

## Tree species diversity along an elevational gradient of Mt. Bokor, a table-shaped mountain in southwestern Cambodia

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**Tree species diversity along an elevational  
gradient of Mt. Bokor, a table-shaped  
mountain in southwestern Cambodia**

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# Preface

This thesis is the main outcome of my Ph.D. study in Ecology Laboratory, the Graduate School of Systems Life Sciences, Kyushu University. My study is focusing on taxonomic and phylogenetic perspectives of species diversity, along the elevational gradient of Mt. Bokor, a table-shape mountain locating in southwest Cambodia.

This research is under the supervision of professor Tatsukazu Yahara and supported by the grant of Japan Society for the Promotion of Science (JSPS) for Global Center of Excellence (GCOE) Program ‘Asian Conservation Ecology’ and also by the Environment Research and Technology Development Fund (S9) of the Ministry of the Environment, Japan.

This thesis includes nine chapters, in which Chapter 1 giving a brief introduction to the research on tree diversity in mountain forest and the purpose of my study; Chapter 2 introducing the geographic characters, climate features and soil compositions of Mt. Bokor, and the field sampling methods of tree species; Chapter 3 discussing about the phylogeny analysis; Chapter 4 describing about elevational tree species richness pattern (this chapter has been published on Journal of Ecological Research, DOI 10.1007/s11284-016-1358-7); Chapter 5 describing and discussing about elevational phylogenetic diversity patterns and phylogenetic structure of tree species (this part has been firstly drafted); Chapter 6 discussing about the evolutionary perspectives for explaining the diversity patterns of Mt. Bokor, and Chapter 7 describing the community structure of the forest of Mt. Bokokr and Chapter 8 providing insights from the neutral theory on tree species richness in Mt. Bokor. Chapter 9 gives a conclusion of this thesis.

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The past Ph.D. study in Kyushu University brought me many changes not only enhancing my research ability but also broadening my horizons and some other aspects. Firstly, Prof. Yahara gave me lots of advice and help in research design, data collection, analysis and especially in scientific paper writing, which much improved my research ability. The manuscript could not be accepted without the great help from Prof. Yahara. In addition, I joined five times of plant field survey in a tropical rainforest of mountain Bokor in Cambodia. The experience of tropical rainforest survey and travel much improved my field survey skills and greatly broadened my personal view-sights. I am also becoming familiar with the society and customs of Cambodia and Southeast Asia. Furthermore, Prof. Yahara and Kyushu University also supported me to attend many important academic conferences, held in Japan and other countries. Through them, I could listen many excellent research reports from different ecological fields and get many useful comments from other researchers, which much improved my academic knowledge. Moreover, study in Japan also provided me a good opportunity to learn Japan's culture, society and language. All of these experiences were particularly valuable for my Ph.D. study and my life.

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I would like to give my sincere gratitude to my parents, wife and family for their love, encouragement and supports. Life experience told me that the purposes of study is not only learning the knowledge itself but also learning how to be a man with good personal accomplishments. Thank everyone who helped me in my life.

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## Abstract

**Background:** Although many studies have been made on extremely high tree species richness in tropical lowland rain forests, patterns of tree species richness in tropical montane forests of Southeast Asia are still remaining relatively poorly understood. In addition, measuring the biodiversity by only using species diversity of taxonomic level is inherently limited and could potentially obscure other diversity patterns. By incorporating other biodiversity dimensions as phylogenetic diversity and structure could give a better understanding of the biodiversity patterns and assemblages. Furthermore, many previous studies documented that plant species richness decreases with increasing elevation or shows a hump-shaped with a mid-peak. However, most of studies did not attempt to standardize the amount of sampling efforts.

**Purpose:** In this thesis I carried on a series uniform sampling effort to quantify tree species richness. I also calculated phylogenetic diversity, phylogenetic structure (NRI and NTI), and diversification rate along the elevational gradient of Mt. Bokor, a table-shaped mountain in southwestern Cambodia and discuss the underlying mechanisms for the tree species richness pattern.

**Location:** Mt. Bokor, a table-shaped mountain in Bokor National Park, locating in southwest Cambodia.

**Methods:** I used two methods to record tree species richness: first, I recorded trees taller than 4 m in 20 uniform plots (5 x 100 m) placed at 266–1048-m elevation; and second, I collected specimens along an elevational gradient from 200 to 1048 m. For both datasets, I applied rarefaction, extrapolation and Chao1 estimator to standardize the sampling efforts. In addition, I used two DNA barcode segments, *rbcL* and *matK*, to construct the phylogenetic tree and calculate the phylogenetic diversity, phylogenetic structure and diversification rate of tree species. I also applied methods of rarefaction and extrapolation to phylogenetic diversity that is underestimated due to the sampling completeness. In addition, I used both generalized linear model (GLM) and linear regression to test the relationships between species richness, phylogenetic diversity, phylogenetic structure, and diversification rate with elevation, and

relationships between species richness with phylogenetic diversity, phylogenetic structure, and diversification rate.

**Results:** I recorded 464 tree species including 82 families and 230 genera (308 tree species from 20 plots and 389 tree species from the general collections) of Mt. Bokor. Species richness observed in 20 plots had a weak but non-significant correlation with elevation. Species richness estimated by rarefaction or Chao1 from both data sets also showed no significant correlations with elevation. Similarly, phylogenetic diversity, phylogenetic structure (NTI) and diversification rate of tree species also showed no significant relationships with elevation. While phylogenetic structure index (NRI) shows a decreasing pattern with elevation. In addition, species richness had no correlations with tree height and d.b.h. Neutral model parameters showed a relatively high rate of speciation and a moderate rate of migration in Mt. Bokor.

**Main Conclusion:** Unlike many previous studies, tree species richness is nearly constant and high values along the elevational gradient of Mt. Bokor where temperature and precipitation are expected to vary. This pattern does not agree with any of the four common patterns between species richness and elevation summarized by McCain and Grytnes (2010). Similarly, phylogenetic diversity, phylogenetic structure (NTI) and diversification rate of tree species also kept constant values along the elevation gradient. Remarkably, the constant tree species richness pattern could be explained by the constant diversification rates of different elevations in Mt. Bokor. I suggested that the table-shaped geography with regional climate features could cause this special evolution history and species richness pattern of Mt. Bokor.

### **Keywords**

Diversification rate, elevational gradient, neutral theory, phylogenetic diversity, phylogenetic structure, rarefaction and extrapolation, species richness pattern, sampling bias, tropical forest.

## **Chapter 1 Introduction**

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## **1.1 Mountain forests and biodiversity**

Mountains are covering about a quarter of Earth land surface (Maselli et al., 2010; [www.cbd.int/mountain](http://www.cbd.int/mountain)) and representing about 23% of Earth forest (Price et al., 2011; Guo et al., 2013). Mountains are playing crucial roles or functions on the Earth ecosystem by harboring the great number of species, providing freshwater, maintaining the Earth climate etc (Price et al., 2011). Within them, particularly, mountain regions are supporting about a quarter of territory biodiversity on the Earth (Maselli et al., 2010; <http://www.cbd.int/mountain>) and harboring more than half (25 of 34) of biodiversity hotspots (Price et al., 2011; Guo et al., 2013; Gradstein et al., 2008; Yahara et al., 2012). Among the biodiversity hotspots, Southeast Asia area (Indo-burma) is one of the largest hot spots (number 14). In addition, mountain forests are also rich in endemism (Maselli et al., 2010; Price et al., 2011).

The tropical rainforests in Southeast Asia are the oldest and consistent forests, and have the last few primary rainforests (<http://www.blueplanetbiomes.org>). Remarkably, there are more unidentified endemic species in tropical mountain forest of Southeast Asia regions (Tagane et al., 2015).

## **1.2 Biodiversity loss in Southeast Asia**

During past decades, the decrease of forest area in global scale is becoming a serious problem, which causes critical threats for biodiversity (Yahara et al., 2012), especially in developing countries (Price et al. 2011). The terrestrial species loss rate is estimated to be 100 times higher than species natural extinction of species (Leadley et al., 2010; Yahara et al., 2012). Tropical forests in Southeast Asia are regarded as one of the centers of species richness in the world (Yahara et al., 2012). However, Southeast Asia is facing with the highest rate of deforestation and biodiversity loss in the earth (<http://www.blueplanetbiomes.org>; Sodhi et al., 2004; Yahara et al., 2012). Three quarters of original forests and up to 42% of biodiversity are estimated to disappear till 2100 in Southeast Asia (Sodhi et al., 2004). In order to obtain more reliable species loss rate and the trends of species diversity, more empirical studies are necessary to be made in Southeast Asia (Yahara et al., 2012).

### **1.3 Biodiversity dimensions**

There are three basic dimensions of diversity as taxonomic diversity (also species richness), functional trait diversity (FD) and phylogenetic diversity (PD) (Willig et al., 2013; Cisneros et al., 2014). Functional diversity and phylogenetic diversity usually increase with taxonomic diversity (Cisneros et al., 2014), in which taxonomic diversity represents the number of the species recorded in a certain space during a specific time (Kim et al., 2011), and Phylogenetic diversity defined by Faith (1992) is the sum of the branch lengths of a phylogenetic tree connecting all the species in the target assemblage, which is the most widely used phylogenetic metric (Willig et al., 2013; Chao et al., 2014). However any analysis only of counting taxonomic diversity data is inherently limited, by incorporating data of functional traits diversity or phylogenetic diversity, we can gain more detailed insights into biodiversity patterns and processes (Bishop et al. 2015). Even though phylogenetic diversity is usually correlated with species richness (Cisneros et al., 2014), some studies also found inconsistent correlations. For an example, Brehm et al (2013) addressed a decreasing phylogenetic diversity pattern of geometrid moths in tropical Andes, whereas the species richness of geometrid moths kept a constant pattern along an elevational gradient from 1020 to 2677 m (Brehm et al., 2003).

### **1.4 Elevational taxonomic diversity**

Understanding patterns of species richness and diversity along elevational gradients is a critical issue for the biodiversity and biogeography research (Körner, 2007; McCain and Grytnes 2010). Therefore, many efforts have been made to describe elevational changes of taxonomic species richness (Rahbek, 1995, 2005; Lomolino, 2001; McCain and Grytnes 2010). As a result, four common patterns of the changes have been recognized: decreasing, low plateau, low plateau with a mid-elevational peak (LPMP), and a mid-elevational peak, among which the mid-elevational peak has been observed most frequently in plant species studies (Rahbek 1995, 2005; McCain and Grytnes 2010; Guo et al., 2013).



## **1.6 Hypothesis for diversity patterns**

Based on those above observations, researchers have proposed some hypotheses to explain the patterns of species richness along elevational gradients, which can be divided into four main categories: climate (temperature and precipitation etc.), space (species area relationship and mid domain effect etc.), evolutionary history (speciation rate and extinction rate etc.) and biotic processes (competition and immigration etc.) (Rahbek 1995; Lomolino 2001; Körner 2007; McCain and Grytnes 2010).

### **1.6.1 Area hypothesis**

The species richness in the sampling plot is determined by the number of species in the species pool (Zobel et al., 1998). The species area relationship (SAR) addressed that larger land areas tends to have larger number of species (Rosenzweig 1995), thus to have larger species pools (Grytnes 2003). The land area usually decreases with elevation, thus the species pool also decreases with the elevation. The species richness pattern predicted by area hypothesis should be decreased with elevation.

### **1.6.2 Climate hypothesis**

Besides land area, climate variables are considered to be one of the most important determinants of species richness (McCain and Grytnes 2010). Within climatic factors, temperature and precipitation are considered to be most important determinants of species richness along the elevational gradients (Rahbek 1995; Lomolino 2001; Hawkins et al. 2003; Körner 2007; McCain and Grytnes 2010; Guo et al. 2013). Among them, temperature decreases average 0.6 °C with per 100 m increase (Barry 2013) and the lower temperature on higher elevational zones may restrict the productivity and then limit the species diversity (McCain and Grytnes 2010). The species richness pattern predicted by temperature hypothesis is the decreasing with elevation. In addition, the most common precipitation pattern is increasing with elevation and species richness is usually positively correlated with precipitation. Thus the precipitation hypothesis predicts that the species richness is positively correlated with elevation. However, in tropical mountains precipitation shows more complex patterns by displaying decreasing or unimodal along the elevational gradients (McCain and Grytnes 2010).

### **1.6.3 Productivity hypothesis**

Species richness is positively correlated with productivity (McCain and Grytnes 2010). The productivity is predicted to be highest in the mid elevation. Thus the species richness pattern is estimated as the hump-shaped pattern.

### **1.6.4 Mid-domain effect**

Mid-domain effect model developed by Colwell and Hurtt (1994) predicts a hump-shaped species richness pattern. Colwell and Hurtt (1994) claimed that a hump-shaped pattern could be arisen by the random placement of species ranges within a bounded domain, even under a uniform environment.

### **1.6.5 Evolution**

Evolutionary history of species richness is also an important factor determining plant species richness along the elevational gradients (McCain and Grytnes 2010). The Evolution hypothesis claims that the species richness is the highest in the mid-altitude zones where have relatively high speciation rates and low extinction rates. Two hypotheses related to evolutionary hypothesis were proposed to explain the commonly found hump-shaped species pattern, the montane species-pump hypothesis and the montane museum hypothesis (Smith et al., 2007; Wiens et al., 2007; Hutter et al., 2013). The montane species-pump hypothesis predicts that the diversification rates are higher in the intermediate altitude than lower and higher altitudes. On the other hand, the montane museum hypothesis argues that the diversification rates keep almost constant along the altitudinal gradient, but the mid-altitudes are firstly colonized and have longer evolutionary time than other elevational zones (Smith et al., 2007; Wiens et al., 2007; Hutter et al., 2013).

## **1.7 The purpose of the study**

The main purposes of this paper are quantifying tree species richness, phylogenetic diversity, and phylogenetic structure patterns along an elevational gradient from the 200 m to 1048 m of Mt. Bokor, using rarefaction and extrapolation methods as well as Hubbell's neutral theory.

Specific questions addressed are as follows. (1) How do species richness, phylogenetic diversity and phylogenetic structure change along the elevation gradient of Mt. Bokor? (2) What are relationships between species richness and phylogenetic diversity with tree density, tree size and tree height? (3) What are the plausible mechanisms behind the species richness pattern of Mt. Bokor?

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## **Chapter 2 Study area, sampling methods, and environmental features**

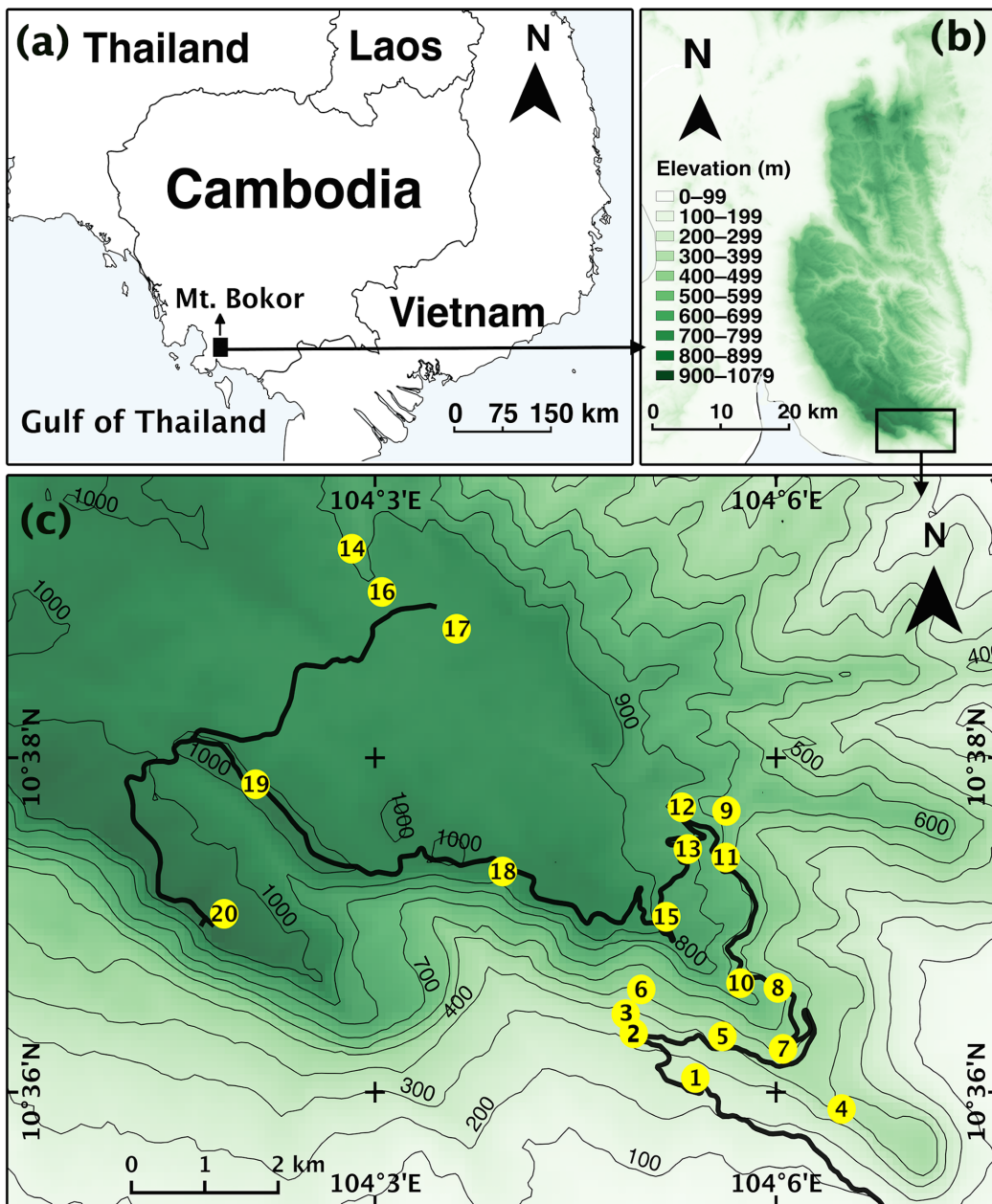
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## 2.1 Geographic characters of Mt. Bokor

We carried out field surveys in Bokor National Park, Kampot Province, locating in southwest Cambodia, which covers approximately 140,000 ha (Fig. 2.1). The mountain range including Bokor National Park and the north neighbouring Cardamon Mountains maintains a large area of the last remaining primary evergreen tropical forest of Cambodia. Mt. Bokor is locating in the southern part of the Bokor National Park, facing towards the Gulf of Thailand and is a table-shaped mountain, with an altitudinal gradient from the seashore to highest peak of 1089 m (Rundel et al. 2003; Stuart and Emmett 2006). The south slope of Mt. Bokor is covered with the wet tropical rainforest, and the top plateau area is covered with a mosaic of grassland and health forest (Rundel et al. 2003; Stuart and Emmett 2006; Tagane et al., 2015). To describe the topology of Mt. Bokor, we calculated areas of each altitudinal interval per 100 m (from 200–299 m to 1000–1089 m) in the whole mountain range of Bokor (Fig. 2.2 and Fig. 2.3). Altitudinal interval areas decreased with altitude ( $R^2 = 0.67$ ;  $P = 0.007$ ), but this pattern was not significant if we excluded the areas above 900 m ( $R^2 = 0.18$ ;  $P = 0.19$ ) (Fig. 2.2b, Zhang et al., 2016).

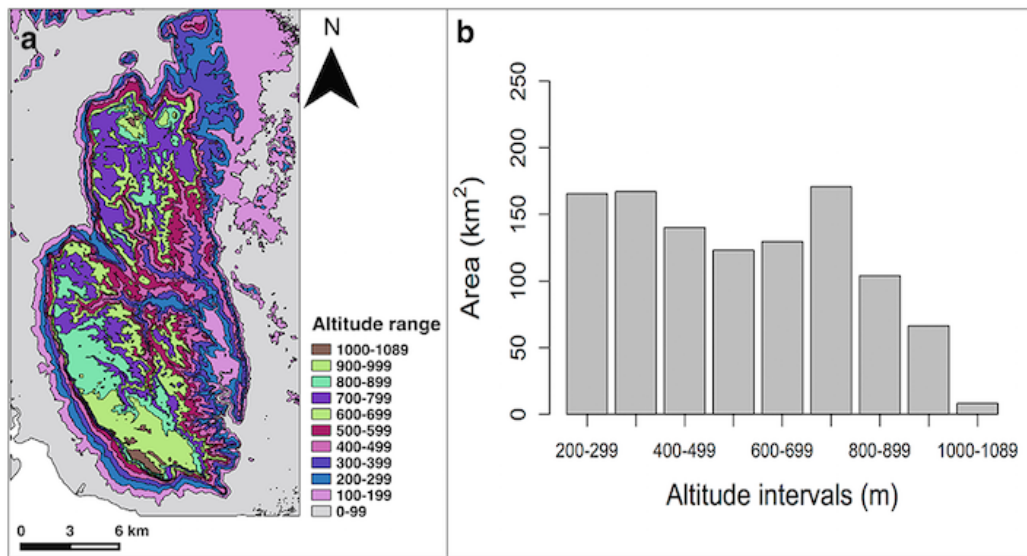
We also carried out field surveys in Mt. Honba, in the vicinity of Nha Trang city in southern Vietnam. The results from Mt. Honba will be published elsewhere, but the relationship between altitude and species richness in Mt. Honba is included here to demonstrate that our method can detect a significant trend. (Zhang et al., 2016).

The geographic map of the Bokor national park and Mt. Bokor (Fig. 2.1, Fig. 2.2a and Fig. 2.3) were created through Quantum GIS 2.4.0–Chugiak (Quantum GIS Development Team 2014). The data of digital elevation models (DEMs) was obtained from the Shuttle Radar Topography Mission (SRTM) 90 m database, which is maintained by the Consultative Group for International Agriculture Research Consortium for Spatial Information (CIGAR–CSI) (Jarvis et al. 2008). We also use SRTM to calculate the land areas for each altitudinal interval (Zhang et al., 2016).

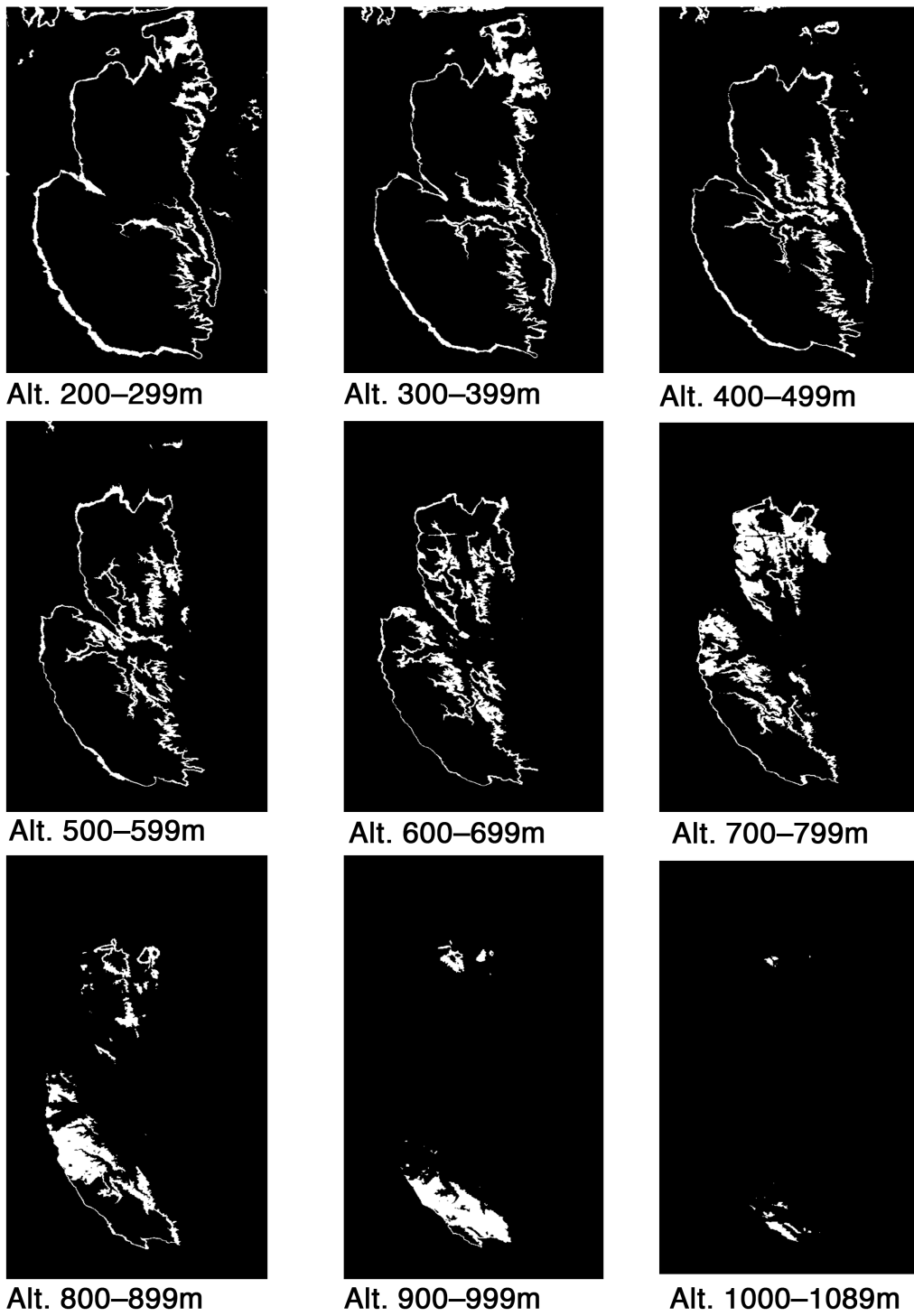


**Fig. 2.1** Location of Bokor National Park (black rectangle) (a), topographic of Bokor National Park (b), the topographic of Mt. Bokor (yellow points, sampling plots; black line, main general specimen collection route; plus mark, cross graticules) (c).





