

## **Supplementary Information**

### **Supplementary Materials and Methods**

#### **Study area**

This study was conducted on Tianping Mountain, northwest Hunan Province, China (N29.714072°–29.787100°, E109.906154°–110.170800°), which includes a core region of the Badagongshan National Nature Reserve. This area supports a rich biodiversity, including approximately 1 500 animal species and 2 300 plant species (Zhu et al., 2020a). Therefore, it is considered a “natural museum” and a “world rare species gene pool”. The study area is ~20 000 ha, three quarters of which is covered by forests (Qiao et al., 2015). The elevation of this region is between 200 to 2 700 m, with the main vegetation changing from crops, evergreen broadleaf forests, to evergreen deciduous broadleaf forests from low, mid, to high elevations (Xiong et al., 1999). This region belongs to the north subtropical monsoon climate. The mean annual temperature is 11.5 °C, and the mean annual precipitation ranges from 2 105 to 2 840 mm (Chen & Li, 2003).

#### **Data acquisition**

Ten transects along an elevational gradient were randomly selected (Supplementary Figure S1). These transects were 200 m long and 2 m wide and were placed near stream tributaries. To reduce spatial correlation, all transects were separated by a

minimum distance of 1.5 km, and by a deep mountain gorge, stream, or other prominent landmark.

We used nocturnal time-constrained visual encounter surveys based on distance sampling, which is considered effective for sampling anurans (Dodd, 2010; Funk et al., 2003). Field work was performed in April, June, August, and October in 2017. These four sampling events were conducted in accordance with spring, early summer, midsummer, and autumn, covering the anuran breeding, foraging, and migration seasons in the study area. Four people systematically walked at a slow pace (about 4 m/min) and intensively searched for anuran species by turning over stones, logs, leaf litter, tree branches, shrubs, and bushes along the transects using 220 lm torches after sunset, with two transects being sampled per night (Khatiwada et al., 2019; Zhu et al., 2020b). In each transect, all individuals encountered were captured and stored in cotton bags (38 cm×21 cm), with one cotton bag containing one individual. Amphibian sampling was conducted in accordance with the Law of the People's Republic of China on the Protection of Wildlife and approved by the Chengdu Institute of Biology Animal Care Committee [CIB2015003]. We took all captured individuals to a nearby dry place, where they were photographed and identified to species and sex following Fei et al. (2009, 2012). All anurans were measured for a set of 15 external morphological traits using a digital caliper to the nearest 0.01 mm, including snout-vent length (SVL), head length (HEL), head width (HW), snout length (SL), eye diameter (ED), nose eye distance (NED), upper eyelid width (UEW),

interorbital space (IOS), internasal space (INS), lower arm and hand length (LAL), hand length (HAL), hindlimb length (HIL), tibia length (TL), tibia width (TW), and foot length (FL) (Supplementary Figure S2). The mass of each individual was measured using a digital scale to the nearest 0.01 g. After measurement, all individuals were released back to their original habitats.

A set of 16 microhabitat variables were measured in each transect during the sampling events in April, June, August, and October, separately. These environmental variables (i.e., air temperature, air humidity, altitude, water depth, water width, leaf litter depth, canopy cover, number of trees, shrub cover, leaf litter cover, rock cover, soil pH, water temperature, water pH, water conductivity, and water velocity) play important roles in shaping anuran assemblages (Grundel et al., 2015; Keller et al., 2009; Khatiwada et al., 2019; Wyman, 1988; Wyman & Jancola, 1992). They were thus considered to have potential effects on anuran functional diversity patterns. Details on measurement methodologies are provided in Zhu et al. (2020b).

### **Functional traits**

Typically, animals display five main ecological functions (e.g., food acquisition, defense against predation, nutrient processing, reproduction, and mobility) in ecosystems, which can be described using relevant functional traits (Villéger et al., 2017). In the present study, we focused on three main functions of amphibians (i.e., food acquisition, defense against predation, and mobility) and profiled them through

