Taxonomic diversity and community structure of invertebrates associated with rodent nests in northeastern Madagascar

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Abstract

Rodents build nests primarily for shelter and reproductive activities. These nests also serve as microhabitats for different groups of invertebrates. In northeastern Madagascar, using a spool-and-line technique of internally unrolling thread bobbins attached between the shoulders of captured and released rodents (one native - *Eliurus minor* and 58 introduced rats - *Rattus rattus*), allowed them to be tracked to their nests. We examined 79 individual nests from across elevational and habitat gradients, as well as seasons, identifying close to 6700 invertebrates from over 40 families. The nest fauna was dominated by mites (59%) and ants (22%). On the basis of multivariate analyses taking into account abundance of individual invertebrates found in the nests, most variability was at the family level, and showed statistical differences related to elevation, site habitat, season, and position of the nest (burrow or in an arboreal position). These results highlight that rodent nests harbor diverse entomological assemblages, driven more by variation in taxonomic composition than by differences in the number of families recorded. This study highlights the ecological role of rodent nests in providing microhabitats for different arthropods on Madagascar and presumably in parallel elsewhere in the tropics.

Keywords: rodent nests, invertebrate diversity, habitats, community structure, Marojejy

Résumé détaillé

Les rongeurs construisent des nids pour se protéger, se reproduire, se reposer et élever leur progéniture. Ces nids, qu'ils soient arboricoles ou souterrains, abritent également une diversité d'invertébrés encore méconnue. Cette étude, réalisée dans le Nord-est de Madagascar, examine la communauté d'invertébrés cohabitant avec les rongeurs endémiques (Nesomyidae) et introduits (Muridae). Afin de localiser et de collecter leurs sites de nidification, différents pièges à rongeurs ont été installés le long d'un gradient altitudinal allant de 80 à 1300 m, incluant des sites agricoles, des forêts secondaires (*savoka*) et des forêts humides intactes du Parc National de Marojejy. Une fois les rongeurs capturés, ils ont été ensuite équipés d'une bobine de fil attachée entre les omoplates (méthode dite du « spool-and-line ») pour les traquer jusque dans leurs nids. Les invertébrés ont ensuite été extraits des nids, conservés en éthanol et identifiés au laboratoire jusqu'au niveau de la famille, et même du genre pour les fourmis.

Au total, 79 nids appartenant à un d'individu de l'espèce endémique (*Eliurus minor*) et 58 individus de l'espèce introduite (*Rattus rattus*) ont été examinés, révélant près de 6700 invertébrés appartenant à plus de 40 familles, dominées par les acariens (59 %) et les fourmis (22 %). Les résultats ont montré que la hauteur des nids par rapport au sol varie en fonction de la saison. Durant la saison humide, les nids arboricoles ont été observés au-dessus du sol en moyenne à 3,4 m, et durant la saison froide et sèche, à 2,1 m. Cette différence de 1,3 m entre les saisons permettrait d'éviter les risques d'inondations et/ou l'exposition au froid. Malgré cette variation saisonnière, les analyses de composition communautaire d'invertébrés

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n'ont pas montré de différence significative sur la richesse taxonomique en fonction de la position des nids (souterrains et arboricoles), mais de nettes différences qualitatives ont été constatées au niveau des familles. Certaines familles et genres (fourmis) ont montré des préférences marquées : les araignées Agelenidae, Lycosidae, Pisauridae et Oonopidae et les fourmis *Pheidole* sont plus fréquentes dans les terriers, tandis que l'Isopode est le seul associé aux nids arboricoles. Ces différences traduisent des adaptations écologiques variées liées à plusieurs facteurs, comme la structure du nid, l'humidité, la température et les matériaux végétaux utilisés par l'hôte, ainsi qu'aux différents modes de vie des invertébrés.

L'analyse de la composition globale des nids révèle une prédominance d'arachnides, notamment d'acariens, qui sont souvent des parasites de vertébrés et sont associés à plusieurs maladies présentant des risques pour l'homme. Les insectes, qui représentent une part importante des invertébrés collectés sont répartis en de nombreux groupes fonctionnels : prédateurs (Staphylinidae, Reduviidae), décomposeurs (Tenebrionidae), nécrophages (Histeridae) et phytophages (Curculionidae) qui jouent des rôles écologiques essentiels. Les larves de diptères et de lépidoptères témoignent de l'importante ressource nutritive constituée par les nids, riches en débris végétaux et matières fécales. Cette diversité fonctionnelle souligne la complexité trophique des communautés d'invertébrés associées aux nids, où coexistent diverses formes d'interactions (prédatrices, parasitaires et compétitives).

Les résultats montrent que si la diversité et l'abondance totales ne varient pas de manière significative suivant les facteurs environnementaux, la richesse taxonomique change nettement, indiquant une sensibilité de certains groupes d'invertébrés aux conditions locales et aux variations microclimatiques. Les nids de rongeurs constituent donc des microhabitats clés pour certains éléments de la faune invertébrée tropicale, favorisant la coexistence d'espèces adaptées à des gradients écologiques spécifiques. Les variations observées en fonction de l'altitude, de la saison et de la position du nid traduisent l'importance de la microstructure et de la dynamique environnementale sur l'organisation des communautés. Ces résultats suggèrent par ailleurs que les nids, arboricoles ou terriers, offrent des conditions stables et abritent une grande diversité de groupes d'invertébrés. En somme, les nids de rongeurs dans les forêts tropicales de Madagascar représentent des refuges et des centres de recyclage de matière organique, contribuant à la biodiversité locale et à la structuration des communautés d'invertébrés.

