



Range Extension of the Western Burrowing Frog, *Sphaerotheca pashchima* (Anura: Dicroglossidae), in Central and Northern India, with an Overview of the Distribution of other Indian Species in the Genus *Sphaerotheca*

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The South Asian endemic burrowing frogs of the genus *Sphaerotheca* comprise ten currently recognized species in

three morphological groups (Dahanukar et al. 2017; Prasad et al. 2019). Five species occur in India: Western Burrowing

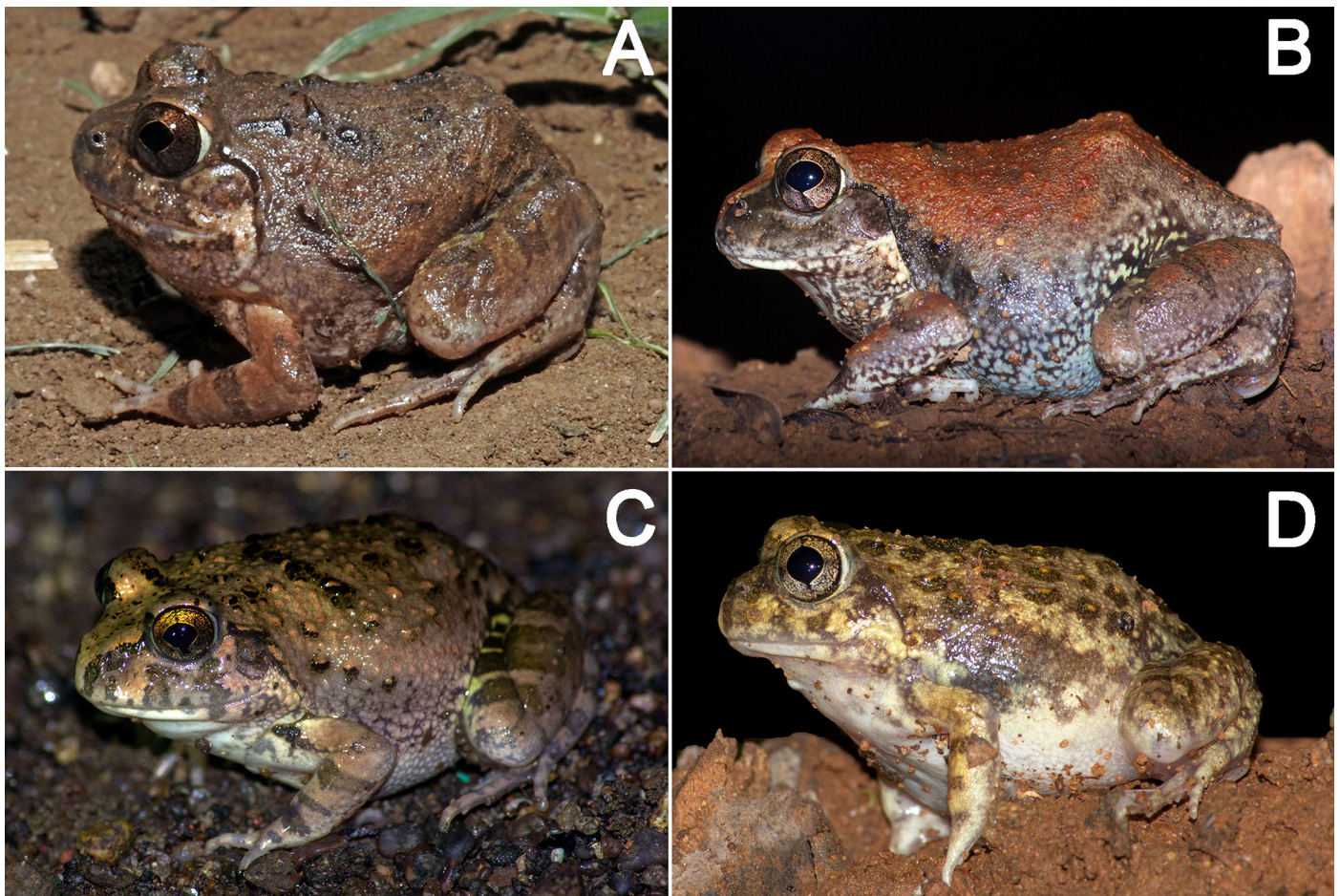


Fig. 1. Frogs of the genus *Sphaerotheca* from northern and eastern Maharashtra, India: Western Burrowing Frogs (*Sphaerotheca pashchima*) from Melghat (A), Chandrapur (B), and Yavatmal (C); a Magadha Burrowing Frog (*S. magadha*) from Chandrapur (D). Photographs by Shubhankar Deshpande (A) and Nikhil Dandekar (B, C, D).