ecomorphological functional traits based on published literature (e.g., Dalmolin et al., 2020; Trochet et al., 2014; Tsianou & Kallimanis, 2016). Specifically, the mass of each individual was log-transformed and other external morphological traits (except SVL) were scaled by SVL (Supplementary Table S3). These 15 ecomorphological traits were unitless ratios that were *a priori* independent of anuran SVL, thus avoiding the effects of animal body size (Winemiller, 1991; Villéger et al., 2010). Therefore, these traits can reflect the main functions that anurans display in ecosystems. Specifically, scaled mass, scaled head length, scaled head width, scaled snout length, and scaled eye diameter were related to anuran food acquisition. Scaled mass, scaled eye diameter, scaled nose eye distance, scaled upper eyelid width, scaled interorbital space, and scaled internasal space reflected anuran ability to defend against predation. Scaled mass, scaled lower arm and hand length, scaled hand length, scaled hindlimb length, scaled tibia length, scaled tibia width, and scaled foot length were related to anuran mobility (Dalmolin et al., 2020; Trochet et al., 2014; Tsianou and Kallimanis, 2016; Supplementary Table S3).

### **Statistical analyses**

All functional traits were scaled (mean of 0 and standard deviation of 1) (Villéger et al., 2008) and used for principal component analysis (PCA). The eigenvalues of the first four synthetic principal components were  $>1$  (PC1=5.94, PC2=3.86, PC3=2.61 and PC4=1.74, respectively), and were used to create a four-dimensional functional

space (PC1=37.15%, PC2=24.12%, PC3=16.32% and PC4=10.85%, respectively; Supplementary Figure S3).

Accounting for functional entity relative biomass in transects, four functional diversity indices, including functional richness, functional evenness, functional divergence, and functional specialization, were selected to describe the complementary components that filled functional space (Mouillot et al., 2013). Specifically, functional richness reflected the proportion of functional space occupied by functional entities. Functional evenness measured the regularity of the distribution of functional entity relative biomass in functional space. Functional divergence reflected the proportion of relative biomass supported by functional entities with extreme functional traits. Functional specialization was the proportion of the relative biomass of functional entities with extreme functional traits in functional space (Mouillot et al., 2013; Schleuter et al., 2010; Vileger et al., 2008). All functional diversity indices were first calculated in each transect for each sampling event. The data from the four sampling events were then pooled to calculate the whole year's functional diversity indices in each transect.

Spatial variation of anuran functional diversity was tested along the elevational gradient using linear regression models with a quadratic term, which was subsequently removed if it was not significant ( $P > 0.05$ ) (Crawley, 2007). Functional diversity indices were log-transformed prior to analyses if needed. We only tested the response of anuran functional diversity indices to elevation after pooling the data from

the four samplings. This is because most transects did not contain sufficient functional entities for calculation in each season (at least five functional entities should be included in the calculation as we used four-dimensional functional space). To identify the potential differences in functional diversity indices among seasons (i.e., four months), Kruskal-Wallis and Wilcoxon rank sum tests were conducted for each index, separately.

A total of eight microhabitat variables, including air humidity, water temperature, number of trees, canopy cover, shrub cover, leaf litter cover, leaf litter depth, and water conductivity, were selected based on our previous study showing that these variables have significant effects on amphibian distribution and species richness (Zhu et al., 2020b). To explore the determination of environmental variables on functional diversity indices, we first constructed generalized linear models (GLMs), including all microhabitat variables for each index. A normal distribution with an identity link function was applied to each index. We compared different GLMs by removing variables one by one from the global model based on Akaike information criterion (AIC) values. The best model was determined based on the lowest AIC value. After that, hierarchical partitioning analyses were used to calculate the relative contribution of each selected environmental variable to the variations in different diversity indices.

All statistical analyses were conducted in R 3.6.1 (R Development Core Team, 2020). Functional diversity indices were calculated based on the *FD* package (Villegger et al., 2008). Linear regression and GLMs were performed using the *lme4* package

(Bates et al., 2015). Hierarchical partitioning was undertaken using the *hier.part* package (Walsh et al., 2004).

## REFERENCES

Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**(1): 1–48.

Chen CD, Li DH. 2003. On the biodiversity and the ecological integrity of Wulingyuan District, Hu'nan Province. *Acta Ecologica Sinica*, **23**(11): 2414–2423.

Crawley MJ. 2007. *The R Book*. New York: Wiley.

Dalmolin DA, Tozetti AM, Pereira MJR. 2020. Turnover or intraspecific trait variation: explaining functional variability in a neotropical anuran metacommunity. *Aquatic Sciences*, **82**(3): 62.

