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Functional diversity patterns reveal different elevations shaping Himalayan amphibian assemblages, highlighting the importance of morphologically extreme individuals

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ABSTRACT

Biological diversity is a concept that contains multiple facets, of which functional diversity is considered to be more integrative to describe the relationship between biodiversity and ecosystem functioning. In recent decades, functional diversity patterns have been assessed along elevational gradients in plants and birds. However, few empirical studies have been conducted to reveal amphibian functional diversity patterns between elevational zonations on mountains. In the present study, we investigated amphibian ecomorphologically functional diversity patterns in different elevational zonations of Nepal Himalaya. Our results indicated that functional space occupied by amphibian assemblages differed in size between elevational zonations, with assemblages in zonation-2 (500–1300 m) occupying the highest proportion. In terms of the position in functional space, assemblages in low elevational zonations (zonation 1 & 2) differed significantly from those in high elevational zonations (zonation 3–5). Distribution of individuals in the five assemblages along the six axes of the functional space was significantly different between most pairs of comparisons, while the difference varied between assemblages along axes. Interestingly, despite morphologically extreme amphibians accounted for small proportions of the total number of amphibians, they filled a large proportion of the six dimensional functional space. More importantly, most of the extreme amphibians were also the most functional uniqueness individuals, and they were widely distributed in all the zonations. Therefore, functional vulnerability of amphibian assemblages exists throughout all the elevational zonations. Accordingly, extreme amphibians with most functional uniqueness should be in particular well protected to maintain amphibian functional diversity. Further research can explore the mechanism underlying the unique amphibian functional diversity patterns in Nepal Himalaya.

1. Introduction

Biodiversity has strong positive effects on ecosystem functioning sparking the concerns of ecologists over hundreds of years (Eisenhauer et al., 2019). However, most previous studies only focused on taxonomic diversity despite biodiversity is a multifaceted concept (Gaston, 1996). Beyond taxonomic, phylogenetic diversity can reflect the evolutionary relationships among species, while functional diversity can describe the range and variability of species traits within a community (Zhao et al., 2018). Accordingly, phylogenetic diversity was used to evaluate the conservation priority areas (Forest et al., 2007; Mendoza and Arita, 2014), and functional diversity was used to better describe the association between human-induced perturbations and biodiversity (Mouillot et al., 2013; Zhao et al., 2019). Given that these two facets can provide complementary information to biodiversity, increasing studies started to quantify phylogenetic and in particular, functional diversity of animal communities at both global and regional scales (Su et al., 2019). For instance, Carmona et al. (2021) examined the geographical distribution

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of amphibian functional diversity, at a macroecological scale, and they found that larger species with slower pace of life were more threatened. And the extinction of threatened species can cause different effects on amphibian functional diversity across realms, probably because of a result of evolution within small units, high reliance on environmental conditions, and/or low dispersal abilities (Toussaint et al., 2021). At regional scales, functional diversity patterns were typically assessed between elevational zonations (or along elevational gradients), which has been widely tested in the animal communities such as birds (Montaño-Centellas et al., 2020). However, few empirical studies have been conducted to reveal the spatial patterns of amphibian functional diversity between elevational zonations on mountains (but see Sun et al., 2021a).

Indeed, amphibians play important functional roles in ecosystems, contributing to some key ecosystem processes. For instance, they can regulate food webs and biogeochemical cycles through predation and competition (Khatiwada et al., 2016). Typically, animal functional roles were reflected by their morphology (Sun et al., 2021a; Villéger et al., 2017). Although morphological traits cannot account for the entire range of functional roles, they can still reflect some key functions performed by animals. For instance, morphological traits of passerine birds were selected to indicate their diet, foraging substrate, and movements (Kennedy et al., 2020). Morphological traits of fish and tadpoles can provide information on their food acquisition and locomotion (Sun et al., 2021b; Zhao et al., 2017, 2014). Similarly, morphological traits of amphibian adults reflected their ability to prey on other species, their mobility, and their ability to defense against predation (Dalmolin et al., 2020; Trochet et al., 2014; Tsianou and Kallimanis, 2016). These traits were biological features related to their contribution to the regulation of food webs and biogeochemical cycles in ecosystems (Oliveira et al., 2017; Zhao et al., 2018). For instance, body size is associated with amphibian nutrient transport capacity, while arm length is related to the burrowing behavior regulating the soil properties (Oliveira et al., 2017). However, the distribution patterns of amphibian morphological traits between elevational zonations were still rare studied.

This is especially true in Nepal Himalaya, where is one of the biodiversity hotspot regions all over the world (Khatiwada et al., 2019). Nepal Himalaya has the world's highest mountain range, in which supports quantitative endemic amphibian species, as well as high diversity of amphibians (Khatiwada et al., 2019). Previous studies have described amphibian community structures in this area, indicating that amphibian species richness declined linearly along an elevational gradient (Khatiwada et al., 2019). Moreover, amphibian assemblages were determined by environmental variables such as surface area and above-ground net primary productivity (represented by normalized difference vegetation index; Khatiwada et al., 2019). Nevertheless, empirical studies are still missing in terms of amphibian ecomorphological functional diversity patterns between elevational zonations in Nepal Himalaya, which can provide important information to biodiversity conservation.

Furthermore, species distribution within traits space (i.e., a multidimentional space created by synthetic axes capturing combined traits; Maire et al., 2015) can also describe their ecological strategies (Su et al., 2019). For animals, traits distribution is typically unimodal at the global scales. Species with general morphological traits values tend to concentrate in the core area, while species with extreme morphological traits values tend to occupy the tails (Carmona et al., 2021). Specifically, the ecomorphologically extreme species exhibit unique strategies in response to environmental conditions (Raffard et al., 2017). Therefore, identification of ecomorphologically extreme species was distinguished in vertebrates to better protect them (e.g., Carmona et al., 2021; Mouillot et al., 2014; Ricklefs, 2012; Toussaint et al., 2016). And the relative contribution of ecomorphologically extreme species (i.e., the percentage of traits space filled by extreme species) to the traits space can be considered as a measure of functional vulnerability of the studied area. Using this approach, Mouillot et al., (2014) reveled that many functions of global coral reef fish were highly vulnerable as these species tended to disproportionately pack into a few particular functions. And Su et al., (2019) reported that functional vulnerability of freshwater fish existed throughout the world, suggesting more attention should be paid to ecomorphologically extreme fish. However, these studies only focused on global scales, studies at the regional scales are still needed to better understand the ecological importance of ecomorphologically extreme species, as well as the general patterns of animals traits distribution between elevational zonations on mountains. More importantly, since some other studies argued that functional vulnerability should also be summarized by functional uniqueness of species (Carmona et al., 2021; Toussaint et al., 2016), identifying the combined information of species traits distribution patterns and species functional uniqueness can help us better recognize the conservation priority of species, and to maintain the functional diversity (Carmona et al., 2021).

