

HIERARCHICAL ANALYSIS OF AMPHIBIAN DIVERSITY IN PRIMARY AND SECONDARY RAIN FORESTS OF CENTRAL VIETNAM

BINH V. NGO^{1,4}, YA-FU LEE², CHUNG D. NGO¹, AND NGHIEP T. HOANG³

¹Department of Biology, University of Education, Hue University, 34 Le Loi Road, Hue 49000, Vietnam

²Department of Life Sciences, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan

³Division of Zoology, Dong Thap University, 783 Pham Huu Lau Road, Cao Lanh 81100, Vietnam

⁴Corresponding author; e-mail: nvbinhsp@hueuni.edu.vn

Abstract.—We assessed species composition, diversity, and distribution patterns of rain forest amphibian assemblages in central Vietnam using a hierarchical analysis of diversity across study areas and different habitat types. We also plotted rarefaction curves to compare unequal assemblages of amphibians and compared the results with our field observations. We recorded 13,529 individuals of 100 species from Anura and Gymnophiona, comprising eight families and 33 genera. There was an overall similarity in species composition of 0.86 between two areas, A Luoi and Nam Dong, Thua Thien Hue Province, central Vietnam; however, the overall similarity in species composition between primary and secondary forests was only 0.03. Only 29 species (29%) of total recorded amphibians were found in both forest types. The relative abundance and species richness were significantly higher in primary forests than in secondary forests. In both areas, rarefaction curves revealed that the expected number of amphibian species in primary forests was significantly richer than that in secondary forests, suggesting that assemblages of amphibians in interior forest habitat may be more vulnerable to alterations caused by disturbances, such as logging. The lognormal model to predict the expected total number of species theoretically available for observation in the entire community (including the rare species not yet collected) indicated that the expected total number of species is approximately 105, with the number of unseen species predicted to fall below the veil line estimated at about 4.48.

Key Words.—abundance; Anura; Gymnophiona; lognormal; rarefaction; species composition

INTRODUCTION

The fate of amphibians is a major and urgent concern of global biodiversity conservation. Nearly a third of the extant 7,793 species are threatened globally. The International Union for Conservation of Nature (IUCN) estimates that 41% threatened with extrapolation to include Data Deficient (DD) species, and more than 160 species may be extinct (IUCN 2018). Multiple factors have been identified that, often collectively or interactively, cause amphibian population declines worldwide (Alford 2011; Palmeirim et al. 2017; Stuart et al. 2004) in both aquatic and terrestrial ecosystems (Alford and Richards 1999; Becker et al. 2007). These include habitat loss and fragmentation (Becker et al. 2007; Brooks et al. 2002; Palmeirim et al. 2017), climate change (Carey and Alexander 2003; Pounds et al. 1999; 2007; Whitfield et al. 2007), epidemic diseases (Berger et al. 1998; Daszak et al. 2003; Pounds et al. 2006), and other factors such as pollutants, invasive species, and over-exploitation (Blaustein and Johnson 2003; Clavero and Garcia-Berthou 2005; Kats and Ferrer 2003; Relyea 2005). The current population declines, already noted in at least 41% of the species, are likely to result in an even greater loss of amphibian diversity in tropical regions

where vast numbers of species reside and which often exhibit high endemism (Alford 2011; Allmon 1991; Beebee and Griffiths 2005; Fauth et al. 1989; Vitt and Caldwell 1994).

To understand ecological and conservation needs, it is critically important to assess amphibian diversity to illustrate patterns of species rarity and distribution as well as the environmental and biological factors that influence these, particularly in tropical regions. The species diversity in any defined geographic region depends on measurements at different levels of resolution (Krebs 1999; Magurran 2004), and recent monitoring programs have promoted additive partitions of species diversity to estimate within-habitat, between-habitat, and within-landscape diversity (Crist et al. 2003; Lande 1996; Loreau 2000; Meynard et al. 2011). Additive partitions of species diversity have been applied to tropical forests (e.g., DeVries and Walla 2001; DeVries et al. 1997, 1999; Summerville et al. 2003); however, hierarchical analyses of amphibian diversity in relation to diverse distribution pattern from tropical regions are still scarce (but see Gardner et al. 2007b; May et al. 2010).

Tropical primary forests harbor many more amphibian species than secondary forests or plantations (Gardner

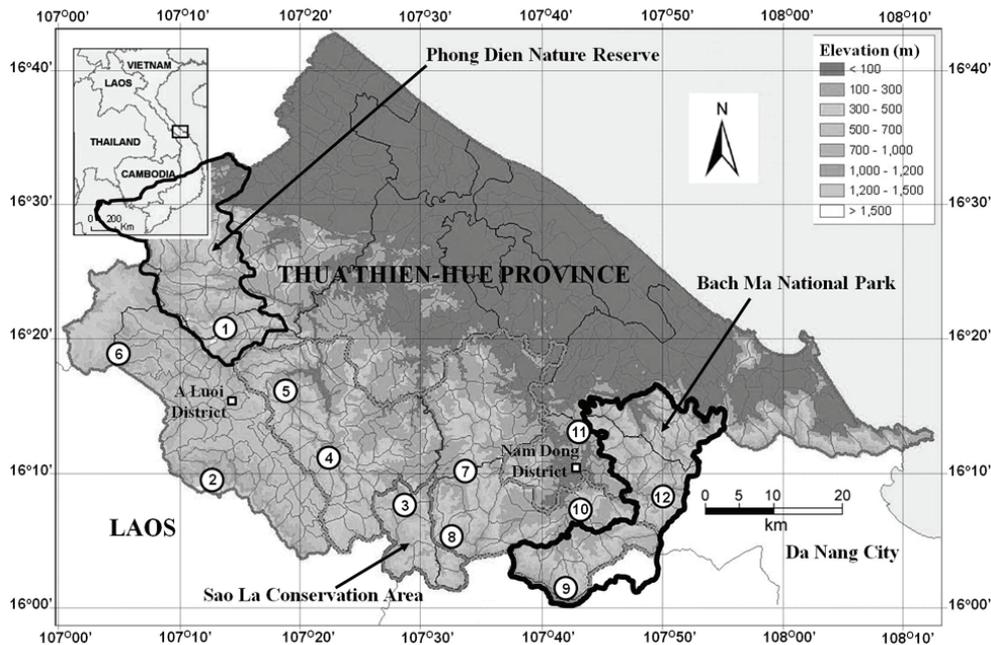


FIGURE 1. Thua Thien Hue Province, Vietnam, showing six stream sites in the A Luoi area (about 1,225 km²) with sites 1–3 in primary forests, sites 4–6 in secondary forests, and six stream sites in the Nam Dong area (about 648 km²), where sites 7–9 are in primary forests and sites 10–12 are in secondary forests.

et al. 2007b; Vallan 2002). As ectotherms, amphibians are generally susceptible to environmental changes and habitat alterations (Vitt and Caldwell 2013; Wells 2007). Fragmented and secondary forests lack spatial heterogeneity and have altered disturbance regimes, traits that diminish and homogenize species composition (Ernst and Rödel 2008; Ernst et al. 2007; Laurencio and Fitzgerald 2010; Neave et al. 1996; Parris 2004).

