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Communication

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Abstract: The dorsal surface of *Duttaphrynus melanostictus* is covered with keratinized warts of various sizes and shapes. Using combinations and patterns of cranial and mid dorsal warts, we attempted to identify individual toads from a natural population as a non-invasive alternative to existing marking techniques based on toe clipping, pit tagging, and subcutaneous elastomer injections to facilitate population estimates. An accuracy of 100% identification was achieved via this method, making it a potent tool for population studies in this species that is faster, cheaper and less disruptive than standard marking techniques.

Keywords: Capture-recapture, *Duttaphrynus melanostictus*, individual identity, population estimation.

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Author Details: Mr. Uddalak Tathagato Bindhani is Project Fellow (Ecology) in the project entitled 'Population Management of species involved in Human-Wildlife Conflict', at the Wildlife Institute of India. He is interested in understanding the effects of anthropogenic activities on the behavioural ecology of wild fauna. Dr. Abhijit Das is Scientist/Faculty with the Department of Endangered Species Management, Wildlife Institute of India. His research interests lie in understanding the evolutionary origin and diversification of Himalayan herpetofauna.

Author Contribution: UTB conducted the field study, undertook photography and analyzed the data. AD designed the study and supervised UTB.

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भारतीय वन्यजीव संस्थान
Wildlife Institute of India

INTRODUCTION

Accurate estimates of population size are essential for the effective management and conservation of species, and capture-recapture methods are frequently employed in an attempt to obtain rigorous population estimates (Begon 1979; Donnelly et al. 1994; Lettink 2012). The ability to recognize individuals within a population is fundamental to most capture-recapture methods. Individuals can potentially be recognized by artificial marks, or for species that exhibit sufficient phenotypic variation, by natural markings (Heyer et al. 1994; Sutherland 2006). Artificially marking animals usually involves capturing and handling, which can stress individuals and/or lead to injury (Bradfield 2004). Marking often creates a wound, which is a potential site of infection. In the case of amphibians, artificial marking usually involves tagging, toe-clipping, branding, tattooing, subcutaneous elastomer injections, or subcutaneous pit tags (Donnelly et al. 1994). Studies of the effects of these artificial marking techniques on behaviour and survival rates have reported conflicting results, indicating that it may be difficult to make broad generalisations about the effects of these techniques on amphibians (Donnelly et al. 1994; Bradfield 2004; Lettink 2012).

Negative effects of artificial marking techniques on amphibians have significant implications for population monitoring via capture-recapture methods. Altered behaviour and increased mortality resulting from marking violate an assumption that underlies most capture-recapture methods, namely that the probability of recapture is not affected by marking (Caughley et al. 1994) and does not jeopardise reproduction and growth. This is especially relevant to studies of threatened species.

The use of natural features or markings to identify individuals within a population is non-invasive, and therefore does not pose the same risk as invasive artificial marking techniques. Individuals or a particular region of their bodies can be either drawn or photographed, and the resulting images compared with the images for all previous captures (Bradfield 2004; Caorsi et al. 2012). The Common Asian Toad *Duttaphrynus melanostictus*, is a widespread amphibian species in the Indian sub-continent that exhibits a preference for human modified habitats and homesteads (Daniels 2005). Each *D. melanostictus* shows a distinct pattern of black, keratinized warts on the dorsal surface. These warts are typically paired except in toads less than 6 months old, and their patterns are not sexually dimorphic (Daniel 2002; Daniels 2005).

The objectives of this study were to determine whether photographic identification of naturally marked animals can be used to identify individual *Duttaphrynus melanostictus*. An earlier observation suggested that individual toads differed from one another in the pattern of dorsal warts, but no description of warts and methodology for identification was provided (Daniels 1994). Thus, we classified dorsal warts with respect to their position and combination and developed a simple method for efficient identification of individuals via visual analysis.

MATERIALS AND METHODS

Study site

The following study was conducted at the Chandrabani Forest Division (30.283^{0}E & 77.974^{0}N), Wildlife Institute of India campus, Dehradun (Fig. 1). The study area is ~3.44ha. The region is characterized with a sub-tropical climate, experiencing cold winters, warm springs, hot summers and a strong monsoon. The average annual rainfall received is usually around ~2073.3mm.

