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A contribution on morphology and distribution of the Rock Toad *Duttaphrynus hololius* (Günther, 1876) with first report on deformity, calling and breeding behaviours (Amphibia: Anura: Bufonidae)

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ABSTRACT

Morphology of a hitherto fore unsampled population of *Duttaphrynus hololius* from Hyderabad in mid-Deccan plateau (outlier), is presented and found consistent with that of populations characterised so far. We re-map its distribution based on further findings, since the last attempt (in 2013) and our revised analysis revealed a better resolution and refinement in the MaxEnt Species Distribution Modelling (SDM) range projections. We present the first ever information on its courtship and acoustic characterization (a mating call and a male release call), based on observations in Chittoor, Eastern Ghats. We also report the first case of deformity-macrophthalmia with red eye syndrome, in this species based on field observations in Hampi, Deccan plateau. Findings of this species in peri-urban areas and with deformity are highlighted in a conservational perspective. Put together, the new information considerably adds to our knowledge on this uncommon species.

Key words: *Duttaphrynus hololius*, calls, courtship, deformity, distribution, MaxEnt, morphology, peri-urban area

INTRODUCTION

The rock toad *Duttaphrynus hololius* (Günther, 1876) is an uncommon species of toad endemic to the Deccan plateau of Indian peninsula (Boulenger, 1890; Frost, 2020). It was first described as *Bufo hololius* based on type specimen purportedly collected from 'Malabar' a wet zone region in the Western Ghats Mountains (Boulenger, 1890). In a subsequent treatment, Thurston (1888) provided a refined description of this species, again based on the same specimens. This was again recast and elaborated by Boulenger (1890), without any new collection or data. For nearly a century since its original description this species remained totally unrecorded. Subsequent observations on this species were very scarce in scholarly treatises on the subject (Daniel, 2002; Daniels, 2005).

Satyamurti (1967) was perhaps the first who reported on further, fresh specimens from Nellore and Udayagiri of the Eastern Ghats, in the holdings of the Madras Govt. Museum (also see Ganesh & Asokan, 2010). Pillai & Ravichandran (1991) were one of the few who added new data on this little-known species. Then Daniels (1992) reported its range extension from Bannerghatta, near Bangalore. Thereafter Radhakrishnan & Ravichandran (1999) provided a further update on its distribution range. Dubois & Ohler (1999) in their morphological revision of toads tentatively allocated

this species to the *Bufo stomaticus* group. Subsequently, Biju (2001) after exhaustive field excursions remarked that such a toad never exists in Malabar and that the stated type locality is probably in error. A molecular study by Bocxaler *et al.* (2009) also included sequences of this species and reported its affinities with the *Duttaphrynus stomaticus*, thereby seconding the view of Dubois & Ohler (1999).

Then, Chandramouli *et al.* (2011) reported this species in parts of Southern Eastern Ghats and first illustrated this species in life. After their important finding, many more sighting reports of this species emerged (Adimallaiah *et al.*, 2012; Kalaimani *et al.*, 2012). Among such studies, were those that brought forth first time information on its life history. Ganesh *et al.* (2013) worked on the tadpoles and metamorphosis of this species. Chandramouli & Kalaimani (2014) further worked on the same aspect but including that of oral ultrastructure and development. Bhargavi *et al.* (2013) again worked on its distribution, based on the locations known until then, with species distribution modelling. Then survey reports from the Eastern Ghats landscape also listed this species in their amphibian records (Ganesh *et al.*, 2018). Here, we, for the first time, report its courtship, detailed call descriptions and spawning behavior along with further new records from Deccan plateau.