Central Vietnam, including Thua Thien Hue Province, is a recognized high biodiversity region in Indochina (Nguyen et al. 2009), and considered to be one of the biodiversity hotspots in the world (Myers et al. 2000). Clear-cut logging of primary tropical rain forests, fragmentation of secondary forests, replanting with commercial monocultures, and rapid conversion to different land uses threaten biodiversity in Thua Thien Hue Province (Nguyen et al. 2004). We investigated species composition, relative abundance, diversity, and community structure of amphibian assemblages in two study areas from central Vietnam and compared those between primary and secondary forests. In addition, we used rarefaction curves to estimate expected number of amphibians. We also determined the lognormal distribution of tropical amphibians in central Vietnam. We expected tropical primary forests to harbor considerably greater numbers of amphibian species than secondary forests or plantations (Gardner et al. 2007b), so we examined whether observed partitions of diversity indices are significantly different between primary and secondary forests. We predicted that amphibian

diversity would be higher in primary forests than in secondary forests.

MATERIALS AND METHODS

Study region.—We conducted fieldwork in the primary and secondary rain forests of Thua Thien Hue Province, central Vietnam (15°59'30"–16°44'30"N, 107°00'56"–108°12'57"E, approximately 5,062.6 km² in size; Fig. 1). We sampled montane rain forests at elevations of 700–1,400 m above sea level and cloud forests above 1,400 m up to summits at 1,774 m. The data are presented as mean ± standard error (SE) unless otherwise noted. The study region has a tropical monsoon climate with annual temperatures of 24.4° ± 0.41° C (ranging between 15.8° C ± 0.52° C in January and 29.7° ± 0.71° C in June). Seasonal monsoons bring an annual mean precipitation of 4,980 ± 377 mm. A relatively dry period extends from January to August (the dry season), with a mean monthly rainfall of 139 ± 25 mm, while most of the rain falls from September to December (monthly mean = 738 ± 96 mm; Nguyen et al. 2004). This study region faces serious threats including rapid clear-cut logging, expanding infrastructure, hydropower development, and gold exploitation.

Field sampling.—We conducted visual searches for amphibian species in 12 stream sites (Appendix 1), including six sites in the A Luoi area and six sites in the Nam Dong area with three sites located in primary

forests (montane streams with elevations > 580 m) and three remaining sites in mature secondary forests with elevations < 480 m (Fig. 1). The primary forest sites had many large trees with dense canopy and was not fragmented. The secondary forest sites had impacts from human activities and a fragmented canopy. Stream selection criteria included the ability to carry out surveys. We conducted surveys only during the dry season when amphibian activity is greatest. Weather conditions during the rainy season in this region are very severe and preclude field work.

We surveyed each stream on one or two nights from 2000 to 2020, once per month from January 2008 to August 2010 and two times per month in January and February from 2015 to 2017. Each night, a team of three people walked slowly with a roughly equal pace along the stream for 5 km, randomly alternating the walking direction among nights. We visually searched for amphibians using spotlights in water where they were visible and reachable, on land up to 15 m away from the stream, and on tree trunks and vegetation. We collected amphibians by hand and identified to species on each site following Frost (Frost, D.R. 2020. Amphibian Species of the World: an Online Reference. Version 6.1. Electronic database accessible at <https://amphibiansoftheworld.amnh.org/index.php>.) and Nguyen et al. (2009) for nomenclature. We tallied the total number; to identify recaptured animals, we used visible implant elastomer tags (Hoffmann et al. 2008; Nauwelaerts et al. 2000) to mark each frog, and then we released them at the place of capture. We only used adult individuals for this study and if a frog was re-caught, it was excluded from the analysis of the diversity indices.

Data analysis.—Simpson's Index depends heavily upon the relative abundance of the more abundant species. Therefore, we cannot know immediately which community has higher diversity if only using Simpson's Index of diversity when observing numbers of species and individuals from two communities (Krebs 1999). We used the converted Simpson Index (Simpson 1949),

$$1 - D = 1 - \frac{\sum [n_i(n_i - 1)]}{N(N - 1)}$$

to assess species heterogeneity (SH), and estimated its 95% confidence intervals (CI) using the jackknife technique, where n_i is the number of individuals in the i^{th} species, and N is the total number of individuals (Magurran 2004). We adopted Smith and Wilson's Index (Smith and Wilson 1996),

$$E_{\text{var}} = 1 - 2/\{\pi \arctan[\frac{\sum (\ln n_i - \sum \ln n_j/s)^2/s}{s}]\}$$

to estimate the species evenness, n_i and n_j represent the individual number in the i^{th} and the j^{th} species,

respectively, $i = 1 \sim s$, $j = 1 \sim s$, and s is the total number of species in the sample (Magurran 2004). We used the Morisita Index

$$(C_\lambda = 2\sum X_{ij}X_{ik}/(\lambda_1 + \lambda_2)N_jN_k)$$

to measure the overall similarity in species composition between primary and secondary forests and between two A Luoi and Nam Dong areas (Krebs 1999; Magurran 2004). For the Morisita Index, X_{ij} and X_{ik} are individual numbers of species i in sample j and sample k , $\lambda_1 = \sum [X_{ij}(X_{ij} - 1)]/N_j(N_j - 1)$, $\lambda_2 = \sum [X_{ik}(X_{ik} - 1)]/N_k(N_k - 1)$, and N_j and N_k are the total individual number in samples j and k , respectively.

In comparing samples, we would not know immediately which community had higher species richness based counts of the number of species and the number of individuals in each collection. To overcome this problem, we standardized all amphibian samples from different communities to a common sample size of the same number of individuals (Krebs 1999). We used rarefaction curves to compare two samples of different size, assuming that individuals were randomly dispersed. We standardized all samples from different communities to the same sample size using Hurlbert's (1971) algorithm:

$$E(S_n) = \sum \{1 - (N - N_i)! / [(n!)((N - N_i) - n)!] / [N! / n!(N - n)!]\}$$

$E(S_n)$ is the expected number of species in a random sample of n individuals drawn without replacement from N individuals, $i = 1 \sim s$, s is the total number of species in the sample, N_i is the number of individuals in the species i^{th} , N is the total individual number in the collection, n is the sample size (the number of individuals) chosen for standardization, $N! / n!(N - n)!$ is the number of combinations of n individuals that can be chosen from a set of N individuals. We calculated the variance of the expected number of species $var(S_n)$ in the random sample of n individuals following Heck et al. (1975; reviewed in Krebs 1999).

We constructed Whittaker plots of species-abundance data (Whittaker 1960) to assess the pattern of commonness and rarity. To predict the number of expected species and estimate the expected total number of species in the study region, we employed the approach of Pielou (1975), following methods of Cohen (1959; 1961) to fit a truncated lognormal model to abundance data (reviewed in Magurran 2004). We also used the method of Pielou (1975) to fit a lognormal distribution to the species abundance data and to divide the class boundary (Krebs 1999; Magurran 2004).

