Effects of sampling on the elevational distribution of nematode-trapping fungi

CURRENT STATUS: UNDER REVISION

Deng Wei
Institute of Eastern-Himalaya Biodiversity Research, Dali University

Wang Jia-Liang
Fu Yang People’s Hospital Infection management section

Matt Scott
Scion (New Zealand Forest Institute)

Fang Yi-Hao
Institute of Eastern-Himalaya Biodiversity Research, Dali University

Liu Shuo-Ran
Institute of Eastern-Himalaya Biodiversity Research, Dali University

Yang Xiao-Yan
Institute of Eastern-Himalaya Biodiversity Research, Dali University

Xiao Wen xiaow@eastern-himalaya.cn
Institute of Eastern-Himalaya Biodiversity Research, Dali University

Corresponding Author
ORCiD: 0000-0002-4951-7524

DOI: 10.21203/rs.2.14154/v1

SUBJECT AREAS
Applied & Industrial Microbiology General Microbiology

KEYWORDS
elevational gradient of species richness; sampling pattern; observation bias; human interference
Abstract

Background Modelling species richness across an elevation gradient has long attracted attention, and at same time places some significant obstacles to research. Many interpretations of patterns and corresponding mechanisms for species distributions are made without consideration of multiple confounding factors. What are factors that affect species richness with elevation? The answer may contribute to better understanding of the elevational distribution patterns and mechanisms. In this study, we performed the research on species richness of nematode-trapping fungi (NTF) across an elevation gradient in Yunnan, China.

Results The results showed that sampling patterns, sampling altitude range, and human disturbance in sampling site could affect the resulting patterns of species richness significantly.

Conclusion The results suggested that future studies on the elevational gradients of species richness should address these factors, and try to adopt the high-sampling patterns to reduce the observation bias.

Background

In mountainous environments, steep environmental gradients can occur with slight variances in elevation. Ecological patterns observed across elevation gradients has contributed to the underpinning of ecological niche theory and the current knowledge of global life zones, species composition and biogeography [1]. Elevational gradients remain a mainstay for contemporary research on many topics in ecology and evolution and are critical to our current understanding of large-scale trends in biodiversity, global change and conservation.

Although extensive research has been conducted to understand the pattern of species
richness and diversity across elevational gradients, beginning with naturalist Carl Linnaeus in 1743, there is still a lack of consensus [2-4]. Debate mainly stems from differences in richness patterns between biogeographical regions or species groups in developing a unifying hypothesis. Species richness patterns across an elevation gradient differs between groups (e.g., plants, mammals, birds and microbes), and biogeographic regions [5-12]. The pattern of species richness across and elevational gradient comprise four principal models [2]: decreasing, low-elevation plateau (LEP), low-elevation plateau with a mid-peak (LPMP), and mid-peak [13-15]. Studies have also recorded bimodal patterns, multi-peak patterns, U-shaped patterns and some irregular patterns [7, 16-17]. The mid-elevation peak is the most common pattern observed [5, 18].

Studies have offered numerous factors that regulate species richness with changing elevation, such as size and scale of the studied site [2, 19]. However, in recent years, researchers have identified other important factors such as sampling pattern, sampling range and human disturbance. Nogués et al (2008) showed that species richness patterns were influenced by the altitude of areas studied and levels of human disturbance [20]; Grytnes (2008) showed that sampling intensity also affected the results of elevational gradient study, using a rarefaction curve to circumvent the resulting errors [21]. However, at present, neither the effects of sampling patterns, sampling range nor the influence of human disturbance have been sufficiently identified.

The Three Parallel Rivers Region is a world hotspot for biodiversity. The region has dramatic extremes in elevation (up to 6000m) from the Nujiang, Lancang and Jinsha Rivers to the upper limits of the adjacent mountain ranges making it an outstanding area for testing theories of elevational gradients. In this study, we examined the vertical distribution pattern of nematode-trapping fungi (NTF) richness at two sites (Gaoligong Mountain and Cangshan Mountain) and identify the regulating factors to richness. We
believed comparing these sites would demonstrate that the absolute elevation range and sampling layout would influence the richness pattern observed.

