously (Dover et al., 1928). Some of our specimens resemble *Ar. kuchingensis* (Fig. 9c, d), although this species was recorded to utilise bamboos and coconut husks for breeding instead of *Nepenthes* pitchers (Barraud, 1934; Rattanarithikul et al., 2010). Further investigations should be conducted to ascertain if this species does indeed utilise *N. rafflesiana* pitchers for breeding, both within and outside of Singapore.

One species of metazoan inquiline that had been found in previous studies in *N. rafflesiana* pitchers was not encountered in this study—*Uranotaenia xanthomelaena* (Mogi & Chan, 1997). This species had been found in *N. rafflesiana* pitchers from Lower Peirce Reservoir (Mogi & Chan, 1997). *Armigeres flavus* is another species that had

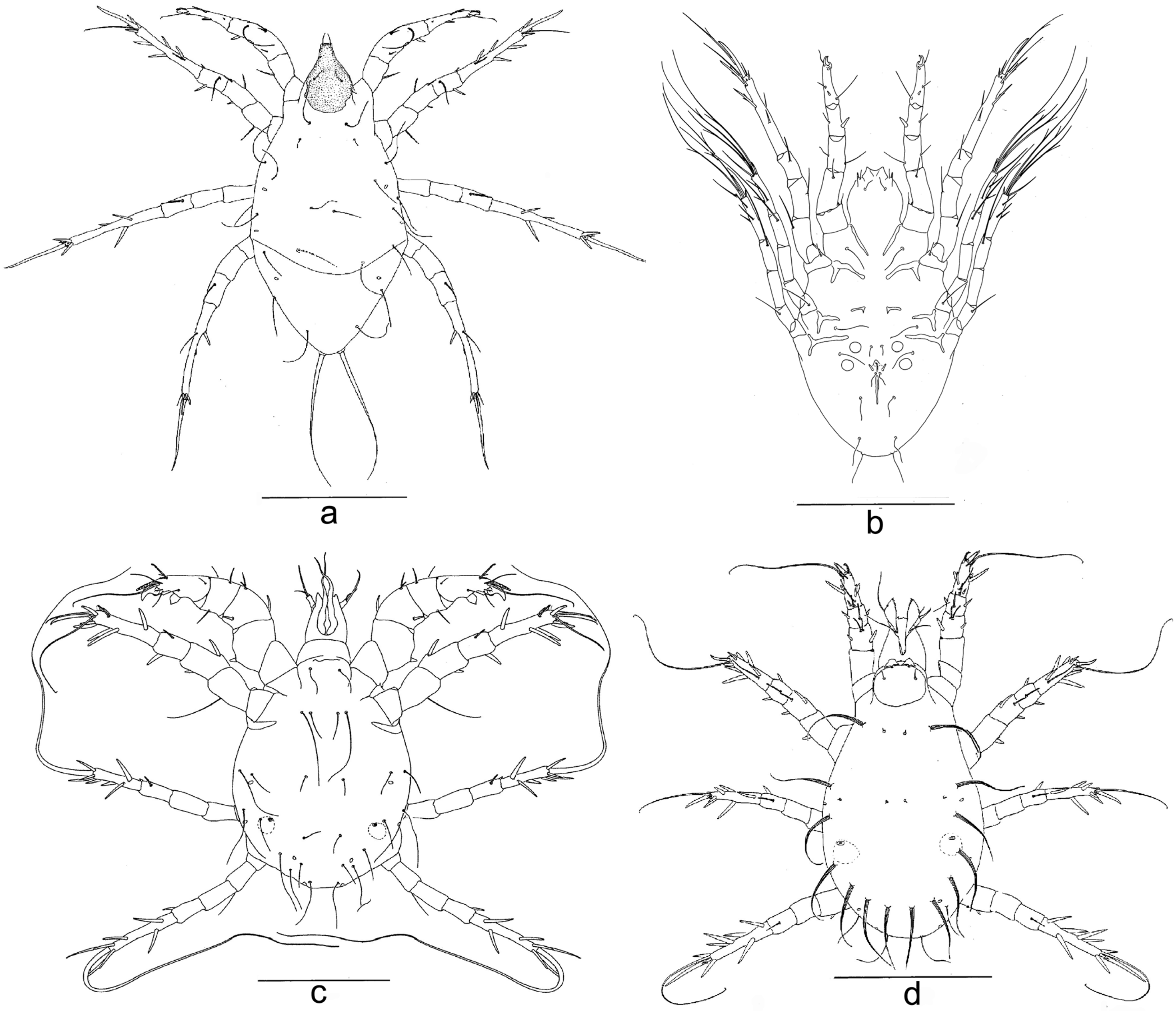

Fig. 10. Illustrations of mite genera inhabiting *Nepenthes rafflesiana* pitchers in Singapore: (a) Undescribed genus (male dorsum), (b) *Creutzeria* sp. (male venter), (c) Zwickia sp. (male dorsum), (d) *Nepenthacarus* sp. (male dorsum). Scale bars represent 150 micrometres. (Illustrations by: Norman J. Fashing).

been recorded from *Nepenthes* pitchers in Singapore, but it is unclear if it was collected from *N. rafflesiana* (Edwards & Given, 1928). It is noteworthy also that *Toxorhynchites acaudatus* was a common occurrence in *N. rafflesiana* before the 1970s (Dover et al., 1928; Barr & Chellapah, 1963), but this was surprisingly not reflected in a later study (Mogi & Chan, 1997). We did not encounter this species in any of the 51 samples in this study from the CCNR, to which *Tx. acaudatus* is confined, but have observed it in *N. rafflesiana* lower pitchers on rare occasions (W. N. Lam & R. J. Y. Lim, pers. obs.). The predator *Tx. acaudatus* is commonly found in *N. ampullaria* instead (Lim et al., 2019).