We performed statistical analyses using STATISTICA 10.0 (StatSoft Inc., Tulsa, Oklahoma, USA) for Windows 2010 and set the significance level at $\alpha = 0.05$. For the

rarefaction analysis, we used the software for Ecological Methodology 7.2 (Exeter Software, Setauket, New York, USA). We employed a three-way Multivariate Analysis of Variance (three-factor MANOVA) to test for differences in numbers of species and individuals among areas, forest types, and sites. We compared the rank/abundance data between two forest types for two areas using the Kolmogorov-Smirnov two-sample tests. The D -value is the largest unsigned difference between the two cumulative relative frequency distributions of the two samples and we calculated it using the absolute value and a largest unsigned difference determined from empirical data. We computed the critical value for an approximate test for two large samples with the required probability ($\alpha = 0.05$) as $n_1 n_2 D_\alpha$, where $D_\alpha = K_\alpha \times \sqrt{[(n_1 + n_2)/(n_1 \times n_2)]}$, $K_\alpha = \sqrt{[1/2(-\ln(\alpha/2))]}$. For the truncated lognormal distribution of species abundance data, we compared the cumulative number of observed species with the cumulative expected without so called unseen species using a Kolmogorov-Smirnov goodness of fit test for the larger sample of 100 species (Magurran 2004).

RESULTS

Species composition.—We recorded 13,529 individuals of 100 amphibian species belonging to 33 genera, eight families, and two orders (Anura and Gymnophiona; Table 1, Appendix 2). These included seven species classified as Near Threatened (NT), eleven

classified as Vulnerable (VU), and two (*Amolops cucae* [no English common name] and the Yunnan Paa Frog, *Nanorana yunnanensis*) classified as Endangered (EN) in the IUCN Red List. Ranidae (five genera and 30 species) and Rhacophoridae (seven genera and 19 species) were the most species-rich families, whereas Ichthyophiidae was represented by a single species (Table 1). The most common species were the Chloronate Huia Frog, *Odorrana chloronota* ($n = 1,035$), Morafkai Frog, *O. morafkai* ($n = 1,026$), Annam Flying Frog, *Rhacophorus annamensis* ($n = 1,031$), and Anderson’s Spadefoot Toad, *Megophrys major* ($n = 1,026$). In contrast, the Gamboja Toad (*Ingerophrynus galeatus*), Big-eared Toad (*I. macrotis*), Annam Spadefoot Toad (*Megophrys intermedia*) Chinese Spiny Frog (*Quasipaa spinosa*), *Odorrana yentuensis* (no English common name), Tiny Bubble-nest Frog (*Gracixalus supercornutus*), Himalaya Flying Frog (*Rhacophorus bipunctatus*), and Hill Garden Bug-eyed Frog (*Theloderma asperum*) were the rarest species (each with a single individual; Appendix 2).

The number of recorded amphibian species from the A Luoi area was 89 species (89% of the total), compared to 84 species (84%) from the Nam Dong area, and 73 species (73%) in both areas. There were 92 species (92%) recorded in primary forests, compared with 37 species (37%) recorded in secondary forests and only 29 species (29%) recorded in both habitat types. Both the mean numbers of species and individuals detected in primary and secondary forests from the A Luoi area were

TABLE 1. The total number of genera (G) and species (Sp) in parenthesis for each amphibian family and its relative abundance (RA, %) collected in primary and secondary forests from the A Luoi and Nam Dong areas in central Vietnam. Species heterogeneity (SH; numbers in parentheses are the 95% confidence intervals) and species evenness (SE), estimated by the converted Simpson’s index and Smith and Wilson’s index, respectively, are provided for each forest type in the respective area.

Family (G, Sp)	A Luoi				Nam Dong			
	Primary		Secondary		Primary		Secondary	
	G, Sp	RA						
Anura								
Bufonidae (3, 5)	3, 5	0.4	1, 1	9.6	2, 3	0.5	1, 1	13.5
Dicroglossidae (7, 14)	5, 10	16.2	4, 7	28.7	5, 11	20.8	5, 6	21.7
Hylidae (1, 2)	1, 2	0.2	0	0	1, 2	0.2	0	0
Megophryidae (5, 14)	5, 14	19.1	2, 4	0.8	4, 12	21.0	2, 4	1.0
Microhylidae (4, 15)	2, 9	0.6	3, 10	38.6	3, 11	0.8	3, 10	46.7
Ranidae (5, 30)	5, 27	47.3	4, 10	15.0	5, 23	42.4	4, 8	13.1
Rhacophoridae (7, 19)	6, 13	16.4	3, 3	6.9	6, 14	14.2	1, 1	3.7
Gymnophiona								
Ichthyophiidae (1, 1)	0	0	1, 1	0.4	1, 1	0.1	1, 1	0.4
Total (33, 100)	27, 80	4965	18, 36	2740	27, 77	3987	17, 31	1837
SH	0.91 (0.90–0.92)		0.94 (0.93–0.95)		0.93 (0.92–0.94)		0.94 (0.93–0.95)	
SE	0.499		0.497		0.486		0.476	

TABLE 2. The average number (mean \pm standard deviation) and range (in parentheses) of amphibian species and individuals detected in each area and in each forest type from Thua Thien Hue Province, central Vietnam.

	A Luoi (n = 44)		Nam Dong (n = 44)	
	Primary	Secondary	Primary	Secondary
Species				
	13.81 \pm 3.86 (8–27)	5.82 \pm 2.23 (2–13)	6.57 \pm 2.63 (2–16)	3.58 \pm 1.54 (1–9)
Individuals				
	43.55 \pm 15.33 (25–99)	24.04 \pm 11.59 (7–64)	34.97 \pm 19.89 (4–110)	16.11 \pm 11.99 (2–65)

larger than those from the Nam Dong area (Table 2). The number of species detected varied significantly between areas (Wilk’s $\lambda = 0.544$, $F_{1,443} = 372.54$, $P < 0.001$) and forest types ($\lambda = 0.471$, $F_{1,443} = 498.93$, $P < 0.001$; area \times forest interaction, $\lambda = 0.811$, $F_{1,443} = 103.22$, $P < 0.001$), and among sites ($\lambda = 0.943$, $F_{2,443} = 13.35$, $P < 0.001$; no interaction effects with area, forest, or both, all P -values > 0.050). The number of individuals encountered also differed between forest types ($\lambda = 0.688$, $F_{1,443} = 201.57$, $P < 0.001$; no interaction effects with area, site, or both, all P -values > 0.400), and between areas ($\lambda = 0.923$, $F_{1,443} = 37.26$, $P < 0.001$) and among sites ($\lambda = 0.921$, $F_{2,443} = 19.18$, $P < 0.001$ with area \times site interaction, $\lambda = 0.981$, $F_{2,443} = 4.209$, $P = 0.010$). Overall, primary forests harbored over twice as many species and almost twice as many individuals compared with secondary forests (Table 1).

Amphibian diversity.—Within habitats, both the heterogeneity and evenness index values varied but fluctuated less in primary forest sites than those in secondary forest sites (Table 1). In both the A Luoi and Nam Dong areas, species heterogeneity is similar across all habitats and sites (secondary forests, A Luoi: SH = 0.94; Nam Dong: SH = 0.94, and primary forests, A Luoi: SH = 0.91; Nam Dong: SH = 0.93). The evenness index for primary and secondary forests in the A Luoi area was higher than those in the Nam Dong area (Table 1).

