

SPECIES DIVERSITY AND HABITAT PREFERENCES OF AMPHIBIAN FAUNA IN GULMI DISTRICT, NEPAL

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ABSTRACT

The study was conducted to measure species diversity and habitat preference of amphibian fauna in Gulmi district, Nepal, from March 2016 to July 2018. The objectives of the study were to: (i) examine the richness and abundance of amphibian species in Gulmi district (ii) evaluate amphibian inventory of species and diversity (iii) compare the assemblage composition of different habitats. Data were collected by quadrate and pitfall sampling method. In total, 674 individual amphibian species belonging to 4 families, 9 genera and 12 species were recorded. The richness of amphibian species in riparian habitat (S= 11) was higher in number compared to the richness of the forest (S=3). Shannon-Weiner diversity index (H') was 1.842 in agricultural field and 2.012 in riparian, whereas Simpson's diversity index (λ) were 0.789, 0.815 in agricultural field and riparian habitat, but lower (0.613) in forest. Margalef's, Menhinick's and Brillouin index (HB) diversity indices salso showed higher diversity in riparian and lower in forest. Comparison of relative abundance and equitability index/Pielou evenness (J) of individuals among the species showed that species diversity of agricultural field (0.359) and riparian (0.336) habitat were abundant and numerically similar to the other habitats. Among habitats, abundance was greatest in the riparian with 286 (42.43%) followed by agricultural fields with 203 (30.11%) individual amphibians. The lowest amphibian abundance was documented in forest with 21 (3.11%) individuals. The presence of exclusive species in forest and wetland reinforces the importance of preserving different habitats for the maintenance of richness and diversity of local species.

KEYWORDS: Species Richness, Species Abundance, Diversity, Diversity Index & Habitats

Received: Nov 09, 2019; Accepted: Nov 29, 2019; Published: Jan 21, 2020; Paper Id.: IJZRJUN20201

INTRODUCTION

Amphibians are paired limbs (except caecilians) and naked skin cold blooded vertebrates. There are currently 8,081 amphibian species, of which 7,131 are frogs and toads, 737 are newts and salamanders, and 213 are caecilians (Frost, 2019). They mainly live in aquatic medium and show a great variety of climatic and ecological zones (Hall and Henry, 1992). Distribution of animals depends on the variations in temperature, rainfall, soil quality, hydrological conditions etc. The main habitat of amphibians includes terrestrial arias like agricultural fields, forest, grasslands, alpine meadows, trees, tunnels, cliffs, rocks, burrowing etc. whereas the aquatic form like rivers, lakes, oxbow lakes, ponds, wetlands, and even houses as macro-habitat and micro-habitat. These diverse habitat types support a significant number of amphibians. They live in both terrestrial and aquatic environments (Schneider et al. 2001; McCallum 2007; Pokhrel & Thakur, 2017).

It is well known that species are not homogeneously distributed in the world. The geographic condition such as altitude slope, latitude, soil condition etc. and climatic condition like temperature, light condition, precipitation humidity etc. determines the richness of species. The amphibians of Nepal have a wide range of both

vertical and horizontal distributions. However, the study of these species is prioritized less than the other vertebrates (Bhattarai et al., 2017). The study on amphibians is still in infancy, their distribution and current status of population is poorly known in Nepal (CEPF, 2005). Historically first zoogeographical analysis of Nepalese herpetofauna was introduced by Swan and Leviton (1962). Foreigners and Nepalese researchers such as Fleming and Fleming (1974), Shah and Giri (1991), Schleich (1993), Shah (1995), Shrestha (2001), Schleich and Kastle (2002) etc. are interested in and thus, have contributed to the field of herpetology. The exact number of amphibian species in Nepal is still unknown. However, a total of 43 species (Shah, 1995), 61 species (Shrestha, 2001), 50 species (Schleich and Kastle (2002), 59 species (Shah and Tiwari (2004) of amphibians have been reported from Nepal. However, the distribution and abundance of herpetofauna are poorly known in Nepal.