Mots clés : nids de rongeurs, diversité des invertébrés, habitats, structure des communautés, Marojejy

Introduction

Many mammal species, including small mammals such as rodents, build nests, which can be burrows in the ground or placed in arboreal positions. These serve several functions for the animal: places to give birth and care for offspring, shelters for resting or hibernating, protection against extreme environmental conditions and refuge against predators (Stones *et al*., 1968; Dawson *et al*., 1973; Flowerdew, 1993; Tecot *et al*., 2012). Recent studies have highlighted that nests of small terrestrial mammals often contain different types of plant or other organic material (Csanády, 2022; Deeming, 2023). Such nests can provide a stable and protected microhabitat for a variety of invertebrates (Krawczyk *et al*., 2015), which include commensal, predatory, parasitic, and non-parasitic species. The presence of certain invertebrates can serve as indicators of environmental conditions inside the nest, for example, a high abundance of springtails suggests a moist environment (Krawczyk *et al*., 2015). Nest structures, beyond their functional role for the primary occupants, influence ecological interactions between vertebrates and invertebrates (Hansell, 2000; Mainwaring *et al*., 2014). In addition, nest-building materials (plant fibers, leaves, grasses, etc.) can influence the composition of invertebrate communities, both through their structure, role as nutrient sources and anti-parasitic properties, and their capacity to retain moisture (Suárez-Rodríguez *et al*., 2013; Deeming & Reynolds, 2015; Yang *et al*., 2020).

Arthropods, which represent more than 80% of all known animal species on Earth, exhibit extraordinary ecological diversity and adaptations, allowing them to colonize a multitude of terrestrial and aquatic habitat (Culliney, 2013). Within vertebrate nests, arthropods can play multiple ecological roles: scavengers and decomposers that recycle organic matter, predators that feed on other invertebrates or even bioindicators whose presence reflects microclimatic conditions (Noriega *et al*., 2018; Menta *et al*., 2020). Some taxa, such as mites, springtails or isopods, are directly involved in the breakdown of plant fiber, thereby influencing nutrient cycling inside the nest (Sogari *et al*., 2019). Others arthropods, including parasitic or hematophagous species, may directly gain nutritive resources from the mammal occupants (Yang & Gratton, 2014).

Although the invertebrate fauna of bird nests has been the subject of numerous studies (Gibson *et al*., 2019), that of rodent nests remain relatively unknown, particularly in tropical areas. Research on bird nests has highlighted the influence of environmental factors such as sun exposure, humidity, and vegetation density on the diversity of associated invertebrates (Zuk & Loye, 1991; Hansell, 2000). It is therefore likely that abiotic and biotic parameters, such as season, elevation, climate, vegetational cover, and/or underground versus arboreal nest height, play a role in the structure of the invertebrate communities (Ghannem *et al*., 2018; Cavaliere *et al*., 2019; Griffiths *et al*., 2021; Naccarato *et al*., 2023). Several of these variables can strongly influence the nest microfauna by modulating temperature, humidity, breeding conditions, and colonisation/dispersal (Cantarero *et al*., 2013).

In this context, our study aims to explore the composition of invertebrate communities present in rodent nests (ground burrows and arboreal positions) in northeastern Madagascar. Combining a descriptive approach and multivariate statistical analyses, we sought to answer the following questions: (1) What is the taxonomic diversity of invertebrates occurring in rodent nests? (2) Do the number of invertebrate families and the total abundance largely at the family level vary according to different ecological parameters related to sampling sites? (3) Do abiotic factors such as elevation, season or nest position (arboreal versus ground burrows) influence the taxonomic richness or abundance of invertebrates?

Methodology

Study site

The study was carried out along an altitudinal gradient ranging from 80 to almost 1300 m, encompassing sites both within and outside the Parc National de Marojejy in northeastern Madagascar. Five of the study sites (Sites 1 to 5) were located in an area modified by human activities around the village of Mandena and a short distance to the park entrance (Herrera *et al*., 2020). These sites included the following habitats: two rice fields (A, B) (Site 1: 14.7983°S, 50.3602°E, 95 to 110 m and Site 4: 14.4713°S, 49.8068°E, 100 to 125 m), vanilla plantation (Site 2: 14.8052°S, 50.3528°E, 80 to 120 m), an area of regenerating secondary forest known in Malagasy as *savoka* (Site 3: 14.4642°S, 49.7987°E, 130 to 160 m), and a mixed agricultural field (Site 5: 14.4693°S, 49.8034°E, 120 to 150 m). The sixth site (Site 6), located within the Parc National de Marojejy, about 9.5 km from the village of Mandena, corresponded to an intact mid-altitude evergreen humid forest (Goodman *et al*., 2023) known as Camp Simpona (14.4370°S, 49.7430°E, 1320 to 1370 m).