Between primary and secondary forests and within a landscape, both β -diversity and γ -diversity were slightly lower in the A Luoi area ($\beta = 0.54$ and $\gamma = 0.91$) than in the Nam Dong area ($\beta = 0.56$ and $\gamma = 0.99$). The similarity index of species composition between primary and secondary forests in the A Luoi area was 0.027 and the Nam Dong area was 0.031. The similarity index of species composition between two A Luoi and Nam Dong areas was 0.86. These results indicated that the species composition of amphibians between primary and secondary forest is very different, while identical between two study areas. The largest unsigned difference (D) in the cumulative relative abundance

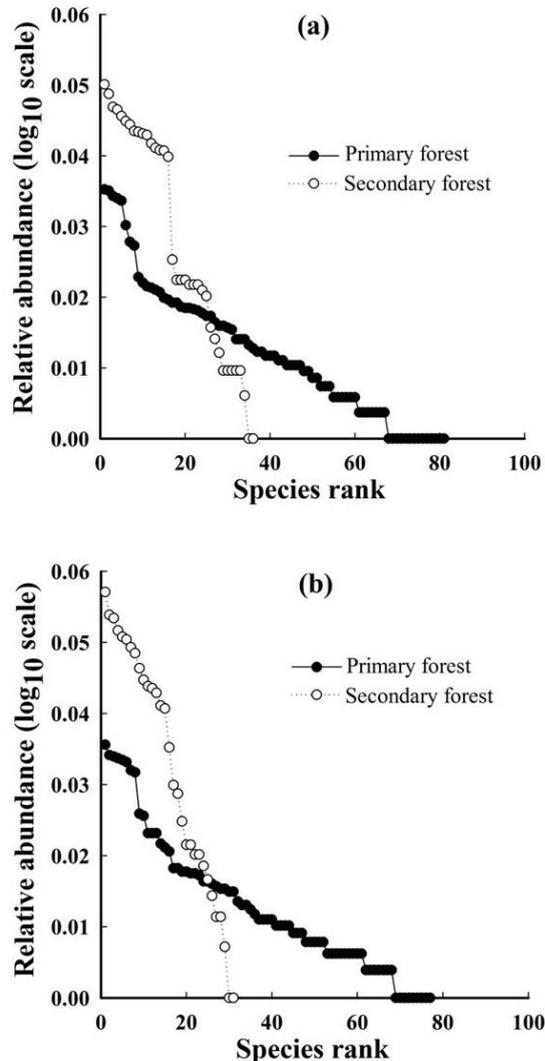


FIGURE 2. Whittaker plots of abundance-rank of amphibians collected in primary and secondary forests in the (a) A Luoi and (b) Nam Dong areas, Vietnam.

between primary and secondary forests was larger in the A Luoi area (0.31) than in the Nam Dong area (0.29). In both areas, the cumulative relative abundance was significantly different between primary and secondary forests (A Luoi: $n_1 = 81$, $n_2 = 36$, $n_1 n_2 D = 895.21$, $P < 0.020$; Nam Dong: $n_1 = 77$, $n_2 = 31$, $n_1 n_2 D = 697.01$, $P < 0.050$; Fig. 2).

Richness estimates.—Our comparison results using rarefaction curves showed that primary forest had significantly higher expected richness of species than the secondary forest in the A Luoi and Nam Dong areas, with the 95% confidence limits for the two forest types in both areas indicating a difference between the amphibian communities (Fig. 3). The differences in the expected number of species from the two forest

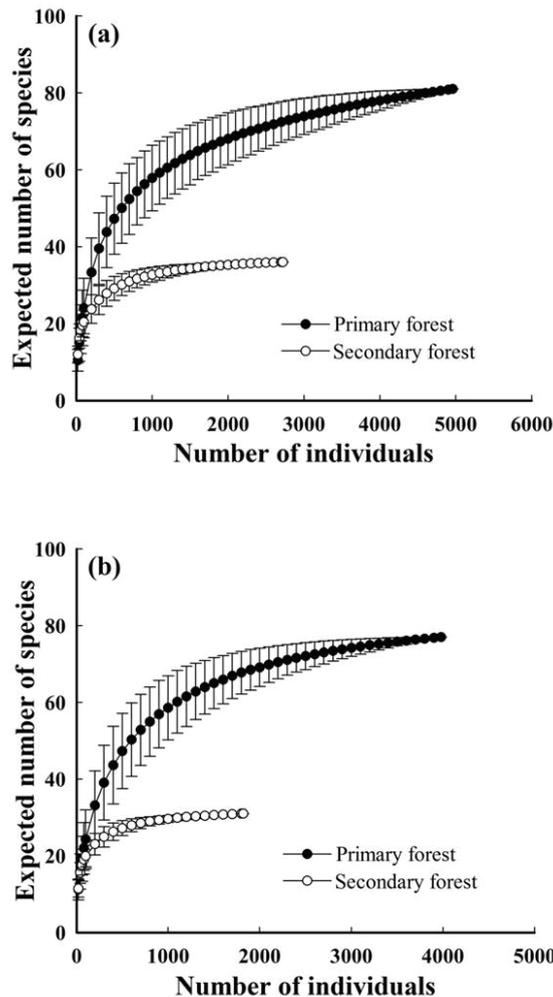


FIGURE 3. Rarefaction curves of species accumulation with increasing number of individuals sampled in primary and secondary forests from the (a) A Luoi and (b) Nam Dong areas, Vietnam.

types were significant at $P < 0.050$ because most of the 95% confidence limits of the expected species accumulation curves did not overlap (Fig. 3). Most lognormal distributions of species abundance data are truncated to the left of the pattern (Fig. 4). After \log_{10} transformation of the data of species abundance, from empirical evidence, we determined the values of the estimates of the mean ($\mu_x = 1.337$) and variance ($V_x = 0.904$) allowing for the truncated lognormal distribution. Note that we assume the truncation point (x_0) to fall at -0.301 ($\log_{10} 0.5$), this being the upper boundary of each class (octave) containing species that lie behind the veil line (Fig. 4, Appendix 3). To calculate the standardized normal variate (z_0) corresponding to the truncation point (x_0) for the distribution, we obtained the value $z_0 = -1.723$ and $p_0 = 0.045$ (here p_0 is proportional to the number of species predicted to be behind the veil line). Finally, we employed the value of p_0 to estimate the

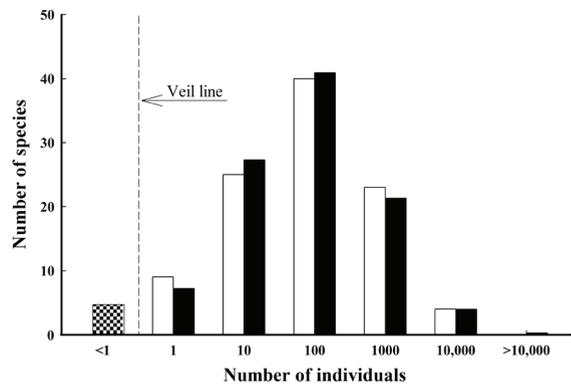


FIGURE 4. The lognormal distribution pattern according to the original method of Pielou using \log_{10} for the amphibian communities assessed in central Vietnam. The number of species observed (white bars) comparing to the number expected (black bars) by the truncated lognormal distribution. For clarity, 0.5 added to the boundaries during the calculation is not included in the graph. The veil line indicates that this class is less than one, and the checkered bar represents the so called unseen species that are predicted to lie behind it. This class represents the rarer species that were absent from the catch for the amphibian communities in the present study.

total species richness of the assemblage of amphibians (the expected total number of amphibian species in the theoretical community), $S^* = S/(1 - p_0) = 100/(1 - 0.045) = 105$ species approximately. Thus, we identified approximately 95.5% (100/105 species) of the species compared with the expected total number of species in the whole community. The expected number of species (about 4.48 species) in class 1 (i.e., octave < 1) corresponds to the number of species predicted to fall below the veil line (Fig. 4, Appendix 3).

