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Plant diets of land snail community members are similar in composition but differ in richness

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ABSTRACT

Herbivore diets are often generalistic, and communities of herbivores tend to share much of their diets. In the tropical lowlands of Malaysian Borneo, tens of different noncarnivorous land snail species are able to coexist in communities on limestone outcrops. We tried to answer the question whether diet differentiation plays a role in their coexistence. We show, with a large metabarcoding study of the plant diet from gut contents of 658 individual snails (from 26 species, with a focus on three of the most common species in the region), that the different snail species indeed share much of their plant diet, but that mean diet richness varies strongly among species (up to 15.3×). These differences are mostly explained by snail size, with larger snails having wider diets. Furthermore, phylogenetic analyses of the plant diet by individual snails showed signs of clustering in *c.* 28% of the individuals, possibly suggesting phylogenetic specialization, although such clustering was weak when diets were considered by species. We discuss how observed trends in diet richness and diet clustering could also be explained by random feeding, with larger species simply eating more or less specifically, and by other, noncompetitive interactions, such as snails avoiding desiccation. Our study shows how to efficiently put the power of metabarcoding to work in unravelling the complex community processes commonly encountered in tropical ecosystems and is thus of substantial relevance to both community ecologists and conservationists.

INTRODUCTION

Herbivore communities often consist of many different species feeding on roughly the same generalist diet (Belovsky, 1986; Gordon & Illius, 1989). Such ecological communities have been a popular study system for a long time (Schoener, 1974), probably because some of the most important species interactions, such as competition and facilitation, take place within herbivore communities (Schoener, 1983; Stachowicz, 2001;

Bruno, Stachowicz & Bertness, 2003). However, the very existence of such trophically similar communities seems paradoxical (Behmer & Joern, 2008). On the one hand, trophically similar species have a tendency to cluster together because they have very similar needs (Leibold & McPeck, 2006); this lies at the very heart of the definition of the community. On the other hand, it is generally accepted that species that are too similar cannot coexist indefinitely due to too strong competition, as formalized in the ‘competitive exclusion principle’ or Gause’s law (Gause, 1934; Hardin, 1960).

The assembly of communities of trophically similar species (‘guilds’) has been described by the unified neutral theory of biodiversity (UNTB; Hubbell, 2001; Rosindell, Hubbell &

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Etienne, 2011). The central axiom of the UNTB is that different species in the community are functionally equivalent (Alonso, Etienne & McKane, 2006). This is in stark contrast with niche theory, which highlights the necessity of functional differences in coexisting species (Elton, 1927). Various publications described efforts to unify both theories in the form of so-called emergent neutrality (DeSantis *et al.*, 2006; Segura *et al.*, 2011; Scheffer, van Nes & Vergnon, 2018; D'Andrea, Gibbs & O'Dwyer, 2020). This could arise when underlying weak stabilizing forces (on the species) and strong equalizing forces (increasing species similarity) work interactively. This 'near-neutrality' would most likely be possible in species-rich communities (Holt, 2006). Behmer & Joern (2008) showed experimentally how dietary and nutritional differences in coexisting herbivorous species, eating the same plant taxa, can be extremely cryptic and thus easily overlooked, again leaving a seemingly neutral footprint. And there are other subtle ways for species to coexist on the same resources, as shown long ago by Hutchinson's seminal work on the 'paradox of the plankton' (Hutchinson, 1961), with co-occurring species differentiating in other niche dimensions than just food or nutrients.

Here, we ask how it is possible that tens of different land snail species can coexist within a community and whether there is a role played by diet differentiation among species. Communities of large mammalian herbivores have been studied thoroughly in this respect, and from these studies it is known that sympatric species can coexist due to (subtle) differences in morphology (e.g. digestive system and body size) and spatial and seasonal differences in their diets (Prins & Olff, 1998; Kartzinel *et al.*, 2015). Similar studies on invertebrates are far less numerous (but see McClenaghan *et al.*, 2015). We studied seemingly neutral land snail communities living on limestone outcrops in the tropical lowland of Sabah, Malaysian Borneo (Schilthuizen, 2011; Hendriks *et al.*, 2019a), and applied metabarcoding of the gut contents of 658 individuals from 26 species, with a focus on three regionally abundant species, to reconstruct both individual- and species-level plant diets (Pompanon *et al.*, 2012; Taberlet *et al.*, 2012; Kartzinel *et al.*, 2015). Based on the observation by Schilthuizen (2011) of the seeming neutrality of these communities, together with an apparent excess of available food, we expected competition for food to be very low or absent. However, these communities are characterized by two striking features that might not be in line with neutrality requirements and we therefore studied these in relation to the diet. First, communities were composed of representatives from three main taxonomic clades (Neritimorpha, Caenogastropoda and Stylommatophora), with a roughly 50:50 proportion of Stylommatophora and non-Stylommatophora (Schilthuizen, 2011). These taxonomic clades are considered ecologically distinct (and functionally different), with Stylommatophora being better dispersers and better adapted to drought and other extreme conditions (Schilthuizen *et al.*, 2002; Schilthuizen, Cabanban & Haase, 2005), which was expected to leave its mark on the diet. Not much is known about the ecology of the Neritimorpha in the region, but their preference for humid limestone (Khalik *et al.*, 2018, 2019) suggests ecological differentiation from at least the Stylommatophora. Second, the range of sizes of sympatric snail species is enormous, covering five orders of magnitude (based on adult shell volume). Furthermore, because diet is first of all dependent on the local availability of food, we also took into account the influence of the location. With this we also want to highlight that most snail species consume both living and dead plant material, which makes them both herbivores and detritivores (Williamson & Cameron, 1976; Speiser & Rowell-Rahier, 1991; Hägele & Rahier, 2001). For correctness, we will refer to the snail community as a plant eater community instead of a herbivore community, but the general idea that food is not limited, resulting in low competition for food, remains the same. Finally, we considered different levels of phylogenetic clustering of the plant diet, corresponding to different plant taxonomic ranks (but without explicit

definition of the rank). Different studies have shown that herbivore–resource interactions can occur at either a low rank (even at the level of genotypic variation; Barbour *et al.*, 2015) or a higher rank (Symons & Beccaloni, 1999).

The snail diet data in our research have already been used in a previous study (Hendriks *et al.*, 2020); hence, the methods sections of both studies are alike. The former manuscript (Hendriks *et al.*, 2020) dealt with the three-way interaction between snail community diversity, their diet and bacterial communities, in combination with environmental variables (such as anthropogenic influences). One of the main findings regarding diet was an absent or weak correlation between snail community diversity and diet. In the current study, we further explore the diet data to investigate the potential role of diet differentiation for species coexistence.

MATERIAL AND METHODS

Study system and sampling

We studied species-rich communities of noncarnivorous land snails on six limestone outcrops in the Lower Kinabatangan Floodplain in Sabah, Malaysian Borneo (Fig. 1; Supplementary Material Table S1). Many community members are known to have a strong preference for calcium carbonate, with the outcrops effectively representing habitat islands for these snails (Schilthuizen *et al.*, 2003), meaning that communities can be defined by such outcrops (from here on referred to as 'locations'). Although on the scale of an entire rock face, the dozens of species that we report on here are often found interspersed, all foraging simultaneously among the microvegetation of the rock face, some species-specific preferences may be discerned. These have been reported upon, albeit anecdotally, by previous workers (Berry, 1961; Schilthuizen, Vermeulen & Davison, 1999). These preferences manifest themselves in clustering at the scale of decimetres or metres at the most. For example, Vertiginidae and certain *Diplommatina* (Diplommatinidae) are often found on dry parts of the rock with sparse lichen coverage, whereas *Plectostoma* (Diplommatinidae) prefer wetter, mossy parts. *Acmella* (Assimineidae), finally, appear to dwell in crevices more than other taxa. Mean location community species richness was 30.3 (range: 24–38; based on data from Hendriks *et al.*, 2020). Snail samples were collected from three plots of 2 m × 2 m per location, with an interplot distance of at least 50 m, measured along the base of the outcrop (Hendriks *et al.*, 2019b). All samples were collected within 1 week to reduce possible seasonal effects. Although we sampled diet data from 26 species, we focused on three regionally abundant target species (not closely related to one another): *Alycaeus jagori* Von Martens, 1859, *Georissa similis* E. A. Smith, 1893 *sensu lato* (in fact a complex of recently described, closely related taxa; Hendriks *et al.*, 2019a; Khalik *et al.*, 2019) and *Plectostoma concinnum* (Fulton, 1901). We aimed to collect 40 individuals/target species/plot (20 individuals for further analyses, plus 20 more as backup and collection vouchers), searching each of four plot quadrants for 30 min. In addition, we collected all other snail species encountered during this search (usually present only in small numbers), with a maximum of 20 individuals/species/plot. Samples were preserved in 98% ethanol and frozen directly in the field, and registered in the BORNEENSIS collection of Universiti Malaysia Sabah, Malaysia (Supplementary Material Table S2). All samples were collected [permit nos JKM/MBS.1000-2/2 JLD.6 (107, 112, 114, 116 and 118)] and exported [permit no. JKM/MBS.1000-2/3 JLD.3 (51)] under permits issued by the Sabah Biodiversity Council, Malaysia.