Dodd Jr CK. 2010. *Amphibian Ecology and Conservation: A Handbook of Techniques*. New York: Oxford University Press.

Fei L, Hu SQ, Ye CY, Huang YZ. 2009. *Fauna Sinica, Amphibia, vol.2, Anura*. Beijing: Science Press. (in Chinese)

Fei L, Ye CY, Jiang JP. 2012. *Colored Atlas of Chinese Amphibians and Their Distribution*. Chengdu: Sichuan Science and Technology Press. (in Chinese)

Funk WC, Almeida-Reinoso D, Nogales-Sornosa F, Bustamante MR. 2003.

- Monitoring population trends of *Eleutherodactylus* frogs. *Journal of Herpetology*, **37**(2): 245–256.
- Grundel R, Beamer DA, Glowacki GA, Frohnapple KJ, Pavlovic NB. 2015. Opposing responses to ecological gradients structure amphibian and reptile communities across a temperate grassland–savanna–forest landscape. *Biodiversity & Conservation*, **24**:1089–1108.
- Keller A, Rödel M-O, Linsenmair KE, Grafe TU. 2009. The importance of environmental heterogeneity for species diversity and assemblage structure in Bornean stream frogs. *Journal of Animal Ecology*, **78**(2):305–314.
- Khawiwada JR, Zhao T, Chen YH, Wang B, Xie F, Cannatella DC, et al. 2019. Amphibian community structure along elevation gradients in Eastern Nepal Himalaya. *BMC Ecology*, **19**: 19.
- Mouillot D, Graham NAJ, Villéger S, Mason NWH, Bellwood DR. 2013. A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, **28**(3): 167–177.
- Qiao XJ, Li QX, Jiang QH, Lu JM, Franklin S, Tang ZY, et al. 2015. Beta diversity determinants in Badagongshan, a subtropical forest in central China. *Scientific Reports*, **5**: 17043.
- R Development Core Team. 2020. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Schleuter D, Daufresne M, Massol F, Argillier C. 2010. A user's guide to functional



- diversity indices. *Ecological Monographs*, **80**(3): 469–484.
- Trochet A, Moulherat S, Calvez O, Stevens VM, Clobert J, Schmeller DS. 2014. A database of life-history traits of European amphibians. *Biodiversity Data Journal*, **2**: e4123.
- Tsianou MA, Kallimanis AS. 2016. Different species traits produce diverse spatial functional diversity patterns of amphibians. *Biodiversity and Conservation*, **25**(1): 117–132.
- Villegger S, Mason NWH, Mouillot D. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, **89**(8): 2290–2301.
- Villéger S, Miranda JR, Hernández DF, Mouillot D. 2010. Contrasting changes in taxonomic vs. functional diversity of tropical fish communities after habitat degradation. *Ecological Applications*, **20**(6):1512–22.
- Villéger S, Brosse S, Mouchet M, Mouillot D, Vanni MJ. 2017. Functional ecology of fish: current approaches and future challenges. *Aquatic Science*, **79**(4):783–801.
- Walsh CJ, Papas PJ, Crowther D, Sim PT, Yoo J. 2004. Stormwater drainage pipes as a threat to a stream-dwelling amphipod of conservation significance, *Austrogammarus australis*, in southeastern Australia. *Biodiversity & Conservation*, **13**(4): 781–793.
- Winemiller KO. 1991. Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecological Monographs*, **61**:343–65.

Wyman RL, Jancola J. 1992. Degree and scale of terrestrial acidification and amphibian community structure. *Journal of Herpetology*, **26**(4):392.