The vegetation is natural and semi-natural represented by a mosaic of natural scrub, woodland, various successional stages of *Shorea robusta* forest including stream bank vegetation and grassy banks. Thirty-three species of herpetofauna inhabit the campus; amphibians: 11 species belonging to four families (Bufonidae, Microhylidae, Rhacophoridae, Dicroglossidae), with two species of toads, and reptiles: 22 species belonging to nine families (Colubridae, Typhlophidae, Elapidae, Agamidae, Varanidae, Natricidae, Trionichydae, Geoemydidae, Scincidae) as listed on the campus database (www.wii.gov.in). All wildernesses are in close proximity to and in certain parts, interspersed with human habitation.

The study was conducted from mid March to the first week of May 2015. Dehradun, having already received its early showers at the end of February and early March, marked the onset of breeding activity of *Duttaphrynus melanostictus*. Tadpoles started appearing at the natal site by the end of March to early April.

Methods

During the study period (18 March 2015 – 28 April 2015), regular night surveys were conducted for 42 days. Usually a set of two digital images were generated for each captured animal, whereby the first image was in

Figure 1. Location of Wildlife Institute of India, Uttarakhand, India.

portrait mode and the second in landscape mode. This was done in order to obtain clear and properly focussed image sets for the dorsal side of each individual. This also helped in negating the problems in analysing the wart patterns due to discrepancies in the position of the animal when being photographed. If an animal exhibited certain distinct marks (such as deformity or scar), features or patterns on any part of the body, a third digital image was generated to showcase the distinctive features. The digital images generated were assigned unique identification codes, affixed with other data of the animal collected, and clear black and white photographs were obtained by printing a single photograph in the complete frame of an A4 sheet. The photographs were then subjected to visual analysis and manual scrutiny in order to determine the distinctive aspects of individuals.

For the purpose of this study the dorsal warts and associated structures were classified with respect to the position of their occurrence on toads as follows (Image 1; Table 1):

Snout warts: Keratinized small tubercles present in between cranial ridges in front of upper eyelids. The position and pattern of distribution with respect to the cranial ridges as well as arrangements of these warts are used as one character. It is a discontinuous character as toads may lack tubercles or warts in this region.

Crown warts: The region of the head from the point where the cranial ridge is notched along the eye to the anterior end of the parotoid glands is termed the crown

Table 1. Depicting the dorsal warts and their coding.

Character	Type	Code
Snout Warts	Discontinuous (not encountered in all individuals)	SW
Crown Warts	Discontinuous	CW
1st Primary Dorsal Wart Pair	Continuous (encountered in all individuals)	1PD
2nd Primary Dorsal Wart Pair	Continuous	2PD
3rd Primary Dorsal Wart Pair	Continuous	3PD
Parotoid Warts	Discontinuous	PW

(sensu stricto Daniel 2002), hence keratinized tubercles present in this region toad are referred to as crown warts. The position of these warts with respect to the cranial ridges, parotoid glands and the first primary dorsal wart pair exhibits great variation, as do their shapes and patterns of appearance. This is a discontinuous character, with some toads lacking crown warts.

Mid-dorsal groove: An associated structure aiding in the classification of dorsal warts and subsequent identification of individuals. A distinct dorsal groove is observed along the vertebral axis of the toad on the dorsal side. It becomes conspicuous along the plane of the anterior portion parotoid glands, just behind the crown, and runs all through the entire length of the body up to the vent. The mid-dorsal groove is usually smooth and usually lacks any tubercles or warts but a few keratinized tubercles might be found in the groove. The region shows distinct lateral undulations.

Primary dorsal wart pairs: Two series of large warts along the middle of the dorsal surface of the toad's body and exhibiting a certain degree of symmetry on either side of the distinct mid-dorsal groove. The primary dorsal warts are considerably enlarged and usually more keratinized in the adults. The primary dorsal warts appear to maintain a constant distance from the mid-dorsal groove. A certain wart of a pair may often be found associated in close proximity to a small secondary or satellite wart alongside it. They show great variability in their position and pattern of distribution. They are commonly found to be oval or spheroid in shape though some individuals do exhibit a conspicuous shape.