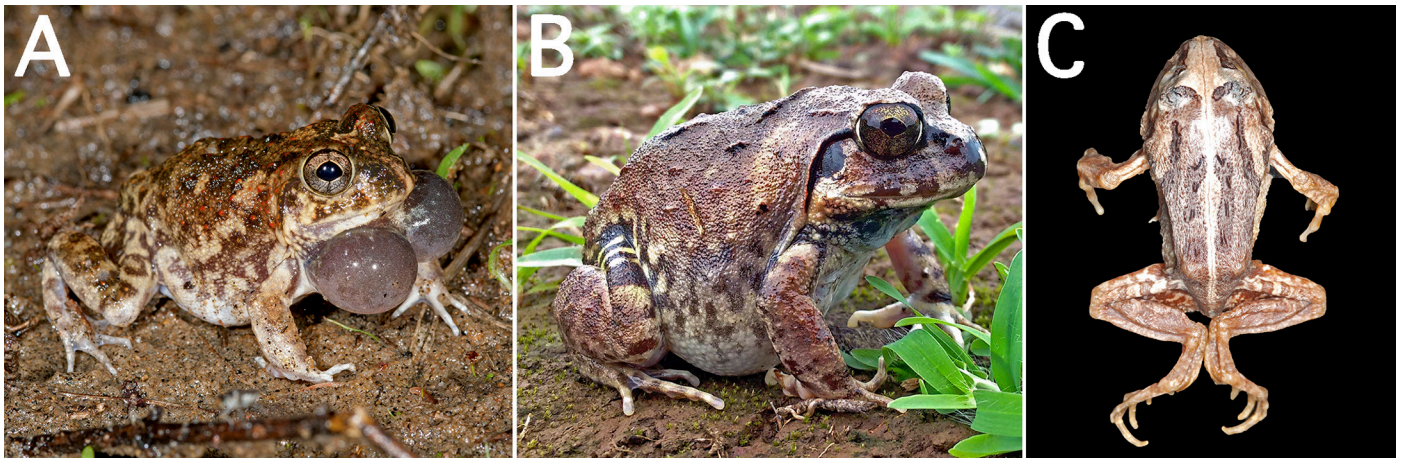


Fig. 2. Frogs of the genus *Sphaerotheca* from India: A Short-headed Burrowing Frog (*Sphaerotheca breviceps*) from Tranquebar (A); a Dobson's Burrowing Frog (*S. dobsonii*) from the Bankot-Mandangad Road, Ratnagiri, Maharashtra (B); and a Jerdon's Burrowing Frog (*S. pluvialis*) from the Tadoba-Andhari Tiger Reserve, Chandrapur, Maharashtra (C). Photographs by Shauri Sulakhe (A & C) and Nikhil Dandekar (B).

Frog, *S. pashchima* Padhye et al. 2017 (Fig. 1A–C); Magadha Burrowing Frog, *S. magadha* Prasad et al. 2019 (Fig. 1D); Short-headed Burrowing Frog, *S. breviceps* (Schneider 1799) (Fig. 2A); Dobson's Burrowing Frog, *S. dobsonii* (Boulenger 1882) (Fig. 2B); and Jerdon's Burrowing Frog, *S. pluvialis* (Jerdon 1853) (Fig. 2C) (Figs. 3–4).

The Western Burrowing Frog, *Sphaerotheca pashchima* Padhye et al. 2017, was described from western peninsular India (mainly the states of Gujarat, Maharashtra, and Karnataka). We

herein report the genetically confirmed occurrence of this species from northern and eastern Maharashtra, representing a substantial range extension from the nearest known locality, and provide an overview of the distribution of all Indian species of *Sphaerotheca*.

We collected Western Burrowing Frogs from roadside pools in semi-evergreen and dry deciduous forests at one locality in Amravati District in northern Maharashtra and one locality each in the Yavatmal and Chandrapur districts of eastern Maharashtra (Fig. 4). Specimens were preserved in