**Fig. 2.2** Altitudinal intervals distribution patterns from 0–99 m to 1000–1089 m (a) and area patterns from 200–299 m to 1000–1089 m.



**Fig. 2.3** Areas extracted for different altitudinal intervals (white colored) from 200–299 m to 1000–1089 m of Mt. Bokor.

## 2.2 Field survey

We carried out 7 field surveys (December 3–23 in 2011; May 8–16, July 14–20, and October 15–26 in 2012; and February 16–17, August 6–13, and December 7–12 in 2013) in the southern slope and top area of Mt. Bokor (Tagane et al. 2015). We used occurrences of tree species recorded by two different sampling methods, i.e. plot-based surveys and general collections of specimens. We employed not only the former but also the latter to compare our results with Grytnes and Beaman (2006) who analyzed specimen data of Mt. Kinabalu. For the former, we placed 20 rectangular plots of 100 m × 5 m along an altitudinal gradient from alt. 266 m to 1048 m and the distances among plots varied from 0.2 to 10 km to avoid the spatial autocorrelation (Fig. 2.1c, Table 2.1). All the plots were chosen in the natural forest with little human disturbance; we did not place any plot below 266 m because forest is highly disturbed or cleared. The start and end points of each plot were georeferenced with GPS. Each plot was divided into 10 subplots of 10 m × 5 m. In each subplot, we recorded species, d.b.h. (diameter at breast height) and height of all trees above 4 m. Tree heights were recorded to the nearest 1 m using a long, flexible length of glass fiber rod with an internode of 1 m and the maximal length of 15 m. Species were distinguished in the field, recorded with field names, and carefully identified later using voucher specimens collected in the plots. Specimens of tall trees were collected using the flexible length of glass fiber rod equipped with a sickle on the top. In addition, as a general survey of the flora, we collected 1225 specimens along the whole altitudinal gradient of Mt. Bokor, including the southern slope, along the main road and the top area. For each species, we counted the number of collection localities in each of 11 altitudinal intervals from 0–99m to 1000–1048m. In plot and general surveys, we collected a total of 3100 specimens (excluding duplicates) for each of which we also collected a piece of leaf as a sample for DNA extraction and dried it with silica-gel. Endemic species of Mt. Bokor are specified based on the most recent taxonomic study of trees of Mt. Bokor (Tagane et al. 2015; Tagane et al. unpublished).

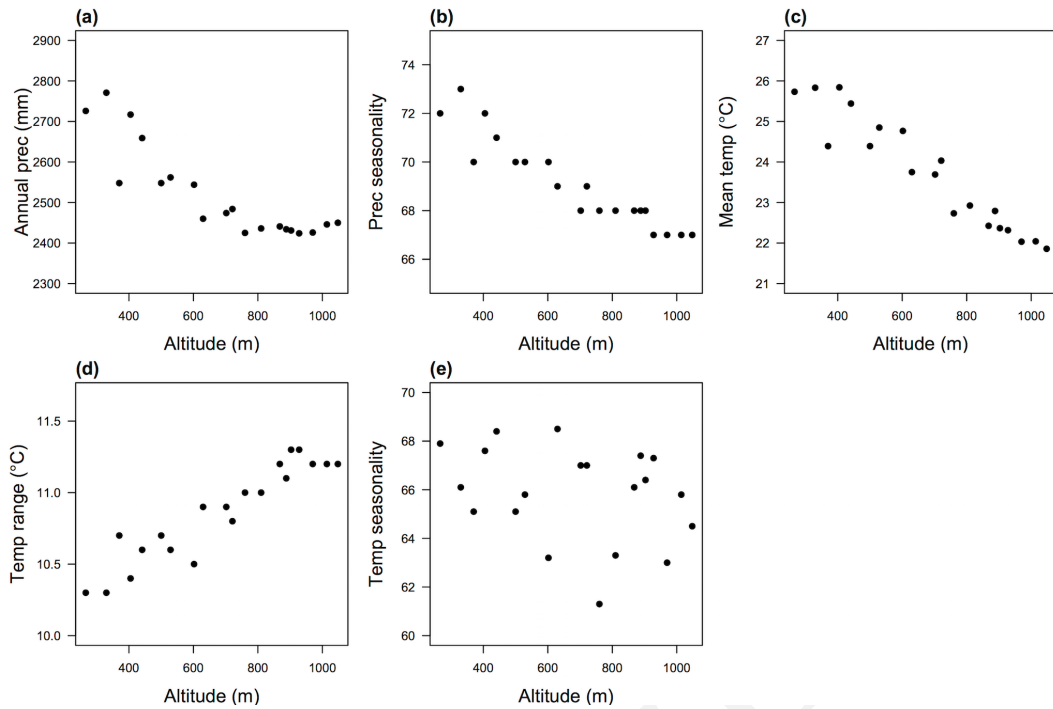
**Table 2.1** The distances (km) between 20 plots, \*: distance < 1 km.

Plot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1																				
2	1.04																			
3	1.27	0.24*																		
4	2.04	2.99	3.2																	
5	0.67*	1.17	1.35	1.89																
6	1.39	0.53*	0.39*	3.16	1.27															
7	1.26	2.01	2.19	1.13	0.84*	2.09														
8	1.64	2	2.1	1.83	0.99*	1.86	0.81*													
9	3.58	3.15	3.03	4.27	3	2.64	3.26	2.46												
10	1.4	1.54	1.62	2.17	0.75*	1.35	1.05	0.51*	2.3											
11	2.96	2.58	2.49	3.7	2.38	2.1	2.66	1.87	0.62*	1.68										
12	3.61	3.01	2.86	4.57	3.1	2.49	3.5	2.74	0.61*	2.47	0.90*									
13	3.05	2.49	2.35	4.05	2.54	1.97	2.96	2.22	0.73*	1.92	0.53*	0.56*								
14	8.46	7.47	7.23	10.01	8.22	7.07	8.88	8.24	6.18	7.84	6.55	5.66	6.08							
15	2.18	1.54	1.41	3.5	1.77	1.02	2.37	1.79	1.63	1.34	1.13	1.47	0.95*	6.51						
16	7.75	6.77	6.53	9.31	7.52	6.36	8.18	7.54	5.53	7.14	5.87	4.99	5.39	0.71*	5.81					
17	6.8	5.86	5.62	8.27	6.52	5.42	7.14	6.48	4.4	6.1	4.77	3.88	4.3	1.78	4.78	1.13				
18	3.8	2.78	2.53	5.6	3.71	2.46	4.49	4.06	3.15	3.56	3.04	2.59	2.54	4.76	2.31	4.07	3.29			
19	7.16	6.13	5.9	9.08	7.19	5.92	8	7.62	6.43	7.12	6.48	5.82	5.95	3.4	5.87	3.08	3.43	3.56		
20	6.78	5.83	5.63	8.81	6.98	5.77	7.83	7.61	6.98	7.1	6.88	6.4	6.38	5.16	6.03	4.79	4.94	3.84	1.78	

## 2.3 Climate

Climate variables are considered to be one of the most important determinants of species richness (McCain and Grytnes 2010). In this study, we extracted five climate variables from the Worldclim database (Hijmans et al. 2005; <http://www.worldclim.org/>), including annual precipitation, precipitation seasonality (coefficient of variation of monthly precipitation), mean temperature, temperature range and temperature seasonality (standard deviation of monthly temperature  $\times$  100) (Fig. 2.4). Annual precipitation of Mt. Bokor decreases with altitude, from 2726 mm at 266 m to 2450 mm at 1048 m (Fig. 2.4a) and precipitation seasonality also decreases from 72 at 266 m to 67 at 1048m with altitude (Fig. 2.4b). Similarly, mean temperature of Mt. Bokor decreases with altitude, from 25.7 °C at 266 m to 21.9 °C at 1048 m (Fig. 2.4c), but temperature range increases with altitude (Fig. 2.4d). While all of those variables are strongly correlated with altitude, temperature seasonality has no significant correlation with altitude ( $R^2 = 0.18$ ;  $P = 0.21$ ; Fig. 2.4e; Table 2.2).

In addition, Rundel et al. (2003) reported that annual precipitation in the top area of Mt. Bokor exceeds 5000 mm and Stuart and Emmett (2006) noted that annual precipitation in Bokor National Park as a whole varies from 3000 mm to 5000 mm because the summer southwestern monsoon from the Gulf of Thailand provides high rainfall on Mt. Bokor. Considering those inconsistencies, the precipitation data from Worldclim seems not reliable enough.



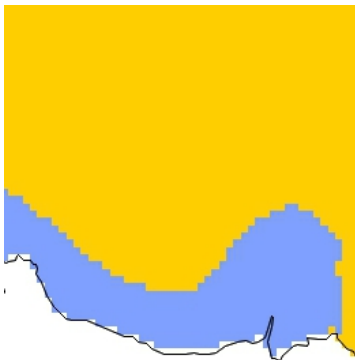
**Fig. 2.4** Relationships between altitude with annual precipitation (a), precipitation seasonality (b), mean temperature (c), temperature range (d), and temperature seasonality (e).

**Table 2.2** Pearson's correlation coefficients among altitude and climatic variables (\* $R > 0.75$ ). Alt: Altitude, Prec: Annual Precipitation, Prec.s: Precipitation seasonality, Temp: Annual mean temperature, Temp.r: Temperature range, Temp.s: Temperature seasonality.

	Alt	Prec	Prec.s	Temp	Temp.r	Temp.s
Alt	1.00					
Prec	-0.87*	1.00				
Prec.s	-0.94*	0.96*	1.00			
Temp	-0.96*	0.92*	0.96*	1.00		
Temp.r	0.94*	-0.91*	-0.95*	-0.97*	1.00	
Temp.s	-0.29	0.36	0.33	0.36	-0.21	1.00

## 2.4 Soil composition

Soil composition is also considered as an important factor for determining the species richness (McCain and Grytnes 2010). According to Harmonized World Soil Database (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012; <http://webarchive.iiasa.ac.at/>) there are two different types of soils along the altitudinal gradient of Mt. Bokor: Orthic Acrisols above alt. 700 m and Eutric Gleysols below alt. 700 m (<http://www.iiasa.ac.at>). Table 2.3 shows that the main composition of soil Orthic Acrisols and Eutric Gleysols (<http://www.iiasa.ac.at>).



**Fig. 2.5** Soil composition along the altitudinal gradient of Mt. Bokor: Orthic Acrisols (above alt. 700 m, yellow color) and Eutric Gleysols (below alt. 700 m, blue color), (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012; <http://www.iiasa.ac.at>).

## 2.5 Reference

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## **Chapter 3 Phylogeny analysis of tree species in Mt. Bokor**

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### 3.1 DNA extracting and sequencing

We collected a total of 3100 specimens from two sampling methods. For each of specimen collected, we cut a piece of leaf (1 cm diameter) as a sample for DNA extraction and dried it with silica–gel. For DNA isolation and sequencing, we followed the protocol of Toyama et al. (2015). We extracted two DNA segments, *ribulose –1, 5– biphosphate carboxylase oxygenase (rbcL*, 513 bp) and *maturase K (matK*, 1058 bp) from the samples. Condoncode aligner V5.1.5 (<http://www.codoncode.com>) was used to check electronpherograms. In addition, MEGA V.6.0.6 (Tamura et al., 2013) was used to arrange the DNA sequences. We used MAFFT V.7 (<http://mafft.cbrc.jp>) to align DNA sequences. We finally sequenced *rbcL* and *matK* for 514 tree species. The sequence data will be finally deposited to the GenBank.

### 3.2 Time calibrated phylogeny

We used the extracted barcodes of *rbcL* and *matK* to reconstruct the phylogenetic tree of 515 taxa collected in Mt. Bokor and estimate their divergence time. Firstly, we analyzed the phylogenetic relationships of 514 taxa by using maximum likelihood method of MEGA V.6.0.6 (Tamura et al., 2013), which was also used to estimate the best–fitting model for each gene segment (Hutter et al., 2014). The GTR + *I* + *Γ* model (general time reversible with proportion of sites invariable and rates at other sites varying according to a gamma distribution) was selected as the best–fitting model of evolution for each gene segment (Table 3.1 for *rbcL*, Table 3.2 for *matK*). Then we used BEAST 1.8.1 (Drummond et al. 2006; Drummond & Rambaut 2007; Drummond et al., 2012) to estimate divergence times. An uncorrelated lognormal relaxed clock model (UCLN) and Yule speciation process were used for these analyses (Drummond et al. 2006). The minimum ages of clades in the tree to prior probability distributions are determined, following Bell et al., (2010). In total, 20 clades are used: *Apiales*, *Aquifoliales*, *Arecales*, *Celastrales*, *Ericales*, *Fabales*, *Gentianales*, *Illiciales*, *Lamiales*, *Laurales*, *Liliales*, *Malvales*, *Magnoliales*, *Malpighiales*, *Myrtales*, *Oxalidales*, *Sapindales*, *Santalales*, *Vitales* and *Proteales* (Table 3.3). The minimum ages of 20 selected clades were set to an exponential distribution prior (Bell et al., 2010; Drummond et al., 2012).

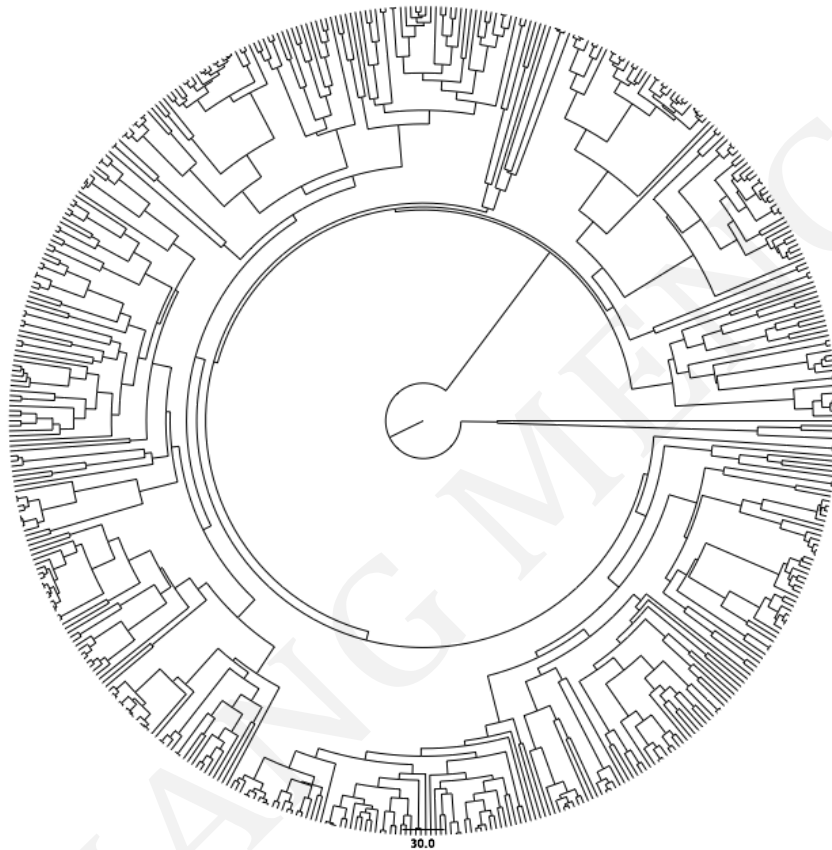
Totally 10000 trees were constructed by Markov–chain Monte Carlo simulations (MCMC) with 10 million times and we discarded first 1000 trees (Toyama et al., 2015). We selected the maximum clade credibility tree from the posterior distribution of 9000 trees by using TREEANNOTATOR V.1.6.1 (Drummond & Rambaut 2007) with a posterior probability limit of 0.5 and median node heights (Toyama et al., 2015).

### **3.3 PD, MPD, MNPD, NRI and NTI**

We firstly calculated Faith's PD of the both plot based and general specimen samples (Faith 1992; Kembel et al. 2010). Similar to species richness, PD is largely affected by the sample size and the observed PD should be underestimated (Chao et al., 2014). For species richness studies, the rarefaction and extrapolation approach was usually used to reduce a bias due to differences in sample size (Colwell et al., 2012). In the previous study, we applied rarefaction and extrapolation (Chao1 estimated richness) for the observed species richness to interpret the tree species richness pattern in Mt. Bokor (Zhang et al., 2016). Here we used a rarefaction and extrapolation of PD to account the sample completeness (Chao et al., 2014). For both of our 20 plots and 10 elevational intervals of general specimen collection, we used the number of specimens collected in both methods as reference samples to extrapolate till 400 specimens.

We next calculated the four other metrics of community phylogenetic structure including MPD (mean pairwise distance), MNPD (mean nearest taxon distance), NRI (net relatedness index) and NTI (Nearest taxon index) (Webb et al., 2002; Kembel et al. 2010). In which, values of NRI and NTI  $> 0$  indicate that the community is phylogenetically clustered, otherwise negative values indicate the community is phylogenetically overdispersed (Webb et al., 2002). NRI is sensitive to phylogeny wide patterns of taxonomy (Webb et al., 2002; Kembel et al. 2010). All above indices were calculated by R 3.2.2 (R Core Team 2014), using the *picante* package (Kembel et al. 2010).

**3.4 Phylogenetic tree of 514 tree species in Mt. Bokor (details are in the additional support Figure S1)**



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**Table 3.1** The best fit evolution model selection of *rbcl* sequence

Model	#Param	BIC	AICc	lnL	Invariant	Gamm R	Freq A	Freq T	Freq C	Freq G	A=>T	A=>C	A=>G	T=>A	T=>C	T=>G	C=>A	C=>T	C=>G	G=>A	G=>T	G=>C	
GTR+G+	1179	40018.24	27751.99	-12691.32	0.44	0.68	1.78	0.26	0.29	0.23	0.22	0.02	0.06	0.15	0.02	0.14	0.05	0.06	0.18	0.04	0.17	0.07	0.04
K2+G+	1172	40046.16	27852.67	-12748.72	0.44	0.68	1.78	0.25	0.25	0.25	0.25	0.04	0.04	0.16	0.04	0.16	0.04	0.04	0.16	0.04	0.16	0.04	0.04
T92+G+	1173	40048.25	27844.37	-12743.56	0.44	0.68	1.78	0.27	0.27	0.23	0.23	0.05	0.04	0.15	0.05	0.15	0.04	0.05	0.18	0.04	0.18	0.05	0.04
HKY+G+	1175	40072.05	27844.37	-12743.05	0.44	0.68	1.76	0.26	0.29	0.23	0.22	0.05	0.04	0.14	0.05	0.15	0.04	0.05	0.18	0.04	0.17	0.05	0.04
TN93+G+	1176	40083.96	27844.37	-12742.80	0.44	0.68	1.77	0.26	0.29	0.23	0.22	0.05	0.04	0.15	0.05	0.14	0.04	0.05	0.18	0.04	0.17	0.05	0.04
GTR+G	1178	40151.71	27844.37	-12764.26	n/a	0.30	1.78	0.26	0.29	0.23	0.22	0.02	0.06	0.15	0.02	0.14	0.05	0.06	0.17	0.04	0.18	0.07	0.04
K2+G	1171	40171.26	27844.37	-12817.48	n/a	0.28	1.77	0.25	0.25	0.25	0.25	0.05	0.05	0.16	0.05	0.16	0.05	0.05	0.16	0.05	0.16	0.05	0.05
T92+G	1172	40178.21	27844.37	-12814.75	n/a	0.29	1.76	0.27	0.27	0.23	0.23	0.05	0.04	0.14	0.05	0.14	0.04	0.05	0.18	0.04	0.18	0.05	0.04
HKY+G	1174	40200.35	27844.37	-12813.41	n/a	0.29	1.75	0.26	0.29	0.23	0.22	0.05	0.04	0.14	0.05	0.15	0.04	0.05	0.18	0.04	0.17	0.05	0.04
TN93+G	1175	40212.56	27844.37	-12813.31	n/a	0.29	1.75	0.26	0.29	0.23	0.22	0.05	0.04	0.14	0.05	0.14	0.04	0.05	0.18	0.04	0.17	0.05	0.04
JC+G+	1171	40861.22	27844.37	-13162.46	0.44	0.70	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
JC+G	1170	40983.83	27844.37	-13229.98	n/a	0.29	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GTR+	1178	41775.04	27844.37	-13575.92	0.49	n/a	1.66	0.26	0.29	0.23	0.22	0.03	0.06	0.14	0.02	0.14	0.05	0.07	0.18	0.04	0.16	0.07	0.05
T92+	1172	41826.31	27844.37	-13638.80	0.49	n/a	1.70	0.27	0.27	0.23	0.23	0.05	0.04	0.14	0.05	0.14	0.04	0.05	0.17	0.04	0.17	0.05	0.04
HKY+	1174	41848.25	27844.37	-13637.36	0.49	n/a	1.68	0.26	0.29	0.23	0.22	0.05	0.04	0.14	0.05	0.14	0.04	0.05	0.18	0.04	0.16	0.05	0.04
K2+	1171	41855.13	27844.37	-13659.41	0.49	n/a	1.68	0.25	0.25	0.25	0.25	0.05	0.05	0.16	0.05	0.16	0.05	0.05	0.16	0.05	0.16	0.05	0.05
TN93+	1175	41875.94	27844.37	-13644.99	0.49	n/a	1.65	0.26	0.29	0.23	0.22	0.05	0.04	0.12	0.05	0.16	0.04	0.05	0.2	0.04	0.15	0.05	0.04
JC+	1170	42636.36	27844.37	-14056.24	0.49	n/a	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GTR	1177	44415.29	27844.37	-14902.25	n/a	n/a	1.65	0.26	0.29	0.23	0.22	0.03	0.06	0.12	0.03	0.16	0.05	0.07	0.2	0.04	0.15	0.06	0.04
T92	1171	44470.29	27844.37	-14967.00	n/a	n/a	1.65	0.27	0.27	0.23	0.23	0.05	0.04	0.14	0.05	0.14	0.04	0.05	0.17	0.04	0.17	0.05	0.04
K2	1170	44470.80	27844.37	-14973.46	n/a	n/a	1.65	0.25	0.25	0.25	0.25	0.05	0.05	0.16	0.05	0.16	0.05	0.05	0.16	0.05	0.16	0.05	0.05
TN93	1174	44483.45	27844.37	-14954.95	n/a	n/a	1.65	0.26	0.29	0.23	0.22	0.05	0.04	0.12	0.05	0.16	0.04	0.05	0.2	0.04	0.15	0.05	0.04
HKY	1173	44484.89	27844.37	-14961.88	n/a	n/a	1.65	0.26	0.29	0.23	0.22	0.05	0.04	0.14	0.05	0.14	0.04	0.05	0.18	0.04	0.16	0.05	0.04
JC	1169	45244.42	27844.37	-15366.48	n/a	n/a	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08



**Table 3.2** The best fit evolution model of *mark* sequence

Model	#Param	BIC	AICc	InL	Invariant	Gamma	R	Freq A	Freq T	Freq C	Freq G	A=>T	A=>C	A=>G	T=>A	T=>C	T=>G	C=>A	C=>T	C=>G	G=>A	G=>T	G=>C
GTR+G+	1135.00	68757.69	57195.25	-27456.09	0.04	1.71	1.07	0.31	0.34	0.17	0.17	0.05	0.05	0.10	0.05	0.08	0.05	0.09	0.16	0.04	0.18	0.09	0.04
GTR+G	1134.00	68769.12	57216.85	-27467.91	n/a	1.42	1.07	0.31	0.34	0.17	0.17	0.05	0.05	0.10	0.05	0.08	0.05	0.09	0.16	0.04	0.18	0.09	0.04
T92+G+	1129.00	68978.81	57477.42	-27603.24	0.04	1.67	1.34	0.33	0.33	0.17	0.17	0.07	0.04	0.10	0.07	0.10	0.04	0.07	0.19	0.04	0.19	0.07	0.04
T92+G	1128.00	68991.15	57499.93	-27615.51	n/a	1.38	1.34	0.33	0.33	0.17	0.17	0.07	0.04	0.10	0.07	0.10	0.04	0.07	0.19	0.04	0.19	0.07	0.04
HKY+G+	1131.00	68992.68	57470.94	-27597.98	0.04	1.69	1.34	0.31	0.34	0.17	0.17	0.07	0.04	0.10	0.06	0.10	0.04	0.06	0.20	0.04	0.18	0.07	0.04
TN93+G+	1132.00	68996.61	57464.69	-27593.85	0.04	1.72	1.34	0.31	0.34	0.17	0.17	0.07	0.03	0.10	0.06	0.11	0.04	0.06	0.22	0.04	0.17	0.07	0.03
HKY+G	1130.00	69004.59	57493.03	-27610.04	n/a	1.40	1.34	0.31	0.34	0.17	0.17	0.07	0.04	0.10	0.06	0.10	0.04	0.06	0.20	0.04	0.18	0.07	0.04
TN93+G	1131.00	69009.45	57487.71	-27606.37	n/a	1.43	1.34	0.31	0.34	0.17	0.17	0.07	0.03	0.10	0.06	0.11	0.04	0.06	0.22	0.04	0.17	0.07	0.03
K2+G+	1128.00	69123.89	57632.67	-27681.89	0.04	1.51	1.40	0.25	0.25	0.25	0.25	0.05	0.05	0.15	0.05	0.15	0.05	0.05	0.15	0.05	0.15	0.05	0.05
K2+G	1127.00	69134.01	57652.97	-27693.05	n/a	1.25	1.40	0.25	0.25	0.25	0.25	0.05	0.05	0.15	0.05	0.15	0.05	0.05	0.15	0.05	0.15	0.05	0.05
JC+G+	1127.00	70370.58	58889.54	-28311.33	0.04	1.53	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
JC+G	1126.00	70380.99	58910.13	-28322.64	n/a	1.27	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GTR+	1134.00	70399.89	58847.63	-28283.29	0.04	n/a	0.98	0.31	0.34	0.17	0.17	0.06	0.06	0.10	0.05	0.08	0.05	0.10	0.16	0.05	0.17	0.09	0.05
GTR	1133.00	70672.85	59130.76	-28425.87	n/a	n/a	0.98	0.31	0.34	0.17	0.17	0.05	0.06	0.10	0.05	0.08	0.04	0.10	0.16	0.05	0.17	0.09	0.05
TN93+	1131.00	70679.99	59158.25	-28441.64	0.04	n/a	1.37	0.31	0.34	0.17	0.17	0.07	0.03	0.10	0.06	0.11	0.03	0.06	0.22	0.03	0.17	0.07	0.03
T92+	1128.00	70681.97	59190.76	-28460.93	0.04	n/a	1.37	0.33	0.33	0.17	0.17	0.06	0.03	0.10	0.06	0.10	0.03	0.06	0.20	0.03	0.20	0.06	0.03
HKY+	1130.00	70684.20	59172.64	-28449.84	0.04	n/a	1.37	0.31	0.34	0.17	0.17	0.07	0.03	0.11	0.06	0.10	0.03	0.06	0.21	0.03	0.19	0.07	0.03
HKY	1129.00	70989.20	59487.81	-28608.44	n/a	n/a	1.38	0.31	0.34	0.17	0.17	0.07	0.03	0.11	0.06	0.10	0.03	0.06	0.21	0.03	0.19	0.07	0.03
TN93	1130.00	70990.37	59478.81	-28602.93	n/a	n/a	1.38	0.31	0.34	0.17	0.17	0.07	0.03	0.10	0.06	0.11	0.03	0.06	0.22	0.03	0.17	0.07	0.03
T92	1127.00	70992.18	59511.14	-28622.13	n/a	n/a	1.38	0.33	0.33	0.17	0.17	0.06	0.03	0.10	0.06	0.10	0.03	0.06	0.20	0.03	0.20	0.06	0.03
K2+	1127.00	71209.44	59728.40	-28730.76	0.04	n/a	1.36	0.25	0.25	0.25	0.25	0.05	0.05	0.14	0.05	0.14	0.05	0.05	0.14	0.05	0.14	0.05	0.05
K2	1126.00	71572.15	60101.29	-28918.22	n/a	n/a	1.35	0.25	0.25	0.25	0.25	0.05	0.05	0.14	0.05	0.14	0.05	0.05	0.14	0.05	0.14	0.05	0.05
JC+	1126.00	72426.52	60955.65	-29345.40	0.04	n/a	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
JC	1125.00	72786.87	61326.19	-29531.68	n/a	n/a	0.50	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

**Table 3.3** The minimum ages of these 20 selected clades (the exponential distribution prior was used, Bell et al., 2010).