In the Marojejy region, daily mean temperatures generally range between 14.0°C and 22.7°C. The coldest period occurs between June and September, when temperatures can drop to 12°C near Camp Simpona, while the warm season spans from December to February; the highest temperatures, exceeding 28.9°C, have been recorded in lowland areas between September and November. Rainfall is strongly seasonal, with an annual average of 1654 mm, of which about 64% falls during the rainy season from November to April, whereas the dry season (May to October) is markedly less humid (Goodman *et al*., 2018; Marline *et al*., 2023).

Nest search and collection

Small mammals were captured using the same protocols employed at Marojejy for a range of recent studies and using a grid system (Herrera *et al*., 2020). To track rodents, including endemic Nesomyidae and introduced Muridae species, we used two different models of thread spools, which unroll from the inside to the outside, manufactured by Culver Textiles Corporation (West New York, New Jersey): the smaller weighing 1.73 g and the larger 4.3 g. After being wrapped in heat-shrink plastic, the spools were attached between the animal's shoulder blades, after the fur was shaved with a hair clipper, with a special type of glue (SureHold Plastic Surgery, manufactured by Barristo Enterprises, Chicago, USA); this technique is often referred to as "spool-and-line" (Miles *et al*., 1981; Woolley, 1989). The spool was positioned so that the thread unspooled in a posterior direction, and largely following the techniques of Boonstra and Craine (1986) and Steinwald *et al*. (2006). We also explored for burrows along banks near cultivated fields, rice fields, and in intact forest near Camp Simpona to increase the number of nests collected.

For each located nest, GPS coordinates were recorded, as well as the height of the site (burrow or arboreal position) and the type of vegetation within a 1 m radius. We distinguished two types of nesting sites: (1) “arboreal nest” generally refers to sites located off the ground, typically in trees or shrubs. If the support was a tree, we recorded the height, diameter at breast height (DBH), and different aspects of placement (e.g. within tree hole, between two branches, etc.); (2) “burrow” refers to an underground nesting site and recorded details included the number of entrances, the presence of bifurcations or chambers and the diameter and shape of the main entrance. For arboreal nests, the entire nest structure (including all plant material used in its construction) was collected. For burrows, we excavated the terminal nest chamber and collected all material inside it (plant lining, loose debris, and associated fine soil). Each sample was placed in a clean and clear plastic bag, labeled with a unique field number, and transported to the field laboratory for direct examination.

Nest examination and invertebrate extraction

At the field Station, each nest was carefully removed from the plastic bag, normally within 60 minutes of collection, examined in detail with the naked eye and/or a binocular scope, and all visible invertebrates were extracted with fine tweezers or brushes, and then preserved in tubes filled with 70° ethanol for subsequent identification.

Laboratory procedures identification

All arthropods were identified with a binocular scope, and using the keys of Borror *et al*. (1970) identified to family level. The exception was for ants, which were determined to the level of genus employing Fisher and Bolton (2016).

Statistical analysis

The term dominance is defined as the total effective number of individuals by family/genera (ants only) with respect to invertebrates identified from all nests combined. We used generalized linear models (GLM) to test the effect of season, altitude, habitat, and nest height on invertebrate taxonomic richness (at the family level with the exception of ants down to genus) and abundance. ANOVA tests were applied to GLMs, PERMANOVA for community composition, and correlation tests for relationships between continuous variables. The exact Fisher-Pitman permutation test was used to analyze variation in the abundance of invertebrate families between nest locations (arboreal vs. burrows). This is a robust nonparametric method for non-normal distributions and small sample sizes. This test provided the means to assess if the average abundances per taxonomic group differed significantly between nest locations.

To analyze variation of nest height among sites, we applied ANOVA, suitable for non-normally distributed data. This test allowed us to assess whether the median nest positions differed significantly between sites. When significant differences were detected, Tukey tests were performed to identify the groups responsible for the variation.

To examine differences among invertebrate community composition across nest types, we used Non-metric Multidimensional Scaling (NMDS) ordination based on the Bray-Curtis dissimilarity index. NMDS is a non-parametric ordination method that reduces the multidimensional complexity of data by representing it in a low-dimensional space (usually 2 dimensions). Unlike linear ordination methods, NMDS does not assume a linear relationship between variables but seeks to preserve the relative distances between samples as closely as possible. The effectiveness of the NMDS is measured by a stress value, which quantifies the difference between the distances observed in the original data and the distances represented in the reduced space. In general, a stress value < 0.2 is considered a diagnostic separation and values between 0.2 and 0.3 are also considered as important, but to a lesser degree with respect to the level of separation.

Results

Invertebrates in rodent nests

During the study, 149 individual rodents, including 135 *Rattus rattus* (Muridae) and 14 *Eliurus* spp. (Nesomyinae) were released and tracked using the spool-and-line method. Of these individuals, 75 nesting sites were located and sampled, including 62 arboreal nests and 17 burrows, associated with 59 individual rodents – one *E. minor* and 58 *R. rattus*. Nine individual rodents were found to have visited a nest multiple times, with seven visiting two nests and two visiting three nests. In addition to the 75 sites mentioned above, and additional four were opportunistically found based on the visual location of arboreal nests and burrows. All rodent nesting sites were composed of different types of plant material.