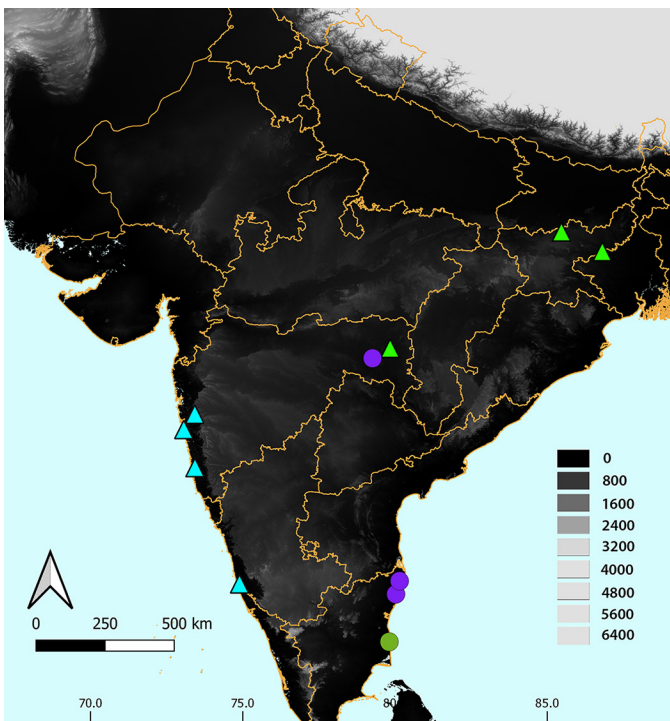


Fig. 3. Map showing the distribution of species in the genus *Sphaerotheca* in India: Short-headed Burrowing Frog (*S. breviceps*) green dot, Jerdon's Burrowing Frog (*S. pluvialis*) purple dots, Magadha Burrowing Frog (*S. magadha*) green triangles, and Dobson's Burrowing Frog (*S. dobsonii*) blue triangles.

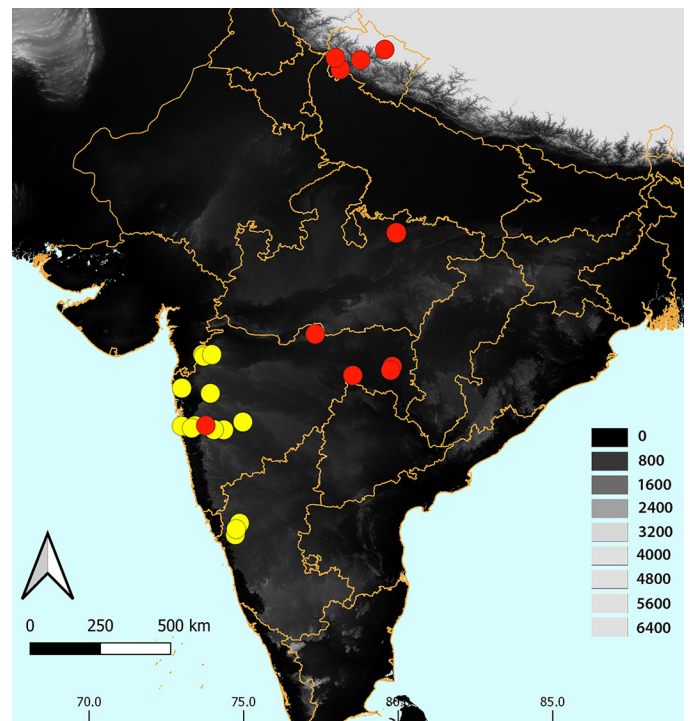


Fig. 4. Map showing the distribution of the Western Burrowing Frog (*Sphaerotheca pashchima*) in India. Locations published in Padhye et al. (2017) are marked by yellow dots; locations published after Padhye et al. (2017) and in the current study by red dots.

Table 1. Specimens examined in this study. Asterisks (*) indicate molecular data generated during this study.

