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A new species of fan-throated lizard of the genus *Sitana* Cuvier, 1829 (Squamata: Agamidae) from northern Karnataka, India

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Abstract. A new species of fan-throated lizard of the genus *Sitana* Cuvier, 1829 is described from northern Karnataka, India. The new species is similar to members of the clade of *Sitana spinaecephalus* Deepak et al., 2016, however, can be distinguished based on morphological as well as molecular data. *Sitana dharwarensis* sp. nov. differs from its sister species, *S. laticeps* Deepak & Giri, 2016 in bearing a much larger dewlap. Data from micro-CT scan of the cranium and jaws further add support to the distinctness of the new species. The rivers, namely Krishna and Tungabhadra, likely act as a biogeographic barrier for terrestrial lizard species.

Key words. Reptilia, mtDNA, molecular phylogeny, micro-CT scan, taxonomy.

INTRODUCTION

Members of the genus *Sitana* Cuvier, 1829 have received considerable attention from the view of systematics, evident from the recent surge in species descriptions in the last five years (Amarasinghe et al. 2015; Deepak et al. 2016a; b; Deepak & Karanth 2017; Sadasivan et al. 2018). The genus currently contains eleven species, however, a recent molecular investigation hints at the presence of additional undescribed species (Deepak & Karanth 2017).

During the course of a herpetological investigation trip to northern Karnataka, a state in south India, we collected specimens of *Sitana*, which resembled *S. laticeps* Deepak & Giri, 2016, and *S. spinaecephalus* Deepak, Vyas & Giri, 2016 based on the dewlap coloration (Deepak et al. 2016a). Molecular data for a specimen was generated which shows that the specimen was genetically related to *S. laticeps*, however, it differed in several aspects with regard to its morphology. Phylogenetic analysis based on existing sequences generated by Deepak & Karanth (Deepak & Karanth 2017) and the sequence generated in the present work recovered two well supported clades; one representing *S. laticeps sensu stricto* occurring north of Krishna river, and a second clade representing specimens from south of the river. The clade containing sequences from south of Krishna river is divergent and exhibits unique set of morphological characters which enables us to describe it as a new species herein based on molecular as well as morphological data, further supported by its allopatric range.

MATERIALS AND METHODS

Morphology. Specimens were collected by hand, euthanized and fixed in 6% formalin. They were later washed and stored in 70% ethanol. Muscle tissue was taken prior to fixation and stored for molecular work. The type specimens are deposited in the collection of the Bombay National History Society, Mumbai (BNHS) and the collection facility of the National Centre for Biological Sciences, Bangalore (NCBS). Specimens were measured using a Mitutoyo™ digital caliper. Descriptive style and morphometric/morphological characteristics were recorded as follows (Sadasivan et al. 2018). The following measurements were taken: snout-vent length (SVL, from tip of snout to anterior border of cloaca), head length (HL, from snout tip to posterior border of tympanum), head width (HW, distance from left to right outer edge of the head at its widest point), head height (HH, dorsoventral distance from top of head to underside of jaw at transverse plane intersecting angle of jaws), snout-eye length (SE, from snout tip to anterior border of orbit), eye to tympanum (ET, from posterior border of orbit to anterior border of tympanum), jaw length (JL, from rostrum to corner of jaw), interorbital width (IO, transverse distance between anterodorsal corners of left and right