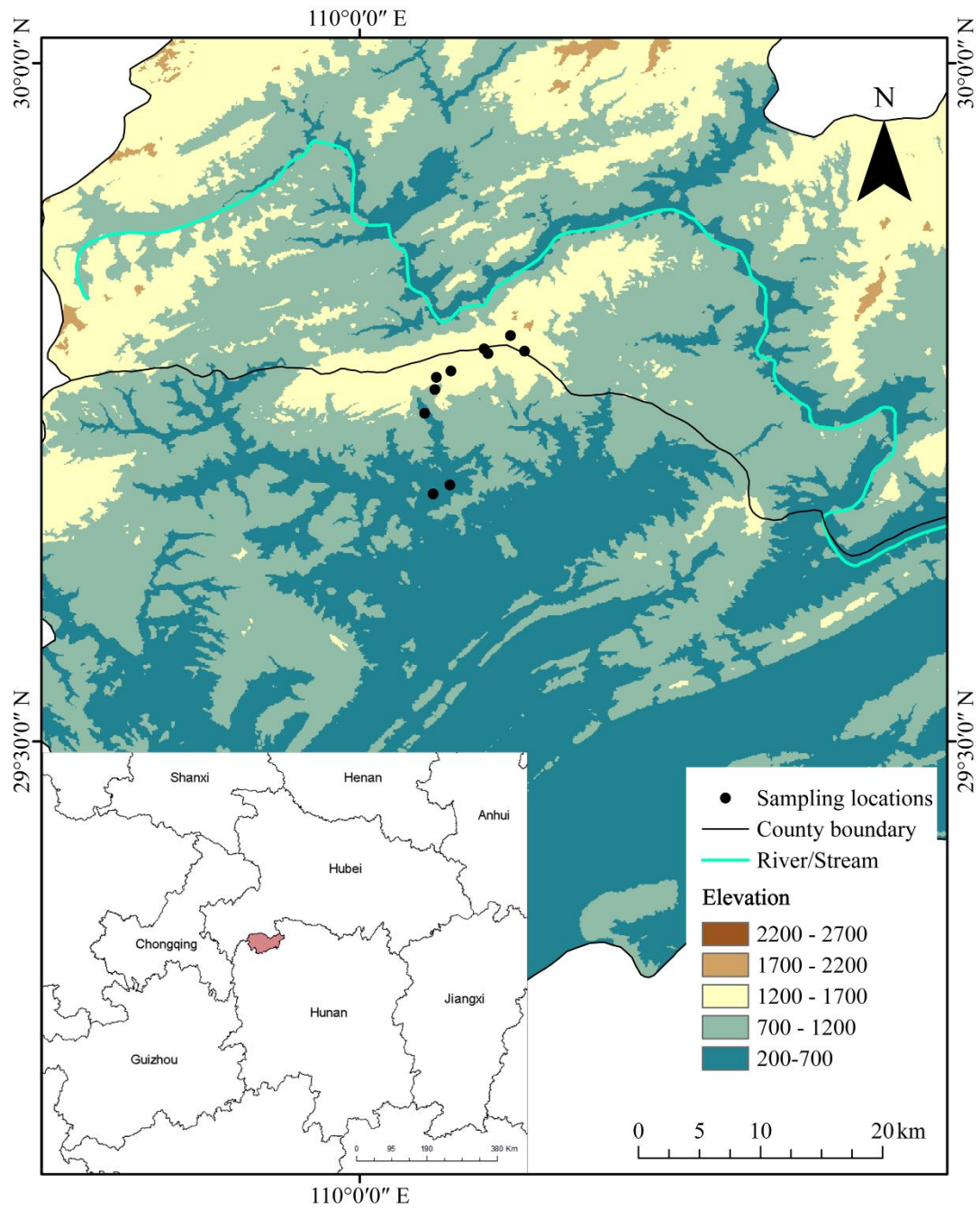
Wyman RL. 1988. Soil acidity and moisture and the distribution of amphibians in five forests of Southcentral New York. *Copeia*, 1988:394–9.

Xiong EG, Zhu KL, You LS, Wang YJ, Fu P, Gu R, et al. 1999. Investigation on butterflies in Sangzhi county and Tianping Mountain natural preservation region. *Journal of Hunan Agricultural University*, **25**(4): 312–317. (in Chinese)

Zhu WB, Zhao CL, He YX, Liao CL, Zhao T, Jiang JP, et al. 2020a. Spatial-temporal patterns of amphibian diversity in the Badagongshan National Nature Reserve, Hunan. *Journal of Ecology and Rural Environment*, **36**(8): 961 – 967. (in Chinese)