In the present study, we investigated amphibian ecomorphologically functional diversity patterns in different elevational zonations of Nepal Himalaya. Specifically, we 1) compared the functional differences of amphibian assemblages between elevational zonations to reveal the existence of functional distinctiveness or convergence across elevational zonations; and 2) calculated the proportion of traits space filled by morphologically extreme amphibians, and identified extreme amphibians with most functional uniqueness to assess the functional vulnerability in each elevational zonation.

2. Materials and methods

2.1. Study area

This study was conducted in the eastern, central, and western part of Nepal Himalaya, extending between 80°04′ − 88°12′ E and 26°22′ − 30°27′ N (Fig. 1). Geographically, Nepal lies in the central part of the Himalayas and can be divided into three major east–west running mountain ranges: the Siwalik Range (1000–1500 m), the Mahabharat range (Lesser Himalaya; 1500–3000 m), and the Greater Himalaya (5000–8000 m; (Hagen, 1969; Manandhar, 2002). Nepal is also characterized by rugged terrain and high climatic variability through seasonal and elevational scales. At the seasonal scale, summer monsoonal rainfalls from Bengal Bay strongly affect the seasonal temperature and precipitation in this region (Bookhagen and Burbank, 2010). The mean annual temperature varies between 7 °C and 26 °C, and most of the precipitation occurs (about 80%) during May to October (Vetaas, 2000). Climate factors also vary at the spatial scale along an elevation gradient, with mean annual temperature declining linearly from 23.4 °C to 7 °C. Mean annual precipitation increases from 1000 to 2600 mm between the lowlands and the summit areas. Therefore, there could be several climatic zonations from subtropical to alpine climate along an elevational gradient (Bhattarai et al., 2004; Carpenter, 2005), with distinct vegetation cover in each climatic zonation. Specifically, field work in eastern Nepal Himalaya was conducted in the catchment of the Koshi basin from May to July in 2014 and 2015, coinciding with the rainy season. A total of 79 transects were established and sampled along the Arun River and the Tamor River, covering an elevational gradient from 78 m to 4200 m. Since Koshi Tappu Wild Reserve, Kanchenjunga Conservation Area, and Makalu Barun National Park are located at this area, amphibians are relatively well protected. Field work in central Nepal Himalaya was carried out in Gandaki river basin from May to July in 2017. Amphibians can be detected in 17 transects located along Marsyandi River, with the elevation changing from 350 m − 4220 m. This river lies in the Annapurna Conservation Area, which is one of the major tourist destinations in Nepal (NTNC, 2020), causing the low abundance of amphibians in the field. In Western part of Nepal Himalaya (Mahakali River basin), a large proportion of landscapes has been transformed over a long period of time by anthropogenic activities such as hydroelectric projects, cultivation, grazing, and extraction of natural resources. These human-induced activities strongly reduced the diversity and quantities of

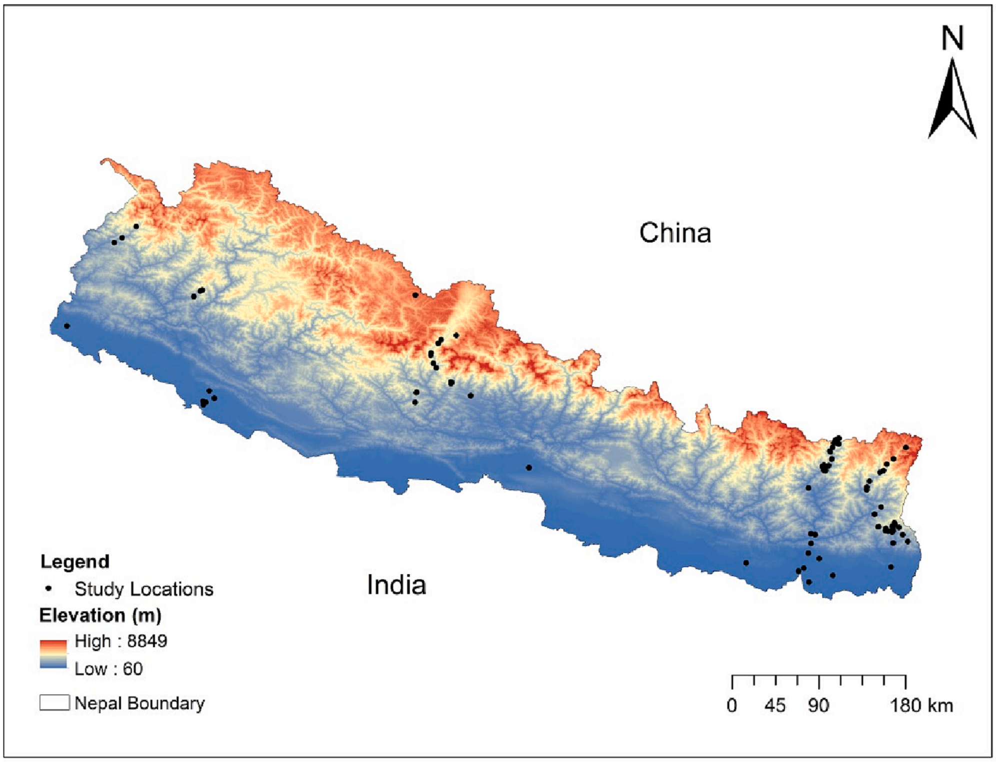

Fig. 1. The map of the study area. Black solid dots indicate the transects.

amphibians, and we only found amphibians in 12 transects during the field work from May to July in 2018. These transects were established along the Mahakali River, covering the elevational gradients from 178 m to 3575 m.

2.2. Amphibians sampling

Amphibians were sampled using the combination of nocturnal time constrained visual encounter and acoustic aids surveys, which were effective methods to cover entire amphibian community including terrestrial, arboreal, aquatic, as well as fossorial and even well-camouflaged species. Specifically, the searches were conducted in the transects from low to high elevations using 220 lm troches between 19:00 h and 23:00 every night. Each transect was sampled only once, and one to three transects were sampled per night during the sampling processes. Details of the sampling protocols have been provided in Khatiwada et al., (2016). All the individuals encountered were captured in the field with the help of native field guides. These individuals were then taken to a nearby dry place where they were identified to species and measured for a set of morphological traits. For individuals that can be identified to species by external morphological traits, they were released back into their original habitats. For individuals that were difficult to identify in the field, they were euthanized by chlorobutanol solutions, tissue sample collected from thigh muscle, which were preserved into 95% ethanol immediately. After that, these individuals were fixed in 4% formalin for 24 h, and then were preserved in 70% ethanol. DNA barcoding analyses based on a fragment of the mitochondrial 16S rRNA gene were used for species identification in the laboratory.