Species (Voucher number)	Source or Date of collection	Locality (latitude °N, longitude °E)	GenBank Accession Number
<i>S. pashchima</i> (BNHS 6011)	Padhye et al. (2017)	Dharwad-Halliyal-Yellapur Rd., Karnataka (15.3494,74.8675)	KY215979
<i>S. pashchima</i> * (WILD-16-AMP-655)	29.xi.2016 ¹	Dharwad-Halliyal-Yellapur Rd., Karnataka (15.3494, 74.8675)	MT798138
<i>S. pashchima</i> (ZSI-WRC A/1549)	Padhye et al. (2017)	Yellapur-Halliyal Rd., Karnataka (15.1602, 74.7587)	KY215980
<i>S. pashchima</i> (BNHS 6012)	Padhye et al. (2017)	Near Yellapur, Karnataka (14.9799,74.7314)	KY215981
<i>S. pashchima</i> * (WILD-16-AMP-656)	29.xi.2016 ¹	Near Yellapur, Karnataka (14.9799, 74.7314)	MT798139
<i>S. pashchima</i> (ZSI-WRC A/1550)	Padhye et al. (2017)	Near Yellapur, Karnataka (14.9799, 74.7314)	KY215982
<i>S. pashchima</i> (BNHS 6013)	Padhye et al. (2017)	Kolad, Raigad, Maharashtra (18.4037, 73.3213)	KY215983
<i>S. pashchima</i> * (WILD-16-AMP-657)	03.xi.2016 ²	Kolad, Raigad, Maharashtra (18.4037, 73.3213)	MT798140
<i>S. pashchima</i> (BNHS 6014)	Padhye et al. (2017)	Talekhar, near the Phansad WLS (18.47, 72.99)	KY215984
<i>S. pashchima</i> * (WILD-16-AMP-658)	03.xii.2016 ³	Talekhar, near the Phansad WLS (18.47, 72.99)	MT798141
<i>S. pashchima</i> (BNHS 6015)	Padhye et al. (2017)	Akole, Sangamner, Maharashtra (19.518, 73.923)	KY215985
<i>S. pashchima</i> (BNHS 6016)	Padhye et al. (2017)	Wada, Thane, Maharashtra (19.687, 73.000)	KY215986
<i>S. pashchima</i> (BNHS 6017)	Padhye et al. (2017)	Salher Fort Road, Maharashtra (20.747, 93.973)	KY215987
<i>S. pashchima</i> (BNHS 6018)	Padhye et al. (2017)	Waghai-Ahwa Road, Dang, Gujarat (20.709, 73.709)	KY215988
<i>S. pashchima</i> (BNHS 6019)	Padhye et al. (2017)	Ahwa-Chinchli Road, Dang, Gujarat (20.765, 73.972)	KY215989
<i>S. pashchima</i> (WILD-16-AMP-641)	Padhye et al. (2017)	Rehekuri WLS, Maharashtra (18.5982, 74.9742)	KY215990
<i>S. pashchima</i> (WILD-16-AMP-642)	Padhye et al. (2017)	Saswad, Pune, Maharashtra (18.3075, 74.083)	KY215991
<i>S. pashchima</i> (BNHS 6003)	Padhye et al. (2017)	Ahwa, Gujarat (20.7644, 73.6757)	KY215992
<i>S. pashchima</i> (WILD-16-AMP-643)	Padhye et al. (2017)	Ahwa, Gujarat (20.7644, 73.6757)	KY215993
<i>S. pashchima</i> (WILD-16-AMP-644)	Padhye et al. (2017)	Tamhini, Pune (18.4773, 73.4267)	KY215994
<i>S. pashchima</i> * (INHER-Amphibia-175)	20.xiii.2016 ⁴	Pune City, Maharashtra (18.487056, 73.774074)	MT773254
<i>S. pashchima</i> * (INHER Amphibia-194)	27.xi.2017 ⁵	Maregaon, Chandrapur, Maharashtra (20.25794, 79.75656)	MT773260
<i>S. pashchima</i> * (INHER Amphibia-201)	08.xiii.2017 ⁶	Ekara, Chandrapur, Maharashtra (20.38215, 79.810225)	MT773261
<i>S. pashchima</i> * (INHER-Amphibia-236)	26.iv.2018 ⁷	Chikhaldara, Melghat, Maharashtra (21.418279, 77.315735)	MT773255
<i>S. pashchima</i> * (INHER-Amphibia-239)	13.xiii.2018 ⁵	Pandharkawda, Maharashtra (20.096232, 78.532176)	MT773257
<i>S. pashchima</i> * (INHER-Amphibia-240)	13.xiii.2018 ⁵	Pandharkawda, Maharashtra (20.096232, 78.532176)	MT773256
<i>S. pashchima</i> (SbSG17HNBGU)	A. Choudhary (2017, unpubl.)	Rawadi, Uttarakhand (30.22177, 78.78434)	KX815440
<i>S. pashchima</i> (SbJM1HNBGU)	Choudhary A. (2017, unpubl.)	Joshimath, Uttarakhand (30.55444, 79.56635)	KX815439
<i>S. pashchima</i> (Sb27HNBGU)	Choudhary A. (2017, unpubl.)	Jageetpur, Haridwar, Uttarakhand (29.91889, 78.12618)	KX815438
<i>S. pashchima</i> (SbHR25HNBGU)	Choudhary A. (2017, unpubl.)	Jageetpur, Haridwar, Uttarakhand (29.91889, 78.12618)	KX815437
<i>S. pashchima</i> (Sb31HNBGU)	Choudhary A. (2017, unpubl.)	Chandrabani, Dehradun, Uttarakhand (30.28476, 77.97365)	KX815435
<i>S. pashchima</i> (Sb32HNBGU)	Choudhary A. (2017, unpubl.)	Chandrabani, Dehradun, Uttarakhand (30.28476, 77.97365)	KX815436
<i>S. pashchima</i> (WIIADA225)	Prasad et al. (2020)	Panna Tiger Reserve, Madhya Pradesh (24.63001, 79.97365)	MN741157
<i>S. pashchima</i> (WIIADA232)	Prasad et al. (2020)	Panna Tiger Reserve, Madhya Pradesh (24.63001, 79.97365)	MN741158
<i>S. dobsonii</i> (INHER Amphibia-86)	Dahanukar et al. (2017)	Tamhini,Pune, Maharashtra (18.4773, 73.4267)	KY215970
<i>S. dobsonii</i> (WILD-16-AMP-651)	Dahanukar et al. (2017)	Devi Hasool, Maharashtra (16.7415, 73.4320)	KY215971
<i>S. dobsonii</i> (BNHS 6008)	Dahanukar et al. (2017)	Devi Hasool, Maharashtra (16.7415, 73.4320)	KY215972
<i>S. dobsonii</i> (BNHS 6010)	Dahanukar et al. (2017)	Bankot-Mandangad Road, Maharashtra (17.9801, 73.0649)	KY215974
<i>S. dobsonii</i>	Kotaki et al. (2010)	Bajpe, Mangalore, Karnataka (12.984187, 74.881343)	AB277305
<i>S. dobsonii</i>	Kotaki et al. (2010)	Bajpe, Mangalore, Karnataka (12.984187, 74.881343)	AB530608
<i>S. breviceps</i> (BNHS 6005)	Dahanukar et al. (2017)	Tranquebar, Tamil Nadu (11.0621, 79.8128)	KY215977
<i>S. breviceps</i> (WILD-16-AMP-645)	Dahanukar et al. (2017)	Tranquebar, Tamil Nadu, 11.0621, 79.8128)	KY215978
<i>S. pluvialis</i> (ZSI A9074)	Vences (2000)	Pattaravakkam Hill, Tamil Nadu (12.696, 80.030)	AF215418
<i>S. pluvialis</i> (ZSI 2681)	Bossuyt (2003)	Sri Lanka	AF249042
<i>S. pluvialis</i> * (INHER-Amphibia-180)	01.xiii.2013 ⁸	Wadala Tukum near Tadoba, Chandrapur, Maharashtra (20.300, 79.262)	MT773262
<i>S. magadha</i> (ZSI/WRC/2179)	Prasad et al. (2019)	Koderma, Jharkhand (24.417985, 85.468000)	MK694738
<i>S. magadha</i> (BNHS 6006)	Dahanukar et al. (2017)	Maithon, Jharkhand (23.7758, 86.8092)	KY215975
<i>S. magadha</i> (WILD-16-AMP-647)	Dahanukar et al. (2017)	Maithon, Jharkhand (23.7758, 86.8092)	KY215976
<i>S. magadha</i> * (INHER Amphibia-189)	15.vii.2017 ⁹	Bramhapuri, Chandrapur, Maharashtra (20.62367, 79.84725)	MT773258
<i>S. magadha</i> * (INHER Amphibia-191)	15.vii.2017 ⁹	Bramhapuri, Chandrapur, Maharashtra (20.62367, 79.84725)	MT773259