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MATERIALS AND METHODS

This paper is based on observations made by the authors in different regions and on different aspects of this species during 2016–2018. The toads sighted were ascertained as *Duttaphrynus hololius* owing to their morphological congruence with previous description accounts (Günther, 1876; Satyamurti, 1967; Pillai & Ravichandran, 1991; Chandramouli *et al.*, 2011; Bhargavi *et al.*, 2013). In one study site (Hyderabad) specimens sighted in the field were observed, their microhabitats noted and were photographed in situ, before being preserved, registered and vouchered in museum deposited at Natural History Museum of Osmania University, Hyderabad (NMH.OU). Measurements were recorded to the nearest mm using vernier calipers (least count 0.1 mm). Snout-vent lengths of live individuals were measured with a standard graduated ruler (least count 1 mm). Morphological features were documented using a magnifying hand lens (X 5 optical zoom). Measurement protocols and body-form nomenclature followed Dubois & Ohler (1999). In two other study sites (Chittoor and Hampi) the toads were not collected or otherwise disturbed, but only visually observed and their behaviours documented. In Chittoor, during 17th–19th July, for three days, during each night, a three-membered team conducted fieldwork between 19:00 and 23:00 h. Thus, based on about 35 man-hours of night surveys in the area, the calling behaviours were documented. Colouration notes of live individuals were taken during field work and based on photographs taken there on. Photographs were taken using Canon EOS 700D and Canon Powershot S3 IS model camera and some are reproduced here as photographic vouchers. Habitat type classification follows Champion & Seth (1968). Geo-coordinates and elevation values were recorded using a hand held Garmin 12 Channel Global Positioning System using a WGS-84 map datum. Ambient temperature (in °C) and relative humidity (in %) readings were scored using a hand-held digital thermohygrometer after one minute of exposure. Frog malformation terminologies are based on descriptions in Meteyer (2000). Call recordings were done using

the Canon Powershot S3 IS camera and then we extracted the audio from the recorded video clip in .wav non-lossy format. Call analyses were done in Raven Pro (oscillogram, spectrogram) and Audacity (power spectrum) software. Species Distribution Modelling was done, including the new additional localities following the methods stated in Bhargavi *et al.* (2013), using the same algorithms and software MaxEnt (Phillips *et al.*, 2006) with topographic and bioclimatic variables in WorldClim database (Hijman *et al.*, 2005).

RESULTS

Duttaphrynus hololius (Günther, 1876)

Description of new specimens from Hyderabad (Figure 1a; Table 1): Snout-vent length of a medium size (32–47mm); body dorso-ventrally depressed; head width (19.46 mm) greater than head length (17.04 mm); flat above; without any cephalic ridges on top; loreal well-defined, with a canthus rostralis; interorbital space (8.71 mm), over two times the width of intermarial space (3.5 mm); nostrils nearly circular (may be the artefact of preservation), situated much closer to tip of snout-tip (1 mm) compared to eye (2 mm); pupil horizontally elongate; tympanum visible, ca. 70% the size of horizontal eye diameter (5.18 mm), sides of head with well-defined paratoid glands that appear as flattened globular structures, located over temporal to shoulder regions; skin smooth, with beset with some small isolated circular glandules, especially along the sides of the torso; no skin folds on sides of head or limbs; underside mostly smooth but becomes rugose when nearing the groin; forelimbs and hind limbs with a few glandular tubercles above, but none beneath; digit-tips, except the first finger, without enlarged discs; ventrolateral grooves not present on dilated tips; fingers lacking webs, toes mildly webbed; webbing confined to the digit base; fourth toe webbing does not exceed the penultimate subarticular tubercle; subarticular tubercles and palmar tubercles present; supramarginal tubercles small, especially in the largest of specimens, being feeble to absent in smaller ones.