2.3. Functional traits

Based on published literatures (e.g., Dalmolin et al., 2020; Sun et al., 2021a; Trochet et al., 2014; Tsianou and Kallimanis, 2016; Zhao et al., 2022), we selected 15 eco-morphological traits as amphibian functional traits, which can reflect three main functions of amphibians displayed in ecosystems (i.e., food acquisition, defense against predation, and mobility). These traits were calculated from 15 external morphological measurements, including snout vent length (SVL), head length (HL), head width (HW), snout length (SL), eye diameter (ED), eyelid-naris distance (ENL), upper eyelid width (UEW), inter orbital width (IOW), internarial distance (IND), tympanum diameter (TYD), arm length (FAL), hand length (HAL), hindlinmb length (THL), tibia length (SHL), and foot length (FOL; Supporting Information Table S1). These morphological measurements were conducted using a digital caliper (to the nearest 0.01 mm). After been measured, these traits (except SVL) were scaled by SVL to reduce the trivial correlation with body size (Villéger et al., 2010; Winemiller, 1991).

2.4. Elevational zonations

Elevational zonations were determined based on the climatic factors of transects. This is because the main vegetation cover can be varied with the change of climate along an elevational gradient on mountains, which can subsequently affect the distribution of amphibian species (Khatiwada et al., 2019; Zhu et al., 2020). Specifically, 19 climatic variables (Supporting Information Table S2)were extracted from Worldclim website (http://www.worldclim.com/) based on the GPS location of each transect. These variables were subsequently used to conduct a Principal Components Analysis, followed by a K-means cluster analysis (conducted using "*NbClust*" function from the "*NbClust*" package in R; Charrad et al., 2014; R Development Core Team, 2011) to determine the climatic zonations, which were considered as different elevational zonations in the present study.

2.5. Statistical analyses

Quantitative studies have been conducted to reveal the importance of intraspecific variability in functional traits studies (e.g., Albert et al., 2012; Cianciaruso et al., 2009; Zhao et al., 2017; Zhao et al., 2014). And they suggested that a shift from "species level" to "individuals level" in functional ecological studies can be stronger to understand ecosystem processes (Bolnick et al., 2011; Violle et al., 2012). Therefore, we followed this claim in the present study, and considered each amphibian individual as a distinct functional entity to do further analyses. Fifteen functional traits from all the individuals sampled in the field were standardized by the SVL values, which were then used to compute a principal component analysis (PCA). Following Maire et al. (2015), the first six PCA axes were selected to create a multidimensional functional space as they can faithfully represent the trait-based functional distance between amphibians, and each individual was ordered in this space for following analyses. The size of functional space occupied by amphibian assemblages in each zonation were calculated by the volume of the convex hull (i.e., observed functional diversity) based on the six trait combinations axes. To test whether observed functional diversity of a zonation was significantly different from functional diversity of a random subset of amphibian assemblages, we used null models based on a random selection of the same number of individuals in each zonation from the regional pool (999 iterations). The standardized effect size (SES) was used to measure the difference between observed functional diversity (FDobs) and random functional diversity (FDrand) from the null model: SES = (FDobs − mean (FDrand))/sd (FDrand). The significance of the difference from null expectations was tested using a two-tailed test ($\propto < 0.05$). Accordingly, the assemblage was considered as functionally clustered when FDobs was lower than 97.5% of the FDrand values ($P < 0.025$). And the assemblage was considered as functionally overdispersed when FDobs is greater than 97.5% of the FDrand values ($P > 0.975$; Toussaint et al., 2016). The distribution of individuals coordinates on the first six PCA axes between elevational zonations was illustrated and compared using transformed raincloud plots and Kolmogorov–Smirnov tests, respectively. PERMANOVA and PERMDISP2 tests were then conducted to compare the average and the variance distributions of individuals in the six-dimensional functional space between the classified elevational zonations.

To calculate the proportion of traits space filled by morphologically extreme amphibians, we followed the definitions from Su et al. (2019) to define the 2.5% of the individuals with the lowest (and the highest) values on each of the six PCA axes as the morphologically extreme individuals (MEI), and the 0.5% of the individuals with the lowest (and the highest) values on each of the six PCA axes as the most morphologically extreme individuals (MMEI). The rest individuals with their trait values among the 95% closet to that of the average among all individuals were defined as the morphologically core individuals (MCI). Finally, the contributions of MEI and MMEI to the value ranges of the six PCA axes and to the six dimensional functional space in each elevational zonation were calculated. Similarly, to test the significance of the observed functional diversity occupied by MEI/MMEI, we also designed null models based on a random selection of the same number of MEI/MMEI in each zonation (999 iterations). A P-value lower than 0.025 indicated that MEI/MMEI was functionally distinct and under-contributed to functional diversity, whereas a P-value higher than 0.975 indicated that MEI/MMEI was over-contributed to functional diversity.

Functional uniqueness of each individual was computed as the functional distance to the nearest neighbor of the individual of interest. Functional uniqueness reflected how “isolated” an individual is in the regional pool. Here we used the Euclidean distance in the six-dimensional functional space to represent the values. Finally, we matched the identities of the first 50 and 233 (same number to the individuals of MMEI/MEI) most functional uniqueness individuals to the MMEI and MEI.

All statistical analyses were performed in R 3.6.1 (R Development Core Team, 2011). The volume of functional space was computed using “*convhulln*” function from the “*geometry*” package (Roussel et al., 2020). “*pairwise.perm.manova*” function from the “*RVAideMemoire*”, and “vegdist”, “*betadisper*” functions from the “*vegan*” package were used to produce the Euclidean distance matrix and conduct the PERMANOVA and PERMDISP2 tests, respectively (Oksanen et al., 2010). The “*ks.test*” function from the “*stats*” package was used to conduct the Kolmogorov–Smirnov tests (R Development Core Team, 2011).

3. Results

Five elevational zonations were determined based on the K-means cluster analyses, including zonation-1 (<500 m), zonation-2 (500–1300 m), zonation-3 (1300–2000 m), zonation-4 (2000–2700 m), and zonation-5 (>2700 m; Supporting Information Fig. S1). In total, 970 individuals were obtained and measured in our field sampling activities. These individuals belonged to 33 species and 6 families, with each species containing 27.7 ± 30.1 individuals. We grouped these sampled individuals into five assemblages based on their distribution in the five elevational zonations. As a result, we found 462 individuals belonging to 20 species and 5 families in zonation-1; 151 individuals belonging to 16 species and 6 families in zonation-2; 157 individuals belonging to 17 species and 5 families in zonation-3; 160 individuals belonging to 8 species and 5 families in zonation-4, and 40 individuals belonging to 5 species and 3 families in zonation-5 (Table 1).