Methods

**Studied subjects**

NTF are a type of predatory Eukaryotic microorganisms that capture nematodes with specialized vegetative mycelia that function as trapping structures. These trapping structures include adhesive network (*Arthrobotrys*), adhesive knobs and branches (*Dactylellina*), and constricting rings (*Drechslerella*). NTF are widespread, occupying a range of aquatic and terrestrial habitats, and are an important natural biocontrol factors [23]. The limited species number and relative ease to identification make NTF ideal to ecological research.

**Studied areas**

This field study was conducted at two sites (Gaoligongshan and Cangshan) in the Three Parallel Rivers region of Yunnan, China.

Gaoligongshan National Nature Reserve (24°56′–28°23′N 98°08′–98°53′E) is located within the Hengduan Mountains (maximum elevation >6000m) along the Sino-Burmese border in northwestern Yunnan province. The nature reserve is situated along the north-south oriented Gaoligong mountain chain within the Nujiang and Dulongjiang River catchments. The vertical difference from the valley bottom to the ridgeline generally increases to the north with a maximum difference of nearly 4000 m. The enormous altitudinal gradient causes distinct climate, soil and vegetation zones.

Cangshan Mountain (25°33′–25°59′N 99°54′–100°12′E) is in the Yuengling Range of the Hengduan Mountains. It is situated in the Langcang River catchment at the intersection of the south subtropical and mid-subtropical climate zones. Cangshan Mountain also has distinct climate and biological zones along an altitudinal gradient.
Sample collection

Soil samples were collected using two modes (“distance interval method”, “elevation interval method”) between 1400 m and 3400 m in Gaoligong Mountain: (1) Samples were collected from 116 sites every one kilometer along the Dulongjiang Highway in the Gaoligong Mountains, Dulong Nationality Autonomous County. In total, 580 soil samples were gathered. The elevational distribution of sampling sites were shown in Fig 1; (2) At Gaoligong, soil samples were collected from 40 sites at 100 m elevation intervals, totaling 200 soil samples. At Cangshan Mountain, soil samples were collected from 30 sites at 100 m elevation intervals between 2100-3500 m, totaling 300 soil samples. Fifteen sites were located on the western face, and 15 were located on the eastern face of Cangshan. Soil samples were collected at each sampling site according to the five-point sampling method and brought back to the laboratory for cryopreservation. Due to the limitation on the geographical situation of the studied areas and the research methods of NTF, we ignored in this study whether the sampling met the standard of species rarefaction curve, only focusing on the sampling altitude range and layout patterns.

Preparation of culture medium

Corn meal agar medium was used to isolate and purify the NTF; Potato dextrose agar medium was used to extract strains for eutrophic culture [22].

The isolation, purification and identification of NTF

Soil sprinkling technique were used to isolate and purify the NTF from the soil samples, and identify the NTF species in terms of morphology and molecular biology [22, 23].

Data treatment

Occurrence frequency (OF) = the quantity of soil sample for individual species/total soil samples x 100%.

The original data was sorted and analysed by EXCEL and SPSS programme.
Results

**Elevational distribution patterns of NTF in Gaoligong Mountain under two sampling patterns**

There were 2 genera and 15 species and 120 strains of NTF isolated and identified from 580 soil samples collected equidistantly along the sampling lines, and 2 genera and 10 species and 45 strains of NTF isolated and identified from 200 soil samples collected along an elevational gradient. The vertical distribution patterns of NTF were shown different in two different sampling modes. The OF of NTF along the elevation in Gaoligong Mountain showed a midpeak pattern in the distance interval sampling method and a decreasing pattern in the elevation interval sampling method along the elevational gradient, see Fig 2. The number of NTF species showed a LPMP pattern in the distance sampling method and a decreasing pattern in the elevation sampling method along the elevational gradient, see Fig 3. The sampling sites were unevenly distributed in the distance sampling methods along the sampling lines, the layout of sampling sites had a significant correlation with species distribution ($r=0.872\ P=0.01$).

**The elevational distribution pattern of NTF in the smaller altitude range of sampling**

Only the OF of NTF was statistically documented for the altitude range of 2100-3500 at both sites. At smaller altitude intervals, the NTF showed some elevational distribution features that the OF and species number of NTF decreased with the increasing elevational gradient, fitting the decreasing pattern, see Fig 4.