¹Collected by Anand Padhye, Shauri Sulakhe, Chaitanya Risbud, Rajgopal Patil. ²Collected by Nikhil Dandekar and Srushti Bhawe. ³Collected by Makarand Ketkar and Mayuresh Kulkarni. ⁴Collected by Makarand Ketkar. ⁵Collected by Nikhil Dandekar. ⁶Collected by Nikhil Dandekar and Disha Sharma. ⁷Collected by Makarand Ketkar and Shubhankar Deshpande. ⁸Collected by Abhijeet Bayani. ⁹Collected by Nikhil Dandekar and Jaydeep Patil.

100% ethanol, later transferred to 70% ethanol for long-term preservation, and deposited in the museum of the Institute of Natural History, Education, and Research (INHER), Pune (Table 1). Harvested thigh-muscle tissues were preserved in absolute ethanol.

Frogs were identified as *S. pashchima* based on interorbital width less than upper eyelid width, snout-to-nostril distance less than half of eye diameter, nostril closer to snout than to eye, internarial distance greater than interorbital distance, snout rounded, dorsum rough and warty, finger 2 equal to or less than finger 4 in length, finger 1 less than finger 3 in length, outer metatarsal tubercle absent, tibiotarsal tubercle absent, and length of inner metatarsal tubercle more than three times inner toe length (Padhye et al. 2017). To further corroborate the morphological diagnosis, we employed mtDNA barcoding. DNA extraction, PCR amplification of 16S rRNA gene, and sequencing protocols followed Padhye et al. (2014). Sequences were analysed by the BLAST tool (Altschul et al. 1990) for similar sequences in NCBI Genbank database (www.ncbi.nlm.nih.gov). Sequences generated in the current study were deposited in GenBank (accession numbers MT798138–MT798141 and MT773254–MT773262; Table 1). New sequences were added to the database used by Dahanukar et al. (2017), Padhye et al. (2017), and Prasad et al. (2019) for genetic analysis.

Generated sequences were cleaned manually in MEGA 7 (Kumar et al. 2016) using chromatograms visualized in Chromas V.2.6.5 (Technelysium PTY. Ltd.). Cleaned and downloaded sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA 7 (Kumar et al. 2016) using default parameters. The final alignment used in phylogenetic analyses contained 52 sequences each of 527 bp length including 2 sequences of *Mimervarya cepfi* (Table 1) used as an outgroup to root the phylogenetic tree.

