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Testing a Hump-Shaped Pattern with Increasing Elevation for Ant Species Richness in Daliang Mountain, Sichuan, China

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Abstract: Ants have long been regarded as ubiquitous insects that are indicators of environmental change and ecosystems. Understanding the patterns of ant species richness along elevational gradients is crucial for elucidating their ecological functions within ecosystems. However, there is currently no comprehensive consensus on the pattern. In this study, we explored the pattern of ant species richness along an elevational gradient in the Mt. Daliang region (Sichuan, China), a biodiversity conservation hotspot in China. The ant species richness was investigated using 115 plots 50 × 50 m in size, distributed across 12 elevation bands of 250 m interval between 750 to 3500 m a.s.l. We identified 157 ant species from 51 genera and seven subfamilies. Myrmicinae was the most diverse subfamily, consisting of 20 genera and 84 species, followed by Formicinae, Dolichoderinae, Ponerinae, Dorylinae, Amblyoponinae, and Proceratiinae. We found a unimodal distribution pattern of ant species richness along the elevational gradient, with the highest ant species richness occurring at mid-elevations. This hump-shaped pattern of ant species richness was presented alongside the temperature variation. Furthermore, our results indicated that ground-foraging ant species were the most abundant in this region and that ants prefer to nest in the soil. Our findings highlight the importance of elevation in influencing ant species richness in Daliang Mountain, Sichuan, China, and provide novel insights into the potential drivers of elevational gradients in ant species communities.

Keywords: ant species richness; elevation; temperature; microhabitats; Daliang Mountain; southwest China

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1. Introduction

Ants (Hymenoptera: Formicidae) are ubiquitous social insects, which inhabit nearly all terrestrial ecosystems except the polar regions and extremely cold areas above the snow line in high mountains [1]. They play multiple roles ecologically, including serving as seed dispersers for plants [2], engaging in mutualistic relationships with sap-sucking insects [3], and acting as hosts for a variety of associated organisms [4]. However, certain ant species can adversely impact human life, damage crops, and spread diseases [5–9]. Ants exhibit remarkable diversity, with 14,312 species across 343 genera and 16 subfamilies [10]. In China, 1113 ant species from 119 genera and 12 subfamilies have been documented [11]. Elevational gradients are recognized as crucial determinants of ant species richness [12]. Ant richness is often regarded as an indicator of forest health and overall biodiversity [13–15]. Their ecological significance and high diversity make ants an ideal model system for studying species richness distribution patterns [16–22]. Previous studies at multiple scales showed that ant species richness does not follow a single pattern along elevational gradients [12,23–37]. Generally, there are three main trends, as follows: (1) a monotonous

decrease with increasing elevation [12,35,36]; (2) low-elevation plateaus [37]; and (3) a hump-shaped pattern, where richness peaks at middle elevation [34,38]. Among these, the hump-shaped pattern of ant species richness has been the most frequently reported [37]. Despite many studies investigating the patterns of ant species along elevational gradients [12,35–38], a consensus on these patterns remains elusive [37]. Therefore, further work is needed in different mountainous regions, particularly those that have not been previously studied. Given the unparalleled significance of montane ecosystems, exploring the distribution pattern of ant species could provide crucial insights for the conservation of species diversity.

Mountains are recognized as biodiversity hotspots [39]. Their intricate topography and diverse climatic conditions have given rise to a unique and diverse fauna [40]. They provide an ideal natural location for testing ecological hypotheses [41–43]. In fact, mountains present compressed versions of regional and elevational gradients in the distribution patterns of species richness [32,44–46]. Such patterns are frequently linked to environmental variables [23,47]. A recent study suggested that elevation's impacts on the species diversity of plants and soil microbes are strongly mediated by thermal variation. This finding emphasizes the importance of elevation and temperature in driving the diversity of plants, bacteria, and fungi in Qinghai–Tibetan alpine wetlands [48]. Temperature has been shown to play a crucial role in shaping ant species richness in relation to elevation [23,37]. A global analysis of the elevational patterns in ant richness indicates that these patterns are driven by a complex interaction of multiple factors, such as temperature and area distribution [12,23,37,49]. The importance of temperature as an explanatory variable for the distribution of ant richness distribution across the mountain could predict species changes under climate change [32,50]. Furthermore, microhabitats can have a significant impact on ant species richness [34,51–53]. Many ant species are functionally associated with their specific microhabitats [32,51,52]. Understanding how ant species richness responds to these environmental factors can, thus, assist in exploring the mechanisms underlying richness patterns. This, in turn, can enhance our comprehension of both the ecological and evolutionary impacts of the elevational gradient on the ant community within a particular mountain [32,50].