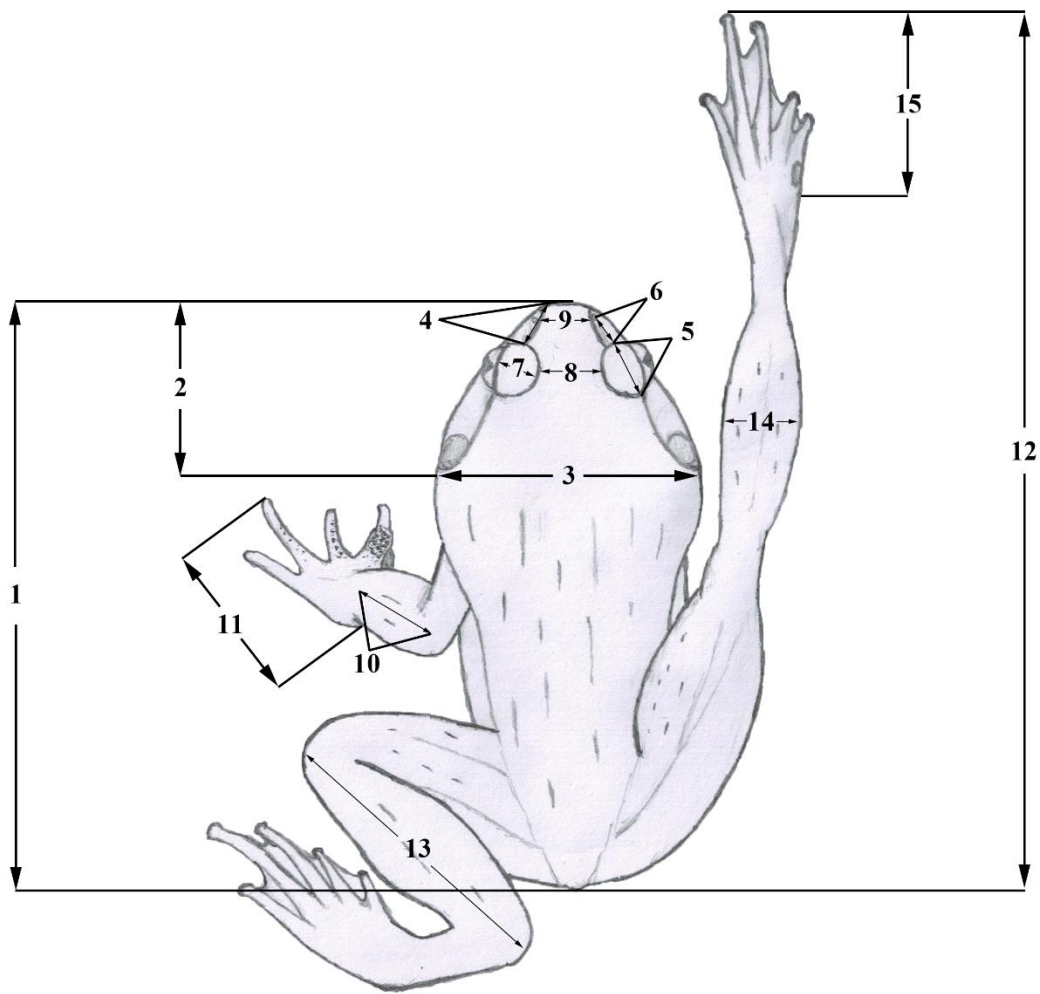
Zhu WB, Zhao CL, Liao CL, Zou B, Xu D, Zhu W, et al. 2020b. Spatial and temporal patterns of amphibian species richness on Tianping Mountain, Hunan Province, China. *Zoological Research*, **41**(2): 182–187.

