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Discovery of a new species of kangaroo lizard (Squamata: Agamidae: *Agasthyagama*) from the southern Western Ghats of India

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Abstract

We describe a new species of *Agasthyagama* from Idukki district, Kerala, India. The new species is distinguished from its sister species *Agasthyagama beddomii* by a combination of scale characters and are also genetically different from each other with 11% uncorrected pairwise difference in ND2 gene and 3% in 16S gene. The two species are also geographically separated, the closest distributional records are approximately 80 km apart.

Keywords

Agamid, endemic, evergreen forest, Kerala, southern India, Tamil Nadu, terrestrial

Introduction

Terrestrial agamids from the forests of southern India and Sri Lanka are now classified into two separate genera, *Agasthyagama* Srikanthan et al., 2021 and *Otocryptis* Wagler, 1830 on genetic and morphological grounds. The genus *Otocryptis* initially included two species: *Otocryptis beddomii* Boulenger, 1885 from the southern Western

Ghats of India and *Otocryptis wiegmanni* Wagler, 1830 from the wet zones of Sri Lanka (Smith 1935). After a long gap, an additional species, *Otocryptis nigristigma* Bahir & Silva, 2005, was described from the dry forests of Sri Lanka. Deepak and Karanth (2018) showed that the kangaroo lizards from Sri Lanka were paraphyletic with those in the Western Ghats. This prompted Srikanthan et al. (2021) to erect the monotypic genus *Agasthyagama*, to accommodate Indian *O. beddomii*, although without any molecular data for *Otocryptis nigristigma*. However, the two species in Sri Lanka are morphologically more similar to each other than to the Western Ghats endemic *Agasthyagama beddomii* (Srikanthan et al. 2021). Despite the two Sri Lankan and the Indian species being placed in different genera, they have some homologous morphological characters. The prominent one being the reduced fifth toe which makes them all poor climbers and likely poor dispersers. The sister genera to *O. wiegmanni* are *Sarada* and *Sitana* which have completely lost the fifth toe (Russell and Rewcastle 1979; Deepak et al. 2016; Deepak and Karanth 2018). Among the four closely related genera the genus *Sitana* is relatively speciose with 14 species while the rest are depauperate (Uetz et al. 2022). While *Sarada* and *Sitana* are found mostly in open grassland habitats, *Agasthyagama* and *Otocryptis* are found in evergreen and deciduous forest floors.

Agasthyagama beddomii was originally described based on a series of five specimens (four at NHM, London and one at ZSI, Kolkata), all of which came from the "Shevagerry Ghat" (also known as Sivagiri hills) in the southern Western Ghats of India. Srikanthan et al. (2021) examined one of the syntypes (ZSI) and two additional specimens while describing the new genus *Agasthyagama*, but did not assign a lectotype. Currently, *A. beddomii* is known to be largely distributed south of the Shencottah Gap and in regions immediately north of the gap (Srikanthan et al. 2021). The type locality Sivagiri hills and the labels on the jar suggest that they were collected by R. H. Beddome at an elevation of 4500 ft (approximately 1372 m). However, *A. beddomii* was never reported from the type locality since its original description but is commonly found in the regions south of it from elevations ranging between 150 m and 1050 m a.s.l. (Inger et al. 1984; Srinivasulu et al. 2014).

Our recent fieldwork in the southern Western Ghats north of the known region of *A. beddomii* resulted in finding a new population from the mid-elevations in the Idukki region of the Periyar river basin. We examined museum specimens, including the type series, and newly collected specimens of *A. beddomii* from across its distributional range, spanning from low to high elevations. We also obtained mitochondrial 16S and ND2 genetic data from the freshly collected specimens from the Idukki population. Our analyses revealed significant, and consistent morphological and genetic differences between the two populations. Additionally, we herein designate a

