Lost, forgotten, and overlooked: systematic reassessment of two lesser-known toad species (Anura, Bufonidae) from Peninsular India and another wide-ranging northern species

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Abstract

We rediscovered two species of toads, *Bufo stomaticus peninsularis* and *Bufo brevirostris*, which were described from Peninsular India 84 and 101 years ago, respectively, but have not been reported since. Because the name-bearing types of both species are either damaged or lost, we provide detailed redescriptions, morphological comparisons, and insights into phylogenetic relationships with closely related members of the genus *Duttaphrynus* sensu lato, based on new material from the type locality of each species. We clarify and validate the identity of *D. brevirostris*, which was rediscovered from multiple localities in the Malenadu and adjoining coastal regions of Karnataka. We also demonstrate that *Bufo stomaticus peninsularis*, which was considered a synonym of *Duttaphrynus scaber*, is a distinct species. *Bufo stomaticus peninsularis* differs from *Duttaphrynus scaber* morphologically and genetically, and is more closely related to members of the *Duttaphrynus stomaticus* group. We also clarify the identity of the namesake species of the *Duttaphrynus stomaticus* group, which is reported widely in India and neighbouring countries, but lacks sufficient taxonomic information due to its brief original description and reportedly untraceable type material. We located and studied the complete syntype series of *D. stomaticus*, probably for the first time in over a century, and we report on the status of available specimens, provide detailed description of a potential type, compare it to related species, and clarify the species' geographical range. Our molecular analyses suggest that *D. stomaticus* is minimally divergent from, and possibly conspecific with, *D. olivaceus*. Our analyses also clarify its relationship to the closely-related *D. peninsularis* comb. nov., with which it was previously confused. Finally, our study provides other insights into the phylogenetic relationships and genetic differentiation among various species of *Duttaphrynus* toads.

Key Words

Amphibia, *Bufo stomaticus peninsularis*, distribution, *Duttaphrynus brevirostris*, *Duttaphrynus stomaticus* group, *Firouzophrynus*, molecular phylogeny, redescription, rediscovery, taxonomy

Introduction

The genus *Duttaphrynus* sensu lato, comprising 26 recognised Asian species, is a widely-distributed and commonly-occurring group of toads, found at elevations from sea level up to 2500 m asl (Frost et al. 2006; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015). The genus is represented by 19 species in India, 16 of which were described with type localities designated in the country. Among the Indian *Duttaphrynus* species, nine occur in Peninsular India and of these, six are endemic to the region. Although the wide-ranging species (*D. melanostictus*, *D. stomaticus*, *D. hololius*, and *D. scaber*) are frequently studied and reported from Peninsular India (Sarkar et al. 1993; Dutta 1997; Biju 2001; Chanda 2002; Van Bocxlaer et al. 2009; Dinesh et al. 2009; Srinivasulu et al. 2013; Ganesh et al. 2020), the taxonomic status of the endemic species has not been thoroughly investigated.
subsequent to their original descriptions (Dubois and Ohler 1999; Biju 2001). These include five recognised species—D. beddomii (Günther, 1876), D. brevirostris (Rao, 1937), D. microtympanum (Boulenger, 1882), D. parietalis (Boulenger, 1882), and D. silentvalleyniensis (Pillai, 1981). Their identities remain somewhat doubtful, due to reasons such as either brief or cursory original descriptions, unavailability of type specimens, or absence of new topotypic collections (Dubois and Ohler 1999; Biju 2001). In addition, identification of Duttaphrynus species is challenging, due to their overall phenotypic similarities and substantial intraspecific morphological variability (Inger 1972; Dubois and Ohler 1999; Biju 2001; Van Bocxlaer et al. 2010; Wogan et al. 2016; Jayawardena et al. 2017). Another four available names from Peninsular Indian regions exist as junior subjective synonyms (Dubois and Ohler 1999). Given such complex nomenclatural histories, misidentifications of Duttaphrynus species in museum specimens (S.D.B., personal observation) and regional biodiversity reports (Ray and Deuti 2008; Gururaja 2012; Hegde 2012; Seshadri et al. 2012; Ganesh et al. 2020) are frequent.

Two Duttaphrynus toads were described by C. R. Narayan Rao (15 August 1882–2 January 1960), who was among the most notable amphibian taxonomist in southern India during the colonial and post-colonial periods of the twentieth century. He described a total of 27 new species of frogs, including subspecies and varieties, largely from the states of Karnataka and Tamil Nadu (Rao 1920, 1922, 1937). However, a large number of his types (19 species; deposited in the Central College, Bangalore) are lost (Dubois 1984; Biju 2001). Seventeen of Rao’s species currently are recognised as valid; nine of these have had their name-bearing type status stabilised through designation of neotype specimens (e.g., Bossuyt and Dubois 2001; Biju et al. 2011, 2014a, 2014b; Garg et al. 2018). Similarly, the fate of Rao’s bufonid species has remained precarious: (1) Bufo brevirostris Rao, 1937 was described based on a single specimen from “Kempholey, Hassan District, Mysore State,” which subsequently was reported to be lost (Dubois 1984; Biju 2001). Hence, this species is known only from its original description. Dubois and Ohler (1999) discussed the problematic taxonomic status of this taxon, and, later Van Bocxlaer et al. (2009) transferred it to Duttaphrynus based on DNA sequences from a single specimen, without further information or discussion. The species continues to be recognised in the literature, albeit in the absence of new reliable records, photographs, or voucher specimens (Dutta 1997, Chanda 2002; Dinesh et al. 2009; Subramanian et al. 2013; Jayawardena et al. 2017). Additionally, (2) Bufo stomaticus peninsularis Rao, 1920 was described as a new variety of “Bufo stomaticus” from “Mavkote and Watekolle, Coorg,” based on a specimen (ZSIC 19176) designated as the holotype by Chanda et al. (2001 “2000”). This taxon was considered a synonym of Duttaphrynus stomaticus (Daniel 1963; Daniels 2005), until Srinivasulu et al.’s (2013) correction of some photograph-based misidentifications of “D. scaber” (not Duttaphrynus stomaticus peninsularis) as “D. stomaticus,” which was implicitly considered as the transfer of Bufo stomaticus peninsularis into the synonymy of Duttaphrynus scaber (Schneider, 1799) by Frost (2021). However, most recently Ganesh et al. (2020) made a curatorial statement referring to the identity of this taxon as “status: incertae sedis” without any clarification.

The confusing taxonomic status of Rao’s variety Bufo stomaticus peninsularis is also undeniably linked to its originally assigned species—Duttaphrynus stomaticus (Lütken, 1864). Although Srinivasulu et al. (2013) reported on misidentifications of D. stomaticus from Peninsular India, no studies to date have provided direct and conclusive evidence for either resolving the identity of Bufo stomaticus peninsularis or clarifying the occurrence of Duttaphrynus stomaticus in Peninsular India. The latter is considered as a widely distributed species in south and southwest Asia, with its range encompassing nearly the whole of India and the neighboring Bangladesh, Nepal, Pakistan, Afghanistan, and Iran (Stöck et al. 2006; Ragtegar et al. 2008; Van Bocxlaer et al. 2009; Shaikh et al. 2014; Portik and Papenfuss 2015; Nepali and Singh 2018; Frost 2021) (Suppl. material 1: Table S1). However, Duttaphrynus stomaticus was originally described from “ostindiske” (= East Indies or East India) (Lütken 1864), where its type locality was subsequently restricted to “Assam” (Boulenger 1891). Since type specimens were reported as untraceable (Dutta 1997), the identification of this species in recent literature is apparently based only on its brief original description, rather than examination of name-bearing types, or detailed redescription of topotypic material.

The present study was undertaken to conclusively resolve the taxonomic identity and stabilise the nomenclatural status of the two lesser-known Duttaphrynus toads from Peninsular India (Bufo brevirostris Rao, 1937 and Bufo stomaticus peninsularis Rao, 1920) and another wide-ranging northern species (Bufo stomaticus Lütken, 1864). We do so based on morphological comparison with original descriptions and available type specimens (except for D. brevirostris), as well as molecular and morphological insights gathered from new topotypic material, arguably rediscovered for the first time since both species’ original descriptions. We also aimed to infer phylogenetic relationships of the focal species, as well as gather insights on patterns of genetic differentiation among all known members of the genus Duttaphrynus that are characterised by known localities, and represented by accompanying voucher molecular data.

Materials and methods

Field study

Surveys were carried out for sampling the target species from regions encompassing their type localities in the Indian states of Karnataka, Andhra Pradesh, Tamil Nadu, and Assam. Additionally, some populations of
\textit{Duttaphrynus stomaticus} were randomly sampled from regions across India to understand intra and interspecific relationships. A total of 15 newly sampled populations are included in the study (Suppl. material 1: Tables S2 and S3). Surveys and sampling were conducted both during day and night hours, mostly during the pre-monsoon and monsoon months (April–August), but occasionally also at other times of the year (March and October). The sampled individuals were photographed to document colouration and characters in life, followed by euthanisation using Tricaine methanesulphonate (MS-222). Tissue samples were taken from the thigh muscle or liver, preserved in absolute ethanol, and stored at -20 °C for molecular studies. Locality information was recorded using a GPS with the WGS84 datum system. Distribution maps were prepared in QGIS version 2.6.1 (http://www.qgis.org).