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orbits), nares to eye (NE, distance from the anterior edge of orbit to posterior edge of naris), snout width/internasal distance (IN, transverse distance between left and right nares), tympanum diameter (TD, greatest diameter of tympanum), orbit diameter (OD, distance between anterior and posterior margins of orbit), lower arm length (LAL, distance from elbow to distal end of wrist, or just underside of forefoot when the limb is flexed), upper arm length (UAL, distance from anterior insertion of forelimb to elbow when the limb is flexed), finger lengths (F1, F2, F3, F4, F5) (e.g., F4 = Distance from juncture of 3rd and 4th digits to distalmost extent of 4th finger including the claw), femur length (FEL, length of femur from groin to knee), crus length (CL, length of crus (tibia) from knee to heel), hind foot length (HFL, distance from proximal end (heel) of hind foot to distal most point of fourth toe), hind limb length (HLL, from groin to tip of fourth toe), toe lengths (T1, T2, T3, T4) (e.g., T4 = Distance from juncture of 3rd and 4th digits to distal end of 4th digit on hind foot), trunk length (TrL, from forelimb insertion to hind limb insertion), trunk height (TrH, depth midway between the fore and hind limb insertions), trunk width (TrW, width midway between the fore and hind limb insertions), tail length (TL, from posterior border of cloacal opening to tip of tail), tail height (TH) and tail width (TW, at tail base),dewlap length (DWL, distance between posterior end of dewlap and tip of lower jaw), and extent of dewlap in trunk (DWLT, measured from the axilla till the end of the dewlap). Meristic characters were counted for multiple individuals per species. The following characters were scored: mid-body scale rows (MBS, number of scale rows around the trunk at midbody), ventral scales (VEN, number of scales from below mental around the base of the dewlap to anterior border of cloaca), fourth toe lamellae (LAM4, number of 4th toe lamellae, from 1st lamella at the digit's cleft to the most distal lamella), dewlap scales (ESD, number of enlarged scale rows on the dewlap), supralabials (SL, posterior end defined by the last enlarged scale that contacts the infralabials at the corner of mouth), infralabials (IL, posterior end defined by the posterior most enlarged scales that contact the supralabials at the corner of the mouth), ventral scales on the belly (VENB, number of scales posterior to the dewlap to the anterior border of cloaca), and vertebral scales (VS, number of scales above the vertebral column counted from the mid-dorsal first nuchal spine to a level directly above the cloacal opening).

Institutional abbreviations

NCBS = National Centre for Biological Sciences, Bangalore
BNHS = Bombay Natural History Society, Mumbai
CES = Centre for Ecological Sciences, Bangalore

Micro-CT scans were generated for three male specimens using a Bruker® Skyscan 1272 (Bruker BioSpin Corporation, Billerica, Massachusetts, USA). Head of the specimens were scanned from 16 to 20 minutes at 15µm. Volume rendering was performed with CTVox (Bruker BioSpin Corporation, Billerica, Massachusetts, USA) and images were edited in Adobe Photoshop CS6. Osteological description is based on volume renders retrieved from CTVox following terminology of the skull described by Evans (Evans 2008).

Molecular analysis: Genomic DNA was extracted from liver tissue following Qiagen DNeasy™ Tissue kits following protocols specified by manufacturers. We amplified partial segment of mitochondrial Nicotinamide Adenine Dinucleotide Dehydrogenase Subunit 2 (*NADH* 2) gene with published primers L4437 5'-AAGCTTTC-GGGCCCATACC-3' and H5540 5'-TTTAGGGCTTT-GAAGGC-3' (Macey et al. 1997). A 12µl reaction was set containing 5µl of Qiagen Taq PCR Master Mix, 4µl of water, 0.5µl of each primer and 2µl template DNA, carried out with an Eppendorf Mastercycler Nexus GSX1. Thermo-cycle profile used for amplification were as follows: 94°C for 15 minutes, (denaturation temperature 94°C for 50 seconds, annealing temperature 59°C for 50 seconds, elongation temperature 72°C for 1 minutes) x 35 cycles, 72°C for 12 minutes, hold at 4°C. PCR product was cleaned using QIAquick PCR Purification Kit and sequenced with a 3730 DNA Analyzer. Sequences were cleaned and edited in Geneious R6 v.6.18. (Kearse et al. 2012) and were also manually checked in MEGA6. Taxon selection for phylogenetic analysis and additional sequences for the nuclear gene G protein-coupled receptor 149 (R35) were taken from Deepak et al. (2017, 2018). Sequences were aligned with ClustalW (Thompson & Gibson 2002) in MEGA6 (Tamura et al. 2013). Aligned data comprised of 856 bp of ND2 and 649 bp of R35 gene which was analyzed with PartitionFinder (Lanfear et al. 2012) for optimal partitioning strategy and evolutionary substitution model. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were employed to infer phylogenetic relationships in RAxML (Stamatakis 2014) and MrBayes 3.2.2. (Ronquist & Huelsenbeck 2003) respectively with data partitioned by codon positions. ML analysis was run for 1000 bootstrap replicates under GTR + G model to assess clade support. BI was run for 10 million generations with a sampling rate of 1000 under GTR + G. The analysis was terminated after the standard split frequency reached below 0.05. GenBank accession numbers for the sequence generated of the holotype is MH399850. GenBank accession numbers for sequences used in the present study are listed in supporting material.