1st primary dorsal wart pair: It is the first large, distinct and keratinized wart encountered after the crown warts. It follows just behind the origin of the mid-dorsal groove, in the hind neck region, and is placed within one-third of the length of the parotoid glands from the anterior end. It often exhibits variability in shape and in the symmetry of its position on either side of the mid-dorsal groove. A satellite wart is found quite rarely. It is a continuous

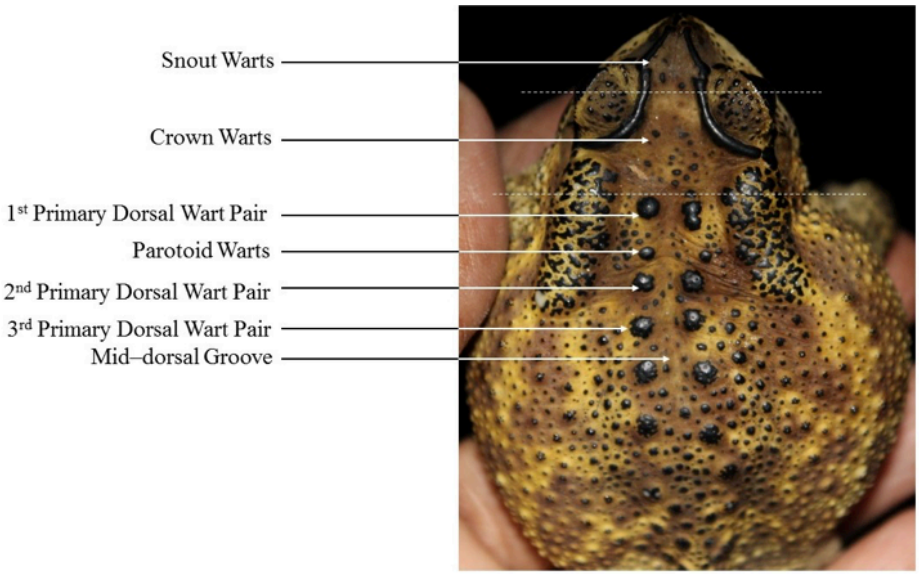

Image 1. Primary dorsal warts on *Duttaphrynus melanostictus*.

character being observed in all individuals.

2nd & 3rd primary dorsal wart pair: These are found closely associated to each other usually towards the distal end with respect to the plane of the parotoid glands. Variability in shape is less conspicuous and they usually are spheroid or ovate. Often found associated with a satellite wart. Varies with respect to position and pattern. It is a continuous character, being encountered in all individuals.

Parotoid warts: These warts are encountered along the parotoid glands on either side of the mid-dorsal groove and in the region between the 1st and 2nd primary dorsal wart pairs. They exhibit great variability with respect to their pattern, shape, position and distribution. It is a discontinuous character as certain toads don't bear these warts.

The above mentioned potential characters may be coded as shown in Table 1.

The photographs of the *Duttaphrynus melanostictus* individuals, obtained during the duration of the nocturnal surveys at the study site, were subjected to rigorous visual scrutiny, matching and analysis. We then attempted to segregate the individual toads based on the combinations of dorsal warts required to effectively distinguish and identify individuals. This allowed us to arrive at a pattern to be followed during visual analysis of photographs while sequestering individual toads to a sub-group.

RESULTS

The 1st primary dorsal wart pair was found to be the initial basis of analyzing the dorsal warts, owing to its apparent consistency in position and shape. The other dorsal wart characteristics were now used in combination to the 1st primary dorsal wart pair to distinguish and identify the individuals. Visual matching and analysis thus led to the development of a combination of dorsal wart characters based on which the individuals were subjected to effective individual identification and subsequent grouping as mentioned in Table 2.

The photographs of the *Duttaphrynus melanostictus* individuals, obtained during the duration of the nocturnal surveys at the study site, were subjected to rigorous visual scrutiny, matching and analysis (Appendix 2). We then attempted to segregate the individual toads based on the combinations of dorsal warts required to effectively distinguish and identify individuals. This allowed us to arrive at a pattern to be followed during visual analysis of photographs while sequestering individual toads to a sub-group.

DISCUSSION

In the wake of the rising concerns of global decline in amphibian populations (Stuart et al. 2004; Whittaker et al. 2013) the need of methods and protocols for sampling natural populations of amphibians has been greatly realized. It is here that mark-recapture techniques of

Table 2. Dorsal wart pattern combinations used for individual identification of toads.