**Morphological study**

Sex and maturity were determined by examining the gonads through a small lateral or ventral incision, or by the presence of secondary sexual characters (such as nuptial pads and vocal sacs in males). The following measurements were taken to the nearest 0.1 mm with digital slide-calipers: SVL (snout-vent length), HW (head width, at the angle of the jaws), HL (head length, from rear of mandible to tip of snout), SL (snout length, from tip of snout to anterior orbital border), EL (eye length, horizontal distance between bony orbital borders), IFE (internal front of the eye, shortest distance between the anterior orbital borders), IBE (internal back of the eyes, shortest distance between the posterior orbital borders), IUE (inner upper eyelid width, the shortest distance between the upper eyelids), UEW (maximum upper eyelid width), IN (internarial distance), NS (distance from the nostril to the tip of the snout), EN (distance from the front of the eye to the nostril), PD (minimum distance between parotoids), PL (maximum parotoid length), PW (maximum parotoid width), TYD (greatest tympanum diameter), TFE (distance from the tympanum to the back of the eye), FAL (forearm length, from flexed elbow to base of outer palmar tubercle), HAL (hand length, from base of outer palmar tubercle to tip of third finger), TL (thigh length, from the vent to the knee), SHL (shank length, from knee to heel), FOL (foot length, from base of inner metatarsal tubercle to tip of fourth toe), TFOL (total foot length, from heel to tip of fourth toe), ITL (inner toe length), OMTL (length of outer metatarsal tubercle), and IMTL (length of inner metatarsal tubercle). Digit number is represented by roman numerals I–V in subscript. All measurements provided in the taxonomy section are in millimetres (mm). Measurements and associated terminology follow Dubois and Ohler (1999) and Biju and Bossuyt (2009). The webbing formulae follow Savage and Heyer (1967) as modified by Myers and Duellman (1982). The amount of webbing relative to subarticular tubercles is described by numbering the tubercles 1–3, starting from the base. For the convenience of discussion, webbing is additionally defined as basal, small, medium, or large, following Garg and Biju (2017).

To ascertain the degree of morphometric differentiation among the three Indian members of the \textit{Duttaphrynus stomaticus} group, a multivariate analysis was performed using 21 morphometric characters from male specimens. The data for each character was expressed as the ratio of the respective SVL so as to reduce the impact of allometry, and subjected to Principal Component Analysis (PCA), a dimensionality reduction technique. Furthermore, Box and Whiskers plots were created for a univariate analysis of SVL and five morphometric characters that yielded the most significant contribution to the PCA, in order to visualise differences among the species. The analyses were performed in R (R Development Core Team 2008) using the package MASS and the plots were made using the ggplot2 and ggfortify packages.

**Molecular study**

Genomic DNA was extracted from the new samples using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer’s protocols. A short fragment of the mitochondrial 16S rRNA (~540 bp) was PCR-amplified using previously published primer sets 16Sar and 16Sbr (Simon et al. 1994). Purified PCR products were sequenced with the same primers using BigDye Terminator v3.1 Cycle Sequencing Kit on ABI 3730 automated DNA sequencer (Applied Biosystems). Raw sequences were checked and assembled in ChromasPro v1.34 (Technelysium Pty Ltd.) and deposited in the NCBI GenBank under accession numbers MZ816170–MZ816184.

We reconstructed phylogenetic relationships among major distinct evolutionary lineages representing known or putative \textit{Duttaphrynus} species (Van Bocxlaer et al. 2009; Portik and Papenfuss 2015). DNA sequences for nine mitochondrial gene regions (12S ribosomal RNA, tRNAVal, 16S ribosomal RNA, tRNALeu, NADH dehydrogenase subunit 1, tRNAIle, tRNAGln, tRNAVal, and NADH dehydrogenase subunit 2) and two nuclear genes (NCX1 and CXCR4) from previously published studies (Biju and Bossuyt 2003; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015; Liedtke et al. 2016) were retrieved from the GenBank and assembled along with selected new sequence data (Suppl. material 1: Table S2). Sequences were aligned using ClustalW in MEGA 6.0 (Tamura et al. 2013). Alignments for coding DNA were checked by comparison with amino acid sequences, whereas the alignment for non-coding sequences was visually optimised and the ambiguously aligned regions were subsequently excluded from phylogenetic analyses. A character set of total 5,737 bp assembled for 18 taxa was used for the Maximum Likelihood (ML) and Bayesian Inference (BI). Appropriate models of sequence evolution were determined for each gene by implementing
Akaike Information Criteria in ModelTest 3.4 (Posada and Crandall 1998). Maximum Likelihood (ML) searches were performed on a partitioned dataset using the GTR+GAMMA model with 2,000 independent runs executed alongside 10,000 rapid bootstrap replicates in RAxML 7.3.0 (Stamatakis et al. 2008) as implemented in raxmlGUI 1.1 (Silvestro and Michalak 2012). Bayesian analyses were performed using the best-fit General Time Reversible (GTR) model with a proportion of invariant sites (+I) and gamma-distributed rate variation among sites (+G) independently for each gene partition, with all parameters estimated. Bayesian searches were executed in MrBayes (Ronquist and Huelsenbeck 2003) with two parallel runs of four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains executed for 10 million generations using uniform priors and sampling frequency of trees after every 1,000 generations. Convergence of the parallel runs was determined by split frequency standard deviations of less than 0.01 and ~1.0 potential scale reduction factors for all model parameters. Bayesian posterior probabilities (BPP) for the clades were summarised after discarding the first 2,500 trees (25 percent) as burn-in from each run (Huelsenbeck et al. 2001).

We further assessed relationships using available homologous mitochondrial 16S rRNA sequences from GenBank and our new samples (Suppl. material 1: Table S3). Sequences were aligned using ClustalW in MEGA 6.0 (Tamura et al. 2013) and the alignment was manually checked for the presence of any ambiguous or doubtful sites. Certain short GenBank sequences and sequences or positions that showed low confidence for homology were excluded from phylogenetic analyses. A character set of 524 bp from 137 taxa, including an outgroup, was subjected to ML and BI analyses. The ML search was executed in RAxML based on 500 independent runs using the GTR+GAMMA model and clade support was assessed through 1,000 rapid bootstrap replicates. The Bayesian analysis was performed with two parallel runs of four MCMCMC chains executed for 10 million generations using the GTR+I+G model, with a sampling frequency of 1,000 and 25 percent burn-in. The resultant ~15,000 trees were summarised to determine clade support (BPP).

Material studied. Duttaphrynus brevirostris (Rao, 1937)

Rediscovery and validation of taxonomic status. This species was described based on a single specimen (“snout to vent, 27.00 mm”) deposited in the Central College, Bangalore (CCB). This original name-bearing type specimen is considered lost (Dubois 1984; Biju 2001) and the species currently is known only from its original description. Rao (1937) enumerated several morphological character states to describe this taxon, but did not provide comparisons with other species. Our collection from a region of Kempholey Ghat in Sakleshpur taluk, that is part of the type locality (Rao 1937), is comparable with the original description with respect to several mentioned characters such as “canthus rostralis angular,” “nostril nearer to the end of the snout than to the eye,” “first finger equal to the second,” “protoid elongate, moderately prominent,” and “upper surface of the skin covered with small uniformly distributed tubercles; with a small row of larger warts on the median line of the back.” The primary inconsistencies between Rao’s described specimen and our new collection involve snout-vent length. SVL 45 mm (vs. “27.00 mm”) and weakly developed or
inconspicuous cephalic ridges (vs. “crown without bony ridge”). The cephalic ridges in our new collection are relatively smooth, depressed, or less conspicuous (Figs 1A, C, 2A) when compared to other species of the Duttaphrynus melanostictus group from Peninsular India. Hence, presence or absence of this character may be considered a matter of interpretation depending on degree of its prominence. Furthermore, the body size disparity between our collection and that of Rao (1937) also suggests that the type specimen he described could have been a subadult. We examined another subadult specimen from Someshwar (SDBDU 4714; SVL 25 mm), previously
Table 1. Morphometric measurements for specimens included in the study. Measurement abbreviations and museum acronyms are provided in the Material and methods section. ST = Syntype; TT = Topotype; RS = Referred specimen. All measurements are in millimeters (mm).