The first six PCA axes explained 70.2% of the total variance among the amphibians in Nepal Himalaya (PC1 = 25.2%, PC2 = 11.5%, PC3 = 10.8%, PC4 = 8.9%, PC5 = 7.4%, PC6 = 6.4%). Specifically, PC1 was mainly contributed by traits related to mobility (e.g., hindlimb length, tibia length, and foot length), with positive values indicating higher values of hindlimb, tibia, and foot on this axis. PC2 was strongly determined by head width and inter orbital width, and the positive values on this axis indicated higher values of these two traits. PC3 was also strongly influenced by traits related to food acquisition, such as snout vent length, head width, eyelid-naris distance, and internarial distance. PC4 showed a strong contribution of snout vent length and eyelid-naris distance. PC5 was mainly determined by upper eyelid width and tympanum diameter. PC6 was strongly contributed by head length and head width, and therefore codes for food acquisition (Supporting Information Table S3).

3.1. Differences in functional diversity between elevational zonations

Observed functional space occupied by the five amphibian assemblages differed in size. Specifically, assemblage in zonation-1 occupied 19.2% of the whole functional space. And assemblages in zonation-2 to zonation-5 occupied 21.7%, 9.9%, 3.2%, and 0.3% of the whole functional space, respectively (Fig. 2). The results of the null models and SES values indicated that most of the observed functional diversity values in the zonations were significantly lower than the expected values (except zonation-2, of which the observed value was higher than the expected value; Fig. 3, Supporting Information Table S4).

The results of PERMANOVA tests indicated that the position in functional space occupied by amphibian assemblages in each zonation was significantly different from each other. However, based on the PERMDISP2 test, the significant differences in the variance of

Table 1
The number of individuals, species, and families, as well as the percentage they accounted for the total number of individuals, species, and families in entire dataset.

	Number of individuals (%)	Number of species (%)	Number of families (%)
Zonation-1	462 (47.6%)	20 (60.6%)	5 (83.3%)
Zonation-2	151 (15.6%)	16 (48.5%)	6 (100.0%)
Zonation-3	157 (16.2%)	17 (51.5%)	5 (83.3%)
Zonation-4	160 (16.5%)	8 (24.2%)	5 (83.3%)
Zonation-5	40 (4.1%)	5 (15.2%)	3 (50.0%)

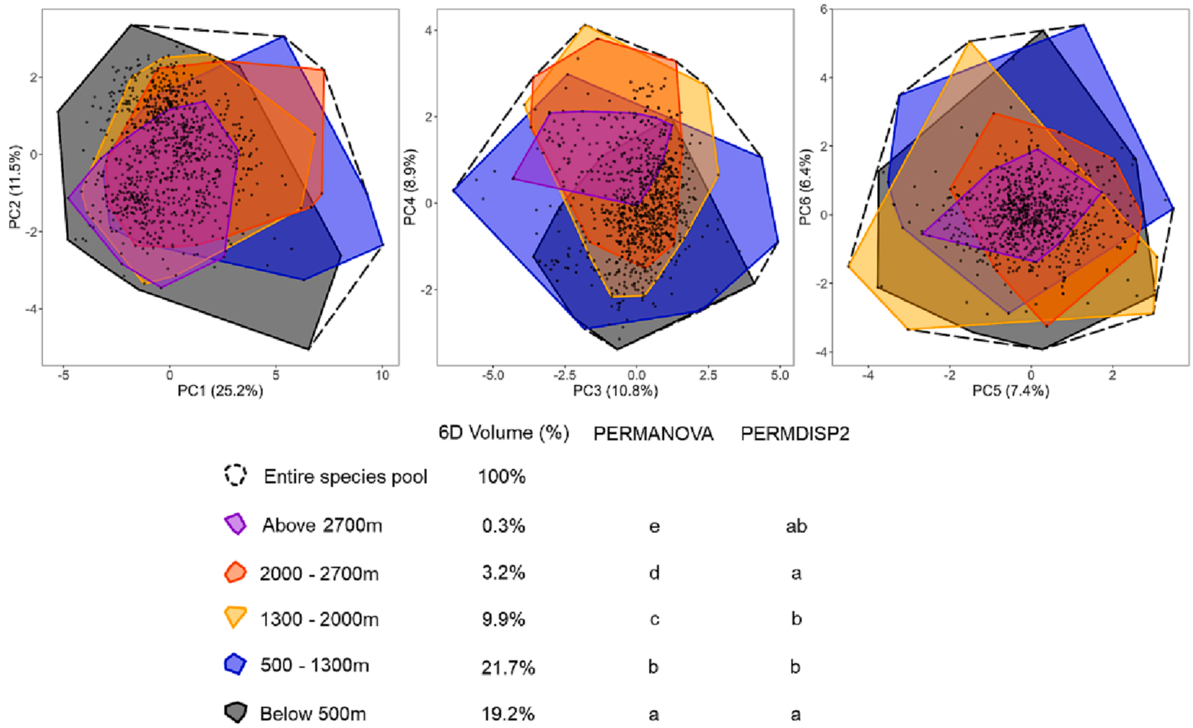

Fig. 2. Distribution of different amphibian assemblages in the six-dimensional functional space in Nepal Himalaya. The values below show the percentage of the six-dimensional functional space volume occupied by each amphibian assemblages. Different letters indicate the significant difference of the average and the variance distributions between amphibian assemblages compared by PERMANOVA and PERMDISP2 tests, respectively.

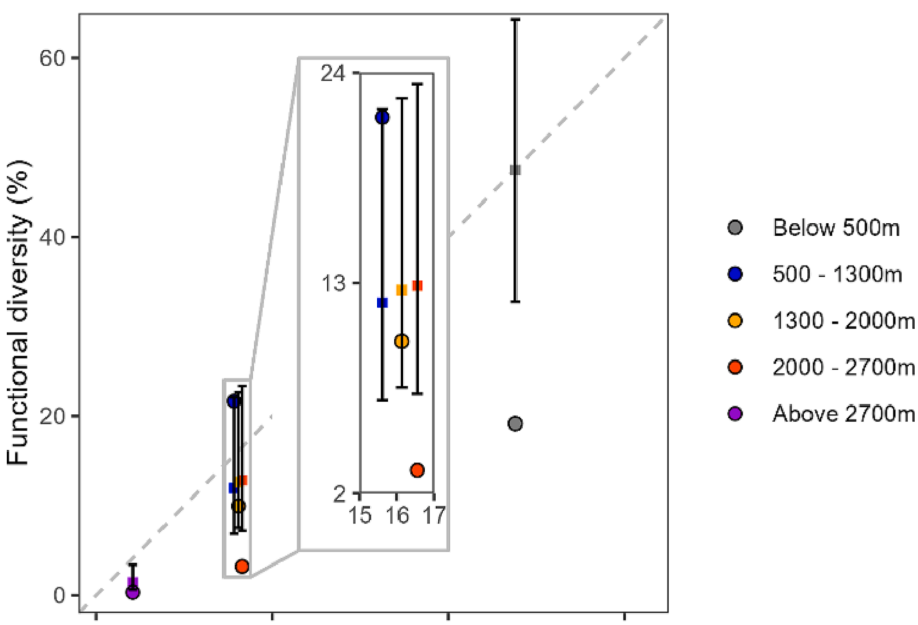

Fig. 3. The number of individuals and functional facets of amphibian diversity in the five zonations. Functional diversity and the number of individuals are presented as the percentage of the total functional and total number of individuals of amphibians. The effects of the number of individuals on the functional diversity is expressed as the mean (colored squares) and associated 95% confidence interval (black whiskers), which is computed by null models with 999 values. The null models can simulate the amphibian assemblages in each zonation by randomly sampling in the regional pool of amphibians. Color circles represent the observed values. Observed functional diversity values above and below the dashed line indicate a significant functional overdispersion and a significant functional clustering, respectively. The dashed line represents the identity line functional diversity = the number of individuals. Different colors represent different zonations.