We examined the cumulative expected distribution of amphibian species excluding so called unseen amphibians that lie behind the veil line compared with the cumulative observed distribution. The total number of species in this study is 100, thus the critical value for D is $D_{0.05} = 0.089$ and $D_{0.01} = 0.104$. The observed D is 0.024, which is < 0.089 . Therefore, the two distributions were not significantly different at $P < 0.050$ and we conclude that the observed distribution is consistent with a truncated lognormal distribution (Fig. 4).

DISCUSSION

The species richness and relative abundance of amphibians in primary forests are significantly greater than that in secondary forests. The similarity in amphibian species composition and relative abundance between two areas was considerably higher than those between two forest types. This is due to the difference in levels of disturbance being much greater between secondary and primary forests than between the A Luoi and Nam Dongng areas. Spatial processes are important

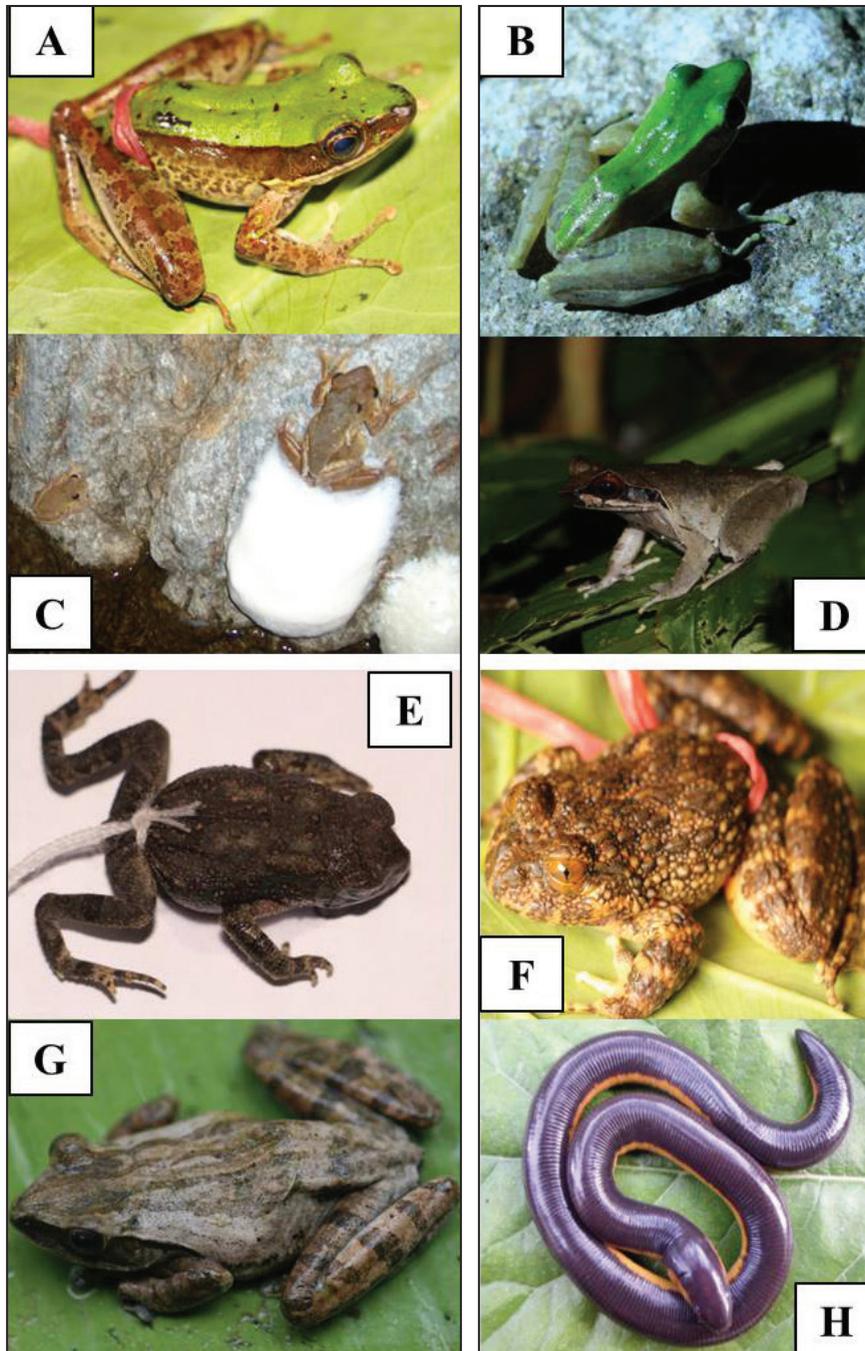


FIGURE 5. The most common species we found in our study in central Vietnam. (A) the Chloronate Huia Frog, *Odorrana chloronota*, (B) Morafkai Frog, *Odorrana morafkai*, (C) Annam Flying Frog, *Rhacophorus annamensis*, (D) Anderson's Spadefoot Toad, *Megophrys major*. The rarest species we found were (E) the Gamboja Toad, *Ingerophrynus galeatus*, (F) Yunnan Paa Frog, *Nanorana yunnanensis*, (G) Burmese Whipping Frog, *Polypedates mutus*, and (H) Mengla County Caecilian, *Ichthyophis bannanicus*. (Photographed by Binh V. Ngo).

for a natural community and influence the number and relative abundance of coexisting species in any given locality at the regional scale (Verhoef and Morin 2010). Approximately 92% of taxa were found in primary forests, whereas only 37% of amphibians were recorded in secondary forests. This rate is lower than the typical study of Gardner et al. (2007b) in northeastern Brazilian

Amazonia, in which 96% of all amphibians occurred in primary forests, but higher than the study of Vallan (2002) in the tropical rain forests of eastern Madagascar (< 81%).

In addition, the contrasting partitions of species richness and relative abundance, the Simpson Index of diversity and Smith and Wilson's Index of evenness, may

also be explained by the pattern of species dominance or rarity (Crist et al. 2003; Krebs 1999; Lande 1996). The most abundant species are widespread and therefore the same common species comprise most of the regional or forest α -diversity. In contrast, the rarest species were all found in primary forests, with low abundance (each species represented by a single individual), resulting in a small contribution to α -diversity (Krebs 1999; Magurran 2004). This explained why both Simpson's Index and Smith and Wilson's Index between two areas were similar. In fact, both communities from the A Luoi and Nam Dong areas had equal numbers of rare species (eight individuals of eight species), and the number of common species was similar (Fig. 5, Appendix 3).

Estimation of species richness using the rarefaction method allows us to solve this problem. In both areas, the value of SH was considerably higher in secondary forests than in primary forests (see results). Based on the number of expected species using the rarefaction method, however, showed that community samples from primary forests have significantly higher species richness than community samples from secondary forests. One essential problem in community ecology that frequently appears in comparing the samples of communities is that they are based on different sample sizes (Hurlbert 1971; Magurran 2004; Sanders 1968; Simberloff 1972).