| Voucher No | Status | SVL (all males, from Karnataka) |
|------------|--------|---------------------------------|
| SDBU 2006.6370 | TT | 50.8 | 18.3 | 14.0 | 3.1 | 5.8 | 4.9 | 1.0 | 12.0 | 3.4 | 1.7 | 6.2 | 4.5 | 3.7 | 11.5 | 10.9 | 19.9 | 17.8 | 18.4 | 28.3 | 1.6 | 1.8 |
| SDBU 2006.4018 | TT | 52.0 | 17.6 | 14.0 | 2.8 | 5.9 | 4.7 | 0.9 | 12.4 | 3.5 | 2.2 | 5.4 | 4.3 | 3.6 | 10.9 | 9.9 | 20.5 | 19.4 | 18.3 | 27.4 | 1.5 | 7.5 |
| SDBU 2006.4019 | TT | 45.2 | 15.5 | 10.4 | 2.3 | 5.2 | 3.9 | 1.0 | 9.8 | 2.9 | 1.8 | 5.0 | 3.6 | 3.0 | 10.6 | 10.0 | 18.1 | 16.8 | 17.2 | 26.1 | 1.4 | 1.8 |
| SDBU 2006.4020 | TT | 47.7 | 16.4 | 11.8 | 2.5 | 5.6 | 4.1 | 1.0 | 10.5 | 3.3 | 1.6 | 5.3 | 3.8 | 3.2 | 10.2 | 9.2 | 18.3 | 17.1 | 17.1 | 26.1 | 1.6 | 1.3 |
| SDBU 2006.4021 | TT | 47.4 | 15.6 | 13.3 | 2.6 | 5.5 | 4.1 | 1.0 | 10.8 | 3.1 | 1.5 | 5.9 | 3.7 | 3.5 | 10.6 | 9.6 | 18.2 | 15.7 | 14.1 | 21.8 | 1.6 | 1.4 |
| Mean | 48.6 | 16.6 | 12.7 | 2.7 | 5.6 | 4.3 | 1.0 | 11.1 | 3.2 | 1.8 | 5.6 | 4.0 | 3.4 | 10.8 | 9.9 | 19.0 | 17.4 | 17.0 | 25.9 | 1.5 | 2.8 |
| SD | 2.7 | 1.1 | 1.6 | 0.3 | 0.4 | 0.0 | 0.11 | 0.2 | 0.3 | 0.5 | 0.4 | 0.3 | 0.5 | 0.6 | 1.1 | 1.4 | 1.7 | 2.5 | 0.2 | 1.7 |

| Voucher No | Status | SVL (all males, from Assam) |
|------------|--------|---------------------------------|
| ZMUC 131136 | ST | 55.0 | 18.9 | 15.3 | 3.1 | 6.3 | 6.3 | 1.7 | 13.7 | 3.5 | 1.8 | 5.7 | 3.6 | 3.5 | 12.2 | 12.2 | 20.5 | 20.3 | 19.4 | 30.5 | 3.7 | 2.3 |
| ZMUC (untagged) | ST | 59.2 | 20.4 | 16.2 | 3.6 | 6.4 | 6.3 | 1.6 | 13.8 | 4.7 | 2.1 | 6.7 | 4.6 | 4.5 | 12.3 | 12.5 | 20.3 | 20.3 | 22.5 | 31.6 | 3.3 | 2.1 |
| SDBU 2018.4109 | RS | 57.6 | 19.7 | 16.9 | 4.2 | 6.5 | 5.4 | 1.5 | 14.4 | 3.4 | 1.8 | 6.2 | 4.1 | 3.9 | 12.2 | 12.4 | 20.8 | 22.5 | 21.4 | 32.9 | 2.1 | 2.9 |
| SDBU 2018.4110 | RS | 69.2 | 23.7 | 18.5 | 4.5 | 6.2 | 6.2 | 1.6 | 14.9 | 5.0 | 2.1 | 6.6 | 5.6 | 4.5 | 13.7 | 13.9 | 27.8 | 27.6 | 24.5 | 36.0 | 3.4 | 2.3 |
| SDBU 2018.4111 | RS | 55.1 | 18.5 | 15.1 | 3.5 | 6.7 | 6.6 | 1.3 | 13.2 | 3.8 | 1.7 | 5.5 | 2.9 | 2.9 | 11.6 | 11.8 | 20.2 | 20.2 | 20.6 | 31.1 | 3.0 | 2.4 |
| Mean | 59.2 | 20.2 | 43.6 | 3.8 | 6.4 | 6.2 | 1.5 | 14.0 | 4.1 | 1.9 | 6.1 | 4.2 | 3.9 | 12.4 | 12.6 | 21.9 | 22.2 | 21.7 | 32.4 | 3.1 | 2.4 |
| SD | 5.2 | 1.8 | 53.7 | 0.5 | 0.2 | 0.4 | 0.1 | 0.6 | 0.6 | 0.2 | 0.5 | 0.9 | 0.6 | 0.7 | 0.7 | 3.0 | 2.8 | 1.7 | 2.0 | 0.5 | 0.3 |

| Voucher No | Status | SVL (all males, from Tamil Nadu) |
|------------|--------|---------------------------------|
| SDBU 2006.4242 | RS | 43.0 | 15.6 | 13.9 | 3.5 | 5.5 | 3.5 | 0.7 | 10.2 | 3.5 | 1.8 | 5.6 | 3.2 | 3.7 | 9.8 | 10.1 | 17.6 | 17.4 | 16.3 | 24.3 | 1.5 | 1.5 |
| SDBU 2006.4243 | RS | 45.2 | 17.1 | 14.5 | 3.8 | 5.4 | 3.7 | 0.8 | 10.1 | 3.6 | 1.7 | 5.4 | 3.1 | 3.7 | 9.9 | 10.2 | 17.5 | 18.8 | 16.8 | 26.9 | 1.5 | 1.4 |
| SDBU 2015.2892 | RS | 46.2 | 17.2 | 13.3 | 3.5 | 5.9 | 3.4 | 0.9 | 10.3 | 3.5 | 1.9 | 5.7 | 3.4 | 3.8 | 9.3 | 9.8 | 17.7 | 17.2 | 16.1 | 22.3 | 1.7 | 1.5 |
| SDBU 2015.2894 | RS | 42.7 | 16.4 | 13.1 | 3.5 | 5.4 | 3.6 | 0.8 | 10.9 | 3.3 | 1.6 | 5.3 | 3.2 | 3.2 | 9.2 | 9.9 | 18.7 | 17.5 | 16.1 | 23.5 | 1.4 | 1.4 |
| SDBU 2015.2895 | RS | 47.0 | 17.5 | 13.2 | 3.4 | 5.9 | 3.5 | 1.9 | 10.2 | 3.2 | 1.8 | 5.5 | 3.5 | 3.8 | 9.9 | 11.2 | 18.9 | 17.8 | 18.6 | 26.5 | 1.7 | 1.4 |
| Mean | 44.8 | 16.8 | 13.6 | 3.5 | 5.6 | 3.5 | 1.0 | 10.3 | 3.4 | 1.8 | 5.5 | 3.3 | 3.6 | 9.6 | 10.2 | 18.1 | 17.7 | 16.8 | 24.7 | 1.6 | 1.4 |
| SD | 1.9 | 0.8 | 0.6 | 0.2 | 0.3 | 0.1 | 0.5 | 0.3 | 0.2 | 0.1 | 0.2 | 0.2 | 0.3 | 0.6 | 0.7 | 0.6 | 1.1 | 2.0 | 0.1 | 0.1 |

Duttaphrynus brevirostris (all males, from Karnataka)
Duttaphrynus peninsularis (all males, from Karnataka and Tamil Nadu)
Duttaphrynus stromaticus (all males, from Assam)
Duttaphrynus hololius (all males, from Tamil Nadu)
Figure 2. Topotype of *Duttaphrynus brevirostris* (Rao, 1937), topotype of *D. peninsularis* (Rao, 1920), and referred specimens of *D. stomaticus* (Lütken, 1864) in life. A. *Duttaphrynus brevirostris* (BNHS 6126) from Kempholey Ghat region in Sakleshpur taluk. B. *Duttaphrynus peninsularis* (SDBDU 6370) from Wattakolli. C–F. *Duttaphrynus stomaticus*: C. SDBDU 2015.2909 from Assam; D. SDBDU 2012.2170 from Rajasthan; E. SDBDU 2012.2172 from Delhi; and F. SDBDU 2012.2268 from Bihar.

reported along with DNA sequence data (Van Bocxlaer et al. 2009), and found some comparable characters such as “a small row of larger warts on the median line of the back,” “a network of dark lines,” and “a dark temporal line extending to the sides,” which can usually also be observed in subadults of *Duttaphrynus melanostictus* group species (S.D.B., personal observations). The Someshwar specimen is genetically identical to our Sakleshpur collection. Together, these two populations are also morphologically and genetically similar to our additional collections from other localities within the Malenadu (Malnad) and adjoining coastal regions of Karnataka (see ‘Material studied’). Altogether, we consider the available morphological and molecular evidence reliable for assigning all the mentioned populations to *D. brevirostris* (Rao 1937).

Since the absence of a name-bearing type has contributed towards poor knowledge and uncertainty regarding the taxonomic identity of this taxon, as evident from the absence of new records, below we provide a detailed description of a newly-collected voucher specimen from the original type locality (Kempholey Ghat region in Sakleshpur taluk, Hassan district, Karnataka State, India: BNHS 6126), which is largely consistent with what is known of the former name-bearing type (Rao 1937). The topotype description provided below, augmented by a range of variation observed in vouched specimens and genetic data from additional localities (Table 1; Suppl. material 1: Tables S3, S4), validate the identity of *D. brevirostris* and also serve as a redescription of this poorly known species for the benefit of future taxonomic work.

**Description of topotype, BNHS 6126** (measurements in mm). A medium-sized, robust adult male (SVL 45.0); head of moderate size, wider (HW 16.9) than long (HL 14.0); snout subovoid in dorsal and ventral view; not pro-
jecting, its length (SL 6.1) longer than horizontal diameter of eye (EL 5.9); loreal region obtuse with sharp canthus rostralis; distance between posterior borders of the eyes (IBE 13.9) 2.2 times the distance between the anterior borders (IFE 6.3); interorbital space 1.2 times wider (IUE 5.1) than upper eyelid width (UEW 4.1); rostril oval without lateral flap of skin, closer to tip of snout (NS 1.7) than to eye (EN 3.2); tympanum distinct (TYD 2.6), vertically oval, 44.1% of eye diameter (EL 5.9), tympanum to eye distance (TYE 0.7); pineal ocellus absent; vomerine ridge and teeth absent; tongue small, oval, entire, median lingual projection absent; parotoid glands present, oval, flat, without spines and warts, longer (PL 6.2) than wide (PW 3.4), shorter than distance between them (PD 8.7); supraorbital and postorbital ridges weakly developed.