**The elevational distribution pattern of NTF in Cangshan Mountain**

At Cangshan, there were 3 genera and 12 species and 57 strains of NTF isolated and identified from 300 soil samples, the OF decreased with increasing elevation, consistent with the decreasing pattern. The species number initially increased and subsequently
decreased, with the peak value biased towards low altitude, conforming to LPMP pattern (Fig 5).

**The elevational distribution of NTF after removal of the human interference range**

The low-altitude areas of eastern slope and the western slope of Cangshan Mountain, to some degree, were disturbed by human activities, but the ecological function areas of Cangshan Natural Reserve were less disturbed. The sampling areas in this study, located at the altitude of 2100-2300 meters, were outside the function areas, thus it was more affected by human activities.

Excluding data outside the altitude range of 2100-2300 meters, rarafaction curve and correlation analysis were carried out for the elevational distribution patterns of NTF in Gaoligong Mountain and Cangshan Mountain. The results showed that both the OF and species number of NTF had a decreasing pattern in Cangshan Mountain, so did the OF and species number at same altitude range in Gaoligong Mountain, see Fig 6. The OF and species number in the two areas were considerably correlated, $\text{OF}\ r=0.907, P=0.005$, species number: $r=0.857, P=0.014$.

**Discussion**

The accuracy of results on studying the vertical distribution patterns of NTF may be affected by sampling patterns, scale of study and post-sampling treatment of data. Geographical limitation, sampling uniformity, habitat fragmentation and human impacts can also influence distribution patterns [8, 20, 24]. Although previous studies have acknowledged these impacting factors, few have been validated by examples. This study on the elevational distribution patterns of NTF in the Three River Parallel Regions, have suggested that sampling pattern, sampling range, human disturbance and multi-dimensionality of biodiversity all affected the research outcome.
Sampling modes affecting the elevational distribution pattern of NTF

In this study, the different sampling methods have obtained different results on the elevational distribution patterns of NTF. In the distance sampling method, the OF of NTF conformed to midpeak pattern, and species number met LPMP pattern; however, the elevation sampling methods obtained a totally different result that the OF and species number of NTF both showed a decreasing pattern. It suggested that sampling methods would affect the observed pattern. Our results emphasize the importance of sampling method in developing species richness and OF models along environmental gradients.

Undoubtedly, in the process of studying the distribution patterns of species, increasing sampling efforts sees a consequent increase to a model’s accuracy [25]. However, when the sampling sites are unevenly distributed, the results will likely be biased. The equidistantly sampling method along the sample line in Gaoligong Mountain resulted in the fact that the sampling points were not averagely distributed on the elevational gradient, but concentrated in the middle altitude range, which probably caused the overestimation on the species richness of NTF in this area. The correlation analysis on the number of species and sample points indicated that they were strongly correlated with each other, thus this sampling pattern in which the sample points were concentrated in the middle altitude areas showed a mid-elevation peak pattern.

When using the evenly sampling method along the elevational gradient in Gaoligong Mountain, the elevational distribution of NTF showed a decreasing pattern, this method would not be affected by the distribution of sampling sites and the scale of studied region, thus the decreasing pattern was probably closer to the true situation of the elevational distribution of NTF. We used the same sampling method in Cangshan Mountain, and got the same results that NTF OF showed a decreasing pattern. This demonstrated that our hypothesis was validated in different regions, and the elevation sampling method can
resolve the observation biases and present more accurate results.

Sampling range affecting the elevational distribution of NTF

When we truncated the sampling range to 2100-3500 meters in the Gaoligong Mountains where the samples were equidistantly collected along the sample lines (distance method), it was found that the original mid-elevation peak pattern (mid-peak pattern for OF, LPMP pattern for species number) changed to a decreasing pattern for the elevation sampling method. Previous studies on truncation and scale effects on the vertical distribution of species have been carried out. Truncating the low-altitude range of the studied region led to the changes of the vertical distribution of species from the midpeak pattern to a decreasing pattern [20]. When the elevational gradient was entirely surveyed, the pattern was hump-shaped, changing eventually to a decreasing pattern as the scale of extent diminished. Likewise, the OF at the same altitude range of Cangshan Mountain (2100-3500 meters) was also in line with the decreasing pattern. This result further supported that gradient truncation significantly affected the research on the elevational distribution patterns, this also confirmed our speculation that the sampling range affected the research and the diminishing of sampling range caused the changes of mid-peak pattern to the decreasing pattern.