Combination of dorsal wart characters	Combination code	Remarks
1st primary dorsal wart pair only	1PD	Based on variability of shape, size, pattern, satellite wart and symmetry.
1st primary dorsal wart pair + 2nd primary dorsal wart pair + 3rd primary dorsal wart pair	1PD + 2PD + 3PD	Based on variability of satellite warts, pattern and position.
1st primary dorsal wart pair + parotoid warts	1PD + PW	Based on variability of pattern, position and size.
1st primary dorsal wart pair + crown warts	1PD + CW	Based on variability of pattern, position and size.
1st primary dorsal wart pair + snout warts	1PD + SW	Based on variability of pattern and position.
1st primary dorsal wart pair + parotoid warts + 2nd primary dorsal wart pair	1PD + PW + 2PD	Based on variability of shape, size, pattern, satellite wart and symmetry.

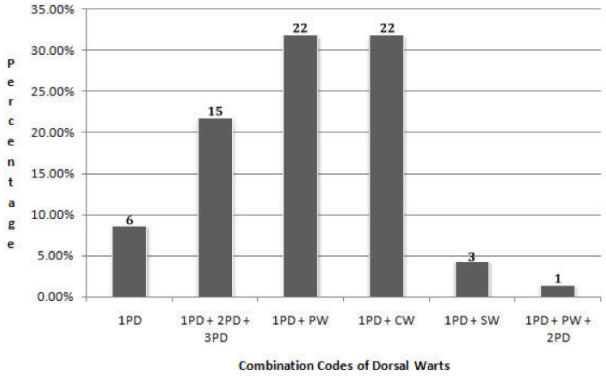

Figure 2. Graph showing the percentage of individuals identified based on the criteria of wart pattern combination used. The numerals atop the bars represent the number of individuals identified via the respective pattern combination. (Also refer Appendix II).

capturing, marking, releasing and recapturing animals have become an indispensable tool to monitor and estimate trends in populations. Mark-recapture techniques are advantageous, being statistically more accurate and robust than uncorrected counts of indices of relative abundance (Lettink 2012).

Visual image matching of natural markings is significantly more accurate than invasive techniques like toe-clipping and computer-assisted image matching, which though useful for large datasets are constrained by the position and posture of the animal, glare, shadows, lighting, background colour, equipment and cumbersome processing protocols to be followed, which can expose animals to prolonged durations of stress and handling (Caorsi et al. 2012; Sanchez et al. 2018). Invasive techniques like toe-clipping, especially for the first finger, might adversely affect amplexus in males owing to the loss of the nuptial callosities on phalanges. Thus there is a need for non-invasive identification techniques, as amphibians are most active during the breeding season (Sutherland 2006).

Dorsal warts were found to be a reliable and cheap way to ascertain and monitor populations in *Duttaphrynus melanostictus*. Thus, the technique may also be used in capture-recapture studies of this species. The study achieved an accuracy of 100%, whereby the digital image sets of the toads successfully distinguished and identified all 69 individuals (Appendix 1).

The results indicate that it should be possible to efficiently process photographs of unidentified captures in a full-scale monitoring programme by using the combination code key to identify and determine the identity of any given capture.

Analysis of photographs of 69 toads identified six combinations which resulted in optimal allocation of individuals into captures and recaptures. The decision as to which of these combinations to use in future studies shall depend on the clarity of the photographs of the dorsal side of the toad taken in the field. It is recommended that the 1st primary dorsal wart pair should be considered initially.

The 2nd primary dorsal wart pair was always found to be in symmetry, pattern and variation with the 3rd primary dorsal wart pair or the parotoid warts if present. Thus, establishment of individual identity was never made based on the 2nd and 3rd primary dorsal wart patterns alone, and thus were considered a separate combined character combination with the 1st primary dorsal wart pair.

It might also be mentioned that there were eight toads (~ 11.59%) that also exhibited certain distinct marks, patterns, wounds or infections. Preliminarily these could be used as a cue for individual identification, especially when in the field, complementing the dorsal wart patterns. But, it was seen that there was no constancy (wounds and infections heal, body marks might be lost during moulting and sloughing of skin etc.) of these characters, and thus they are unsuitable for application to individual identification in the long run.