Forelimbs short; forearm length (FAL 10.8) shorter than hand length (HAL 11.3); fingers rather thin, FL₃ nearly equal to FL₄, FL₅, longest (6.3); relative length of fingers: I=II<IV<III; tips of fingers rounded; subarticular tubercles prominent, single on fingers I, II, IV, double in finger III, oval, all present; prepollex oval, distinct; single rounded prominent palmar tubercle; numerous supernumerary tubercles irregularly set on palm.

Hind limbs relatively long and thin, thigh length (TL 17.8) shorter than Shank length (SHL 18.8) and foot length (FOL 18.5); relative length of toes: I=II<IV<III<II; tips of all toes rounded, without discs; webbing between toes present, small: I–II=III–1II–3–41V3–2V; well-developed dermal fringes present on all toes; subarticular tubercles rather distinct, oval, all present; inner metatarsal tubercle present, prominent, its length (IMT 1.6) nearly half the length of outer metatarsal tubercle (OMT 3.1); numerous supernumerary tubercles irregularly set on foot.

Skin. Dorsal and lateral surfaces of head and snout, and skin between eyes relatively smooth; anterior and posterior parts of back with flat and smooth glandular projections; flanks glandular without horny spinules or warts; dorsal surfaces of thigh, shank, and tarsus with smooth glandular warts. Ventral surfaces of throat, chest, belly, and thighs glandular.

Secondary sexual character. Male: light brown granular projections on lateral surfaces of fingers I, II, and III. Colour in preservation. Dorsum and limbs slate grey to buff coloured; lateral surfaces of head, flank, and groin slightly lighter than dorsum; ventral surfaces (including limbs) off-white; throat with a faint light bluish-grey calling patch (Fig. 1). Colour in life: dorsum uniformly golden yellow with a brown tinge; limbs darker than dorsum; ventral surfaces white with a prominent bluish-yellow calling patch on throat.

Variation. Adult size range: SVL 45–49 mm. Morphometric data from three adult males, including the described topotype, is given in Table 1. Dorsal colour varies from dark brown to golden yellow with a brown or reddish tinge; prominence of cephalic ridge varies from being inconspicuous to rather prominent; parotoid glands more prominent in life and relatively flattened in preservation; dorsal skin texture varies from having smooth glandular projections to glandular warts.

Comparisons. Duttaphrynus brevirostris differs from other congeners that have relatively prominent cephalic ridges (D. chandai, D. himalayanas, D. kiphirensis, D. mamitensis, D. manipulateus, D. melanostictus, D. microtympanum, D. mizoramensis, D. nagalandensis, D. parietalis, D. scaber, D. silentvalleyensis, D. stomaticus, D. crocus, D. kotagamai, D. noellerti, and D. total) by its relatively smooth and inconspicuous cephalic ridges (vs. prominent and often with carotenoid margins or spinules), and smooth glandular dorsal skin (vs. presence of prominent glandular warts with horny spinules). Specifically, it also differs from the Indian species by the following characters: from D. chandai, by its shorter male snout-vent length, SVL 45–49 mm (vs. longer, SVL 67–89 mm), absence of canthal, parietal, and cranial ridges (vs. present), and distinct tympanum (vs. inconspicuous externally); from D. himalayana, D. kiphirensis, D. mamitensis, D. manipulateus, D. melanostictus, D. microtympanum, D. mizoramensis, D. nagalandensis, D. parietalis, D. scaber, D. silentvalleyensis, and D. wokhaensis, by absence of canthal, preorbital, and supratympanic ridges (vs. present), relatively flat parotoid glands (vs. prominently raised), and ventral surfaces of hand, fingers, foot, and toes with smooth tubercles (vs. raised and spinular tubercles); and from D. beddomei, D. hololius, D. peninsularis, and D. stomaticus by the presence of supraorbital and postorbital ridge (vs. absent). Duttaphrynus brevirostris specifically also differs from D. beddomei by its finger and toe tips lacking expanded discs (vs. with weakly-expanded discs), relatively reduced foot webbing, I–II=III–2III–3IV3–3V (vs. extensive, I=II–I1III–I1II=1V1–1V1–1V); and from D. hololius, by its robust body (vs. dorso-ventrally flattened body), absence of mid-dorsal line (vs. present), sharp canthus rostralis (vs. rounded), snout rounded in lateral view (vs. acute), and more extensive foot webbing, I–II–III–II2–3IV3–3V (vs. rudimentary); from D. stomaticus, by its shorter male snout-vent length, SVL 45–49 mm (vs. longer, SVL 54–69 mm), snout subovoid in dorsal view (vs. rounded), canthus rostralis sharp (vs. rounded), and relatively reduced foot webbing, I–II=II–III–2III–3IV3–3V (vs. more extensive: I–II1III–1III1–1II1–IIV1–1V); and from D. peninsularis, by its canthus rostralis sharp (vs. rounded), snout length longer than eye diameter, SL/EL ratio 1.2–1.3 mm (vs. nearly equal), and relatively reduced foot webbing, I–II–III–II2–3IV3–3V (vs. more extensive: I–II1III–I1III–1III1–1III1–1III1–1V).

Phylogenetic relationships and genetic distances. Duttaphrynus brevirostris is a member of the Duttaphrynus melanostictus group (Fig. 3), within which it is more closely related to D. melanostictus, D. cf. microtympanum (D. “sp”, Van Bocxlaer et al. 2009), and D. parietalis (Fig. 3). All populations of D. brevirostris exhibit intraspecific distances of 0–0.2% in 16S. The sequence
Figure 3. Phylogenetic relationships and genetic differentiation in the genus Duttaphrynus. A. Maximum Likelihood phylogenetic tree based on 5,737 bp DNA comprising nine mitochondrial gene regions and two nuclear genes, showing phylogenetic relationships between the major species-level lineages. Values above and below the branches indicate Bayesian Posterior Probabilities (BPP) and RAxML Bootstrap Support (BS), respectively; B. Maximum Likelihood barcoding tree based on 524 bp of the mitochondrial 16S rRNA sequences. BPP and BS support values are indicated above and below the branches, respectively. Coloured vertical bars outside the terminal node labels indicate putative species delimited in the bPTP analysis; C. Median-Joining haplotype network based on 42 haplotypes recovered from 133 sequences of the 16S gene (420 bp). Size of the coloured circles is proportional to the number of haplotypes; black circles indicate median vectors; each branch represents a single mutation step; additional mutational steps are indicated by values in parentheses; photo credits: D. crocus (Guinevere O. U. Wogan), D. olivaceus (Parham Beyhaghi), and D. dhufarensis (Todd W. Pierson).

Divergence for *D. brevirostris* from other members of the *Duttaphrynus melanostictus* group was as follows: 2.1–3.3% from *D. melanostictus*, 2.2–2.6% from *D. cf. microtympanum*, 2.8–3.2% from *D. parietalis*, 3.0–4.3% from *Duttaphrynus* sp. 1, and 2.4–5.6% from *Duttaphrynus* sp. 2 (Suppl. material 1: Table S4).

**Distribution and natural history.** *Duttaphrynus brevirostris* is endemic to the Western Ghats, where it currently is known only from the State of Karnataka. Here, we report this species from Hassan district (Sakleshpur taluk, encompassing the type locality Kempholey Ghat), Kodagu district (Bhagamandala), and Udupi district (Someshwara and Manipal). Furthermore, we confirm the following available DNA sequences for this species: Someshwara (FJ882786, Van Boeckel et al. 2009), specimen examined herein; Bajipe (AB530640) and Shirva (AB530642), specimen vouchers unavailable and reportedly released (Hasan et al. 2014); and another sample...
**Duttaphrynus peninsularis** (Rao, 1920), comb. nov.

Figs 1–5; Table 1; Suppl. material 1: Tables S1–S5

Peninsular Toad

**Original name and description.** *Bufo stomaticus peninsularis* Rao, 1920. Rao, C. R. N. 1920. Some South Indian batrachians. “Journal of the Bombay Natural History Society” 27: 119–127. **Holotype.** ZSIC 19176, SVL 45.1 mm (designated by Chanda et al. 2001 “2000”), from “Mavkote and Wattekolle, Coorg,” Karnataka State, India. **Current status of specific name.** Valid name, as *Duttaphrynus peninsularis* (Rao, 1920), comb. nov.

**Material studied.** **Topotype.** An adult male, SDBDU 6370 (SVL 50.8 mm), collected by S. D. Biju, from Wattakolli, Karnataka State. **Other referred specimens.** Four adult males, SDBDU 4018 (SVL 51.8 mm), SDBDU 4019 (SVL 45.5 mm), SDBDU 4020 (SVL 49.5 mm), and SDBDU 4021 (SVL 46.5 mm), from Coimbatore, Tamil Nadu State.