Recent works on amphibians have reported 117 species of Nepal (Poudel et al., 2011), over 50 species (Schleich & Rai, 2012) and 53 species of amphibians (Bhattarai et al. 2017). Some researchers studied amphibians and reptiles regionally, according to which, 8 species of amphibians are found to be representing three families (Duttaphrynus, Ranidae and Dicroglossidae) in Manasolu conservation area (Shrestha & Shah, 2017), 11 amphibian species in Mustang (resource mapping report–2014), 12 species in Parsa (Bhattarai, 2017), 3 species in Nagarjun forest (Pokhrel, et al., 2011), 29 species in eastern Nepal Himalaya (Khatiwada, et al., 2019) etc.

Very few species have been described from disturbed habitats, indicating a diminished species composition when compared with the original habitat (Molur, 2008). The basic idea of a diversity index is to obtain a quantitative estimate of biological variability that can be used to compare biological entities, composed of discrete components, in space or in time (Help et al., 1998). The quality of amphibian habitat is influenced by the amount and type of vegetation in the water body, wetland or stream and surrounding terrestrial habitat. Besides, the hydro-period, water quality, the presence of predators and competitors, the prevalence of diseases and the nature and frequency of human disturbances influence the quality of amphibian habitat (Hamer & McDonnell, 2008).

The distribution of biodiversity is influenced by altitudinal pattern on species richness and diversity (McCain, 2007). Because of their unique life cycles, amphibians often require both aquatic and terrestrial habitats. They can be found in almost all habitat types like wetland, riparian, agricultural field forest as well as human habitat. Their population distribution is determined by habitat features (Balaji et al., 2014). The distribution, abundance and rich patterns of organisms determined by altitudinal gradients cause variation in climatic, biological and the physical environment as the prime factors (Lomolino, 2001; Whittaker et al., 2001).

Variation in sampling intensity in space can result in incorrect species richness and distribution measures. Higher sampling intensity results in a higher probability of species detection (Elphick, 1997, Williams et al. 2002). Well-sampled areas appear to be richer in species than poorly sampled areas (Reddy & Da valos, 2003).

They are especially sensitive to ecosystem changes due to direct and constant contact with their environment. They utilize both terrestrial and aquatic environments which plays a role in healthy ecosystem (Schneider et al. 2001; McCallum 2007, and Leduc, 2012). All adult amphibians act as consumers and predators for many harmful insects and other pests. They are essential components of their food webs and their larvae mainly feed on algae and bits of dead animals in water, diatoms, planktons or other small organisms. Thus, they play an important role in biological pest control and healthy ecosystem.

The objectives of the study were to (i) examine amphibian species richness abundance in Gulmi districts (ii) evaluate amphibian list of species and diversity (iii) compare the assemblage composition of different habitats types: forest, agricultural field, riparian, human habitat and wetland. The purpose of this study was to improve our understanding of how amphibians respond at the species-specific level and (sub) order levels to habitat characteristics associated with different habitat. Thus, this study aimed at contributing to a better understanding of the current situation of amphibians in a severely transformed region of high biological relevance, and to help determine the importance to the conservation of biodiversity at the regional level. Site specific information on amphibian diversity and their habitat preference was also very few. Keeping this in mind, the present study was conducted on amphibian diversity and their habitat preferences.

MATERIALS AND METHODS

Study Area

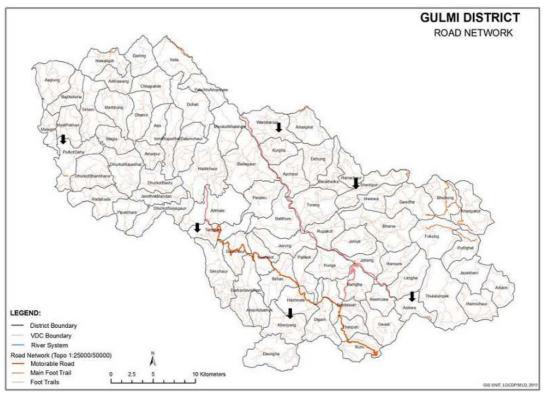


Figure 1: Gulmi District Showing different Sampling Stations in Study Area.