A total of 6677 invertebrates were extracted from the 79 nests and ranging from a minimum of two to a maximum of 595 individuals per nest. Arthropods belonging to the following groups were found: Arachnida: spiders, mites, and pseudoscorpions; Entognatha: springtails; Insecta: Coleoptera, Diptera, Hemiptera, Hymenoptera, Psocoptera, Dermaptera, Lepidoptera, Mantoptera, Siphonaptera, and Thysanoptera. The most numerous taxonomic groups included Arachnida (Acarina - 59%, 3972 individuals) and Insecta (Hymenoptera - 22%, 1199 individuals) (Figure 1). The invertebrates identified in the single nest of the endemic rodent *E. minor* include (Table 1): Psocoptera, non-identified family (n = 3), Hymenoptera (one genera, Formicidae) (n = 3), Aranae, family Gnaphosidae (n = 1), Coleoptera, families Ptiliidae and Ptiliidae (n = 18), Collembola, non-identified family (n = 1), and Acarina, non-identified family (n = 12).

Arachnids

A total of 4102 individual arachnids were collected from rodent nests/burrows (Table 1), representing around 36% of all identified invertebrates. Acarina (mites) were the most numerous with 3972 individuals (97% of all arachnids). Araneae (spiders) represent around 2% of this group, with 87 individuals belonging to 16 families. The most abundant families were Clubionidae (23 individuals) and Prodidomidae (11 individuals). Finally, Cheliferidae (pseudoscorpions) included 43 individuals or about 1% of the arachnids collected (Table 1).

Entognathes

Collembola (springtails) were the only Entognatha identified in the nests, with 180 individuals (3%) of the total identified invertebrate fauna (Table 1).

Insects

Insects made up the largest part of the invertebrate fauna collected from the nests, with 2395 individuals (61%) (Tables 1 & 2). They are divided into 32 families belonging to 14 orders. Among the most represented groups: Hymenoptera, mainly ants (Formicidae), were by far the most abundant with 1488 individuals (divided into 14 genera), representing 62% of all insects (Table 2). Beetles were notably diversified, including families such as Staphylinidae (92 individuals), Ptiliidae (32 individuals), and Cucujidae (30 individuals). Cockroaches (Blattidae, Blaberidae, etc.) are also represented, with a total of 128 individuals in four families. Other orders such as Psocoptera, Hemiptera, Dermaptera, Isopoda, and even a representative of the Mantodea were identified. Diptera and Lepidoptera larvae are also well represented, with 184 and 73 individuals, respectively.

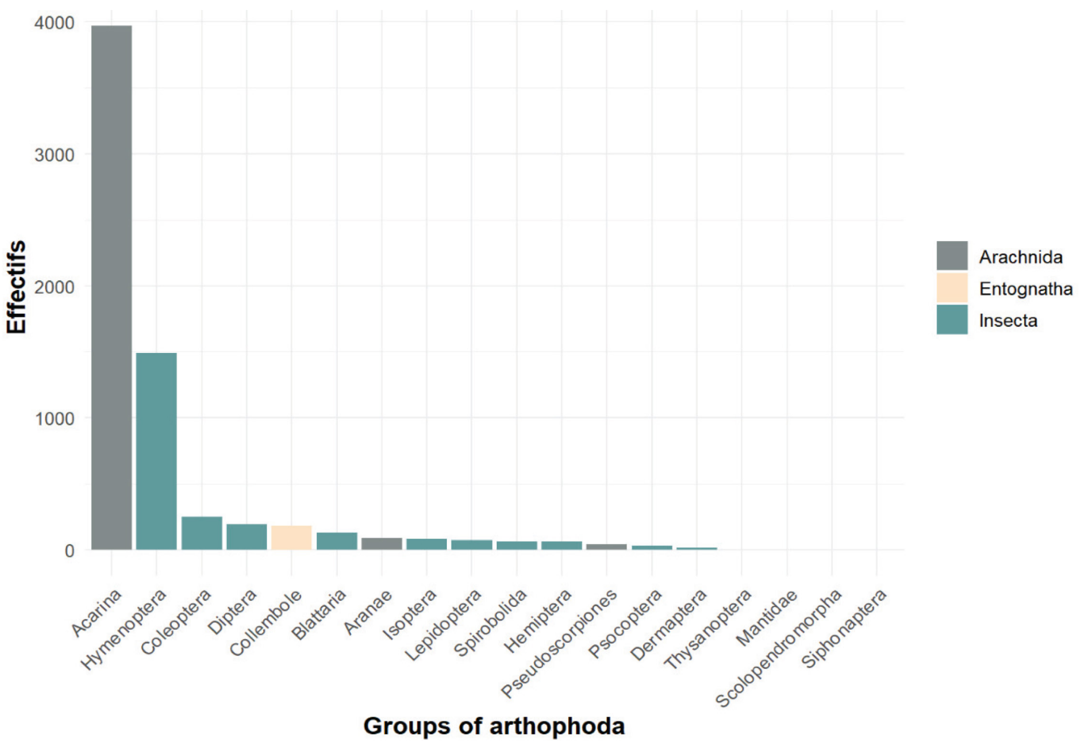

Figure 1. Numbers of Arthropods collected from rodent nests in and around the Parc National de Marojejy.

Table 1. The invertebrates collected from rodent burrows and nests of *Eliurus minor* and *Rattus rattus* in and around the Parc National de Marojejy. (*): invertebrate parasite of rodents at the level of order.