assemblage distributions were only detected between zonation-1 and 4, and between zonation-2 and 3 (Fig. 2). Distribution of individuals in the five different assemblages along the six axes of the functional space was significantly different between most pairs of comparisons, while the difference varied between assemblages along axes (K–S tests, $P < 0.05$; Fig. 4, Supporting Information Table S5). Specifically, assemblage in zonation-1 differed significantly from that in zonation-2 along PC1 – PC4, in zonation-3 along PC1, PC2 and PC4 – PC6, in zonation-4 along all the six axes, and in zonation-5 along PC3 – PC5. Moreover, assemblage in zonation-2 differed significantly from that in zonation-3 along PC2 – PC4, in zonation-4 along PC3 – PC6, in zonation-5 along PC1, PC3, and PC4. In addition, assemblage in zonation-3 differed significantly from that in zonation-4 along PC3 – PC6, in zonation-5 along PC3, PC4, and PC6. Finally, assemblage in zonation-4 differed significantly from that in zonation-5 along PC1, PC3, PC5 and PC6.

3.2. Morphologically extreme individuals between elevational zonations

Of the 970 individuals considered in the present study, 233 individuals (24.0% of the total number of individuals) were identified as

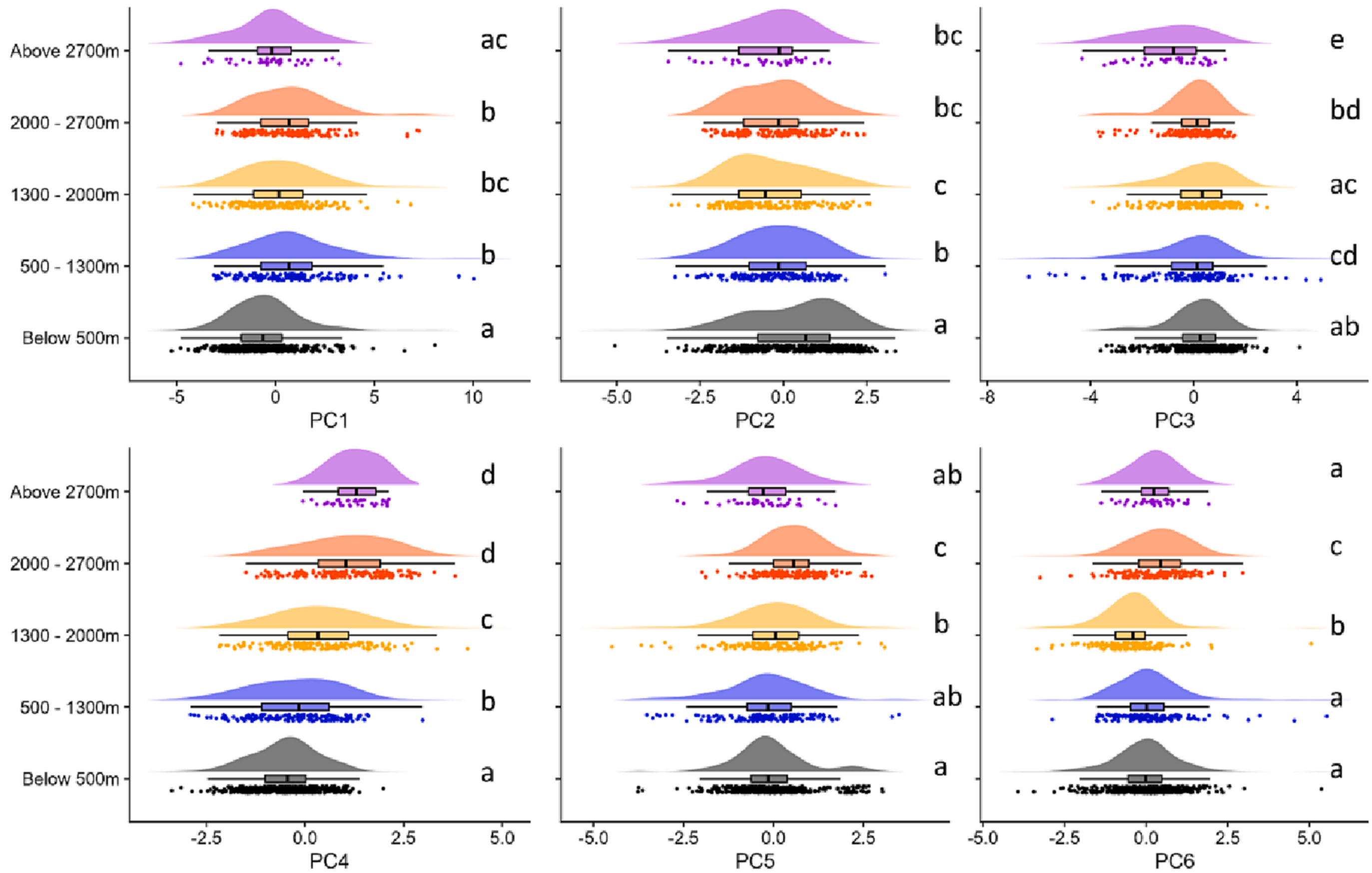

Fig. 4. Density distribution of amphibian traits on the first six PCA axes in each elevational zonation. Significant difference compared by Kolmogorov–Smirnov tests are shown beside each plot item.