This synchronized scientific method is simple to

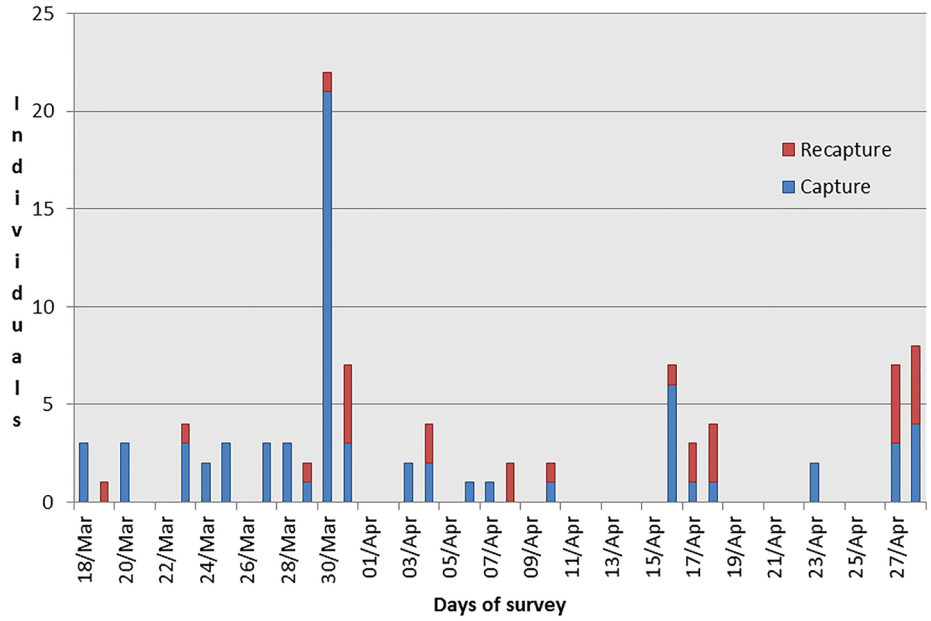

Figure 3. Graph showing the number of captures and recaptures of toads during each night survey of the study period.

follow and easy to implement, and thus can even be utilized by laymen in the field of biology to monitor toads in their backyards. The study also holds great value, both scientific and economic, in keeping tabs of toad populations threatened from road related mortality. It thus shows great potential to be successfully utilized and implemented in citizen science programmes aimed at studying amphibians.

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Appendix 1. Data sheet table, as prepared, for the creation of dorsal wart pattern combination codes and thus, distinctly identifying individuals of *D. melanostictus* from the Wildlife Institute of India campus, where '1' represents the character being used and '0' represents that the character wasn't utilized for identifying the individual:

Individual Id.	1 – PDW	2 – PDW	3 – PDW	SW	CW	PW
#1.	1	0	0	0	0	0
#2.	1	0	0	0	0	1
#3.	1	0	0	0	1	0
#4.	1	0	0	0	0	1
#5.	1	1	1	0	0	0
#6.	1	0	0	0	1	0
#7.	1	0	0	0	1	0
#8.	1	0	0	0	0	1
#9.	1	0	0	0	0	1
#10.	1	0	0	0	0	0
#11.	1	0	0	0	0	1
#12.	1	0	0	0	0	1
#13.	1	0	0	0	1	0
#14.	1	0	0	0	0	1
#15.	1	0	0	0	1	0
#16.	1	0	0	0	0	1
#17.	1	1	1	0	0	0
#18.	1	1	1	0	0	0
#19.	1	0	0	0	0	1
#20.	1	1	1	0	0	0
#21.	1	0	0	0	1	0
#22.	1	1	1	0	0	0
#23.	1	0	0	0	1	0
#24.	1	0	0	0	1	0
#25.	1	0	0	0	0	0
#26.	1	0	0	0	1	0
#27.	1	0	0	0	1	0
#28.	1	0	0	0	1	0
#29.	1	0	0	0	0	1
#30.	1	0	0	0	0	1
#31.	1	1	1	0	0	0
#32.	1	0	0	1	0	0
#33.	1	1	1	0	0	0
#34.	1	1	0	0	0	1
#35.	1	1	1	0	0	0
#36.	1	1	1	0	0	0
#37.	1	0	0	0	0	1
#38.	1	0	0	0	1	0
#39.	1	1	1	0	0	0
#40.	1	1	1	0	0	0
#41.	1	0	0	0	1	0
#42.	1	0	0	0	1	0
#43.	1	0	0	0	0	1

