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Distribution of colour and pattern polymorphism in montane frogs of Sri Lanka

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Abstract
Amphibian body colouration and pigment patterns play a vital role on their survival in the environment. Anuran polymorphism were studied in and around the five lentic water bodies of within and outside the Horton Plains National Park from January 2017 to November 2017 on three consecutive days per month. A total number of 694 amphibians, belonging to 3 families and 11 species were recorded during the study. Colour polymorphism were recorded only in *Taruga eques*, in this species six and three dorsal colours were recorded respectively within and outside the park. The most prevalent dorsal colourations of *Taruga eques* were Green and Dark brown. Distinct hour glass pattern was the highest prevalent pattern polymorphism of *Taruga eques* irrespective to the locality type. Moreover, three different dorsal patterns were recorded in *Minervarya greenii*. The present study indicated that endemic *Taruga eques* possess diverse colour and pattern polymorphism as other tree frogs in the world. Most of the recorded polymorphism in the present study were not previously recorded in *Taruga eques* and *Minervarya greenii*. Presence of dorsal pigment patterns may be a selective advantage for amphibians, specifically for tree frogs which may disrupt the expression of bright dorsal colouration for visually oriented predators. However, further field studies are warranted to understand the selective advantages of polymorphism in amphibians.

Keywords: Polymorphism, *Taruga eques*, *Minervarya greenii*, Horton plains National Park

1. Introduction
Polymorphism is defined as "arising of two or more distinct forms simultaneously in the same habitat of a species in such proportions that the rarest of them cannot be maintained by recurrent mutation'' [15].

The existence of two or more distinctly colour / pattern phenotypes among individuals of an interbreeding population is known as colour / pattern polymorphism [15], including presence or absence of colours and patterns (dorsal marks, stripes and dots). Vertebrates which are in the lower level of the hierachy express numerous body colours due to different distributions and combinations of six kinds of chromatophores. Of which, melanophores, xanthophores and iridophores have been found as main pigment cells that responsible for creating various colour mixtures of amphibian skin. Anuran polymorphism exhibit variations in body colour and dorsal pattern which are known as polymorphic amphibians.

In amphibians, particularly this phenomenon is pervasive among anurans, but rare or absent among salamanders and caecilians, respectively [15]. Many anuran species exhibit striking colour or pattern polymorphism which have been recorded in about 225 species representing 35 genera and 11 families [19]. Hartl and Jones (1998) [18], stated that four sources contribute to phenotypic variations as,

a) Genetic variation- where the phenotype of each individual is genetically determined
b) Environmental variation- where the phenotype of each individual is set by differences in environments
c) Genotype-environment variation- where the phenotype is assigned by interaction of genotype and enviroment
d) Genotype-environment association - where the phenotype is assigned by association of genotype and enviroment.

Animal colouration plays an important role in many aspects of animal ecology [23] and have important implications for animal fitness [2]. Body colouration primarily used for defense against predators and camouflage is considered as the most commonly used and wide spread defensive colouration [22]. Colouration also provides valuable information about sex, maturity

or availability for reproduction and fitness [21,41]. Colouration is significant for ectothermic animals, such as amphibians and reptiles, in which some colour anomalies may have an effect on thermoregulation [39].

Many polymorphism studies have been done worldwide related to amphibians. Some are genetic studies [1, 4-9, 27, 34]. In some studies, scientists have investigated polymorphism with the amphibian developmental stages in laboratories [20].

In addition to these laboratory studies, many field based surveys have been carried out within particular regions of countries, for the identification of the variations in colour and patterns of amphibian species [14, 23, 26, 28, 30, 36, 43].

However, no polymorphism studies on amphibians have been conducted in Sri Lanka. Therefore, it is very important to conduct an amphibian polymorphism study within and outside the Horton Plains National Park (HPNP) to fill the gap of field studies pertinent to amphibian polymorphism, in natural ecosystems of Sri Lanka.

2. Materials and Methods

2.1 Study site

The study was conducted within and outside the HPNP from January to November 2017. HPNP is located between 6°47'-6°50' northern latitudes and 80°46'- 80°50' eastern longitudes [17] which occupies an area of 3,160 ha and is contiguous with Peak Wilderness Sanctuary to the west.