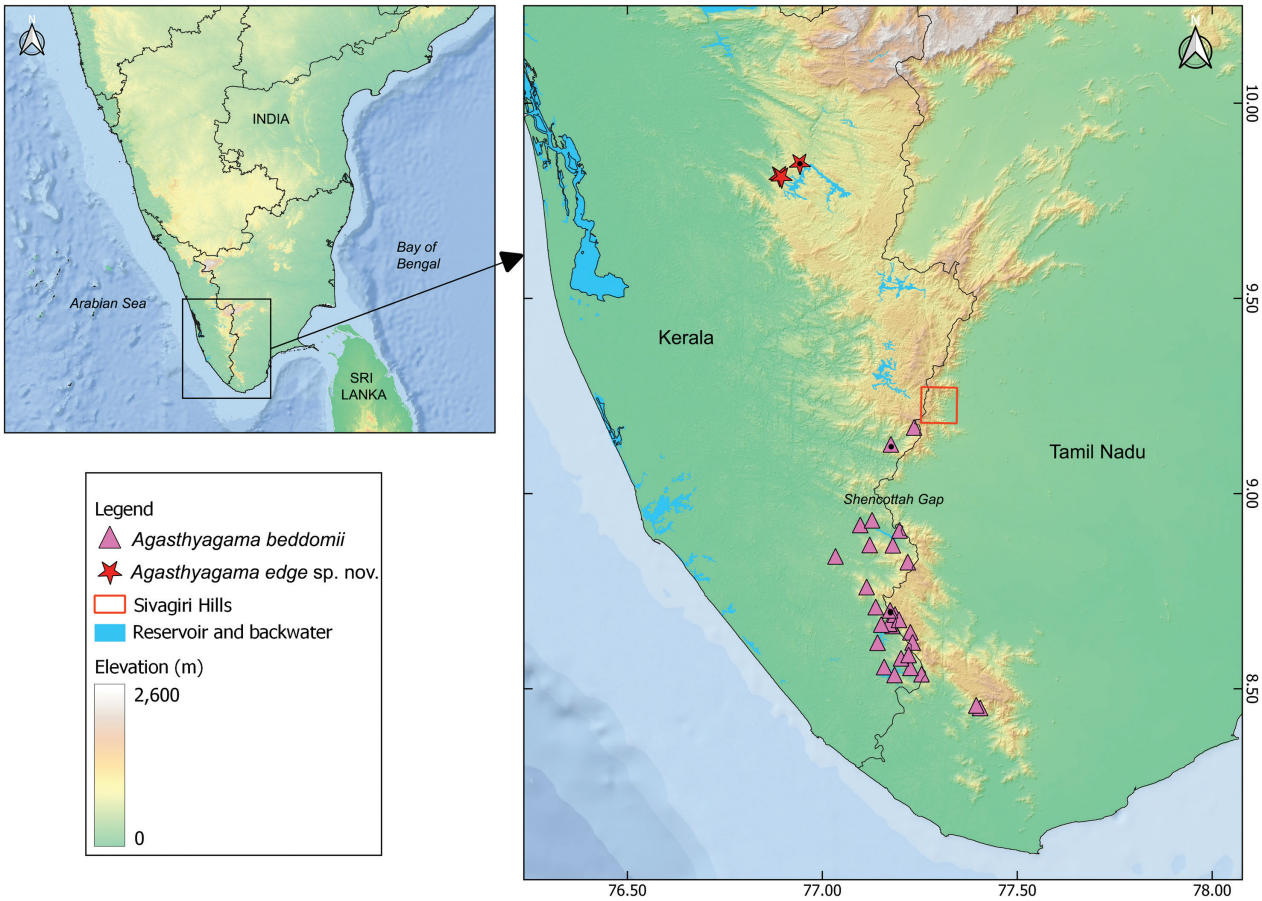

Figure 1. Map showing the distribution of *Agasthyagama edge* **sp. nov.** and *A. beddomii* in the Western Ghats of India. Locations for the samples used for molecular analyses are marked with a black circle within the respective symbols.

lectotype and provide a redescription and diagnosis for *A. beddomii*. Further, based on the results of the morphological and molecular analyses, we describe the Idukki population as a new species.

Materials and methods

Molecular phylogeny

We generated DNA sequences for two specimens of *Agasthyagama* viz, ZSI/WRC/R/1165 and ZSI/WRC/R/1173 from Kulamavu, Idukki district, Kerala, India (Fig. 1). Specimens were euthanized, fixed in 4% formalin, rinsed with distilled water and transferred to 70% ethanol for preservation. All specimens collected in this study are deposited in the Zoological Survey of India, Western Regional Centre, Pune with the following registration numbers ZSI/WRC/R/1165 to ZSI/WRC/R/1173 and in the museum of the Bombay Natural History Society with registration numbers: BNHS 3220 to BNHS 3223.

We amplified partial sequences of two mitochondrial 16S (1029 bp) and ND2 (464 bp) genes. PCR amplification for both markers was done following the existing primers and protocols (ND2: MetF1 (Forward) and H5934 (Reverse) (Macey et al. 1997); 16S: primers 16Sar-L (Forward) and 16Sbr-H (Reverse) (Palumbi et al. 1991). PCR conditions were as follows: Fragments of 16S were amplified using an initial denaturation at 95°C for 5 min, followed by 39 cycles of denaturation at 95°C for 45 sec, annealing at 50.4°C for 45 sec and extension at 72°C for 1 min 30 sec. Final extension was at 72°C for 10 min. Fragments of ND2 gene were amplified using an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at 51°C for 45 sec (16S), 57°C for 45 sec (ND2) and extension at 72°C for 55 sec. Final extension was at 72°C for 10 min. PCR reactions were carried out in 25µl reaction containing 11µl of Takara emerald RR310B mastermix, 12µl of PCR grade H_2O, 0.5µl of each forward and reverse primers and 1µl (60–80 ng) of template DNA. PCR amplifications were carried out in S1000TM Thermal Cycler (Bio-Rad, USA). Amplified PCR products were run on a 2% agarose gel and viewed with an Essential V4 (UVITEC Cambridge, UK) gel documentation system to confirm the PCR amplification. PCR products were purified and Sanger sequenced in both directions at Barcode Biosciences (Bangalore, India).

Bidirectional sequences were manually checked using the CHROMAS 2.6.6 software (http://technelysium.com.au/wp/chromas) and aligned using ClustalW (Thompson et al. 1994) with default prior settings implemented in MEGA 7 (Kumar et al. 2016). For the protein coding gene (ND2), we checked for unexpected stop codons by translating the sequence to amino acids in MEGA7 (Kumar et al. 2016). The new sequence generated in this study was aligned with data for 10 other agamid species downloaded from Genbank (Table S1).

The dataset was partitioned by gene and codon positions using PartitionFinder v2 (Lanfear et al. 2017) with default settings to find the best-fit model of sequence evolution implemented within the Phylosuite (Zhang et al. 2020). The best-fit scheme comprised four partitions, by gene and by codon position, GTR+G: 16S; TVM+I: ND2_position1; HKY+I: ND2_position2; HKY+G: ND2_position3. Maximum Likelihood (ML) analysis for the final dataset was carried out using the GUI version of the IQTREE (Nguyen et al. 2015) implemented within the Phylosuite. A Bayesian Inference (BI) analysis was carried out using the program MrBayes 3.2 (Ronquist et al. 2020), with default prior settings. Four separate runs were set up with eight Markov chains each initiated from random trees and allowed to run for 10 million generations, sampling every 100 generations. Analyses were terminated when the standard deviation of split frequencies was less than 0.001, the first 25% of trees were discarded as "burn-in", and trees were constructed under 50% majority consensus rule. We obtained ESS values using the Tracer software (Rambaut et al. 2018) and confirmed the convergence for all the priors (ESS>200). Support for the internal branches for the ML and BI was quantified using 1000 replicates of standard bootstrap (BS) (Felsenstein 1985) and posterior probabilities (PP), respectively. The resulting tree was edited in Figtree (http://tree.bio.ed.ac.uk/software/figtree). Uncorrected pairwise genetic distances were calculated in MEGA 7 using the default setting selecting a pairwise deletion option.