Node	Exponential (myr)	Lognormal (myr)
1 Apiales	49 (39–61)	53 (42–63)
2 Aquifoliales	88 (85–93)	87 (85–101)
3 Arecaceae, Arecales	31 (25–38)	33 (21–36)
4 Celastrales	71 (60–80)	76 (62–89)
5 Ericales	92 (92–102)	92 (92–102)
6 Fabales	79 (68–88)	83 (73–94)
7 Gentianales	65 (56–74)	69 (54–78)
8 Illiciales	–	–
9 Lamiales	69 (61–76)	74 (68–78)
10 Laurales	112 (108–121)	119 (107–133)
11 Liliales	86 (72–99)	114 (105–123)
12 Malvales	78 (70–87)	83 (70–89)
13 Magnoliales	69 (50–90)	76 (58–96)
14 Malpighiales + Celastrales	98 (98–103)	102 (95–109)
15 Myrtales	89 (89–95)	89 (89–99)
16 Oxalidales	89 (78–98)	93 (82–105)
17 Sapindales	71 (70–73)	71 (70–75)
18 Santalales	91 (76–104)	99 (81–115)
19 Vitales	–	–
20 Proteales	110 (101–120)	116 (103–131)

**Chapter 4 Constant tree species richness along  
an elevational gradient of Mt. Bokor**

ZHANG MENG

## **Abstract**

Some previous studies along an elevational gradient on a tropical mountain documented that plant species richness decreases with increasing elevation. However, most of studies did not attempt to standardize the amount of sampling effort. In this paper, we employed a standardized sampling effort to study tree species richness along an elevational gradient on Mt. Bokor, a table-shaped mountain in southwestern Cambodia, and examined relationships between tree species richness and environmental factors. We used two methods to record tree species richness: first, we recorded trees taller than 4 m in 20 uniform plots (5 × 100 m) placed at 266–1048-m elevation; and second, we collected specimens along an elevational gradient from 200 to 1048 m. For both datasets, we applied rarefaction and a Chao1 estimator to standardize the sampling efforts. A generalized linear model (GLM) was used to test the relationship of species richness with elevation. We recorded 308 tree species from 20 plots and 389 tree species from the general collections. Species richness observed in 20 plots had a weak but non-significant correlation with elevation. Species richness estimated by rarefaction or Chao1 from both data sets also showed no significant correlations with elevation. Unlike many previous studies, tree species richness was nearly constant along the elevational gradient of Mt. Bokor where temperature and precipitation are expected to vary. We suggest that the table-shaped landscape of Mt. Bokor, where elevational interval areas do not significantly change between 200–900-m, may be a determinant of this constant species richness.

## **Keywords**

Elevational gradient, rarefaction, sampling bias, species richness pattern, tropical forest

## **Introduction**

Terrestrial plant diversity varies with both latitude and elevation (Guo et al. 2013). Tropical areas are well known to harbor the highest levels of plant diversity in the world along an elevational gradient (Whitmore 1999; Kreft and Jetz 2007; Kerkhoff et al. 2014). Ecologists have had a continuing interest in this high species richness in tropical lowland rain forests (Whitmore 1999; Hubbell et al. 2008). While most studies related to the species richness in tropical areas, particularly of plants, have been conducted in lowlands, some studies along an elevational gradient on a tropical mountain have documented that plant species diversity decreases with increasing elevation (see Aiba and Kitayama 1999 for a review). However, most of previous elevational studies did not attempt to standardize the amount of sampling effort (Rahbek 1995; Guo et al. 2013). Grytnes (2003) and Carpenter (2005) made a pioneering transect survey using uniform sampling to standardize sampling effort. Subsequently, Grytnes and Beaman (2006) applied a rarefaction model to densely collected specimen data of Mt. Kinabalu, a prominent mountain on the island of Borneo in Southeast Asia, and estimated total species richness for each elevational interval. The result showed that a weakly hump-shaped elevational pattern of species richness exists, peaking in the interval between 600 and 900 m or between 900 and 1200 m.

A hump-shaped pattern of species richness with a mid-elevational peak has been observed in many studies made on various organisms worldwide (McCain and Grytnes 2010; Guo et al. 2013). This pattern agrees with the prediction of the “mid-domain effect” model developed by Colwell and Hurtt (1994) who claimed that a hump-shaped pattern can arise from the random placement of species ranges within a bounded domain, even under uniform environmental conditions. This model triggered a series of empirical studies on species richness along elevational gradients. By reviewing those studies, Currie and Kerr (2008) concluded that observed broad-scale patterns of species richness are not consistent with the mid-domain hypothesis.

Additional empirical studies are needed using a standardized method to examine the pattern of species richness and identify major factors determining variation of species richness with elevation. Thus, we are conducting a series of field surveys in many

mountainous areas of tropical Southeast Asia by recording plant species richness within 5- × 100-m plots placed at different elevations on a mountain (Yahara et al. 2012; Tagane et al. 2015). While many studies of tropical lowland forests have used fewer and larger plots, our strategy employs placing smaller plots in many more locations than have been employed in many previous studies of the elevational patterns of plant species richness (Gentry et al. 1995; Grytnes 2003; Sanchez-Gonzalez and Lopez-Mata 2005; Kluge et al. 2006). While our previous studies using 5- × 100-m plots revealed significant changes of species richness along an elevational gradient in most mountains we studied, including Mt. Honba in southern Vietnam, we found a non-significant correlation between species richness and elevation on a table-shaped mountain, Mt. Bokor, in southwestern Cambodia. Mt. Honba and Mt. Bokor are located approximately 400 km apart on the southern Indochina Peninsula, where annual rainfall is very high (Rundel et al. 2003; Stuart and Emmett 2006; Tagane et al. 2015). It would be interesting to know why two geographically proximate mountains under similar climatic conditions would show a notable difference in patterns of species diversity along an elevational gradient.

The main purpose of this study is to describe a pattern of plant species richness along an elevational gradient from 200 to 1048 m in Mt. Bokor, Cambodia. Additionally, we compare these observations with elevational changes of tree density and discuss plausible factors determining the plant species richness pattern along the elevation gradient of Mt. Bokor.

## **Methods**

### **Elevational land area and species pool**

In this study, sampling area had no direct effect on species richness because all plots sampled were the same size (500 m<sup>2</sup>) along the elevational gradient (Grytnes 2003). However, species richness in a sampling plot is influenced by the number of individuals sampled and the number of species in the species pool (Zobel et al. 1998). The species area relationship indicates that a larger area tends to have a larger number of species (Rosenzweig 1995), and thus has a larger species pool (Grytnes 2003). Here, we calculated the areas of elevational intervals per 100 m (from 200–299 m to 1000–1079 m) in the entire mountain range of Mt. Bokor, starting at the Shuttle Radar

Topography Mission layer, but excluding the areas below 200-m elevation (Fig. 2a). Elevational interval areas decreased with elevation ( $R^2 = 0.67$ ;  $P = 0.007$ ), but this pattern became non-significant if we excluded the areas above 900 m ( $R^2 = 0.18$ ;  $P = 0.19$ ) (Fig. 2a).

## **Data analysis**

### **Rarefaction, extrapolation and Chao1 richness estimator:**

Reducing sampling limitations and biases are the challenge for ecologists to compare species richness and analyze diversity patterns (Chao et al. 2005; Colwell et al. 2012). To meet this challenge, models of rarefaction and extrapolation have been proposed. The rarefaction approach could reduce a bias due to differences in sample size. Extrapolations enable us to estimate species richness expected in a larger sample (Colwell et al. 2012). In our study, for plot-based sampling, we used a species richness dataset from 10 successive subplots of  $10 \times 5 \text{ m}^2$  to extrapolate 20 extra samples. Meanwhile, for general specimen sampling, we used the number of specimens collected in each of 11 altitudinal intervals as reference samples to extrapolate till 400 specimens. In addition, we calculated Chao1 with confidence interval (95%), a widely used estimator of total species richness (Colwell et al. 2004; Colwell 2013) for all the plots and altitudinal intervals. All of the above approaches were processed by the version 9.1.0 of EstimateS with a set of 100 randomizations for estimators (Colwell 2013).

### **Species richness patterns:**

Species richness observed in the 20 plots and nine elevational intervals was compared as “point diversity” (Carpenter 2005; Kluge et al. 2006). Species richness was considered to have a Poisson distribution because of its discrete values (McCullagh and Nelder 1989). Thus, simple scatter diagrams and a generalized linear model (GLM) up to the second polynomial with quasi-Poisson regression with a logarithmic link were explored to illustrate the changes of species richness and tree density as a function of elevation as well as the relationship between species richness and tree density. A quasi-Poisson distribution was used to account for overdispersion.

### **Spatial autocorrelation and environmental variables analysis**

Initially, we calculated Moran's  $I$  (Fortin et al. 2002; Paradis et al. 2004), one of the most widely used coefficients of spatial autocorrelation, to check for the significance of two-dimensional spatial autocorrelation in our species richness within 20 plots. We calculated Moran's  $I$  of all 20 plots and also for neighbors at a geographic distance less than 2 km. In addition, because environmental variables were also highly correlated ( $R > 0.75$ ) with each other (Table 2.2 in Chapter 2), we used General Least Squares (GLS) model, which could efficiently incorporate spatial autocorrelation effects and manage spatial dependence of variables (Dormann et al. 2007; Ortiz-Yusty et al. 2013), to determine correlations of field observed and estimated species richness (Chao1) of 20 plots with climatic variables. Furthermore, to determine whether soil had important effects on species richness, we also used GLMM to test whether there were differences of tree species richness between altitude above 700 m and below 700 m.

All the above calculations and illustrations were made with R 3.0.2 (R Core Team 2014), using the *ape* package (Paradis et al. 2004) for Moran's  $I$  test, *lem4* (Bates et al. 2014) for GLMM analysis, *nlme* (Pinheiro et al. 2015) for GLS analysis.

## **Results**

### **Plots and specimen data**

In the 20 plots, we recorded 3029 individual trees with height above 4 m, including 308 species of 178 genera and 76 families (Table 1). Among them, only two species, *Gironniera subaequalis* and *Toxicodendron succedaneum*, were deciduous. *G. subaequalis* was found from 266 to 970 m (58 individuals) and *T. succedaneum* was found above 1000 m (two individuals). The four most abundant species were *Archidendron quocense* (154 individuals), *Lithocarpus elephantum* (67) and *Macaranga andamanica* (66) and *Mallotus paniculatus* (112). Tree density varied from 65 individuals per plot (500 m<sup>2</sup>) at 266 m to 348 at 1014 m. Table 3 summarizes the GLM analysis results based on plot data. A significantly positive correlation was observed between elevation and tree density (Fig. 2b, Table 3;  $P = 0.013$ ); however, note that tree density records at 1014 m (348) and 1048 m (295) were much higher than 188 individuals at 970 m, 138 at 928 m and other density records at lower



elevations. If the data collected at 1014 m (348) and 1048 m (295) were excluded, the correlation became non-significant (Table 3;  $P = 0.148$ ). Species richness varied from 27 at 266 m to 70 at 970 m (Fig. 3); a weak but non-significant correlation existed between species richness and elevation (Table 3;  $P = 0.064$ ). Moreover, the second polynomial regression also showed species richness had no significant correlation with elevation (Table 3; for elevation,  $P = 0.267$ ; for elevation squared,  $P = 0.396$ ). Additionally, the correlation between tree density and species richness was not significant (Fig. 2c, Table 3;  $P = 0.077$ ). The number of families and genera was also minimal at 266 m and maximal at 970 m. However, on Mt. Honba, a significant increase in species richness was found from 225 to 1200 m with species richness decreasing at 1336 m and 1500 m (Fig. 3, Table 3; fitted to a quadratic curve, for elevation,  $P = 0.008$ ; for elevation squared,  $P = 0.014$ ). As a general floristic survey, we collected 1120 specimens of tree species which represented 389 species of 200 genera and 79 families (Table 2). The number of collected specimens per 100-m interval varied from 45 at 300–399 m to 223 at 900–999 m. Species richness per 100-m interval varied from 42 at 300–399 m to 150 at 900–999 m. The number of specimens was positively correlated with elevation (Dev. = 96.62,  $P = 0.021$ ). However, observed species richness was not significantly correlated with elevation (Fig. 4b; Dev. = 69.3,  $P = 0.094$ ). The numbers of endemic species collected from the highest to the lowest interval were 12, 29, 11, 6, 2, 0, 2, 2, and 0, respectively.

### **Rarefaction extrapolation and richness estimator**

According to rarefaction curves of 20 plots, if 65 individuals (minimal sample size in Plot 1) are assumed to be sampled, species richness and its 95% confidence interval vary from 20.91 (CI range: 16.23–25.6) at 330 m to 41.3 (CI range 35.01–47.58) at 888 m (Fig. 5a). Extrapolated species richness at 30th subplots was maximal at alt. 702 m, and minimal at alt. 928 m. However, the 95% confidence intervals of the Chao1 richness estimator (Fig. 4a) overlapped with each other except in the following two cases: (1) expected richness at 928 m was significantly lower than that in the neighboring plots at 903 m and 970 m, and (2) expected richness at 330 m was significantly lower than that in the neighboring plot at 370 m.

Rarefaction and extrapolated curves for all nine elevational intervals (Fig. 5b) also showed curves that fell within a narrow interval. For species richness and its 95%

confidence interval, rarefaction from the minimum number of specimens (45), varied from 38.5 (CI range: 34.25–43.64) at 1000–1048 m to 43.61 (CI range 34.07–53.15) at 200–299 m (Fig. 5b). The Chao1 estimated species richness value peaked at the 400–499-m elevational interval of and was the least at the interval of 1000–1048 m. The 95% confidence intervals of the Chao1 richness estimator (Fig. 4b) did not overlap between the neighboring elevational intervals in one case: between 900–999 m and 1000–1048 m.

### **Spatial autocorrelation and environmental variables analysis**

Based on Moran's  $I$  of the complete set of 20 plots (obs. =  $-0.01$ ; exp. =  $-0.05$ ; SD =  $0.06$ ;  $P = 0.51$ ) no spatial autocorrelation of species richness was observed among the 20 plots. Additionally, Moran's  $I$  between the plots with a geographic distance of less than 2 km (obs. =  $-0.01$ ; exp. =  $-0.05$ ; SD =  $0.16$ ;  $P = 0.77$ ) also indicated no spatial autocorrelation between them. GLS regression results showed that no significant relationships were found between observed species richness with environmental variables except for annual precipitation ( $P = 0.01$ ), while all of the environmental variables had no significant effects on Chao1 estimated richness (Table 3). In addition, GLS model efficiently managed the spatial correlations ( $R < 0.75$ ) among the environmental factors (Table S1, S2)

## **Discussion**

### **Constant species richness along the elevational gradient**

In this study, we quantified the pattern of tree species richness of a wet tropical rainforest along an elevational gradient on a table-shaped mountain, Mt. Bokor. The observed species richness of 20 plots had no significant pattern along the elevational gradient from 266–1048 m. However, species richness data from Mt. Honba, obtained using the same plot size, showed a hump-shaped pattern with a significant increase in species richness from 225–1200 m. Also, species richness estimates by Chao1 from the plot data (266–1048 m) showed no significant correlations with elevation (Fig. 4a), and rarefaction estimates standardized for a minimal sample size (65 individuals) showed no significant difference among plots (Fig. 5a). For the general collection data summed for nine elevational intervals (200–299 m to 1000–1048 m), observed species richness also had no significant correlations with elevation (Fig. 4b). In

addition, species richness estimates by Chao1 from the general collection data showed no significant correlations with elevation (Fig. 4b) and rarefaction estimates standardized for a minimal sample size (45 specimens) showed no significant difference (Fig. 5b). This constant richness pattern agreed neither with the “mid-domain effect” model (Colwell and Hurtt 1994) nor with any of the four common patterns summarized by McCain and Grytnes (2010), where the hump-shaped pattern is considered to be the most common pattern among several elevational gradient research studies involving plants (Rahbek 1995, 2005; McCain and Grytnes 2010; Guo et al. 2013). Thus, our findings in Mt. Bokor provide a unique opportunity to understand the relationship of species richness with several factors that can vary with elevation.

### **Plausible mechanisms behind the constancy of species richness**

The fact that species richness is not significantly correlated with elevation implies three possibilities. First, our sample size may be too small to detect the relationship between species richness and elevation. Second, neither temperature nor precipitation had any significant effect on species richness in Mt. Bokor. Third, effects of temperature and precipitation may cancel each other out completely.

Because our survey is based on a small plot size (500 m<sup>2</sup>), observed species richness is not saturated within this plot size (Fig. 5), and also because the relationship between species richness and elevation was not significant ( $P = 0.064$ ), further surveys using larger plots may detect a correlation of species richness with elevation. However, a data set from Mt. Honba using the same plot size showed a hump-shaped pattern with the significant increase in species richness from 225–1200 m (Fig. 3). Thus, we can conclude that the correlation between species richness and elevation in Mt. Bokor is, if any, weaker than the significant correlations previously observed on other mountains.

For the second possibility, temperature and precipitation have been considered to be important determinants of species richness along elevational gradients in other situations (Rahbek 1995; Lomolino 2001; Hawkins et al. 2003; Körner 2007; McCain 2007; McCain and Grytnes 2010; Guo et al. 2013). The relationship between tree species richness and temperature is generally hump-shaped (O’Brien et al. 1998).

However, our study found no significant change in tree species richness with elevation while the temperature decreases by 0.8 °C with every 100-m increase of elevation (Rundel et al. 2003), implying that temperature alone is not a significant factor restricting species richness in Mt. Bokor. Tree species richness is known to decrease with annual precipitation at a global scale (Francis and Currie 2003, Hawking et al. 2003) and also in tropical Southeast Asia (Slik et al. 2009). While annual precipitation on top of Mt. Bokor exceeds 5000 mm (Rundel et al. 2003), annual precipitation in Bokor National Park as a whole varies from 3000–5000 mm (Stuart and Emmett 2006). Thus, it is likely that annual precipitation is higher at higher elevations, although the precipitation data extracted from the Worldclim database shows the reverse trend. Because our study showed no significant change of tree species richness with elevation, it is unlikely that a precipitation gradient alone constrains species richness.

We cannot exclude the third possibility that the combined effects of decreasing temperature and increasing annual precipitation with elevation would have negative and positive effects on species richness, respectively, and therefore could cancel each other. To test this possibility, we need more reliable annual precipitation data from different elevations of Mt. Bokor.

### **An implication for the land area hypothesis**

One of the difficulties in studying patterns of species richness along an elevational gradient is that many factors change with elevation creating confounding conditions with each other (Körner 2007). Temperature decreases with elevation by an average of 0.6 °C for each 100-m increase (Barry 2013). However, land area usually decreases with elevation (Körner 2007). Therefore, when studying the correlation between elevation and species richness, it is usually difficult to determine whether the correlation reflects a direct coupling or the results of the combining effects of several other factors (Rahbek 1995; Körner 2007). If land area per elevational zone is a major determinant of species richness, then species richness is expected to not decrease with elevation on a table-shaped mountain. This prediction is consistent with our findings on Mt. Bokor that neither Chao1 nor land area per elevation zone significantly vary with elevation below 900 m but Chao1 is significantly lower on the top of the plateau above 1000 m where land area per elevation zone is very limited.

However, the decrease of Chao1 above 1000 m may be associated with the unique environment on the plateau where the landscape is very flat (Fig. 1). We found unique features in the two plots on the plateau (1014 m and 1048 m). Tree density was significantly higher (Fig. 2a) while tree height was lower on the plateau. Those observations suggest that some of the forest that has developed on the top of the plateau of Mt. Bokor can be considered to be a kind of kerangas (heath) forest that develops under frequent flooding on a flat landscape (Proctor et al. 1983). In addition, endemism peaks on the plateau, suggesting that the environments on the plateau have been historically unique and have driven adaptive speciation.

## **Conclusion**

We found that Mt. Bokor, an isolated, table-shaped mountain, shows a nearly constant pattern of tree species diversity along an elevational gradient. This pattern does not agree with any of the four common patterns between species richness and elevation summarized by McCain and Grytnes (2010). We suggest that the table-shaped landscape may be a determinant of this constant species richness. In this study, we focused on trees because the tree species had been mostly completely identified. Subsequent taxonomic studies related to vines, shrubs and herbs that are currently in progress will enable us to examine whether species richness patterns are similar among different life forms. Further studies on other mountains are also needed to deepen our understanding of the patterns of species richness in the tropical rain forests of Southeast Asia.