This quintessence in terms of the area of this research was Gulmi district, (Lati: 270 55' N to 280 27' N, Long: 830 10' E to 830 35' E). It is located in Province 5, Nepal. It covers an area of 1149 km2 and altitude ranges from 465 m to 2690 m (District profile, 2007). This district is unique in geographically, culturally as well as plant and animal diversity. It has great altitudinal and climatic variation. Bimonthly surveys were conducted during field research period from March 2016 to July 2018 and sampling effect was kept constant for each habitat. The amphibian abundance was determined using quadrate visual encounter surveys (Veith et al., 2004; Phochayavanich et al., 2010) on 20×20m by assistant worker for 30 minute per quadrate, pitfall, random search etc. along with forest, riparian, wetland, agricultural field and human habitat. Quadrates were selected systematically in such a way that equal number of quadrates were laid in all the habitats and 20 quadrates in each habitat in each station.

Six study sites were chosen that covered and represented whole district's geography, vegetation and climatic

condition. Sampling stations were Tamghs-Resunga (Resunga municipality) (280 01'.655' N & 830 11' 185" E), Purkoot-Mahelpokhari (Madane rural municipality)(280 10' 355" N & 830 03' 395" E), Kharjeng-Mankot (Chatrakot rural municipality) (270 57'. 995' N & 830 20' .965' E), Aslewa (Satyawati rural municipality) (270 58' .15' N & 830 28' .085' E, 500 to 999 m), Santipur (Chandrakot rural municipality) (28006' 50. 78" N & 830 24' 46.83" E, 1054 to 1938 m, and Wami (Musikot rural municipality) (28010'40. 98" N & 83016' 57. 06" E, 724 to 1029 m).

Data Collection

This study design included 6 sites or stations. Each sampling site was split into five sub-habitat sites in which 20 cell quadrates were sampled in each sub-habitat and altogether 100 quadrates in each site. The habitat type was first represented by forest that included community forest, governmental forest and personal forest. The elements of forest included unprotected clusters of trees of various size and quality of native forest fragments, bushes and shrubs. The second habitat types were agricultural fields that included cultivated maize, rice, wheat, seasonal and off-seasonal vegetables, pulses etc. Third type of habitat included the riparian type. The fourth habitat type sampled was human habitat including urban, small markets and rural areas, where the human population was dense. The fifth sampled area was wetland including small ponds, lake, marshy, and wet area. The pitfall bucket traps were conducted in riparian and side of wetland habitat. The pitfalls were checked each in morning and evening for captured animals.

The essential information such as date, time, longitude and latitude (obtained by GPS), altitude, microhabitat, and circumstances of capture were recorded at the time of capture or observation. As a further aid to taxonomic identification, the acoustic repertoire of some amphibians was recorded.

Data Analysis

The researcher performed all the statistical tests, and built all the figures, using the software R package 3.6.1 in R Studio v.3.1.0 (R Development Core Team 2013). Microsoft Excel 2013 and SPSS software IBM 23 version were also used to build figures. Species richness (the number of species) and abundance (number of individuals) of each taxon was estimated by using software R package 3.6.1. Data was tested by using Menhinick's index (D=nN- \sqrt{N}), Margalef's index (D=n-1lnN). The Shannon-Wiener Index [H' = - \sum (pi ln pi)] was used to determine the diversity of species heterogeneity (where, H' = species diversity, and pi = proportional frequency of the ith species). Simpson's Index (λ) λ DS= \sum ni (ni-1N(N-1)=1- \sum ni(ni-1N(N-1)) and Pilou evenness (J) (J=H'Hmax=H'ln S) were tested in R package.