Morphology

Morphometric measurements (in millimetres) were taken with a digital caliper to the nearest 0.1 mm, with the aid of a dissecting microscope. Morphometric and meristic characters collected in this study are as follows: Head length (**HL**) measured from tip of snout to mandible, head width (**HW**) at its widest point, head height (**HH**) at its highest point, interorbital distance (**IO**) at the anterior most point between the eyes, jaw length (**JL**) at tip of snout to end of jaw, naris to eye distance (**NE**) from posterior point of nostril to anterior point of orbit, snout to orbit distance (**SO**) snout tip to anterior point orbit, snout-width (**SW**) outermost edges of the rostrum, orbit diameter (**OD**) maximum diameter of the orbit, finger and toe lengths measured from the joint of the adjacent finger; 3rd finger length (**3FL**) excluding claw, 4th finger length (**4FL**) excluding claw, 4th toe length (**4TL**) excluding claw, 5th toe length (**5TL**) excluding claw, femur length(**FL**) length of femur from groin to knee, Crus length(**CL**) length of crus/tibia from knee to heel, heel length(**HeL**) base of palm to distal most point of the fourth toe, lower arm length(**LAL**) distance from elbow to proximal end of the wrist or just the underside of the forefoot, upper arm length(**UAL**) distance from anterior insertion of forelimb to elbow, palm length (**PL**) base of palm to distal most point of forelimb. snout-vent length (**SVL**), tail length (**TL**), tail width (**TW**) maximum tail width, taken posterior to the vent, trunk length (**TrL**) measured from axilla to groin.

Mid body scales (**MBS**) circummarginal scale rows at mid body length, dorsal scales (**DS**) counted from behind occiput to before cloacal ending, abdominal ventral scales (**AVEN**) number of scales on abdomen after dewlap till vent, dewlap ventral (**DVEN**) number of scales from mentum to last dewlap scale, ventral scales (**VS**) number of scales from mentum till vent, supralabials (**SL**) number of SL scales, infralabials (**IL**) number of IL scales.

Catalogue numbers of reported specimens bear the institutional prefix **NHMUK** (formerly **BMNH**): Natural History Museum, London, UK; **FMNH**: Field Museum of Natural History, Chicago, USA; **ZSI**: Zoological Survey of India, Kolkata, India; **BNHS**: Bombay Natural History Society, Mumbai, India; **CES**: Centre for Ecological Sciences, Indian Institute of Science, Bangalore. Measurements and counts for all BNHS, CES and ZSI specimens including the ones collected for this study were collected by Saunak Pal and for NHMUK and FMNH specimens this data was collected by V. Deepak.

Results

Phylogenetic relationship

Both ML and BI analyses recovered similar tree topologies. *Agasthyagama* and *Otocryptis* were recovered as paraphyletic with the former being the basal to the clade containing *Otocryptis* + *Sitana* + *Sarada*, as previously reported (Fig. 2) (Deepak and Karanth 2018; Pal et al. 2018; Srikanthan et al. 2022). *Agasthyagama* is recovered as a strongly supported (BS 100, PP 1.0) monophyletic clade containing *A. beddomii* and the new species described below. The new species described here is sister to the *Agasthyagama beddomii* with strong support (BS 100, PP 1.0), in both analyses.

The uncorrected pairwise sequence divergences (p distances) are as follows. Among the sequences available, a sample of new species (ZSI/WRC/R/1165) is 11% divergent from the sample of *A. beddomii* (CESL 032) from