RESULTS

Molecular phylogenetics based on a fragment of mitochondrial *ND2* and nuclear R35 gene recovered *Sitana* specimens from northern Karnataka embedded within a clade containing *S. laticeps* and *S. spinaecephalus* and as the sister taxon to *S. laticeps* with high support from ML (bootstrap 100) & BI (posterior probability 1.0). Among the 856 sites of *ND2* gene, 750 sites are conserved, 106 are variable and 40 parsimony informative sites. Morphological data (see diagnosis below) and molecular data support recognition of the population of *Sitana* from northern Karnataka as a distinct species, which is descried here.

***Sitana dharwarensis* sp. nov.**

Sitana laticeps Deepak & Karanth 2018: 56–57 (in part) Figs 1–4, Table 1

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Holotype. ♂ adult (NCBS-AL142); India, Karnataka, Bagalkot; 16.139744° N, 75.672671° E; alt. 590 m; 14 Apr. 2018; M. Ambekar, A. Murthy & Z. Mirza leg.

Paratypes. 2 ♀♀ adult (BNHS 2510, NCBS-AL143); 1 ♂ adult (BNHS 2509); same data as for holotype.

Diagnosis. *Sitana dharwarensis* sp. nov. is a large sized species in relation to members of the *Sitana spinaecephalus* clade, males reaching SVL of 52 mm. Dewlap large, coloration in breeding males cream to off-white, extending up to 47% of the trunk. Parietal bone with a subtle indentation on the anterior border, maxillary bone short in its length and covers a smaller area of the snout, squamosal long and slender gradually tapering at both ends in a sharp tip and, quadrate robust and stout.

Sitana dharwarensis sp. nov. differs from most known species within the genera *Sitana* and *Sarada* Deepak et al., 2016 in bearing a white colored moderately large dewlap (vs. dewlap in shades of red, blue and black in *Sarada* spp., *Sitana visiri* Deepak, 2016, *S. attenboroughii* Sadasivan et al., 2018, *S. marudhamneydhal* Deepak et al., 2016, *S. bahiri* Amarasinghem et al. 2015, *S. devakai* Amarasinghe et al., 2015). The new species is similar to *S. laticeps* and *S. spinaecephalus* in sharing a white dewlap. It differs from *S. laticeps* in bearing a much larger dewlap, dewlap extending to about 47% of