2.2 Census of amphibians

The census was conducted in five lentic water bodies with equal effort to each sampling site, associated with all the habitats types located within and outside the HPNP. GPS (Global positioning system) points of these sampling sites were recorded using Garmin etrex Euro hand held GPS receiver. Sampling sites, within the HPNP (Figure 1) (Sampling site A- 6°50'20.3"N, 80°48'43.0"E, Sampling site B- 6°50'01.3"N, 80°48'32.4"E, Sampling site C- 6°48'04.2"N, 80°48'24.2"E, Sampling site D- 6°51'01.2"N 80°49'03.3"E, Sampling site E- 6°48'18.6"N 80°54'28.2"E)

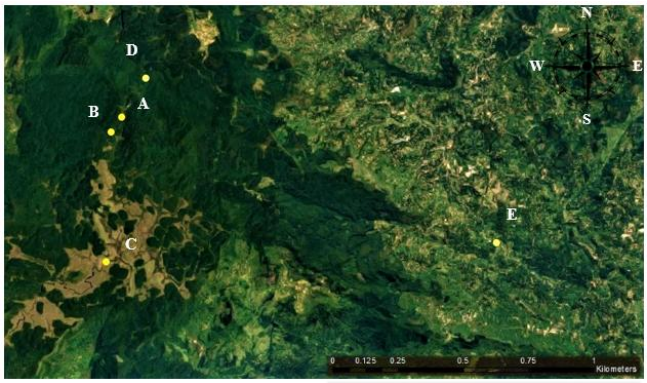

Fig 1: Locations of sampling sites within and outside the HPNP

Six quadrates each 1m x 2m were placed within each sampling site for the sampling of amphibians (Figure 1). Three quadrates were randomly placed in the area 1 m from the outside the pond bank and three quadrates were randomly placed inside the pond in the area of 1 m from the pond bank [13]. Anuran species (metamorphs, juveniles and adults) in these quadrates were surveyed during the morning to after noon (08:00hrs – 16:00hrs) and night (18:00hrs – 20:00hrs).

Moreover, vegetation within each plot was searched even when slight movement was detected. Head lamps and torches were used for nocturnal searches.

Metamorphic anurans were captured with a dip net or by hand [33] and adult amphibians were captured with a dip net or by hand [40, 42].

Small amount of water was added to the container to prevent overheating and desiccation [42]. Overcrowding based on the container size and T_{amb} was avoided [24]. At the end of the survey they were released back in to their captured habitat.

Amphibian species were identified using the amphibian guides of Dutta and Manamendra-Arachchi (1996) [11]; Manamendra-Arachchi and Pethiyagoda (2006) [25]; de Silva (2007) [10]. When an amphibian was captured, following data were recorded.

(a) Amphibian species

(b) Presence or absence of polymorphism (If polymorphism were found, they were recorded with the count and polymorphism type).

2.3 Identification of amphibian polymorphism

Amphibian pattern and colour variations were identified and differentiated by the standard observer [31] with the assist of Dutta and Manamendra-Arachchi (1996) [11]; Manamendra-Arachchi and Pethiyagoda (2006) [25] and de Silva (2007) [10] amphibian guides.

2.4 Data analysis

Graphical representations were created by using Microsoft excel 2013 software.

3. Results

A total of 694 amphibians belonging to 4 families and 11 species were recorded during the study in the five lentic water bodies studied. 511 individuals were examined inside the HPNP and 183 individuals were examined outside the HPNP (Table 1).

Table 1: Amphibian abundance in and around the lentic water bodies within and outside the HPNP

Locality type	Family	Species	Abundance
HPNP	Dicroglossidae	*Minervarya greeni*	135
	Microhylidae	*Microhyla zeylanica*	11
		Ramanella palmata	03
		Philautus alto	02
	Rhacophoridae	*Philautus frankenbergi*	01
		Philautus microtypanum	12
		Taruga eques	347
OHPNP	Bufonidae	*Duttaphrynus melanostictus*	06
		Euphlyctis cyanophlyctis	04
	Dicroglossidae	*Fejervarya kirtisinghei*	08
		Fejervarya limnocharis	18
	Rhacophoridae	*Taruga eques*	147

Colour polymorphism were recorded only in *T. eques* species. Six dorsal colours were recorded in the HPNP and three colour variations were recorded outside the HPNP (Figure 2). Most prevalent dorsal colourations of *T. eques* inside and outside the HPNP were green (93.08%) (Figure 2A) and Dark brown (62.59%) (Figure 2B) respectively. Outside the HPNP 29.25% was green dorsal coloured and 8.16% was yellow dorsal coloured (Figure 2C). Dark orange (Figure 2D), cream (Figure 2E) and purple dorsal (Figure 2F) coloured *T. eques* were recorded only inside the HPNP.