Nomenclature and Taxonomy

The species were identified in the field using diagnostic keys given by various books and literatures of Smith (1981), Dixon (2000), Schleich and Kaestle, (2002), and Rai (2003). Amphibian specimens were identified with the help of the literature; Bossuyt and Dubois (2001), and Dutta and Manamendra-Arachchi (1996). The species encountered were identified by using field guides and color photographs (Shah & Tiwari, 2004).

RESULTS AND DISCUSSIONS

Species Richness

A total of 12 species of amphibians representing 9 genera in 4 families were recorded. More number of species from the family Ranidae (8 species) followed by Bufonidae (2 species), Microhylidae (1 species), and Rhacophoridae (1 species) were recorded (Table 1). The species richness of amphibians was greater in riparian habitat (S= 11). The other species

found in the habitat of agricultural field (S=9), human habitat (S=7) and wetland (S=7) were lesser in number compared to the number of riparian habitat, whereas the forest was the least in having just three species (Table 2). Shrestha (2001) reported that one species of Salamander, one species of Caecilians, 59 species of toad and frogs and Schleich & Rai (2012) reported 50 species of amphibians in which 1 species of Salamander, 1 species of Caecilians, 48 species of toad and frogs in Nepal.

Table 1: Amphibian Species in Different Habitat of Gulmi District

Family	Scientific Name	Common Name	Habitat Forest	Agriculture	Riparian	Human Habitat	Wetland	Total	%
Bufonidae	Bufo melanostictus (Schneider, 1799)	Common Asian toad	9	19	21	30	4	83	12.17
	Bufo stomaticus (Luken, 1862)	Marbled toad	3	16	28	28	7	82	12.02
Microhylidae	Microhyla ornate (Dumeril & Bibron, 1841)	Ornate narrow mouthed frog	0	5	6	0	0	11	1.61
Ranidae	Amolops marmoratus (Blyth, 1855)	Marbled cascade frog	0	0	10	0	0	10	1.47
	Euphlyctis cyanophlyctis (Schneider, 1799)	Skittering frog	0	78	102	17	51	248	36.37
	Hoplobatrachus crassus (Jerdon, 1853)	Jerdon's bull frog	0	12	18	0	5	35	5.14
	Hoplobatrachus tigerinus (Daudin, 1802)	Indian bull frog	0	4	17	0	6	27	3.96
	Paa liebegii (Gunther, 1860)	Liebig's paa frog	0	0	4	0		4	0.59
	Sphaerotheca maskeyei (Schleich and Andres, 1998)	Maskey's burrowing frog	0	12	16	0	0	28	4.11
	Zakerana nepalensis (Dubois, 1975)	Crikate frog	0	26	17	0	6	49	7.18
	Zakerana teraienesis(Dubois, 1975)	Crikate frog	0	31	47	1	9	88	12.90
Rhacophoridae	Polypedates leucomystax (Gravenhorst, 1829)	Java whipping frog	9	0	0	0	0	9	1.32
Total			21	203	286	76	88	674	
%			3.116	30.1186	42.449	11.2759	13.0563	100	