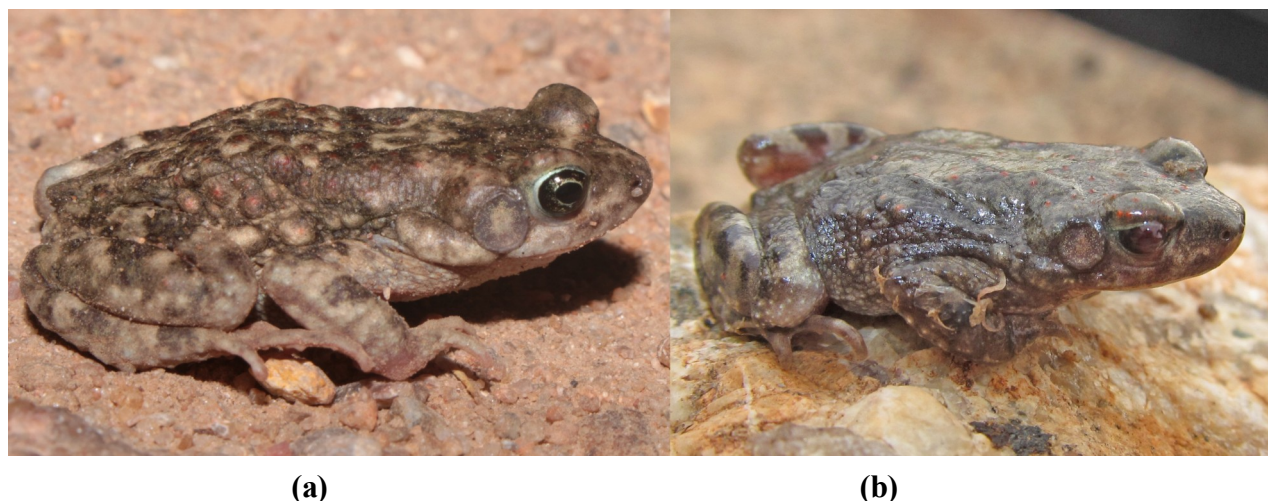


Figure 1. *Duttaphrynus hololius*: (a) NHM.OU.AMP.DH.1, in life from Hyderabad Photo: GCK, (b) live individual with macrophthalmia and red-eye syndrome, from Hampi, Photo: SRG.

Table 1. Morphological details of Hyderabad specimens (NHM.OU.AMP.DH.1-3), measurements taken in mm.

Characters	DH1	DH2	DH3	Mean
Snout to vent length	46.33	35.24	32.8	38.12
Head Width	19.46	14.49	13.26	15.73
Head Length	17.04	12.19	13.77	14.3
Mandible edge to nostril distance	15.99	10.59	9.25	11.94
Mandible edge to front of eye distance	12.5	7.59	6.35	8.81
Mandible edge to back of eye distance	8.58	3.74	3.65	5.32
Distance between front of eyes	8.71	6.45	5.58	6.91
Distance between back of eyes	12.45	11.25	9.9	11.2
Forelimb Length	10.16	8.2	7.95	8.7
Hand Length	11.69	7.86	8.33	9.29
Length of 3 rd finger	4.67	4.37	4.9	4.64
Tibia length	17.73	13.82	13.17	14.9
Foot length	15.82	9.88	11.65	12.45
Length of 4 th toe	10.3	6.29	8.01	8.2
Internarial distance	3.5	3.49	2.46	3.15
Distance of eye to nostril	3.31	3.03	3.24	3.19
Eye length	5.18	3.87	2.94	3.9
Tympanum diameter	3.66	3.28	3.1	3.34
Tympanum to eye distance	0.66	0.43	0.7	0.59
Length of inner metatarsal tubercle	2.93	1.41	1.69	2.01
Inner toe length	3.7	1.58	2.19	2.49
Metatarsal tubercle to 4 th & 5 th toe web distance	7.83	6.18	5.96	6.65
Metatarsal tubercle to 3 rd & 4 th toe web distance	7.37	6.35	6.15	6.62
Length of webless part of 4 th toe from 3 rd toe	8.26	4.85	5.5	6.2
Length of webless part of 4 th toe from 5 th toe	7.51	4.42	5.19	5.70
Length of tarsus and foot	18.36	12.51	13.77	14.8
Femur length	16.56	12.46	12.57	13.86
Tibia width	4.27	3.36	2.02	3.21
Distance between upper eyelids	5.47	4.39	4.57	4.81
Maximum width of upper eyelid	3.75	3.52	3.18	3.18
Distance from nostril to snout tip	1.49	1.8	0.86	0.86
Distance from eye to snout tip	5.6	5.07	4.67	5.1