Acarine inquiline taxa. Histiostomatidae is a large family of astigmatine mites containing species that inhabit a wide diversity of moist or wet habitats (OConnor, 2009). Schatz et al. (2011) recorded 59 genera and 567 species, and since that time more have been described. Many species are inhabitants of the water-filled cavities of plants (a.k.a. phytotelmata) (Kitching, 2000), including the pitchers of *Nepenthes* spp. where they can be present in large numbers (Fashing, 2002; Walter & Proctor, 2013). The ecology of histiostomatid *Nepenthes* pitcher mites remains poorly studied, and recent studies have only been able to show that one mite taxon (*Creutzeria* spp.) competes with inquiline microbes for detrital resources without significantly increasing nutrient availability to pitchers via excretion (Lam et al., 2020b) and that predatory inquiline species such as *Corethrella calathicola* (Diptera: Corethrellidae) prey on these mites (Lam et al., 2020a).

Four histiostomatid genera were identified from subsamples of the 78 *N. rafflesiana* pitchers: an undescribed genus (Fig. 10a), *Creutzeria* sp. Oudemans (Fig. 10b), *Zwickia* sp. Oudemans (Fig. 10c), and *Nepenthacarus* sp. Fashing (Fig. 10d). The undescribed genus is most probably the species described as "*Zwickia nepenthesiana*" from specimens collected from the pitchers of *N. ampullaria* in Singapore (Hirst, 1928). Although Hirst's description is brief and his illustrations incomplete, it is obvious from the illustrations