Species Abundance and Composition in different habitats

During field visits, a total of 674 individual amphibians were recorded. Skittering frog (Euphlyctiscyanophlyctis) preferred aquatic habitat being most abundant followed by Cricket frogs (Zakeranateraienesisand Zakerananepalensis) that were found in riparian habitat. Jerdon's bull frog (Hoplobatrachuscrassus) and Indian bull frog (Hoplobatrachustigerinus) showed aquatic habitats which were relatively less abundant in study area. The common Indian toad (Bufomelanostictus) and marbled balloon frog (Bufostomaticus) were found throughout the entire area: human settlement areas, agricultural land and forest areas. The least abundant species were Liebig's Paa frog (Paaliebegi) was recorded from small streams. Java whipping frog (Polypedatesleucomystax) was found in arboreal form in the forest. Maskey's burrowing frog (Sphaerothecamaskeyei) was found in burrowing form, while marbled cascade frogs (Amolopsmarmoratus) were recorded from small streams. However, ornate narrow mouthed frogs (Microhyla ornate) were found only in the forest and rocky areas (fig. 2). Among habitats, abundance was greatest in the riparian with 286 (42.43%) followed by agricultural fields with 203(30.11%) individual amphibians. The lowest amphibian abundance was documented in forest with 21(3.11%) individuals (Table 1). During study period, Bufomelanostictus and Bufostomaticus were found in all habitats. Amolopsmarmoratus, Paaliebegi and Polypedatesleucomystax were found in single habitats of riparian and forest; respectively (Figure 2). Similar results were reported by Pokharel and Thakuri (2010). The amphibians were all over due to the presence of ponds (breeding sites) and abundant food as the main reasons of encountering their higher species richness in the grassland habitats. A study by Brothertonet al. (2004) found that most amphibians migrated from wetland habitat to bogs, open ponds, road sides, grassland and woodland for breeding, foraging and hibernation.

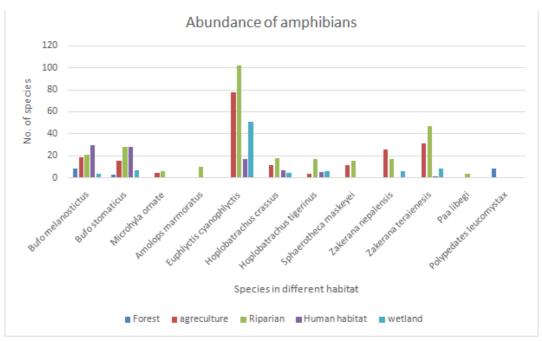


Figure 2: Abundance of Amphibian in Gulmi District.

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S. No.	Diversity Index	Forest	Agriculture Field	Riparian	Human Habitat	Wetland						
1	Species richness (S)	3	9	11	6	7						
2	Menhinick's index	0.65	0.63	0.65	0.64	0.75						
3	Margalef's index	0.66	1.51	1.77	1.12	1.34						
4	Rarefaction (Rarefy)	2.88	5.10	5.50	4.02	4.17						
5	Brillouin index (HB)	0.86	Inf	Inf	1.36	1.30						
6	Species abundance	21	203	286	88	88						
7	Shannon-Wiener Index (H')	1.01	1.84	2.01	1.46	1.42						
8	Simpson's Index (λ)	0.61	0.79	0.82	0.74	0.63						
9	Pilou evenness (<i>J</i>)	0.56	0.36	0.34	0.41	0.33						
10	Hill's ratios (Ea:b)	0.61	0.25	0.21	0.35	0.27						
11	Effective diversity no.	2.73	6.31	7.45	4.32	4.13						

Table 2: Diversity Index of Amphibian at Gulmi

Species Diversity

Biodiversity indices were calculated to investigate the differences in amphibian diversity and abundance among different habitats (Table 2). Simpson index ranges from 0 to 1. Mature and stable communities have high diversity value (0.6 to 0.9), while the communities under stress conditions, exhibiting low diversity, usually show close to zero value (Dash, 2003). The Simpson diversity index was 0.612, 0.789, .814, 0.736 and 0.633 in different habitats of forest, agricultural field, riparian, human habitat and wetland; respectively. Shannon–Wiener, Simpson, Margalef's diversity index and Menhinick's index had the similar results. A higher Simpson's index as well as a higher Shannon-Wiener's index also indicated great species evenness in the riparian and agricultural areas. The index value (H) was 1.842 in agricultural field and 2.013in riparian, which were more than 1.50. This indicates the higher diversity, but values of diversity index of forest, human habitat and wetlands were 1.0042 and 1.464 and 1.420; respectively less than 1.50. So, these habitats showed less diversity (Table 2). Shannon-Weiner and Simpson diversities increase as richness increase for a given pattern of evenness, and increase as evenness increases for a given richness (Shah & Pandit, 2013). Primary and secondary riparian habitat presented the highest values of species diversity. The reproductive resources played a significant role in accumulation of