Human disturbance affecting the study on the elevational distribution of NTF

Areas rich in biodiversity often overlap with areas of high human populations, and it’s generally accepted that human disturbance can affect the distribution of biodiversity [26-28]. Surprisingly the influence from human disturbance on species richness models has been long ignored. In recent years, some researchers have given this some attention, pointing out that human populations are generally based around low elevations, and therefore human disturbance is not evenly distributed across most elevations [29-30]. The OF of NTF in Cangshan Mountain was in the decreasing pattern, while the species
number showed the LPMP pattern. In eastern slope of Cangshan Mountain, below the altitude of 2200 meters is residential area, in west slope of Cangshan Mountain, below 2400 meters is farmland, the boundary of Cangshan Nature Reserve is in the range of 2100-2300 meters, thus the areas outside the boundary may be affected by varying degrees of human disturbance [31]. When omitting the data collected in the range of 2100-2300 meters of Cangshan Mountain, we found both the OF and species number of NTF in a decreasing pattern, which evidently proved that human disturbance affected the vertical distribution of NTF. The declined biodiversity in the low-altitude areas because of human disturbance led to the peak of biodiversity in the middle-altitude areas. This was probably the reason why the species number of NTF in Cangshan Mountain showed a LPMP pattern when including the human-disturbed areas.

**Prospects and suggestions**

When we used different indicators (OF and species number) to identify microbial biodiversity on the altitude gradient in this study, the same set of studies presented different results, it probably meant that our previous studies did not reflect the full picture of biodiversity. Johnathan et al (2018) pointed out that because of multidimensional and scale-dependent characteristics of biodiversity, it would be better to describe its change from multiple perspectives [32]. Multidimensionality made the study of biodiversity at different time and different space more challenging than other variables in ecology [33]. In some studies, elevational distribution patterns present by used different biodiversity indicators (species number, species density, evenness, biodiversity index) are different [17, 34-35], thus the distribution patterns shown by different biodiversity indicators seemed to be interlaced, and different indicators showed different dimensions and different levels of biodiversity, to describe the biodiversity more fully from multiple dimensions needs more research and exploration.
Despite of the viewpoints from Nogues et al (2008) that the removal of high altitude areas had little effect on the research of the vertical distribution of species [20], Three River Parallel Region in China is characterized by vast elevation span, vertical climate and vertical vegetation, the alpine mudstone beach and yearly snow-covering in the high-altitude areas may cause steep fall of biodiversity. In this study, the altitude range was not large enough to completely cover the whole range of “the Three Rivers Parallel Region” and to carry out the exploration of the impact on the vertical distribution in the high-altitude areas. Future research should also focus on the integrity of the altitude range and further extend the studied areas.

The results in this study suggested that future study should address the sample-laying patterns and ensure not only the sampling evenness at each altitude, but also the consistency of the altitude range between the studied areas. Whenever and wherever possible, rarefaction curve should be used to determine the rationality of the sampling, at the same time, attention should also be paid to the impact of human disturbance on the elevational distribution patterns of species. We also noted that the OF was frequently used in the microbiology study as an indicator of species density which was relatively less affected, but the large animals might be more affected if the number of species was used as an indicator, therefore, the species inconsistency which was in the studies on the vertical distribution may be related to sampling patterns. Of course, the environmental heterogeneity caused by altitude was more than the difference of temperature, precipitation and vertical area. In the future, we need to systematically carry out the research by integrating environment, biological groups, sampling patterns and data analysis to obtain the real situation of the vertical distribution of species, which is crucial to understand the forming mechanism, maintaining mechanism and large-scale distribution of microorganisms.
Conclusions

The results showed that sampling patterns, sampling altitude range, and human disturbance in sampling sites do affect the understanding on NTF elevational distribution. Our study suggested that future studies should concern more on sampling design, agreement on biodiversity elevational distribution can be achieved only after agreement on sampling is made.