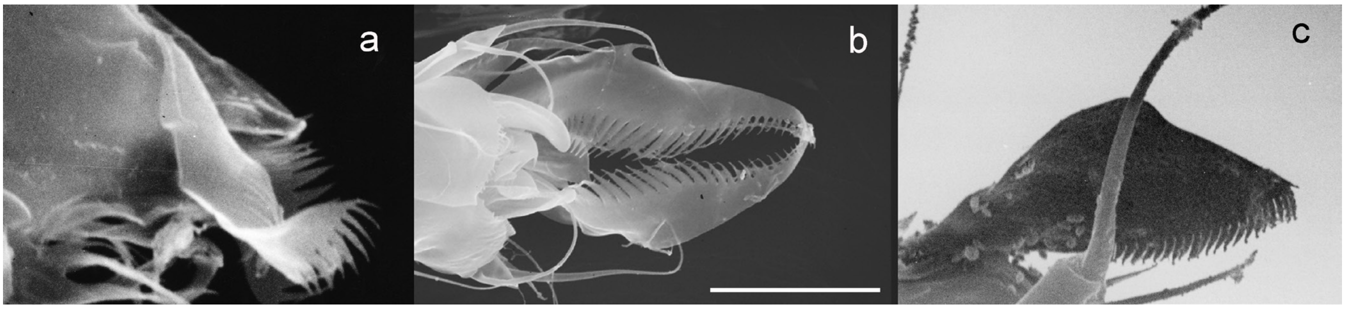

Fig. 11. Scanning electron microscope (SEM) photographs exemplifying morphological differences in the chelicerae of *Nepenthes* histiostomatid mites: (a) *Creutzeria* sp., (b) *Zwickia* sp., (c) *Nepenthacarus* sp. Scale bar = 10 micrometres. (Photographs by: Norman J. Fashing).

that the species does not belong to the genus *Zwickia* nor any other extant genus. The undescribed genus in this study does, however, closely resemble the idiosomal shapes of Hirst's illustrations and it is commonly found in pitchers of all three Singapore *Nepenthes* species.

Bittleston et al. (2016) and Bittleston (2018), using metabarcoding of pitcher fluid samples, reported two additional genera present in Singapore *N. rafflesiana*: *Hormosianoetus* Fain and *Rostrozetes* Sellnick. It should be noted that this is the first report of the two genera being inquilines of *Nepenthes* pitchers, and that neither genus has been reported from existing *Nepenthes* studies utilising slide mounted specimens collected in Singapore or any other locality. That barcoding did not reveal the presence of any of the four genera observed in this study suggests that the database used for the barcoding analysis did not contain sufficient data from relevant taxa to adequately identify mite inquilines. It is therefore unlikely that *Hormosianoetus* and *Rostrozetes* are inquilines of *Nepenthes* pitchers.

Our study indicates that a degree of specialisation may occur according to pitcher type or geographic location. The genus *Zwickia* was found only outside of the CCNR (Table 2), perhaps indicating a preference for adinandra belukar, but even then it was only found in four pitchers, therefore more sampling is necessary to confirm this. The predominant presence of *Creutzeria* sp. (five samples) and the undescribed genus (six samples) in lower pitchers when compared to upper pitchers (no samples of either *Creutzeria* sp. or the undescribed genus) indicates a possible degree of specialisation for pitcher type. The prevalence of lower pitchers with mites, however, could also be due to a pitcher preference for the inquiline hosts on which the mite genera are phoretic.

Mites were observed in only 14 (17.9%) of the 78 *N. rafflesiana* pitchers sampled, whereas significantly more pitchers of *N. ampullaria* sampled contained mites (53 of 147; 36.1%) ($\chi^2 = 7.412$, df = 1, p-value = 0.006) using an identical sampling methodology across similar sites. The difference may be due to the difference in pitcher lifespan for the two species; *N. rafflesiana* pitchers are relatively short lived, with mean half-lives of just 1.1 months while those of *N. ampullaria* survive much longer, with mean half-lives of 2.4 months (Osunkoya et al., 2008). There is therefore considerably more time for acarine inquilines to colonise *N. ampullaria* pitchers.