Trait data were collected from various literature sources on snail taxonomy (Vermeulen, 1991; Vermeulen, Liew & Schilthuizen, 2015; Liew, 2019a, b). For each species, we scored maximum shell height and width, and used these to calculate shell volume (as $\frac{1}{3} \times \text{height} \times \text{width}^2$) as a proxy for snail size (Supplementary Material Table S3). In addition, we calculated species abundances

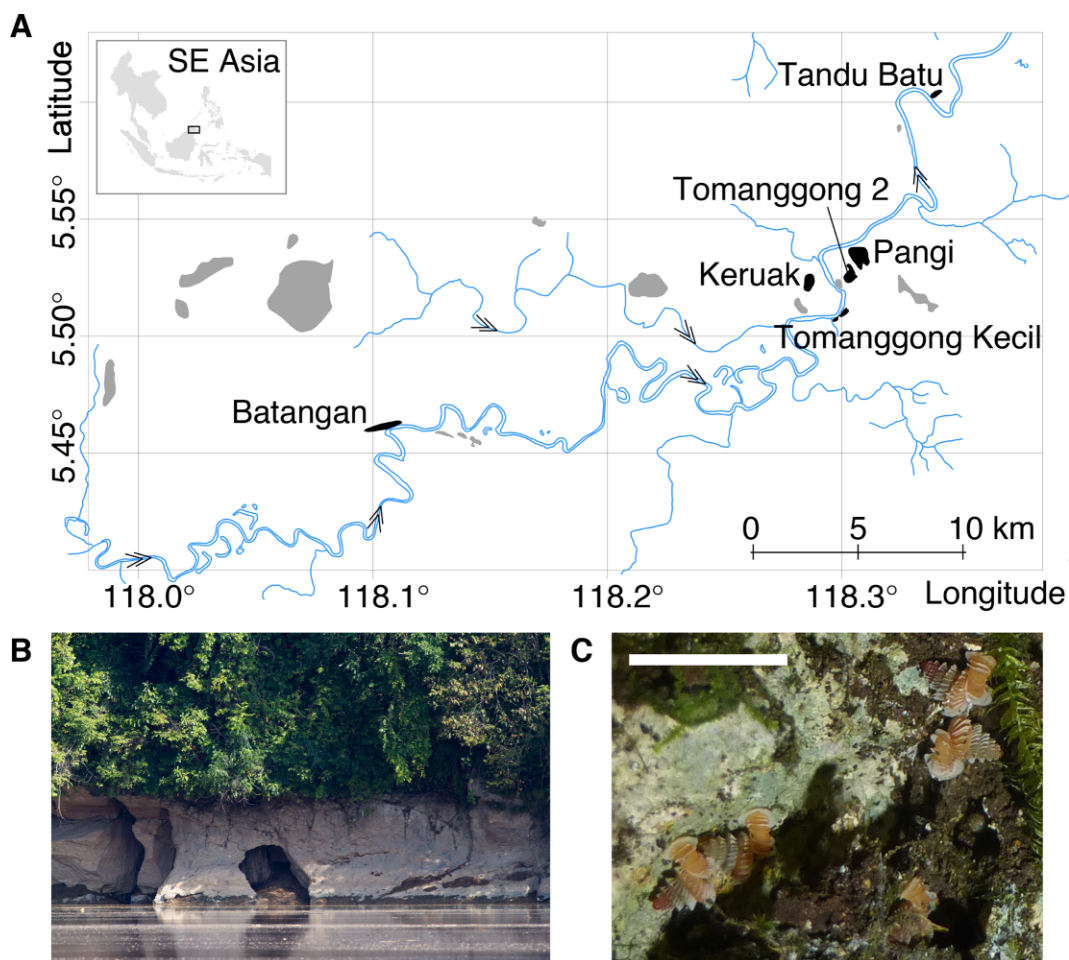


Figure 1. **A.** Map of limestone outcrops in the Lower Kinabatangan Floodplain, Sabah, Malaysian Borneo. The six outcrops in black were sampled for this study. The black square in the inset in the left corner highlights the region of Borneo where we sampled. **B.** Batangan, one of the outcrops visited for sampling, as seen from the river (March 2015). **C.** *Plectostoma concinnum*, one of the target species, feeding on limestone rock (Tandu Batu, April 2016). Scale bar = *c.* 5 mm. Images credits: Kasper P. Hendriks.

from the census data reported by [Hendriks et al. \(2020\)](#) (Supplemental Material Table S4).

Metabarcoding library preparation

The process of obtaining metabarcoding plant diet data from the snail gut was described in detail by [Hendriks et al. \(2020\)](#) and is only summarized here. Using Omega's E.Z.N.A.® Mollusc DNA Kit, we extracted genomic DNA from the gut contents of each snail or from the whole snail after removing the shell (i.e. when snails were too small to extract the gut; e.g. *G. similis s. l.*). Gut preparations were done in a Petri dish filled with 70% ethanol to prevent outside contamination; to prevent cross-contamination, both dishes and tools were sterilized in between preparations. Library preparation involved two amplification steps. First, the 110-bp chloroplast *rbcL* region was amplified in 40 PCR cycles using the primer pair Z1f/19bR ([Hofreiter et al., 2000](#)) with Illumina adapter overhang to the primers. Successfully amplified products (i.e. showing a band of target product on a gel) were purified with 0.9× NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel). Second, we ran another ten PCR cycles to add sample-specific multiplex Nextera XT indexes by which samples could be demultiplexed during subsequent bioinformatics. We measured concentrations for each sample using the QIAxcel Advanced System with QIAxcel DNA Screening Kit (Qiagen). We equimolarly normalized and pooled samples

into three pools of *c.* 280 samples each (including three negative controls) using a QIAgility robot (Qiagen), and checked the quality and concentration of the final pools on a Bioanalyzer using a high-sensitivity chip (Agilent). The pools were sequenced paired-end on a MiSeq PE 300bp by BaseClear, Leiden, the Netherlands, using 50% PhiX Control v3 to reduce effects of low library diversity.

Metabarcoding bioinformatics

We combined raw sequence data (fastq files) from the three sequencing pools and used QIIME 2 v. 2017.12 ([Bolyen et al., 2019](#)), with the DADA2 philosophy and routine ([Callahan et al., 2016](#)), to denoise, apply quality control and export representative amplicon sequencing variants (ASVs). Taxonomic assignment of ASVs (to the lowest rank with a confidence value >0.70) was performed by blasting data against our custom-built classifier, based on the seed plant (Spermatophyta) *rbcL* database from [Bell, Loeffler & Brosi \(2017\)](#). Nonplant ASVs and ASVs that could not be aligned were removed. We aligned the remaining 778 true plant ASVs using MAFFT v. 1.3.5 ([Katoh & Standley, 2013](#)) and built a maximum likelihood plant diet phylogeny using FastTree v. 1.0 ([Price, Dehal & Arkin, 2009](#)), with default settings, in Geneious v. 9.1.6 (<https://www.geneious.com>).

Table 1. Sample sizes by higher-order classification, snail species and location.

Classification	Taxon	Batangan	Keruak	Pangi	Tandu Batu	Tomanggong 2	Tomanggong Kecil	Total
Neritimorpha	<i>Georissa kinabatanganensis</i> Khalik, Hendriks, Vermeulen & Schilthuizen, 2018		8					8
	<i>Georissa similis</i> E. A. Smith, 1894 s. l.	3	26	25	6	20	25	105
Caenogastropoda	<i>Sulfurina martensi</i> (Issel, 1874)	9	2				1	12
	<i>Acmella cyrtoglyphe</i> Vermeulen, Liew & Schilthuizen, 2015		3				3	
	<i>Acmella striata</i> Vermeulen, Liew & Schilthuizen, 2015				7			7
	<i>Alycaeus jagori</i> Von Martens, 1859		1	56	39	21	43	160
	<i>Chamalycaeus</i> sp.			1			1	2
	<i>Diplommatina calvula</i> Vermeulen, 1993			3				3
	<i>Diplommatina gomantongensis</i> (E. A. Smith, 1894)			1				1
	<i>Diplommatina rubicunda</i> (Von Martens, 1864)			1		1	4	6
	<i>Japonia kinabaluensis</i> (E. A. Smith, 1895)	3						3
	<i>Japonia</i> sp.	3	2					5
	<i>Leptopoma pellucidum</i> (Grateloup, 1840)				1			1
	<i>Leptopoma sericatum</i> (Pfeiffer, 1851)	12	1	1	1			15
	<i>Plectostoma concinnum</i> (Fulton, 1901)	45	70	36	34	45	53	283
	<i>Plectostoma simplex</i> (Fulton, 1901)				13			13
	<i>Pterocyclos</i> sp.	1			2		5	8
Stylommatophora	<i>Everettia</i> sp.						2	2
	<i>Kaliella accepta</i> (Smith, 1895)		3	1		1	1	6
	<i>Kaliella barrakporensis</i> (Pfeiffer, 1852)					2		2
	<i>Kaliella calculosa</i> (Gould, 1852)					1		1
	<i>Kaliella scandens</i> (Cox, 1872)		1			2		3
	<i>Microcystina appendiculata</i> (Von Moellendorff, 1893)						1	1
	<i>Macrochlamys tersa</i> (Issel, 1874)				1	1	2	4
	<i>Videna metcalfei</i> (Pfeiffer, 1845)	2	1					3
	<i>Videna</i> sp.						1	1
	Total		78	120	123	104	94	139

Grey shading indicates the three target species for which sample sizes are especially high because of targeted sampling. For sample sizes by plot, see Supplementary Material Table S5. Note that for analyses of individual samples, those samples with <150 raw reads were removed, leaving a total of 554 samples.