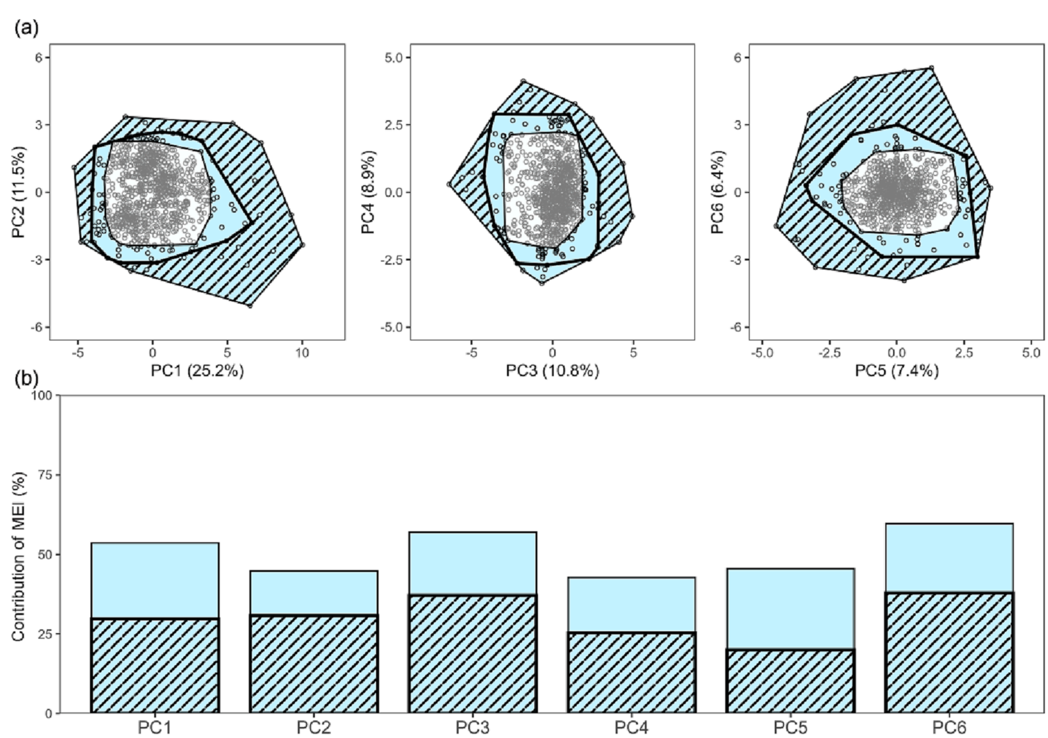

Fig. 5. Contributions of morphologically extreme amphibians to functional diversity in Nepal Himalaya. (a) The whole convex hull, including 970 individuals, is delimited by the external black line. Blue-filled areas and hatched parts show the functional space volume filled by MEI and MMEI, respectively. The white-filled areas represent the functional space volume filled by MCI. (b) The contributions of MEI (blue) and MMEI (hatched) to the first six PCA axes, respectively.

the MEI. These individuals belonged to 28 species, but most of them were concentrated into several species such as *Nanorana liebigii*, *Duttaphrynus melanostictus*, *Hoplobatrachus tigerinus*, *Microhyla nilphamariensis*, *Euphlyctis kalasgramensis*, *Minervarya nepalensis*, *Duttaphrynus himalayanus*, and *Nanrona blanfordii*. 50 individuals (5.2% of the total number of individuals) belonged to 21 species were identified as the MMEI, of which four species contained $\geq$ 4 individuals (i.e., *D. melanostictus*, *E. kalasgramensis*, *M. nepalensis*, and *N. liebigii*). Moreover, MEI filled more than 40% of the range of each PCA axis. This value is even up to 59.6% for PC6, which was mainly determined by head length and head width. In terms of MMEI, they filled more than 25% of the range of each PCA axis (except PC5 = 19.98%). Overall, MEI and MMEI filled a large proportion of the six dimensional functional space (92.1% and 73.8%, respectively), while the MCI only packed into the rest 7.9% of the functional space (Fig. 5; Table 2). The results of the null models and the SES values indicated that the observed functional diversity occupied by MEI/MMEI was significantly higher than expected values in almost all the zonations (Supporting Information Table S4).

MEI and MMEI were distributed in all of the five zonations. However, most of MEI was concentrated in zonation-1 (107), which accounted for 23.2% of the total number of individuals in zonation-1. The number of MEI in zonation-2, zonation-3, zonation-4, and zonation-5 was 38, 36, 38, and 14, respectively, and they accounted for 25.2%, 22.9%, 23.8%, and 35.0% of the total number of individuals in each zonation, respectively (Table 3). The contributions of MEI to the range of each PCA axis in zonation-1 and zonation-2 exceeded 35% (except PC4 in zonation-1). And MEI also had important contributions to the range of some PCA axes in zonation-3, zonation-4, and zonation-5. Likewise, MEI filled more than 77% of the six dimensional functional space in each zonation (Fig. 5). For the distribution of MMEI, five zonations contained 19, 14, 10, 4, and 3 individuals, respectively, from low to high elevations, and they accounted for 4.1%, 9.3%, 6.4%, 2.5%, and 7.5% of the total number of individuals in each zonation, respectively (Table 3). The contribution of MMEI to the range of each PCA axis in zonation-2 exceeded 26%. And they also exhibited important contributions to PCA axes in other zonations. In addition, MMEI filled more than 28% of the six dimensional functional space in each zonation, and this number was even up to 80.4% in zonation-2 (Fig. 6).

Among the first 50 most functional uniqueness individuals (the same number to the individuals of MMEI), 28 individuals (56.0%) were MMEI. These individuals belonged to 17 species and six families, such as four *Duttaphrynus melanostictus*, four *Euphlyctis kalasgramensis*, and three *Megophrys zhangi*. They were widely distributed in all the zonations, including ten in zonation-1, nine in zonation-2, five in zonation-3, three in zonation-4, and one in zonation-5 (Supporting Information Table S6). When focusing on the first 233 most functional uniqueness individuals (the same number to the individuals of MEI), 131 individuals (56.2%) were MEI. These individuals belonged to 27 species and six families, such as 16 *Duttaphrynus melanostictus*, 14 *Minervarya nepalensis*, 12 *Nanrona blanfordii*, and 10 *Duttaphrynus himalayanus*. They were also distributed in all the zonations, including 43 in zonation-1, 34 in zonationa-2, 26 in zonation-3, 17 in zonation-4, and 11 in zonation-5 (Supporting Information Table S6).