The trophic instars of species in the family Histiostomatidae are typically ambulatory, moving about on the substrate. This is certainly the case for the undescribed genus found in *N. rafflesiana*; however, the other three genera deviate from this typical mode of locomotion. Members of the genus *Creutzeria* are completely natatorial, with legs modified for swimming (see Fashing et al., 1996; Walter & Proctor, 2013). Members of the genus *Nepenthacarus* have been observed moving about in or on the water surface film, and occasionally crawling about on arthropod parts or debris floating on the water surface (see Fashing, 2002). Members of the genus *Zwickia* are typically observed using legs I and II to move about on substrate at the bottom of the pitchers; however, they are occasionally observed using backward movements of legs III with their extremely long setae to propel themselves for a short distance through the fluid. They can be considered both natatory and ambulatory; however, swimming appears to be a much more laborious task for *Zwickia* spp. and is less utilised than walking (see Fashing, 2004).

Although histiostomatid mites are small, observations on mouthpart morphology using scanning electron microscopy coupled with observations on overall behaviour using a stereomicroscope can be used to gain insight into feeding behaviour (e.g., see Fashing, 1998). Histiostomatid mouthparts are typically highly modified for filtering microorganisms and small organic material from the habitat in which they live (OConnor, 2009); the chelicerae in particular are used to aid in gathering such items by brushing or scraping. Both cheliceral morphology (Fig. 11) and behaviour suggest that resource partitioning does occur between the genera of nepenthebiont mites. The chelicerae of *Creutzeria* spp. (Fig. 11a) resemble brushes and are flexible rather than ridged. Individuals swim in the water column and appear to be brushing the surface of decomposing insect cuticle, most likely feeding on the film of microbes growing on the prey carcasses. Lam et al. (2020b) found that they are unlikely to filter microbes from the pitcher fluid. The chelicerae of the undescribed genus are somewhat similar to those of *Creutzeria*; however, it is an ambulatory species and brushes microbes from the insect remains at the bottom of the pitcher. The chelicerae of *Zwickia* (Fig. 11b) narrow distally to a point and appear to be more ridged. It is possible they are used to extract the microbial film growing in crevices as well as by brushing from the surfaces. The chelicerae of *Nepenthacarus* (Fig. 11c) are quite ridged with short, rake-like teeth. It is possible that these are used to rake microbes and small debris from the surface film. It is speculated that inquiline mite species coexist by specialising in different habitat strata, utilising morphological and behavioural modifications for feeding on microbial growth. The four genera therefore appear to partition the habitat not only by their means of locomotion but also by their feeding behaviour.

Saprotrophic inquiline taxa. Phorid larvae, particularly those of *Endonepenthia schuitemakeri*, were also very abundant and commonly encountered in the pitchers of *N. rafflesiana* (Table 2). Phorid inquiline larvae are known to consume large amounts of prey rapidly and pupate and emerge relatively quickly, and thus process nutrients in large-bodied prey efficiently (Leong et al., 2018; Lam et al., 2019). Phorids were more frequently encountered in *N. rafflesiana* than in *N. ampullaria* (present in 34 of 56 [61%; Table 2] *N.rafflesiana*, and 14 of 147 [10%; Lim et al., 2019] *N. ampullaria* lower pitchers) (χ^2 = 29.7, df = 1, *p*-value < 0.001), but *Dasyhelea* species, which often take longer to grow and pupate (W. N. Lam, pers. obs.) were more common in *N. ampullaria* than in *N. rafflesiana* (present in 80 of 147 [54%; Lim et al., 2019] *N. ampullaria*, and 10 of 78 [13%; Table 2] *N. rafflesiana* lower pitchers) (χ^2 = 16.6, df = 1, *p*-value < 0.001). *Nepenthes rafflesiana* pitchers are known to trap large volumes of prey in stochastic mass capture events (Bauer et al., 2015) within just a few days of pitcher opening (Bauer et al., 2009), and their pitchers are relatively short lived (Osunkoya et al., 2008). This contrasts strongly with the slow resource acquisition strategy of *N. ampullaria*, whose pitchers are longer-lived and seldom experience surges in prey capture volumes comparable to those of *N. rafflesiana* (Moran et al., 2010). While acknowledging many other ecological and physicochemical differences in the pitchers of *N. rafflesiana* and *N. ampullaria*, it is likely that the relative abundances of these saprotrophic inquilines in the pitchers of these two *Nepenthes* species reflects this difference in resource acquisition strategies between *Dasyhelea* and Phorid inquiline species.

