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Biometrical Analysis and Distribution of *Duttaphrynus melanostictus* (Anura: Bufonidae) in Dehradun region of Western Himalaya

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Abstract
Duttaphrynus melanostictus, known as common Indian toad is a widely distributed species of south Asia including Indian subcontinent region. This study was performed to investigate the biometry of this toad in Dehradun region of Western Himalaya. A total of 44 samples comprising 19 males and 25 females were observed and analyzed for their morphometric and qualitative characters during breeding season. Morphological characteristics among the samples were more expressed in morphometric rather than qualitative characters. Sexual size dimorphism was observed biased towards female in terms of snout vent length (SVL) with other characters. SVL was ranged from 43.25mm to 59.21mm and from 53.65 to 77.43mm in males and females respectively. A significant positive correlation (p<0.01) was observed between 11 morphometric parameters during the present study.

Keywords: Bufonidae, *Duttaphrynus melanostictus*, morphometry, Western Himalaya

1. Introduction
Bufo is a cosmopolitan genus of about 225 species of true toads with species in all regions of the world except Antarctic, Arctic, Australia, New Guinea and neighbouring islands. The species *Duttaphrynus melanostictus* (Schneider, 1799) belongs to genus *Duttaphrynus* within Bufonidae family. Previously it was known as *Bufo melanostictus* but in 2006, a review of amphibian taxonomy placed *Bufo melanostictus* in a new genus, *Duttaphrynus* [1]. It is one of the most widespread species in its genus and distributed widespread across Asia. It inhabits Taiwan and southwestern and southern China, including Hainan Island, southward through Southeast Asia to Indonesia, and westward to India and Sri Lanka [2]. It has a large distribution in plains as well as high altitudes (2500 m a.s.l.) and populates entire Dehradun, Uttarakhand [3].

It is terrestrial and nocturnal species, reported in diverse habitats, including from tropical to subtropical, temperate areas where it is found up to 1,800-2,000 m a.s.l. [4, 5]. It inhabits a wide range of environments, including forests to urban areas, associated with human habitations near cultivated field, construction sites and breeds opportunistically in various bodies of freshwater, including gutters, puddles and even cement cisterns in parks [6].

It has been remained the subject of numerous studies such as ovarian activity, metabolic switching and phylogenetic relationship [7-9]. Several studies on anuran morphometry have been reported worldwide viz., [10, 11] and in India as well [12-14]. However, morphometric data of this amphibian species from Uttarakhand, Western Himalaya is scarce. Hence, the basic purpose of this study is to establish regional variation in external morphology of adults within population of common Indian toad across Dehradun region of Western Himalaya.

2. Materials and Methods
The present study area is located in Dehradun region of Uttarakhand and lies between latitudes 29˚58'-31˚2'N and longitudes 77˚34'-78˚18'E. This region is covered by outer Himalaya on north and Shivalik foothills on south. The sampling stations (Table 1) where *D.Melanostictus* populations were investigated were located along a longitudinal E-W transect during their breeding period March to September 2014-15. It was based on the standard procedure [15, 16].

Table 1: Sampling sites details with their geographical coordinates & altitudes.

Site(s)	Locality	Coordinates	Altitude (m asl)	Habitat	N Total (N=44)
DD01.	Balawala	30°15' N 78°06' E	607	Pond	14
DD02.	Raipur	30°18' N 78°05' E	682	Water tank	12
DD03.	Mussoorie	30°27' N 78°04' E	1817	Road side pools	7
DD04.	Jhakhan	30°21' N 78°03' E	774	Garden	11