#44.	1	1	1	0	0	0
#45.	1	0	0	0	0	1
#46.	1	0	0	0	1	0
#47.	1	0	0	0	0	1
#48.	1	1	1	0	0	0
#49.	1	0	0	0	1	0
#50.	1	0	0	0	1	0
#51.	1	0	0	0	0	1
#52.	1	0	0	0	1	0
#53.	1	0	0	1	0	0
#54.	1	0	0	0	0	1
#55.	1	0	0	0	0	0
#56.	1	0	0	0	0	1
#57.	1	1	1	0	0	0
#58.	1	0	0	0	1	0
#59.	1	0	0	0	0	1
#60.	1	0	0	1	0	0
#61.	1	0	0	0	0	1
#62.	1	0	0	0	0	1
#63.	1	0	0	0	1	0
#64.	1	0	0	0	0	0
#65.	1	0	0	0	1	0
#66.	1	1	1	0	0	0
#67.	1	0	0	0	1	0
#68.	1	0	0	0	0	1
#69.	1	0	0	0	0	0

Appendix 2. Colour plates depicting the 69 individuals encountered during the study period, denoted as captures and recaptures with the date of encounter:

#1. 18/03/15. C

#2. 18/03/15. C

#3. 18/03/15. C

#3. 19/03/15. R

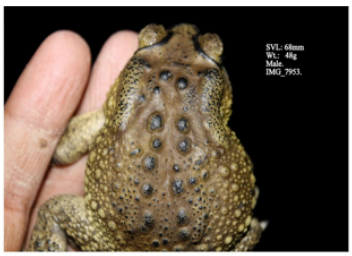
#4. 20/03/15. C

#5. 20/03/15. C

#6. 20/03/15. C

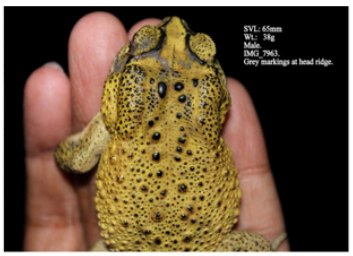
#6. 23/03/15. R

#7. 23/03/15. C

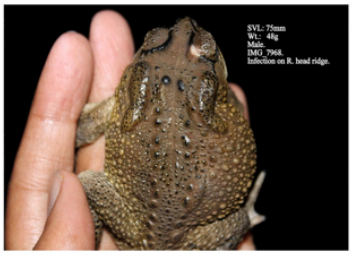


#8. 23/03/15. C

#9. 23/03/15. C



#10. 24/03/15. C

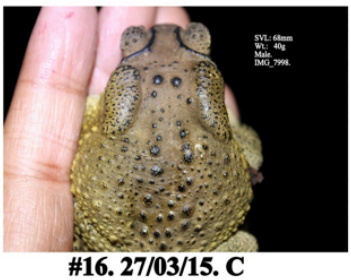


#11. 24/03/15. C

#12. 25/03/15. C

#13. 25/03/15. C

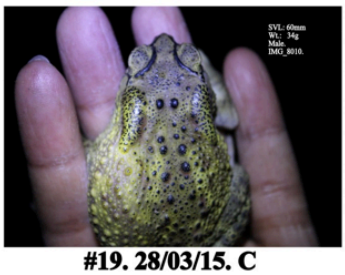
#15. 27/03/15. C



#16. 27/03/15. C

#17. 27/03/15. C

#18. 28/03/15. C

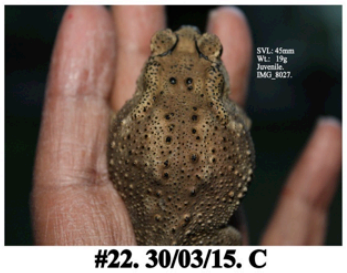


#19. 28/03/15. C

#20. 28/03/15. C

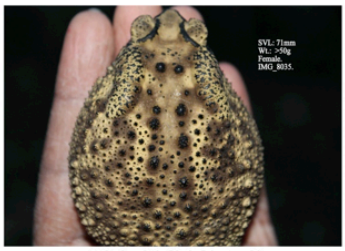
#13. 29/03/15. R

#21. 29/03/15. C



#22. 30/03/15. C

#23. 30/03/15. C



#24. 30/03/15. C

#25. 30/03/15. C

#26. 30/03/15. C

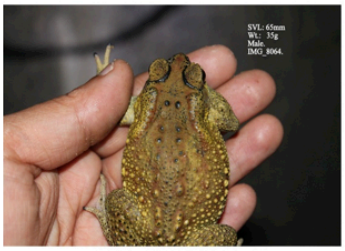
#27. 30/03/15. C

#28. 30/03/15. C

#29. 30/03/15. C

#30. 30/03/15. C

#31. 30/03/15. C



#32. 30/03/15. C

#33. 30/03/15. C