Using the rarefaction curve for species richness could allow for comparisons in terms of the expected number of species in a fixed number of amphibians by moving vertically through Figure 3 at any fixed number. The comparison can be taken at the point at which the amphibian abundance level of the larger community matches the level in the smaller one. This comparison is appropriate because sampling methods are similar for the two areas. Previous studies indicate that the expected species curves for the two samples can intersect (Gardner et al. 2007a,b; Peet 1974; Sanders 1968; Simberloff 1972). Our estimated results of species richness are consistent with several previous studies, in which amphibian communities from primary forests were significantly more diverse and had higher species richness than secondary forests (Gardner et al. 2007b; Ribeiro-Junior et al. 2008; Sanders 1968). Again, the contribution of rare species was demonstrated using the rarefaction curves.

One characteristic feature of natural communities is that they contain relatively few species that are common and relatively large numbers of species that are rare (Fisher et al. 1943; Krebs 1999; Pielou 1975, 1977; Preston 1948; Williams 1947). It is relatively easy to determine for any given area the number of species in the area and the number of individuals in each of these species; however, to enumerate all of the species in a natural community is extremely difficult and costly,

if not impossible (Krebs 1999; Pielou 1975; Preston 1948). In this study, we attempted to collect and analyze a large assemblage of amphibian species living in the tropics, using a log-normal pattern of species abundance (\log_{10}) to estimate the expected total number of species theoretically available in the whole amphibian community. The expected number of species predicted to fall below the veil line in this study was about 4.48 species and the total number of species theoretically available was approximately 105 species compared to 100 observed species. The value of theoretically available species obtained from the lognormal distribution model is necessary to scale the expected distribution of abundances even when the model of the distribution is absent (Krebs 1999; Magurran 2004; Pielou 1975). We identified approximately 95% of the number of species theoretically available.

Amphibians are seldom detected with accuracy, especially in the case of rare species, regardless of the methods employed, physiological characteristics of species, study periods, and collecting approaches (MacKenzie et al. 2002; Ngo 2015; Roloff et al. 2011; Walls et al. 2011; Wells 2007). Non-detection of one or several species in a region does not mean an absence of these species unless under there is a detection probability of 100%. This applies to some organisms that in general are easier to detect than amphibians, such as birds or insects (Preston 1948). Additionally, not all species present in a community are equally easy to detect (MacKenzie et al. 2002; Preston 1948; Verhoef and Morin 2010). If an amphibian assemblage contains species so rare to that community, should theoretically contain only a fraction of the species, it is most likely that those species will be unrepresented in the collection (Preston 1948, 1962a,b). On average, those uncommon species with a theoretical representation appreciably less than one individual will be missed from the sample.

Although our observations only detected 100 species compared to about 105 theoretical species in the entire community, some rare species (e.g., the Yunnan Firebelly Toad, *Bombina maxima*, Malayan Giant Toad, *Phrynoidis asper*, Boulenger's Paa Frog, *Quasipaa boulengeri*, and Tonkin Bug-eyed Frog, *Theloderma corticale*) are known to occur in this region and nearby with the Truong Son mountain range (near A Sap Stream, site 2) and its vicinity in Quang Nam Province (near Thuong Nhat Stream, site 9) during the main rainy season (from September to December; Ngo 2015). Photographs and interviews with native hunters indicate that some rare species occur in the two study areas (e.g., the Gordon's Bug-eyed Frog, *Theloderma gordonii*, *Philautus abditus*, and Nguyen's Caecilia, *Ichthyophis nguyenororum*). Of the non-detected species, according to Nguyen et al. (2009), seven occur irregularly in the two study areas. The remaining 94 are regular breeders in the

study region. Thus, the total of 105 amphibian species according to the lognormal model is an appropriate finding based on species-abundance data of the study region with the large sample.

In the two study areas, logged forests (secondary forests) have large gaps in the canopy and fewer big trees than unlogged forests (Ngo 2015; Nguyen et al. 2013). The transformation of intact tropical rain forests into secondary vegetation or into exploited forests leads to the loss of microhabitats for many amphibians (e.g., Gardner et al. 2007b; Vallan 2002). The presence of a primary forest canopy that better regulates forest temperature and soil moisture is crucial in determining amphibian survival, reproduction, and movements (Inger and Colwell 1977; Lee et al. 2006; May et al. 2010). Many anuran juveniles prefer habitats with forested canopies compared with open-vegetation types (Rothermel and Semlitsch 2002). Moreover, flowing waters of streams may transform into stagnant waters if a change from the primary forest into secondary forest takes place (Ngo 2015). The combination of these processes leads to habitat loss or habitat degradation (Inger and Colwell 1977; Ngo 2015; Vallan 2002), which may influence amphibian survival and movement patterns because there are fewer niches that amphibian species may occupy. Therefore, we should safeguard remaining primary forests and enhance secondary forests to provide essential habitats and sustain diversity of amphibians.

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BINH V. NGO is an Associate Professor of Biology at the College of Education, Hue University, Vietnam, where he has taught Zoology, Ecology, and Animal Behavior over the last 5 y. He received his Ph.D. in Zoology from National Cheng Kung University, Taiwan (2015). His research focuses primarily on behavioral ecology, bioacoustics, population biology, community ecology, conservation ecology, log-normal distributions, and site occupancy of amphibians and reptiles living in tropical regions. (Photographed by Nhan T. Dang).



YA-FU LEE is a Professor of Biology at National Cheng Kung University, Taiwan. He received his Ph.D. in Ecology and Evolutionary Biology from the University of Tennessee, Knoxville, USA (1999). His primary research interests include behavioral ecology, community ecology, evolutionary ecology, conservation ecology. His research interests lie in animal behavior, ecology, their interrelationships and evolutionary contexts, and implications for conservation. He studies how interspecific and intraspecific individual differences in behavior, life history, and eco-morphological traits shape roles of organisms through various processes within populations or assemblages and which affects community structures and diversity. (Photographed by Yen-Min Kuo).



CHUNG D. NGO is a Professor of Biology at the College of Education, Hue University, Vietnam, where he has taught Zoology, Developmental Biology, Vertebrate Comparative Anatomy, General Biology, Herpetology, and Vertebrate Practice over the last 30 y. He received his Ph.D. in Zoology from the College of Education, Hanoi National University, Vietnam (1991). His primary research interests include behavioral ecology, population biology, community ecology, and taxonomy of herpetofauna living in the tropics. (Photographed by Binh V. Ngo).



NGHIEP T. HOANG is a Lecturer in the Department of Biology, Dong Thap University, Cao Lanh, Vietnam. She received her Ph.D. in Zoology from the College of Education, Hue University, Vietnam (2012). Her primary research interests include the systematics, life history, diversity, ecology, and biogeography of herpetofauna found in the Mekong Delta of Southeast Vietnam. (Photographed by Oanh T. Nguyen).

APPENDICES

APPENDIX 1. Coordinates of the 12 localities where we collected adult frogs for the present study, including the A Luoi area (about 1,225 km²) and the Nam Dong area (about 648 km²). Abbreviations are PF = primary forest and SF = secondary forest. One asterisk (*) is Dong Ngai Stream in Phong Dien Nature Reserve, two asterisks (**) is A Pat Stream in Sao La Conservation Area and three asterisks (***) is Thuong Nhat and Huong Loc Streams in Bach Ma National Park, central Vietnam.