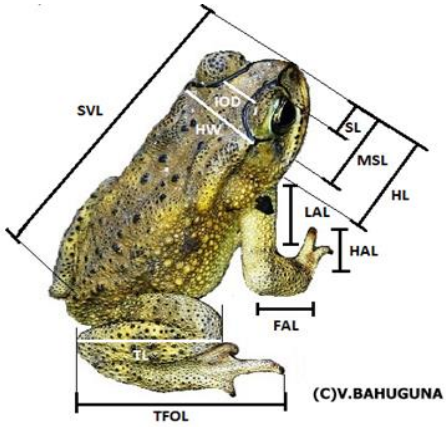

Fig 1: *Duttaphrynus melanostictus*

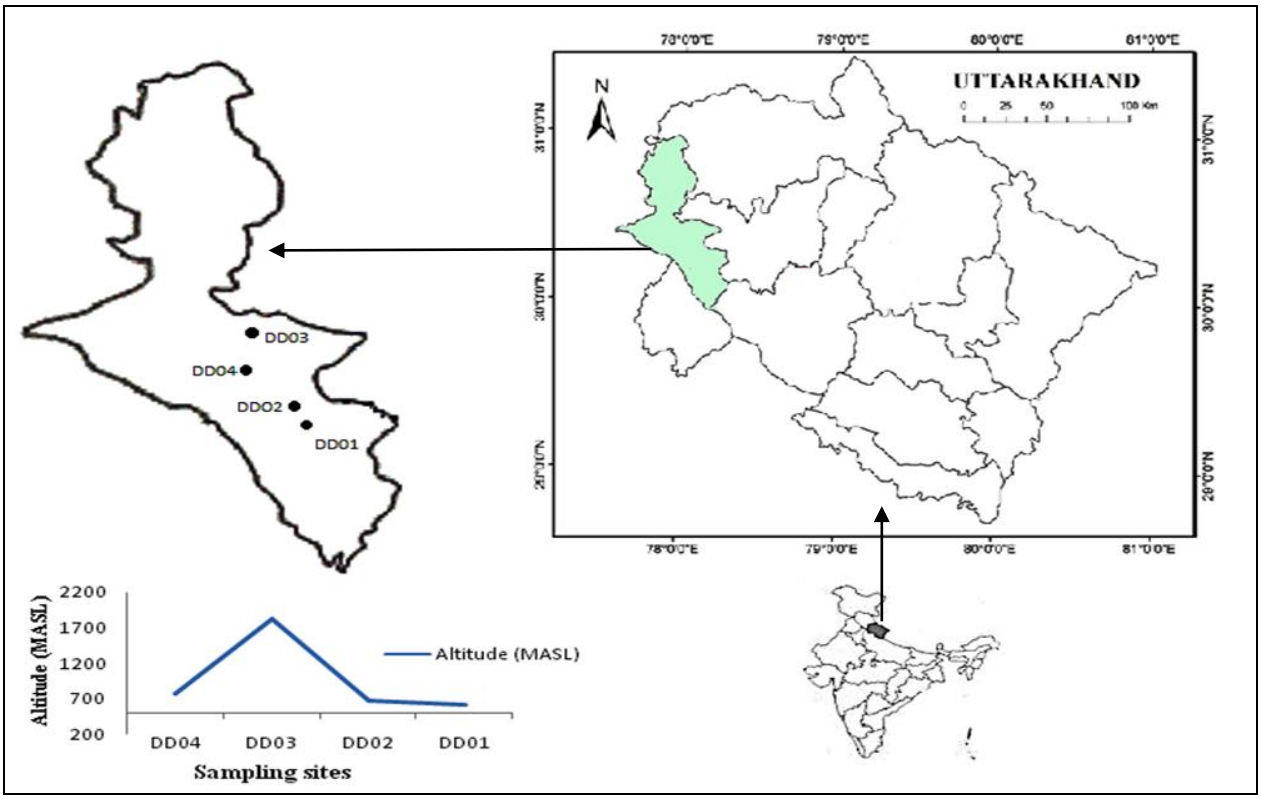

Fig 2: Location map of study area

Fig 3: Views of the sampling stations *DD01, DD02, DD03, DD04.*

2.1 Morphometric Measurements

Thirty morphometric measurements were recording using digital Vernier caliper (Aerospace) to the nearest 0.1 mm accuracy according to Pichandi *et al.* [16] Chowdhary *et al.* [17] and Bahuguna *et al.* [18]; as follow: SVL-Snout-vent length, HL- Head length (from back of mandible to tip of snout), HW-Head width (left side back of mandible to right side back of mandible), STL -Snout-tympanum length (Tip of snout to front of tympanum), MSL-Mouth angle-snout length (Tip of snout to end of mouth opening), NS -Nostril- snout length (Distance from nostril to tip of snout),SL-Snout length, NTL-Nostril tympanum length (Distance between nostril and front of tympanum) EN-Distance from front of eyes to nostril, TEL-Tympanum eye length (distance between end of eye to front of tympanum),TD-Tympanum diameter, MFE-Distance from back of mandible to front of eye, MBE-Distance from back of mandible to back of eye, MN-Distance from back of mandible to nostril, IN-Internarial space (Distance between both nostrils), EL-Eye length (greatest diameter of the eye including upper eyelids), IOD-Interorbital distance, UEW-Maximum width of upper eyelid, HAL-Hand length (from base of outer palmer tubercle to tip of third finger), FAL-Fore arm length (from elbow to base of outer palmer tubercle),

LAL-Lower arm length, HLL-Hind limb length, THIGHL-Thigh length, TL-Tibia length, FOL-Foot length (from base of inner metatarsal tubercle to tip of fourth toe),TFOL-Length of tarsus and foot (from base of tarsus to tip of fourth toe), ThreeFL-Third finger length, OneFL-First finger length, FourTL-Fourth toe length, ITL-Inner toe length. According to Berger & Rybacki, 1994 [19], smaller individuals are likely not sexually mature that why only specimens larger than 42 mm snout-vent length were consider for analysis. Morphometric measurements of the samples during the survey were taken in the field conditions and specimens were immediately released after recording the observations. Statistical analysis was performed on the recorded data from the individuals to establish the correlation between the studied parameters.