4. Discussion

This study revealed that the different functional diversity patterns between elevational zonations can shape the distinct Himalayan amphibian assemblages, and highlighted the importance of morphologically extreme individuals. Our observations are consistent with previous studies reporting that more amphibians distribute in tropical areas, probably because these places have greater historical lineages and higher environmental heterogeneity than temperate and cold zones (Jansson et al., 2013; Stevens, 2011). However, despite 47.6% of the individuals were obtained in zonation-1, they occupied smaller functional space than those in zonation-2 (500–1300 m), which only contained 15.6% of the total number of amphibians. This indicated that individuals in zonatin-1 were less functional divergent compare to zonation-2. Moreover, amphibians in zonation-3 and zonation-4 occupied only 9.9% and 3.2%, respectively, of the total functional space, despite similar number of amphibians were sampled in these two zonations (157 and 160 individuals, respectively) when comparing with that in zonation-2 (i.e., 151 individuals). This could be attributed to some species that were only distributed in zonation-2, such as *Microhyla nilphamariensis*, *Minervarya pierrei*, *Minervarya syhadrensis*, *Polypedates teraiensis*, and *Sphaerotheca maskeyi*. Amphibians cannot easily disperse across their distribution range because of their thermal tolerance ability (Khatiwada et al., 2020b). Species within each zonation were involved in independent evolution, leading to distinct ecological niche utilization and functional traits (e.g., habitat; Khatiwada et al., 2019; Zhu et al., 2020). For instance, *P. teraiensis* was only found in the bushes just above the temporary or permanent pools and rice fields between 150 and 800 m, while *Amolops mahabharatensis* was only detected in the torrent habitat with high canopy cover between 700 and 1400 m (Khatiwada et al., 2021; Khatiwada et al., 2020a). Forty individuals belonging to five species were detected in zonation-5, which occupied extremely low percentage (0.3%) of the total functional space, suggesting that these amphibians (both within and between species) were functional similar entities. This is not surprise, as the extreme environmental conditions (e.g., low temperature, low vegetation coverage, and high Ultraviolet-B radiation) in area >2700 m only allowed some specific amphibians to live in (Khatiwada et al., 2019; Sun et al., 2021b). When controlling the effects of the number of individuals, the results showed that most of the observed functional diversity patterns were different from the expected patterns from the null models. And functional clustered was observed in most of the zonations except zonation-2. These results supported the

Table 2
Contributions of MMEI and MEI to each PCA axis and six-dimensional functional space in each zonation and the regional pool, respectively. MMEI: most morphologically extreme individuals. MEI: morphologically extreme individuals.

	Zonation-1 (%)	Zonation-2 (%)	Zonation-3 (%)	Zonation-4 (%)	Zonation-5 (%)	Total (%)
MMEI – PC1	32.3	34.8	8.6	5.8	14.5	29.7
MMEI – PC2	30.7	29.0	3.7	0.0	13.0	30.7
MMEI – PC3	16.4	42.5	0.0	0.0	15.6	37.1
MMEI – PC4	13.8	26.7	24.3	17.0	0.0	25.3
MMEI – PC5	21.7	29.7	15.9	0.0	0.0	20.0
MMEI – PC6	37.4	36.2	41.9	23.9	0.0	37.9
MMEI – 6D functional space	58.5	80.4	58.8	29.4	28.5	73.8
MEI – PC1	46.7	47.0	39.4	33.3	28.8	53.5
MEI – PC2	44.8	36.0	24.3	13.4	26.7	44.8
MEI – PC3	37.9	58.0	30.2	15.4	29.3	57.0
MEI – PC4	25.5	37.5	37.4	29.7	0.0	42.8
MEI – PC5	36.1	46.0	49.6	30.6	18.7	45.5
MEI – PC6	60.2	60.8	63.3	43.5	19.3	59.6
MEI – 6D functional space	86.4	92.4	82.3	78.0	82.7	92.1

Table 3
The number of MEI/MMEI, species of MEI/MMEI, and families of MEI/MMEI, as well as the percentage (in parentheses) they accounted for the total number of individuals, species, and families in each zonation.

	No. of MEI (%)	Species of MEI (%)	Families of MEI (%)	Number of MMEI (%)	Species of MMEI (%)	Families of MMEI (%)
Zonation-1	107 (23.2%)	16 (80.0%)	5 (100.0%)	19 (4.1%)	9 (45.0%)	4 (80.0%)
Zonation-2	38 (25.2%)	12 (75.0%)	6 (100.0%)	14 (9.3%)	6 (37.5%)	5 (83.3%)
Zonation-3	36 (22.9%)	12 (70.6%)	5 (100.0%)	10 (6.4%)	6 (35.3%)	4 (80.0%)
Zonation-4	38 (23.8%)	5 (62.5%)	3 (60.0%)	4 (2.5%)	3 (37.5%)	2 (40.0%)
Zonation-5	14 (35.0%)	5 (100.0%)	3 (100.0%)	3 (7.5%)	3 (60.0%)	3 (100.0%)