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## Figure Legends

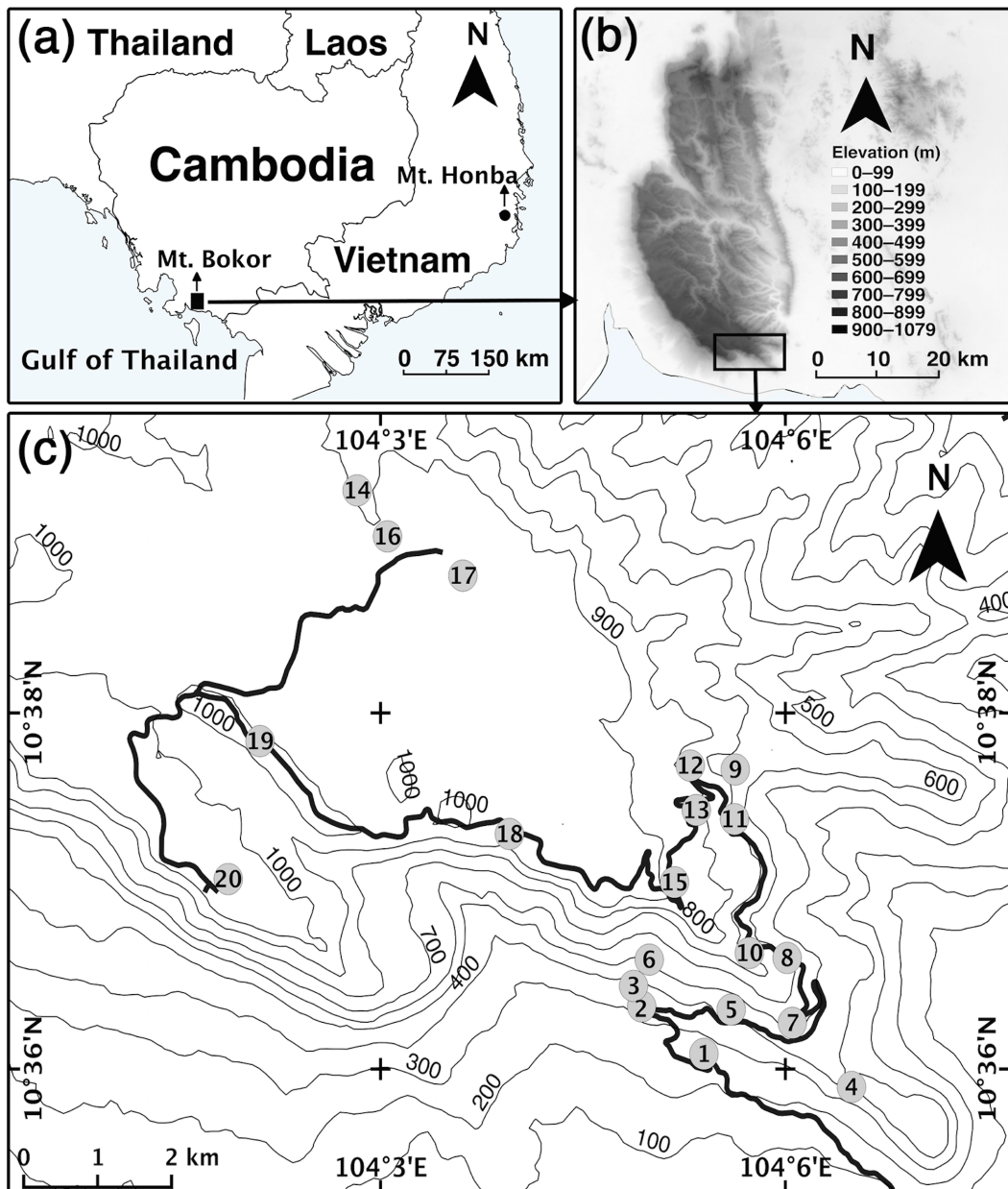
**Fig. 1** Study area: (a) Locations of Bokor National Park (black rectangle) and Mt. Honba (black solid circle), (b) topography of Bokor National Park, (c) the locations of plots in the southern slope and top area of Mt. Bokor (circles with numbers, sampling plots; thick line, main route along which we made general specimen collection; thin lines, contours; plus mark, cross graticules)

**Fig. 2** (a) Distribution of land area per elevation zone, (b) the relationship between tree density and elevation ( $P = 0.013$ ), and (c) the relationship between species richness and tree density ( $P = 0.077$ )

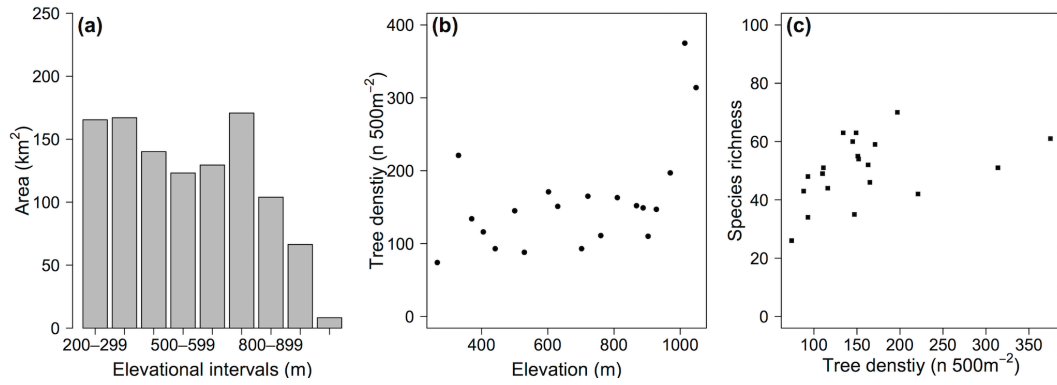
**Fig. 3** Relationships between observed species richness and elevation in Mt. Honba (solid circles, species richness; firm curve line, fitted by generalized linear regression (GLM) with the 95% confidence interval shown by dash lines, for elevation,  $P = 0.008$ ; for elevation squared,  $P = 0.014$ ) and Mt. Bokor (solid square, the relationship was not significant,  $P = 0.064$ )

**Fig. 4** Field observed species richness and Chao1 estimated richness for the (a) 20 plots and (b) nine elevational intervals. Open circles/squares, field observed richness; solid circles/squares, point estimates; bars, confidence intervals (95%)

**Fig. 5** (a) Rarefaction curves for 20 plots and (b) nine elevational intervals; a dash line in (a) indicates 65, the minimum of individuals recorded, and a dash line in (b) indicates 45, the minimum of specimens collected. Legends refer to 20 plots or nine elevational intervals. For simplicity, confidence intervals are not illustrated. Units in both parts of the figure are in m.

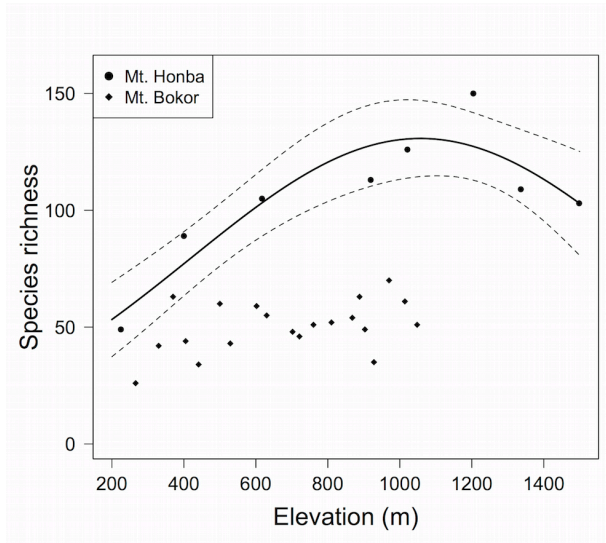


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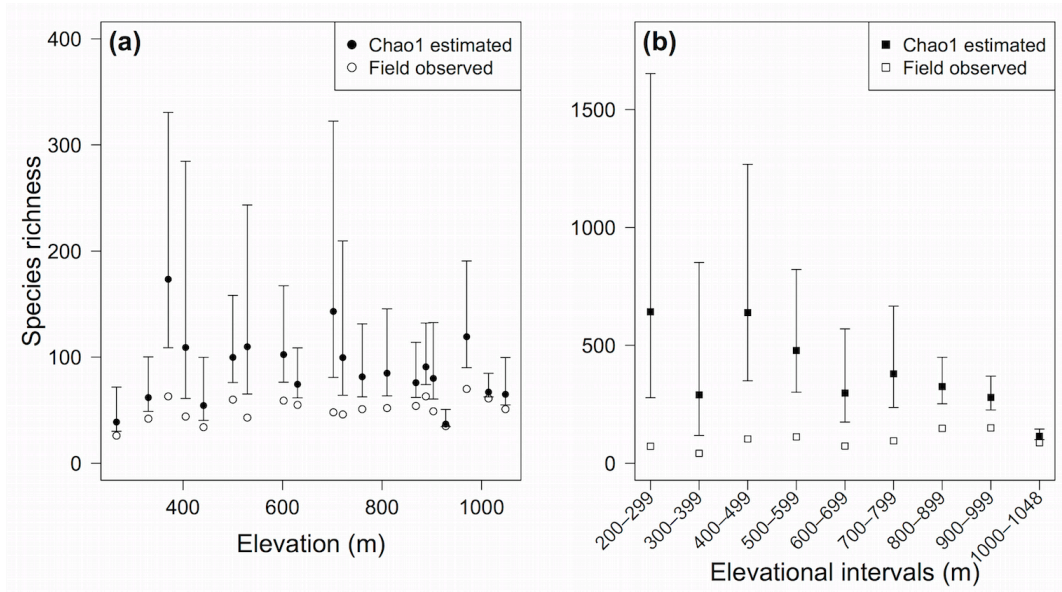
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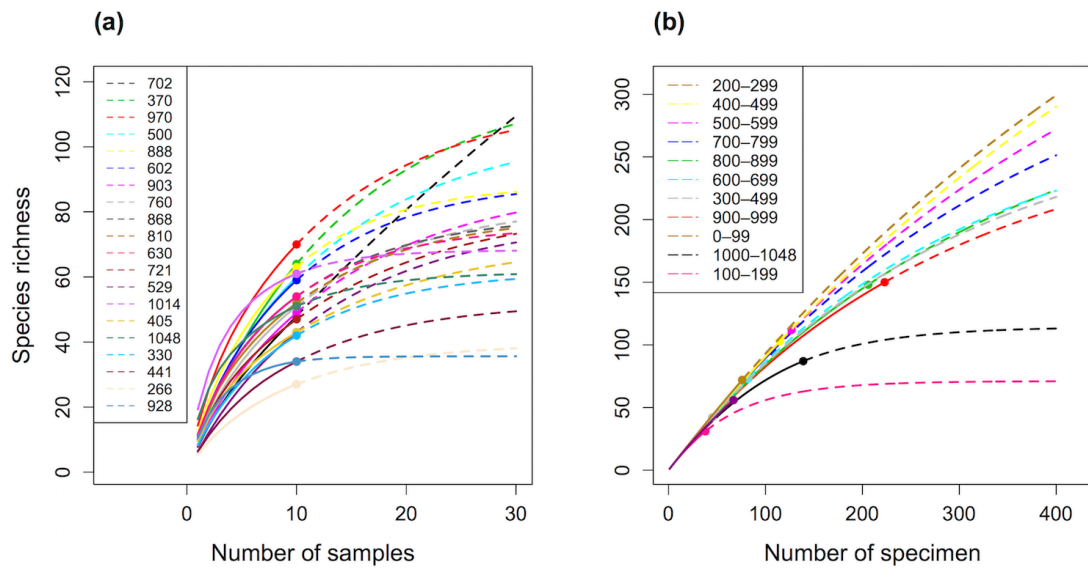
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**Fig. 4** Field observed species richness and Chao1 estimated richness for the (a) 20 plots and (b) nine elevational intervals. Open circles/squares, field observed richness; solid circles/squares, point estimates; bars, confidence intervals (95%)

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**Fig. 5** Rarefaction (solid lines) and extrapolation curves (dashed lines) for 20 plots (a) and 11 elevational intervals (b) of Mt. Bokor. The legend follows the order of species richness expected by extrapolation. Legends refer to 20 plots or 11 elevational intervals. For simplicity, confidence intervals are not illustrated. Units in both parts of the figure are in m.

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## Tables

**Table 1** Elevations, tree density, the number of families, genera, and species in 20 plots in Mt. Bokor

Plot	Elevation (m)	Individuals	Families	Genera	Species
1	266	65	20	25	27
2	330	221	25	40	42
3	370	134	32	54	64
4	405	115	26	36	43
5	441	93	25	34	34
6	500	145	35	48	60
7	529	95	30	39	43
8	602	145	32	52	59
9	630	142	32	48	54
10	702	93	30	44	48
11	721	136	32	43	47
12	760	111	31	44	51
13	810	163	33	46	52
14	868	152	30	44	54
15	888	140	30	45	63
16	903	110	32	42	49
17	928	138	20	29	34
18	970	188	36	53	70
19	1014	348	33	50	61
20	1048	295	27	41	51



**Table 2** Tree diversity revealed by general collections of specimens in nine elevational intervals on Mt. Bokor.

Elevational interval (m)	Specimens	Families	Genera	Species
200–299	76	35	54	72
300–399	45	21	31	42
400–499	115	37	72	103
500–599	127	48	79	112
600–699	82	36	56	73
700–799	107	41	70	95
800–899	206	57	102	148
900–999	223	51	95	150
1000–1048	139	39	65	87

**Table 3** Summary of statistical tests using a generalized linear model for: (1) the relationships between observed species richness and species density as a function of elevation, and the relationship between species richness and tree density of plot data on Mt. Bokor; and (2) the relationship between species richness and elevation on Mt. Honba.

Study sites	Indicators	Order	Predictors	d.f.	Dev.	<i>P</i>
Mt. Bokor	Richness	2	Elevation	17	38.04	0.267
		2	Elevation <sup>2</sup>	17	38.04	0.396
	Density	1	Elevation	18	39.76	0.064
		1	Density	18	40.86	0.077
		1	Elevation	18	388.76	0.013*
		1	Elevation.e	16	200.84	0.148
Mt. Honba	Richness	2	Elevation	5	9.29	0.008*
		2	Elevation <sup>2</sup>	5	9.29	0.014*

Richness, species richness; Density, species density; Density.e, species density excluding the data above 1000 m; Elevation<sup>2</sup>, elevation squared; Elevation.e, elevation below 1000 m; d.f., degree of freedom; Dev., residual deviance; \*,  $P < 0.05$  indicates significance.

## Electronic supporting information

Table S1 Correlation coefficients among environmental variables of observed species richness GLS analysis (\* $R > 0.75$ ).

	Intercept	Alt	Prec	Prec.s	Temp	Temp.r
Alt	-0.07					
Prec	0.14	-0.38				
Prec.s	-0.46	0.05	-0.71			
Temp	-0.40	0.45	0.02	-0.41		
Temp.r	-0.91*	-0.10	0.19	0.10	0.53	
Temp.s	0.48	0.00	-0.24	0.17	-0.56	-0.67

Table S1 Correlation coefficients among environmental variables of Chao 1 estimated richness GLS analysis (\* $R > 0.75$ ).

	Intercept	Alt	Prec	Prec.s	Temp	Temp.r
Alt	-0.23					
Prec	0.04	-0.31				
Prec.s	-0.33	0.17	-0.76			
Temp	-0.62	0.42	0.19	-0.37		
Temp.r	-0.90*	0.00	0.28	-0.05	0.74	
Temp.s	0.55	-0.06	-0.28	0.17	-0.65	-0.71

Alt: Altitude, Prec: Annual Precipitation, Prec.s: Precipitation seasonality, Temp: Annual mean temperature, Temp.r: Temperature range, Temp.s: Temperature seasonality.

ZHANG MENG

## **Chapter 5 Phylogenetic diversity and structure of tree species in Mt. Bokor**

ZHANG MENG

## **Abstract**

Although numerous studies of taxonomic species diversity patterns along the elevational gradients have been investigated in different mountains, measuring the biodiversity by only using species diversity of taxonomic level is inherently limited and could potentially obscure other diversity patterns such as phylogenetic diversity. In addition, quantifying phylogenetic structure could provide a useful tool to understand the species assemblage in community. In a previous study, we observed a constant altitudinal tree species richness of Mt. Bokor, a table-shaped mountain, locating in the southwest Cambodia. In this study, we used two DNA barcode segments, *rbcL* and *matK*, to construct the phylogenetic tree, calculate the phylogenetic diversity and phylogenetic structure of tree species along an elevational gradient of Mt. Bokor, southwest Cambodia. We also applied a rarefaction and extrapolation to the analysis of phylogenetic diversity considering the sampling completeness. Our results showed that observed phylogenetic diversity (PD) did not significantly change along elevational gradient. Nearest Taxon Index (NTI) also had no significant relationships with elevation, but Net Relatedness Index (NRI) had a significantly decreasing correlation with elevation. In addition, the rarefied and extrapolated PD also showed no significant trends with elevation. In summary, we found nearly constant phylogenetic diversity constant phylogenetic structure pattern along the elevational gradient of Mt. Bokor. The table-shaped geography with regional climate features could play an important role for the constant phylogenetic diversity and phylogenetic structure of tree species in Mt. Bokor.

## **Keywords**

Elevational gradient, phylogenetic diversity, phylogenetic structure, rarefaction and extrapolation, sampling bias, tropical forest

## Introduction

Elevational biodiversity patterns have long been interesting to biogeographers and numerous studies of taxonomic species diversity patterns along the elevational gradients have been made in different mountains of the world (see McCain and Grytnes 2010 for a review). Taxonomic species diversity often shows monotonically decreasing or hump-shaped patterns with a mid-peak along the elevational gradients (Rahbek 1995; McCain and Grytnes 2010; Guo et al., 2013). However, measuring the biodiversity only using species diversity of taxonomic level is inherently limited and could potentially obscure other diversity patterns (Bishop et al. 2015). By incorporating other dimensions of phylogenetic diversity or functional trait diversity could give us a better understanding of the biodiversity patterns and assemblages (Tallents et al., 2005; Brehm et al., 2013; Cisneros et al., 2014; Bishop et al., 2015).

Faith (1992) defined phylogenetic diversity (PD) as the sum of the branch lengths of a phylogenetic tree connecting all the species in the target assemblage, which is the most widely used phylogenetic metric (Chao et al., 2014). Although PD is usually correlated with species richness (Cisneros et al., 2014), some studies also found inconsistent correlations. For examples, Brehm et al (2013) addressed a decreasing phylogenetic diversity pattern of geometrid moths in tropical Andes, whereas the species richness of geometrid moths kept a constant pattern along an elevational gradient from 1020 to 2677 m (Brehm et al., 2003). Cisneros et al (2014) addressed that the species richness of bat in Manu Biosphere Reserve of Peru showed a nonlinearly decreasing pattern with elevation, but the phylogenetic diversity did not show a significant trend with elevation. Tallents et al (2005) found an increasing phylogenetic diversity of forest trees in Usambara mountains of Tanzania, contrasting with the species richness remaining constant with elevation. In addition, similar to species richness, the Faith's PD tends to increase with the sampling size (Chao et al., 2014). A rarefaction and extrapolation model of phylogenetic diversity is proposed to account for the sample incompleteness (Chao et al., 2014). We also apply this model for our dataset of the plot based samples and general specimen collection.

Furthermore, neutral and niche-based processes are considered as two main processes for determining species distribution and assemblage (Ndiribe et al., 2013; Qian et al.,

2014). Neutral process argues that stochastic events (e.g. species dispersion limitation, speciation and local extinction), cause a randomly composed pattern of species in a community (Hubell, 2001; Ndiribe et al., 2013; Qian et al., 2014), while niche process predicts that interspecific interactions (e.g. competition and predation) and environmental filtering determine species assemblage (Ndiribe et al., 2013; Qian et al., 2014). Niche processes are influenced by the species evolutionary history. Thus quantifying phylogenetic relatedness could provide a useful tool to understand the species assemblage in community (Webb et al., 2000; Webb et al., 2002; Ndiribe et al., 2013; Qian et al., 2014). Phylogenetic clustering expects that filtering by the local environment, the closely related species assemblage in similar positions, while phylogenetic overdispersion can be driven by competition or facilitation (Webb et al., 2002; Ndiribe et al., 2013; Qian et al., 2014). Several studies of phylogenetic relatedness among species have been investigated along elevational gradients (Brehm et al., 2013; Qian et al., 2014; Smith et al., 2014). Brehm et al (2013) reported that phylogenetic relatedness of geomerid moths was significantly increased with elevation. Qian et al (2014) found that angiosperm were more phylogenetic clustered in higher elevations. Smith (2014) found a significant phylogenetic clustering of ants in highest elevation cloud forest.

Although understanding biodiversity patterns and assemblage from phylogenetic perspectives has been increasingly investigated (Webb 2000; Cadotte et al., 2010), phylogenetic diversity and structure in tropical mountains so far are still seldom reported (Brehm et al., 2013). In a previous study, we recorded 464 tree species including 82 families and 230 genera and tree species richness kept a constant pattern along the elevation of Mt. Bokor, a table-shaped mountain, southwest Cambodia (Zhang et al., 2016). In this study, we extracted two DNA barcodes, *rbcL* and *matK*, and constructed the phylogenetic tree and calculated the phylogenetic diversity and structure of the tree species in Mt. Bokor. Phylogenetic diversity are usually correlated with species richness (Cisneros et al., 2014), thus here we predicted that PD distribution pattern of tree species in Mt. Bokor are corresponding tree species richness pattern.

The main purpose of this study is to quantifying the phylogenetic diversity and structure of tree species along the elevational gradient of Mt. Bokor, in southwest



Cambodia. We also ask followings questions (1) how about phylogenetic diversity and phylogenetic structure of tree species changing with elevation? (2) how about species richness related to the phylogenetic diversity of the different elevations?

## **Methods**

### **Rarefaction and extrapolation of Phylogenetic diversity**

We firstly calculated Faith's PD of the both plot based and general specimen samplings (Faith 1992; Kembel et al. 2010). Similar to species richness, PD is largely affected by the sampling size and the observed PD should be underestimated (Chao et al., 2014). For species richness studies, the rarefaction and extrapolation approach was usually used to reduce a bias due to differences in sample size (Colwell et al., 2012). In the previous study, we applied rarefaction and extrapolation (Chao1 estimated richness) for the observed species richness to interpret the tree species richness pattern in Mt. Bokor (Zhang et al., 2016). Here we used a rarefaction and extrapolation of PD to account the sample completeness in which PD was defined as the total length of a phylogenetic tree from any point fixed on its main trunk (Chao et al., 2014). For both of our 20 plots and 10 elevational intervals of general specimen collecting, we used the number of specimens collected in both methods as reference samples to extrapolate till 400 specimens.

### **MPD, MNTD, NRI and NTI**

Firstly we calculated MPD (mean pairwise distance), MNTD (mean nearest taxon distance). We then measured the standardized effect size ( $SES_{mpd/mntd}$ ) of observed MPD and MNTD by using a null model of randomly shuffling tip labels across the tips of the phylogeny with 999 runs (Kembel 2010). Then we calculated two widely used phylogenetic structure metrics; Net Relatedness index (NRI) and Nearest Taxon Index (NTI), where NRI and NTI equals  $-1$  times  $SES_{mpd}$  and  $SES_{mntd}$  separately (Webb 2002; Kembel 2010). In which, values of NRI and NTI  $> 0$  indicates that MPD is lower than estimating by null model thus the community is phylogenetic clustered, otherwise negative values indicate the community is phylogenetic overdispersed (Webb et al., 2002).

All of phylogenetic indices were calculated by R 3.2.2 (R Core Team 2014), using the *picante* package (Kembel et al. 2010).

### **Quantitative analysis**

Linear regression was used to illustrate the relationships between phylogenetic diversity, Chao1 estimated phylogenetic diversity and phylogenetic structure indices (MPD, MNTD, NRI and NTI) with elevation and the relationships between phylogenetic diversity, MPD and MNTD with species richness. All of calculations were used R 3.2.2 (R Core Team 2014).

## **Results**

### **Species diversity and phylogenetic diversity**

The phylogenetic tree of 20 plots shows in Fig. 1. The observed PD of 20 plots shows no significant relationships with altitude (Fig. 2a,  $R^2 = 0.076$ ,  $P = 0.13$ ). While the species richness is strongly correlated with PD of 20 plots (Fig. 3a,  $R^2 = 0.83$ ;  $P < 0.001$ ). For of 10 altitudinal intervals, we find a positive correlation between observed PD and altitude (Fig. 2b,  $R^2 = 0.63$ ;  $P = 0.003$ ), and species richness has a strong relationship with observed PD of 10 altitudinal intervals (Fig. 3b,  $R^2 = 0.93$ ;  $P < 0.001$ ).

### **Phylogenetic diversity of rarefaction and extrapolation**

The PD rarefaction and extrapolation curves of 20 plots are lying in a narrow interval (Fig 4a). The Chao1 estimated PD value of 20 plots was maximal of 6085.436 at 529 m and minimal of 2828.762 at 928 m. And the 95% confidence intervals of Chao1 richness estimated PD (Fig. 2a) overlap with each other except for the following three cases: (1) estimated PD at 928 m was significantly lower than that of neighboring plots at 903 m and 970 m, (2) estimated PD at 970 m was significantly higher than that of the neighboring plot at 1014 m and 1048 m, and (3) estimated PD at 330 m is significant lower than PD at 370m.

The PD Rarefaction and extrapolation curves of 10 altitudinal intervals also showed all curves are lying near with each other (Fig. 4b). The Chao1 estimated PD value varied from 2180.33 at the altitudinal interval of 100–199 m to 8911.287 at the interval of 800–899 m. The 95% confidence intervals of Chao1 richness estimated PD did not overlap between the neighboring altitudinal intervals in two cases: (1) between 100–199 m and 200–299 m and (2) between 900–999 m and 1000–1048 m (Fig. 2b).

### **MPD, NRI, MNTD and NTI**

The MPD value of 20 plots was maximal of 274.95 at 928 m and minimal of 206.46 at 330 m (Table 1). The observed MPD of 20 plots was significant increasing with elevation (Fig. 5a,  $R^2 = 0.49$ ,  $P < 0.001$ ) and the species richness also had a positive correlation with MPD (Fig. 5b,  $R^2 = 0.08$ ,  $P = 0.012$ ). NRI showed a positive correlation with elevation (Fig. 5c,  $R^2 = 0.26$ ,  $P = 0.013$ ), in which 18 plots showed phylogenetic clustering and only 2 plots (928 m and 1014 m) showed phylogenetic overdispersion (Fig. 5c, Table 1).

The MNTD value of 20 plots was maximal of 68.19 at 370 m and minimal of 145.08 at 266 m (Table 1). The observed MNTD of 20 plots decreased with elevation (Fig. 5d,  $R^2 = 0.22$ ,  $P = 0.02$ ) and the species richness had a negative correlation with MNTD (Fig. 5e,  $R^2 = 0.56$ ,  $P < 0.001$ ). NTI had no significant relationships with elevation (Fig. 5f,  $R^2 = 0.078$ ,  $P = 0.12$ ), while 10 plots showed phylogenetic clustering and 10 plots showed phylogenetic overdispersion (Fig. 5c, Table 1).

### **Discussion**

This study attempts to quantifying the phylogenetic diversity and structure patterns of tree species along the elevation gradient of Mt. Bokor and the relationships between phylogenetic diversity and species richness. Similarly to tree species richness pattern, observed phylogenetic diversity of 20 plots and Chao 1 estimated PD of both 20 plots and 10 elevational intervals showed nearly constant patterns in Mt. Bokor. In addition, phylogenetic structure pattern (NRI) also showed a decreasing relationship with elevation, which indicates that tree species showed a phylogenetic clustered pattern in Mt. Bokor. While NTI showed no relationships with elevation. These results are inconsistent from many previous studies, in which PD was found higher in high elevation (Tallents et al., 2005; Gonzalez-Caro et al., 2014) and phylogenetic structure was often observed as more phylogenetic clustered in higher elevations (Brehm et al., 2013; Qian et al., 2014; Smith et al., 2014).