We used maximum-likelihood (ML) and Bayesian inference (BI) methods for phylogenetic analysis. The dataset of 16S region was not partitioned per codon position and the best substitution model for phylogenetic analysis was determined using PartitionFinderV.1.1.1 (Lanfear et al. 2012). Model search was performed using the Bayesian Information Criterion (BIC) (Schwarz 1978). Maximum-likelihood analysis was performed in RaxmlGUI (Silvestro and Michalak 2011) under the GTR + G model of sequence evolution. Branch support was tested using 1,000 non-parametric bootstrap pseudo-replicates. Bayesian trees were generated using MrBayes V.3.2.6 (Ronquist et al. 2012). The best substitution model for phylogenetic analysis was determined using PartitionFinder V.1.1.1 (Lanfear 2012). Model search was performed using the Bayesian Information Criterion (BIC) with a greedy search algorithm. The model of sequence evolution was SYM + G. Two simultaneous, independent analyses were run starting from different random trees. Three heated

and one cold chain was used in the analysis. Markov chains were sampled every 150 generations for 2.5 million generations. At the end of the run, the standard deviation of split frequencies was less than 0.005 and the analyses were not continued further. The absence of bimodal distribution indicated clear convergence (Tracer v.1.7; Rambaut et al. 2003) and all ESS values were above 200. A total of 25% of trees were discarded as burn-in. The tree representing the best evolutionary hypothesis was selected using a 50% majority consensus rule. Species delimitation analysis was performed on the BI tree using Bayesian Poisson Tree Process using 500000 Markov chain Monte Carlo (MCMC) generations with thinning parameter of 100 and burn-in of 0.1 (Zhang et al. 2013).

Delimitation analysis unambiguously confirmed the identification of five species: *S. pashchima*, *S. breviceps*, *S. dobsonii*, *S. magadha*, and *S. pluvialis* from India with strong posterior probability values (Figs. 5–6).

Few records of *S. pashchima* have been published since its description by Padhye et al. (2017). Prasad et al. (2019) extended the range of *S. pashchima* to the lower Himalayas in the state of Uttarakhand based on genetic sequences submitted to GenBank (accession numbers KX815435–KX815440) (Table 1). Our delimitation analysis reconfirmed this species to be *S. pashchima*. Prasad et al. (2020) genetically confirmed the presence of the species in the Panna Tiger Reserve in Madhya Pradesh. Our study also confirmed the occurrence of *S. pashchima* in eastern Maharashtra, extending the species' range into central India (Fig. 4). Dahanukar et al. (2017) mentioned that “The sequence GU191122 for specimen identity as *S. rolandae* from Rajasthan is not of good quality and with several gaps; however, partial sequence comparison suggests that the species is misidentified and is likely to be *S. pashchima* sp. nov. indicating its presence in Rajasthan.” However, due to the lack of a precise locality, we could not include it on the map; thus the presence of *S. pashchima* in Rajasthan needs to be confirmed. Frogs collected for this study were found mostly in roadside puddles in forested areas or areas with good tree cover, but only a few individuals were seen at any one location. Based on our data and that from the literature, *Sphaerotheca pashchima* occupies a variety of habitats in the semi-evergreen forests in the Western Ghats, the deciduous forests in central India, the rainshadow of the Deccan Plateau, the evergreen forests in the western Himalayan region, and might be widely distributed throughout central, western, and northern India.

Sphaerotheca magadha was described from the eastern state of Jharkhand (Prasad et al. 2019). In this study we confirm its presence in the Vidarbha region of Maharashtra, extending the species' range in central India by more than 700 km from the nearest previously reported locality (Fig. 3). Similarly *S. pluvialis*, which was considered to be restricted