Statistical analyses

Metabarcoding data (ASV table, taxonomic assignments and meta-data) and trait data were imported into R v. 3.6.0 (R Core Team, 2018) and combined using the R package phyloseq v. 1.24.2 (McMurdie & Holmes, 2013). Upon inspection, 40 out of 778 true plant ASVs were found in one or more negative controls and considered to possibly originate from contamination; these were therefore removed from all samples before downstream analyses, with associated tip labels removed from the plant diet phylogeny.

We first created an overview of all the plant families found in the total dataset ($n_{\text{samples}} = 658$). For each individual, we scored the occurrence of plant diet families and calculated percentages of occurrence by target species and location for each plant diet family. Data for all nontarget species were pooled because sample sizes for nontarget species were low (ranging from 1 to 15; Table 1).

In the analyses described below, all ASVs identified to originate from seed plants were included in the analyses, including ASVs for which explicit identification down to plant family level was not possible. We kept these data because we were primarily interested in genetic variation within the diet and not necessarily the taxonomy

of the plants. To exclude the effects of poor sequencing libraries, 104 samples with less than 150 raw reads (selected as the lower threshold based on rarefaction curves of individual diets) to represent their diets were removed prior to analyses at the level of the individual. In analyses where data were pooled by species, location or plot, all 658 samples were included. We used the Chao1 estimator (to account for different sample sizes, i.e. different numbers of reads per individual snail sample from metabarcoding; Chao, 1984) to calculate plant diet richness (counted as the number of ASVs) for each individual snail; we sorted the results by species and, for target species, by location. We pairwise tested target species diet richness differences using a Wilcoxon signed rank test with Bonferroni correction for pairwise testing. Subsequently, using generalized linear mixed modelling (GLMM) with the R package glmmTMB v. 0.2.3 (Brooks *et al.*, 2017), we tested for the correlation between the response variable ‘mean Chao1 diet richness’ (per species) and ‘shell volume’ (used as a proxy for snail size; see above), with ‘location’ as the random effect variable. In addition to this basic model, we ran models including also the fixed variable ‘classification’ (with options Neritimorpha, Caenogastropoda and Stylommatophora). We

modelled mean Chao1 diet richness (being count data) using a log link function and performed model selection [based on the Akaike information criterion (AIC)] from the full model (which included interactions between shell volume and classification). In an alternative GLMM, we tested for correlation between mean Chao1 diet richness with regional ‘species abundance’ (where we used regional census data from Hendriks *et al.* 2020; Supplementary Material Table S4) instead of shell volume. This served as a proxy for ‘regional success’ of the species (shell volume and abundance were themselves not significantly correlated; linear model results: $R^2 = 0.052$, $P = 0.282$). Both models were rerun with response variable data not represented by the Chao1 estimate of the original ASVs but by Chao1 estimates of richness from two agglomerated versions of the plant diet phylogeny; in the latter two analyses, all ASV-tip label taxa with a tip cophenetic distance smaller than 0.05 and 0.10, respectively, were pooled together (Supplementary Material Fig. S1). These datasets represent deeper plant diet phylogenetic ranks without simply resorting to plant taxonomic ranks, such as family, which in different clades might have a different phylogenetic meaning (Symons & Beccaloni, 1999).

To study the possibility of species and/or individual preferences for specific plant taxa, we tested for phylogenetic nonrandomness in the diet, applying theory from community ecology (Webb *et al.*, 2002). At both the individual and species levels, we calculated standardized effect sizes (SEs) for mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD), using the R package *picante* v. 1.8 (Kembel *et al.*, 2010), with ‘taxa’ defined by plant diet phylogeny tip labels and tip swapping as the null model. In this sense, negative SES values indicate phylogenetic diet clustering (i.e. a possible sign of phylogenetic specialization with a diet consisting of specific taxonomic plant diet clusters); positive values indicate overdispersion (here diets are more phylogenetically diverse, e.g. to gather diverse nutrients, or spread the intake of plant-specific metabolites) and values around zero indicate randomness (no phylogenetic specialization). While MNTD describes diversification among recently diverged taxa/lineages, MPD does so for deeper relationships (Mazel *et al.*, 2016). As before, we ran these analyses not only with the original plant diet but also with the agglomerated versions of the phylogeny because we expected phylogenetic diet clustering could be sensitive to the taxonomic rank of the diet items (Symons & Beccaloni, 1999; Barbour *et al.*, 2015). We first calculated SES values for data pooled by species for the whole region (for the three target species plus a bin group for all nontarget species) to represent the ‘species diet’. Subsequently, we calculated values by individual (including individuals from all species) and applied one-sample *t*-tests to confirm mean values being different from zero. Because individual snails can only eat what is available around them, SES-MPD and SES-MNTD analyses by individual were carried out on datasets pruned to the location (i.e. all diet taxa from outside the individual’s location were removed first). We used an ANOVA to model the influence on SES results of the explanatory variables ‘target species’ (including a pooled group of nontarget species), ‘location’ and ‘agglomeration’. We performed model selection based on AIC and used the R package *relaimp* v. 2.2-3 (Grömping, 2006) to calculate the relative importance of the explanatory variables, using metric LMG (partitioned R^2 values, as suggested by—and named after—Lindeman, Merenda & Gold, 1980).

We studied target species and location diet differences as both Sørensen and unweighted UniFrac distances (with the latter taking diet phylogenetic distances into account), using nonmetric multidimensional scaling (NMDS) and permutational analysis of variance (PERMANOVA; Anderson, 2017); analyses were done using the R package *vegan* v. 2.5-5 (Oksanen *et al.*, 2017). A Sørensen distance of 0 meant two samples with exactly the same diet, while 1 meant no shared diet items. Similarly, a UniFrac distance of 0 indicated exactly the same diet and thus that all branches from the diet phylogeny are shared, while 1 meant that no branches are shared.

To keep PERMANOVA possible, because it is computationally demanding on large datasets, we pooled data by target species–plot combinations. We studied the explanatory variables ‘target species’ and ‘location’, and used 4,999 permutations. To test for possible differences in variance among groups, we ran BETADISPER on the same data with the same number of permutations.

Data accessibility: All sample vouchers were deposited in the BORNEENSIS collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia. DNA extracts are stored at $-80\text{ }^{\circ}\text{C}$ at the Naturalis Biodiversity Center, Leiden, the Netherlands, for future reference. Museum IDs and location details for samples are given in Supplementary Material Table S2. Metabarcoding data were deposited to the NCBI GenBank Sequence Read Archive database as project PRJNA530120 and can be retrieved from <https://www.ncbi.nlm.nih.gov/sra/PRJNA530120>. A complete R project zip file with raw data and scripts to recreate all models presented in this paper, as well as figures and tables, has been deposited on Figshare (<https://doi.org/10.6084/m9.figshare.16917760.v1>).

RESULTS

We collected metabarcoding plant diet data for 658 individual snail samples (554 after removal of 104 samples with <150 raw reads per sample), distributed over 26 snail taxa and covering each of three main higher-level taxonomic groups present in the region, that is the Neritimorpha (3 species), Caenogastropoda (14 species) and Stylommatophora (9 species) (Table 1). As anticipated, we found the highest sample sizes for the three target species: *Alycaeus jagori* (160 samples), *Georissa similis s. l.* (105 samples) and *Plectostoma concinnum* (283 samples), with sample sizes for the nontarget species ranging from 1 to 15.

A generalist diet

Combining all rbcL read data (ASVs that could be identified to originate from seed plants) from all individual snails, 65.4%, representing 32 plant families, could be identified with $>70\%$ confidence to family rank (Fig. 2). In total, we found 17 plant families from the diets shared in all the target species and 11 plant families in all six locations. The top-five was made up of large, well-known plant families, such as Fabaceae (bean family), Asteraceae (composite family), Brassicaceae (mustard family) and Moraceae (fig family), each present in 15–50% of individuals. In contrast, some plant families were just found in a single target species/location and then always in only one or two individuals ($<1\%$).