Order	Family	Invertebrates present in *E. minor* arboreal nest	Number of invertebrates in *R. rattus* burrows	Number of invertebrates in *R. rattus* arboreal nests	Total number of invertebrates in all nests	Dominance (%) in all nests
ARANEAE	Agelenidae	0	4	0	4	0.06
	Clubionidae	0	0	23	23	0.34
	Dictynidae	0	0	1	1	0.01
	Filistatidae	0	1	0	1	0.01
	Gnaphosidae	1	1	0	3	0.03
	Linyphiidae	0	2	1	3	0.04
	Lycosidae	0	2	0	2	0.03
	Mysmenidae	0	0	1	1	0.01
	Oonopidae	0	4	1	5	0.07
	Pisauridae	0	2	0	2	0.03
	Prodidomidae	0	2	9	11	0.16
	Salticidae	0	3	3	6	0.09
	Theridiidae	0	0	2	2	0.03
	Thomasidae	0	2	3	5	0.07
	Zodaridae	0	1	1	2	0.03
	Non-identified Areneae	0	1	16	17	0.25
PSEUDOSCORPIONES	Cheliferidae	0	8	35	43	0.64
ACARINA*		12	641	3319	3972	59.49
COLEOPTERA	Bostrichidae	0	0	1	1	0.01
	Carabidae	0	0	1	1	0.01
	Cerambycidae	0	0	1	1	0.01
	Cucujidae	0	3	27	30	0.45
	Curculionidae	0	2	1	3	0.04
	Coccinelidae	0	2	13	15	0.22
	Cryptophagidae	0	0	1	1	0.01
	Histeridae	0	1	0	1	0.01
	Nitidulidae	0	0	7	7	0.10
	Ptiliidae	13	3	16	32	0.48
	Scydmaenidae	0	0	1	1	0.01
	Staphylinidae	5	2	85	92	1.38
	Tenebrionidae	0	0	12	12	0.18
	Throscidae	0	1	2	3	0.04
	Non-identified Coleoptera larva	0	8	42	50	0.75
BLATTARIA	Blaberidae	0	0	24	24	0.36
	Blattidae	0	7	43	50	0.75
	Cryptocercidae	0	0	15	15	0.22
	Polyphagidae	0	7	32	39	0.58

Table 1. (continued)

Order	Family	Invertebrates present in *E. minor* arboreal nest	Number of invertebrates in *R. rattus* burrows	Number of invertebrates in *R. rattus* arboreal nests	Total number of invertebrates in all nests	Dominance (%) in all nests
DIPTERA	Muscidae	0	2	8	10	0.15
	Non-identified Diptera larva	0	8	176	184	2.76
HYMENOPTERA	Figitidae	0	0	4	4	0.06
	Formicidae	3	540	945	1488	22.29
	Non-identified Hymenoptera larva	0	0	2	2	0.03
PSOCOPTERA	Caeciliusidae	0	1	4	5	0.07
	Lepidopsocidae	0	3	5	8	0.12
	Non-identified Psocoptera	3	4	12	19	0.28
HEMIPTERA	Anthocoridae	0	3	12	15	0.22
	Reduviidae	0	3	8	11	0.16
	Non-identified juvenile Hemiptera	0	14	20	34	0.51
SPIROBOLIDA	Spirobolidae	0	4	58	62	0.93
SCOLOPENDROMORPHA	Scolopendromorpha	0	0	1	1	0.01
ISOPODA	Trachelipodidae	0	21	30	51	0.76
	Non-identified Isopoda	0	14	16	30	0.45
MANTODAE	Mantidae	0	0	1	1	0.01
DERMAPTERA	Labiidae	0	1	14	15	0.22
SIPHONAPTERA*	Pulicidae	0	1	0	1	0.01
THYSANOPTERA	Phlaeothripidae	0	1	1	2	0.03
LEPIDOPTERA	Gelechiidae	0	1	0	1	0.01
	Non-identified Lepidoptera larva	0	6	67	73	1.09
COLLEMBOLA		1	18	161	180	2.70
1) Total		**38**	**1355**	**5284**	**6677**	**100**

Table 2. Formicidae genera collected from rodent burrows and nests of *Eliurus minor* and *Rattus rattus* in and around the Parc National de Marojejy.

Genera	Ants present in *E. minor* arboreal nest	Number of ants in *R. rattus* burrows	Number of ants in *R. rattus* arboreal nests	Total number of ants in all nests	Dominance (%)
Anochetus	0	0	9	9	0.60
Camponotus	0	8	21	29	1.95
Crematogaster	0	0	3	3	0.20
Hymenopteres	0	0	1	1	0.07
Hypoponera	3	0	2	5	0.34
Monomorium	0	1	43	44	2.96
Nylanderia	0	0	29	29	1.95
Paratrechina	0	0	31	31	2.08
Pheidole	0	524	295	819	55.04
Plagiolepsis	0	1	0	1	0.07
Strumigenys	0	0	1	1	0.07
Tapinoma	0	0	293	293	19.69
Technomyrmex	0	0	157	157	10.55
Tetramorium	0	6	60	66	4.44
Total	**3**	**540**	**945**	**1488**	**100**

Influence of abiotic factors associated with nest position

Variation in nest height related to site elevation and season

Nest height varied significantly between sites (ANOVA, F = 4.58, P = 0.001) (Figure 2). This difference is best explained by local vegetation structure. Post-hoc comparisons indicate that nests are significantly higher in the Rice field and *Savoka* sites compared to the Vanilla field (Tukey, P = 0.013; P = 0.012, respectively), while other differences between sites are not significant. These results suggest that local conditions or habitat structure influence the height at which rodents build their nests. With the different sites combined (Figure 3), season also influences nest height (ANOVA, F = 10.99, P = 0.0014) of *Rattus rattus*, which are placed in higher positions during the rainy season (on average at 3.4 m), as compared to the dry season (on average at 2.1 m) (Tukey, P = 0.001), probably in response to soil moisture or possible temporary flooding. Camp Simpona was visited only once and data on seasonal differences are not available.