Locality and habitat: On 19th June 2017, three specimens of *D. hololius* were collected at 21.30h (Indian Std. Time), from small ephemeral puddles formed with the onset of rains on the rocky surfaces at TSFA Telangana State Forest Academy (17°32'38"N 78°27'32"E; 595 m asl), Telangana uplands, Peninsular India. Also this region is not quite surrounded by typically vast formations of rocky hillocks encompassed with low-lined rock-boulders but in fact has human settlements nearby, being present one the northern fringes of a big capital city—Hyderabad. This locality is one of the dry deciduous forest patches within the city precincts. Subsequent visits also revealed the breeding of this species with the sightings of tadpoles in the water puddles on the rocky surfaces where the individuals first sighted.

A case of deformity (Figure 1b): On 11th Oct. 2016, during fieldwork in Hampi hillocks (15.332°N, 76.467°E; 460 m asl) in western parts of Deccan plateau,

we encountered a live subadult *D. hololius* with an eye deformity. Its right eye was deformed with a condition known as macrophthalmia with red eye syndrome presenting itself with a large, inflated, blood-shot appearance. Its left eye was normal. The toad apparently responded to visual cues (e.g. waving hand, approaching / moving objects) shown at its left, but not at the right side, indicating visual impairment of the affected right eye.

Courtship and calling behavior (Figure 2): Between 17th and 19th July 2017, we observed *D. hololius* in situ within Rishi Valley School (13.633°N, 78.458°E; 770 m asl) campus and its outskirts.

On 17th, active adults were sighted hopping about on rocks, from as early as 19:10 hrs. Calls were heard but despite attempts to locate the calling toads, none was seen. After careful examination, their calls were found to be intermixed with the synchronously calling louder frog *Sphaerotheca* sp. The ponds were full of *Sphaerotheca*



Figure 2. *Duttaphrynus hololius*: (a) calling male, (b) male and female in accompaniment, (c) & (d) adult male and female in amplexus, Photos: SRG.

sp. and other frogs such as *Euphlyctis* spp. Two female toads (determined after examining gular and carpal regions for vocal sac and nuptial pad respectively) were sighted inside a puddle. None of them were calling and were not sighted in a place where the toad calls were heard. Instead, *Sphaerotheca* sp. and *E. cyanophlyctis* calls were heard and were also seen in the same pond. Till around 23:00 h, acoustic surveys were conducted throughout the campus in every rock-pool formation.

On 18th, calling male toads were located. Individuals were located by tracing calls and toads with distended vocal sacs were seen in ephemeral pools and puddles on rock formations. The calling toads were very shy and immediately stopped calling as soon as spot-lights focused on them. From a survey of 23 such ponds in the vicinity, a total of 8 adults were sighted. From toads that were sighted, calls were recorded using Canon SX130 camera and audio extracted and saved in .wav, non-lossy format. At 20:20 h, two toads mounted on one another were sighted. Two more toads, both calling individuals, were also sighted nearby. At first glance, it appeared to be an amplexant pair, but then both the mounting and the mounted toads were calling. When a different type of squeaky call was emitted by the mounted toad, the mounting toad realized that it too was a male and released the grip. Subsequently, both the individuals were examined and determined to be males, based on a whitish single mid-gular vocal sac and nuptial thumb pads.

On 19th, an amplexant pair was sighted at 19:45 h. This pair hopped away into a crevice and could not be located subsequently. At 20:45 h, two adult toads were

seen in accompaniment, one calling while the other silently sitting nearby. The calling toad (male) approached the silent (female) toad and the female resisted the male toad's advancements. By 21:00 h, the male mounted on the female and the copulation started. The female first tried to hop away from the male, but then the male started holding on to the female and mounted. Amplexus was observed. It was axillary, with sometimes the male sliding backwards as the female hopped forward. The female toad with the male on top, slowly hopped into water and stretched out the limbs. The male was holding on to the female and quick successive jerky movements were observed. Spawning happened very quickly and within seconds the female oviposited in water and hopped out. In as far as visible in the murky water disturbed by the ovipositing female, the eggs appeared as creamy white beady rounded structures. From 21:00 h, the amplexus lasted till 21:35 h, at which time, the male descended from the female and parted.