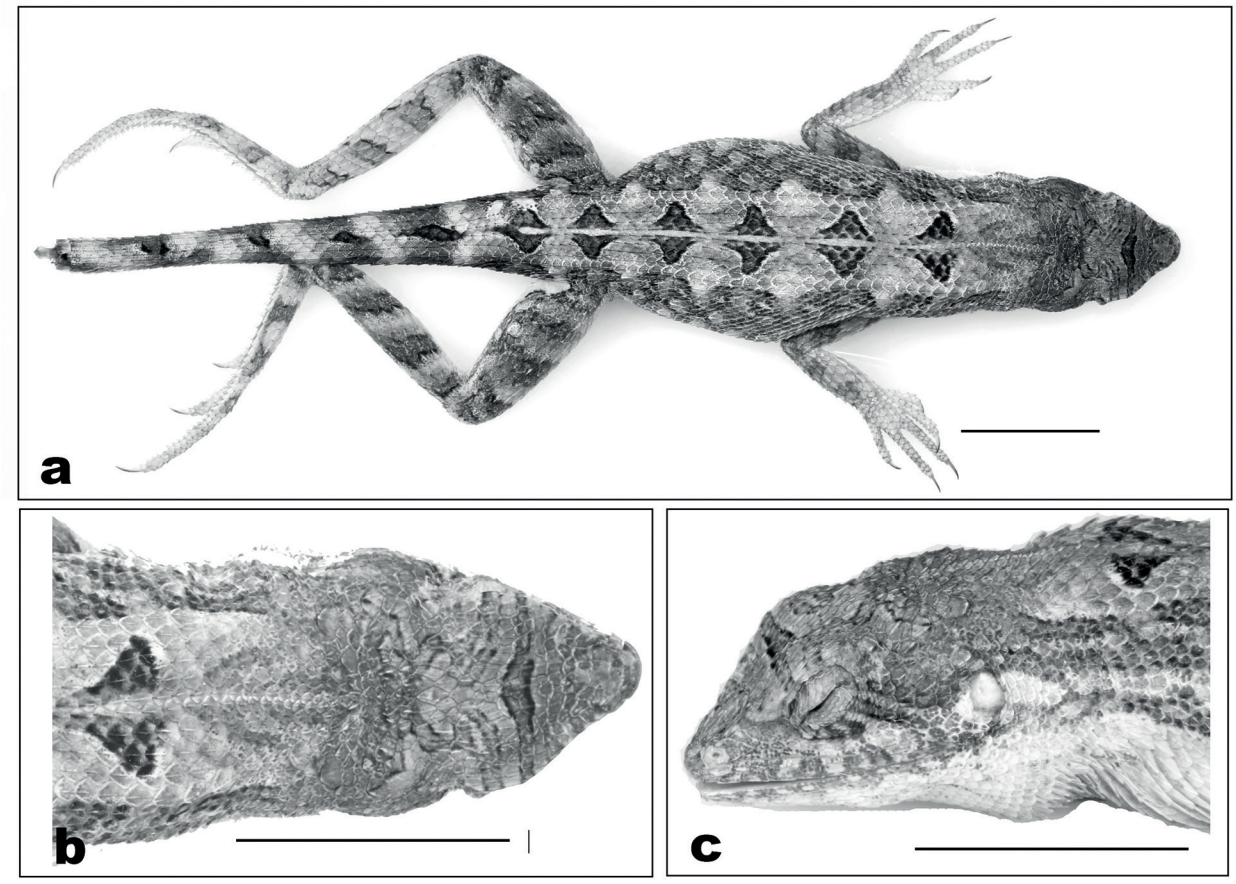

Fig. 1. *Sitana dharwarensis* sp. nov., holotype, ♂ (NCBS-AL142). **a**. Dorsal view of the specimen. **b**. Dorsal view of head. **c**. Lateral view of head. Scale bar: 10 mm.

Table 1. Measurements and morphological details of type specimens of *Sitana dharwarensis* sp. nov. in millimeters

	Holotype NCBS-AL142	Paratype BNHS 2509	Paratype NCBS-AL143	Paratype BNHS 2510
Sex	Male	Male	Female	Female
SVL	52.1	44.8	42.0	39.8
HL	14.1	13.2	12.4	12.2
HW	10.2	9.3	9.1	8.9
HH	9.5	8.2	6.7	7.0
SE	5.5	5.2	5.0	4.8
ET	3.6	3.3	3.1	3.0
JL	15.4	13.9	12.6	12.5
IO	8.0	7.3	6.8	6.6
NE	3.5	2.8	2.6	2.5
IN	2.9	1.9	2.4	2.4
TD	2.1	1.5	1.6	1.5
OD	3.3	2.8	2.9	2.6
LAL	8.5	6.7	6.7	6.4
UAL	10.7	6.6	6.4	6.3
F1	2.0	1.5	1.5	1.5
F2	3.1	2.5	2.5	2.5
F3	4.8	4.0	4.0	3.6
F4	4.5	3.8	3.6	3.3
F5	2.9	2.6	2.3	2.2
FEL	15.7	13.0	12.5	11.7
CL	19.6	15.8	16.6	14.6
HFL	21.9	19.5	19.2	18.0
T1	2.0	1.7	1.6	1.4
T2	3.0	2.8	2.8	2.6
T3	6.0	5.0	4.9	4.9
T4	12.2	10.0	9.2	9.1
TrL	21.3	19.4	19.0	18.8
TrH	9.7	7.5	6.9	6.0
TrW	14.2	12.9	12.6	10.6
TL	-	118.5	112.4	-
TH	4.0	3.1	2.7	2.7
TW	5.4	3.8	3.4	2.9
DWL	33	27.9	–	–
DWLT	9.9	6.9	–	–

the trunk (vs. 29% in *S. laticeps,* 45% in *S. spinaecephalus*). *Sitana dharwarensis* sp. nov. further differs from *S. laticeps* in bearing a subtle indentation on the anterior border of parietal (vs. a deep indentation in *S. laticeps*, Fig. 2), the maxillary bone is short and covers a smaller area of the snout (vs. much longer and covering a larger area of the snout in *S. laticeps*), the squamosal is long and slender gradually tapering at both ends in a sharp tip (vs. squamosal short, abruptly ending in a blunt tip at both ends), quadrate is robust and stout (vs. slender in *S. laticeps*, Fig. 2).