Influence of environmental factors on invertebrate community composition

We subsequently examined if differences in nest height were associated with variation in invertebrate community, taxonomic diversity, and composition. Of the 79 nests analyzed, 17 were from burrows and 62 were from arboreal sites. Analysis of the composition of invertebrate families revealed no significant difference at the family level between the arthropod communities identified from burrows and arboreal positions (PERMANOVA, Bray-Curtis distance, F = 1.31, R^2 = 0.017, P = 0.207). NMDS ordination (stress value = 0.284; Figure 4) illustrates a strong overlap between the two nest types, confirming the absence of a clear general separation in associated invertebrates at the family level related to nest height.

Regarding diversity indices, taxonomic richness (number of families per nest), Shannon index and Simpson index did not differ significantly between ground and above-ground nests (Wilcoxon tests: P = 0.559, P = 0.280, and P = 0.221, respectively). These results suggest that nest height (burrows vs. arboreal) does not significantly influence the taxonomic composition at the family level of invertebrate communities.

Number of groups, abundance, and biological communities of invertebrates

As mentioned above, several factors influenced nest location, the number of taxonomic groups or the abundance of individuals within families or genera (only for ants) and based on a Chi-square tests these aspects did not show any statistically significant aspects between sites. The same pattern was found for variables such as altitude, season, and nest height, which were not statistically significant with reference to diversity or total abundance.

In contrast, the composition of invertebrate communities (the families present in each nest) did vary significantly according to altitude, season,

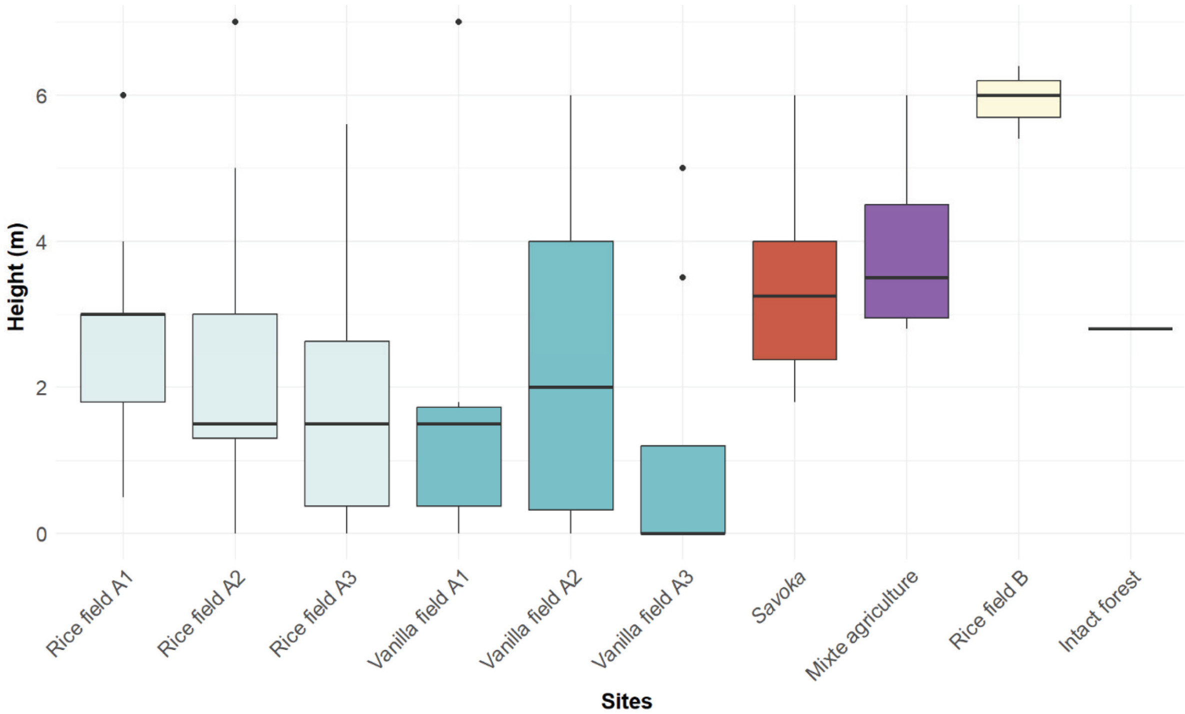

Figure 2. Rodent nest height by study site in and around the Parc National de Marojejy.