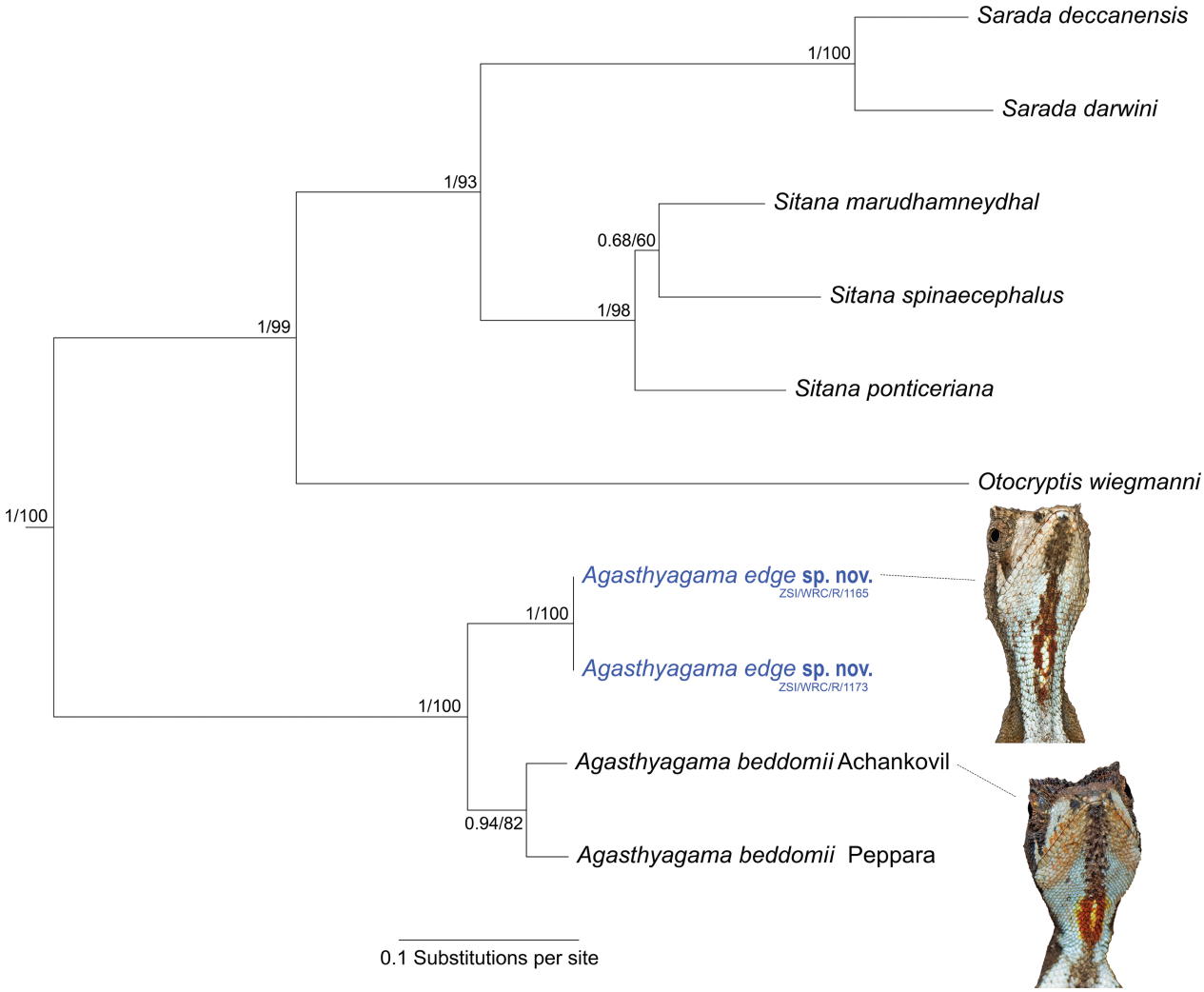

Figure 2. Maximum likelihood phylogeny for *Agasthyagama* and its three sister genera using the concatenated 16S and ND2 genes. Numbers on internal branches are Bayesian posterior probability/ML bootstrap support values. See Appendix 1 for data sources. Inset images of the two *Agasthyagama* species showing differences in throat coloration and pattern.

Peppara, in the mitochondrial ND2 gene. Within the 16S gene, both the samples of the new species are identical and two samples (CESL 268, CESL 032) of *A. beddomii* are 1.2% divergent from each other. The new species is 3% divergent from both samples (CESL 268, CESL 032) of *A. beddomii* in 16S gene. We have only obtained ND2 sequences for the holotype, and our multiple attempts to amplify ND2 sequences for other samples failed consistently.

Morphology

We identified a few non-overlapping meristic characters (number of dorsal scales and dewlap ventral scales) which are diagnostic characters to separate the two *Agasthyagama* species (see Diagnosis and comparison subsection in the new species description section). Breeding colouration on the throat of the two species are different, *A. beddomii* has broad dark stripe from the mentum, forming an elongated brick red circle at the throat, bordered with bright yellow scales on the outside and with a bright yellow to orange blotch in the centre. The new species have grey brown stripe from the mentum, gradually forming a brick red elongated circle with pale bluish-white scales at the centre.

Hemipenis of the two species are overall similar in shape (Fig. 3), bilobed, as long as wide, sulcus spermaticus well defined and shallowly forked. Sulcal lips raised and papillate, sulcus smooth originating from the base. Apex covered with small calyces and large calyces at the middle and base of the hemipenis. On the asulcal side both the lobes were covered with calyces but the base was nude with folds, this is true for both the species. Up to six flounces were present (Fig. 3B) in two of the *A. beddomii* checked at FMNH (FMNH 217760, 217765) as well as the new species (Fig. 3A) (ZSI/WRC/R/1165).

Systematics

Agasthyagama beddomii (Boulenger, 1885)

Figures 3B, 4, 5

Chresonymy.

Otocryptis beddomii Boulenger, 1885: 272

Otocryptis beddomii — Smith (1935: 147), Wermuth (1967: 74), Das (1996: 44), Pal et al. (2018)

Otocryptis beddomi — Inger et al. (1984), Jose et al. (2007)

Otocryptis beddomei — Manthey (2010: 114), Deepak and Karanth (2018)

Agasthyagama beddomii — Srikanthan et al. (2021)

Lectotype (by present designation). NHMUK 1882.5.22.101, adult female collected from "Head of Sevagherry Ghat, 4500 ft" (now "Sivagiri Hills", in the borders of Kerala and Tamil Nadu state, India) by R. H. Beddome (Figs 4 and 5).

Paralectotypes. ZSI 15733 (former syntype), adult female; NHMUK 1882.5.22.102–104 (former syntypes), one female and two male specimens, collected along with the lectotype.

Other material examined. Four males (FMNH 217757, FMNH 217760, FMNH 217767, FMNH 217768) and four females (FMNH 217764, FMNH 217765, FMNH 217773, FMNH 217775) collected by R. F. Inger and H. B. Shaffer from Trivandrum district, Kerala; two males (BNHS 3220, BNHS 3221) collected from Shendurney, Kollam district, Kerala.

Comment. The original description of *Agasthyagama beddomii* included mention of five specimens with measurement data for one specimen. Four of these specimens are now housed at NMHUK, London and one at ZSI, Kolkata, India. Among the specimens in NHMUK three are juveniles (two males, one female), one adult female (NHMUK 1882.5.22.101), and one housed at ZSI (ZSI 15733), an adult female (Boulenger 1885). Among these, one adult female (NHMUK 1882.5.22.101) matched with the measurements provided by Boulenger (1885) and was well preserved (see Table 1). Therefore, due to the well-preserved condition of the specimen as well as the historic significance, we herein, designate NHMUK 1882.5.22.101 as a lectotype, instead of a male specimen which is usually the case in agamid descriptions.