Genetic divergence. Genetic divergence (un-corrected p-distance) between populations of *S. dharwarensis* sp. nov. was 1–2% whereas it was 3–4% from *S. laticeps*.

Description. The holotype male (NCBS-AL142) is in generally good condition, with an incision on the thigh made to remove muscle tissue. The entire tail is broken from its base and is preserved separately.

Adult male SVL 52.8 mm. Head relatively long (HL/SVL ratio 0.27), wide (HW/HL ratio 0.72), not depressed (HH/HL ratio 0.67), distinct from neck (Fig. 1a). Snout moderately long (SE/HL ratio 0.39) bluntly conical; longer than eye diameter (OD/SE ratio 0.45) (Fig. 1c). Eye large (OD/HL ratio 0.56); pupil round, eyelids covered with small pentagonal and hexagonal scales, supraciliaries short. Snout obtusely pointed when viewed dorsally, rostral much wider than deep, bordered posteriorly by two supralabials, prenasal and dorsally by three small scales. Canthus rostralis and supraciliary edge moderately sharp consisting of nine scales. Nostrils positioned in the centre of a large, undivided nasal plate, bordered by eight scales (right side), including one prenasal, two postnasals and one supranasal, and separated from rostral by prenasal and supralabials. Ten rectangular, weakly keeled supralabials, bordered above by a single row of slightly smaller, rectangular, keeled scales. Loreal region concave, scales of the loreal region heterogeneous in size, flat, keeled, some roughly hexagonal. Scales on postorbital and temporal region homogenous, imbricate, strongly keeled, and directed posteriorly and dorsally. Orbital scales small but not granular. Tympanum naked. Canthals enlarged, overlapping, becoming slightly smaller along subimbricate supraciliaries, protruding slightly laterally on supraorbital ridge. Scales on dorsal surface of snout, forehead, interorbital, and occipital region heterogeneous in size, and shape; mostly elongate, imbricate, strongly keeled longitudinally; those on snout smaller, rhomboidal, those on forehead largest, greatly elongate; supraorbital scales increase in size becoming more elongate from supraciliaries to inner edges of orbits, of which the enlarged scales follow the curvature of the orbit posterolaterally; occipital region with slightly smaller, less elongate; imbricate, and keeled scales. Parietal plate with pineal eye, the plate slightly larger than adjacent scales. Mental shield narrower than rostral; gular scales keeled. Dewlap moderately large, extends posteriorly over 47%