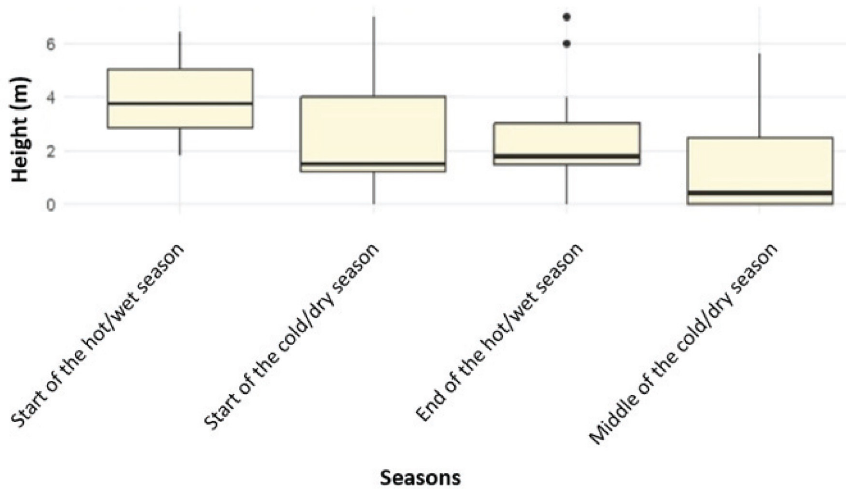

Figure 3. Rodent nest height during different seasons in and around the Parc National de Marojejy. All nest types are combined here.

and nest height (PERMANOVA, Jaccard distance, F = 1.95, R^2 = 0.12, *P* = 0.001). This indicates that while overall taxonomic richness and abundance remain stable, the families represented changed with respect variation for these three parameters. Such differences suggest that invertebrates at least at the family level associated with rodent nests are sensitive to local ecological factors, including more tolerant of humidity or temperature variation, or perhaps preference for particular plants incorporated into nests.

We assessed, for each invertebrate family and ant genera, whether abundance differed significantly between nests located in burrows as compared to arboreal positions. The results of a series of Fisher-Pitman tests indicate that some families and genera have marked preferences associated with nest position. Among the groups showing a significant difference included four spider families Agelenidae (*P* = 0.009), Lycosidae (*P* = 0.045), Pisauridae (*P* = 0.041), and Oonopidae (*P* = 0.023) and the Formicidae genus *Pheidole* (*P* = 0.014) prefer burrows. In contrast, only the Isopoda show an affinity for arboreal nests (Fisher-Pitman test, *P* = 0.039). The remaining families and genera (for ants) showed no significant difference in terms of nest position choice, whether arboreal or burrows.

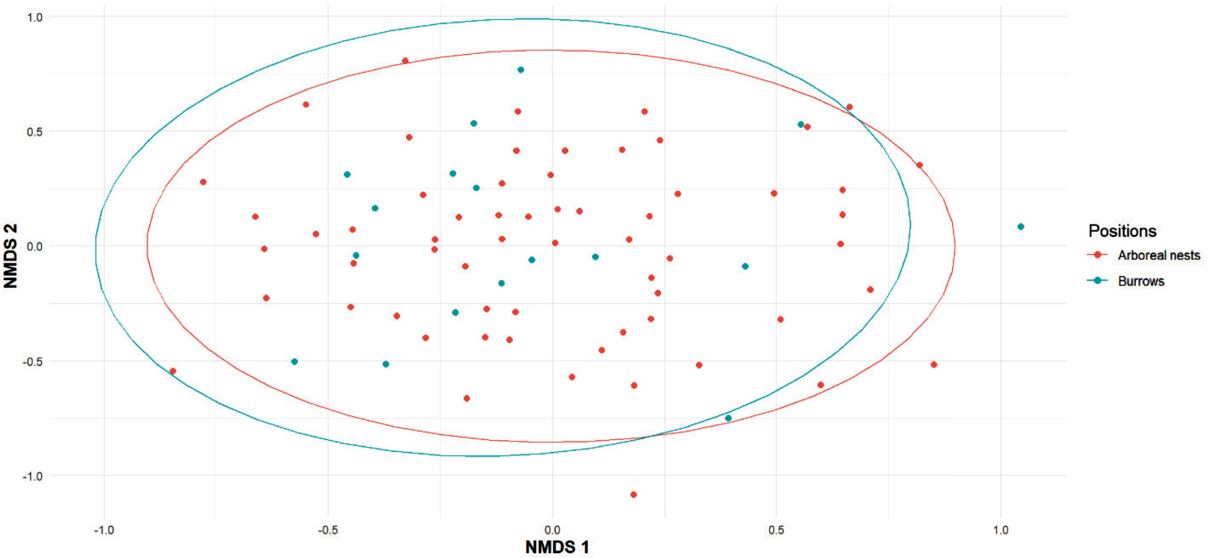

Figure 4. Non-metric Multidimensional Scaling ordination (Bray-Curtis distance) representing the composition of invertebrate communities in rodent borrows and arboreal nests. The ellipses correspond to the 95% confidence interval.