The Daliang Mountain is located in the transition zone between the Tibetan Plateau, the Yunnan–Guizhou Plateau, and the Sichuan Basin, which is made up of the Sichuan–Yunnan Plate and the South China Plate, and has a subtropical semi-humid climate [34,54–56]. Despite the remarkable geographical and ecological diversity of Mt. Daliang, there is currently still very little information available on the richness of ant species in this region. Moreover, a thorough examination of the ant richness distribution pattern and its driving factors in Mt. Daliang has not yet been conducted. Therefore, the aim of this study was to comprehensively investigate the number of ant species, and to determine the ant species richness pattern by testing the following hypothesis: ants exhibit a hump-shaped pattern with maximum species richness at the middle elevation along an elevation gradient of Daliang Mountain, Sichuan, China.

2. Materials and Methods

2.1. Sampling Design

The Daliang Mountain is located in Liangshan Yi Autonomous Prefecture, Sichuan Province, China. It lies at the junction of Sichuan and Yunnan provinces and on the southeastern edge of the Qinghai–Tibet Plateau, serving as the watershed of Jinshakou and Mabian River. The terrain in this region is intricate, characterized by significant elevation variations, and a highly diverse climate, which has led to rich biodiversity [57]. Elevational gradients are considered an important factor for understanding ant species richness because

of the extensive variations in climate and habitat [8,12]. Our study was conducted along an elevation gradient in Daliang Mountain (Figure 1). In this study, a total of 115 sample plots (50 m × 50 m) were established along the elevational gradient. We examined elevational gradients between 750 m a.s.l. to 3500 m a.s.l. with 250 m elevation intervals in Mt. Daliang. The elevation ranges of species was divided into 12 elevational bands. In each sample plot, five independent squares (1 m × 1 m) were established. Due to the complexity of the field environment, there may be some deviation when choosing specific sample plots. However, the error range was maintained within ±50 m a.s.l. The sample sites and vegetation types of this study are presented in Figure 2 and Table S1.

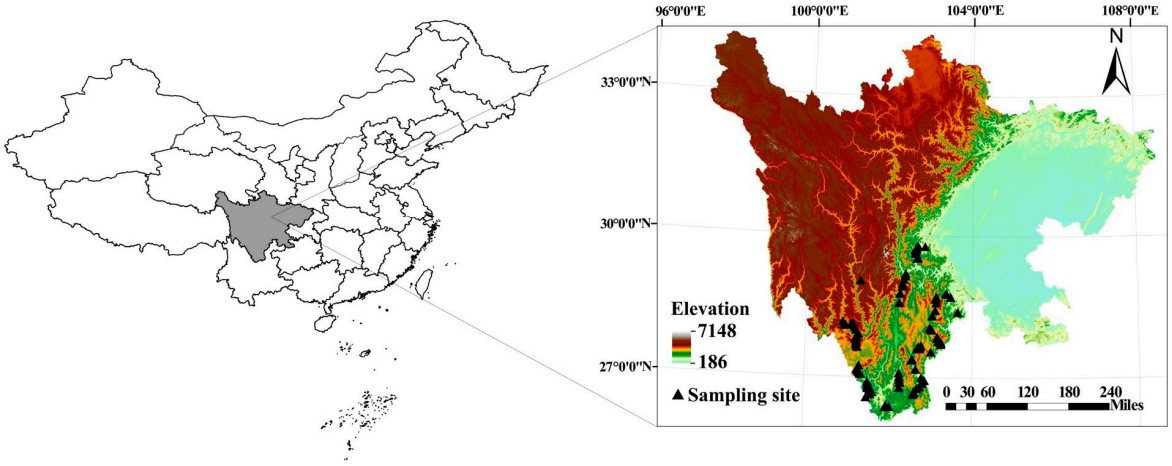


Figure 1. Location of the studied plots in Daliang Mountain, Sichan Province, China.