Several possible causes could explain these special phylogenetic diversity and nearly phylogenetic clustered structure patterns. Firstly, because the PD was highly significant with species richness and the species richness keeps a nearly constant

value along the altitudinal gradient in Mt. Bokor, which caused a constant PD patterns in Mt. Bokor. Secondly, constant PD patterns also reflect that a uniform evolutionary history of tree species might be shaped along the elevational gradient in Mt. Bokor. In addition, the uniform evolutionary history of tree species richness could be caused by special geographic and climatic characters of Mt. Bokor, in which the land area of different elevational intervals did not significantly decrease with elevation only except the elevation above 900 m. And the annual precipitation in Mt. Bokor was reported more than 5000 mm (Rundel et al. 2003).

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## Figure Legends

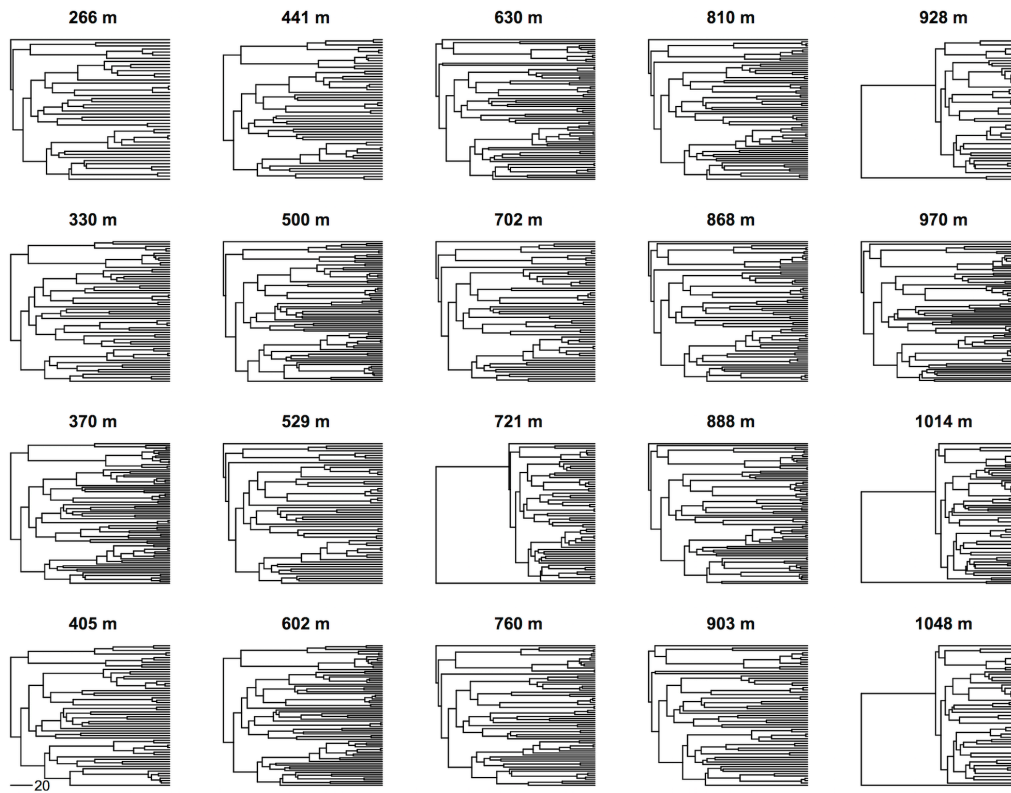
**Fig. 1** Phylogenetic lineage relationships of tree species from 20 plots.

**Fig. 2** Relationships between altitude with field observed species and Chao1 estimated phylogenetic diversity of 20 plots (a) and 9 altitudinal intervals (b). Open circle/square: field observed phylogenetic diversity, Solid circle/square: point estimate phylogenetic diversity, bar: confidence interval (95%).

**Fig. 3** Strong relationships between phylogenetic diversity with species richness of 20 plots ( $R^2 = 0.82$ ;  $P < 0.001$ ) (a), and species richness of 10 altitudinal intervals ( $R^2 = 0.92$ ;  $P < 0.001$ ) (b).

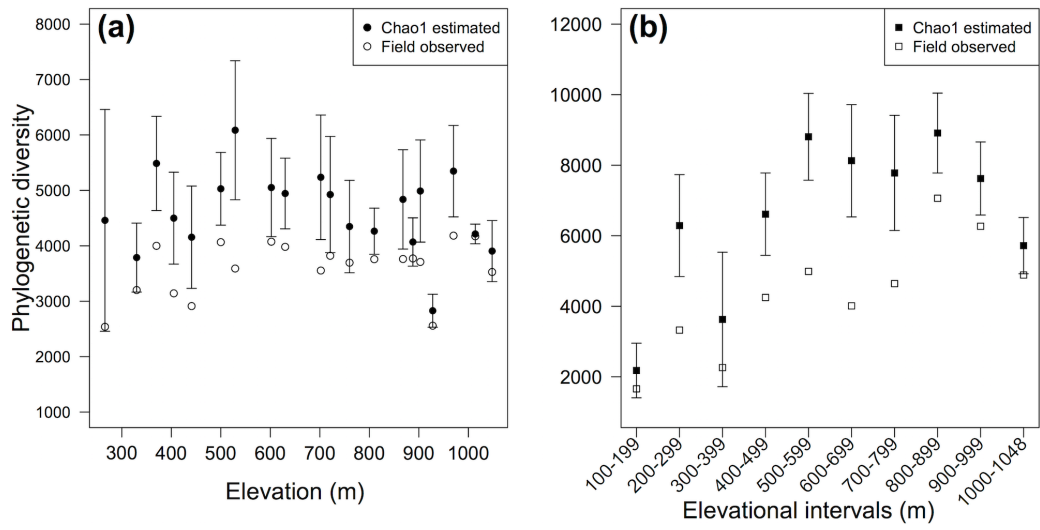
**Fig. 4** Phylogenetic diversity rarefaction and extrapolation curves for 20 plots (a) and 9 altitudinal intervals (b); Legends refer to 20 plots or 9 altitudinal intervals. For clarify, confidence intervals are not illustrated.

**Fig. 5** Relationships between MPD (observed mean pairwise distance) (a,  $R^2 = 0.49$ ,  $P < 0.001$ ), NRI (Net Relatedness index) (c,  $R^2 = 0.26$ ,  $P = 0.013$ ), MNTD (mean nearest taxon distance) (d,  $R^2 = 0.22$ ,  $P = 0.02$ ) and NTI (Nearest Taxon Index) (f,  $R^2 = 0.078$ ,  $P = 0.12$ ) with elevation. Relationships between MPD (b,  $R^2 = 0.08$ ,  $P = 0.012$ ) and MNTD (e,  $R^2 = 0.56$ ,  $P < 0.001$ ) with species richness.

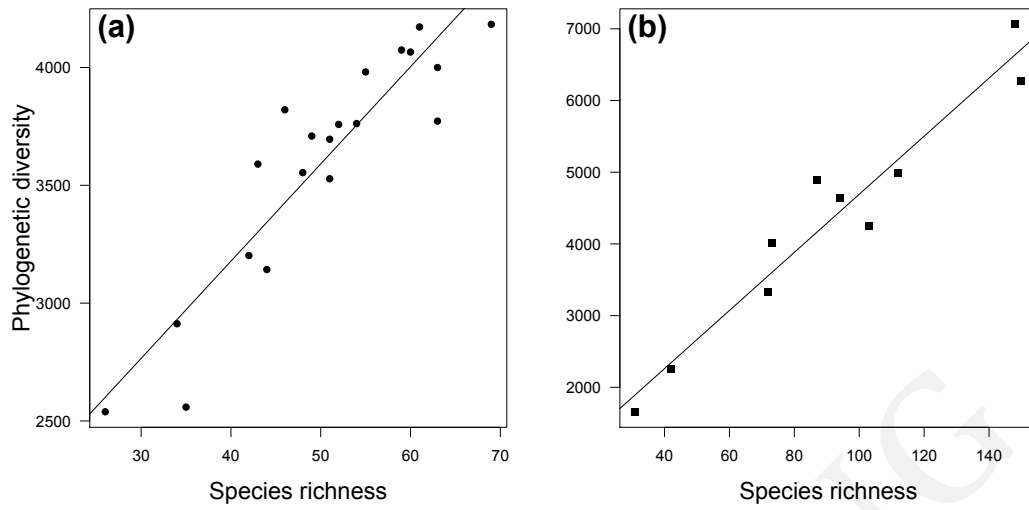


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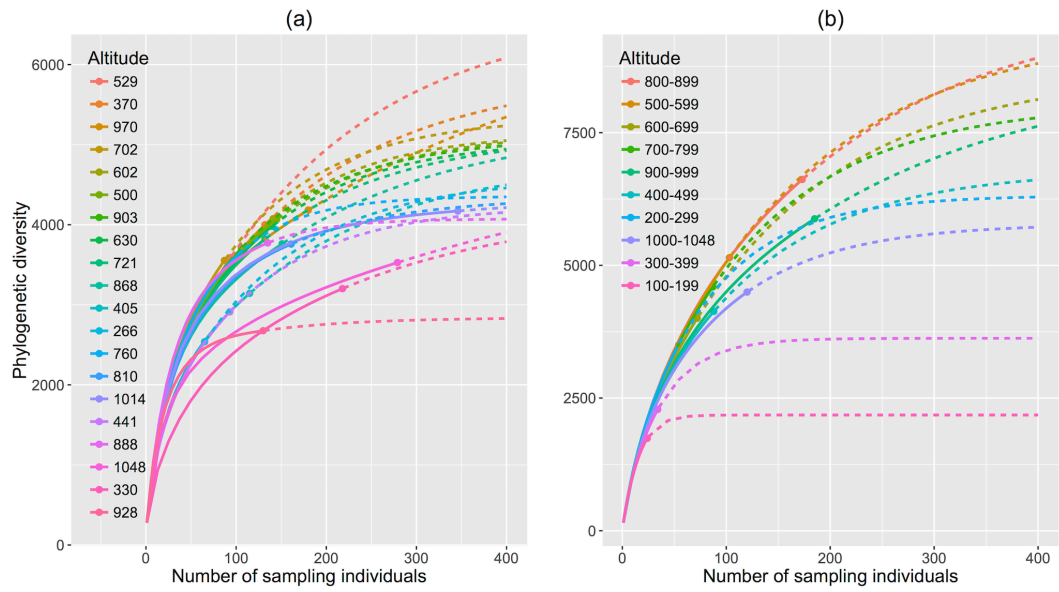




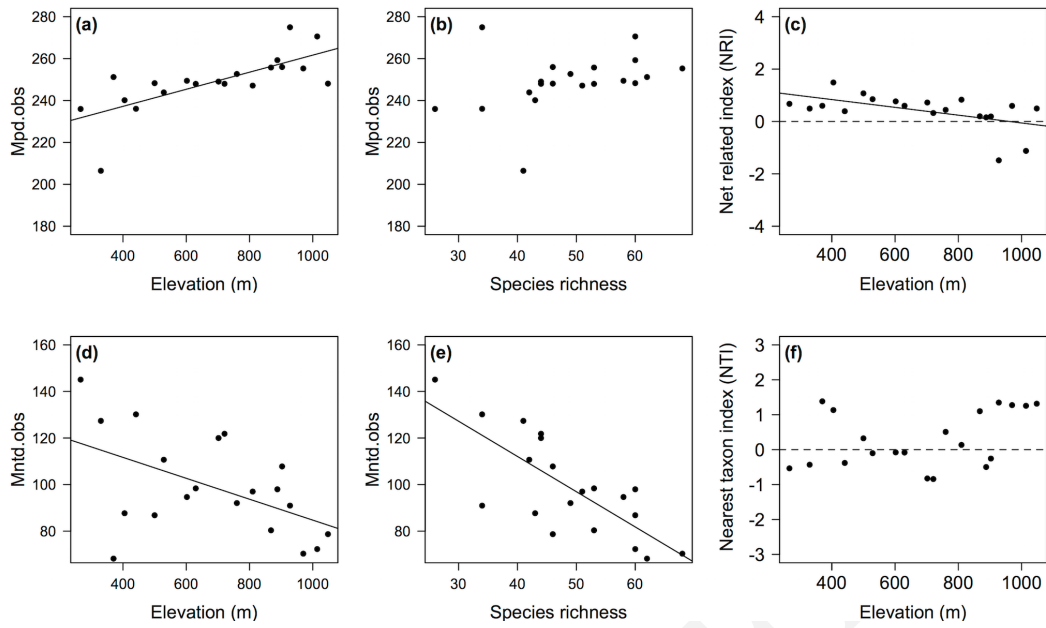
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## Tables

**Table 1** Elevation, Species richness (SR), phylogenetic diversity (PD), extrapolated phylogenetic diversity (PD.e) and observed mean pairwise distance (MPD), Net Relatedness index (NRI), and mean nearest taxon distance (MNTD) and NTI (Nearest Taxon Index) of 20 plots in Mt. Bokor.

Elevation (m)	SR	PD	PD.e	MPD	NRI	MNTD	NTI
266	26	2538.845	4459.383	235.97	0.673	145.08	-0.535
330	42	3201.852	3788.089	206.46	0.492	127.35	-0.432
370	63	3999.797	5486.512	251.19	0.598	68.19	1.383
405	44	3142.411	4498.911	240.15	1.488	87.68	1.133
441	34	2912.424	4155.097	236.05	0.391	130.15	-0.381
500	60	4065.656	5028.979	248.28	1.073	86.81	0.324
529	43	3590.113	6085.436	243.87	0.851	110.66	-0.102
602	59	4074.187	5051.115	249.42	0.767	94.65	-0.078
630	55	3981.137	4944.1	247.94	0.600	98.36	-0.083
702	48	3553.898	5236.705	249.03	0.723	119.98	-0.825
721	46	3820.517	4924.913	247.97	0.322	121.81	-0.842
760	51	3695.546	4347.458	252.66	0.442	92.05	0.508
810	52	3758.32	4264.02	247.12	0.831	96.99	0.135
868	54	3762.066	4837.642	255.74	0.196	80.34	1.100
888	63	3772.433	4068.96	259.26	0.156	97.96	-0.498
903	49	3709.085	4988.612	255.98	0.190	107.79	-0.256
928	35	2558.741	2828.762	274.95	-1.485	90.95	1.348
970	70	4183.252	5347.398	255.30	0.595	70.32	1.275
1014	61	4171.854	4214.229	270.56	-1.123	72.28	1.255
1048	51	3527.749	3904.91	248.07	0.495	78.69	1.316

**Table 2** Elevational intervals of 10 altitudinal intervals in Mt. Bokor, with information of species richness, phylogenetic diversity, and extrapolated phylogenetic diversity

Elevational interval (m)	Species richness	Phylogenetic diversity	Extrapolated phylogenetic diversity
100–199	31	4479.043	5688.055
200–299	72	5940.885	7822.954
300–399	42	6551.236	8867.292
400–499	103	4730.302	8033.593
500–599	112	4075.178	8028.631
600–699	73	4890.296	8681.594
700–799	94	4173.298	6969.757
800–899	148	2169.327	3654.342
900–999	150	3530.94	6271.935
1000–1048	87	1722.935	2402.508

**Chapter 6 Evolutionary causes for the constant tree species richness pattern of Mt. Bokor**

ZHANG MENG

## **Abstract**

Tropical rainforests in Southeast Asia are harboring extremely high species richness. Although altitudinal species richness patterns have been studied in a few mountains of Southeast Asia, the evolutionary causes of species distribution patterns have not been reported. In a previous study, we observed a constant altitudinal tree species richness of Mt. Bokor, a table-shaped mountain, locating in the southwest Cambodia. Here, we test a hypothesis that tree species diversification rate does not significantly change along the altitudinal gradient of Mt. Bokor. We used two DNA barcode segments, *rbcL* and *matK*, to construct the phylogenetic tree, estimated divergence times. Our results show that diversification rate keeps a constant pattern in Mt. Bokor. Remarkably, the constant tree species richness pattern could be explained by the constant diversification rates of different altitude in Mt. Bokor. We suggested that the table-shaped geography with regional climate features could cause this special evolution history and species richness pattern of Mt. Bokor.

## **Keywords**

Altitudinal gradient, diversification rate, species richness pattern, tropical forest.



## Introduction

Understanding the species distribution patterns along altitudinal gradients and the underlying mechanisms is a critical issue for biodiversity and biogeography (Rahbek 1995; Körner 2007; McCain and Grytnes, 2010; Guo et al., 2013; Hutter et al., 2013). Numerous studies have reported about the species richness patterns of various organisms along altitudinal gradients in many different mountains of the world (Rahbek, 1995; McCain and Grytne, 2010; Guo et al, 2013). As results, the hump-shaped pattern with a mid-altitudinal peak is found as the most common one (McCain and Grytnes 2010; Guo et al. 2013). Various hypotheses have been proposed to explain altitudinal diversity patterns, e.g. land area, climate, mid-domain and evolutionary history (Rahbek 1995; Lomolino 2001; Körner 2007; McCain 2007; McCain and Grytnes 2010). However, many factors usually vary with altitude and interact with each other (Körner 2007), and the underlying mechanisms are still not fully explored (Körner 2007; Wiens et al., 2007; McCain and Grytne, 2010; Hutter et al., 2013). Furthermore, because most studies focus on the environmental perspectives of species richness, the evolutionary causes are often ignored (Ricklefs, 2006; Smith et al., 2007; Sebastian et al., 2013). Environmental factors may play critical roles in shaping species richness, but evolutionary processes of the balance between speciation and extinction could directly determine species richness (Ricklefs, 2007; Smith et al., 2007; Wiens et al., 2007; Hutter et al., 2013). Thus estimating diversification rates (speciation rate minus extinction rate) is vital for understanding how evolutionary processes have shaped patterns of species richness (Ricklefs, 2007; Wiens et al., 2007; Hutter et al., 2013).

Two hypotheses related evolutionary perspectives were proposed to explain the commonly hump-shaped species pattern, which are the montane species-pump hypothesis and the montane museum hypothesis (Smith et al., 2007; Wiens et al., 2007; Hutter et al., 2013). The montane species-pump hypothesis predicts that the diversification rates are higher in the intermediate altitude than lower and higher altitudes. On the other hand, the montane museum hypothesis argues that the diversification rates keep almost constant along the altitudinal gradient, but mid-altitudes are firstly colonized and have longer evolutionary time than other altitudinal zones (Smith et al., 2007; Wiens et al., 2007; Hutter et al., 2013). Some efforts have

been investigated to test these two hypotheses by using amphibian data, e.g. Wiens et al. (2007) found that tropical bolitoglossine salamanders colonized the mid–altitude habitats earlier which supported the montane museum hypothesis. Similarly, one study of tropical Andean glassfrogs (Hutter et al., 2013) also supported the montane museum hypothesis. While Smith et al. (2007) reported that treefrogs in middle America supported both of hypotheses. Nevertheless, no studies have been documented about the evolutionary causes for the tree species of tropical mountains in Southeast Asia.

Tropical forests in Southeast Asia are harboring extremely high species richness particularly in montane regions, but the deforestation rate is higher than other tropical regions, which threatens the biodiversity in Southeast Asia (Sodhi et al., 2004; Yahara et al., 2012). Identifying the causes for the species diversity patterns in Southeast Asia is crucially necessary. Hence we are carrying on a series of field survey in many different tropical mountains of Southeast Asia (Yahara et al. 2012; Tagane et al. 2015, Zhang et al., 2016). Because most of previous altitudinal studies did not attempt to standardize for the sampling effort (Rahbek 1995; Guo et al. 2013), we take a strategy to record plant richness by using uniformly standard plots (100 m x 5 m) that are placed in different altitudes of a mountain (Yahara et al. 2012; Tagane et al. 2015). In addition, Grytnes and Beaman (2006) applied a rarefaction method on a densely collected specimen data of Mt. Kinabalu and found a weakly humped altitudinal pattern of species richness with the peak between altitude 900 and 1200 m. Thus we used both standard plot based sampling and general specimen collecting during our field survey.

In a previous study, we recorded 464 tree species including 82 families and 230 genera. We found no correlation between species richness and altitude in a table–shaped mountain, Mt. Bokor in southwestern Cambodia (Zhang et al., 2016). Mt. Bokor is facing with Thailand Gulf and locating in the most rain–rich area of the Indo–china peninsula (Rundel et al. 2003; Stuart and Emmett 2006; Tagane et al. 2015). The southwester monsoon in summer from the Gulf of Thailand brings high rainfall for Mt. Bokor (Stuart and Emmett 2006). Consequently, Mt. Bokor is not affected by severe dry climate even in the lower altitude. The top the Mt. Bokor shows a relatively flat terrain and is covered by a mosaic of grassland with health

forest (Rundel et al. 2003; Stuart and Emmett 2006). In addition, the land area of each altitudinal range does not decrease between altitude 200 m and 900 m (Zhang et al., 2016). These climatic and geographic features of Mt. Bokor should play important roles for the constant tree species richness pattern (Zhang et al., 2016). In this study, we use two DNA segments, *rbcL* and *matK*, to construct the phylogenetic tree, estimate divergence times of the tree species in Mt. Bokor.

The main purpose of this study is to quantifying the diversification rates of tree species along the altitudinal gradient of Mt. Bokor. We test a hypothesis that diversification rates of tree species does not change significant along the altitudinal gradient. Moreover, we also ask followings questions (1) how about the diversification rates and the time of the first colonized changing with altitude? (2) how about species richness related to the time of the first colonized of the different altitudes?

## **Methods**

### **Diversitfication rates estimate**

We use the package GEIGER (Harmon et al., 2008) to calculate to estimate the diversification rate of 20 plots by using the method of moments estimator for stem group ages (Magallon and Sanderson 2001) with a moderate relative extinction rate ( $\epsilon = 0.45$ ). We also used  $\epsilon$  values of 0.90 and 0, but both of them gave similar results with the value 0.45.

### **Quantitative analysis**

Linear regression was used to illustrate the relationships between diversification rate and first colonization time with elevation and the relationships between diversification rate, first colonization time, phylogenetic diversity with species richness. All of calculations were used by R 3.2.2 (R Core Team 2014).

## Results

### Diversification rates and first colonization time

In 20 plots, diversification rate increases from 0.011 at 928 m to 0.024 at 370 m and 970m (Table 1). We find no significant relationship between diversification rates of 20 plots with altitude (Fig. 1a,  $R^2 = 0.13$ ;  $P = 0.06$ ). The species richness showed no relationships with diversification rate (Fig. 1c,  $R^2 = 0.045$ ;  $P = 0.19$ ). In addition, the first colonization time varied from 107.48 Myr at 330 m to 277.51 Myr at 721 m and 1048 m (Table 1). We find a positive correlation between the first colonization time and altitude (Fig. 1b,  $R^2 = 0.29$ ;  $P < 0.008$ ). While there is no significant relationship between species richness and the first colonization time (Fig. 1d,  $R^2 = 0.05$ ;  $P = 0.78$ ).

## Discussion

In this study, we found a nearly constant diversification rate pattern along the altitudinal gradient of Mt. Bokor, which did not agree with neither montane species–pump hypothesis nor the montane museum hypothesis. The diversification rates are relatively lower than other plots in four plots (721 m, 928 m, 1014 m and 1048 m). Because we observed four Gymnosperm species in these four plots, including *Dacrycarpus imbricatus*, *Dacrydium elatum*, *Nageia wallichiana* and *Podocarpus pilgeri*, which belongs to the Order of Pinales. In addition, we also observed these four species in three elevational intervals (800–899 m, 900–999 m and 1000–1048 m), which indicates that Pinales species colonized above 700 m. The age of Pinales was estimated as around 250 Myr, which is much earlier than Angiosperms (about 150 Myr), indicating that the upper part of Mt. Bokor may be firstly colonized. However the species richness in top area is relatively lower than the neighbor plots which indicates that the top area was facing relatively higher environment pressure such as the lower temperature, the frequently flooding effects caused by the heavy rain, the much of year surrounded by the thick fog and the strong wind from the Gulf of Thailand (Rundel et al., 2003; Stuart and Emmett 2006).