3. Results and Discussion

A total of 44 samples comprising 19 males and 25 females were observed for their morphometric and qualitative characters during breeding season. During the present study, univariate statistics such as minimum, maximum and mean ±SD values for all the variable of the morphometric parameters were analyzed (Table 2). SVL was ranged from 43.25mm to 59.21mm and from 53.65mm to 77.43mm in males and females respectively. A significant positive correlation at $p<0.01$ was obtained between 11 important morphometric parameters (Table 3). Along with the quantitative morphological measurements, some qualitative characters, including body shape and color, snout shape, dermal ridge and median stripe were also observed. Morphological characteristics among the samples were more expressed in morphometric rather than qualitative characters.

3.1 Diagnosis

D. melanostictus is a stocky, medium to large sized toad with relatively short hind limbs and small head easily recognized by the presence of black cornified cranial crests on head and prominent black warts on the body. Crowns of head concave in shape. Tympanum nearer to eyes, large at least more than half diameter of eyes. Upper eyelid bigger in size than snout. Interorbital space broder than upper eyelid. The ridge on head, maxillary edges, paratoids, tubercles and tip of finger and toe often with dark brown cornified substances. Paratoids are elliptic or kidney shaped. Upper surface of the body with less and more spiny warts with black tips. A double series of round cornified warts present on both side of vertebral line. Forelimb moderate and first finger usually extending beyond second, second finger slightly shorter than fourth. Third finger longest. Hind limbs are short and thick web present between toes. First toe, smallest and third toe longer than fifth but fourth longest.

3.2 Coloration: Body color usually highly variable, dorsum olive brown or dark brown to dull red in colour. Ventral surface of body immaculate or less or more spotted or with network of brown color. The tips of paratoids glands are tubercular or sometime with black spines. Black lines behind the eye present in both male and female. A male turns yellowish green color during the breeding season of monsoons. The juveniles have a very inconspicuous eardrum and lack warts.

Table 2: Descriptive statistics of morphometric parameters of *Duttaphrynus melanostictus* in Dehradun region of Western Himalaya

	Male(N=19)			Female(N=25)		
Parameters	**Minimum (mm)**	**Maximum (mm)**	**Mean± S.D (mm)**	**Minimum (mm)**	**Maximum (mm)**	**Mean± S.D (mm)**
SVL	43.25	59.21	52.42±6.0	53.65	77.43	65.23±8.9
HL	12.16	15.56	14.28±1.2	14.49	19.34	16.63±1.5
HW	13.94	18.87	16.62±2.1	15.41	24.15	19.17±2.9
STL	10.33	12.02	11.19±0.6	10.71	15.65	12.61±1.5
MSL	6.51	11.43	9.64±2.0	9.29	16.09	12.98±2.32
NS	0.36	0.99	0.74±0.2	0.69	2.67	1.43±0.6
SL	4.77	6.05	5.78±0.6	5.26	7.61	6.53±0.7
NTL	9.97	11.03	10.44±0.3	10.02	12.98	11.18±0.8
EN	3.91	5.13	4.52±0.4	4.16	6.16	5.29±0.6
TEL	0.32	1.06	0.74±0.3	0.93	2.4	1.57±0.5
TD	3.27	4.01	3.69±0.3	3.53	4.95	4.19±0.4
MN	10.62	15.24	12.75±1.6	13.01	17.96	15.46±1.6
MFE	7.27	11.01	9.89±1.5	9.7	12.89	11.16±0.9
MBE	3.67	5.05	4.24±0.52	4.03	6.89	5.41±1.0
IN	3.14	3.92	3.57±0.3	3.39	5.06	4.19±0.5
EL	4.55	6.99	5.77±0.99	5.98	10.88	8.27±1.7
IOD	4.43	6.24	5.59±0.6	5.18	8.01	6.67±0.9
UEW	3.23	4.65	4.01±0.66	4.7	7.63	6.11±1.2
HAL	9.63	12.76	11.34±1.2	11.3	19.81	14.56±2.7
FAL	9.25	12.65	10.80±1.2	10.51	17.56	13.73±2.4
LAL	8.37	12.3	10.35±1.4	9.91	17.11	13.28±2.5
HLL	58.21	76.12	67±7.5	65.01	107.11	86.40±15.0
THIGHL	16.46	22.14	19.68±2.7	18.69	31.91	25.28±4.2
TL	16.84	24	20.72±3.0	19.99	32.12	26.16±4.2
TFOL	22.72	35.11	26.54±4.99	23.76	44.58	33.18±7.3
3FL	4.49	6.99	5.73±1.0	4.99	11.75	8.24±2.0
1FL	3.72	4.65	3.99±0.6	4.13	8.33	5.93±1.6
4TL	7.61	13.01	10.41±1.9	10.74	19.17	14.47±3.3
ITL	1.43	2.98	2.06±0.5	1.83	4.41	3.01±0.99