Fig 2: Colour polymorphism of *T. eques* recorded within and outside the HPNP

Percentages of Dark orange dorsal coloured (1.73%), cream dorsal coloured (0.29%) and purple dorsal coloured (0.58%) *T. eques* were very low (Figure 3).

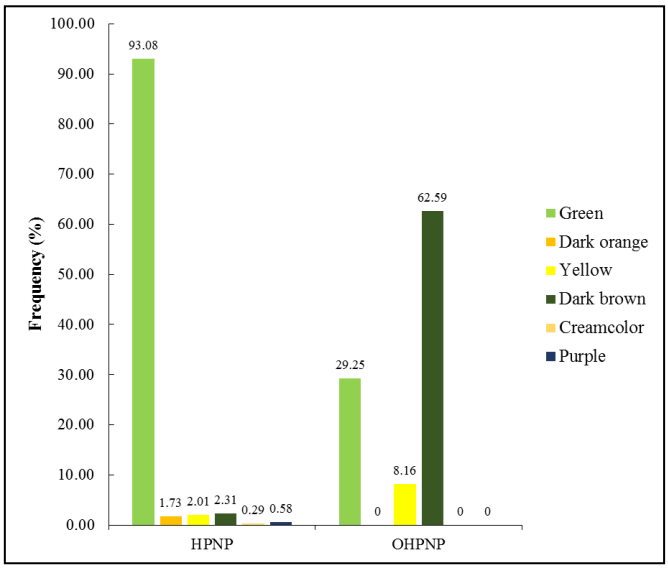

Fig 3: Percentage of different coloured *T. eques* recorded within and outside the HPNP

Pattern polymorphism consisted of colour (pigment) pattern variations which include presence, absence and variations of dorsal spots, stripes and other characteristic patterns. Highest pattern polymorphism were recorded in *T. eques* species. Six and four different pattern polymorphism were recorded from *T. eques* **(**Figure 4) inside and outside the HPNP.

Distinct hour glass pattern (Figure 4A) was the highest prevalent pattern polymorphism of *T. eques* species, in both localities 77.52% in HPNP and 52.38% outside HPNP. However various deviations of the distinct hourglass pattern were recorded. These included, aberrant hour glass pattern (Figure 4B), without hour glass pattern (Figure 4C), yellow line encircled hour glass pattern (Figure 4D) and dorsal spots (Figure 4E).

Fig 4: Variations of pattern polymorphism in *T. eques* species

Yellow line encircled hour glass pattern was the rarest pattern and accounted for only 1.73%. Yellow line encircled hour glass pattern was recorded only inside the HPNP (Table 2).

Table 2: Variations of pattern polymorphism in *T. eques* species within and outside the HPNP

Type of the locality	Type of pattern polymorphism	Percentage (%)
HPNP	Aberrant hour glass	09.80
	Distinct hour glass	77.52
	Dorsal spots	02.59
	Without hour glass	10.95
	Yellow line encircled hour glass	01.73
OHPNP	Aberrant hour glass	28.57
	Distinct hour glass	52.38
	Dorsal spots	14.28
	Without hour glass	19.05

Three different dorsal patterns were recorded in *M. greeni* (Figure 5) inside the HPNP, which are Vertebral Line (Figure 5A), Deformed Vertebral Line (Figure 5B) and Without Vertebral Line (Figure 5C). Three different dorsal patterns were recorded in *F. limnocharis* [Broad Vertebral Line (Figure 5D), Thin Vertebral Line (Figure 5E) and Without Vertebral Line (Figure 5F)] outside the HPNP.

Two types of pattern polymorphism were observed *F. kirtisinghei* (Figure 6) [Vertebral Line (Figure 6A) Deformed Vertebral Line (Figure 6B)] outside the HPNP. Pattern polymorphism were not recorded in the other species observed.

Fig 5: Variations of pattern polymorphism in *M. greeni* and *F. limnocharis* species

Highest frequency of pattern polymorphism was present as a distinct vertebral line in both *M. greeni* and *F. kirtisinghei* species. Percentages were 82.23% and 62.5% respectively. However absence of vertebral line was recorded in high frequency in *F. limocharis* species (Table 3).