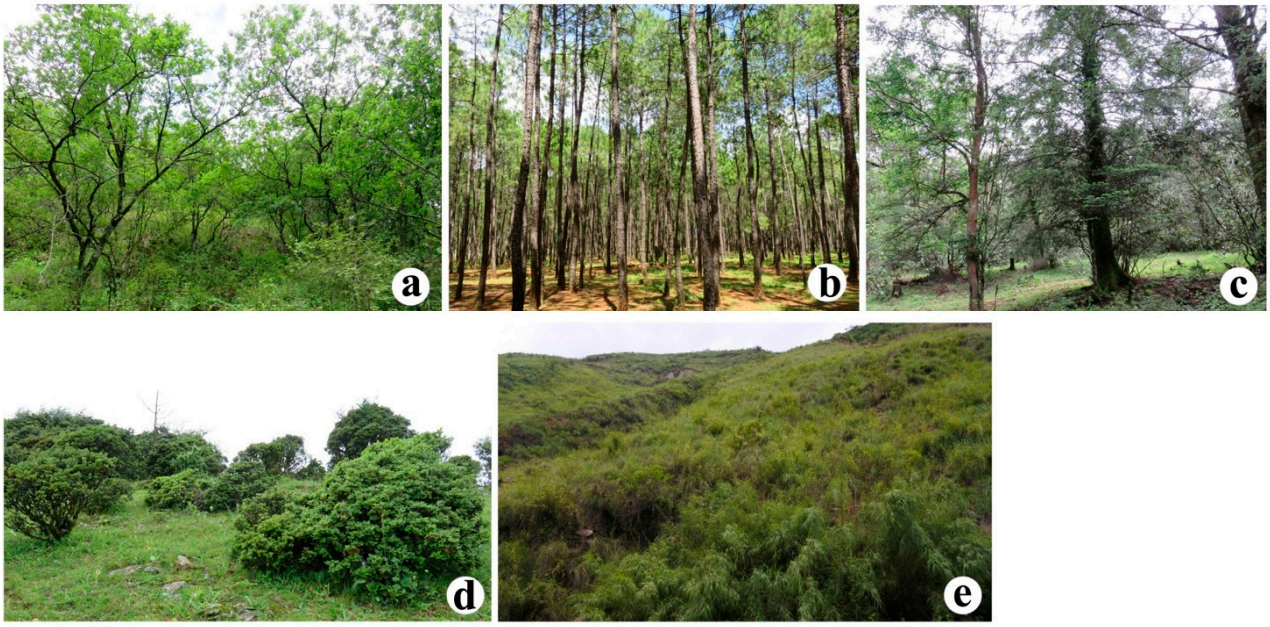


Figure 2. The letters (**a**–**e**) show the vegetation types in the studied plots: (**a**) broad-leaved forest; (**b**) coniferous forest; (**c**) mixed coniferous and broad-leaved forest; (**d**) shrublands; (**e**) grasslands.

2.2. Survey Method

In this study, ant species were collected using a standardized plot-based sampling methodology to maximize the number of ant species [58]. Firstly, five 1 × 1 m quadrats were systematically established within each 50 m × 50 m plot using the diagonal sampling method, maintaining the 10 m intervals between sampling points. All visible ant species within each quadrat were counted and collected. Subsequently, soil cores (20 cm depth)

were excavated using a shovel, and sieved through handheld mesh pans to collect and record subterranean ants. Secondly, a 2 m × 2 m white sheet was deployed beneath target vegetation. One researcher vigorously shook the vegetation over the sheet for 60 s to dislodge arboreal species. Displaced species were immediately examined, collected, and labeled. Thirdly, comprehensive habitat surveys were conducted for 60 min across seven microhabitats, namely ground surface, under stone, tree canopy, rotten wood, moss, trunk, and litter. Ants were collected using aspirators and soft forceps and counted. Meanwhile, the air temperature was recorded for each plot using a mini temperature humidity meter (UNI-T, UT333). Finally, after finishing the above investigation, the information of sample plot location, geographical location, elevation, vegetation type, microhabitats, and investigated time and collector were recorded, and then photos of the sample plots were taken. These specimens were preserved in 2 mL cryopreservation tubes containing 100% ethanol, and then subsequently transported to the laboratory for taxonomic identification and analysis.