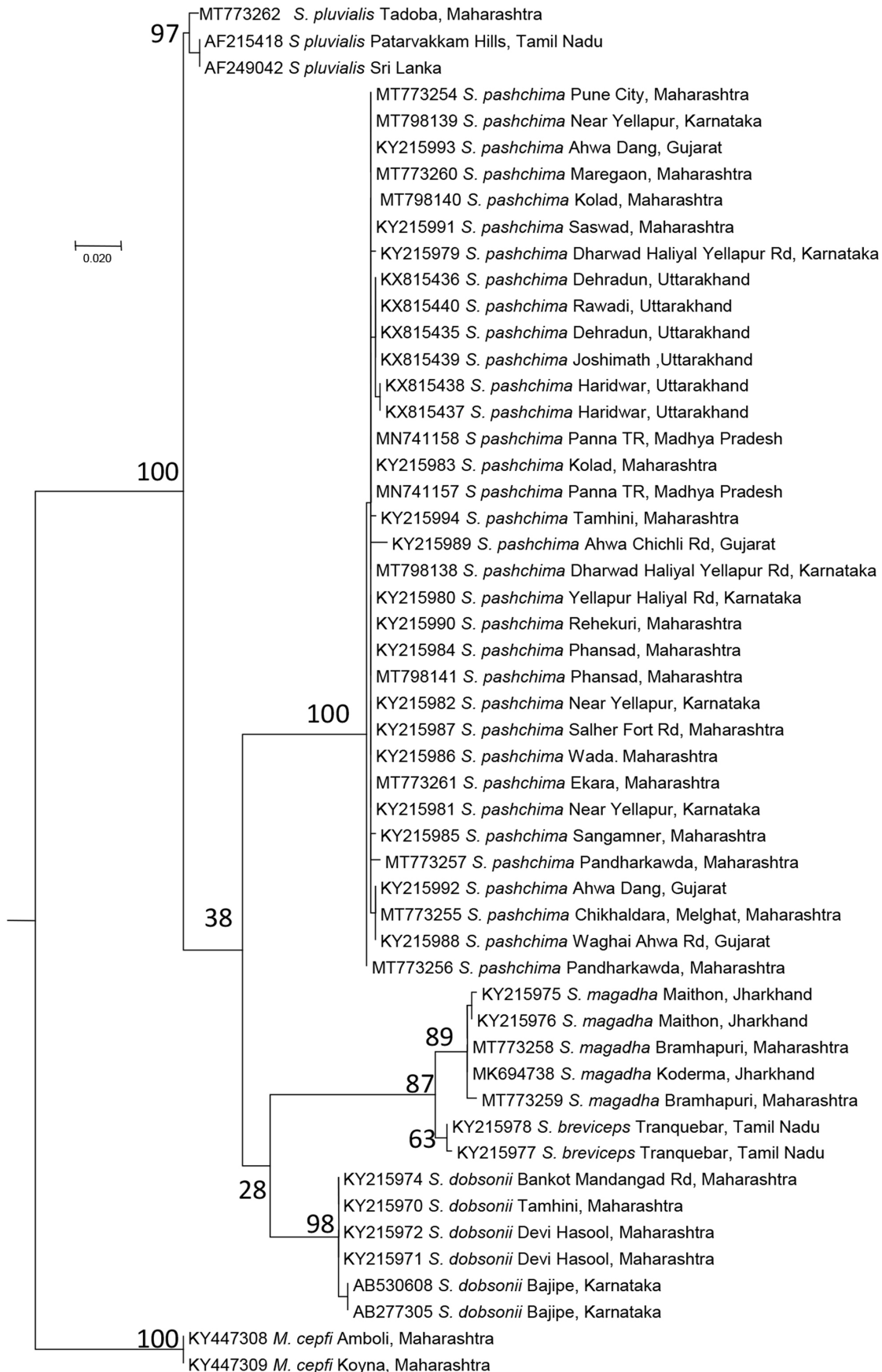


Fig. 5. Maximum-likelihood tree of 16S rRNA partial gene sequences of *Sphaerotheca* spp. Values along the nodes are percent bootstraps for 1,000 iterations.