The freshly collected specimens from Shendurney are morphologically the same as those of the type series in

Figure 3. Asulcal view of hemipenis: **A** *Agasthyagama edge* **sp. nov.** (Holotype ZSI/WRC/R/1165); **B** *A. beddomii* (FMNH 217760). Photographs by Saunak Pal and V. Deepak.

Table 1 – part 1. Morphometric and meristic characters for *Agasthyagama beddomii* examined during this study. "—" missing data, "na" character not available, "M" male and "F" female, "*" indicates an incomplete tail. Note: Some of the characters for the samples from CES were measured differently from the ones collected during this study (see methods in Pal et al. 2018).

Voucher	**NHMUK 1882.5.22.101**	**NHMUK 1882.5.22.102**	**NHMUK 1882.5.22.103**	**NHMUK 1882.5.22.104**	**ZSI 15733**	**FMNH 217773**	**FMNH 217775**	**FMNH 217765**	**FMNH 217764**	**FMNH 217757**
location	Head of Sevagherry Ghat	Head of Sevagherry Ghat	Head of Sevagherry Ghat	Head of Sevagherry Ghat	Head of Sevagherry Ghat	Trivandrum, Kerala, India	Trivandrum, Kerala, India	Trivandrum, Kerala, India	Trivandrum, Kerala, India	Trivandrum, Kerala, India
Sex	F	F	M	M	F	F	F	F	F	M
Type status	Lectotype	paralectotype	paralectotype	paralectotype	paralectotype	No	No	No	No	No
HL	13.9	11	10.6	9.3	9.2	12.2	13.1	12.1	13	11.6
HW	8.6	7	6.6	6.5	7.0	7.7	8.4	7.8	8.3	8.4
HH	7.6	6.5	6	5.4	6.2	6.4	6.9	6.2	6.7	7
IO	5	4.3	4.5	4.3	7.1	4.5	5.4	4.7	4.9	5
JL	10.2	8	8	7	—	9	10	9.3	8.6	10
NE	3.4	2.3	3	2.4	3.7	2.8	3.2	2.5	2.7	2.7
SO	5	4	4.3	3.7	5.4	4.3	4.9	3.9	4.4	4.5
SW	2.6	1.8	2	1.9	2.8	0.9	1.3	1.1	1.3	1.2
OD	4	3.3	3	2.7	2.0	2.9	2.9	3.1	3.3	3.2
3FL	4.8	3.2	3.3	3.2	4.7	3.8	3.7	3.7	3.9	4.7
4FL	5.14	3.9	4.1	3.8	5.3	4.3	4.2	4	4.8	5.1
4TL	10.4	7	8	7.2	8.6	9.7	8.8	8.3	8.8	10.8
5TL	1.74	1.4	1.1	1.6	1.5	1.7	1.8	1.4	1.6	1.9
FL	14.7	11.3	10	9	13.2	12.8	12	11.7	12.8	14.5
CL	15.2	10.9	12	9.4	13.1	14.1	14.5	12.7	14.7	14.9
HeL	19.5	13.4	14.5	14.1	—	17	17.6	17.1	16.2	20.2
LAL	8	6.3	6.9	5.1	6.1	6.8	7	6.8	7.7	7.5
UAL	7.8	5.5	5.6	5.2	6.3	6.6	7	6.4	7.5	6.9
PL	7.9	5.2	6.1	6	—	6.9	6.8	6.5	6.4	7.1
SVL	44.7	31.3	31	28.5	40.6	39.5	43.6	38.5	41.7	41.7
TL	72.7	38	53	53	—	60.3	66	25*	59.9	72.2
TW	3.7	3.8	3	2.7	—	4.9	4.6	3.8	3	4.3
TrL	19.3	11.9	11.2	13	18.4	18.1	21	15.9	19.6	14.4
MBS	79	78	74	75	66	78	80	75	65	85
DS	67	65	62	62	—	65	65	60	61	60
AVEN	*na*	*na*	43	40	*na*	*na*	*na*	*na*	*na*	39
DVEN	*na*	*na*	40	42	*na*	*na*	*na*	*na*	*na*	37
VS	78	79	83	82	84	74	79	70	74	76
SL (left,right)	10,11	9,10	9,9	9,10	—	9,9	9,9	9,9	10,9	9,9
IL (left,right)	11,11	9,9	9,10	9,10	—	9,10	11,10	9,10	10,10	10,9

Table 1 – part 2. Morphometric and meristic characters for *Agasthyagama beddomii* examined during this study. "—" missing data, "na" character not available, "M" male and "F" female, "*" indicates an incomplete tail. Note: Some of the characters for the samples from CES were measured differently from the ones collected during this study (see methods in Pal et al. 2018).