Discussion

On the basis of 6677 identified individual invertebrates, including 18 orders, 40 families, and 14 genera (ants only), our study revealed an important taxonomic diversity within rodent nests (one native - *Eliurus minor* and 58 introduced rats - *Rattus rattus*) in northeastern Madagascar. The dominance of Acarina (59%), followed by Hymenoptera (22%), underscore the role of rodent nests as multifunctional microhabitats for these groups and comparable to that described for mites in bird nests (Hansell, 2000). Many mite species are hematophagous parasites (Stoehr *et al*., 2000), relying on the presence of vertebrates hosts for nutrition, while other species use nests as resting, developmental or breeding sites. We do not have any measures of the taxonomic diversity of mites at least at the level of genera associated with rodent nests, but they are able to exploit organic matter and different aspects the structures provide, such as food, humidity, and protection (Seniczak *et al*., 2020), which would explain their dominance. Also, Acarina play different eco-epidemiological roles as pathogen vectors (Moro *et al*., 2005).

Six groups of invertebrates were identified in the single nest of *Eliurus minor*, with few individuals of each. This reduced taxonomic composition contrasts sharply with that observed in the multiple nests of *R. rattus*, with considerable invertebrate taxonomic representation. Several factors need to be considered to explain differences in invertebrates found in the nests of *R. rattus*, including the specific location of the general habitat (in natural forest and outside of natural forest) or individual behavioral differences (nest material selection or nest maintenance). Another aspect to consider microhabitat specificity for certain invertebrate taxa, particularly microclimate.

In this study, insects accounted for more than 36% of all invertebrates identified from rodent nests and exhibited considerable taxonomic diversity and presumably ecological function in a range of guilds they represent, from predators (Staphylinidae, Reduviidae) and decomposers (Tenebrionidae, Isopoda) to necrophagists (Histeridae), phytophagists (Curculionidae), and occasional pollinators (Coccinellidae). At the level of genera, Formicidae accounted for nearly two-thirds of the individual identified Insecta, highlighting the strong interactions between these social insects and rodent nests. Larvae, presenting about 5% of Insecta identified, particularly Diptera and Lepidoptera, presumably associated with the role of nests as nutritive resources and stable sites for reproductive conditions presumably linked to organic matter (feces, associated with plant remains). Surprisingly, Siphonaptera represented a very small portion of the identified invertebrates. We are unaware of any specific ecological interactions involving small mammals occupying these nests and the associated invertebrates, with the exception of the presence of two parasitic groups (Siphonaptera and Acarina). Moreover, the presence of diverse arthropod orders, such as Scolopendromorpha, Spirobolida, and Thysanoptera, illustrates the trophic complexity of these microecosystems, where predatory, saprophagous and detritivorous species coexist.

On the basis of current data, the number of invertebrates per nest and the overall number of represented families and genera (for ants) are not significantly influenced by altitude, season or nest height, suggesting notable ecological plasticity. However, taxonomic composition, families and genera (for ants) represented in each nest, varied significantly based on these same three parameters.

During the rainy season, the average arboreal nest position tended to be higher than the dry season, presumably associated with adaptive strategies of rodents to avoid soil humidity or flooding (Cantarero *et al*., 2013; Deeming & Reynolds, 2015). Our results suggest the positioning by rodents of their nests are related to decisions based on environmental cues, while differences in the taxonomic representation in different types of nests (burrows or arboreal positions) to broad-scale environmental parameters.

The diversity of trophic guilds detected within the invertebrates identified from nests indicates a seemingly complex network of ecological interactions. Predators presumably regulate smaller arthropod populations, while scavengers and decomposers recycle organic matter, and certain mites are presumably hematophagous and establish direct links with the occupying rodents. This mosaic of predatory, competitive, parasitic, and possibly mutualistic relationships highlights the nest as a sort of microecosystem.

Aspects of differences in community structure related to the vertical position of nests is presumably related to daily or seasonal shifts in temperature, humidity, and light, as is known to occur in other invertebrate communities on Madagascar and elsewhere in the world (Rajaonarivelo *et al*., 2019; Tobisch *et al*., 2023). More specifically, in our data set, burrows were preferentially occupied by Araneae families including Agelenidae, Lycosidae, Pisauridae, and Oonopidae and the Formicidae genus *Pheidole*, consistent with their ground-dwelling foraging and nesting strategies. In contrast, only Isopoda show an affinity for arboreal nests, reflecting the ability of these specific taxonomic groups to exploit particular microhabitats and suggesting that nest height acts as a selective filter linked to factors to explain the distribution of these organisms and associated life-history characteristics (Basset *et al*., 2003; Hawes & Bale, 2007; Wardhaugh, 2014; Mawan *et al*., 2022). In contrast, the majority of the other identified taxonomic groups showed no vertical preference.

In summary, rodent nests in northeastern Madagascar harbor notably diverse and numerous trophic guilds associated with their invertebrate communities. While overall measures of taxonomic representation at the family and generic (for ants) levels remain stable in rodent nests across sites and environmental gradients, taxonomic composition shows notable turnover and is flexible and shaped by altitude, season, and nest height. This dual pattern of stability and variability underscores the ecological role of rodent nests as invertebrate refuges. Based on current data, the location of nests (arboreal vs. burrows) does not influence the taxonomic composition of co-occurring invertebrates. This suggests that, contrary to what might be expected, in this study nest height is not a major structuring factor in the composition of invertebrate communities. Future research should explore the trophic dynamics of these communities and with greater fineness to taxonomic determinations, as well as their interactions with mammalian hosts, using molecular or stable isotope tools to better understand the ecological and possible epidemiological processes underlying nest-associated assemblages.

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