**Reassessment and validation of taxonomic status.** Rao (1920) described a new variety of *Bufo stomaticus* from “Mavkote and Wattekolle, Coorg” as “*Bufo stomaticus peninsularis* var. nov.” The original description mentioned two specimens (“Type and syntype in the Indian Museum”) and subsequently Chanda et al. (2001 “2000”) proposed ZSIC 19176 to be the holotype. Currently a single specimen is available in the ZSIC (Kolkata) collection (S.D.B., personal observation). It is noteworthy that, prior to describing this taxon, Rao (1920) took an opinion from Boulenger (then Curator, British Museum Natural History, London), who was not in favour of separating this collection from *D. stomaticus*. However, Rao being unconvinced mentioned “no doubt about their being racially distinct” in the original description and went on to formally describe *Bufo stomaticus peninsularis* as a new variety of *D. stomaticus*. This nomen was considered to be a synonym of *Bufo stomaticus* (= *Duttaphrynus stomaticus*) by Daniel (1963), without any justification or comparison, other than considering the characters mentioned by Rao (1920) as variation, based on examination of *D. stomaticus* specimens from Bombay. This action was followed by Dubois (1974) and Dutta (1997). In later years, regional anuran lists reported *Duttaphrynus stomaticus* from Peninsular India based on earlier reports and photographs, without citing any voucher specimens (Hegde 2012; Ramachandra et al. 2012; Seshadri et al. 2012). Srinivasulu et al. (2013) identified the “captioned-photographs” of Seshadri et al. (2013) and Hegde (2012) as belonging to *D. scaber*, a species that is widely distributed in Peninsular India (Dutta 1997; Chanda 2002; Daniels 2005; Dinesh et al. 2009; Padhye et al. 2013). Srinivasulu et al.’s (2013) notes concerning the misidentifications of *D. scaber* as *D. stomaticus* (and not *D. peninsularis*) was by implication considered as a synonymisation action of *Bufo stomaticus peninsularis* with *D. scaber* by Frost (2021).

In order to verify the above, we compared the type specimen and the original description of *Bufo stomaticus peninsularis* Rao, 1920. Although the holotype (ZSIC 19176) was found to be in a severely damaged and dehydrated condition (Fig. 1), the head portion was relatively better preserved. Diagnostic morphological characters, such as absence of prominent cephalic ridges, weakly developed parotoid glands, distinct tympanum (about 63% of the eye), and the relatively smooth skin texture of the head and dorsum, match with the original description of *Bufo stomaticus peninsularis* Rao, 1920. Additionally, Rao (1920) clearly stated six differences between his new variety and the typical form of *Bufo stomaticus* from “Indian Museum nos., 16067, 16068, 17254 and 17274” (see the detailed comparison section), which we further re-examined to confirm distinctness of the two taxa.

We examined specimens from two populations of *Duttaphrynus* “stomaticus,” sampled from different localities (including Wattakolli) in Peninsular India, which were found to be comparable to the original description and type specimen of *Bufo stomaticus peninsularis* Rao, 1920 with respect to snout-vent length, absence of cephalic ridges, weakly developed parotoid glands, and relatively smooth skin. Based on re-examination of the holotype and assessment of newly-collected material, and molecular data, we conclude that *Bufo stomaticus peninsularis* Rao, 1920 and *Bufo stomaticus* Lütken, 1864 represent two distinct species, both individually diagnosable from other Indian congeners and each other. Hence, we formally resurrect *Bufo stomaticus peninsularis* Rao, 1920, as a distinct species: *Duttaphrynus peninsularis* (Rao, 1920), comb. nov. Furthermore, since the holotype is poorly preserved, we also provide a detailed redescription of this species, based on new totopytic material from Wattakolli, which matches the original description and the type.

**Description of toptype, SDBDU 6370** (measurements in mm). A medium-sized, robust adult male (SVL 50.9); head of moderate size, wider (HW 18.0) than long (HL 14.0); snout truncate in dorsal and ventral view, rounded in lateral view, projecting beyond the mouth, its length (SL 5.8) nearly equal to horizontal diameter of eye (EL 5.7); loreal region acute with rounded canthus rostralis; distance between posterior borders of the eyes (IBE 13.9) 1.6 times the distance between the anterior...
Figure 4. Geographical distribution of Duttaphrynus brevirostris (dark grey), D. peninsularis (blue), and D. stomaticus (orange).

Borders (IFE 8.2); interorbital space about 1.4 times wider (IUE 6.2) than upper eyelid width (UEW 4.5); nostril oval without lateral flap of skin, closer to tip of snout (NS 1.7) than eye (EN 3.2); tympanum distinct (TYD 3.1), vertically oval, about 56.4% of eye diameter (EL 5.5), tympanum to eye distance (TYE 1.0); pineal ocellus absent; vomerine ridge and teeth absent; tongue small, oval, entire, median lingual projection absent; parotoid glands present, oval, flat, without spines and warts, slightly longer (PL 10.4) than wide (PW 5.5), distance between them (PD 6.2) more than the width.

Forelimbs short; forearm length (FAL 11.5) longer than hand length (HAL 10.9); fingers rather thin, FL_I longer than FL_II, FL_III longest (5.6); relative length of fingers: II<IV<I<III; tips of fingers rounded; subarticular tubercles prominent, single, all present; prepollex oval, distinct; single rounded prominent palmar tubercle; numerous supernumerary tubercles irregularly set on palm.

Hind limbs relatively long and thin, thigh length (TL 19.7) longer than shank (SHL 17.8) and foot (FOL 18.4) length; relative length of toes: I<II<V<III<IV; tips of all toes rounded, without discs; webbing between toes present, small: I1−2II1−3 III1½−3IV3−1½V; dermal fringes present on all toes; subarticular tubercles rather weakly developed, oval; inner metatarsal tubercle present, prominent, its length (IMT 1.6) shorter than outer metatarsal...
tubercle (OMT 1.8); numerous weakly developed super-numerary tubercles set on foot.

**Skin.** Dorsal and lateral surfaces of head and snout, and skin between eyes relatively smooth to sparsely granular; anterior and posterior parts of back with flat and smooth glandular projections; flanks glandular without horny spinules orwarts; dorsal surfaces of thigh, shank, and tarsus with smooth glandular warts. Ventral surfaces of throat, chest, belly, and thighs glandular.

**Male secondary sexual character.** Light brown granular projections on the lateral surfaces of fingers I, II, and III.

**Colour in preservation.** Dorsum and limbs greyish-brown without any prominent markings; lateral surfaces of head, flank, and groin slightly lighter than dorsum; ventral surfaces (including limbs) greyish-white, throat with a faint light blue calling patch (Fig. 1). **Colour in life:** dorsum yellowish-brown with reddish patches; limbs yellowish brown; ventral surfaces white with a prominent bluish-yellow calling patch on throat (Fig. 2).

**Variation.** Adult size range: male SVL 45–52 mm. Morphometric data from five adult males, including the described type, is given in Table 1. The dorsal colour is highly variable in life: SDBDU 4018: light brown with light grey patches, SDBDU 4019: light brown with reddish blotches, and SDBDU 4020: uniformly olive green.

**Comparisons.** *Duttaphrynus peninsularis* differs from the Indian congeners: *D. chandai*, *D. himalayanus*, *D. kiphirensis*, *D. mampionensis*, *D. melanostictus*, *D. microtypanum*, *D. mizoramensis*, *D. nagalandensis*, *D. parietalis*, *D. silentvalleyensis*, *D. scaber*, *D. stuarti*, and *D. wokhaensis*, and species from other regions: *D. crocus* (Myanmar), *D. kotaga-mai* and *D. noellerti* (Sri Lanka), and *D. totol* (Indonesia), by the absence of conspicuous cephalic ridges (vs. present), absence of prominent or raised parotoid glands (vs. present), and dorsal skin without distinct glandular warts or horny spinules (vs. present in all species). Due to the lack of conspicuous cephalic ridges *D. peninsularis* could be confused with four Indian species *D. beddomii*, *D. brevirostris*, *D. hololius*, and *D. stomaticus*. However, it differs from *D. beddomii* in having a relatively larger tympanum (vs. smaller), finger and toe tips without discs (vs. with weakly developed discs), relatively reduced foot webbing, I1–2II1–3 III1–2IV3–1½V (vs. extensive, I1–III1–II1–1IV2–1V), and absence of prominent glandular warts or horny spinules on dorsum (vs. present). *Duttaphrynus peninsularis* differs from *D. hololius* by its robust body (vs. dorso-ventrally flattened), absence of mid-dorsal line (vs. present), snout rounded in lateral view (vs. acute), tympanum smaller than eye diameter (vs. nearly equal), and more extensive webbing between toes, I1–2II1–3 III1½–2IV3–1½V (vs. rudimentary). *Duttaphrynus peninsularis* differs from *D. stomaticus* by its relatively shorter snout-vent length, male SVL 45–52 mm (vs. longer, male SVL 54–69 mm), its snout truncate in dorsal and ventral view (vs. rounded), snout longer than eye diameter (vs. nearly equal), dorsal skin granulation relatively smooth (vs. with prominent glandular warts), and relatively reduced foot webbing, I1–2II1–3 III1½–2IV3–1½V (vs. more, I1–III1–II1–1IV3–1V). For comparisons to *D. brevirostris*, see the respective comparison section.