Type of the locality	Amphibian species	Type of pattern polymorphism	Percentage (%)
HPNP	*Minervarya greeni*	DVL	13.33
		VL	82.23
		WVL	04.44
OHPNP	*Fejervarya kirtisinghei*	DVL	37.5
		VL	62.5
	Fejervarya limnocharis	BVL	11.11
		TVL	33.33
		WVL	55.56

BVL- Broad Vertebral Line VL- Vertebral line
DVL- Deformed Vertebral line WVL- without Vertebral Line
TVL- Thin Vertebral Line

4. Discussion

Amphibian body colouration and pigment patterns play a vital role on their survival in the environment. Amphibian key traits such as body colour and pigment patterns are stabilized between the 30-40 Gosner stages [16]. Hence, metamorphic stage is used to detect polymorphism in polymorphism studies of amphibians [20]. Most of the pattern and colour polymorphism types that were recorded in the field are concordant with the data of Hoffman and Blouin (2000) [19], excluding the deformed vertebral line.

Highest number of colour and pattern variations are discovered in *Taruga eques*. This result is further reinforced by the worldwide findings of amphibian variations in family Hylidae and Ranidae (genus- Rana), since all Hylidae, Rhacophoridae and some of Ranidae frogs are tree frogs that are known to have wide colour and pattern variations.

Taruga eques is considered as an hour-glass tree-frog, due to them having a characteristic hour-glass mark on the dorsum [10]. However, the present study revealed a novel pattern polymorphism of *Taruga eques* which was not known to science as "Without hourglass pattern". Present study was also the first to discover *Taruga eques* without characteristic hourglass pattern. These discoveries, indicate that common name of *Taruga eques* has not been convincingly entitled and it cannot be broadly classified or confined as an "hour-glass frog".

In addition to these endemic frogs, *Dendropsophus ebraccatus* (belongs to family Hylidae) is recognized as an hour-glass tree-frog distributed from Mexico to northern Colombia regions [32]. According to the polymorphism study of *Dendropsophus ebraccatus*, most of colour pattern variations that were detected in *Taruga eques* were resembled with encompassed colour pattern categories of *Dendropsophus ebraccatus* amphibians [32].

In *Minervarya greeni*, two new vertebral line pattern polymorphisms could be noticed as deformed vertebral line and without vertebral line. Previous research on habitat utilization of *M. greeni*, revealed that they prefer to inhabit short plants, submerged plant cover, decaying plant matter and leaf litter as substrates for protection from predators through camouflage [35]. This finding is further strengthened by Hoffman and Blouin (2000) [19], who observed that striped phenotype is a selective advantage for some amphibians which inhabit ponds and forested areas with emergent vegetation. For that reason amphibians with deformed vertebral line or without vertebral line, are easily noticeable to visual predators due to having non- matching morphs with the environment. Therefore, *M. greeni* with vertebral line acquires selective advantage through the natural selection.

Pattern polymorphism recorded in *Fejervarya limnocharis* and *Fejervarya kirtisinghei*, have been previously observed [10, 11].

Greyish-brown, orange-yellow, reddish-brown are the most common dorsal colour mixtures that previously recorded in *Taruga eques* with hourglass pattern [10, 11, 25]. However, dark brown, yellow and cream dorsal colourations, without colour combinations spread throughout the dorsal region were observed in some *Taruga eques* individuals which were not possessed hourglass pattern. Also Cream and pink dorsal colourations has not been previously recorded colour polymorphisms in *Taruga eques*.

Most of the amphibians recorded within the HPNP were with green dorsal colouration. Green dorsal colouration is mainly resulted by combination of xanthophores and iridophores placed above melanophores [7]. Melanophores is responsible for brown colouration and dominance of xanthophores produce yellow and orange dorsal colouration of tree frogs [41]. Lack of iridophores results duller and darker overall colouration [21]. Bagnara *et al.* (1978) [3], states that uncommon blue colouration of amphibians is due to physical effects with the absence of yellow overlying pigments.

Scientists speculate that diverse potential causes may govern the colour and pattern variations of amphibians [37]. However, major conclusion drawn from the field and experimental findings is, that natural selection results in these polymorphism [19] with respect to the predation. Moreover, colour and pattern polymorphism result due to strong

selection by visually oriented predators [12, 19]. According to the Touchon and Warkentin (2008) [38], experimental data suggests that larval stages are induced to produce different cryptic colourations against different aquatic predators of tadpoles and Morey (1990) [29], experimentally reveals that predators attacked to cryptic morphs of amphibians less often. Some tree frogs are also known to be able to change the body colouration against light and dark backgrounds to reduce the colour contrast as anti-predator device [31]. However, these polymorphism studies are lab based experiments rather than field studies. Though field based studies are difficult to conduct, well design studies are remunerative to analyze the effects of predation on morph frequencies. Therefore, it is productive to examine about how amphibian colour pattern variations results in survival of them.