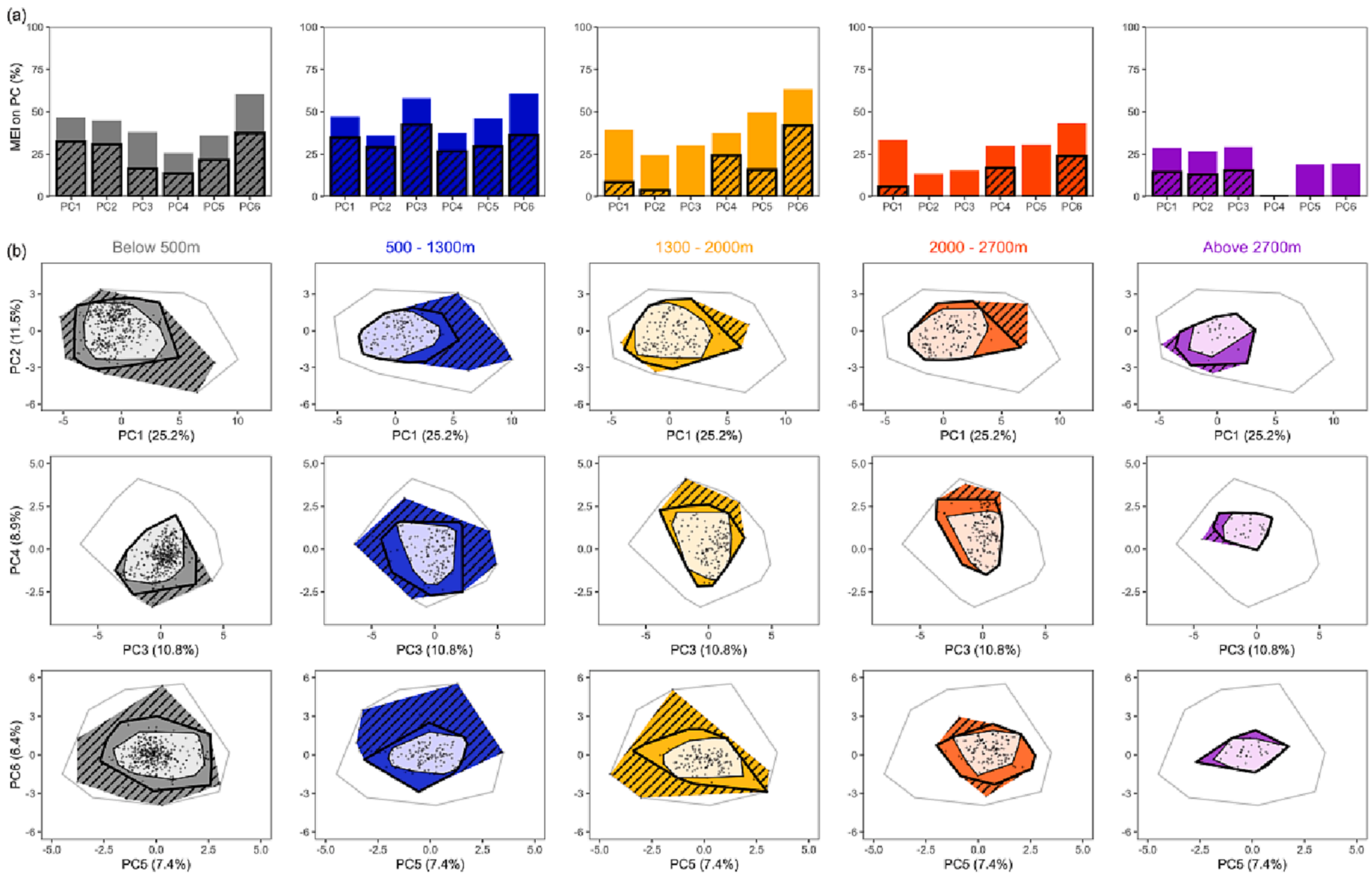

Fig. 6. Contributions of morphologically extreme amphibians to functional diversity in each elevational zonation. (a) Bars show the contributions of MEI to each PCA axis. The hatched parts of the bars show the contributions of MMEI. (b) The whole convex hull contributed by 970 individuals is in grey. Colour-filled areas and hatched parts represent the functional space volume filled by MEI and MMEI, respectively, in each elevational zonation. The white filled areas represent the functional space volume filled by MCI. Black dots indicate the individuals sampled in each elevational zonation.

hypothesis of environmental filtering that shaped the amphibian assembly processes in the studied area (Zhao et al., 2022; Sun et al., 2023).

Interestingly, amphibians' traits were gradually shifted to functional space with lower PC1 and higher PC4 values from low to high elevations. This demonstrated that amphibians can have shorter hindlimb, shorter tibia length, shorter foot length, but larger snout vent length and eyelid-naris distance with the increasing of elevations. One can expect that amphibians inhabited higher elevational habitats should have weaker locomotivity, but larger size and stronger defense ability (e.g., *S. ghunsa* and *Duttaphrynus himalayanus*). Functional distinctiveness can be also attributed to the environmental conditions of high elevations (e.g., low temperature). For instance, amphibians in colder habitats could have larger body size based on Bergman rules (Liao and Lu, 2012). Moreover, weaker locomotion ability induced them to be inactive animals, which can help them to adapt to extreme conditions in high elevational areas by reducing metabolism and energy consumption (Kiss et al., 2009). When considering the distribution of trait values for each PCA axis, we found that amphibian ecomorphological functional traits were continuously distributed, showing a unimodal pattern for all the PCA axes. These results were consistent with the trait distribution patterns of all the vertebrates at the global scale (Carmona et al., 2021). However, these observations were contrast to plants, which had a bimodal pattern of trait distribution (Díaz et al., 2016). Therefore, vertebrates should exhibited distinct ecological strategies comparing with plants to adapt to the living environment.

Based on the distribution of traits, we found 233 individuals belonging to 28 amphibian species were MEI, while 50 individuals belonging to 21 species were MMEI. These results indicated that MEI and MMEI were widely existed in many amphibian species, suggesting that the neglected of intraspecific traits variability (i.e., using species mean traits values) in functional studies could cause bias in identifying the real extreme amphibians (Raffard et al., 2019). However, MEI and MMEI were concentrated into several species, in particular *D. melanostictus*, *E. kalasgramensis*, *M. nepalensis*, and *N. liebigii*. Interestingly, all of these were widely distributed species (Khatiwada et al., 2021), which were easily sampled in the field (117, 117, 54, and 83 individuals obtained in our field work). These results thus supported the previous findings demonstrating that individual specialization in traits was widely existed within species to reduce intraspecific competition (Zhao et al., 2014). More importantly, it provided evidences showing that habitat with specific environment was not the unique driver, whereas individual specialization also could be a considerable factor for the formation of MEI and MMEI.

MEI and MMEI filled a large proportion of the functional space, despite the number of them is limited. And this proportion is significantly higher than the expected in almost all the zonations based on null models. All of these above results suggested that the richness of functional space was mainly supported by some extreme amphibians (Carmona et al., 2021), and the extinction of them can cause dramatically loss of amphibian functional diversity. Importantly, about 60% of the MEI and MMEI were overlapped with the most functional uniqueness individuals, suggesting that MEI and MMEI supported not only the most part of the functional diversity, but also a large part of functional uniqueness. Since both functional uniqueness and extreme traits are important to estimate the functional vulnerability of assemblages (Carmona et al., 2021; Ripple et al., 2017; Toussaint et al., 2016), we argue that MEI and MMEI with the most functional uniqueness identified in the present study should be paid more attention. After considering the small population, narrow distribution, and weak thermal tolerance, we suggested that species like *S. ghunsa*, *Polypedates taeniatus*, and *Uperodon globulosus* should be the priorities to conduct protecting activities in future studies. In addition, MEI and MMEI were widely distributed in all of the five elevational zonations in Nepal Himalaya. However, their relative abundance varied between zonations, and the highest value occurred in zonatian-5 (Table 3). This can be attributed to the amphibians with specific traits to adapt to the extreme environmental conditions in high elevational locations of mountains (Wang et al., 2022). Therefore, habitats >2700 m in Nepal Himalaya should be in particular well protected to support the abundance and richness of extreme amphibians.

Overall, the present study quantified the functional diversity of amphibians in Nepal Himalaya. Our results indicated the existence of functional distinctiveness between elevational zonations, which may be driven by the diverse of environmental conditions in each zonations. Accordingly, environmental filtering should be the mechanism underlying the amphibian assembly processes in the studied area. Moreover, such functional distinctiveness was also strongly contributed by morphologically extreme amphibians located in each zonation. More importantly, extreme amphibians with most functional uniqueness should be more concerned in future studies and conservation activities. However, since functional diversity was calculated only based on ecomorphological traits in the present study, other traits could be incorporated in future studies.

5. Data accessibility statement

Data used in this study are uploaded in Figshare (https://figshare.com/s/c5483fd6e5fd4d6983b2).

CRediT authorship contribution statement

Tian Zhao: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **Guohuan Su:** Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Validation, Writing – original draft, Writing – review & editing. **Jianping Jiang:** Funding acquisition, Project administration, Investigation, Resources. **Na Li:** Data curation. **Chunlin Zhao:** Investigation. **Zijian Sun:** Investigation. **Janak Raj Khatiwada:** Data curation, Investigation, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have sshared a link to my data in the maintext.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110260.

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