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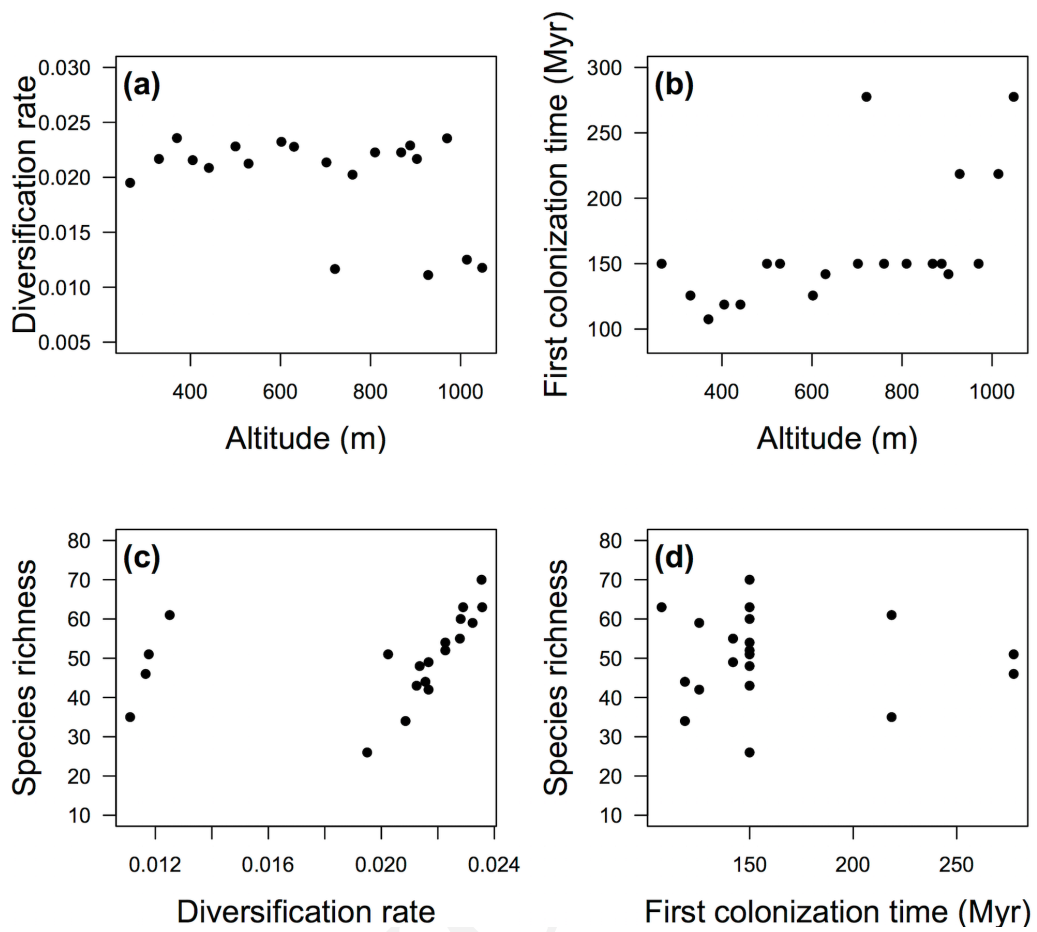
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## Figure Legends

**Fig. 1** Relationships between altitude with diversification rate ( $R^2 = 0.13$ ;  $P = 0.06$ ) (a) and first colonization time ( $R^2 = 0.29$ ;  $P < 0.008$ ) (b), Relationships between species richness and diversification rate ( $R^2 = 0.045$ ;  $P = 0.19$ ) (c) and first colonization time ( $R^2 = 0.05$ ;  $P = 0.78$ ) (d).

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**Fig. 1** Relationships between altitude with diversification rate ( $R^2 = 0.13$ ;  $P = 0.06$ ) (a) and first colonization time ( $R^2 = 0.29$ ;  $P < 0.008$ ) (b), Relationships between species richness and diversification rate ( $R^2 = 0.045$ ;  $P = 0.19$ ) (c) and first colonization time ( $R^2 = 0.05$ ;  $P = 0.78$ ) (d).

## Tables

**Table 1** Altitudes of 20 plots in Mt. Bokor, with information of species richness, diversification rate, first colonization time and phylogenetic diversity, and extrapolated phylogenetic diversity

Plot (m)	Species richness	Diversification rate	First colonization Time
266	26	0.019	149.965
330	42	0.022	125.640
370	63	0.024	107.483
405	44	0.022	118.749
441	34	0.021	118.749
500	60	0.023	149.965
529	43	0.021	149.965
602	59	0.023	125.640
630	55	0.023	141.969
702	48	0.021	149.965
721	46	0.012	277.514
760	51	0.020	149.965
810	52	0.022	149.965
868	54	0.022	149.965
888	63	0.023	149.965
903	49	0.022	141.969
928	35	0.011	218.550
970	70	0.024	149.965
1014	61	0.013	218.550
1048	51	0.012	277.514

**Chapter 7 Community structure of tree species  
in Mt. Bokor**

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## **Abstract**

The main purpose is to discuss about the changing of tree species composition and the relationships between species richness with tree height and basal area along the elevational gradient of Mt. Bokor. We found that elevation, temperature, precipitation and soil significant affected the tree species composition along the elevational gradient of Mt. Bokor. There was no significant difference of tree height distribution between plots 1–17 and plots 18–20. Similarly, there was no significant difference of d.b.h. distribution between plots 1–9 and plots 10–20. In addition, tree species richness decreased with the proportion of trees above 10 m, while there were no significant correlations between species richness and the sum of basal area. Our results indicate the constant productivity along the altitude gradient may play an important role for shaping the tree specie richness pattern.

## **Introduction**

The main purpose is to discuss about the changing of tree species composition along the elevational gradient of Mt. Bokor. We ask followings questions; (1) how about tree height and tree size changing with elevation? (2) how about relationships between species richness with tree height and tree size?

## **Method**

Simple scatter diagrams and linear regression analyses were explored to illustrate the changes of species richness and tree density as a function of altitude, and the relationships of species richness with tree density, the percentage of trees above 10 m (as an indicator of forest height) and the sum of basal area (as an indicator of biomass). Average and distribution of tree height and d.b.h. in each plot were shown by box plots. Chi square test was used to test the difference in the proportion of tree height among 20 plots. General linear mixed model (GLMM) was applied to determine the difference of tree height and d.b.h distribution among plots and altitudinal intervals. A rank abundance distribution curve for all the 20 plots of Mt. Bokor was fitted by the log-normal model using maximum likelihood estimation. Detrended correspondence analysis (DCA) was used to summarize the variation in the tree composition among 20 plots (Hill and Gauch 1980). Furthermore, we chose three important environmental variables, i.e. altitude, temperature seasonality and proportion of topsoil organic carbon (as an indicator of soil), to interpret the DCA ordination (Oksanen et al. 2014).

All the above calculations and illustrations were made with R 3.2.2 (R Core Team 2014), using *lme4* (Bates et al. 2014) for GLMM analysis, and *vegan* (Oksanen et al. 2014) for the rank abundance curve and DCA analysis.

## **Results**

### **Rank abundance curve and DCA analysis**

In the rank abundance curve (Fig. 1a), the proportions of singletons and doubletons were 20.8% and 11.1%, respectively. The four most abundant species were

*Archidendron quocense* (154 individuals), *Mallotus paniculatus* (112), *Lithocarpus elephantum* (67) and *Macaranga andamanica* (66). DCA analysis (Fig. 1b) showed that DCA1 was associated positively with altitude, and negatively with temperature, precipitation and soil. Factors associated with DCA2 are not specified.

### **Relationships between species diversity and tree height and d.b.h.**

Average tree height varied from 4.95 m at alt. 1048 m to 11.58 m at alt. 702 m, and median tree height varied from 5 m at alt. 1048 m to 9 m at alt. 266 m (Fig. 2a). Maximal tree height exceeded 20 m in plots 1–17 but was lower than 20 m in plots 18–20 that were located in the top area (Fig. 2a). There was significant difference in tree height distribution between plots 1–17 and plots 18–20 ( $\chi^2(1) = 7.94$ ;  $P = 0.005$ ). All the median values were below 10 m, but in most plots, there were many outliers larger than upper quartiles, indicating that tree height distributions were skewed to 10 m or lower and tailed to higher values. Thus, we calculated the proportion of trees above 10 m as an indicator of tree height distribution. This proportion varied from 0 % at 1048 m to 47 % at 266 m, but there was no significant difference in the proportion among plots ( $\chi^2(306) = 320$ ;  $P = 0.28$ ). Moreover, tree species richness decreased with the proportion of trees above 10 m (Fig. 2c,  $R^2 = 0.2$ ,  $P = 0.03$ ).

Average d.b.h. varied from 5.78 cm at alt. 1014 m to 12.49 cm at alt. 529 m, and median d.b.h. varied from 3.15 cm at alt. 868 m to 8.76 at alt. 529 m (Fig. 2b). Trees with d.b.h. larger than 100 cm were found in plot 1 (266 m), 3 (370 m), 5 (430 m) and 9 (630 m), which were all locating in the lower altitude. However, there was no significant difference of d.b.h. distribution between plots 1–9 and plots 10–20 ( $\chi^2(1) = 2.11$ ;  $P = 0.14$ ). As in tree height, there were many outliers above upper quartiles and the d.b.h. distributions were tailed to larger values. In addition, we calculated the sum of basal area, which varied from 1.77 m<sup>2</sup> at 888 m to 4.61 m<sup>2</sup> at 370 m and there was no significant correlation between species richness and the sum of basal area (Fig. 2d,  $R^2 = -0.05$ ,  $P = 0.81$ ).

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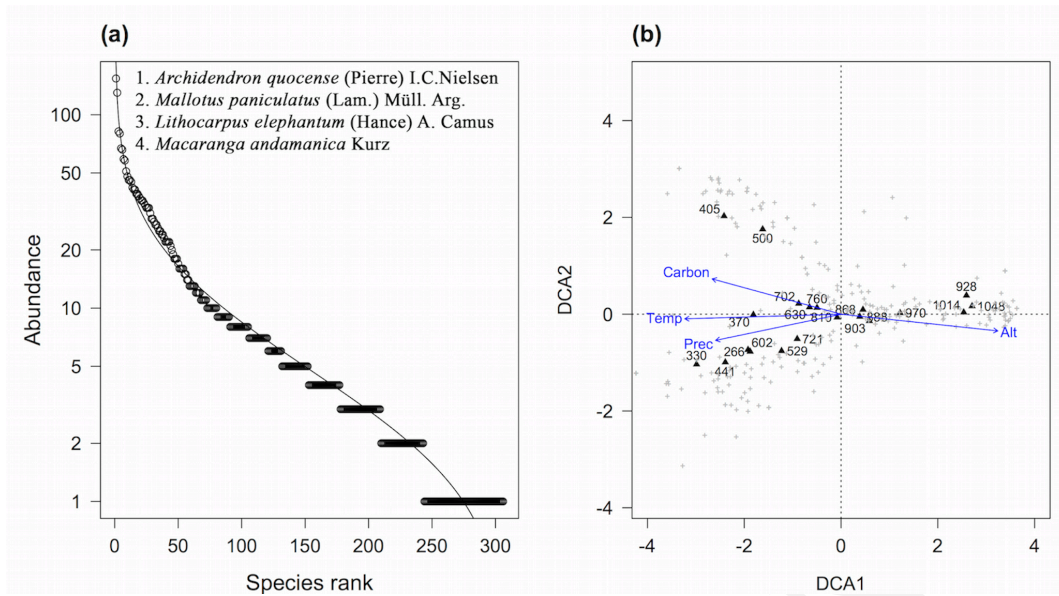
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## Figure Legends

**Fig. 1** The rank abundance curve (a) and DCA ordination of tree composition among 20 plots (b). In (b), triangle: plot, number: altitude, plus: tree species; Alt: Altitude, Carbon: proportion of organic carbon in topsoil, Temp: mean temperature, Prec: annual precipitation.

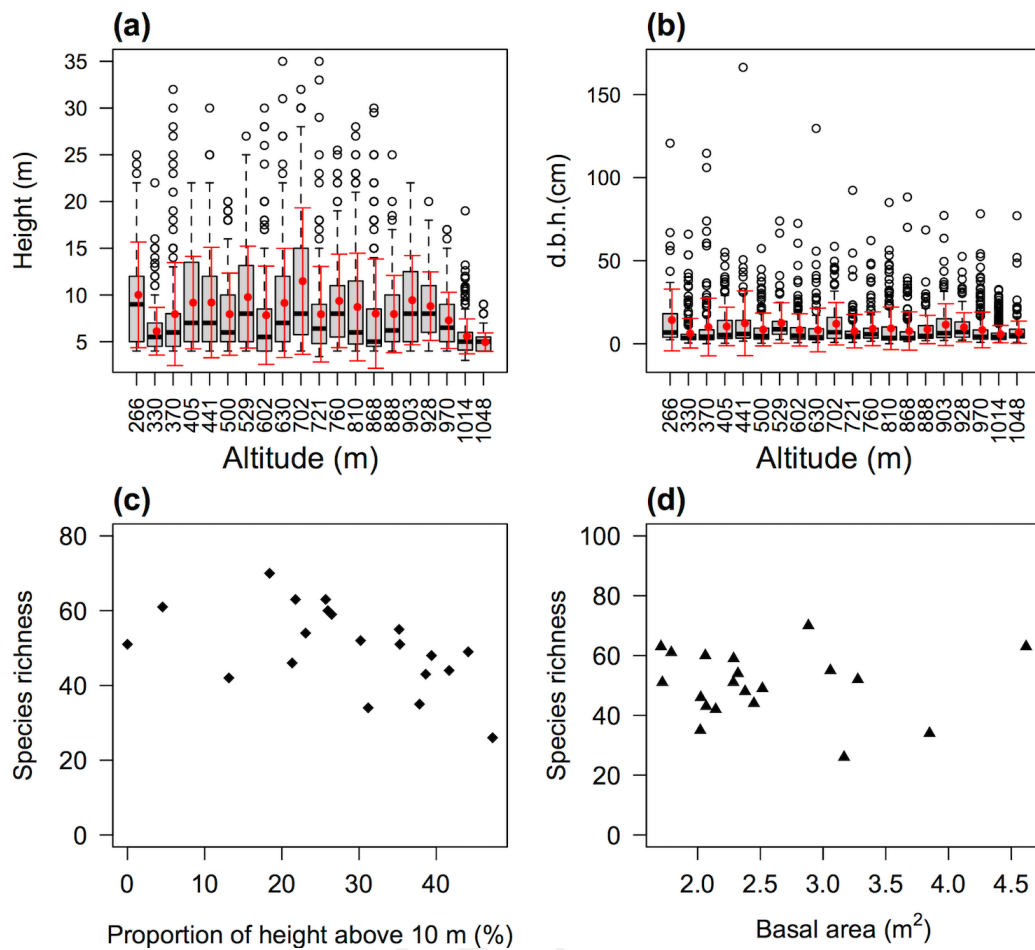
**Fig. 2** Top: distribution of tree height (a) and d.b.h. (b) in 20 plots locating at different altitudes of Mt. Bokor. Box plots show medians (thick bars), interquartile ranges (shaded boxes), and outlier values (open circle). Red circles and lines indicate averages and SD ranges. Bottom: relationships of species richness with the percentage of trees above 10 m (c) and average d.b.h. (d). Solid rhombus/triangle: point estimate, bar: confidence interval.





**Fig. 1** The rank abundance curve (a) and DCA ordination of tree composition among 20 plots (b). In (b), triangle: plot, number: altitude, plus: tree species; Alt: Altitude, Carbon: proportion of organic carbon in topsoil, Temp: mean temperature, Prec: annual precipitation.

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**Fig. 2** Top: distribution of tree height (a) and d.b.h. (b) in 20 plots locating at different altitudes of Mt. Bokor. Box plots show medians (thick bars), interquartile ranges (shaded boxes), and outlier values (open circle). Red circles and lines indicate averages and SD ranges. Bottom: relationships of species richness with the percentage of trees above 10 m (c) and average d.b.h. (d). Solid rhombus/triangle: point estimate, bar: confidence interval.

**Chapter 8 Neutral Theory applied for Mt.  
Bokor.**

ZHANG MENG

## Abstract

The main purpose is to quantify two core parameters of Hubbell's neutral theory, representing processes of speciation ( $\theta$ ) and migration ( $m$ ). We found that if Mt. Bokor region is considered as a whole metacommunity, the estimate of the biodiversity parameter  $\theta_{E2005}$  (a product of speciation rate and metacommunity size) was 105.2, indicating that high species richness in Mt. Bokor is partly explained by relatively higher speciation rate, comparing with other mountains, in this region. This finding agrees with the fact that there are as many as 20 tree species endemic to Mt. Bokor (Tagane et al. 2015). The estimate of migration rate  $m_{E2005}$  was 0.39, indicating that a metacommunity of Bokor is moderately structured under migration limitation. If we divided Mt. Bokor into three different elevation intervals (266-602 m, 630-888 m and 903-1048 m), the estimates of  $\theta$  were equivalent in all three intervals, while the estimates of  $m$  in 903-1048 m are lower than in two lower intervals, which may cause the relatively lower species richness on the top area of Mt. Bokor.

## Introduction

Studying the altitudinal gradient of species richness in Mt. Bokor, we found that tropical forest harbors very high diversity of tree species. To characterize this diversity, we determined rank abundance relationship and two core parameters of Hubbell's neutral theory (Hubbell 2001; Rosindell et al. 2011) representing processes of speciation ( $\theta$ ) and migration ( $m$ ). These two parameters have been successfully applied to analyze and compare tropical forest and other species-rich communities, providing insights for the mechanisms underlying species diversity patterns in spite that no ecological communities are really neutral (Latimer et al. 2005; McGill et al. 2007; Rosindell et al. 2011).

While rarefaction and extrapolation models enable us to estimate species accumulation curve and total species richness, those models do not provide us any mechanistic insight for species richness. On the contrary, Hubbell's neutral theory (Hubbell 2001; Rosindell et al., 2011) models species richness in meta-community with its two core parameters representing processes of speciation ( $\theta$ ) and migration ( $m$ ). This model has been successfully applied to analyze and compare species richness in tropical forest and other species-rich communities, providing insights for the mechanisms underlying species diversity patterns (Latimer et al., 2005; McGill et al., 2006; Rosindell et al., 2011). We applied the neutral theory to our data set from Mt. Bokor and evaluated relative importance of speciation and migration upon shaping the species richness observed. When we apply the neutral theory to our data set, we assumed that the whole mountain represents single metacommunity. To examine the validity of this assumption, we subsequently used two-stage neutral parameters estimation (Etienne, 2009) for different elevation intervals of mountain Bokor.

## Method

The neutral ecological theory (Hubbell, 2001) has been widely used to explain the biodiversity patterns in tropical rain forests (Etienne, 2005, 2009; Volkov et al., 2006; Latimer et al., 2005; Munoz et al., 2007). Hubbell's neutral model describes species richness in a metacommunity as a function of two mechanistic parameters: the

biodiversity parameter  $\theta$  representing the process of speciation in a metacommunity and  $m$  representing the process of immigration from the metacommunity (Latimer et al., 2005; Etienne, 2009). Etienne (2005) developed an exact sampling formula for the neutral model with dispersal limitation, which makes it possible to simultaneously estimate two neutral parameters,  $\theta_{E2005}$  and  $m_{E2005}$ , by using maximum likelihood estimation (MLE). In addition, Etienne (2009) proposed an approximate two-stage approach that could estimate parameters of multiple separated local samples with different degree of the dispersal limitation. Therefore, we use the exact formula to estimate the natural parameters of all 20 plot based samples composing together in Mt. Bokor. We alternatively used the two-stage approach for different elevational plot-based samples in Mt. Bokor. Here we divide the elevational gradient in Mt. Bokor to three elevational interval groups; plot 1-8 (266-602 m), plot 9-15 (630-888 m) and plot 16-20 (903-1050 m). All of the neutral estimations are processed through the pari/gp 2.5.3 (PARI Group, 2013).

## Results

### High speciation and migration in Mt. Bokor region

Maximum likelihood estimates of two neutral model parameters (Etienne, 2005)  $\theta_{E2005}$  and  $m_{E2005}$  for the Bokor metacommunity were 104.1 and 0.55, respectively (Table 1). For comparison, we also estimated those parameters for 5 other tropical forest plots (BCI, Yasuni, Korup, Pasoh and Sinharaja; data from <http://ctfs.si.edu>) coordinated by the Center for Tropical Forest Science of the Smithsonian (Table 1). The  $\theta_{E2005}$  of Bokor (104.1) is lower than Yasuni (205.4) and Pasoh (192.6), but higher than BCI (46.5), Korup (52.7) and Sinharaja (27.49). The  $m_{E2005}$  of Bokor (0.55) is lower than the maximal record in Sinharaja (0.66) but higher than 4 other plots.

We subsequently used two-stage neutral parameter estimation (Etienne, 2009) for three different elevation intervals of Mt. Bokor (Table 2). Because tree density is higher and tree height is lower in the top area (although the differences are not statistically significant), we treated plots 16-20 (903-1048 m) as a unique interval and then divided other plots to two groups; plots 1-8 (266-602 m) and plots 9-15 (630-888

m). The estimates of  $\theta_{E2009}$  (42.9) were equivalent in all three intervals and lower than  $\theta_{E2005}$ , while the estimates of  $m_{E2009}$  were 0.57 in 903-1048 m and 0.999 in two lower intervals, which is higher than the single-stage estimate for the whole Bokor (0.55).

## Discussion

### High speciation and migration in Mt. Bokor region

In addition to land area and environmental variables (Rahbek 1995; Lomolino 2001; Körner 2007; McCain 2007; McCain and Grytnes 2010), evolutionary history species richness is also an important factor determining plant species richness along the altitudinal gradients (McCain and Grytnes, 2010). Neutral model is helpful to infer the effect of evolutionary history on species richness by considering the long-term balance of speciation and extinction under the migration limitation. Thus, we estimated two neutral parameters for our dataset from Mt. Bokor using the formulae of Etienne (2005). The estimate of the biodiversity parameter  $\theta_{E2005}$  (a product of speciation rate and metacommunity size) was 105.2, lower than Yasuni (205.4) and Pasoh (192.6), but higher than BCI (46.5) and two other forest plot (Table 1), indicating that high species richness in Mt. Bokor is partly explained by high speciation rate in this region. This finding agrees with the fact that there are as many as 20 tree species endemic to Mt. Bokor (Tagane et al. 2015). The estimate of migration rate  $m_{E2005}$  was 0.39, indicating that a metacommunity of Bokor is moderately structured under migration limitation, which may cause the relatively lower species richness on the top area of Mt. Bokor.

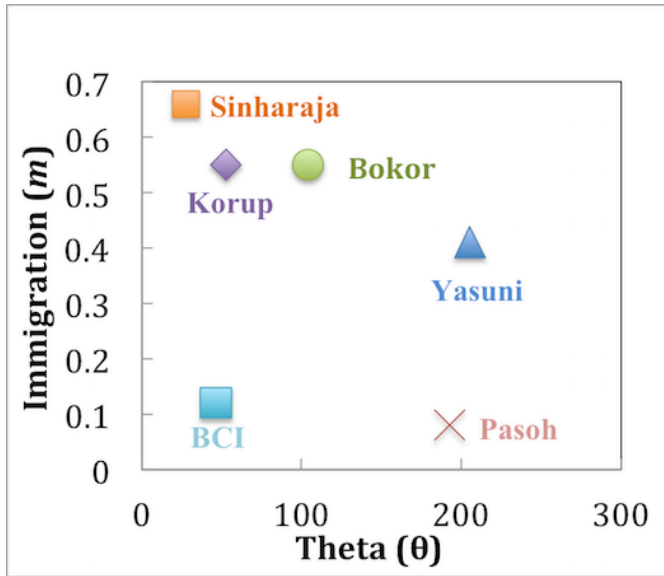
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## Figure Legends

**Fig. 1** Neutral Estimated parameters (theta  $\theta$  and immigration  $m$ ) from 6 tropical forest plots (a) and the relationship between elevation and Fisher's alpha estimated from 20 plots (b).



**Fig. 1** Neutral Estimated parameters (theta  $\theta$  and immigration  $m$ ) from 6 tropical forest plots (a) and the relationship between elevation and Fisher's alpha estimated from 20 plots (b).

## Tables

**Table 1** Comparison of parameter estimates of neutral parameters using the approach of Entienne 2005.

Site	$J$	S	$\theta_{E2005}$	$m_{E2005}$
Bokor, Cambodia (This study)	3173	228	104.1	0.55
Yasuni, Ecuador (Ref)	17,546	821	205.4	0.41
Pasoh, Malaysia	28,279	671	192.6	0.08
Korup, Cameroon	24,591	308	52.7	0.55
BCI, Panama	20,484	227	46.5	0.12
Sinharaja, Sri Lanka	16,776	171	27.5	0.66

$J$  is the sample size; S is species richness;  $\theta$  and  $m$  are the biology parameters of Hubbell neutral model.

**Table 2** Estimates of neutral parameters for different elevational intervals using two stage approach of Entienne 2009.

Elevation	$J$	S	$\theta_{E2009}$	$m_{E2009}$
266-602	1049	172	42.9	1.00
630-888	982	169	42.9	1.00
903-1048	1142	133	42.9	0.568

$J$  is the sample size; S is species richness;  $\theta$  and  $m$  are the biology parameters of Hubbell neutral model.

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## Chapter 9 Conclusion

Unlike many previous studies, tree species richness is nearly constant along the elevational gradient of Mt. Bokor. This pattern does not agree with any of the four common patterns between species richness and elevation summarized by McCain and Grytnes (2010). Similarly, phylogenetic diversity, phylogenetic structure (NRI and NTI) and diversification rate of tree species also keep nearly constant patterns with elevation. Remarkably, the constant tree species richness pattern could be explained by the constant diversification rates of different altitude in Mt. Bokor. I suggested that the table-shaped geography with regional climate features could cause this special evolution history and species richness pattern of Mt. Bokor.