## Supplementary Figures

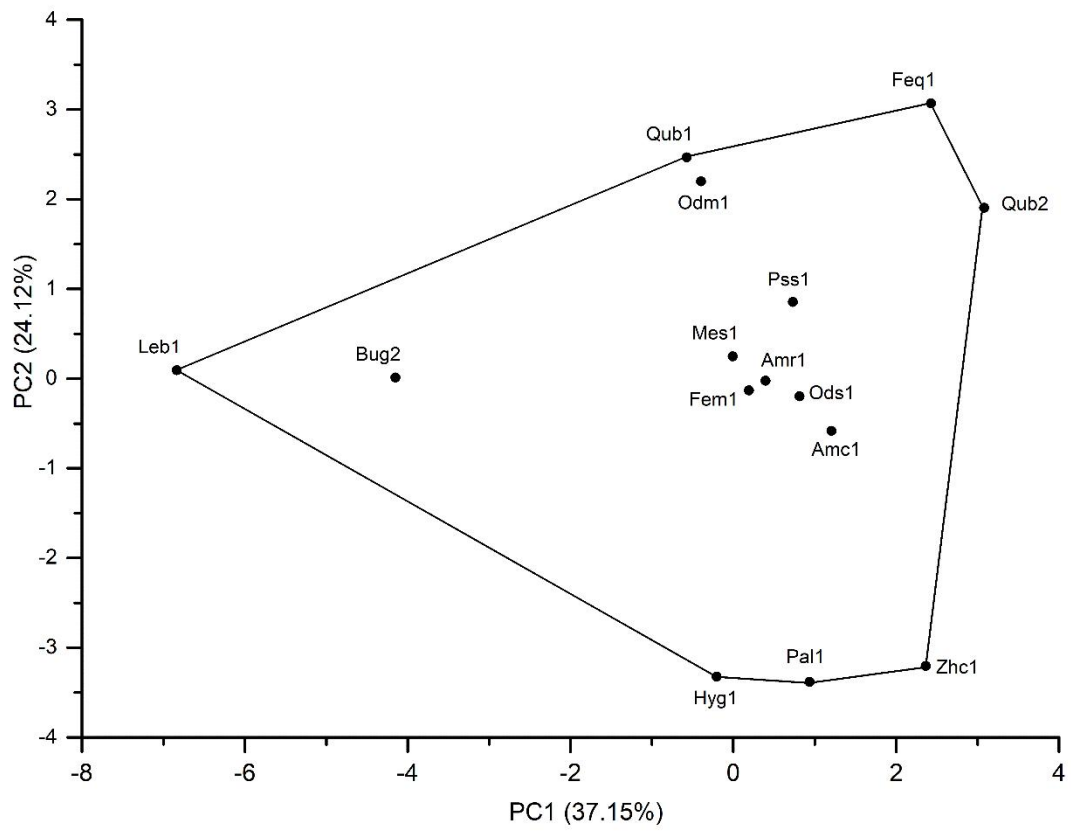


**Supplementary Figure S1** Map showing study area on Tianping Mountain, China.

Black dot denotes transects used in present study.



**Supplementary Figure S2** Measurement of 15 external morphological traits: 1: snout-vent length (SVL); 2: head length (HEL); 3: head width (HW); 4: snout length (SL); 5: eye diameter (ED); 6: nose eye distance (NED); 7: upper eyelid width (UEW); 8: interorbital space (IOS); 9: internasal space (INS); 10: lower arm and hand length (LAL); 11: hand length (HAL); 12: hindlimb length (HIL); 13: tibia length (TL); 14: tibia width (TW); 15: foot length (FL).



**Supplementary Figure S3** Species distribution in two-dimensional functional space.

Abbreviations are available in Supplementary Table S1.

## Supplementary Tables

**Supplementary Table S1** Species and functional entities (and abbreviations) captured on Tianping Mountain.

Oder	Family	Species	Functional entities	Abbreviation	
Anura	Bufonidae	<i>Bufo gargarizans</i>	Juvenile <i>Bufo gargarizans</i>	Bug2	
		Ranidae	<i>Amolops chunganensis</i>	Adult <i>Amolops chunganensis</i>	Amc1
	<i>Amolops ricketti</i>		Adult <i>Amolops ricketti</i>	Amr1	
	<i>Odorrana margaretae</i>		Adult <i>Odorrana margaretae</i>	Odm1	
	<i>Odorrana schmackeri</i>		Adult <i>Odorrana schmackeri</i>	Ods1	
	<i>Pseudorana sangzhiensis</i>		Adult <i>Pseudorana sangzhiensis</i>	Pss1	
	Dicroglossidae		<i>Fejervarya multistriata</i>	Adult <i>Fejervarya multistriata</i>	Fem1
			<i>Feirana quadranus</i>	Adult <i>Feirana quadranus</i>	Feq1
		<i>Quasipaa boulengeri</i>	Juvenile <i>Quasipaa boulengeri</i>	Qub2	
			Adult <i>Quasipaa boulengeri</i>	Qub1	
	Rhacophoridae	<i>Zhangixalus chenfui</i>	Adult <i>Zhangixalus chenfui</i>	Zhc1	
	Hylidae	<i>Hyla gongshanensis</i>	Adult <i>Hyla gongshanensis</i>	Hyg1	
	Megophryidae	<i>Paramegophrys liui</i>	Adult <i>Paramegophrys liui</i>	Pal1	
		<i>Leptobrachium boringii</i>	Adult <i>Leptobrachium boringii</i>	Leb1	
		<i>Megophrys sangzhiensis</i>	Adult <i>Megophrys sangzhiensis</i>	Mes1	