Diet richness depends on snail size

Chao1 plant diet richness (for the original dataset with plant taxa represented by ASVs) varied strongly among individuals and species. The diet richness of the target species by location showed a consistent order between the three species, with the largest species (*A. jagori*) having the widest diet and the smallest species (*G. similis s. l.*) the narrowest (Fig. 3A; differences were often significant based on a Wilcoxon signed rank test and after Bonferroni correction for pairwise testing). At the regional scale, mean plant diet richness by species differed by up to $15.3\times$ (based on ASV; for the 0.05 and 0.10 agglomerated datasets, differences were still $10.3\times$ and $9.0\times$, respectively). Differences among the three target species were highly significant at the regional scale (Fig. 3B). The basic GLMM model (including all species) showed that mean Chao1 diet richness (best fitted by a lognormal distribution) and shell volume are significantly and positively correlated (Fig. 3C and Supplementary Material Table S6). Agglomerated datasets showed a more

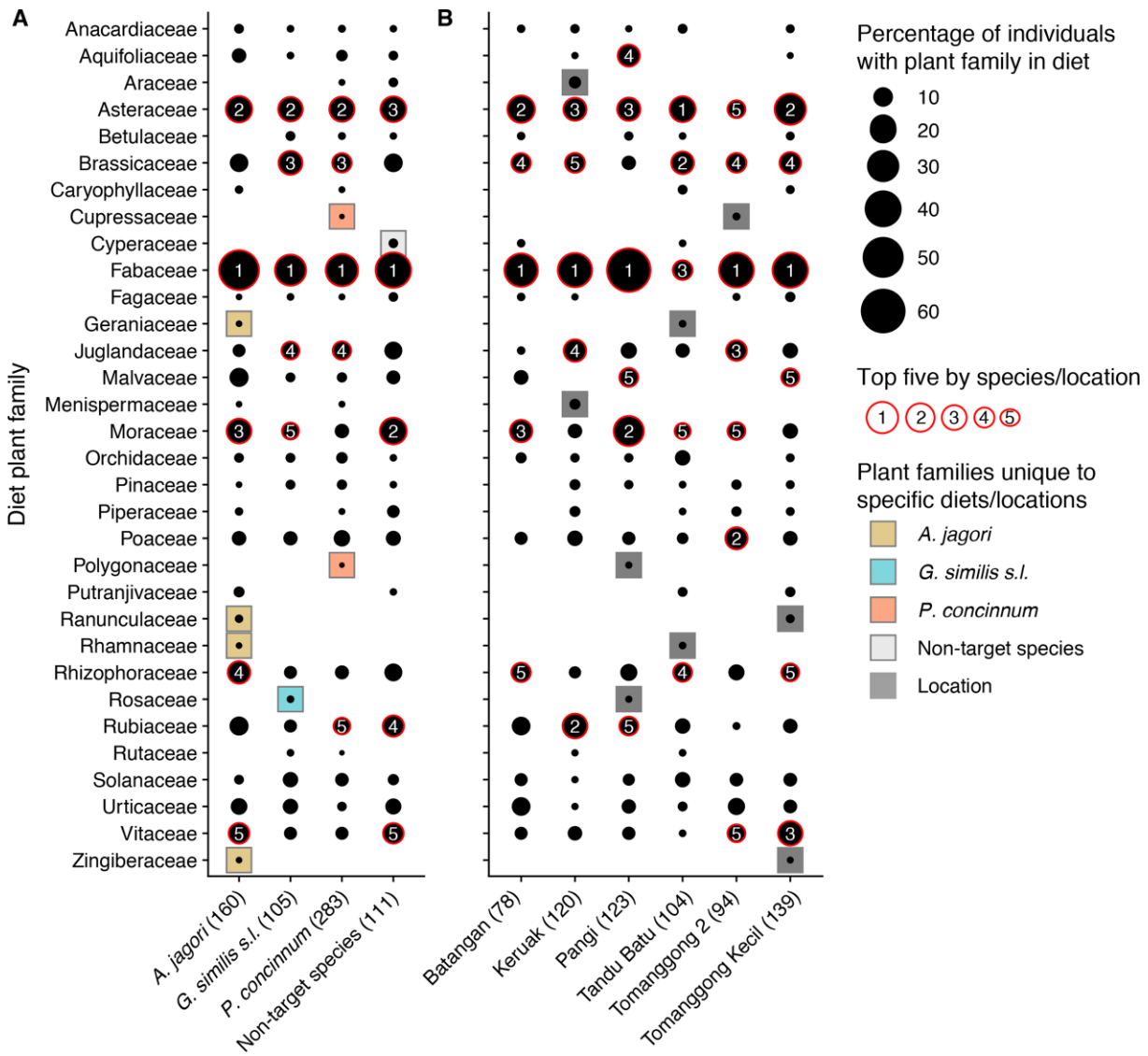


Figure 2. Plant families found in the snail diet, after combining data from all samples per target species and location. **A.** Sorted by target species. **B.** Sorted by location. Sample sizes (i.e. number of individual snails) per species and location are given within parentheses. Dot area scales with fraction of individuals per species and location in which a plant family was found. Additional red outlines and a number highlight the top five most eaten plant families per species and location; ties for position 5 occurred in three locations. Coloured squares highlight plant families eaten by a single species or found from a single location only. Reads that could be assigned to the seed plants (Spermatophyta), but not to plant family rank (i.e. ‘unassigned’), were included in further analyses, but excluded from this graph.

nanced trend, where Chao1 diet richness correlated significantly and positively with shell volume, but the best models highlighted different trends between the three classification groups, with Caenogastropoda the only group in which this trend is present (Supplementary Material Tables S7, S8).

Basic GLMM models (i.e. excluding the effect of ‘classification’) showed that mean Chao1 diet richness and species abundance are significantly and negatively correlated (Supplementary Material Tables S7, S8). However, the best models (based on AIC) did not include the effect of ‘abundance’ and included the effect of fixed variable ‘classification’, but trends in these best models were not significant. Variation in diet richness was thus not well explained by species abundance.

Signs of diet clustering

SES values for the MPD and MNTD by species were significantly negative for *G. similis s. l.*, a possible sign of phylogenetic

specialization in this species (Supplementary Material Table S9, but see the ‘Discussion’ section; clustering seems to be towards the more recent plant taxa, see Supplementary Material Fig. S1). SES-MNTD values were significantly negative for the other target species, too, indicating overall clustering of recent plant taxa. No such clustering was detected in the agglomerated datasets, except in the SES-MPD one for *G. similis s. l.* (Supplementary Material Table S9).

The analyses of SES-MPD and SES-MNTD by individual (instead of by species) showed for many individuals (27.7% in SES-MPD and 28.9% in SES-MNTD) significant negative SES values, indicating diet clustering by individual (Fig. 4A). Furthermore, mean values (from all individuals) were negative and significantly different from zero, as indicated by the one-sample *t*-test (MPD: $t = -10.3$, $P < 0.001$; MNTD: $t = -14.2$, $P < 0.001$; for the results of the agglomerated datasets, see Supplementary Material Fig. S2). Thus, these results show an overall trend towards plant diet