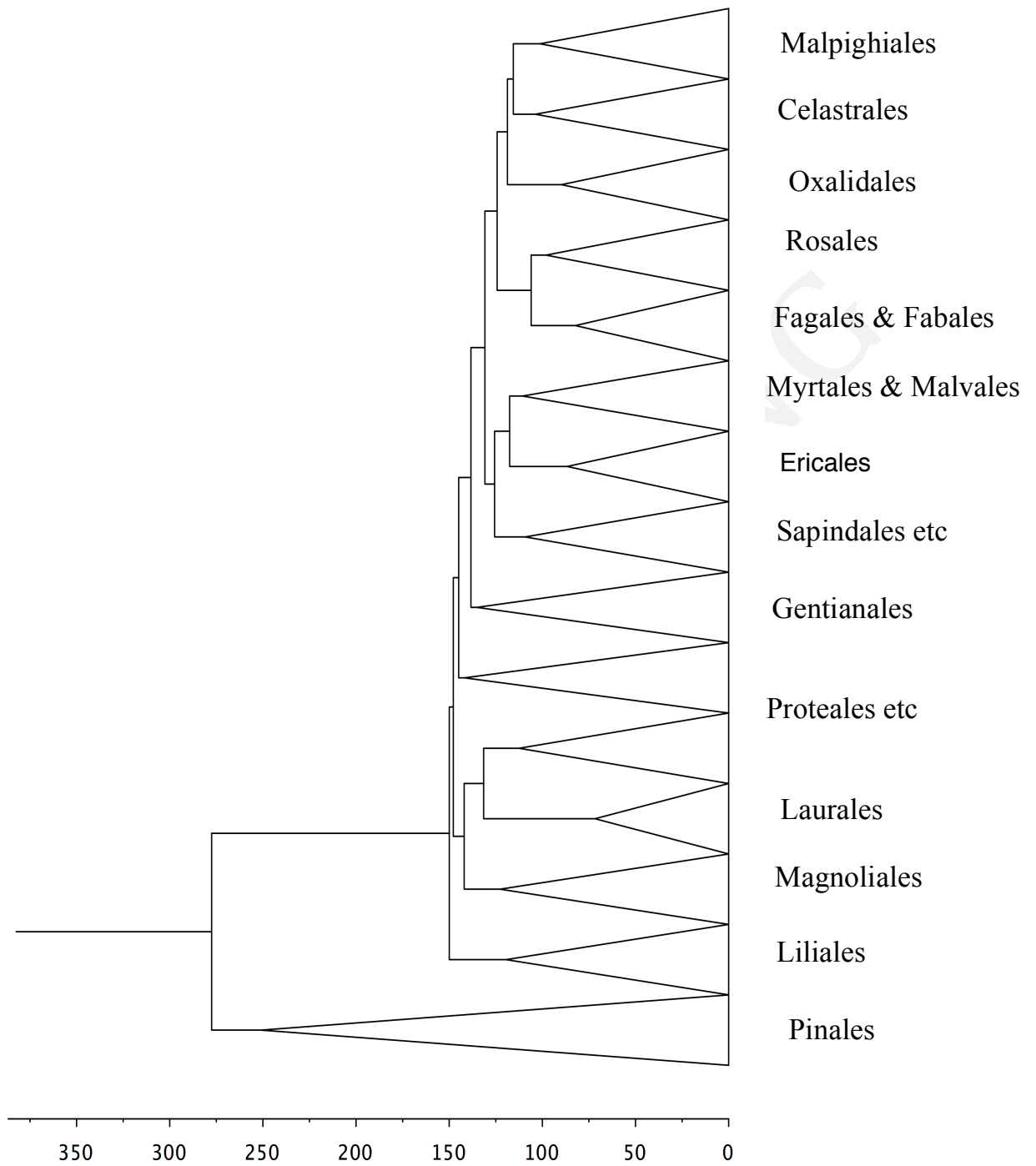
Future study of functional traits diversity along the elevational gradient of Mt. Bokor will give full dimensions of biodiversity patterns of Mt. Bokor, and subsequent taxonomic studies related to vines, shrubs and herbs that are currently in progress will enable us to examine whether species richness patterns are similar or difference among different life forms. Also studying biodiversity patterns from other mountains in Southeast Asia will give a better understanding of elevational diversity patterns in tropical Southeast Asia.

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## **Additional Support Information**

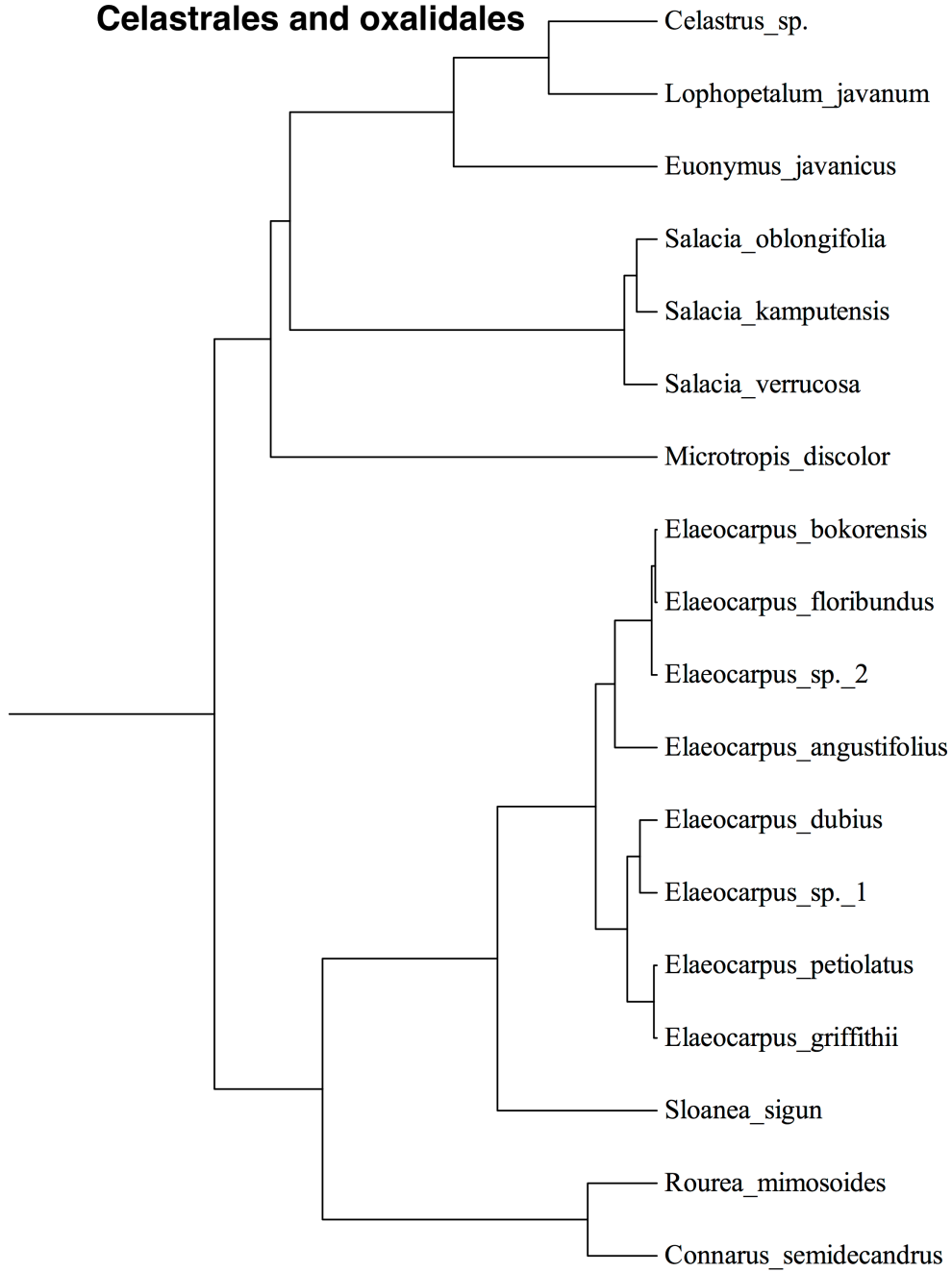
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# Phylogenetic tree of tree species in Mt. Bokor



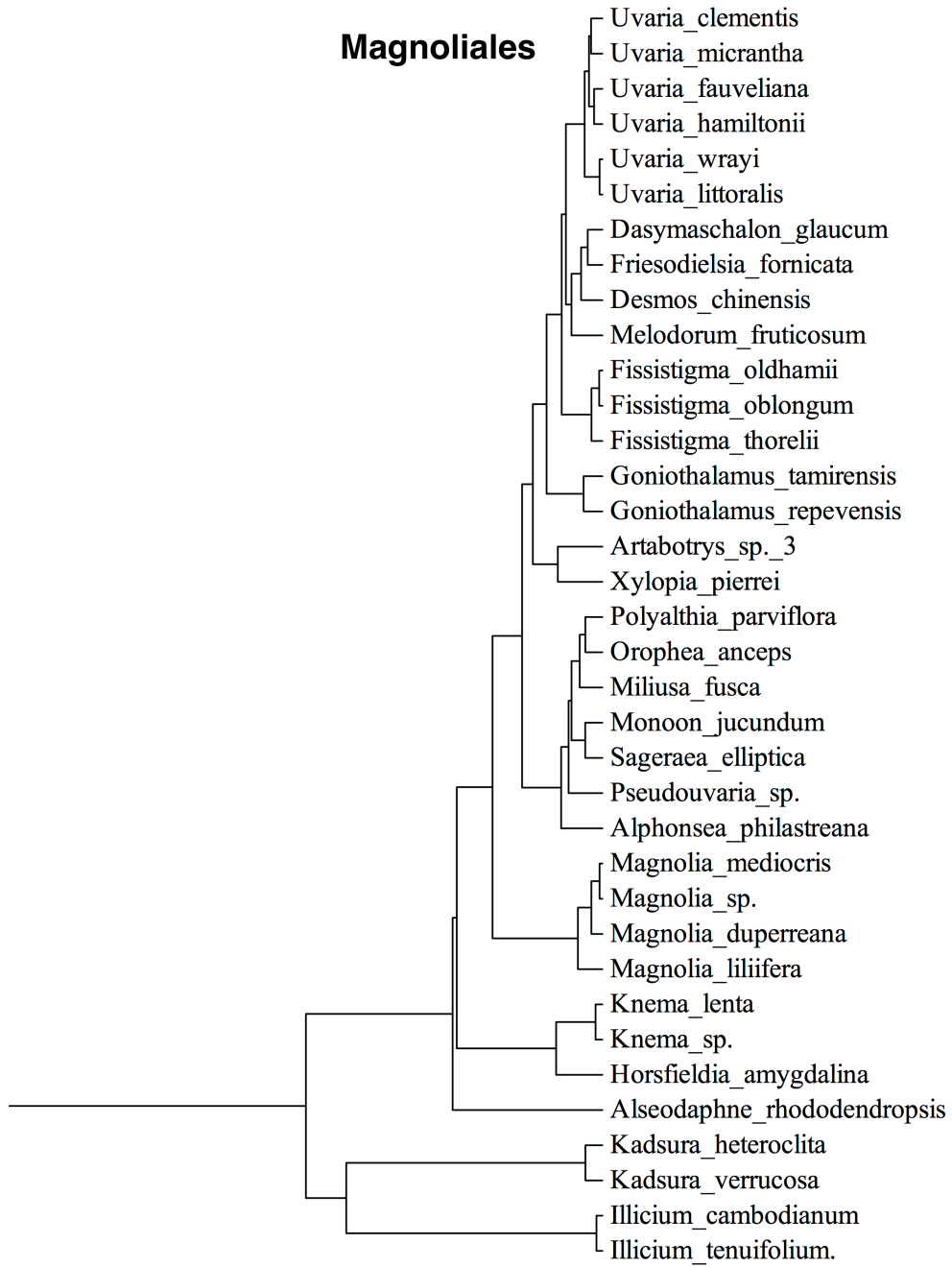


**Celastrales and oxalidales**



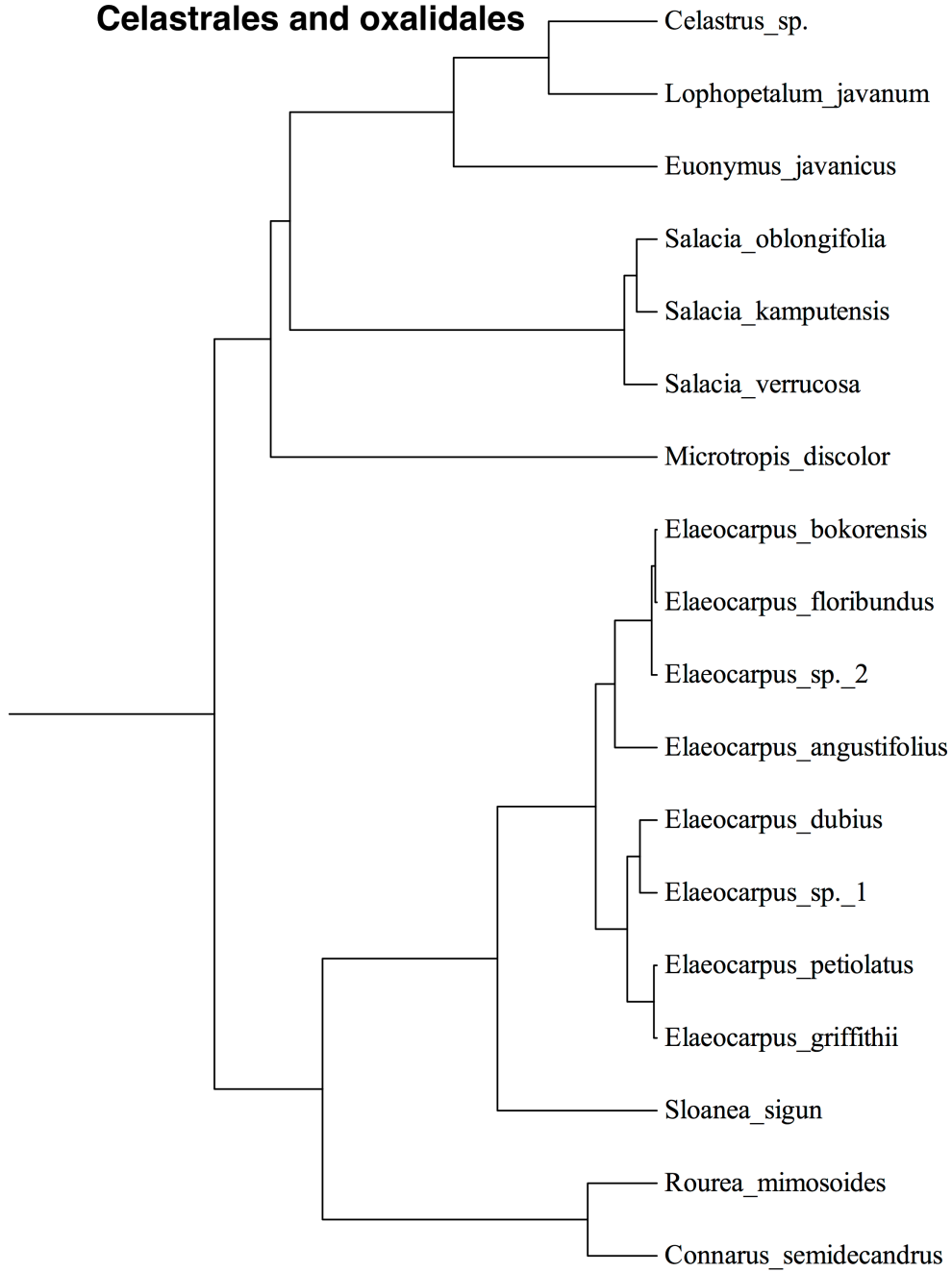
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**Magnoliales**

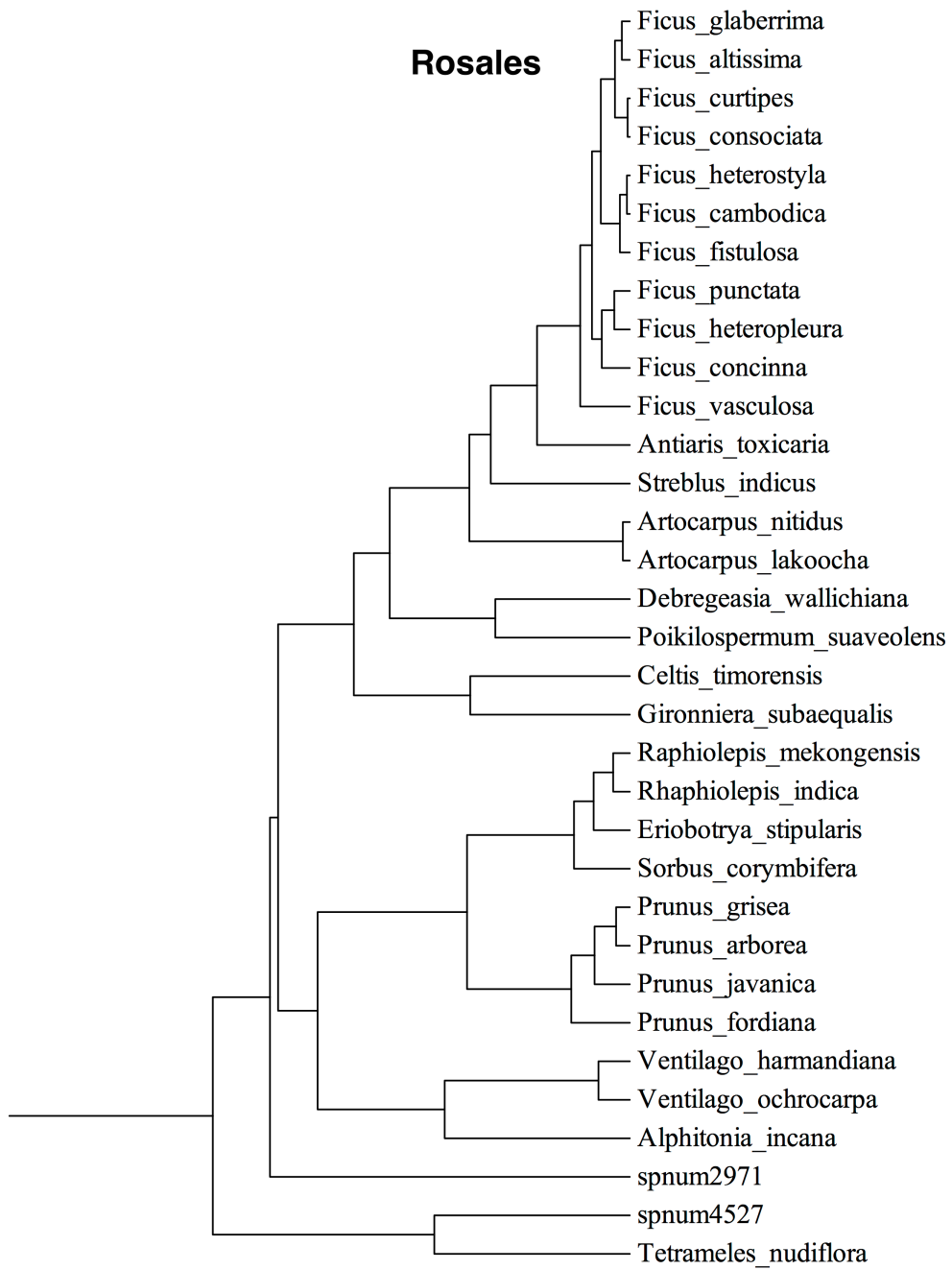


20.0

**Celastrales and oxalidales**

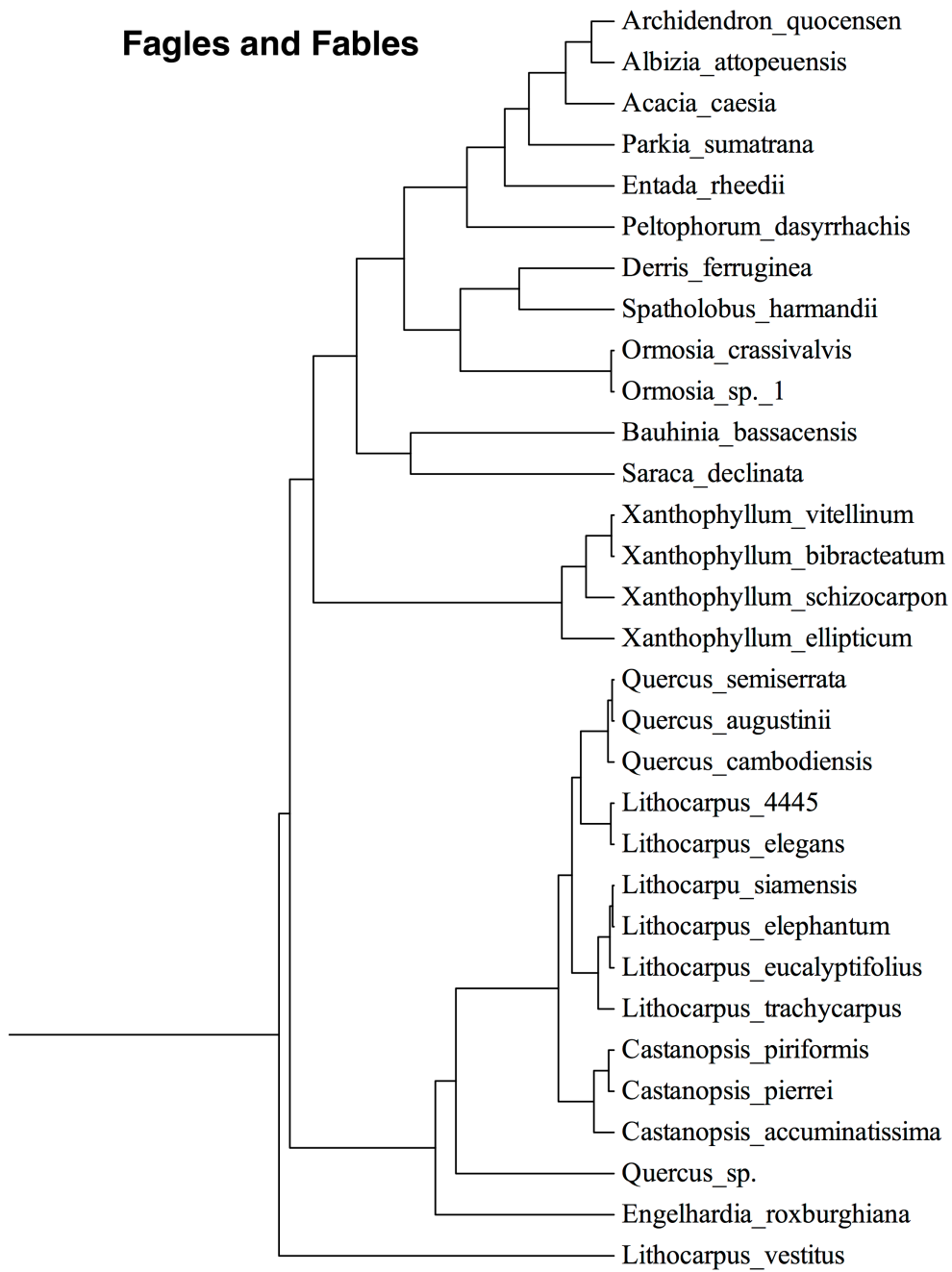


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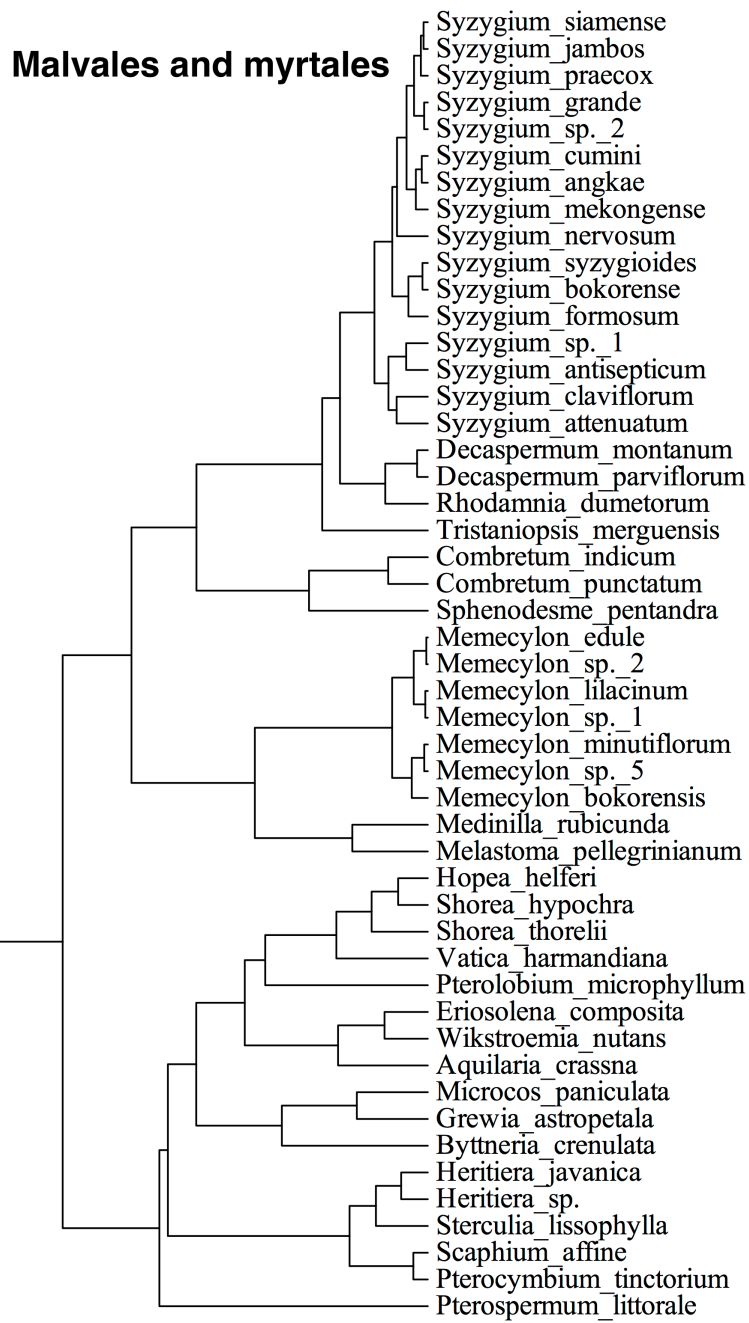