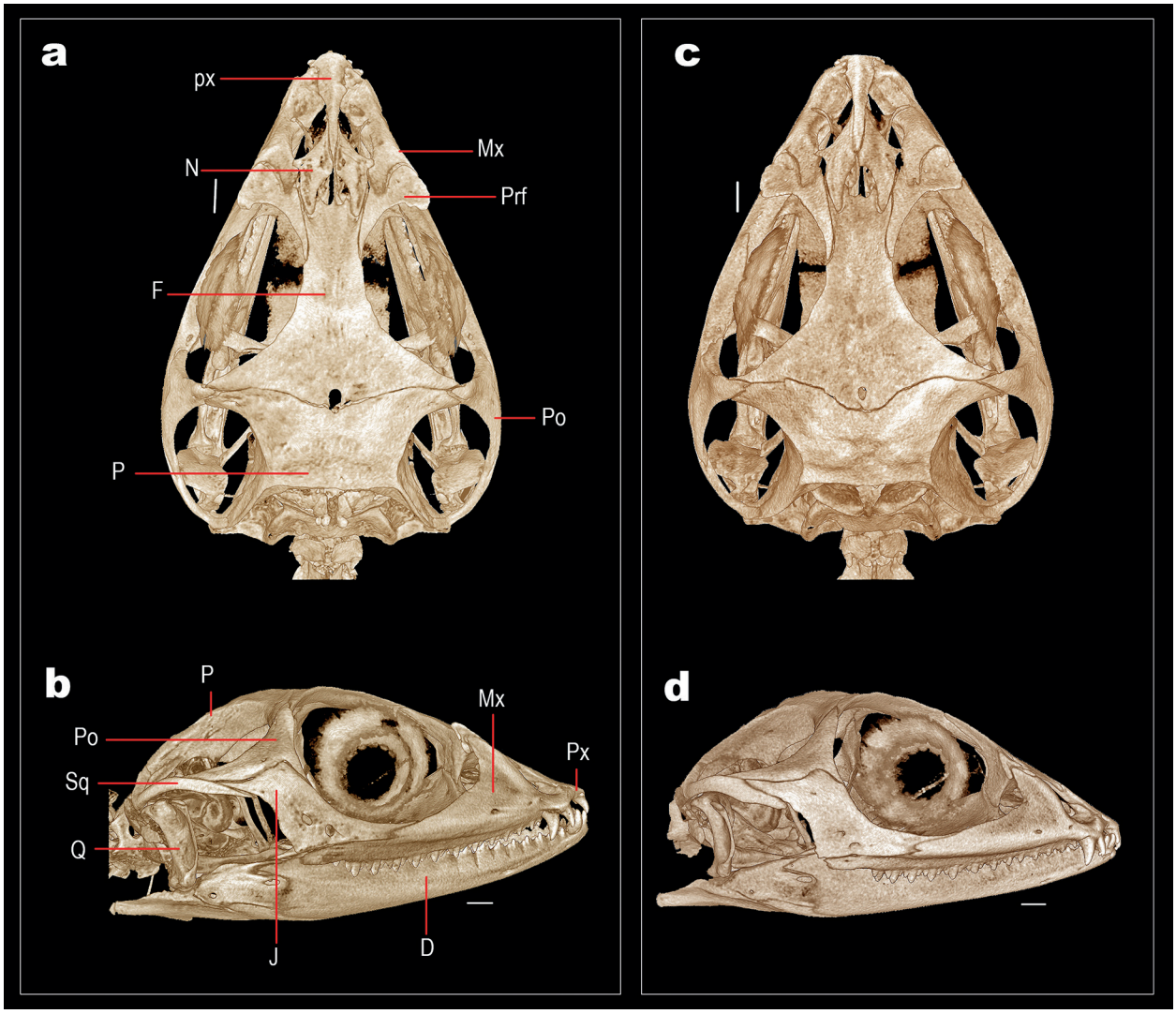

Fig. 2. Micro-CT scan image of the cranium and jaws of *Sitana dharwarensis* sp. nov. (a–b) and Sitana laticeps (c–d). **a, c**. Dorsal view of skull. **b, d**. Lateral view. Scale bar: 1 mm. Abbreviations: D = dentary, F = frontal, J = jugal, Mx = maxilla, N = nasal, parietal, Po = postorbital, prf = prefrontal, Px = premaxilla, Q = quadrate, Sq = squamosal (Evans 2008).

of trunk length, with posterior scales extending slightly beyond axila, not extending to mid-venter, approximately four to five rows of anteriodorsal dewlap scales smaller, elongate, pointed, keeled, remainder of scales much larger, keeled, lanceolate, bluntly pointed, gradually increasing in size towards margin, single marginal row largest with many more pointed scales. 17 enlarged rows of scales on dewlap. Nuchal and dorsal crest absent. Scales on nuchal region smaller, less than half the size of those on interorbital region, imbricate, strongly keeled. Body slender, 59 rows of scales around midbody, of these 10–12 rows of scales on back, from occiput to pectoral region homogenous in size, shape, slightly larger than those on neck, imbricate, pointed, keeled, and directed posteriorly forming regularly arranged longitudinal rows; those on flanks heterogeneous in size, shape, smaller than those on back, obtusely pointed, keeled, with irregularly scattered, slightly larger, pointed, keeled scales; scales of upper rows directed backwards and upwards; ventral rows backwards and downwards; ventral scales subimbricate, keeled, homogenous in size, shape, arranged in 65 rows; no precloacal or femoral pores. 48 scales in a row from nape to the cloaca. Fore and hind limbs relatively slender, tibia short (CL/SVL ratio 0.37); digits moderately long, ending in strong, elongate, slightly recurved claw; inter-digital webbing absent; subdigital lamellae entire, tri-mucronate, 22 subdigital lamellae on toe IV; relative length of fingers 4>3>2>5>1, toes 4>3>2>1. Fore and hind limbs covered above and below with regularly arranged, enlarged, pointed, strongly keeled scales. Enlarged projecting scale on thigh present. Tail entire; tail base swollen; tail uniformly covered

Fig. 3. *Sitana dharwarensis* sp. nov., holotype, ♂ (NCBS-AL142) in life.