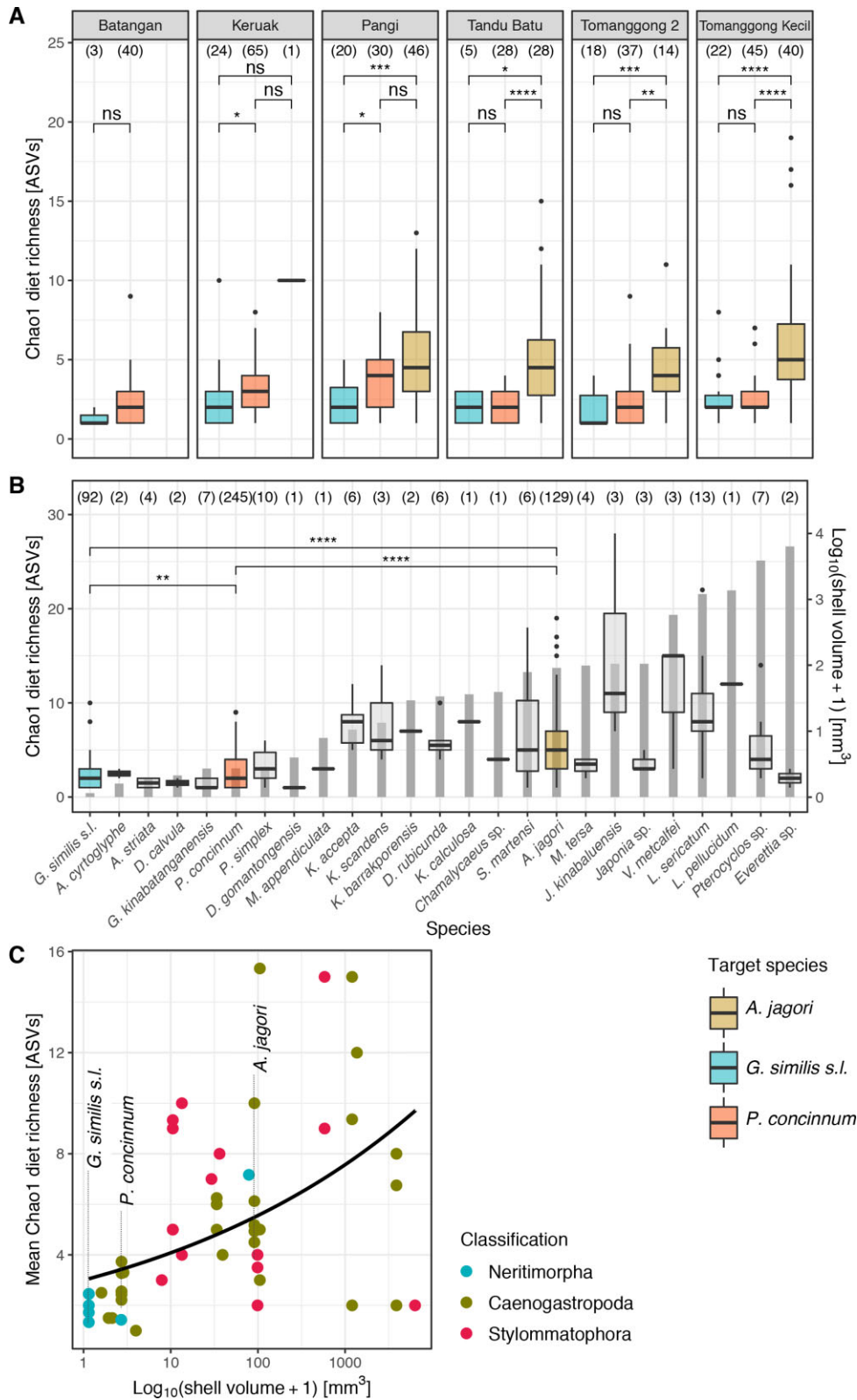


Figure 3. A. Chao1 plant diet richness (original data at ASV level) by individual snail, with results sorted by target species and location and sample sizes in parentheses. Wilcoxon signed rank test results, with Bonferroni correction for pairwise testing, are given above boxplots, with P -values defined as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. **B.** Same data as in panel (A), but results are for whole region combined and for all species in our study. Additionally, grey bars indicate shell volume (as proxy for snail size), with values printed on secondary y -axis. **C.** Mean Chao1 diet richness per species and location (same data as in panel B) as a function of ‘shell volume’ and snail ‘classification’. The solid black line represents a prediction from the best GLMM model with ‘location’ as random effect variable. All results in these graphs based on the dataset from which samples with poor data (number of raw reads <150) were removed first.

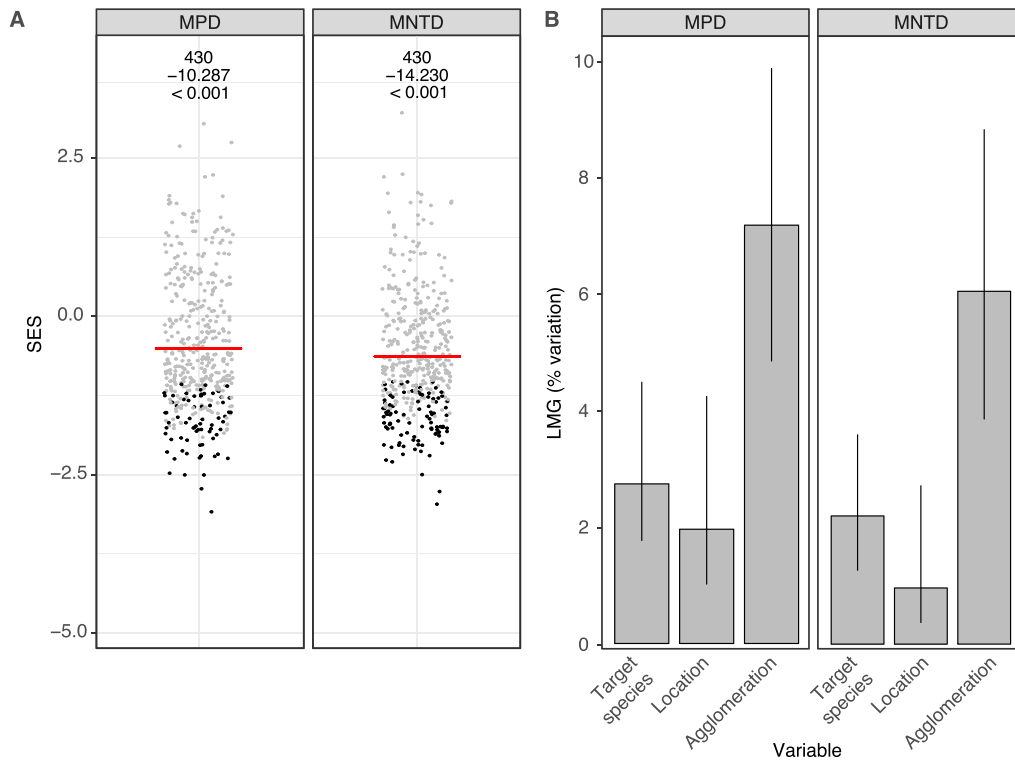


Figure 4. A. Jitter plots of SES for MPD and MNTD for all individual snail samples (each represented by a dot). Significant individual SES results in black ($P < 0.05$); nonsignificant results in grey. Red horizontal lines show the mean values for the jitter group (with data from both significant and nonsignificant individual results included). Top labels show P -values for the difference of the mean from zero, based on a single-sample t -test. Thus, while there is a large spread in individual SES values, there is an overall trend of individual diet clustering. **B.** Relative importance of the explanatory variables tested against SES values using ANOVA (Supplementary Material Table S9). Vertical bars indicate 95% confidence intervals from 1,000 bootstraps.

clustering and individual phylogenetic specialization (cf. Keck & Kahlert, 2019; Kartzinel & Pringle, 2020), and this differs markedly from the species-level results reported in Supplementary Material Table S9. ANOVA models for response variables SES-MPD and SES-MNTD showed that each of the three explanatory variables, ‘target species’, ‘location’ and ‘agglomeration’, explained part of the variance and that the interaction between the first two was significant (Supplementary Material Figs S2, S3; Supplementary Material Tables S10, S11). LMG relative importance values showed that the agglomeration explained most of the variation, followed by the target species and the location, for both SES-MPD and SES-MNTD (Fig. 4B).

Signs of diet differentiation

Target species diets could not be reliably segregated in NMDS analyses (pooled by target species and plot) based on Sørensen distance (stress = 0.35), while this seemed at least partly possible based on unweighted UniFrac distance (stress = 0.20; a stress value larger than 0.20 is considered to indicate that a safe distinction between groups is lacking). Most notably, the UniFrac diet distances between *A. jagori* and *G. similis s. l.* showed little overlap (Fig. 5). PERMANOVA results for these data show that only ‘location’ explains part of the variation and that this is the case only when analyses are based on the UniFrac distance ($P = 0.019$; BETADISPER: $P = 0.535$; i.e. no significant differences in dispersion among groups; Table 2).

DISCUSSION

We studied species-rich land snail communities with an abundance distribution being well described by the UNTB

(Schilthuizen, 2011). Together with a seemingly excess food availability, this led us to expect interspecific competition for food to be very low or absent. Schilthuizen (2011), while having noted the apparent neutrality of these snail communities, hinted at difficulties in interpretation. Neutrality within communities assumes functional equivalence of its members and that all members belong to the same trophic guild (Rosindell et al., 2011). However, these snail communities appear not to comply with these assumptions: they contain members from several widely different taxonomic groups, each with their own ecological characteristics, and adult snail size (based on shell volume) covers five orders of magnitude. Here, we show the following: (1) differences in mean plant diet richness among species are large, varying up to 15.3 \times ; (2) there is a significant positive correlation between snail size and diet richness; and (iii) there are weak signs of individual diet clustering, with significant phylogenetic clustering in the diet in $c.$ 28% of all individuals (from SES values of MPD and MNTD), suggesting some level of phylogenetic specialization (but see below), although this trend is weak when the diet is assessed by species.