10.0

## Fagles and Fables

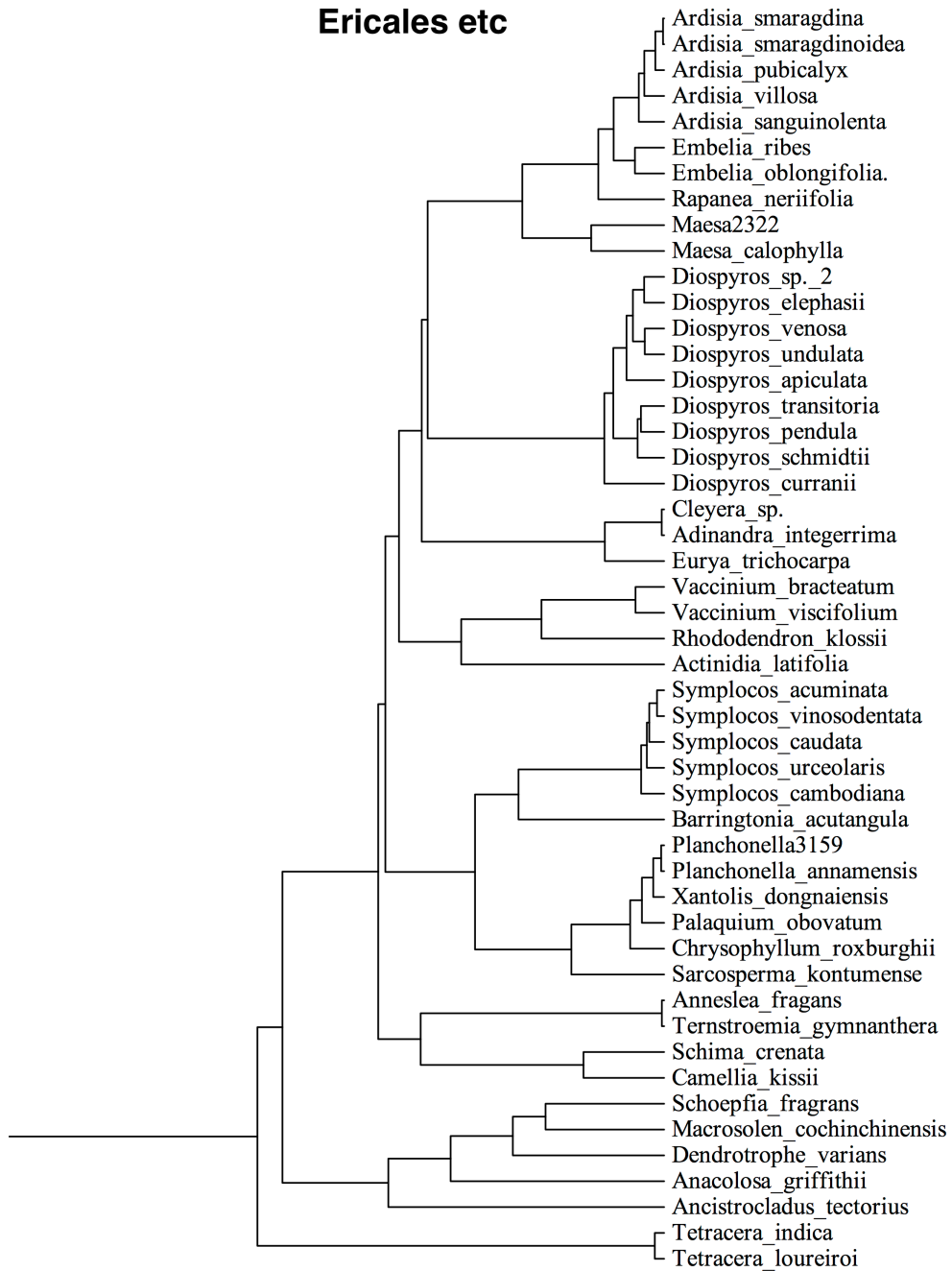


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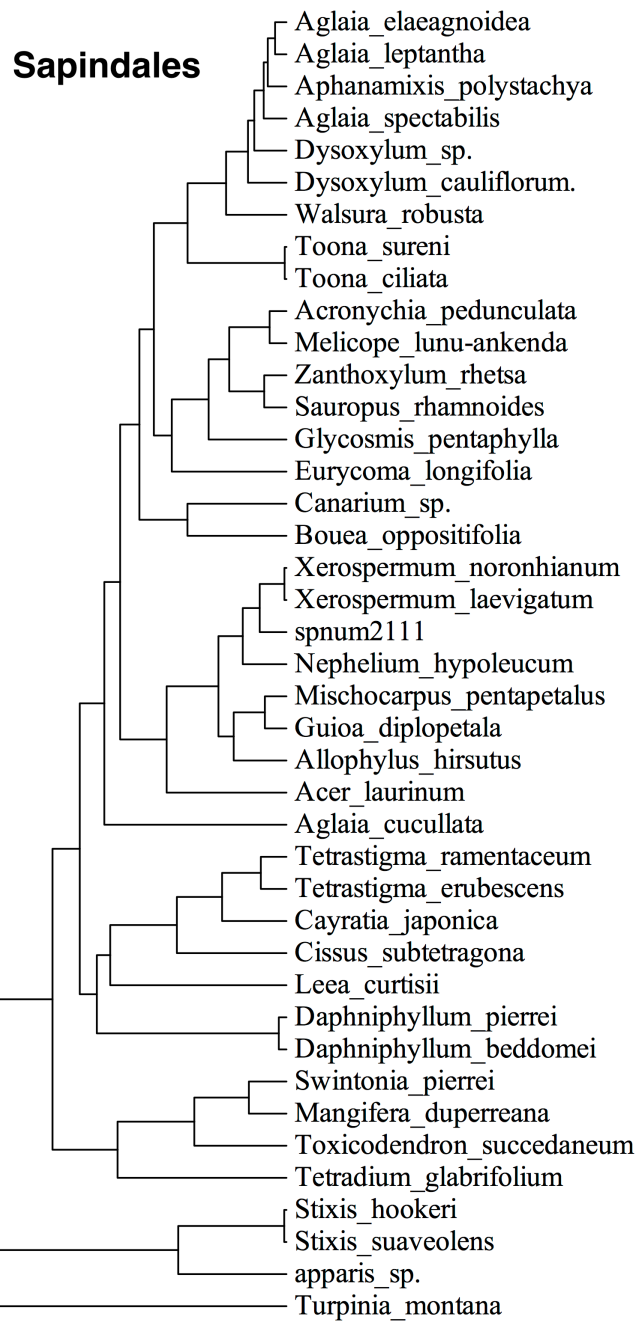


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## Ericales etc



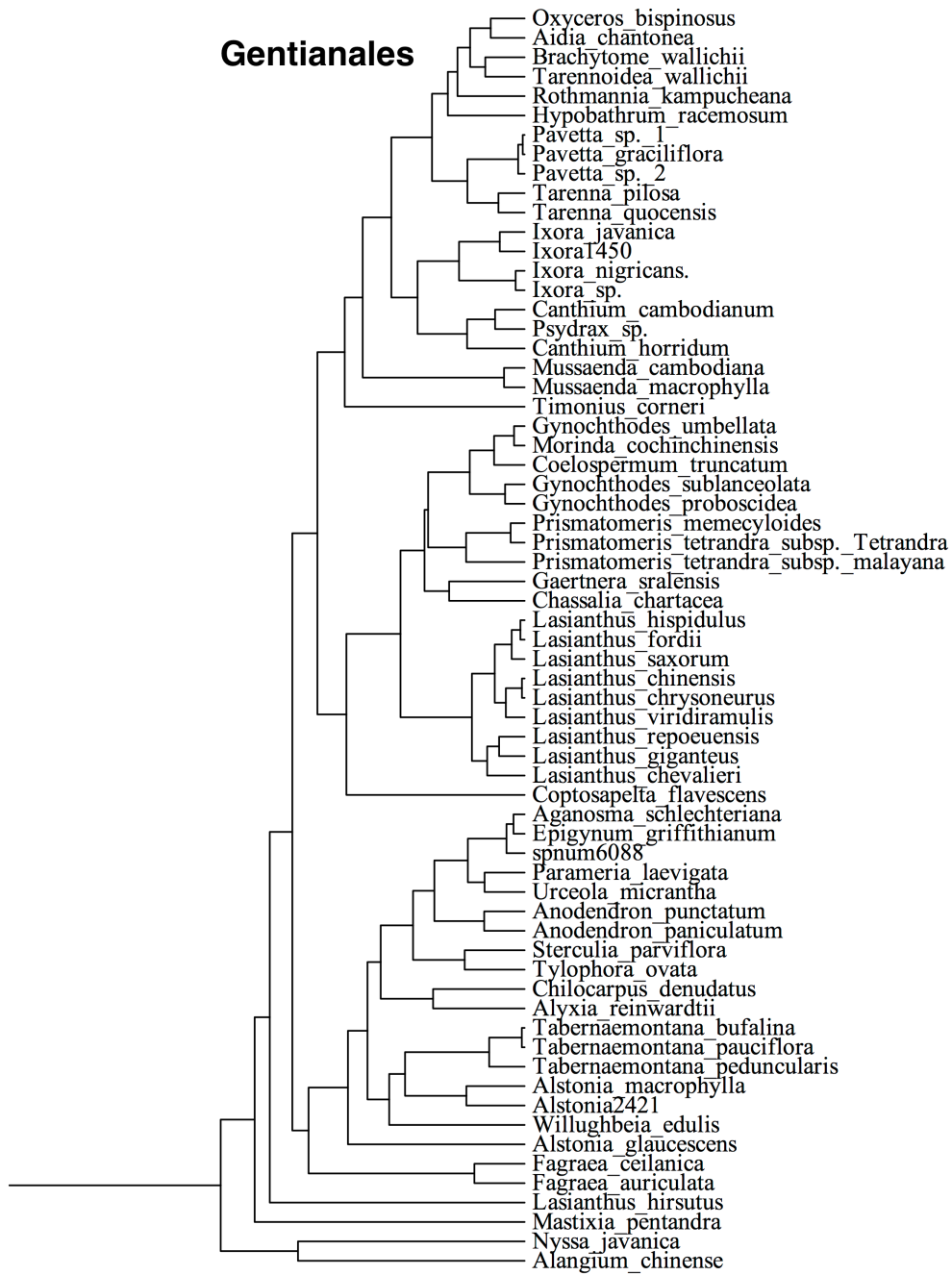
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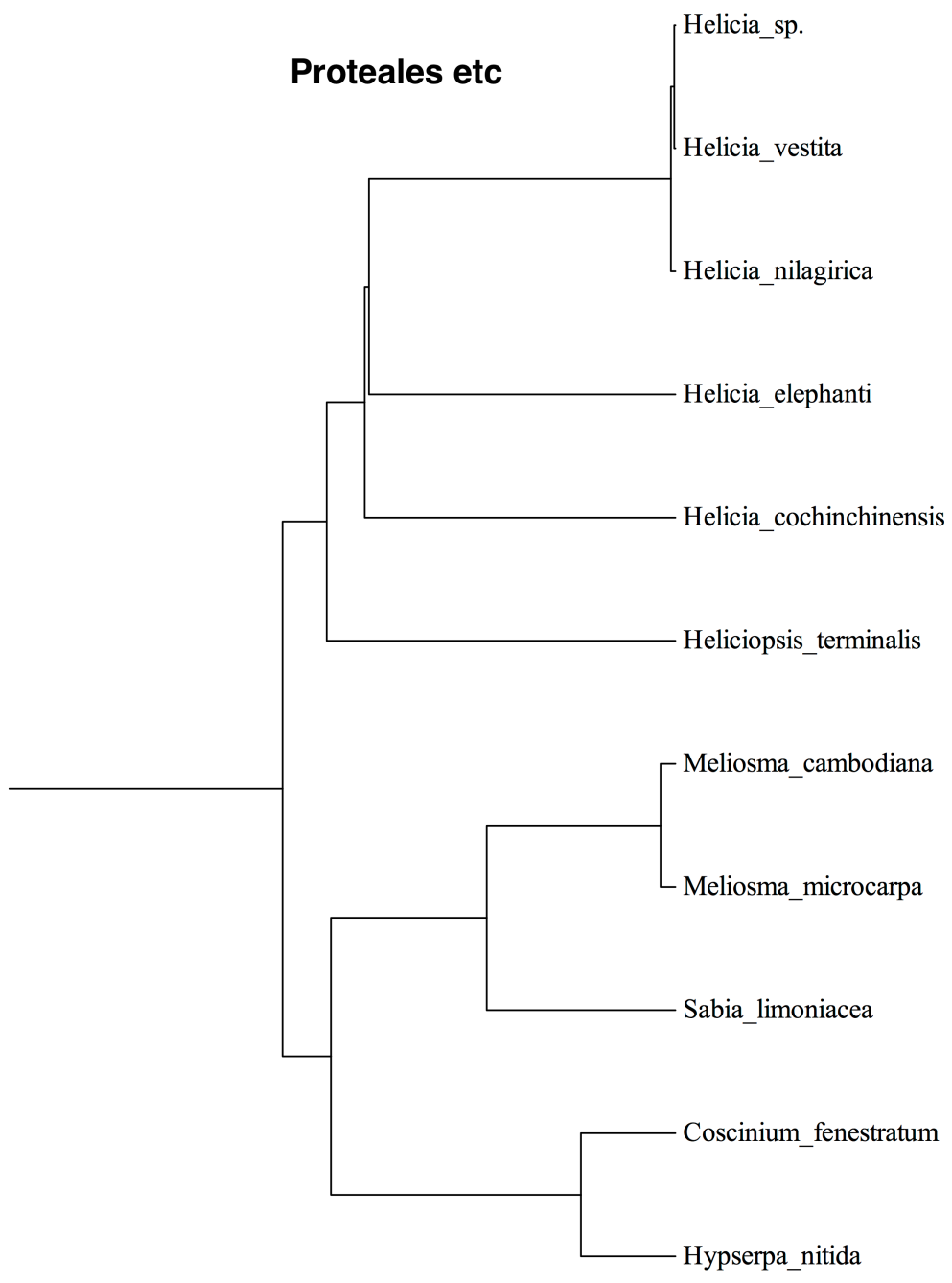
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**Gentianales**

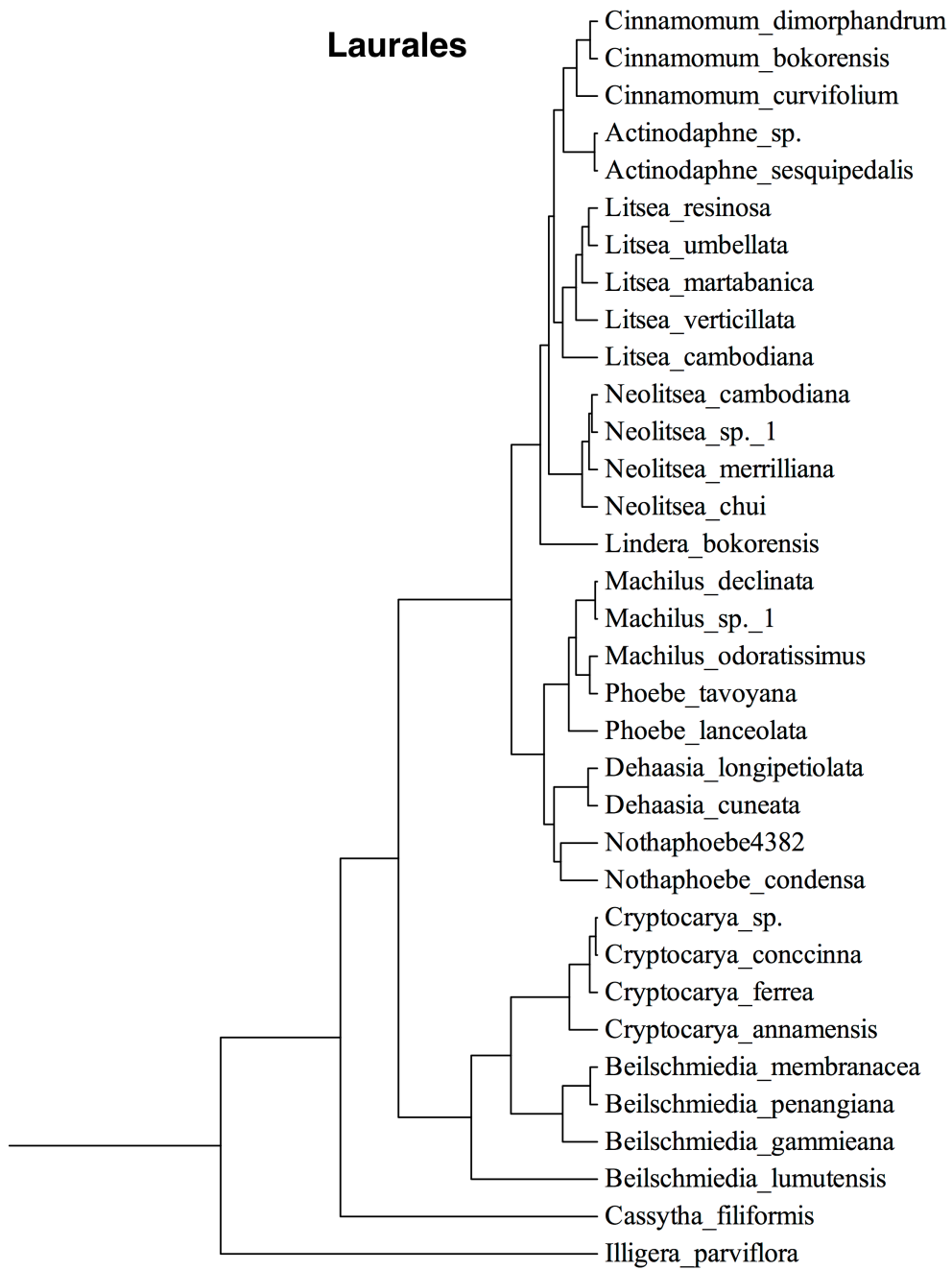


8.0



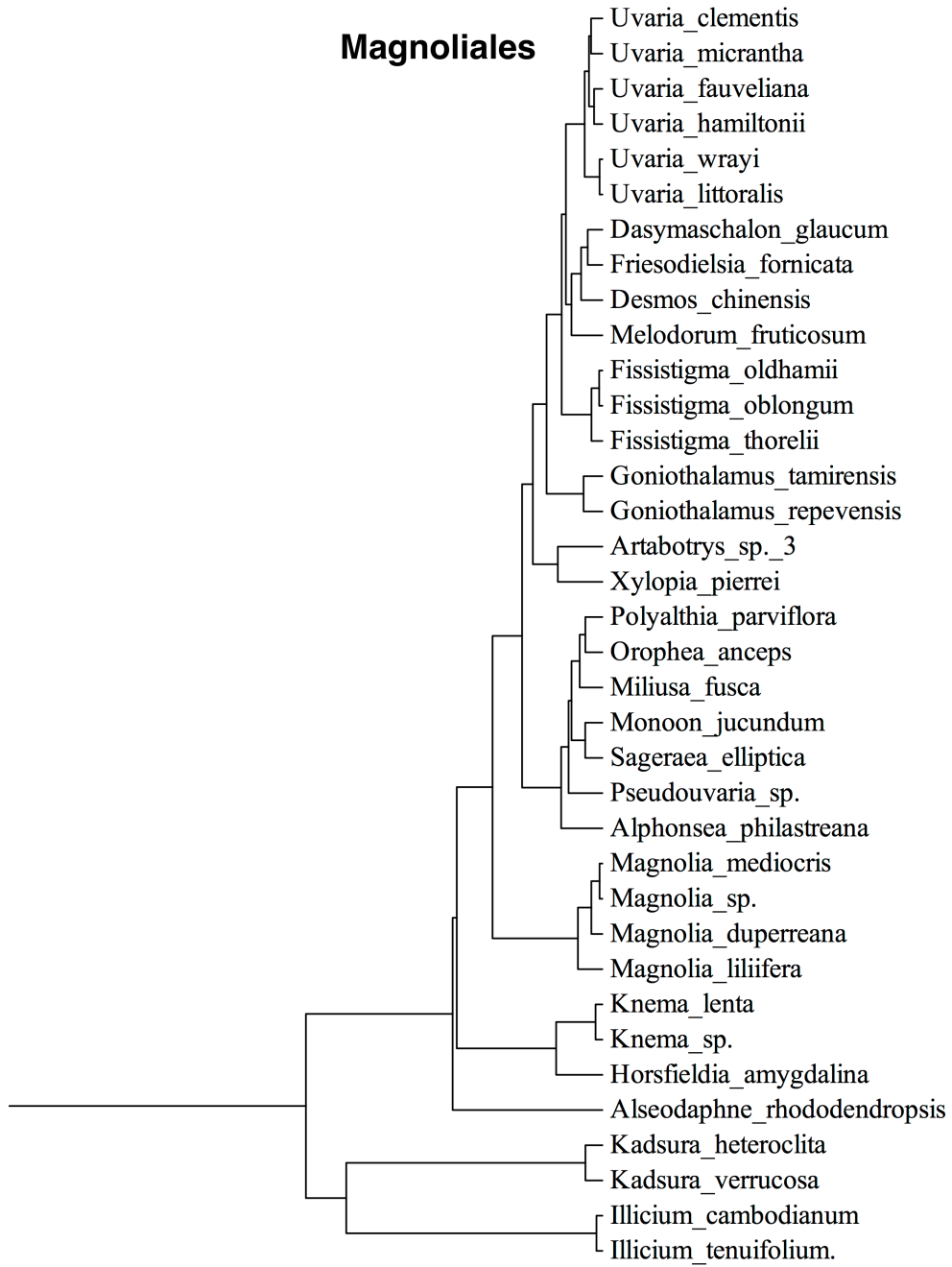
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**Laurales**

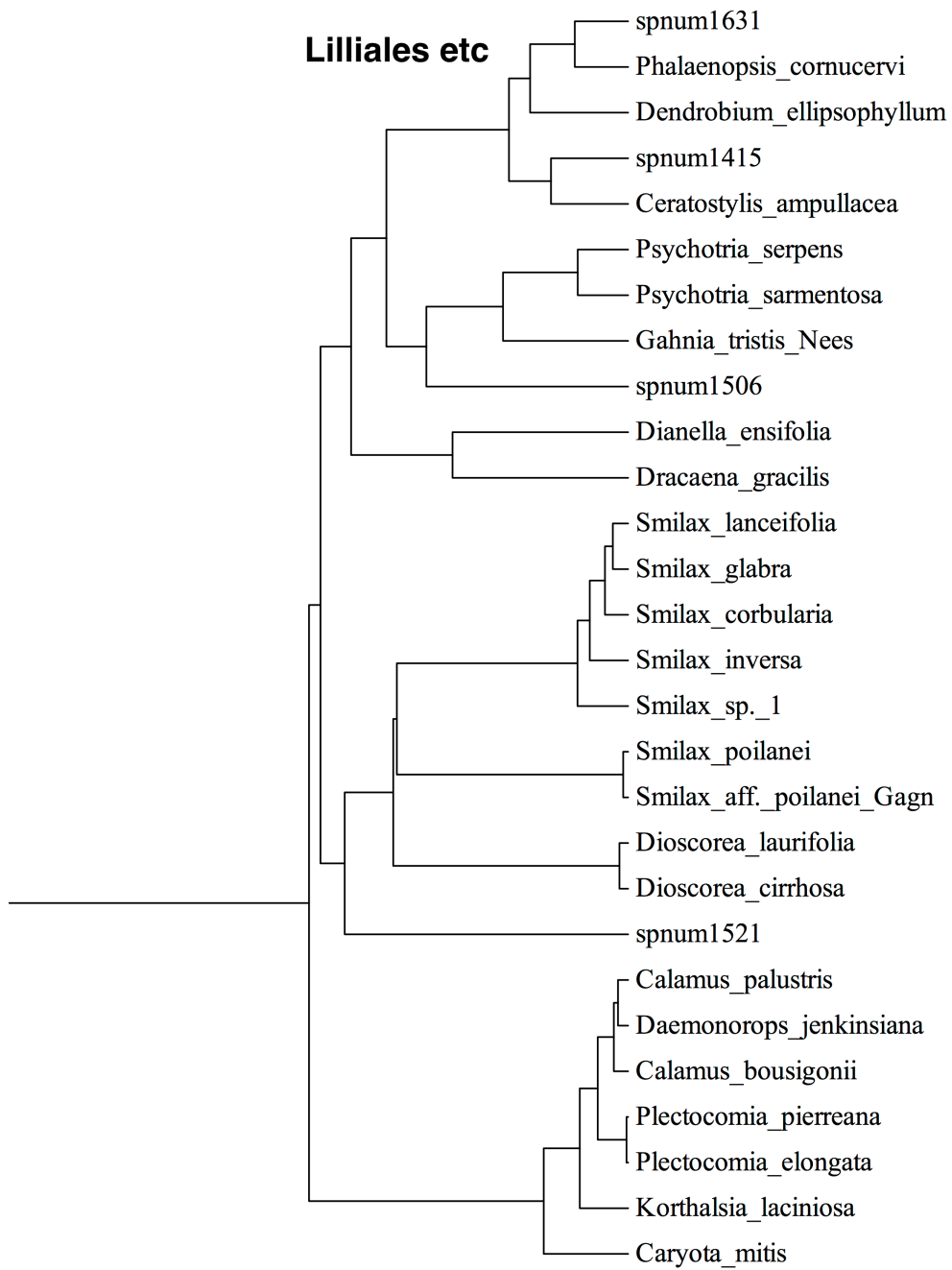


20.0

**Magnoliales**



20.0



20.0