List Of Abbreviations

NTF: Nematode-trapping fungi; LEP: Low-elevation plateau; LPMP: Low-elevation plateau with a mid-peak; OF: Occurrence frequency

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Authors’ contributions
DW, WJL, YXY and XW conceived the research. WJL, FYH collected sample and performed the experiments. DW and WJL performed data analysis. DW wrote the manuscript. MS and LSR edited the manuscript. All authors approved the manuscript.

Availability of data and materials
The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Competing Interests
The authors declare that they have no conflict of interest.

Funding
This work was funded by the National Natural Science Foundation of China (31360013; U1602262;31760126).

Acknowledgments

We are grateful to Zhu Xiao-ming from Dali University for supplying many constructive suggestions.

Author details

1 Institute of Eastern-Himalaya Biodiversity Research, Dali University, Dali, Yunnan, 671003, China. 2 Collaborative Innovation Center for Biodiversity and Conservation in the Three Parallel Rivers Region of China, Dali, Yunnan, 671003, China. 3 The Provincial Innovation Team of Biodiversity Conservation and Utility of the Three Parallel Rivers Region, Dali University, Yunnan, 671003, China. 4 Fu Yang People’s Hospital Infection management section, Fuyang, Anhui, 236000, China. 5 Scion (New Zealand Forest Institute), Christchurch, 8011, New Zealand

References

1. McCain CM, Grytnes JA. Elevational gradients in species richness. In: Cullen K Encyclopedia of Life. New York: John Wiley & Sons; 2010. p. 1-10.

2. Lomolino, Mark. V. Elevation gradients of species-density: historical and prospective views. Global Ecology and Biogeography. 2001; 10, 3-11.

3. Heaney L R . Small mammal diversity along elevational gradients in the Philippines: an assessment of patterns and hypotheses. Global Ecology & Biogeography, 2010; 10(1):15-39.

4. Sanders N J . Elevational gradients in ant species richness: Area, geometry, and Rapoport's rule. Ecography, 2002; 25(1):25-32.

5. Mccain C M. Global analysis of reptile elevational diversity. Global Ecology &
Biogeography, 2010; 19(4):541-553.

6. Sanders N J , Rahbek C . The patterns and causes of elevational diversity gradients. Ecography, 2012; 35(1):1-3.

7. Guo Q , Kelt D A , Sun Z , Liu H , Hu L , Ren H , et al. Global variation in elevational diversity patterns. Scientific Reports. 2013; doi: 10.1038/srep03007

8. Rahbek C . The role of spatial scale and the perception of large-scale species-richness patterns. Ecology Letters; 2005, 8(2):224-239.

9. Miyamoto Y , Nakano T , Hattori M , Nara K. The mid-domain effect in ectomycorrhizal fungi: range overlap along an elevation gradient on Mount Fuji, Japan. Isme Journal Multidisciplinary Journal of Microbial Ecology; 2014, 8(8):1739-1746.

10. Wang J , Soininen J , Zhang Y , Yang B , Wang X , Yang X , Shen B . Contrasting patterns in elevational diversity between microorganisms and macroorganisms. Journal of Biogeography; 2011, 38(3):595-603.

11. Bryant J A , Lamanna C , Morlon H , Kerkhoff A J , Enquist B J , Green J Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. Proceedings of the National Academy of Sciences 2008; 105 Suppl 1:11505-11511.

12. John T. Longino , Michael G. Branstetter The truncated bell: an enigmatic but pervasive elevational diversity pattern in Middle American ants. Ecography; 2019, 42:272-283.

13. Bahram M , Pölme S , Kõljalg U , Zarre S , Tedersoo L . Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. New Phytologist; 2015, 193(2):465-473.

14. Singh D , Takahashi K , Kim M , Chun G , Adams J M . A Hump-Backed Trend in Bacterial Diversity with Elevation on Mount Fuji, Japan. Microbial Ecology; 2012,
15. KrMer T, Kessler M, Gradstein S R, Acebey A. Diversity patterns of vascular epiphytes along an elevational gradient in the Andes. Journal of Biogeography; 2005, 32(10):1799-1809.