We found the plant diets of snails to be wide, generalistic and generally overlapping between the three target species, with all species eating roughly from the same 32 plant families. Wide herbivore diets were explained by Freeland & Janzen (1974) as possibly originating from inflation, as a result of individuals trying to reduce the intake of single plant-specific toxic metabolites, but this does not explain the widely varying (up to 15.3 \times in plant taxa from ASVs) difference in mean diet richness among the 26 snail species in our study. We found a significant positive correlation between mean diet richness (by species) and snail size (with ‘location’ included as a random effect variable). Is this proof of non-neutrality? Probably not, because it is also possible that larger snails simply eat more

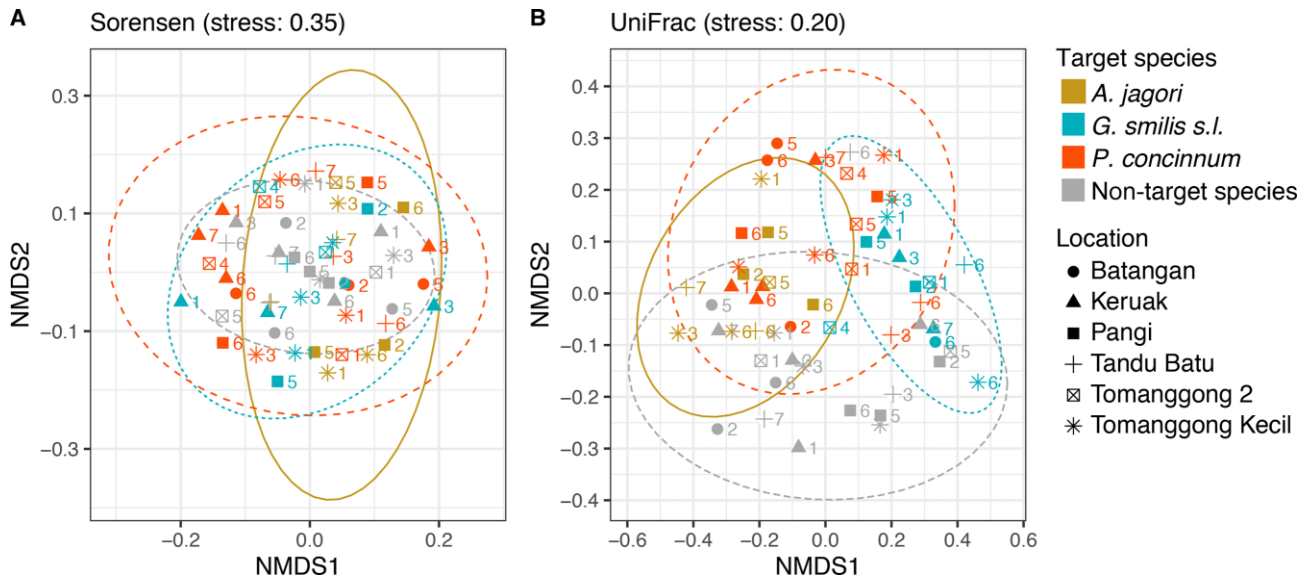


Figure 5. NMDS of plant diet. **A.** Plot based on Sørensen distance. **B.** Plot based on UniFrac distance. Each sample point represents pooled diet taxa data (ASVs) from all samples of a target species from a plot. Stress levels >0.20 are generally considered to indicate no safe distinction between groups. Numbers refer to plots within locations, as shown in Supplementary Material Table S1.

Table 2. PERMANOVA and BETADISPER results from models explaining variation in Sørensen and unweighted UniFrac distances based on ‘target species’ and ‘location’.

Response	Explanatory variable	df	SS	PERMANOVA			BETADISPER		
				Pseudo- <i>F</i>	<i>R</i> ²	Pr (> <i>F</i>)	SS	Pseudo- <i>F</i>	Pr (> <i>F</i>)
Sørensen distance	Target species	3	-0.041	-1.025	-0.085	0.995	0.018	2.295	0.086
	Location	5	-0.132	-1.961	-0.271	1.000	0.007	0.361	0.877
	Target species × location	13	0.189	1.083	0.389	0.441			
	Residuals	35	0.470		0.967				
	Totals	56	0.486		1.000				
UniFrac distance	Target species	3	2.069	3.143	0.148	<0.001	0.032	5.170	0.003
	Location	5	1.500	1.367	0.107	0.019	0.011	0.821	0.535
	Target species × location	13	2.727	0.956	0.195	0.664			
	Residuals	35	7.679		0.549				
	Totals	56	13.975		1.000				

Sample data are based on pooled diet taxa data (ASVs) from all samples of a target species from a plot. Importantly, a significant PERMANOVA result is only informative in combination with a nonsignificant BETADISPER result because the variation among groups could otherwise still be explained solely by differences in group dispersions. Hence, only combinations of significant results for PERMANOVA and nonsignificant results for BETADISPER are in bold.

(because of larger mouthparts and digestive systems) and are able to move faster (in an absolute sense), thereby covering more ground (and plants), or that larger species unintentionally eat plants growing close to or epiphytically on their preferred diet plants. While such a direct size–diet richness correlation was rejected for large mammalian herbivores (Mysterud, 2000; Kartzinel *et al.*, 2015), we do not know of studies testing this simple correlation in snails or other invertebrates.

Our SES results from phylogenetic diet clustering in individual snails (for all three plant taxonomic levels studied and for both MPD- and MNTD-based datasets) suggest nonrandom diet clustering. The same analysis at the species level (i.e. data pooled by species for the whole region) showed such diet clustering only for the full dataset and specifically only for the more recent plant taxa (i.e. clustering only in MNTD, not in MPD). Thus, the patterns found for individual snails are not reflected at the species level. Although snails are often assumed to feed at random, several experimental findings showed that snails can have real food preferences, both

when interspecific competition is present (Hatzioannou, Eleutheriadis & Lazaridou-Dimitriadou, 1994; Byers, 2000; Riley, Dybdahl & Hall, 2008) and when it is absent (Pennings, Nadeau & Paul, 1993; Wakefield & Murray, 1998; O’Rourke *et al.*, 2016). An alternative explanation comes from apparent diet clustering as the by-product of other, nondietary (selection) pressures, such as the preference for plants that provide shelter/concealment from predators (Watanabe, 1984; Alexander & Covich, 1991; Levri, 1998; Nyström & Pérez, 1998) or for plants that offer a humid environment and thus help to avoid desiccation (Chang, 1991; Lee & Silliman, 2006). Finally, signs of diet differentiation were weak, with PERMANOVA of target species diets and models of diet distances between all species showing little diet differentiation. Hence, signs of diet differentiation, and thus possible competition, were present at the individual level, and not at the species level.

Snails, being sessile, can eat only what is present where they live. We accounted for this by including ‘location’ as an explanatory variable in relevant models (SES of MPD and MNTD, and

PERMANOVA of diet distances) and indeed found the location to explain part of the variation in the response variables (roughly of the same order as for ‘species’). An improved, but labour intensive, method would be to sample the complete plant community from the plots, in addition to the snails, and perform plant barcoding, which would allow the assessment of how much of the plant variation available was truly eaten by the snails. While previous studies on snail community diet used rather broad categories (e.g. Schamp, Horsák & Hájek, 2010), we collected data at a very high resolution, possibly even including genetic variation within plant species and plants not previously recorded in the region (Azmi, 1998; Boonratana, 2000). As a result of our metabarcoding marker choice of rbcL, we could not consider any nonseed plant diet items. Therefore, our estimates of diet richness and differentiation are probably too conservative. For example, Barker & Efford (2004) list diets for various pulmonate families (of which several are included in our study) and, apart from ‘live plant material’, these include fungi, detritus, algae, bacteria and (dead) animals. Ideally, our metabarcoding of snail diets would be extended with genetic markers to pick up these food sources as well. Another limitation of using rbcL metabarcoding is that we cannot distinguish the status of the plant material (i.e. living or decaying), although niche differentiation might also occur in this direction. Furthermore, the diets we reconstructed from metabarcoding data are snapshots in time, because snails digest their food within 2–3 d (Dobson & Bailey, 1982; Flari & Lazaridou-Dimitriadou, 1996); we accounted for this in part by using large sample sizes. Snails can also change their diets throughout the year (Hatzioannou *et al.*, 1994) or throughout their lifetime, and it would be worthwhile to repeat our study during different seasons and years because functional differences (and competition for food) might in fact be seasonal (Hutto, 1985; DuBow, 1988).

Despite these possible caveats, our approach is clearly valuable to exploit the power of modern metabarcoding techniques to quickly and efficiently reconstruct individual and species diets, study differences in diets and search for signs of diet clustering and competition. Our approach is easily extendable to larger sample sizes, a broader diet assessment, different trophic levels and different regions or biomes.