Locality	Geographical coordinates	Elevation (m)	Forest
A Luoi			
Dong Ngai*	16°21'04"N, 107°14'03"E	1129	PF1
A Sap	16°09'29"N, 107°12'56"E	1292	PF2
A Pat**	16°07'13"N, 107°29'20"E	1026	PF3
Huong Phong	16°11'34"N, 107°22'23"E	207	SF1
Hong Ha	16°16'09"N, 107°19'13"E	375	SF2
Hong Thuy	16°19'42"N, 107°04'07"E	479	SF3
Nam Dong			
Huong Son	16°10'43"N, 107°43'32"E	587	PF4
Thuong Quang	16°05'21"N, 107°32'03"E	929	PF5
Thuong Nhat***	16°01'09"N, 107°41'55"E	709	PF6
Thuong Lo	16°07'38"N, 107°43'31"E	335	SF4
Huong Phu	16°13'57"N, 107°43'19"E	339	SF5
Huong Loc***	16°08'54"N, 107°50'24"E	297	SF6

APPENDIX 2. List of amphibian species (100 total) observed in the tropical rain forests of central Vietnam from 2008 to 2010 and 2015 to 2017. Abbreviations are AL = A Luoi area (89 species), ND = Nam Dong area (84 species), PF = primary forest (92 species), SF = secondary forest (37 species), IUCN = International Union for Conservation of Nature, NT = Near Threatened, VU = vulnerable, and EN = endangered.

Scientific name	Common name	AL	ND	PF	SF	Total region	IUCN
Order ANURA							
Family Bufonidae							
<i>Bufo cryptotympanicus</i>	Earless Toad	7	10	17	-	17	NT
<i>Bufo pageoti</i>	Tonkin Toad	8	7	15	-	15	NT
<i>Duttaphrynus melanostictus</i>	Doubtful Toad	264	251	4	511	515	
<i>Ingerophrynus galeatus</i>	Gamboja Toad	1	-	1	-	1	
<i>Ingerophrynus macrotis</i>	Big-eared Toad	1	-	1	-	1	
Family Hylidae							
<i>Hyla annectans</i>	Assam Treefrog	7	6	13	-	13	
<i>Hyla simplex</i>	Annam Treefrog	2	3	5	-	5	
Family Megophryidae							
<i>Leptobrachium banae</i>	Spadefoot Toad	73	11	84	-	84	VU
<i>Leptobrachium chapaense</i>	Chapa Spadefoot Toad	14	60	74	-	74	
<i>Leptobrachium mouhoti</i>	No common name	2	-	2	-	2	
<i>Leptobrachium pullum</i>	Vietnam Spadefoot Toad	2	2	4	-	4	
<i>Leptobrachium xanthospilum</i>	No common name	1	1	2	-	2	

APPENDIX 2 (continued). List of amphibian species (100 total) observed in the tropical rain forests of central Vietnam from 2008 to 2010 and 2015 to 2017. Abbreviations are AL = A Luoi area (89 species), ND = Nam Dong area (84 species), PF = primary forest (92 species), SF = secondary forest (37 species), IUCN = International Union for Conservation of Nature, NT = Near Threatened, VU = vulnerable, and EN = endangered.

Scientific name	Common name	AL	ND	PF	SF	Total region	IUCN
<i>Leptobranchella pelodytoides</i>	Thao Asian Toad	52	23	75	-	75	
<i>Leptobranchella tuberosa</i>	Granular Toad	26	46	72	-	72	VU
<i>Megophrys gerti</i>	Gert's Mountain Toad	34	99	123	10	133	
<i>Megophrys hansii</i>	No common name	60	61	117	4	121	
<i>Megophrys intermedia</i>	Annam Spadefoot Toad	1	-	1	-	1	VU
<i>Megophrys major</i>	Anderson's Spadefoot Toad	602	424	1006	20	1026	
<i>Megophrys microstoma</i>	Asian Mountain Toad	33	99	125	7	132	
<i>Megophrys pachyproctus</i>	Zhushihe Mountain Toad	14	5	19	-	19	
<i>Zhangixalus pachyproctus</i>	No common name	55	23	78	-	78	
Family Microhylidae							
<i>Glyphoglossus guttulatus</i>	Burmese Squat Frog	107	150	4	253	257	
<i>Glyphoglossus yunnanensis</i>	Yunnan Squat Frog	11	8	-	19	19	
<i>Kaloula baleata</i>	Muller's Narrowmouth Toad	6	16	-	22	22	
<i>Kaloula pulchra</i>	Beautiful Kaloula	306	182	-	488	488	
<i>Microhyla annamensis</i>	Vietnam Rice Frog	2	1	3	-	3	VU
<i>Microhyla berdmorei</i>	Pegu Rice Frog	15	8	21	2	23	
<i>Microhyla butleri</i>	Butler's Rice Frog	145	90	3	232	235	
<i>Micryletta erythropoda</i>	Mada Paddy Frog	13	4	-	17	17	
<i>Microhyla fissipes</i>	Ornamented Pygmy Frog	163	180	9	334	343	
<i>Microhyla heymonsi</i>	Taiwan Rice Frog	170	112	5	277	282	
<i>Microhyla marmorata</i>	Marble Pigmy Frog	3	1	4	-	4	
<i>Microhyla nanapollexa</i>	No-thumb Pigmy Frog	-	3	3	-	3	
<i>Microhyla picta</i>	Painted Rice Frog	1	1	2	-	2	
<i>Microhyla pulchra</i>	Guangdong Rice Frog	144	133	5	272	277	
<i>Micryletta inornata</i>	Deli Paddy Frog	-	2	2	-	2	
Family Dicroglossidae							
<i>Euphlyctis cyanophlyctis</i>	Studded Frog	12	6	-	18	18	
<i>Fejervarya limnocharis</i>	Indian Cricket Frog	218	70	12	276	288	
<i>Hoplobatrachus rugulosus</i>	Asian Peters Frog	204	69	-	273	273	
<i>Limnonectes khammonensis</i>	Khammon Wart Frog	12	2	14	-	14	
<i>Limnonectes kuhlii</i>	Kuhl's Wart Frog	96	77	3	170	173	
<i>Limnonectes limborgi</i>	Taylor's Frog	33	38	71	-	71	
<i>Limnonectes poilani</i>	Poilane's Frog	554	370	924	-	924	NT
<i>Nanorana aenea</i>	Doi Chang Asian Frog	9	4	13	-	13	
<i>Nanorana yunnanensis</i>	Yunnan Paa Frog	10	5	15	-	15	EN
<i>Occidozyga lima</i>	Lurid Houlema	144	144	18	270	288	
<i>Occidozyga martensii</i>	Marten's Oriental Frog	111	57	7	161	168	
<i>Occidozyga vittata</i>	Degen's Toad	18	-	-	18	18	
<i>Quasipaa spinosa</i>	Chinese Spiny Frog	-	1	1	-	1	VU
<i>Quasipaa verrucospinosa</i>	Granular Spiny Frog	168	385	553	-	553	NT

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APPENDIX 2 (continued). List of amphibian species (100 total) observed in the tropical rain forests of central Vietnam from 2008 to 2010 and 2015 to 2017. Abbreviations are AL = A Luoi area (89 species), ND = Nam Dong area (84 species), PF = primary forest (92 species), SF = secondary forest (37 species), IUCN = International Union for Conservation of Nature, NT = Near Threatened, VU = vulnerable, and EN = endangered.