16. Song-Shuang D, Pei-Xi S U. Altitudinal Variation Characteristics of the Plant Community on the Upper Reaches of Heihe River in the Qilian Mountains. Journal of Glaciology & Geocryology; 2010, 32(4):829-836.

17. Yeh C F, Soininen J, Teittinen A, Wang J. Elevational patterns and hierarchical determinants of biodiversity across microbial taxonomic scales. Molecular Ecology; 2019, 28:86-99.

18. Colwell R K, Lees D C. The mid-domain effect: Geometric constraints on the geography of species richness. Trends in Ecology & Evolution; 2000, 15(2):70-76.

19. Arrhenius O. Species and Area. Journal of Ecology; 1921, 9(1):95-99.

20. Nogués-Bravo, D, Araújo, M. B, Romdal T, Rahbek Scale effects and human impact on the elevational species richness gradients. Nature; 2008, 453(7192):216-219.

21. Grytnes J A, Romdal T S. Using Museum Collections to Estimate Diversity Patterns along Geographical Gradients. Folia Geobotanica; 2008, 43(3):357-369.

22. Zhang K Q, Hyde K D. Nematode-Trapping Fungi. Berlin: Springer-Verlag;

23. Li T F, Zhang K Q, Liu X Z. Nematophagous fungi taxonomy. Beijing: China Science and Technology Press;

24. Mccain C M. Could temperature and water availability drive elevational species richness? A global case study for bats. Global Ecology & Biogeography; 2010, 16(1):1-13.

25. Gotelli NJ and Colwell RK. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters; 2001, 4: 379-
26. Kessler M. Pteridophyte species richness in Andean forests in Bolivia. Biodiversity and Conservation; 2001, 10(9):1473-1495.

27. Marini L, Prosser F, Klimek S, Mars R H. Water-energy, land-cover and heterogeneity drivers of the distribution of plant species richness in a mountain region of the European Alps. Journal of Biogeography; 2008, 35(10):1826-1839.

28. Marini L, Gaston K J, Prosser F, Hulme P E. Contrasting response of native and alien plant species richness to environmental energy and human impact along alpine elevation gradients. Global Ecology & Biogeography; 2010, 18(6):652-661.

29. Marini L, Bona E, Kunin W E, Gaston K J. Exploring anthropogenic and natural processes shaping fern species richness along elevational gradients. Journal of Biogeography; 2011, 38(1):78-88.

30. Paudel P K, Sipos J, Brodie J F. Threatened species richness along a Himalayan elevational gradient: quantifying the influences of human population density, range size, and geometric constraints. Bmc Ecol; 2018, 18(1):6.

31. Sun M. Cangshan mountain chronicle. Kunming: Yunnan national publishing house; 2008.

32. Chase J M, Mcgill B J, Mcglinn D J, May F, Blowes S A, Xiao X, et al. Embracing scale-dependence to achieve a deeper understanding of biodiversity and its change across communities. Ecology Letters; 2018, 21: 1737-1751.

33. Magurran A E. Species Abundance Distributions: Pattern or Process?. Functional Ecology; 2010, 19(1):177-181.

34. He L X, Liu B H. Study on Diversity of Plant Community in Alpine-cold Meadow at Different Altitude. Chinese Qinghai Journal of Animal and Veterinary Sciences; 2005, 35(5):1-4.

35. Tao X, Cui S, Jiang S, Chu H, Li N, Yang D. Reptilian fauna and elevational patterns of
the reptile species diversity in Altay Prefecture in Xinjiang, China. Biodiversity Science; 2018, 26(6):578-589.

Figures

Figure 1

The elevational distribution of sampling sites.
Figure 2

Elevational distribution patterns of NTF OF in Gaoligong Mountain under two sampling pattern.
Figure 3

Elevational distribution patterns of NTF species numbers in Gaoligong Mountain under two sampling patterns.
Figure 4

The elevational distribution pattern of NTF in the smaller altitude range of sampling.
Figure 5

The elevational distribution pattern of NTF in Cangshan Mountain.
Figure 6

Elevational distribution patterns of NTF in two regions of 2300-3500m and their correlation.