Voucher	**FMNH 217767**	**FMNH 217768**	**FMNH 217760**	**BNHS 3220**	**BNHS 3221**	**CESL025**	**CESL099**	**CESL268**	**CESL703**
location	Trivandrum, Kerala, India	Trivandrum, Kerala, India	Trivandrum, Kerala, India	Shendurney, Kerala, India	Shendurney, Kerala, India	Peppara WLS, Kerala, India	Peppara WLS, Kerala, India	Achankovil, Kerala, India	Peppara WLS, Kerala, India
Sex	M	M	M	M	M	F	M	M	M
Type status	No	No	No	No	No	No	No	No	No
HL	12.2	12.9	12.7	11.3	11.6	8.8	9.1	8.9	8.7
HW	7.6	7.7	8.0	7.4	7.5	9.0	7.2	8.2	8
HH	6.5	6.4	6.6	6.4	6.5	8.1	6.7	7.4	7.1
IO	4.4	5.0	4.7	4.8	4.6	6.0	5.1	5.6	5.8
JL	9.4	9.5	9.8	8.1	8.4	13.2	12.7	12.5	12.7

Voucher	FMNH 217767	FMNH 217768	FMNH 217760	BNHS 3220	BNHS 3221	CESL025	CESL099	CESL268	CESL703
NE	2.6	2.8	2.8	2.9	2.8	3.7	3.4	3.6	3.5
SO	4.4	4.6	4.5	4.5	4.6	5.3	5.2	5.4	5.3
SW	1.1	1.3	1.3	2.2	2.4	—	—	—	—
OD	3.4	3.5	3.4	3.2	2.9	3.8	3.7	3.8	4.1
3FL	3.7	4.2	4.1	3.9	3.3	4.3	3.9	4.1	4
4FL	4.4	4.7	4.5	4.2	3.9	5	4.8	5.2	4.9
4TL	9	11.1	10.9	9.6	9.3	10.8	9.5	9.9	10.4
5TL	1.7	2	1.5	1.4	1.6	2.3	2	2.3	2.5
FL	14.5	12.9	13.7	11.9	11.7	13.2	13.8	13.1	14.2
CL	14.7	15.6	16	13	12.5	12.4	12.8	12.9	13.6
HeL	17.4	18.9	18	17.7	17.2	16.7	16.1	16.1	17.1
LAL	7.4	7.4	7.2	6.9	6.7	8	7	7.3	7.3
UAL	7	7.5	7.5	6.2	6.6	7.3	6	7	6.7
PL	6.5	7	7	6.5	6.4	6.8	6	6.7	6.9
SVL	38.9	42.7	41.6	35.4	34.3	37.9	38.1	37.2	40.1
TL	63.2	66.4	41*	40*	53*	38	53	28*	66
TW	4.1	2.9	3.3	3.8	3.4	3.8	5.3	4.9	4.7
TrL	15.4	18.2	15.5	12.7	12.2	15.6	16	14.8	14.8
MBS	87	72	75	74	73	81	81	77	77
DS	62	65	63	62	61	63	63	63	63
AVEN	45	38	38	46	47	*na*	—	—	—
DVEN	44	39	37	41	39	*na*	—	—	—
VS	89	77	75	87	86	71	74	78	73
SL (left,right)	9,9	11,11	9,10	10,10	10,9	9,9	10,10	11,10	10,11
IL (left,right)	10,10	12,12	10,10	11,11	10,9	9,10	10,11	11,10	11,10

Figure 4. Dorsal and ventral view of the lectotype of *Agasthyagama beddomii* (BMNH 1882.5.22.101) at the Natural History Museum, London. Photographs by V. Deepak.

NHM, London and the one in ZSI, Kolkata. However, the type locality and the elevation provided for the specimens are vague. The freshly collected specimens in this study are from an elevational range of 160–1200 m a.s.l. The type materials are the only specimens reported from the high elevations (ca. 1372 m a.s.l.). Additional sampling from the mountains of Sivagiri Hills is required to clarify this issue. Morphometric and meristic data are provided in Table 1, and live photos of the species are shown in Fig 5.

Diagnosis. A small-sized agamid lizard, SVL 29–44.7 mm (n = 19), characterised by laterally compressed body with heterogenous dorsolateral scales, small overlapping scales with larger often trihedral scales; 65–87 rows of scales around midbody; head with unequal keeled scales, a small antehumeral pit present, tympanum concealed; nuchal and dorsal crests absent, 60–67 dorsal scales from behind occiput to above cloacal ending; ventral scales strongly keeled, larger than lateral scales, 71–89 ventrals from mentum to cloaca; males with a small dewlap from mentum to forelimb insertion, gular scales small, keeled, 37–44 scales from mentum to last dewlap scale in males; limbs slender, covered with larger keeled scales; 4th digit on pes much longer than others, 5th digit on pes much reduced. Uniform olive to brown body, with lighter enlarged scales laterally; breeding coloration of males with pale grey to white mid dorsum; white throat with a broad

Figure 5. *Agasthyagama beddomii* in life, all uncollected individuals from various localities: **A** adult male from Kanayar, Achankovil; **B** female from Mararmalai, Kanyakumari District; **C** male from Manalar, Achankovil; **D** female from Rockwood, Shendurney Wildlife Sanctuary; **E** male from Devermala, Achankovil; **F** female from Arippa, Trivandrum. Photographs A and C–F by Sandeep Das and B by Jude. D.

dark brown stripe on dewlap with brick red circle bordered with bright yellow scales on the outside and with a bright yellow to orange blotch at the centre.

Description of the lectotype (NHMUK 1882.5.22.101) (Fig. 4) Specimen is in good condition. A small sized lizard, SVL 44.7 mm, head distinct from neck, longer than width (HeadW/HeadL = 0.62), snout longer than orbital diameter (OD/SO = 0.81), supraciliary and canthal ridge sharp, tympanum subdermal, supralabials 10 and 11 on right and left side respectively, infralabials 11 on both sides. Body laterally compressed, 67 dorsal scales, 79 rows of scales around trunk at midbody, 78 ventrals, strongly keeled. Scales on dorsum smaller than most ventral scales, lateral scales intermixed with unevenly spaced large differently coloured protruding scales. Scales on hindlimbs largest among body scales. Tail rounded, TailL 72.7 mm, gradually tapering posteriorly.

Colouration in preservative. Overall body pale grey to white with patches of light brown to amber on the snout and posterior part of head; dorsum with pale amber; tail dorsally and ventrally light brown, blotches, prominent laterally; limbs and tail dull brown speckled with light grey to white markings ventrally from gular region to abdomen pale white.