Predatory inquiline taxa. One species of *Nepenthosyrphus* was also encountered in this study. This is the same species as that collected from *N. ampullaria* pitchers in Lim et al. (2019), which feeds primarily on phorid larvae, and facultatively on culicid larvae and pitcher prey carcasses (Lam et al., 2019). The species appears to utilise *N. rafflesiana* pitchers as a primary host, and *N. ampullaria* pitchers as a secondary host, as larvae are found more frequently and in higher abundances in pitchers of the former. A more detailed taxonomic study of this *Nepenthosyrphus* species is currently being conducted.

It is noteworthy that no crab spider inquilines were found in any of the pitchers sampled in this study. *Misumenops nepenthicola* (Arachnida: Thomisidae) is known to inhabit *N. rafflesiana* upper pitchers in Brunei (Karl & Bauer, 2020), where it preys on both pitcher visitors and aquatic or emerging inquilines (Chua & Lim, 2012; Lim et al., 2018; Karl & Bauer, 2020). In Singapore, *M. nepenthicola* and another inquiline crab spider species, *Thomisus nepenthiphilus*, are both found almost exclusively in the pitchers of *N. gracilis* and its hybrids, but not in those of *N. rafflesiana*. The reasons for this are unclear, and are discussed in detail in Lam et al. (2020a).

Prey taxa. Differences in prey capture between upper and lower pitchers of *N. rafflesiana* are well documented (Moran, 1996; Di Giusto et al., 2008), and this study reinforces these findings with data of a higher taxonomic resolution. It was evident that *N. rafflesiana* lower pitchers tend to trap more small ant prey, arachnid taxa, and large crawling hemipteran species (*Metochus* cf. *uniguttatus* [Rhyparochromidae] outside of the CCNR, and *Lisarda* spp. [Reduviidae] within the CCNR), while upper pitchers trap more large ant prey (mainly *Polyrhachis* spp. and *Dinomyrmex gigas*), cockroaches, lepidopterans, orthopterans, and coleopterans (Table 4; Fig. 8). Such a resource partitioning strategy may reduce the effect of intraspecific competition between plants of different ontogenetic stages, and furthermore allow plants to maximise prey capture in the microhabitat in which pitchers of each ontogenetic stage are most often found (i.e., in the undergrowth, for lower pitchers, and in the canopy, for upper pitchers).

Differences in composition of ant prey species between upper and lower pitchers may also reflect varying nesting preferences and foraging strategies among the ants, which influence the latter's probability of encountering either pitcher type. For example, ant species frequently occurring in lower pitchers are known to be ground-nesting and mostly epigeaic foragers (e.g., *Carebara* cf. *affinis* [see Moffett, 1988]; *Dolichoderus thoracicus* [see Way & Khoo, 1991]; *Pheidole aglae* [see Eguchi et al., 2004]). *Pheidole aglae*, in particular, has been observed to have a very limited foraging range of about 0.22 m from nest entrances in soil (Eguchi et al., 2004). It may be energetically too expensive for small, ground-dwelling ants to frequent relatively higher vegetation strata where upper pitchers are located, thus ant prey caught in upper pitchers tend to be larger solitary forager species such as *Diacamma* sp. and *Polyrhachis illaudata* (Table 4). The abundance of ant prey in upper pitchers may also be influenced by foraging strategy other than size, however, as is seen in the high local (per pitcher) abundance of *Carebara* cf. *affinis*, despite its few occurrences among upper pitchers (Table 5). This species derives from the now-invalid genus *Pheidologeton*, comprising ants which are known for their group-hunting or raiding behaviour (Moffett, 1988). These ants nest in the ground but build 'trunk trails' to foraging areas; discovery of food resources, even on higher vegetation, can stimulate swarm raids which originate from the trunk trails (Moffett, 1988). Another notable ant genus occurring frequently as prey among both upper and lower pitchers is *Philidris* (Table 4). Little is known about the biology of this genus, but some species have been observed to be closely associated with myrmecophytes, such as the epiphyte *Dischidia major* (Peeters & Wiwatwitaya, 2014). A similar mutualistic association may exist between *N. rafflesiana* and