**Supplementary Table S2** Best model selected by GLMs. Significant *P*-values are in bold. Abbreviations of microhabitat variables are: WT: water temperature; TN: number of trees; CC: canopy cover; SC: shrub cover; LLC: leaf litter cover; LLD: leaf litter depth; WCON: water conductivity.

Functional diversity indices		Estimate	Std.Error	<i>P</i>
FRic	<b>Intercept</b>	-0.653	1.014	0.566
	<b>WT</b>	0.065	0.075	0.447
	<b>TN</b>	0.005	0.004	0.249
	<b>CC</b>	-0.011	0.008	0.279
	<b>SC</b>	-0.019	0.019	0.389
	<b>LLC</b>	0.080	0.057	0.254
	<b>WCON</b>	-0.003	0.003	0.415
FEve	<b>Intercept</b>	2.491	0.405	<b>0.003</b>
	<b>WT</b>	-0.112	0.021	<b>0.006</b>
	<b>TN</b>	-0.005	0.001	<b>0.009</b>
	<b>CC</b>	0.005	0.002	0.057
	<b>SC</b>	0.007	0.006	0.318
	<b>LLC</b>	-0.042	0.018	0.087
FDiv	<b>Intercept</b>	0.957	0.098	<b>&lt; 0.001</b>
	<b>WT</b>	-0.057	0.006	<b>&lt; 0.001</b>
	<b>CC</b>	0.013	0.001	<b>&lt; 0.001</b>
	<b>SC</b>	0.019	0.005	<b>0.016</b>
	<b>LLD</b>	-0.363	0.071	<b>0.007</b>
	<b>WCON</b>	0.004	< 0.001	<b>&lt; 0.001</b>
FSpe	<b>Intercept</b>	0.153	0.163	0.417
	<b>WT</b>	0.023	0.012	0.153
	<b>TN</b>	0.003	< 0.001	<b>0.022</b>
	<b>CC</b>	-0.004	0.001	0.051
	<b>SC</b>	-0.013	0.003	<b>0.022</b>
	<b>LLC</b>	0.039	0.009	<b>0.023</b>
	<b>WCON</b>	-0.002	< 0.001	<b>0.030</b>

**Supplementary Table S3** List of 15 functional traits and their related ecological functions

<b>Functional traits</b>	<b>Measure</b>	<b>Ecological functions</b>
Mass	log(M)	Food acquisition, defense against predation, and mobility
Scaled head length	HEL/SVL	Food acquisition
Scaled head width	HW/SVL	Food acquisition
Scaled snout length	SL/SVL	Food acquisition
Scaled eye diameter	ED/SVL	Food acquisition and defense against predation
Scaled nose eye distance	NED/SVL	defense against predation
Scaled upper eyelid width	UEW/SVL	defense against predation
Scaled interorbital space	IOS/SVL	defense against predation
Scaled internasal space	INS/SVL	defense against predation
Scaled lower arm and hand length	LAL/SVL	mobility
Scaled hand length	HAL/SVL	mobility
Scaled hindlimb length	HIL/SVL	mobility
Scaled tibia length	TL/SVL	mobility
Scaled tibia width	TW/SVL	mobility
Scaled foot length	FL/SVL	mobility

Abbreviations: SVL: snout-vent length, HEL: head length, HW: head width, SL: snout length, ED: eye diameter, NED: nose eye distance, UEW: upper eyelid width, IOS: interorbital space, INS: internasal space, LAL: lower arm and hand length, HAL: hand length, HIL: hindlimb length, TL: tibia length, TW: tibia width, FL: foot length. Details of the measurement are provided in Figure 2.