5. Conclusion

Present study further strengthen that tree frogs possess diverse colour and pattern polymorphisms and most of them were not previously recorded in *Taruga eques*. Spatial variations in some environmental variables may be a major reason for dissimilar distribution of different colour and pattern polymorphism between two localities.

Presence of dorsal pigment patterns may be a selective advantage for amphibians specially tree frogs for disrupt the expression of bright dorsal colouration for visually oriented predators. However, well design field based studies are essential to study the effects of predation on various colour and pattern variations for amphibian survival through avoiding abnormalities.

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7. References

1. Anderson SC, Volpe EP. Burnsi and kandiyohi genes in the leopard frog *Rana pipiens*. Science. 1958; 127(3305):1048-1050.
2. Andren C, Nilson G. Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. Biological Journal of the Linnean Society. 1981; 15(3):235-246.
3. Bagnara JT, Frost SK, Matsumoto J. On the development of pigment patterns in amphibians. American Zoologist. 1978; 18(5):301-3012.
4. Baker AS. A study of the expression of the burnsi gene in adult *Rana pipiens*. Journal of Experimental Zoology Part A. Ecological Genetics and Physiology. 1951; 116(2):191-229.
5. Blouin MS. Inheritance of a naturally occurring colour polymorphism in the ornate chorus frog, *Pseudacris ornata*. *Copeia*. 1989; 19(4):1056-1059.
6. Browder LW, Davison J. Spotting variations in the leopard frog: A test for the genetic basis in the *Rana pipiens* "burnsi" variant. Journal of Heredity. 1964; 55(5):234-241.
7. Browder LW. Genetic and embryological studies of albinism in *Rana pipiens*. Journal of Experimental Zoology Part A. Ecological Genetics and Physiology. 1972; 180(2):149-155.
8. Casler E. Pattern variation in isogenic frogs. Journal of Experimental Zoology Part A. Ecological Genetics and Physiology. 1967; 166(1):121-135.
9. Davison J. Gene action mechanisms in the determination of colour and pattern in the frog (*Rana pipiens*). *Science (New York, NY)*. 1963; 141(3581):648.
10. De Silva A. Amphibians of Sri Lanka: A Photographic Guide to Common Frogs, Toads and Caecilians. Privately published, Kandy, 2007.
11. Dutta SK, Manamendra-Arachchi K. The Amphibian Fauna of Sri Lanka. Wildlife Heritage Trust of Sri Lanka, Colombo, 1996.
12. Endler JA. A predator's view of animal colour patterns. *Evolutionary Biology*. 1978; 11(3):319-364.
13. Faruk A, Belabut D, Ahmad N, Knell RJ, Garner TW. Effects of oil- palm plantations on diversity of tropical anurans. Conservation Biology. 2013; 27(3):615-624.
14. Fishbeck DW, Underhill JC. Distribution of stripe polymorphism in wood frogs, *Rana sylvatica* (Leconte), from Minnesota. *Copeia*. 1971, 253-259.
15. Ford EB. Polymorphism. Biological Reviews. 1945; 20(2):73-88.
16. Gosner KL. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 1960; 16(3):183-190.
17. Green MJB. IUCN directory of South Asian protected areas. IUCN. 1990, 216-219.
18. Hartl DL, Jones EW. Genetics: Principles and Analysis, 4th ed, Jones and Bartlett, Sudbury, 1998.
19. Hoffman EA, Blouin MS. A review of colour and pattern polymorphisms in anurans. *Biological Journal of the Linnean Society*. 2000; 70(4):633-665.
20. Hoppe DM, Pettus D. Developmental features influencing colour polymorphism in chorus frogs. Journal of Herpetology. 1984; 18(2):113-120.
21. Jablonski D, Alena A, Vicek P, Jandzik D. Axanthinism in amphibians; A review and the first record in the wide spread toad of the *Bufotes viridis* complex (Anura: Bufonidae). Belgian Journal of Zoology. 2014; 144(2):93-101.
22. Kang C, Kim YE, Jang Y. Colour and pattern change against visually heterogeneous backgrounds in the tree frog Hyla japonica. Scientific Reports. 2016; 6(3):1-12.
23. Kolenda K, Najbar B, Najbar A, Kaczmarek P, Kaczmarski M, Skawiński T. Rare colour aberrations and anomalies of amphibians and reptiles recorded in Poland. Herpetology Notes. 2017; 10(3):103-109.
24. Lunde KB, Johnson PT. A practical guide for the study of malformed amphibians and their causes. Journal of Herpetology. 2012; 46(4):429-441.
25. Manamendra-Arachchi K, Pethiyagoda R. Sri Lankawe Ubhayajeevin. WHT publications (Pvt) Ltd, Colombo, 2006.
26. Merrell DJ. The distribution of the dominant burnsi gene in the leopard frog, Rana pipiens. Evolution. 1965; 19(1):69-85.
27. Merrell DJ. Laboratory studies bearing on pigment pattern polymorphisms in wild populations of *Rana pipiens*. Genetics. 1972; 70(1):141-161.
28. Milstead WW, Rand AS, Stewart MM. Polymorphism in cricket frogs: An hypothesis. Evolution. 1974; 28(3):489-491.