amphibian species. The mountain streams with intermittent small pools in Bilsa Biological Station (BBS) provided the only suitable breeding habitat and contributed to the high abundance and diversity of amphibians species (Jongsma, et al. 2014). Margalef index value was (> 1.50) in habitat of riparian (1.768) and agricultural field (1.506) that showed the higher species diversity, but in other habitats, values were less than 1.50, thus, the lesser diversity.

The Brillouin index (HB) is similar to that of Shannon, and the use of this index instead of Shannon is recommended when randomness of a sample cannot be guaranteed (Magurran 1988). Brillouin index value (B > 1.20) clearly showed high species diversity in all habitats except forest. Equitability (J) measures the evenness. Pielou evenness (J) constrained between 0 and 1.0 and the more variation in abundances between different taxa within the community, the lower the J. It depended on sample size and was also highly sensitive to rare taxa. Pielou evenness was 0.557, 0.359, 0.339, 0.410 and 0.326 in the habitats of forest, agricultural field, riparian, human habitat and wetland; respectively. Comparison of relative abundance and equitability index (J) of individuals among the species showed that species diversity of agricultural field and riparian habitats were more abundant and numerically more equal than other areas. The exponential of the Shannon's diversity index was computed to get the effective diversity of species. The Shannon diversity index (H') and the effective diversity of species (eH') were comparatively higher in agricultural field (6.313) and riparian areas (7.484) (Table 2). The diversity indices as Simpson index (1-D > 0.80), Shannon-Weiner (H > 2.20), and Brillouin index (B > 2.0), found the areas with high diversity. The relative abundance, equitability index (J > 0.80) and evenness (e^H/S) > 0.70) of individuals among the species show that species diversity were more abundant (Gixhari, et al., 2016).

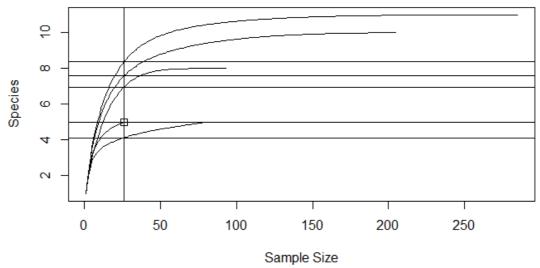


Figure 3: Species Accumulation Curves.

Rarefaction curve was made with a plot of the number of species as a function of the number of samples. The curves were steep sloppy at first and became flatter to the right. More equal abundances between species resulted in a steeper rarefaction curve and it quickly reached an asymptotic level. More intensive samplings were likely to yield only few additional species (Figure.3). The rarefaction curves showed an increase in expected functional diversity (Ricotta, et al. 2012). With the same sampling efforts, riparian habitat is more diverse than other habitats based on the rarefaction curve.

The low forest diversity as indicated by Shannon, Margalef and Menhinick indices, results from the lesser number of species and environmental degradation due to anthropogenic pressures and other biotic factors (Ravera, 2001). Abiotic

factors such as temperature, humidity, elevation, distance from water and/or habitat type influenced species richness and abundance (Santori & McManus, 2014).

The distribution pattern of species richness and diversity on amphibians were the highest in riparian and the lowest in forest. Species of Euphlyctis and Zakerana lived in a mutual association (Manamendra-Arachchi and Pethiyagoda 2006) and this mutual association was clearly observed in the study areas. Canopy cover was the most important predictor for both amphibian richness and abundance (Balaji, et al., 2014). Amphibians were considered generally good indicator species due to their sensitivity to habitat alteration (Pollet & Bendell-Young, 2000). Riparian and agricultural field had similar amphibian species richness and higher diversity. The human populated areas, human disturbance area i.e. forest and wetlands showed considerable declines in richness and diversity. According to Jorgensen et al., (2005), when the disturbances increase, the diversity decreases.