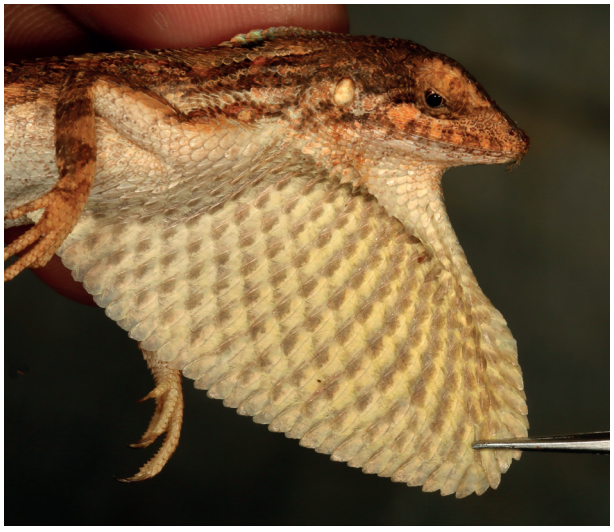

Fig. 4. *Sitana dharwarensis* sp. nov., holotype, ♂ (NCBS-AL142) dewlap in life.

with similar sized, keeled, weakly pointed, regularly arranged, posteriorly directed imbricate scales, no enlarged subcaudal row.

Coloration in preservative (Fig. 1). Coloration much more faded, overall background coloration more yellowish. Rhomboidal marks turn much paler and are almost diffused towards the flank. Blue coloration on the lower chin turns black.

Coloration in life (Figs 3–4). Dark-brown above with five dark rhomboidal markings on the trunk, first mark present just posterior to the neck and the last one on the flank. Each rhomboidal blotch has a light colored line running through it along the vertebral column. Limbs brown, banded with alternating dark and light bands. Head coloration same as the body, labials banded with light and dark bands. Dewlap yellowish white throughout with a steel-grey to blue line running from mental to a few scales below it. The colored line does not enter the dewlap and terminates just before the enlarged dewlap scales. Ventrally white.

Etymology. The specific epithet refers to the Dharwar Craton where the species is distributed.

Variation. The paratypes resemble the holotype in most aspects except for ventral belly scale number. The paratype male possesses 31–32 scales. Other morphometric and meristic characteristics are presented in Table 1.

Natural history. A species inhibiting open dry scrub and rock terrain in northern Karnataka. The type locality is a barren hillock adjacent to a seasonal river. The locality is heavily disturbed from activities relating to stone quarrying. The species is common at the type locality and is found in gardens in the town. Other sympatric reptiles observed are *Eutropis* cf. *carinata*, *Hemidactylus parvimaculatus* Deraniyagala, 1953 and *Hemidactylus*