We quantitatively assessed the degree of morphometric differentiation of *Duttaphrynus peninsularis* from the other two Indian members of the *Duttaphrynus stomaticus* group (*D. hololius* and *D. stomaticus*). An ordination of the first two principal components resulted in formation of three distinct clusters, what we consider to be three species (Fig. 5). The first two principal components (PC) accounted for 50.73% of the total variance, of which PC1 was able to explain 32.08%, and PC2 explained 18.65% of the variation in the dataset. Variables with the highest factor loadings for PC1 were HW, TYD, EL, IUE, and IN, while PC2 was highly loaded for UEW. The third and fourth principal components (PC3 and PC4) accounted for 9.37% and 9.07% of the total variance, respectively, taking the cumulative variance for the first four components to 69.17% (Suppl. material 1: Table S5). The Box and whiskers plots of the five most significant characters recovered from PCA showed diagnostic differences between the three species (Fig. 5). Of the three species, *D. hololius* was more distinct for all the studied characters, whereas *D. peninsularis* and *D. stomaticus* could be clearly delineated based on SVL, EL/SVL, TYD/SVL, and IN/SVL.

**Phylogenetic relationships and genetic distances.** *Duttaphrynus peninsularis* is a member of the *Duttaphrynus stomaticus* group (Fig. 3), within which it is more closely related to *D. stomaticus* and *D. ‘olivaceus’* than to *D. dhufarensis* and *D. hololius*. The studied populations of *D. peninsularis* exhibit intraspecific distances of 0–0.4% in 16S. The sequence divergence of *D. peninsularis* from other members of the *Duttaphrynus stomaticus* group was as follows: 2.3–3.8% from *D. dhufarensis*, 5.2–5.4% from *D. hololius*, 1.3–2.6% from *D. stomaticus*, and 1.0–1.5% from *D. ‘olivaceus’* (Suppl. material 1: Table S4).

**Distribution and natural history.** *Duttaphrynus peninsularis* is currently known only from the Peninsular Indian States of Karnataka, Tamil Nadu, and Maharashtra. Genetically confirmed records are from Karnataka: Kodagu district (Wattakolli); Tamil Nadu: Coimbatore district (Coimbatore); and Maharashtra: Solapur district (Barsi and Solapur). We have also observed this species at Namakkal district (Kolli Malai) of Tamil Nadu. DNA sequences of this species were previously reported as *D. stomaticus* (FJ882787, Van Bocxlaer et al. 2009). Another genetically identical sample from an unknown locality in India is currently available (EU071742, Shouche and Ghate, unpublished GenBank data). Given that this species currently has a disjunct distribution based on available genetically confirmed records, it is likely to be more widely distributed in the intervening regions of Peninsular India (Kerala, Tamil Nadu, and Karnataka, up to southern Maharashtra). Furthermore, its most closely related congener *D. stomaticus* is frequently and widely reported in Peninsular India, which could be misidentifications of *D. peninsularis*; hence the identity of all ‘D.
stomaticus’ records from this region require further verification. Based on the present study, the geographical boundary between *D. peninsularis* (southern species) and *D. stomaticus* (northern species) could lie in the northern Western Ghats regions of Maharashtra state, where we have observed and genetically confirmed the presence of both these species (see Distribution and Natural History section of *D. stomaticus*). Further extensive sampling will be necessary to understand the patterns of population structure and delineate the ranges of these two species, using integrative approaches focusing on quantified ranges of phenotypic variation, traditional morphology, biocoustics, ecological information, and phylogeny.

Most individuals reported here were located during night searches (between 17:00–21:00 hours) largely in vegetated urban areas. The species were also found in secondary forest patches adjacent to human settlements. Ganesh et al. (2020) reported this species as *D. stomaticus* from Tuticorin, Tamil Nadu.

**Duttaphrynus stomaticus** (Lütken, 1864)

Figs 1–5; Table 1; Suppl. mater. 1: Tables S1–S5

Marbled Toad

**Original name and description.** *Bufo stomaticus* Lütken, 1864 “1863.” Nogle ny Krybyr og Padder. Vidsenskablel Meddelelser fra Dans Naturhistorisk Forening i København, Serie 2, 4: 292–311. **Syntypes.** Three adult females, ZMUC 131137 [ex 196], ZMUC 131365 [ex 198], and one unnumbered, from “Assam;” two adult males, ZMUC 131136 [ex 195] and one unnumbered (SVL 59.2 mm), from “Assam;” and three subadults, ZMUC 131366 [ex 199] (SVL 26.4 mm) from “Hoogly,” ZMUC 131363 [ex 193] (SVL 33.4 mm) from “Calcutta” (Kolkata), and ZMUC 131364 [ex 194] (SVL 30.0 mm), from “Culcutta” (Kolkata). **Other referred specimens:** three adult males, SDBDU 2018.4109 (SVL 57.6 mm), SDBDU 2018.4110 (SVL 69.2 mm), and SDBDU 2018.4111 (SVL 55.1 mm), from Sonitpur district, Assam State; two adult males, SDBDU 2018.3717 (SVL 56.2 mm) and SDBDU 2018.3750 (SVL 54.2 mm), from Dehradun, Uttarakhand State; an adult female, SDBDU 2012.2172 (SVL 67.5 mm), from Delhi; an adult female, SDBDU 2012.2269 (SVL 68.7 mm), from Kattha in Banka district, Bihar State; an adult male, SDBDU 2012.2170 (SVL 51.0 mm), from Jaipur, Rajasthan State.

**Taxonomic history of Bufo stomaticus** Lütken, 1864. In the original description, Lütken (1864) mentioned that the Zoological museum, Copenhagen received six specimens of a toad from “Hr. Grosserer Westerman” (= Mr. Wholesman Westermann) from “ostindiske” (= East India). Subsequent researchers stated the type locality of this species to be ‘East India’ where it was later restricted to Assam (Boulenger 1891). Dutta (1997) stated that the type specimens are untraceable. We (SDB and SG) studied the types that are available at ZMUC, Copenhagen, and found a total of eight specimens (see ‘Other material studied’). According to the museum catalogue and bottle labels, all the adult animals are from “Assam,” one juvenile from “Hoogly,” and two juveniles from “Calcutta.” All the specimens belong to the same species and the morphological characters were in agreement with the brief original description. Boulenger (1891) had mentioned after examining the syntypes that the exact locality from where these were procured

**Material studied.** **Syntypes:** Three adult females, ZMUC 131137 [ex 196] (SVL 60.9 mm), ZMUC 131365 [ex 198] (SVL 55.2 mm), and one unnumbered (SVL 61.4 mm), from “Assam;” two adult males, ZMUC 131136 [ex 195] (SVL 55 mm) and one unnumbered (SVL 59.2 mm), from “Assam;” and three subadults, ZMUC 131366 [ex 199] (SVL 26.4 mm) from “Hoogly,” ZMUC 131363 [ex 193] (SVL 33.4 mm) from “Calcutta” (Kolkata), and ZMUC 131364 [ex 194] (SVL 30.0 mm), from “Culcutta” (Kolkata). **Other referred specimens:** three adult males, SDBDU 2018.4109 (SVL 57.6 mm), SDBDU 2018.4110 (SVL 69.2 mm), and SDBDU 2018.4111 (SVL 55.1 mm), from Sonitpur district, Assam State; two adult males, SDBDU 2018.3717 (SVL 56.2 mm) and SDBDU 2018.3750 (SVL 54.2 mm), from Dehradun, Uttarakhand State; an adult female, SDBDU 2012.2172 (SVL 67.5 mm), from Delhi; an adult female, SDBDU 2012.2269 (SVL 68.7 mm), from Kattha in Banka district, Bihar State; an adult male, SDBDU 2012.2170 (SVL 51.0 mm), from Jaipur, Rajasthan State.

**Figure 5.** Morphometric analyses for Indian members of the Duttaphrynus stomaticus group. A. Principal component analysis showing distinct clusters for three species in a scatter plot of the first two principal components; B–G. Box and whiskers plots depicting the most significant diagnostic characters for the three species.
is unknown and believed they originated from Assam or “they are perhaps from Bengal.” However, while describing *Bufo stomaticus* Lütken (1864) provided four measurements from two specimens, without mentioning the voucher numbers—“en Han” (one male) and “en Hun” (one female)”Fra Snudspidsen til Gattet” (= from snout to cloacal) 54 mm and 61 mm, respectively. Among the eight located syntypes, two similar-sized specimens were found bearing small tags on the hind limbs stating ‘type’.

Based on the available information, it is apparent that only two specimens, ZMUC 131137 [ex 196] and ZMUC 131136 [ex 195], were used for Lütken’s (1864) description of *Bufo stomaticus*; hence only these can be considered as potential syntypes. However, since the type series contains both adult and subadult specimens originating from different localities, it has led to confusion regarding the type locality and type status (Boulenger 1891). In order to clarify the taxonomic status of *B. stomaticus*, we provide a detailed redescription for one potential syntype, ZMUC 131137 [ex 196], an adult female, SVL 60.9 mm, from “Assam.” The below redescription, along with live photographs, interspecific comparisons, and enumeration of diagnostic characters, may be useful for differentiating this taxon from other known *Duttaphrynus* species. We also provide additional information on new totopotypic material, including live photographs, genetic data, inferred phylogenetic relationships, and extended geographical records, based on morphologically-characterised and genetically-confirmed records—all of which shows that *D. stomaticus* (as understood here) is consistent with what is known of the name-bearing types.