Table 5. Mean number of each prey species trapped per pitcher in which the prey taxon was found. Columns **Outside CCNR** and **Within CCNR** display the means for pitchers found outside of or within the Central Catchment Nature Reserve (CCNR) only, respectively, while **Mean of all samples** and **SD of all samples** summarise this information for all pitchers, regardless of their locations. **Total number of observations** indicates the number of pitchers in which each prey taxon was found. Only taxa in which a mean of more than 2 prey individuals were trapped per pitcher are displayed. Unidentified taxa and plant material are also excluded from this list.

Taxon	Outside CCNR	Within CCNR	Mean of all samples	SD of all samples	Total number of observations
Pheidole singaporensis	–	648.00	648.00	–	1
Carebara* cf. *affinis	–	433.25	433.25	610.16	12
***Carebara* spp.**	453.67	65.50	298.40	539.06	5
Carebara diversa	315.00	6.00	160.50	218.50	2
***Plagiolepis* spp.**	185.00	5.00	131.00	376.08	10
Dolichoderus thoracicus	–	121.92	121.92	251.73	12
Pheidole aglae	5.33	38.43	32.59	75.19	17
Paratrechina longicornis	24.00	25.00	24.80	33.97	5
Nasutitermitinae	1.00	22.62	21.07	38.58	14
Crematogaster reticulata	18.00	–	18.00	9.90	2
***Philidris* sp.**	19.86	13.67	17.43	31.25	23
***Nylanderia* spp.**	21.00	7.25	16.00	17.39	11
Iridomyrmex anceps	–	14.00	14.00	–	1
***Tetramorium* sp.**	9.75	7.00	9.20	11.90	5
Mrymicinae spp.	–	8.50	8.50	11.12	4
***Pheidole* spp.**	1.00	9.33	7.25	8.10	4
***Crematogaster* spp.**	5.00	6.00	5.50	0.71	2
Tapinoma melanocephalum	5.50	–	5.50	7.68	4
Odontomachus rixosus	7.20	1.00	5.43	7.16	7
Other Ponerinae spp.	5.25	–	5.25	5.97	4
Pscoptera	–	5.00	5.00	–	1
Oecophylla smaragdina	4.50	–	4.50	3.54	2
Tetramorium* cf. *floricola	–	4.00	4.00	–	1
Ctenidae	–	3.00	3.00	–	1
Isopoda	–	3.00	3.00	–	1
Meranoplus malaysianus	3.00	–	3.00	2.77	7
***Polyrhachis saevissima* complex**	3.00	–	3.00	–	1
Polyrhachis illaudata	3.14	1.00	2.88	2.64	8
Moth	3.00	2.00	2.71	1.50	7
Elateridae	–	2.50	2.50	2.12	2
Mite	–	2.20	2.20	0.84	5
Unidentified Blattodea	1.50	2.21	2.13	1.45	16
Sciaridae	2.11	–	2.11	2.62	9

Philidris spp. (Lam et al., 2018), but there is no compelling evidence to prove this at the point of this study.

Differences in prey composition between lower and upper pitchers of *N. rafflesiana* may also be driven by interactions between prey taxa. Resource partitioning and competitive exclusion may occur between the different ant or termite taxa as they compete over the nectar resources of pitchers, and the outcome of such competition may furthermore be mediated by environmental factors such as pitcher location in vegetation strata, as discussed above. However, such competition over pitcher nectar resources is more likely to occur only with eusocial prey taxa, whose members may fall prey to pitchers in exchange for considerable supplies of the nectar secreted by pitchers (Joel, 1988; Merbach et al., 2001; Lam & Tan, 2018). The role of between-prey interactions in shaping pitcher prey assemblages is a promising area of future research.

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