29. Morey SR. Microhabitat selection and predation in the Pacific treefrog, *Pseudacris regilla*. Journal of Herpetology. 1990; 24(3):292-296.
30. Nevo E. Adaptive colour polymorphism in cricket frogs. *Evolution*. 1973; 27(3):353-367.
31. Nielsen HI, Dyck J. Adaptation of the tree frog, *Hyla cinerea*, to coloured backgrounds, and the role of the three chromatophore types. Journal of Experimental Zoology Part A. Ecological Genetics and Physiology. 1978; 205(1):79-94.
32. Ohmer ME, Robertson JM, Zamudio KR. Discordance in body size, colour pattern, and advertisement call across genetically distinct populations in a Neotropical anuran (*Dendropsophus ebraccatus*). Biological Journal of the Linnean Society. 2009; 97(2):298-313.
33. Ouellet M, Bonin J, Rodrigue J, Desgranges JL, Lair S. Hindlimb deformities (Ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. *Journal of Wildlife Diseases*. 1997; 33(1):95-104.
34. Pettus D. Colour inheritance in *Pseudacris triseriata*. Herpetologica. 1966; 22(4):269- 275.
35. Prabath MC. Habitat utilization of *Fejervarya greeni* (Amphibia; Dicroglossidae) in and around lentic water bodies at Horton Plains National Park, Sri Lanka, B.Sc. Thesis, University of Sri Jayewardenepura, 2016.
36. Rabbani M, Zacharczenko B, Green DM. Colour pattern variation in a cryptic amphibian, Anaxyrus fowleri. Journal of Herpetology. 2015; 49(4):649-654.
37. Summers K, Cronin TW, Kennedy T. Variation in spectral reflectance among populations of *Dendrobates pumilio*, the strawberry poison frog, in the Bocas Del Toro Archipelago, Panama. Journal of Biogeography. 2003; 30(1): 35-53.
38. Touchon JC, Warkentin KM. Fish and dragonfly nymph predators induce opposite shifts in colour and morphology of tadpoles. *Oikos*. 2008; 117(4):634-640.
39. Trullas SC, vanwyk JH, Spotila, JR. Thermal melanism in ectotherms. Journal of Thermal Biology. 2007; 32(5):235-245.
40. Urbina G, Jenny C, Galeano SP. Abundance, activity patterns and microhabitat of *Rhinella macrorhina*, an endemic toad from the cloud forests of the Colombian Central Andes. The Herpetological Journal. 2009; 19(1):35-40.
41. Vitt LJ, Caldwell JP. *Herpetology:* An Introductory Biology of Amphibians and Reptiles. Elsevier Science Publishing, San Diego, 2009, 2014. 52-53.
42. Wheeler CA, Welsh Jr HH. Mating strategy and breeding patterns of the foothill yellow-legged frog (*Rana boylii*). *Herpetological Conservation and Biology*. 2008; 3(2):128-142.
43. Wollenberg KC, Veith M, Noonan BP, Lotters S. Polymorphism versus species richness systematics of large Dendrobates from the Eastern Guiana Shield (Amphibia: Dendrobatidae). *Copeia*. 2006; 2006(4):623-629.