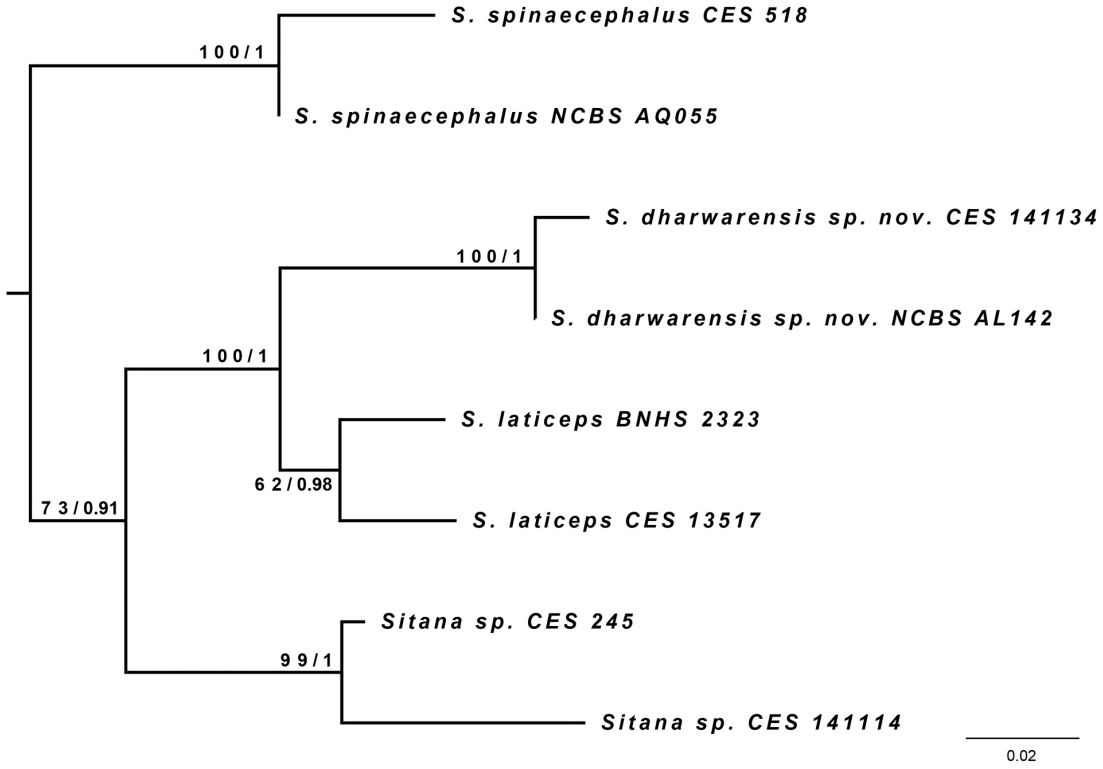

Fig. 5. Maximum Likelihood phylogeny based on 1011bp of ND2 and 649np of R35 gene for selected *Sitana* species. Numbers at nodes indicate ML/BI support.

Fig. 6. Map of south western India showing distribution of *Sitana laticeps* (black circle) and *S. dharwarensis* sp. nov. (asterisk). Inset map shows main map highlighted by the red square.

vijayraghavani Mirza, 2018. Based on available mtDNA *ND2* gene sequences, the species appears to be distributed south of a tributary (Panchgana) of Krishna River at the following localities: Bagalkot, Tumarguddi and Koppal.

DISCUSSION

Molecular phylogenetics based on a fragment of mitochondrial *ND2* and nuclear R35 gene recovered *Sitana dharwarensis* sp. nov. embedded within a clade containing *S. laticeps* and *S. spinaecephalus* and was recovered as sister taxon to *S. laticeps* with high support from ML (bootstrap 100) & BI (posterior probability 1.0) (Fig. 5). The new species is 3–4% (un-corrected p-distance for *ND2* gene) divergent from available sequences of *S. laticeps*. Additionally, the new species is distributed south of Panchganga River, a tributary of Krishna River, whereas *S. laticeps* is distributed north of the river (Fig. 6). The new species appears to be restricted to the area encompassed by the two major rivers Krishna and Tungabhadra. A parallel case appears to be that of the newly described *Hemidactylus vijayraghavani* (Mirza, 2018) from the same locality. These rivers likely act as biogeographic barriers for terrestrial lizards as seen in species of the genus *Sitana* (Deepak et al. 2016; Deepak & Karanth 2017). The sequences of specimens CES 245 and CES

141114 used in the phylogenetic analysis potentially represent a new species.

Description of yet another species of the genus *Sitana* is not surprising as recent studies provided hints on the presence of immense genetic diversity across sampled populations (Deepak & Karanth 2017). With additional sampling, the number of *Sitana* species in the subcontinent will certainly rise. Morphologically cryptic species currently considered conspecific with broadly distributed species, a common case with most lizard species recorded from India (Agarwal et al. 2014; Agarwal & Ramakrishnan 2017; Deepak & Karanth 2017; Mirza et al. 2018), represent a major subset of the reptilian diversity of the country and hence dedicated efforts must be made to document and describe these. Many of these newly described species occur outside of protected areas and in most localities, local populations are at risk from being wiped out. A management plan for non-protected area, especially open and scrublands that are otherwise termed wasteland and considered less biodiverse must be devised to ensure protection of species and habitats.

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