Call descriptions (Figure 3 a, b): The analysed calls were recorded from a 65 mm long male, at a distance of 1 m from the calling individual. During the recording, the ambient temperature ranged 24.4–25.8°C and the humidity ranged 69–76%. Two calls — type-I that is the typical default mating call of this species and a type-II call that is the squeaky distress call of male, mainly during male-male physical interactions were documented. In type-I call (by single calling male), the individual pulsed notes were very frequent and with much shorter inter-note intervals. On the contrary, in type-II call (by multiple males in physical contact), the duration of an individual pulsed

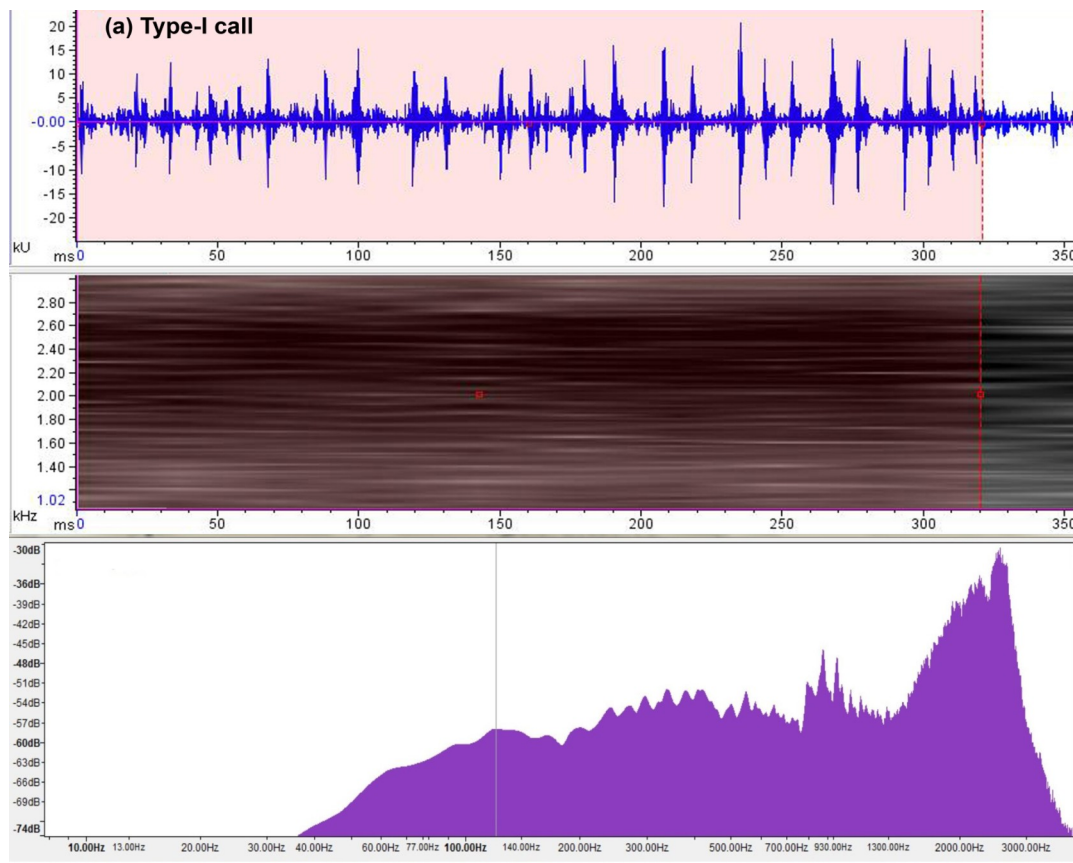


Figure 3a. Type I call of *Duttaphrynus hololius*, oscillogram, spectrogram and power spectrum