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REFERENCES

- ALEXANDER, J.E. & COVICH, A.P. 1991. Predation risk and avoidance behavior in two freshwater snails. *Biological Bulletin*, **180**: 387–393.
- ALONSO, D., ETIENNE, R.S. & MCKANE, A.J. 2006. The merits of neutral theory. *Trends in Ecology & Evolution*, **21**: 451–457.
- ANDERSON, M.J. 2017. Permutational multivariate analysis of variance (PERMANOVA). In: *Wiley StatsRef: statistics reference online* (N. Balakrishnan, T. Colton, B. Everitt, W. Piegorisch, F. Ruggeri & J.L. Teugels, eds). Available at: <https://doi.org/10.1002/9781118445112.stat07841>. Accessed 1 April 2018.
- AZMI, R. 1998. *Natural vegetation of the Kinabatangan Floodplain. Part 1: an introduction to its natural vegetation, including a preliminary plant checklist of the region*. World Wide Fund for Nature, Kuala Lumpur, Malaysia.
- BARBOUR, M.A., RODRIGUEZ-CABAL, M.A., WU, E.T., JULKUNEN-TIITTO, R., RITLAND, C.E., MISCAMPBELL, A.E., JULES, E.S. & CRUTSINGER, G.M. 2015. Multiple plant traits shape the genetic basis of herbivore community assembly. *Functional Ecology*, **29**: 995–1006.
- BARKER, G.M. & EFFORD, M.G. 2004. Predatory gastropods as natural enemies of terrestrial molluscs and other invertebrates. In: *Natural enemies of terrestrial molluscs* (G.M. Barker & M.G. Efford, eds), pp. 279–403. CABI Publishing, Wallingford, UK.
- BEHMER, S.T. & JOERN, A. 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proceedings of the National Academy of Sciences of the USA*, **105**: 1977–1982.
- BELL, K.L., LOEFFLER, V.M. & BROSI, B.J. 2017. An rbcL reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Applications in Plant Sciences*, **5**: 1600110.
- BELOVSKY, G.E. 1986. Generalist herbivore foraging and its role in competitive interactions. *Integrative and Comparative Biology*, **26**: 51–69.
- BERRY, A.J. 1961. The habitats of some minute cyclophorids, hydrocenids and vertiginids on a Malayan limestone hill. *Raffles Bulletin of Zoology*, **30**: 101–105.
- BOLYEN, E., RIDEOUT, J.R., DILLON, M.R., BOKULICH, N.A., ABNET, C.C., AL-GHALITH, G.A., ALEXANDER, H., ALM, E.J., ARUMUGAM, M., ASNICAR, F. & BAI, Y. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, **37**: 852–857.
- BOONRATANA, R. 2000. A study of the vegetation of the forests in the lower Kinabatangan region, Sabah, Malaysia. *Malayan Nature Journal*, **54**: 271–288.
- BROOKS, M.E., KRISTENSEN, K., VAN BENTHEM, K.J., MAGNUSSON, A., BERG, C.W., NIELSEN, A., SKAUG, H.J., MACHLER, M. & BOLKER, B.M. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal*, **9**: 378–400.
- BRUNO, J.F., STACHOWICZ, J.J. & BERTNESS, M.D. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, **18**: 119–125.
- BYERS, J.E. 2000. Competition between two estuarine snails: implications for invasions of exotic species. *Ecology*, **81**: 1225–1239.
- CALLAHAN, B.J., McMURDIE, P.J., ROSEN, M.J., HAN, A.W., JOHNSON, A.J.A. & HOLMES, S.P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, **13**: 581.
- CHANG, H.-W. 1991. Activity and weight loss in relation to solar radiation in the polymorphic land snail *Cepaea nemoralis*. *Journal of Zoology*, **225**: 213–225.
- CHAO, A. 1984. Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, **11**: 265–270.
- D’ANDREA, R., GIBBS, T. & O’DWYER, J.P. 2020. Emergent neutrality in consumer–resource dynamics. *PLoS Computational Biology*, **16**: e1008102.
- DESANTIS, T.Z., HUGENHOLTZ, P., LARSEN, N., ROJAS, M., BRODIE, E.L., KELLER, K., HUBER, T., DALEVI, D., HU, P. & ANDERSEN, G.L. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, **72**: 5069–5072.
- DOBSON, D. & BAILEY, S. 1982. Duration of feeding and crop fullness in *Deroceras reticulatum*. *Journal of Molluscan Studies*, **48**: 371–372.
- DUBOWY, P.J. 1988. Waterfowl communities and seasonal environments: temporal variability in interspecific competition. *Ecology*, **69**: 1439–1453.
- ELTON, C.S. 1927. *Animal ecology*. Sidgwick & Jackson, London.
- FLARI, V. & LAZARIDOU-DIMITRIADOU, M. 1996. Evolution of digestion of carbohydrates in the separate parts of the digestive tract of the edible snail *Helix lucorum* (Gastropoda: Pulmonata: Stylommatophora) during a complete 24-hour cycle and the first days of starvation. *Journal of Comparative Physiology B*, **165**: 580–591.