2.3. Ant Specimen Processing and Identification

For each species we mounted a minimum of 9 ant individuals on triangular paper points. Ant specimens were identified based on the external morphological traits of workers of ants, such as the head, antennae, eye, mesosoma, mandible, pronotum, etc. [11]. Moreover, these ant specimens were carefully verified by an ant taxonomist, Professor Zheng-Hui Xu (Southwest Forestry University). Voucher specimens were deposited in the Southwest Forestry University Herbarium, Yunnan, China.

2.4. Statistical Analysis

The relationships of ant species richness with elevation and temperature were analyzed using logistic regressions. To conduct this analysis, the regression analyses were performed with the average of ant species richness across all plots for each elevation band as the response variable and elevation and temperature as explanatory variables. Differences in ant species number in different microhabitats were analyzed by an independent samples *t*-test. All statistical analyses were performed in R v.4.2.2 [59].

3. Results

In total, 112,328 individual ants were collected across 115 sampling plots in the Daliang Mountain, Scichuan Province, China. A total of 157 ant species belonging to 51 genera and 7 subfamilies (Amblyoponinae, Proceratiinae, Ponerinae, Dorylinae, Myrmicinae, Dolichoderinae, and Formicinae) were identified (Figure 3 and Table S2). Within the Daling Mountain region, Myrmicinae was the most diverse subfamily, comprising 20 genera and 84 species. It was followed by Formicinae (12 genera and 39 species), Dolichoderinae (7 genera and 14 species), Ponerinae (7 genera and 13 species), Dorylinae (3 genus and 4 species), Amblyoponinae (1 genus and 2 species), and Proceratiinae (1 genus and 1 species). Among these genera, *Pheidole* and *Tetramorium* were the most species-rich, with each containing 12 species. Among 112,328 individuals, the 2 ant species with the highest number of individuals in this area were *Paraparatrechina aseta* (Forel) and *Pheidole nietneri* Emery. Furthermore, we found a hump-shaped distribution of ant species richness along the elevational gradient in the Daliang Mountain region, with the peak richness occurring at mid-elevation (Figure 4a, Table S3). Similar to the relationship between the elevational gradient and ant species richness, the mean temperature also exhibited a unimodal association with ant species richness (Figure 4b).

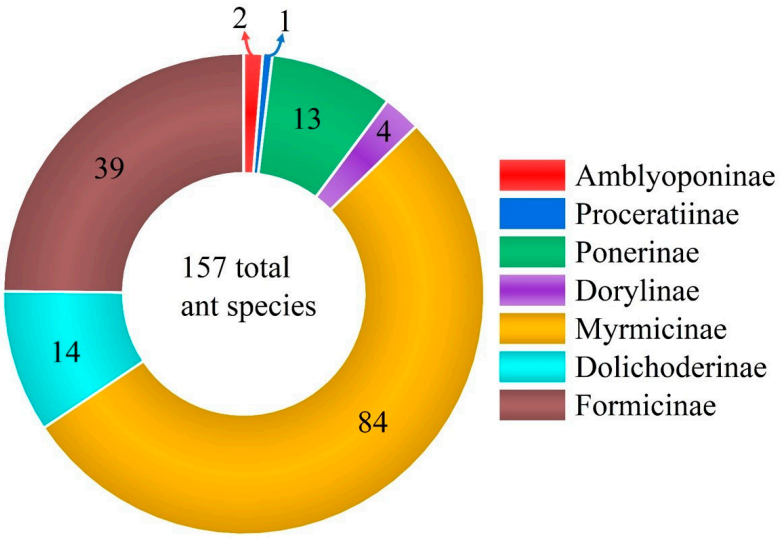


Figure 3. The members of subfamilies are given as the species number of a total of 157 ant species occurrences in the Daliang Mountain, Sichan Province, China.