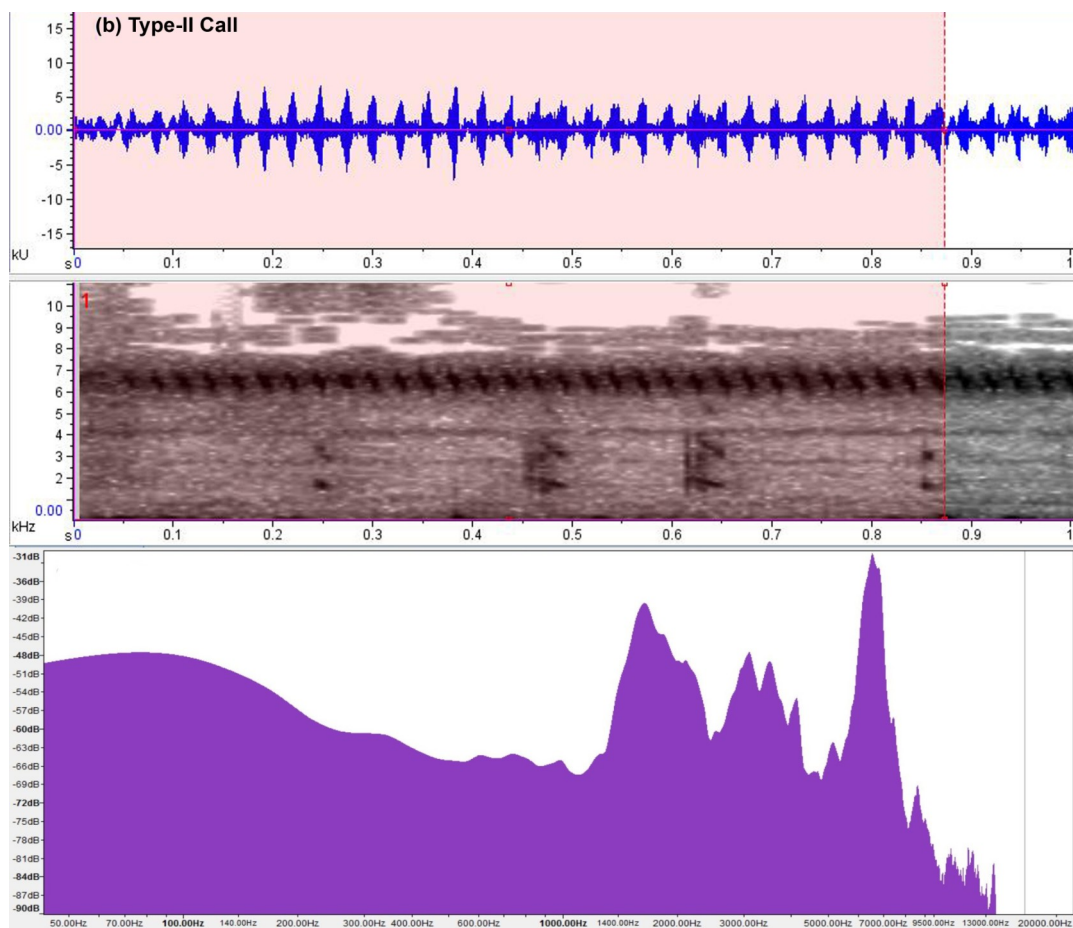


Figure 3b. Type II call of *Duttaphrynus hololius*, oscillogram, spectrogram and power spectrum

note is more or less equal to the inter-note interval. Both the calls are described herein.

Type-I call: Call syllable: *ttrrre-ttrree-ttrre*. A 4 s long portion comprising of a series of multi-pulsed notes was analysed. A single call comprised of 14–17 notes ($n = 9$ notes), many of which are either single or multi-pulsed. Duration of pulsed notes ranged from 0.002–0.007 s with an average of $0.004 \text{ s} \pm 0.0016$; amplitude ranged from 8.34–18.41 kU, with an average of $13.081 \text{ kU} \pm 3.155$; inter-note pulse interval ranged from 0.002–0.032 s, with an average of $0.0163 \text{ s} \pm 0.006$; frequency ranged from 2550–2724 Hz, with an average of $2620.043 \text{ Hz} \pm 764.635$; power ranged from 69.9–83.3 dB, with an average of $77.29 \text{ dB} \pm 6.491$.