- FREELAND, W.J. & JANZEN, D.H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *American Naturalist*, **108**: 269–289.
- GAUSE, G.F. 1934. *The struggle for existence*. Williams & Wilkins, Baltimore, MD.
- GORDON, I.J. & ILLIUS, A.W. 1989. Resource partitioning by ungulates on the Isle of Rhum. *Oecologia*, **79**: 383–389.
- GRÖMPING, U. 2006. Relative importance for linear regression in R: the package relaimpo. *Journal of Statistical Software*, **17**: 1–27.
- HÄGELE, B.F. & RAHIER, M. 2001. Determinants of seasonal feeding of the generalist snail *Arianta arbustorum* at six sites dominated by Senecioneae. *Oecologia*, **128**: 228–236.
- HARDIN, G. 1960. The competitive exclusion principle. *Science*, **131**: 1292–1297.
- HATZHOANNOU, M., ELEUTHERIADIS, N. & LAZARIDOU-DIMITRIADOU, M. 1994. Food preferences and dietary overlap by terrestrial snails in Logos area (Edessa, Macedonia, northern Greece). *Journal of Molluscan Studies*, **60**: 331–341.
- HENDRIKS, K.P., ALCIATORE, G., SCHILTHUIZEN, M. & ETIENNE, R.S. 2019a. Phylogeography of Bornean land snails suggests long-distance dispersal as a cause of endemism. *Journal of Biogeography*, **46**: 932–944.
- HENDRIKS, K.P., BISSCHOP, K., KAVANAGH, J.C., KORTENBOSCH, H.H., LARUE, A.E.A., RICHTER MENDOZA, F.J., SCHILTHUIZEN, M. & ETIENNE, R.S. 2019b. Fieldwork to sample microsnails for diet and microbiome studies along the Kinabatangan River, Sabah, Malaysian Borneo. *Malacologist*, **72**: 33–38.
- HENDRIKS, K.P., BISSCHOP, K., KORTENBOSCH, H.H., KAVANAGH, J.C., LARUE, A.E.A., PHUNG, C.-C., BONTE, D., DUIJM, E.J., SALLES, J.F., PIGOT, A.L., RICHTER MENDOZA, F.J., SCHILTHUIZEN, M., ANDERSON, M.J., SPEKSNIJDER, A.G.C.L. & ETIENNE, R.S. 2020. Microbiome and environment explain the absence of correlations between consumers and their diet in Bornean microsnails. *Ecology*, **102**: e03237.
- HOFREITER, M., POINAR, H.N., SPAULDING, W.G., BAUER, K., MARTIN, P.S., POSSNERT, G. & PÄÄBO, S. 2000. A molecular analysis of ground sloth diet through the last glaciation. *Molecular Ecology*, **9**: 1975–1984.
- HOLT, R.D. 2006. Emergent neutrality. *Trends in Ecology & Evolution*, **21**: 531–533.
- HUBBELL, S.P. 2001. *The unified neutral theory of biodiversity and biogeography*. Princeton University Press, Princeton, NJ.
- HUTCHINSON, G.E. 1961. The paradox of the plankton. *American Naturalist*, **95**: 137–145.
- HUTTO, R.L. 1985. Seasonal changes in the habitat distribution of transient insectivorous birds in southeastern Arizona: competition mediated? *Auk*, **102**: 120–132.
- KARTZINEL, T.R., CHEN, P.A., COVERDALE, T.C., ERICKSON, D.L., KRESS, W.J., KUZMINA, M.L., RUBENSTEIN, D.I., WANG, W. & PRINGLE, R.M. 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences of the USA*, **112**: 8019–8024.
- KARTZINEL, T.R. & PRINGLE, R.M. 2020. Multiple dimensions of dietary diversity in large mammalian herbivores. *Journal of Animal Ecology*, **89**: 1482–1496.
- KATOH, K. & STANDLEY, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**: 772–780.
- KECK, F. & KAHLERT, M. 2019. Community phylogenetic structure reveals the imprint of dispersal-related dynamics and environmental filtering by nutrient availability in freshwater diatoms. *Scientific Reports*, **9**: 1–8.
- KEMBEL, S.W., COWAN, P.D., HELMUS, M.R., CORNWELL, W.K., MORLON, H., ACKERLY, D.D., BLOMBERG, S.P. & WEBB, C.O. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**: 1463–1464.
- KHALIK, M.Z., HENDRIKS, K.P., VERMEULEN, J.J. & SCHILTHUIZEN, M. 2018. A molecular and conchological dissection of the “scaly” *Georissa* of Malaysian Borneo (Gastropoda, Neritimorpha, Hydrocenidae). *ZooKeys*, **773**: 1–55.
- KHALIK, M.Z., HENDRIKS, K.P., VERMEULEN, J.J. & SCHILTHUIZEN, M. 2019. Conchological and molecular analysis of the “non-scaly” Bornean *Georissa* with descriptions of three new species (Gastropoda, Neritimorpha, Hydrocenidae). *ZooKeys*, **840**: 35–86.
- LEE, S.C. & SILLIMAN, B.R. 2006. Competitive displacement of a detritivorous salt marsh snail. *Journal of Experimental Marine Biology and Ecology*, **339**: 75–85.
- LEIBOLD, M.A. & McPEEK, M.A. 2006. Coexistence of the niche and neutral perspectives in community ecology. *Ecology*, **87**: 1399–1410.
- LEVRI, E.P. 1998. Perceived predation risk, parasitism, and the foraging behavior of a freshwater snail (*Potamopyrgus antipodarum*). *Canadian Journal of Zoology*, **76**: 1878–1884.
- LIEW, T.-S. 2019a. *Bornean terrestrial molluscs*. Available at: <http://borneanlandsnails.myspecies.info>. Accessed 28 May 2019.
- LIEW, T.-S. 2019b. *Opisthostoma* and *Plectostoma* (family Diplommatinidae). Available at: <http://opisthostoma.myspecies.info>. Accessed 28 May 2019.
- LINDEMAN, R.H., MERENDA, P.F. & GOLD, R.Z. 1980. *Introduction to bivariate and multivariate analysis*. Scott Foresman & Co., Glenview, IL.
- MAZEL, F., DAVIES, T.J., GALLIEN, L., RENAUD, J., GROUSSIN, M., MÜNKEMÜLLER, T. & THUILLER, W. 2016. Influence of tree shape and evolutionary time-scale on phylogenetic diversity metrics. *Ecography*, **39**: 913–920.
- McCLENAGHAN, B., GIBSON, J.F., SHOKRALLA, S. & HAJIBABAEI, M. 2015. Discrimination of grasshopper (Orthoptera: Acrididae) diet and niche overlap using next-generation sequencing of gut contents. *Ecology & Evolution*, **5**: 3046–3055.
- McMURDIE, P.J. & HOLMES, S. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*, **8**: e61217.
- MYSTERUD, A. 2000. Diet overlap among ruminants in Fennoscandia. *Oecologia*, **124**: 130–137.
- NYSTRÖM, P. & PÉREZ, J.R. 1998. Crayfish predation on the common pond snail (*Lymnaea stagnalis*): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobiologia*, **368**: 201–208.
- OKSANEN, J., BLANCHET, F.G., KINDT, R., LEGENDRE, P., MINCHIN, P.R., O'HARA, R.B., SIMPSON, G.L., SOLYMOS, P., STEVENS, M.H.H. & WAGNER, H. 2017. *Vegan: community ecology R package, v. 2.5-5*. Available at: <https://cran.r-project.org/web/packages/vegan/index.html>. Accessed 1 April 2018.
- O'RORKE, R., HOLLAND, B.S., COBIAN, G.M., GAUGHEN, K. & AMEND, A.S. 2016. Dietary preferences of Hawaiian tree snails to inform culture for conservation. *Biological Conservation*, **198**: 177–182.
- PENNINGS, S.C., NADEAU, M.T. & PAUL, V.J. 1993. Selectivity and growth of the generalist herbivore *Dolabella auricularia* feeding upon complementary resources. *Ecology*, **74**: 879–890.
- POMPANON, F., DEAGLE, B.E., SYMONDSON, W.O.C., BROWN, D.S., JARMAN, S.N. & TABERLET, P. 2012. Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, **21**: 1931–1950.
- PRICE, M.N., DEHAL, P.S. & ARKIN, A.P. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, **26**: 1641–1650.
- PRINS, H.H.T. & OLFF, H. 1998. Species-richness of African grazer assemblages: towards a functional explanation. In: *37th Symposium of the British Ecological Society: dynamics of tropical communities* (D.M. Newbery, H.H.T. Prins & N.D. Brown, eds), pp. 449–490. Blackwell Science, Oxford.
- R CORE TEAM. 2018. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- RILEY, L.A., DYBDAHL, M.F. & HALL, R.O. 2008. Invasive species impact: asymmetric interactions between invasive and endemic freshwater snails. *Journal of the North American Benthological Society*, **27**: 509–520.
- ROSINDELL, J., HUBBELL, S.P. & ETIENNE, R.S. 2011. The unified neutral theory of biodiversity and biogeography at age ten. *Trends in Ecology & Evolution*, **26**: 340–8.

- SCHAMP, B., HORSÁK, M. & HÁJEK, M. 2010. Deterministic assembly of land snail communities according to species size and diet. *Journal of Animal Ecology*, **79**: 803–810.
- SCHEFFER, M., VAN NES, E.H. & VERGNON, R. 2018. Toward a unifying theory of biodiversity. *Proceedings of the National Academy of Sciences of the USA*, **115**: 639–641.
- SCHILTHUIZEN, M. 2011. Community ecology of tropical forest snails: 30 years after Solem. *Contributions to Zoology*, **80**: 1–15.
- SCHILTHUIZEN, M., CABANBAN, A.S. & HAASE, M. 2005. Possible speciation with gene flow in tropical cave snails. *Journal of Zoological Systematics and Evolutionary Research*, **43**: 133–138.
- SCHILTHUIZEN, M., CHAI, H.-N., KIMSIN, T.E. & VERMEULEN, J.J. 2003. Abundance and diversity of land-snails (Mollusca: Gastropoda) on limestone hills in Borneo. *Raffles Bulletin of Zoology*, **51**: 35–42.
- SCHILTHUIZEN, M., TERAVAINEN, M., TAWITH, N.F.T.K., IBRAHIM, H., CHEA, S., CHUAN, C.P., DAIM, L.J., JUBAIDI, A., MADJAPUNI, M.J. & SABEKI, M. 2002. Microsnails at micro-scales in Borneo: distributions of Prosobranchia versus Pulmonata. *Journal of Molluscan Studies*, **68**: 255–258.
- SCHILTHUIZEN, M., VERMEULEN, J.J. & DAVISON, G.W.H. 1999. A note on the ecology of West-Malaysian calcicolous snails (Mollusca: Gastropoda: Diplommatinidae, Cyclophoridae, Vertiginidae, Streptaxidae). *Malayan Nature Journal*, **53**: 351–354.
- SCHOENER, T.W. 1974. Resource partitioning in ecological communities. *Science*, **185**: 27–39.
- SCHOENER, T.W. 1983. Field experiments on interspecific competition. *American Naturalist*, **122**: 240–285.
- SEGURA, A.M., CALLIARI, D., KRUK, C., CONDE, D., BONILLA, S. & FORT, H. 2011. Emergent neutrality drives phytoplankton species coexistence. *Proceedings of the Royal Society B*, **278**: 2355–2361.
- SPEISER, B. & ROWELL-RAHIER, M. 1991. Effects of food availability, nutritional value, and alkaloids on food choice in the generalist herbivore *Arianta arbustorum* (Gastropoda: Helicidae). *Oikos*, **62**: 306–318.
- STACHOWICZ, J.J. 2001. Mutualism, facilitation, and the structure of ecological communities. *Bioscience*, **51**: 235–246.
- SYMONS, F.B. & BECCALONI, G.W. 1999. Phylogenetic indices for measuring the diet breadths of phytophagous insects. *Oecologia*, **119**: 427–434.
- TABERLET, P., COISSAC, E., POMPANON, F., BROCHMANN, C. & WILLERSLEV, E. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, **21**: 2045–2050.
- VERMEULEN, J.J. 1991. Notes on the non-marine molluscs of the island of Borneo 2. The genus *Opisthostoma* (Gastropoda Prosobranchia: Diplommatinidae). *Basteria*, **55**: 139–163.
- VERMEULEN, J.J., LIEW, T.-S. & SCHILTHUIZEN, M. 2015. Additions to the knowledge of the land snails of Sabah (Malaysia, Borneo), including 48 new species. *ZooKeys*, **531**: 1–139.
- WAKEFIELD, R.L. & MURRAY, S.N. 1998. Factors influencing food choice by the seaweed-eating marine snail *Norrisia norrisi* (Trochidae). *Marine Biology*, **130**: 631–642.
- WATANABE, J.M. 1984. Food preference, food quality and diets of three herbivorous gastropods (Trochidae: *Tegula*) in a temperate kelp forest habitat. *Oecologia*, **62**: 47–52.
- WEBB, C.O., ACKERLY, D.D., McPEEK, M.A. & DONOGHUE, M.J. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics*, **33**: 475–505.
- WILLIAMSON, P. & CAMERON, R.A.D. 1976. Natural diet of the land-snail *Cepaea nemoralis*. *Oikos*, **27**: 493–500.