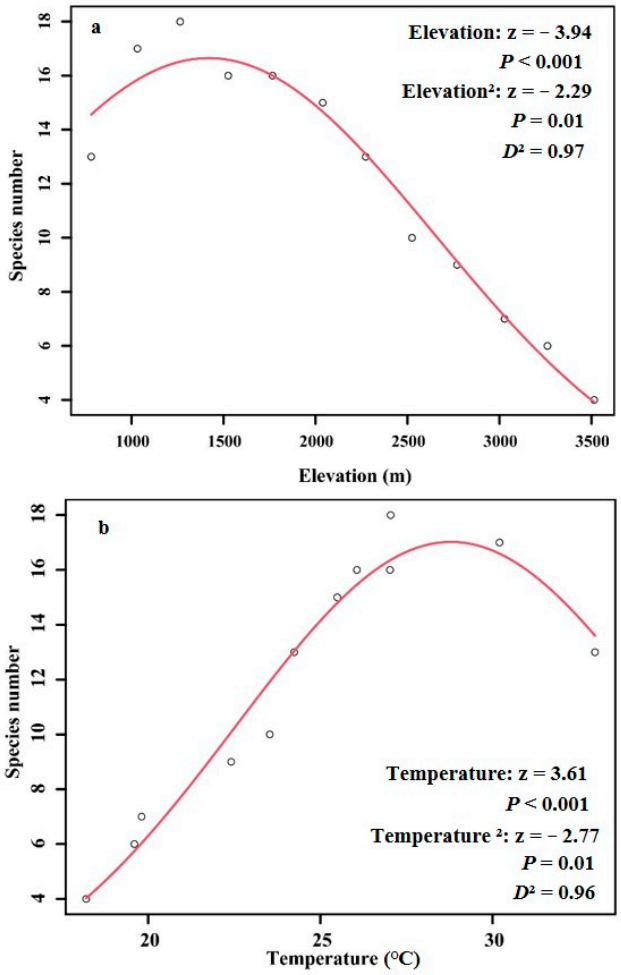


Figure 4. Relationship of species number with elevation (m) (**a**) and mean temperature (°C) (**b**) for every 250 m elevation from 750 m to 3500 m across 115 sampling plots in the Daliang Mountain, Sichan Province, China. The empty circles represent the means after the same elevation was averaged. The graph was visualized according to ant species richness. The fitted lines display the predicted probabilities of ant species as fit by the logistic regression model.

A total of eight types of ant foraging sites (with the tree crown not depicted in Figure 5) were observed, as shown in Figure 5 and Table S4. The number of ant species engaged in foraging on the ground surface was significantly greater than that of ant species in the soil, beneath stone, within the tree crown, on rotten wood, in moss, on the trunk and in the littler, respectively (Figure 6a). However, no significant differences were found among the number of foraging ant species from beneath stone, within the tree crown, on rotten wood, in moss, on the trunk, and in the litter ($p > 0.05$). Moreover, five types of ant nesting sites were detected (Figure 6b). The number of ant species nesting in the soil was significantly higher than that of the number of ant species from the ground surface, under stones, on rotten wood, and in the littler, respectively (Figure 6b).