Scientific name	Common name	AL	ND	PF	SF	Total region	IUCN
Family Ranidae							
<i>Amolops compotrix</i>	No common name	3	3	6	-	6	
<i>Amolops cremnobatus</i>	Lao Sucker Frog	49	18	67	-	67	NT
<i>Amolops cucae</i>	No common name	289	539	828	-	828	EN
<i>Amolops ricketti</i>	Chinese Sucker Frog	20	78	80	18	98	
<i>Amolops spinapectoralis</i>	Spinyback Torrent Frog	3	1	4	-	4	
<i>Hylarana attigua</i>	Similar Frog	42	22	64	-	64	VU
<i>Hylarana erythraea</i>	Red-eared Frog	15	4	3	16	19	
<i>Hylarana lateralis</i>	Kokarit Frog	-	10	10	-	10	
<i>Hylarana macrodactyla</i>	Guangdong Frog	111	38	14	135	149	
<i>Hylarana taipehensis</i>	Taipei Frog	10	-	10	-	10	
<i>Indosylvirana milleti</i>	Dalat Frog	-	6	6	-	6	
<i>Nidirana adenopleura</i>	Olive Frog	118	53	-	171	171	
<i>Nidirana chapaensis</i>	Chapa Frog	9	3	12	-	12	
<i>Odorrana absita</i>	No common name	22	14	36	-	36	
<i>Odorrana andersonii</i>	Golden Crossband Frog	30	14	44	-	44	
<i>Odorrana banaorum</i>	No common name	7	2	9	-	9	
<i>Odorrana chloronota</i>	Chloronate Huia Frog	738	297	1011	24	1035	
<i>Odorrana graminea</i>	Large Odorous Frog	198	355	536	17	553	
<i>Odorrana junlianensis</i>	Junlian Odorous Frog	18	16	34	-	34	VU
<i>Odorrana khalam</i>	No common name	5	3	8	-	8	
<i>Odorrana morafkai</i>	Morafkai Frog	752	274	1020	6	1026	
<i>Odorrana nasica</i>	Tonkin Huia Frog	20	15	35	-	35	
<i>Odorrana orba</i>	No common name	7	-	7	-	7	
<i>Odorrana tiannanensis</i>	Dawei Frog	37	-	37	-	37	
<i>Odorrana yentuensis</i>	No common name	1	-	1	-	1	EN
<i>Pulchrana glandulosa</i>	Sarawak Frog	11	-	11	-	11	
<i>Rana johnsi</i>	Johns's Frog	28	15	41	2	43	
<i>Sylvirana guentheri</i>	Gunther's Amoy Frog	142	124	11	255	266	
<i>Sylvirana maosonensis</i>	Mao-Son Frog	35	25	57	3	60	
<i>Sylvirana nigrovittata</i>	Black-striped Frog	41	-	37	4	41	
Family Rhacophoridae							
<i>Chirixalus nongkhorensis</i>	Nongkhor Asian Treefrog	-	4	4	-	4	
<i>Feihyla vittata</i>	Degen's Toad	9	22	30	1	31	
<i>Gracixalus supercornutus</i>	Tiny Bubble-nest Frog	-	1	1	-	1	NT
<i>Kurixalus appendiculatus</i>	Philippine Flying Frog	4	-	4	-	4	
<i>Kurixalus banaensis</i>	Bana Bubble-nest Frog	4	-	4	-	4	
<i>Kurixalus verrucosus</i>	Boulenger's Bushfrog	63	21	84	-	84	
<i>Polypedates megacephalus</i>	Hong Kong Whipping Frog	187	70	6	251	257	
<i>Polypedates mutus</i>	Burmese Whipping Frog	-	2	2	-	2	

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Scientific name	Common name	AL	ND	PF	SF	Total region	IUCN
<i>Raorchestes gryllus</i>	Langbian Bubble-nest Frog	19	25	44	-	44	VU
<i>Raorchestes parvulus</i>	Karin Bubble-nest Frog	2	-	2	-	2	
<i>Rhacophorus annamensis</i>	Annam Flying Frog	629	402	1028	3	1031	VU
<i>Rhacophorus bipunctatus</i>	Himalaya Flying Frog	-	1	1	-	1	
<i>Rhacophorus exechopygus</i>	Spinybottom Tree Frog	-	5	5	-	5	VU
<i>Rhacophorus kio</i>	Black-webbed Treefrog	4	-	4	-	4	VU
<i>Rhacophorus orlovi</i>	Orlov Frog	32	18	50	-	50	
<i>Rhacophorus rhodopus</i>	Red-webbed Treefrog	-	4	4	-	4	
<i>Rhacophorus robertingeri</i>	No common name	6	42	48	-	48	NT
<i>Theloderma asperum</i>	Hill Garden Bug-eyed Frog	1	-	1	-	1	
<i>Theloderma truongsongense</i>	No common name	40	17	57	-	57	
Order GYMNOPIHIONA							
Family Ichthyophiidae							
<i>Ichthyophis bannanicus</i>	Mengla County Caecilian	10	11	4	17	21	
Total number of individuals:		7,705	5,824	8,952	4,577	13,529	21

APPENDIX 3. The fitting of a truncated lognormal distribution to the species abundance data of amphibians and estimation of the needed parameters is described in the text, which also gives the column headings: (1) Class upper boundary; (2) results of logarithm with base 10 from column 1; (3) standardized form of upper boundary was used to calculate normal probability integrals for column 4; (4) cumulative number of expected species; (5) cumulative expected without so called unseen species; (6) cumulative number of observed species; (7) containing values of $F_{0.5}$ for a Kolmogorov-Smirnov goodness of fit test; and (8) the absolute value of the differences between (5) and (7) columns. The bold value is $g_{\max,0.5}$ used to obtain the Kolmogorov-Smirnov test statistic [$D = (\text{largest difference} + 0.5)/S$]: $\bar{x} = 1.427$; $\sigma^2 = 0.749$; $\gamma = 0.251$; $\theta = 0.052$; $\mu_x = 1.337$; $V_x = 0.904$; $\sqrt{V_x} = 0.951$; $z_o = -1.723$; $p_o = 0.045$; $S^* = 105.33$; $S = 100$; $N = 13,529$.

Class	(1)	(2)	(3)	(4)	Frequency		(7)	(8)
					Expected	Observed		
1	0.5	-0.30103	-1.72265	4.48	4.48	0	--	--
2	1.5	0.17609	-1.22096	11.71	7.23	9	8.5	1.267
3	10.5	1.02119	-0.33235	39.01	34.53	34	33.5	1.028
4	100.5	2.00217	0.69914	79.93	75.45	74	73.5	1.949
5	1000.5	3.00022	1.74857	101.24	96.76	97	96.5	0.262
6	10,000.5	4.00002	2.79986	105.21	100.73	100	100.5	0.230
7	∞	∞	∞	105.33	100.98	100	100.5	0.480