The presence of predators and competitors particularly non-native species, in water bodies, wetlands and streams often resulted in a decrease in diversity of amphibians and richness (Hamer and McDonnell, 2008). However, this study showed their higher richness and diversity in riparian and agricultural field because of their temporary breeding pools and less disturbed habitats.

CONCLUSIONS

The study showed the record of a total of 674 individual species, 12 species representing 9 genera in 4 families of amphibians. The higher species richness and abundance of amphibians were found in riparian habitats and relatively lower in forest habitat. *Euphlyctiscyanophlyctis* was the most abundant in the study area and less abundant species was *Paaliebegi*. Family Ranidae (8 species), Bufinidae (2 species), Microhylidae (1 species), and Rhacophoridae (1 species) were recorded. Among habitats, abundance was greatest in the riparian with 282 (42.43%) followed by agricultural fields with 203(30.11%) individual amphibians. The lowest amphibian abundance was found in forest with 21(3.116) individuals. The study reflects that this area has a greater potential and needs further exploration.

Simpson index, Shannon-Weiner, Brillouin index were in riparian and agricultural field therefore; diversity were more in those habitats. These habitats provided more food and space for reproduction. These indexes were lesser in human habitat and forest so there was less diversity. Human disturbance, pasture areas and less availability of food caused the less diversity. Margalef and Menhinick indices were dependent on the number of species. The information depicted by majority of diversity indices was used to determine habitat qualities, quantitative analysis (species richness, evenness) and their interactions with the biotic and abiotic factors prevailing in the area. Many diversity indices had high diversity values for stable communities, while unstable ones had low values due to environmental degradation.

ACKNOWLEDGMENT

This research was supported by University Grant Commission, Nepal. It is our humble pleasure to acknowledge, deep senses of gratitude to Prof. Karan Bahadur Shah for identification of species, preservation technique and guidance and Mr. MuktaBahadur Nepali and Neelam Nepal: Lecturers Tribhuvan Multiple Campus, Palpa for editing language. We would express our gratitude and respect to Mr. Dhak Bahadur Thapa, Thakur Prasad Poudel, Deepak Khanal and Kamal Gautam for their help in the field.

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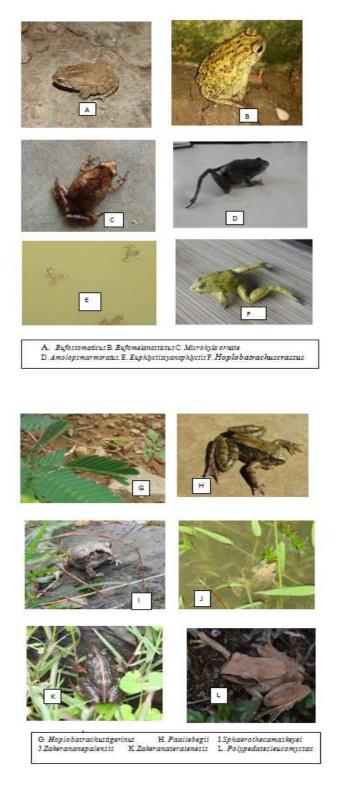
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APPENDIX 1