#34. 30/03/15. C

#35. 30/03/15. C

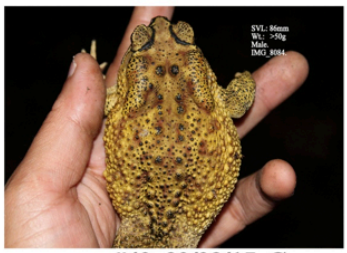
#36. 30/03/15. C

#17. 30/03/15. R

#37. 30/03/15. C

#38. 30/03/15. C

#39. 30/03/15. C

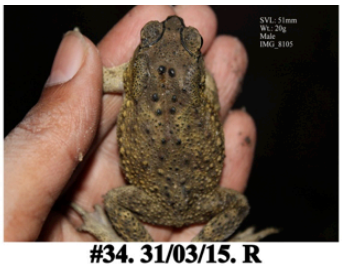


#40. 30/03/15. C

#41. 30/03/15. C

#42. 30/03/15. C © uTb.

#43. 31/03/15. C



#34. 31/03/15. R

#35. 31/03/15. R

#32. 31/03/15. R

#13. 31/03/15. R

#44. 31/03/15. C

#45. 31/03/15. C

#46. 03/04/15. C

#47. 03/04/15. C © uTb.

#35. 04/04/15. R

#34. 04/04/15. R

#48. 04/04/15. C

#49. 04/04/15. C

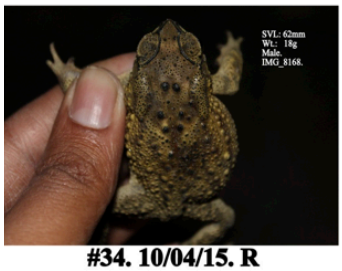
#50. 06/04/15. C

#51. 07/04/15. C

#48. 08/04/15. R

#34. 08/04/15. R

#52. 10/04/15. C © uTb.



#34. 10/04/15. R

#53. 16/04/15. C

#54. 16/04/15. C

#55. 16/04/15. C

#56. 16/04/15. C

#57. 16/04/15. C

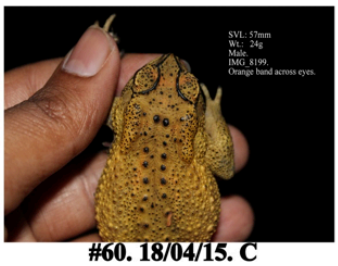
#58. 16/04/15. C

#21. 16/04/15. R

#59. 17/04/15. C © uTb.

#38. 17/04/15. R

#55. 17/04/15. R



#60. 18/04/15. C

#58. 18/04/15. R

#35. 18/04/15. R

#38. 18/04/15. R

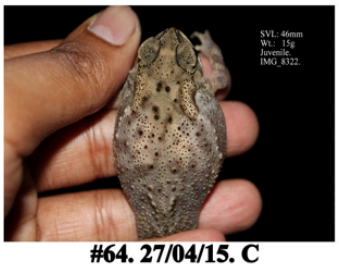
#61. 23/04/15. C

#62. 23/04/15. C

#55. 27/04/15. R © uTb.

#7. 27/04/15. R

#63. 27/04/15. C



#64. 27/04/15. C

#65. 27/04/15. C

#13. 27/04/15. R

#57. 27/04/15. R

#62. 28/04/15. R

#54. 28/04/15. R

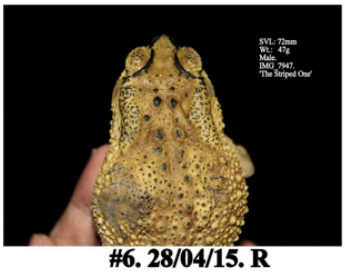
#66. 28/04/15. C © uTb.

#67. 28/04/15. C

#68. 28/04/15. C

#69. 28/04/15. C

#56. 28/04/15. R



#6. 28/04/15. R

#14. 25/03/15. C

Key:
#Individual Identification Number. Date (dd/mm/yy). C or R where, C: Capture and R: Recapture.

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