Table 3: Correlation coefficient (r) between some morphometric measurements of *D. melanostictus* in Dehradun region of Western Himalaya

	SVL	HL	SL	TEL	UEW	LAL	HLL	3FL	1FL	4TL
HL	0.973									
SL	0.925	0.939								
TEL	0.990	0.968	0.890							
UEW	0.977	0.947	0.862	0.988						
LAL	0.988	0.970	0.930	0.976	0.950					
HLL	0.990	0.954	0.908	0.989	0.972	0.989				
3FL	0.976	0.964	0.910	0.976	0.954	0.986	0.985			
1FL	0.931	0.890	0.890	0.923	0.913	0.936	0.943	0.919		
4TL	0.991	0.955	0.925	0.976	0.957	0.988	0.987	0.967	0.955	
ITL	0.969	0.925	0.910	0.951	0.928	0.979	0.975	0.954	0.953	0.986

Significant at (p<0.01)

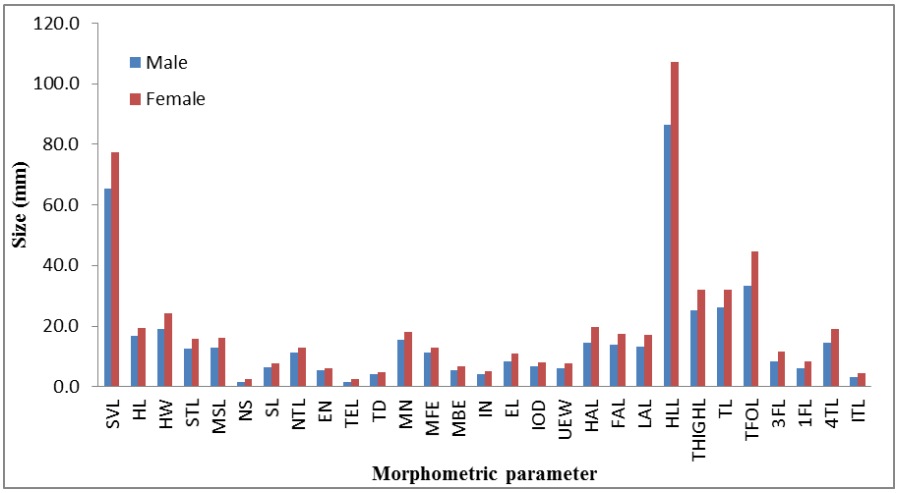

Fig 4: Comparative Morphometric variation among male and female specimens of *D. melanostictus*

Generally in case of anurans morphology, female is of bigger size than the male [20]. Our study also shows a greater degree of female-biased sexual size dimorphism because we found that most of the female samples were 1/4th greater than males in terms of SVL. Our observations are in agreement with previous study of this species from other localities [3, 21]. The most used explanation of the sexual dimorphism is the body size. This has the advantage that bigger female producing a greater number of eggs [18, 22-24]. Wake & Dickie [25] also confirmed that the fecundity in the same species increases with an increasing body size.

In the present study we observed the distribution of this species altitude ranged between 607 m a.s.l. to 1817 m a.s.l. It prefers disturbed lowland habitats such as forest margins, riparian areas and human-dominated agricultural and urban areas. Our result got supports from the findings of Bahuguna & Bhutia [3] as they had reported in India, toad widely distributed in plains and also found at high altitudes (2500 m). In Dehradun, toad population is more concentrated toward Shivalik foothills.

The present study added important information about morphometric analysis could allow a better understanding of the origin and rate of these differences among populations of Dehradun, Uttarakhand region of Western Himalaya. Future studies in this regard would facilitate further understanding of immediate ecological impacts on the anuran morphology.

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