Type-II call: Call syllable: *pip-pi-pi-pippii*. An 8 s long portion comprising of a series of 35 multi-pulsed notes was analysed. These calls were uttered in random fashion often as individual squeaks or a series thereof ($n=6$ calls). Duration of pulsed notes ranged from 0.01–0.022 s, with an average of $0.013 \pm 0.002 \text{ s}$; amplitude ranged from 3.60–7.49 kU, with an average of $4.808 \text{ kU} \pm 0.80$; inter-note pulse interval ranged from 0.007–0.017 s, with an average of $0.014 \text{ s} \pm 0.002$; frequency ranged from 6704–7193 Hz, with an average of $6953.583 \text{ Hz} \pm 128.021$; power ranged from 58.4–81.5 dB, with an average of $68.879 \text{ dB} \pm 6.112$.

Species Distribution Modelling Analyses (Figure 4):

Our mapping and Species Distribution Modelling (SDM) using MaxEnt maximum entropy algorithm revealed a much better, refined picture of its distribution. The new analyses produced projected occurrence maps in Hot to Cold colour scheme, showing high probabilities ranging 77–92% principally in the Mysore plateau, surrounded by low probability ranging 23–31% areas in the surrounding areas south of Cauvery (11°N) and north of Penner (13°N) Rivers. The northern block is again split into a western and eastern cluster separated by low-lying Thungabhadra-Krishna ($14\text{--}15^\circ\text{N}$ $76\text{--}78^\circ\text{E}$) river systems. Most of the projected area is characterized by a probability ranging at 38–69%. Despite being hilly, the Western Ghats were projected to be totally unsuitable zone ($< 8\%$), as revealed by abrupt demarcation of colour schemes that fully excludes these hills from its realized range.

The map revealed comparatively lesser probability $< 31\%$ in the periphery of its range, including from where the current record hails (Hyderabad), as well as the previous new, northernmost regional record (Visakhapatnam) when the earlier SDM was done. Additionally, the map projected the leeward foothills of the central Western Ghats and Sahyadris as low probability regions, which are places devoid of actual sighting records. Apart from the southerly Mysore plateau, other records and projected regions form distinct clusters or units that piece together satellite units. Finally, our projections brought out the hilly ($> 500 \text{ m asl}$), dry zone region bounded by Bangalore-Tirupati-Salem to be of the hottest colour schemes, indicating the highest probability ($> 85\%$) of its occurrence. This is more consistent with on-ground observations and is a vital refinement in its distribution (pers. obs.).

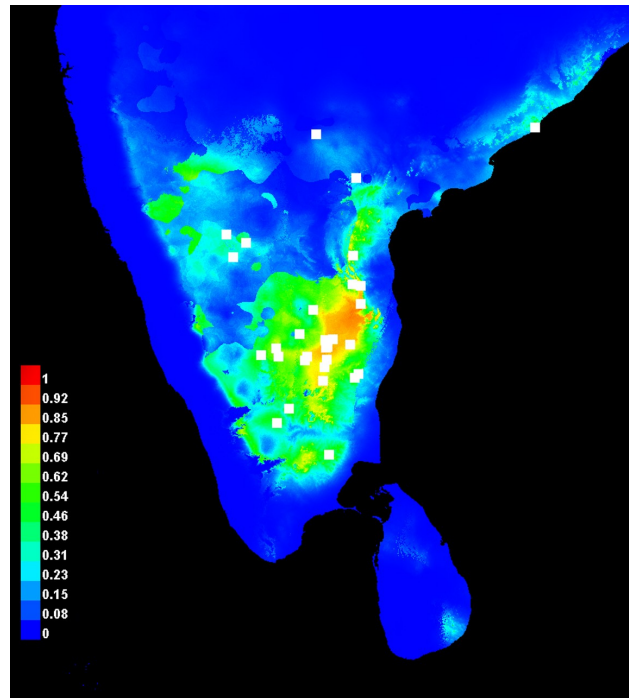


Figure 4. MaxEnt SDM Map projection for *Duttaphrynus hololius*.