Natural History and Distribution. *Agasthyagama beddomii* has a wide distribution (Fig. 1) in the southern Western Ghats. The species is fairly common in its distribution range between 140–1379 m elevation (Table S2). They are largely terrestrial mostly in areas with dense leaf litter cover but rarely seen climbing smaller shrubs, roots or branches less than a metre in height. They are found in different habitat types including southern secondary moist mixed deciduous forests, west coast semi-evergreen forests, west coast tropical evergreen forests, southern hilltop tropical evergreen forests as well as in and around *Myristica* swamp forests (Jose et al. 2007; present study). Young ones were observed during the months of July to November unlike reported previously (Jose et al. 2007). Even though no breeding/mating observations are reported till date, breeding colouration in males, yellow to orange coloured blotch in the throat region was predominantly observed between March and June over a period of seven years (2012, 2013, 2014, 2015, 2018, 2019, 2023).

Agasthyagama edge sp. nov.

https://zoobank.org/DAEF594F-012A-4576-BFF4-89EB8DE-22A7B

Figures 6–9, 9B, Table 2

Holotype. ZSI/WRC/R/1165, an adult male (SVL 42.5 mm) (Fig. 6) collected from roadside disturbed tropical semi-evergreen forest patch in Kulamavu, Idukki district, Kerala, India (9.814150°N, 76.889926°E; 808 m a.s.l) by Sandeep Das, Muhamed Jafer Palot and K. Subin on 29 October 2021

Paratypes. ZSI/WRC/R/1166, ZSI/WRC/R/1167 (adult males) collection details same as holotype and ZSI/WRC/R/1168 and ZSI/WRC/R/1169 (adult males), ZSI/WRC/R/1170 (adult female) collected by the same team on the same day as the holotype from a similar disturbed forest patch (9.845741°N, 76.942100°E; 856 m a.s.l) on the roadside near to Kuyilimala bus stop, Idukki District, Kerala, India which is approximately seven km aerial distance from the holotype locality; ZSI/WRC/R/1171, ZSI/WRC/R/1172 (adult males), ZSI/WRC/R/1173 (subadult male) collected near Uppukunnu Government Tribal School, Idukki District (9.845061°N, 76.881476°E; 636 m) by Muhamed Jafer Palot and Sandeep Das on 25 July 2017. BNHS 3222 and BNHS 3223 (subadult males) collected by Muhamed Jafer Palot and Sandeep Das, on 26 July 2017 from Idukki.

Comment. There are no previous records of *Agasthyagama* from the mountains in Idukki district. Sivagiri hills according to a map provided by Smith (1935) is on the Eastern slopes of the Western Ghats immediately north of the Shencotta/ Shencottah gap.

Etymology. The species is named after the Zoological Society of London's EDGE of Existence Program who supported SD and RKP, and several early career conservation researchers across the globe in their projects on evolutionarily distinct species. EDGE is an acronym for Evolutionarily Distinct and Globally Endangered. The specific epithet "edge" is a patronym as a noun in apposition. We suggest common name "northern kangaroo lizard" and വടക്കൻ കങ്കാരു ഓന്ത് (vadakan kangaroo oanth) as a regional Malayalam name.

Diagnosis and comparison. A small-sized agamid (SVL 30–42.5 mm; n = 11) lizard characterised by the presence of heterogenous dorsolateral scales, oriented backwards, small overlapping scales with larger often trihedral scales; larger scales roughly forming 5 or 6 chevron shaped ridges on mid dorsum from nuchal region to above cloaca; strongly keeled enlarged scales randomly scattered laterally, 76–82 rows of scales around midbody; head with unequal keeled scales, supraciliary edge sharp; a small antehumeral pit present, tympanum concealed; nuchal and dorsal crests absent; ventral scales strongly keeled, larger than lateral scales, 73–80 ventrals from mentum to cloaca; gular scales at the mid-line nearly equal to the adjacent scales; males with a small dewlap, 32–35 scales from mentum to last dewlap scale; limbs slender, covered with larger keeled scales; 4th digit on pes much longer than others, 5th digit on pes much reduced. Uniform olive-brown body, with lighter, enlarged scales laterally, mid dorsum paler; breeding males with pale peach to grey mid dorsum; dewlap in breeding males with pale bluish-white centre flanked by reddish-brown stripe.

Agasthyagama edge **sp. nov.** is the second species from the recently recognised genus *Agasthyagama*. It is

superficially similar to *A. beddomii* in overall shape, size and colour but can be distinguished by combination of the following characters: 49–58 dorsal scales from behind occiput to above cloacal ending (vs. 60–67 dorsal scales in *A. beddomii*) and 32–35 dewlap ventral scales in males (vs. 37–44 dewlap ventral scales in males of *A. beddomii*). Additionally, dewlap stripe in breeding males of *A. edge* **sp. nov.** grey brown from mentum, gradually forming a brick-red elongated circle with pale bluish-white scales at the centre (vs. darker stripe with brick-red circle bordered with bright yellow scales on the outside and with a bright yellow to orange blotch at the centre in breeding males of *A. beddomii*).