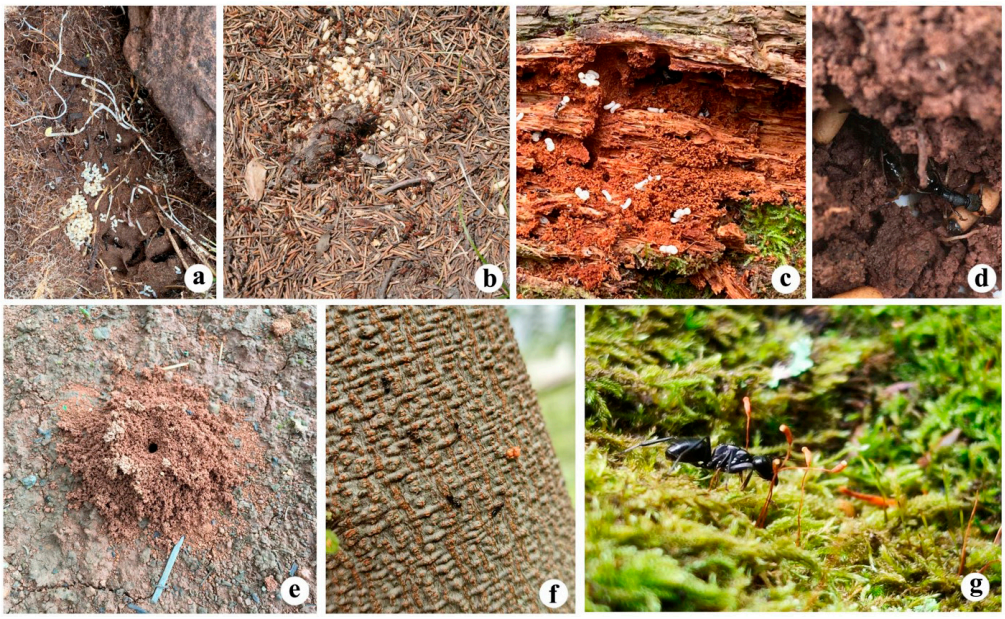


Figure 5. Foraging and nesting sites of ant species. (**a**) Under stone; (**b**) litter; (**c**) inside rotten wood; (**d**) soil; (**e**) ground surface; (**f**) trunk; (**g**) moss.

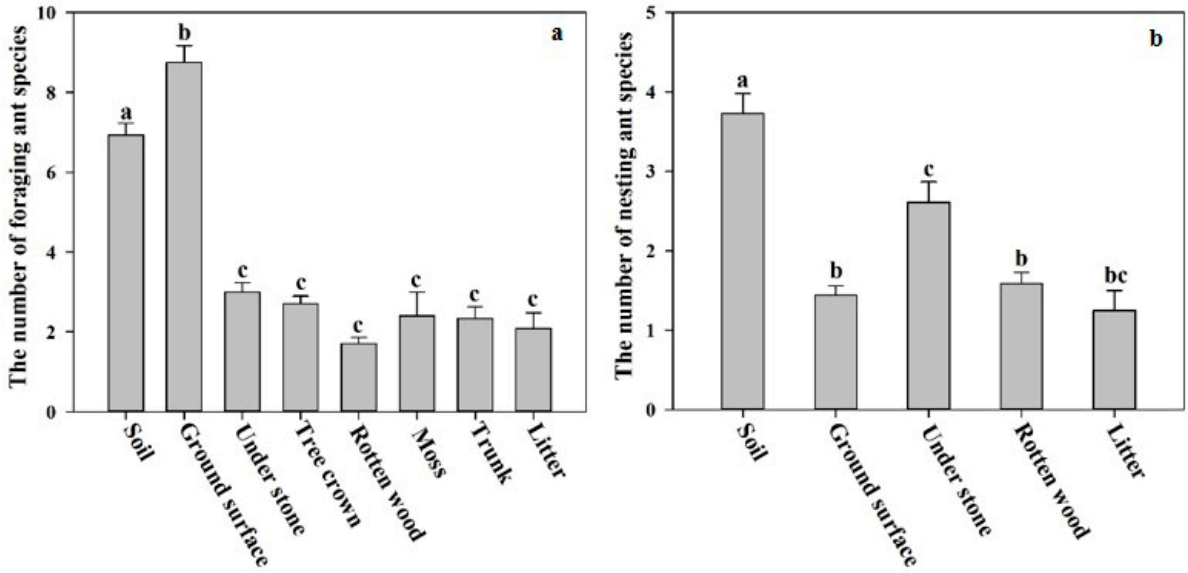


Figure 6. The mean value of ant species number for the foraging (**a**) and nesting ant species number (**b**). Error bars represent standard error (SE). Different letters indicate statistical significance at $p < 0.05$.