DISCUSSIONS

To the best of our knowledge, this is the first ever documentation of both courtship and calling behavior in this little-known species. Though the tadpole morphology and developmental cycle has been documented earlier (Ganesh *et al.*, 2013; Chandramouli & Kalaimani, 2014), no information exist till now on its calling and courtship. The same holds true for the report on deformity as well. We also provide a significant additional morphological characterization of an unsampled outlier population from mid-Deccan (Hyderabad) (also see Adimallai *et al.*, 2013; Bhargavi *et al.*, 2013). Our new SDM analyses have further refined the results of Bhargavi *et al.* (2013). In the following we elaborate on these various aspects of our findings in detail.

Our finding from a peri-urban locality (just north of Hyderabad) is comparable only to a three decades old report from Bannerghatta, just south of Bangalore (Daniels, 1992). Even here, as it is an old record and as Bannerghatta continuously has a Protected Area within, it is unclear if *D. hololius* was sighted practically in a synanthropic setting, like this find from Hyderabad. Hyderabad is one of the records from the highest of latitudes, comparable only with that of Bhargavi *et al.* (2013) report from Visakhapatnam. The habitat of TSFA campus is of the southern tropical dry deciduous type intermingled with scrub vegetation (Champion and Seth, 1968), predominant in the Deccan trap of south-central part of Indian peninsula.

Our mapping and Species Distribution Modelling analyses also adds much to the existing knowledge on the distribution of this species, compared to that reported earlier (Bhargavi *et al.*, 2013) in projecting the rocky landscapes of southeastern India as its realized range with high probability, surrounded by cold colour zones

that sharply ends at the Western Ghats. In keeping with previous results by Bhargavi *et al.* (2013), the Western Ghats are shown to be extremely unsuitable areas. This quantitative analysis further bolsters to refute the erroneous claims of 'Malabar' being its type locality. But unlike in Bhargavi *et al.* (2013) map, in the new analysis even the windward sides of Western Ghats and flood-plain belts of almost all river systems were also coded as unsuitable zones. This is much closer to the on-ground species occurrence pattern.

Reports on amphibian abnormalities are rather rare in India (see Ganesh & Arumugam, 2015; Gurushankara *et al.*, 2007; Hippargi *et al.*, 2010; Modak *et al.*, 2013; Narayana *et al.*, 2017). Certainly, no deformities or any pathogenic impact has so far been reported in this species. Thus, the current report forms the first published instance of deformity recorded in this species, despite a spate of wild sightings in the last decade (see Chandramouli *et al.*, 2011; Ganesh *et al.*, 2018). Our report from the outskirts of Hyderabad city also forms the first report of this species from a predominantly synanthropic setting and thereby exposed to anthropogenic impacts. This situation calls for continued monitoring and surveys of this species in other such cityscapes known to potentially harbour this species, such as peri-urban Bangalore (see Daniels, 1992; Fig. 4 in our work).

As for the breeding observation and call descriptions, these integral natural history details are presented herein for the first time for *D. hololius*. Very few south Asian toads have their calls characterized (Grosjean & Dubois, 2001). Oddly enough, our observations of the association of *D. hololius* with *Sphaerotheca* sp. during the breeding season, is in support of previous such observations by Vivek *et al.* (2014). Vivek *et al.* (2014) observed inter-specific interactions during breeding, between *D. stomaticus* and *Sphaerotheca breviceps* in Aravalli foothills of Rajasthan, with uncanny resemblance to our case. Taken together, our new information on the occurrence, range projections, threats such as deformities and vital natural history traits like breeding and calling have filled in lacunae on what is perhaps the only amphibian endemic to Indian peninsula's dry zone – *Duttaphrynus hololius*.

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