Photos



AUTHORS PROFILE



Mr. Pit Bahadur Nepali, Lecturer in Tribhuvan Multiple Campus, Palpa. PhD Scholar of Central Department of Zoology, Tribhuvan University (TU), Nepal (Title: Species diversity and habitat preference in Lumbini region, Nepal). MSc. in Zoology, specialization in ecology (TU). Mini Research: Ethno Medicine of Gaine people submitted to University Grand Commission- 2004, Nepal. published Articles: Status of herpetofauna in Rupandehi and Arghakhanchi districts, Nepal, J. Nat. Hist. Mus. Vol. 30, 2016-18, 221-233; Ethnography of Gaineat Arghakhanchi district published by DNF in monthly publication MuktikoAawaj, 2058, Ashad (3): 5; Management of insect pest in Arghakhanchi district in PragykPrabaha 2068, Nepal PradhyapakSangh, TUTA, T. M. Campus Unit Committee; A taxonomic study of *Ichthyophis*; legless amohibian in PragykPrabaha 2069, Nepal PradhyapakSangh, TUTA, T. M. Campus Unit Committee; Physio-chemical characteristics of fresh water (Tinahu river) in Palpa district in Scientia journal 2014 Institute of Science and Technology, T. M. Campus, Palpa; Identification of various endo-parasite of human in Palpa district in Ujagaran 2070, Progressive Teacher Association, T. M. Campus, Palpa. Published Report: A manual of proposal and report writing for B.Sc. students submitted to Tribhuvan Multiple Campus, Palpa. Dissertation: Ethno-biology of Gaine People: (A case study of Arghakhanchi district).Membership: Nepal Red Cross Society, Arghakhanchi district (member) and Nepal family planning association, Palapa (Member).



Nanda Bahadur Singh, (BSc-Bio; BA-Maj. Eng; B. Ed-Eng. Ling; MSc-Zoology: ecology-gold medalist) as a full professor at the Central Department of Zoology, Tribhuvan University (T.U.), Kathmandu, Nepal for the last 24 years (since 1995 to present). He did his PhD under Japanese government fellowship (Monbukagakusho) in the field of ethnogenetics (human genetics/molecular anthropology/population genetics) and molecular ethnobiology from the University of Tokyo, Japan in 2002. Conducted his research on eight gene systems of nine genetic markers among highly isolated six Nepalese ethnic groups (Chepang, Chidimar, Gurung, Munda, Raute and Thakali). Supervision: about 60 dissertations of MSc students, specifically on ethnobiology of 45 different Nepalese ethnic groups, and five PhD scholars as well. He has published numerous articles/book reviews in national as well as international journals and magazines and a book entitled "The Endangered Raute Tribe: Ethnobiology and Biodiversity" in 1997 and a co-author of a book on" Nepal Tomorrow: Voices and Visions" edited by D. B. Gurung (https://www.vedamsbooks.com/no36418.htm). A visiting professor of ethnobiology at Yamagata University of Arts and Desgin, Yamagata in Japan (1997) as well as a visiting professor of medical genetics at the College of Medicine, Henan University, P. R. China under the bilateral agreement signed between Tribhuvan University, Nepal and Henan University, China to teach MBBS students in the academic year of Sep, 2005~ July, 2006.

Dr. Singh's abstract entitled"Ethno-molecular surgery of human resistant genes against malaria and HIV/AIDS infections among six indigenous populations in Nepal" was awarded doctoral student fellowship for the poster presentation at the 5th Human Genome Organization (HUGO) Pacific Meeting & 6th Asia-Pacific Conference on Human Genetics held in Biopolis, Singapore, Nov. 17~20, 2004. Achievements: His Majesty late King Birendra Bir Bikram Shah Dev and the then His Majesty King Gyanendra Bir Bikram Shah Dev of Nepal have conferred "MahendraVidhya Bhuvan "B" and "A" (Nepal Vidhya Bhuvan "B" and "A") class medals on Dr. Singh for his academic excellence in 1996 and 2003; respectively. The then His Royal Highness Crown Prince Paras Bir Bikram Shah Dev has handed over the "RONAST (now NAST) Talent Scientist Award 2005" for his novel innovative ethno-genetic and molecular ethnobiology research among six indigenous populations in Nepal. Membership: Secretary for the year of 2008~2009 and vice-president for the year of 2009-2010 at the Rotary Club of Jawalakhel, Kathmandu, Nepal.