**Description of syntype, ZMUC 131137 [ex 196]** (measurements in mm). A medium-sized, robust adult female (SVL 60.9). Head of moderate size, wider (HW 22.7) than long (HL 17.8); snout rounded in lateral, dorsal, and ventral view, projecting beyond the mouth, its length (SL 6.8) longer to horizontal diameter of eye (EL 6.0); loreal region acute with rounded canthus rostralis; distance between posterior borders of the eyes (IBE 16.2) 1.8 times the distance between the anterior borders (IFE 9.2); interorbital space concave, 1.3 times wider (IUE 6.6) than upper eyelid width (UEW 5.0); nostril oval without lateral flap of skin, closer to tip of snout (NS 1.8) than to eye (EN 3.5); tympanum distinct (TYD 3.6), rounded, 58.1% of eye diameter (EL 6.2), tympanum to eye distance (TYE 1.6); pineal occulus absent; vomerine ridge and teeth absent; tongue small, oval, entire, median lingual projection absent; parotoid glands present, oval, elongate, without spines and warts, longer (PL 13.9) than wide (PW 6.5) and distance between them (PD 10.0) wider than their width; cephalic ridges absent.

Forelimbs short; forearm length (FAL 11.5) shorter than hand length (HAL 13.7); fingers rather thin, FL₁ longer to FL₄, FL₄ longest (7.1 mm); relative length of fingers: I<II=IV<III; tips of fingers rounded; subarticular tubercles prominent, single, all present; prepollex oval, distinct; single rounded prominent palmar tubercle; numerous supernumerary tubercles irregularly set on palm.

Hind limbs relatively long and thin, thigh length (TL 21.3) shorter than shank (SHL 21.8) and foot (FOL 22.6) length; relative length of toes: I<II<IV<III<IV; tips of all toes rounded without discs; webbing between toes present, small: I–II–I–II–III–IV–I; dermal fringes present on all toes; subarticular tubercles rather well-developed, oval, inner metatarsal tubercle present, prominent, its length (IMT 3.1) shorter than outer metatarsal tubercle (OMT 3.7); numerous weakly developed supernumerary tubercles set on foot.

**Skin.** Dorsal surfaces of head sparsely granular; lateral surfaces of head shagreened with scattered tubercles; upper eyelids with glandular warts possessing horny spinules; anterior and posterior parts of back with glandular warts possessing horny spinules, larger warts towards posterior back; flanks glandular without warts or horny spinules; dorsal surfaces of thigh, shank, and tarsus glandular. Ventral surfaces of throat, chest, belly, and thighs with fine glandular projections without horny spinules or warts.

**Secondary sexual characters.** Female (ZMUC 131137): ova white, pigmented on pole (diameter 0.8–1.0 mm, N = 20); Male (SDBDU 2018.4111): light brown granular projections on the lateral surfaces of fingers I, II, and III. **Colour in preservation:** dorsum surfaces of head and body uniformly fawn, some spines brown; dorsal surface of fore-and hind limbs light fawn; ventral surfaces of head, body, and limbs light grey (Fig. 1). **Colour in life** (based on other material studied): dorsum yellowish-brown, straw, light brown, or olive green, with or without grey or brown patches; and a pair of faint discontinuous dorsolateral lines; ventral surfaces greyish-white (Fig. 2).

**Variation.** Adult size range: male SVL 54–69 mm, female SVL 60–72 mm. Morphometric data from five adult males, including the described syntype, is given in Table 1. Dorsal colouration varies from light grey or brown to olive green; the amount and degree of prominence of granulation on dorsal skin variable.

**Comparisons.** *Duttaphrynus stomaticus* differs from the Indian species: *D. chandai*, *D. himalayanus*, *D. kiphiresinis*, *D. mamiensis*, *D. manipurensis*, *D. melanostictus*, *D. microtypanum*, *D. mizorimensis*, *D. nagalandensis*, *D. parietalis*, *D. silentvalleyensis*, *D. scaber*, *D. stuar-ti*, and *D. wokhaensis*, and other species found outside: *D. crocus* (Myanmar), *D. kotagam* and *D. noellerti* (Sri Lanka), and *D. toto* (Indonesia), by the absence of cephalic ridges, absence of prominent or raised parotoid glands, and absence of distinct glandular warts or horny spinules (vs. present in all species). Due to the absence of cephalic ridges *D. stomaticus* could be confused with three Indian species *D. beddomii*, *D. hololius*, and *D. pen-insularis*. However, *D. stomaticus* differs from *D. bed-domii* in having a tympanum larger than eye diameter (vs. smaller), finger and toe tips lacking expanded discs (vs. with weakly-expanded discs), relatively reduced foot webbing, I–II–I–III–III–V–I (vs. more extensive, I–II–I–III–I–III–V–I), and less prominent glandular
warts or horny spinules on dorsum (vs. more prominent); from *D. hololius*, in having a stout body (vs. flattened or dorso-ventrally compressed), absence of a prominent or broad mid-dorsal line (vs. present), snout rounded in lateral view (vs. acute), dorsum with relatively more prominent smooth or spinular warts (vs. less prominent and scattered smooth tubercles), and moderate foot webbing, I1–III1–2 III1–IV3–1V (vs. rudimentary). For comparisons to *D. brevirostris* and *D. peninsularis*, see the respective comparison sections of those species.

**Phylogenetic relationships and genetic distances.** *Duttaphrynus stomaticus* is a member of the *Duttaphrynus stomaticus* group (Fig. 3), within which it is more closely related to *D. ‘olivaceus’* and *D. peninsularis* than to *D. dhufarensis* and *D. hololius*. The studied populations of *D. stomaticus* exhibit intraspecific distances of 0–0.4% in 16S. The sequence divergence of *D. stomaticus* from other members of the *D. stomaticus* group is as follows: 0.2–0.6% from *D. ‘olivaceus’*, 1.3–2.6% from *D. peninsularis*, 1.5–3.0% from *D. dhufarensis*, and 3.4–5.6% from *D. hololius* (Suppl. material 1: Table S4).

**Relationships within *Duttaphrynus stomaticus* group.** The close phylogenetic relationship of *Duttaphrynus stomaticus* with *D. dhufarensis*, *D. hololius*, *D. olivaceus*, and *D. peninsularis* is well-supported (Van Bocxlaer et al. 2009; Portik and Papenfuss 2015; present study). Martin (1972) also discussed the absence of conspicuous cephalic ridges as a potential morphological synapomorphy for these species. Within this group, subsequently referred to as the *Duttaphrynus stomaticus* group (Inger 1972; Dubois and Ohler 1999; Silva and Mendelson 1999; Van Bocxlaer et al. 2009), the taxonomic identity of *D. olivaceus* has been questionable due to the lack of sufficient morphological distinctness (Dubois 1984; Balletto et al. 1985; Minton 1966) as well as shallow genetic divergence (Portik and Papenfuss 2015; present study). Eiselt and Schmidtler (1973) regarded *D. olivaceus* as the subspecies of *D. stomaticus*. However, subsequent workers treated *D. olivaceus* as a distinct species closely related to *D. stomaticus* with relatively weak and variable morphological diagnostic characters, such as differences in the size of parotoid glands, number of subarticular tubercles on finger III, and weakly or well-developed tubial gland and tarsal folds (Schmidtler and Schmidtler 1969; Khan 1987; Auffenberg and Rehman 1997). The available genetic data for *D. stomaticus* and *D. olivaceus*, along with new samples reported in this study for various *D. stomaticus* populations from India (including topotypic samples) show a shallow divergence of 0.2–0.6% between the two species (Fig. 3).

Recently, Safaei-Mahroo and Ghaffari (2020) discussed the taxonomic status of *D. olivaceus* (Frost 2021). This study also proposed a new genus name *Firouzophrynus* Safaei-Mahroo & Ghaffari, 2020 to accommodate a single species *Duttaphrynus olivaceus* (Blanford 1874), which rendered the genus *Duttaphrynus* paraphyletic (Frost 2021). Subsequently, based on phylogenetic evidence from selected taxa, Dubois et al. (2021) redelimited *Firouzophrynus* as a genus, while also stating the possibility of considering it as a subgenus, to include members of the *Duttaphrynus stomaticus* group as defined by Inger (1972) and Dubois and Ohler (1999). However, as noted by Frost (2021), there continues to be lack of clarity regarding the morphological and phylogenetic affinities of some other members of the group, which may have implications on the monophyly of *Firouzophrynus*. The composition of *Duttaphrynus stomaticus* species group and its phylogenetic position have been discussed by numerous studies (Inger 1972; Martin 1972; Maxson 1981; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015). However, only five species (*D. stomaticus*, *D. dhufarensis*, *D. hololius*, *D. olivaceus*, and *D. peninsularis*) currently are included in this group based on morphological (Inger 1972; Martin 1972; Dubois and Ohler 1999; present study) and phylogenetic analyses (Frost et al. 2006; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015; this study). At least two other species from Indonesia, *D. valhallae* and *D. sumatranus*, that are known to lack cephalic ridges, a characteristic of the group (Inger 1972; Dubois and Ohler 1999), require further studies to establish their systematic relationships. Although we do not doubt that *Firouzophrynus* could be recognised as a genus or subgenus, we currently consider the taxonomic status of this taxon uncertain, pending additional studies which may provide clarity, because of its cursory description and lack of a clear definition. Because it is beyond the scope of the present work to address this question, we have provisionally referred our focal taxa to the genus *Duttaphrynus*, sensu lato, and make use of previously defined species-groups, which could easily be adopted to an alternate classification, as more evidence concerning the recognition of *Firouzophrynus* becomes available.