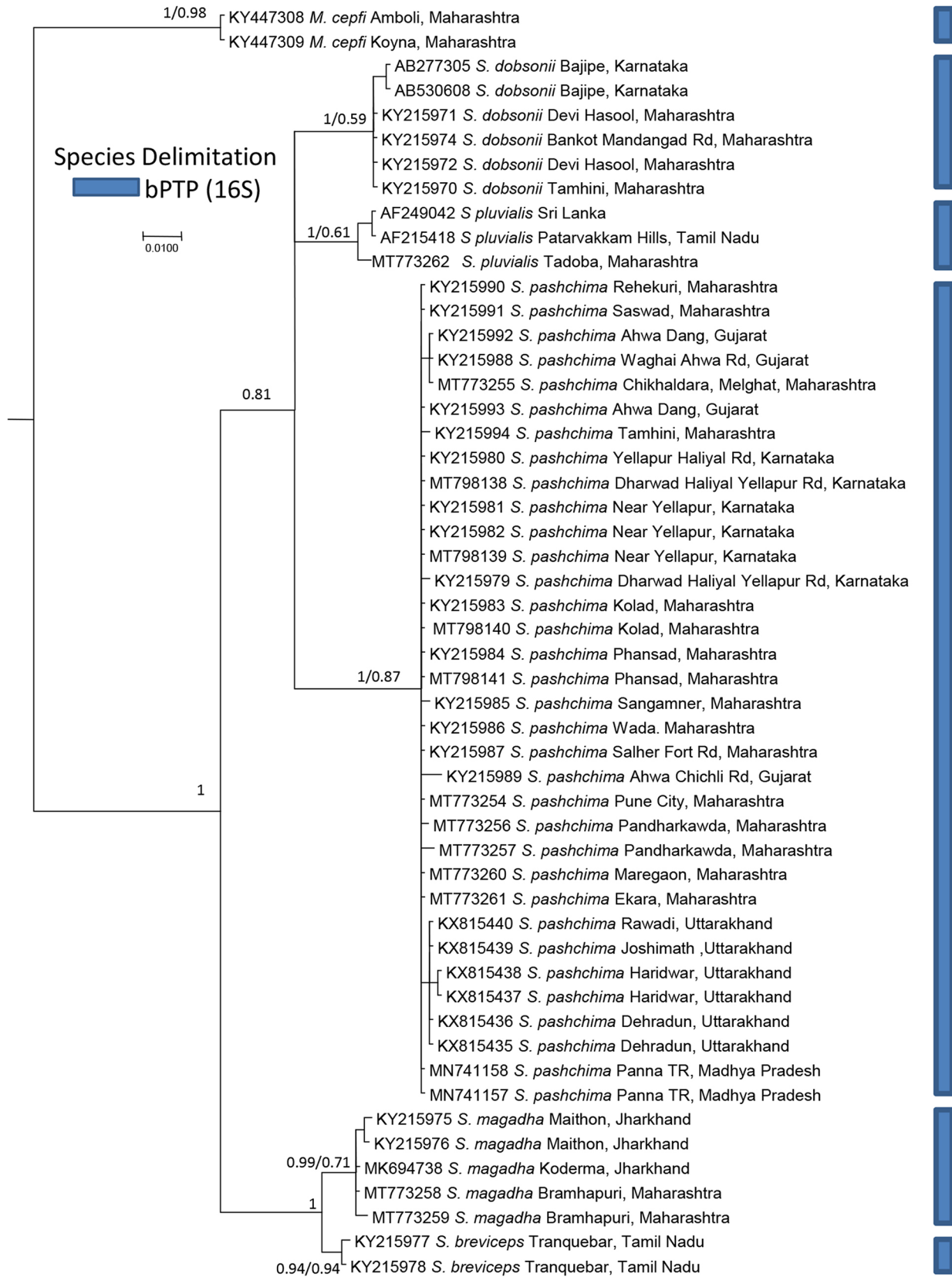


Fig. 6. Bayesian phylogenetic tree of 16S rRNA partial gene sequences of *Sphaerotheca* spp. Values along the nodes are Bayesian posterior probabilities for Bayesian Inference and Bayesian Poisson Tree Process (bPTP), respectively.

to the southern Indian state of Tamil Nadu and Sri Lanka (Prasad et al. 2019), was found in the Vidarbha region of Maharashtra (Fig. 3). Dahanukar et al. (2017) mentioned that specimens of *S. pluvialis* from southern India (ZSI 125151) and from Sri Lanka (ZSI 2681) lacked accurate locality data. The new record extends the range of *S. pluvialis* by more than 800 km from the nearest previously documented locality. We encountered large numbers of *S. magadha* and *S. pluvialis* in the agricultural areas during early monsoon showers.

So, three species of *Sphaerotheca* (*S. pashchima*, *S. magadha*, and *S. pluvialis*) appear to be sympatric in central India. *Sphaerotheca magadha* possesses tarsal tubercles, the presence of which also is used to identify *S. rolandae*. When we examined the entire collection of Indian *Sphaerotheca* in the museum of the ZSI in Kolkata, we found multiple specimens with tarsal tubercles that had been collected both in central India and from southern states. Unfortunately, no genetic sequence of *S. rolandae* from the type locality in Sri Lanka, which is essential to confirm its presence in India, is available for comparison.

Prasad et al. (2019) raised doubt about possible speciation in populations of *S. dobsonii* from higher and lower elevations. The delimitation analysis clearly showed that the specimens mentioned by Dahanukar et al. (2017) from all the locations in the states of Karnataka and Maharashtra are of the same species.

Little information documents the occurrence of different species of *Sphaerotheca* in the northern and southern states of India. That deficiency in part might be attributable to the difficulty of identifying species based on morphological characters alone (Prasad et al. 2019). The new records presented herein provide additional information about the distribution of species of *Sphaerotheca* and highlight the importance of using an integrated taxonomic approach and a need for more data to accurately assess the conservation status of these species.

Acknowledgements

We are thankful to Makarand Ketkar, Jaydeep Patil, Abhijeet Bayani, Disha Sharma, and Shubhankar Deshpande for their help with fieldwork. Special thanks to Srushti Bhawe for technical help in preparing the manuscript. Authors are thankful to the President of INHER, Pune, for institutional support, partial funding, and encouragement. We also thank all the anonymous reviewers for their help and comments.

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