4. Discussion

Ants are critical components of terrestrial food webs, serving as a keystone taxa in ecological interactions and mediating multiple ecosystem processes. Their remarkable sensitivity to environmental changes makes them an ideal indicator for monitoring abiotic, biotic, and functional changes [15]. This study represents the comprehensive ant inventory in the Mt. Daliang region of China. Such inventories are fundamental to biodiversity conservation for formulating and implementing conservation strategies [60]. Among the identified genera, *Pheidole*, consisting of 23,721 individuals across 12 species, and *Tetramorium*, with 5325 individuals and 12 species, emerged as the most diverse. Among all the species surveyed, *Paraparatrechina aseta* had the highest number of individuals (12190). Additionally, twenty-two genera were represented by only a single species within our collection. In the Mt. Daliang region, similar to the Western Sichuan Plateau in China [34,35], the richness of Palaearctic genera, like *Myrmica* and *Lasius*, surpassed that of the Oriental genera, such as *Prenolepis* and *Leptogenys*. However, this richness pattern differed from Yunnan, China [61], and Nepal [36]. These disparities might be attributed to such factors as research objectives, sampling methods, and the surveyed area. Our findings are in line with ant species lists from China's neighboring regions and countries. This similarity is particularly evident in terms of the dominant subfamilies and commonly occurring genera, as seen in southern China [62], southwest China [34,35,63], Yunnan, China [61], northwestern Siwalik, India [64], Jammu and Kashmir, India [65], and in Nepal [36]. Moreover, our results align with earlier research that has recognized *Pheidole* as one of the three most species-rich genera globally (*Camponotus*, *Crematogaster*, and *Pheidole*) [36,66].

Ant species richness patterns typically show three distinct trends along elevational gradients. The most frequently observed is a hump-shaped pattern with maximum species richness at the middle elevation, followed by a richness plateau at low elevation and a monotonous decrease with elevation in the number of ant species [12,28,35–37,67–72]. The Mt. Daliang region is a subtropical semi-humid climate zone, with a section of the Jinsha River's dry and hot valley [73]. In the present study, we found that ant species richness peaks at the middle elevation, exhibiting a hump-shaped pattern with increasing elevation in the Daliang Mountain region. The spatial distribution of the variation of ant species was consistent with a recent study conducted in the Central and Northern Parts of the Western Sichuan Plateau, China [34]. This study confirms that the unimodal pattern of species richness along the elevation gradient would be typical in lower latitude and dry mountain systems [37,74–79]. It is noteworthy that the ant species richness gradient also displayed a unimodal pattern with increasing temperature. Given that temperature decreases by an average 6 °C for each 1000 m increased in elevation [80], temperature could regulate local species richness, with a threshold occurring at specific elevations. However, when this threshold is surpassed, ant species richness becomes constrained by either low or high temperature, thereby leading to reduced ant species richness [37,50]. As a consequence, the highest species richness is likely to be found in middle elevation bands with a moderate temperature [15].

Ant species richness may be further modulated by microhabitat heterogeneity [81–83]. In our study, it was found that ant species foraging on the ground surface and within the soil were relatively the most abundant, and the number of ant species building nests in soil was the highest. This finding is in line with previous investigations [34,53,56,84–90]. As an open area with rich food resources, ample sunlight, relatively high temperatures, and comparatively low humidity, the ground surface was a favored foraging site for ant species [53]. The majority of ant species choose to nest in soil. This is because temperatures and humidity in the soil are more stable compared to exposed above-ground locations [91].

In mountainous environments, nests beneath stones are common microhabitats. Stones provide stability and strength and are able to absorb sufficient sunshine to maintain a warm nest environment [92–94]. The regulation of temperature in brood chambers is facilitated by building chambers in the soil nest [90]. Moreover, numerous other factors, such as food availability, evolutionary history, biogeography, competition, dispersal ability, have the potential to impact the selection of nesting sites [22,91,92,95–97].

5. Conclusions

Our results support the hypothesis that the pattern of ant species richness along the elevational gradients in the Mt. Daliang region is hump-shaped, with peak richness observed at mid-elevations. Furthermore, the distribution pattern of ant species richness is also influenced by temperature and microhabitats. Additionally, this investigation recorded 157 ant species belonging to 51 genera and 7 subfamilies, highlighting the remarkable richness of ant fauna in Mt. Daliang. Our findings suggests that future research should consider more factors to determine the key mechanisms underlying the elevational pattern of ant species richness.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050308/s1, Table S1: Sample plot location in Daliang Mountain, Sichuan, China; Table S2: The occurrence of ant species in each band of elevation; Table S3: The mean of ant species richness were from every 250 m increase in elevation across 115 sampled plots; Table S4: Ant community composition and microhabitats in the Daliang Mountain, Sichuan, China.

Author Contributions: X.-M.Z. and Z.-H.X. conceived the ideas for the study; S.-J.Y. and X.-M.Z. collected the data; S.-J.Y. and X.-M.Z. analyzed the data and wrote the paper. All authors have read and agreed to the published version of the manuscript.

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