**Distribution and natural history.** *Duttaphrynus stomaticus* is one of the most widely-distributed species of the genus, occurring between elevations of sea-level to 2500 m asl in India (through Indo-Gangetic Plains, upper and lower Indus Valleys) and the neighbouring Bangladesh, Nepal, Pakistan (Balochistan), Afghanistan, and Iran (Suppl. material 1: Table S1). This species is known to occur in varying climatic conditions and habitats, ranging from dry scrub forests, arid and semi-arid regions, hot and humid mixed forests, plains, and grasslands to drier and colder regions, montane woodlands and forests (Choudhury et al. 2001; Mehta 2005; Deuti et al. 2014; Safaei-Mahroo et al. 2015). Genetically confirmed records of this species exist from India, Afghanistan, and Pakistan (Suppl. material 1: Table S3). In the present study, we specifically confirm the presence of *D. stomaticus* in the Indian States of Assam, Bihar, Delhi, Punjab, Rajasthan, and Uttarakhand (Suppl. material 1: Table S3) and also clarify the identity of some previously published DNA sequences from Peninsular India (Van Bocxlaer et al. 2009; Shouche and Ghaete 2007, unpublished GenBank data) as belonging to *D. peninsularis*. Hence, records of *D. stomaticus* from Peninsular India (south
of Maharashtra and possibly Odisha) are currently presumed to be doubtful and will require verification of all known populations (see *D. peninsularis* for discussion). The reports of *D. stomaticus* from Karnataka and Tamil Nadu States (Hegele 2012; Ramachandra et al. 2012; Seshadri et al. 2012; Ganesh et al. 2020) likely refer to *D. peninsularis*. A report of *D. olivaceus* from Gurgaon, India (Ray and Deuti 2008) is also questionable (Hedayari and Rastegar-Pouyani 2010) and considered to represent *D. stomaticus* based on our fresh collections from Delhi and surrounding North Indian regions.

*Duttaphrynus stomaticus* is predominantly a nocturnal species. In this study, we found individuals of this species in urban, rural, and secondary forested areas during the breeding season (usually between May–August). Calling and breeding activities were observed in agricultural fields and temporary puddles in urban and rural landscapes, whereas inside secondary forests breeding was observed in shallow parts of flowing streams.

**Phylogenetic relationships and genetic differentiation in the genus *Duttaphrynus***

Our reanalysis of the multilocus data derived from previous studies (primarily Van Boeckxlaer et al. [2009] and Portik and Papenfuss [2015]), with 16S data for our newly-sampled populations, support the monophyly of the *Duttaphrynus melanostictus* group and the *Duttaphrynus stomaticus* group (Fig. 3A), as shown in these previous studies. Among the focal taxa of our study, *D. brevirostris* was nested in the *Duttaphrynus melanostictus* group, with high support for the recovered phylogenetic position, whereas *D. peninsularis* and *D. stomaticus* were recovered in the *Duttaphrynus stomaticus* group with variably-supported relationships (weak or high) in the ML and BI analyses. The genetic differentiation at the species level, based on an expanded mitochondrial 16S rRNA dataset, however, is relatively shallow as compared to other wide-ranging anuran groups in South Asia, such as dicroglossids, microhylids, ranids, and rhacophorids (Biju et al. 2014b, 2020; Vijaykumar et al. 2014; Dinesh et al. 2015; Garg and Biju 2017; Garg et al. 2018, 2019). The maximum intraspecific divergence within the recognised or putative species reaches up to 2.1% in the *Duttaphrynus melanostictus* group (Fig. 3B; Suppl. material 1: Table S4). At the same time, low intraspecific distances of 1.0–6.0% are observed in both species groups. The interspecific divergence between *D. stomaticus* and *D. olivaceus* species is rather shallow (0.2–0.6%) but, together, these two taxa are more extensively differentiated from their sister species *D. peninsularis* (1.0–2.6%). In general, interspecific divergences among some members of the *Duttaphrynus stomaticus* group (*D. stomaticus + D. olivaceus, D. dhufarensis, and D. peninsularis*) trend towards the lower extent of the spectrum (1.0–1.5%) of genetic divergences observed in other *Duttaphrynus* species groups (Fig. 3B; Suppl. material 1: Table S4).

Our species delimitation analyses for the *Duttaphrynus stomaticus* group recovered only four species: *D. dhufarensis*, *D. hololius*, and *D. peninsularis*, and *D. stomaticus + D. olivaceus* (as a single species) (Fig. 3). Hence, our results indicate the need for a future comprehensive phenotypic assessment for all members of the group from its entire range, in order to clarify the taxonomic status of unsupported populations of ‘*D. olivaceus*’, for which specimens were not available in our study for imparting a conclusive morphological evaluation. Furthermore, the results of species delimitation also suggest the presence of additional putative species among other known members of the genus *Duttaphrynus* (Fig. 3B): within the *Duttaphrynus melanostictus* group, one additional putative species was recovered, apart from two previously known and unidentified taxa (*Duttaphrynus* sp. 1 and *Duttaphrynus* sp. 2); within the *Duttaphrynus scaber* group, three putative species were recovered; finally, the *D. himalayanus* lineage comprised of three potential candidate species. These results indicate the possible presence of potentially undescribed cryptic species diversity within the genus, which requires further investigation.

The mitochondrial 16S gene median-joining network, however, did not show sharing of any haplotypes among the studied populations of various recognised or putative species of the genus *Duttaphrynus* (Fig. 3C). The *Duttaphrynus stomaticus* and *D. melanostictus* groups formed distinct species clusters separated by nine mutation steps. At the species-level, members of *D. stomaticus* group were separated by a minimum of one to five mutation steps between *D. olivaceus–D. stomaticus* and *D. peninsularis–D. olivaceus*, respectively, and a minimum of 15 steps between *D. hololius* and the remaining species of the group. Within the *Duttaphrynus melanostictus* group, the putative *Duttaphrynus* spp. 1 and 2 were separated by three mutation steps, followed by four steps between *D. melanostictus–D. parietalis* and *D. melanostictus–D. cf. microtympanum*, and up to a minimum of 10 steps between *D. melanostictus–D. sp. 1*. All other known members of the genus—*D. scaber* group species (*D. cf. atukoralei* and *D. scaber*), *D. himalayanus*, *D. stuarti*, and *D. crocus*—were separated from species of the *D. melanostictus* group and *D. stomaticus* group by at least eight mutation steps (Fig. 3C).

Altogether, our various analyses were congruent with respect to the distinctness and phylogenetic position of *D. brevirostris* and *D. peninsularis*. We suggest a further detailed population-level investigation of the *D. stomaticus + D. olivaceus* clade, for which the name *D. stomaticus* (Lütken 1864) holds priority, if *D. olivaceus* (Blanford 1874) is confirmed to be conspecific by evaluation of phenotypic data. Our results also shed light on the degrees of mitochondrial differentiation among members of the *D. stomaticus* group, as well as the other known species of the genus; these and other data will facilitate future taxonomic and phylogenetic studies on toads of the genus *Duttaphrynus*. 
Conclusions

The results of this study resolve long-standing uncertainty regarding the identities and taxonomic status of two toad species described from Peninsular India. *Bufo brevirostris* Rao, 1937 was considered a problematic taxon, because its original name-bearing types are lost. *Bufo stomaticus peninsularis* Rao, 1920 was long forgotten as an available name for Peninsular Indian populations closely related to *Duttaphrynus stomaticus*. We substantiate *D. peninsularis* to be a distinct species, which is both morphologically diagnosable and phylogenetically distinct. Taxonomic redefinition of both of these species was achieved not just by examining the original literature and available types, but also through an effort to rediscover new material from each species’ respective type locality. The redescription of *Bufo brevirostris* Rao, 1937 based on new toptotypic material, along with detailed comparisons to related taxa, objectively clarifies its identification for future reference. Similarly, toptotypic material for *Bufo stomaticus peninsularis* Rao, 1920 enabled a detailed re-evaluation of its taxonomic status in the absence of a well-preserved type. Altogether, our results emphasise that new collections from type localities of historically available names should be attempted when taxonomic resolution is not feasible on the basis of original descriptions or type specimens (Bailey 1933; Garg and Biju 2016).

The present work clarified the taxonomic identity of another species, *Duttaphrynus stomaticus*, which was overlooked due to its presumed wide distribution. This taxon was known only from its brief original description, and the available, original name-bearing types remained unexamined due to literature-based misconceptions concerning their untraceability (Dutta 1997; Ganesh et al. 2020). We located the well-preserved eight original type specimens, and clarified the status of name-bearing types and the identity of this species, which we redescribed to facilitate future taxonomic studies. This action also aided our objective of resolving the taxonomic status of *D. peninsularis*, which was originally defined as a variety of *D. stomaticus*. Our results have important implications concerning the taxonomy and geographical ranges of the two species. Hereafter, *D. stomaticus* should be considered as a species found in the northern regions of South Asia, whereas its sister taxon *D. peninsularis* should be recognised as a Peninsular Indian form (Fig. 4; Suppl. material 1: Table S3). Detailed redescriptions provided in this study will enable proper identification and range delineation, and serve as the basis for future conservation action. Knowledge of phenotypic variation and phylogenetic affinities of both species will also facilitate a better understanding of patterns of genetic differentiation within the genus, particularly among the species of the *Duttaphrynus stomaticus* group.

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Supplementary material 1

Supplementary tables S1–S5

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Data type: species data

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