Description of the holotype (ZSI/WRC/R/1165). The holotype is generally in good condition; hemipenis everted, exposed and visible on both sides when viewed dorsally (Fig. 3A). Tail entire, curved towards the left; head slightly tilted towards the left a small incision of ca. 4 mm to extricate tissue, are artefacts of preservation. An adult male (SVL: 42.5 mm), morphometric and meristic data are summarised in Table 2. General habitus moderately compressed. Head moderately large (HL/SVL = 0.30), elongate (HW/HL = 0.63), maximum height less than maximum width, not depressed (HH/HL = 0.58). In profile, snout pointed, steeply tapering to a pointed tip; snout moderately long (SO/HL = 0.43), longer than orbital diameter (OD/SO = 0.73). Orbit large (OD/HL = 0.31); pupil round, eyelids covered with small rounded scales, a single row of scales bordering eyelids slightly elongate; four supraciliaries on each side, elongate, similar in size, with single anterior most supraciliary smallest. Canthals and supraciliaries imbricate, laterally forming a distinctly protruding supraorbital ridge. Rostral rectangular, approximately three times wider (1.6 mm) than high (0.6 mm), bordered by seven scales including first supralabial. Nostrils circular, positioned in upper half of a single roughly pentagonal nasal shield; nasal bordered by seven scales on each side, including one prenasal, one supranasal, two postnasals, with two subnasals and the first supralabial on left side, and three subnasals on right side; separated on both sides from rostral by prenasal (Fig. 6D). Supralabials roughly rectangular, more or less equal-sized, posterior-most being longest, bordered above by a row of scales starting behind prenasal; eight supralabials on the left, nine on the right side. Loreal region with irregularly arranged, flat to partially keeled scales; scales surrounding orbit, small, granular. Scales on postorbital and temporal region heterogeneous in shape and size, flat. Dorsal forehead and snout scales heterogeneous in size, weakly tubercular at the edges, becoming keeled on the supraorbital region and rear of the head. Scales on occipital region heterogeneous in shape and size; smaller scales weakly tubercular, larger ones with distinct keels. Parietal eye oval, longitudinally oriented, partially visible through a roughly rhomboid parietal which is subequal in size to contacting scales, with much smaller posteriorly. Single small spinose scale along the upper posterior temporal region, another small sharply keeled, partly spinose scale in the post tympanic region on each side; tympanum concealed. Mentum subpentagonal, longer than wide, slightly narrower than rostral; bordered laterally by a first infralabial and posteriorly by a pair of elongated postmentals, subequal in length to the mentum but do not contact each other, bordered posteriorly by a row of two enlarged chin shields, postmental pairs flat; 10 infralabials on each side. Remaining gular scales imbricate to subimbricate, subequal in size, partially keeled scales towards the side while central gular scales strongly keeled. Posterior gular region with enlarged, flat, strongly keeled, pointed, imbricate scales; anterior gular scales smaller, subimbricate; all gular scales directed posteromedially except a few median rows which are directed posteriorly. A small but distinct gular pouch present up to anterior to the forelimb insertion (Fig. 6E). Ventral scales larger than scales on posterior gular region, enlarged, flat, strongly keeled, pointed, imbricate, fairly homogenous; arranged in regular longitudinal rows directed posteriorly, 34 scales from mentum to last dewlap scale, 40 scales from posterior end of dewlap to cloacal opening. (Fig. 6E). Nuchal and dorsal crest absent (Fig. 6C). Scales on the nuchal region are smaller, less than half the size of those on the interorbital region, imbricate, strongly keeled. Body slender, relatively long (TrL/SVL = 0.40); 79 rows of scales around midbody; dorsal scale row with 53 scales, five transverse chevron shaped marking directed backwards from back of neck until groin along the vertebral region composed of three or four larger, sharply keeled trihedral dorsal scale rows on either side, separated by smaller dorsal scales; scales on flanks heterogeneous, smaller than those on back, oriented posteroventrally, partially keeled, intermixed with scattered larger, strongly keeled scales (Fig. 7B). Fore and hindlimbs relatively slender, tibia short (CL/SVL = 0.33); digits moderately long, ending in strong, elongate, slightly recurved claw; inter-digital webbing absent; subdigital lamellae entire, bi-mucronate, 14 subdigital lamellae on digit IV of manus and 17 subdigital lamellae on digit IV of pes including claw sheath; relative length of digits on manus 4 > 3 > 2 > 5 > 1, of pes 4 > 3 > 2 > 1> 5. Fore and hindlimbs covered above and below with regularly arranged, enlarged, pointed, strongly keeled scales. Tail moderately long (TL/SVL = 1.86), entire, base swollen, uniformly covered with similar sized, keeled, pointed, regularly arranged, backwardly directed imbricate scales; subcaudal scales keeled, weakly pointed near base, becoming pointed posteriorly.

Colour in life. Uniform dull olive-brown body with slightly darker head, few light and darker enlarged scales scattered laterally; mid dorsum paler, forming a broad pale peach band from behind the head continuing till above tail, flanked by a thin almost broken stripe, most prominent above shoulder and above groin where it turns orangish (Fig. 9 A, B, 10B). Body colouration paler towards lower flanks and much darker near the shoulder and around the antehumeral pit region, laterally continuing to the orbit. Head laterally darker with a pale white, roughly triangular patch from behind nasal plate extending below the midorbit; another narrow, conical pale stripe from posteroventral margin of orbit extending to the last labials; nasal shield darker followed by small al-

ternating dark speckles on the supralabials, labials white to pale grey, interrupted by darker speckles. Head above uniform with a dark brown transverse band across the forehead, connecting supraorbitals. Abdomen pale off-white to buff in colour with few brown speckles on some scales. Forelimbs and hindlimbs darker suffused with alternating bands, a distinct pale peach band across upper arm of forelimb and a narrow light band just below elbow on the dorsal side; hind limbs with alternating light and dark brown bands, a distinct pale white to buff marking at the knee junction; digits roughly banded with light and dark markings. Tail uniform darker with few obscure lighter patches laterally, roughly forming dark and light bands towards latter half of the tail. Gular region with a central dark brown stripe starting from posterior half of the mentum, gradually becoming elongated brick red stripe abruptly ending at posterior half of neck, the latter half of stripe forming an elongated oval shape with a row three to four bluish-white scales (Fig. 2). Throat scales along both sides of the stripe pale bluish-white, all the

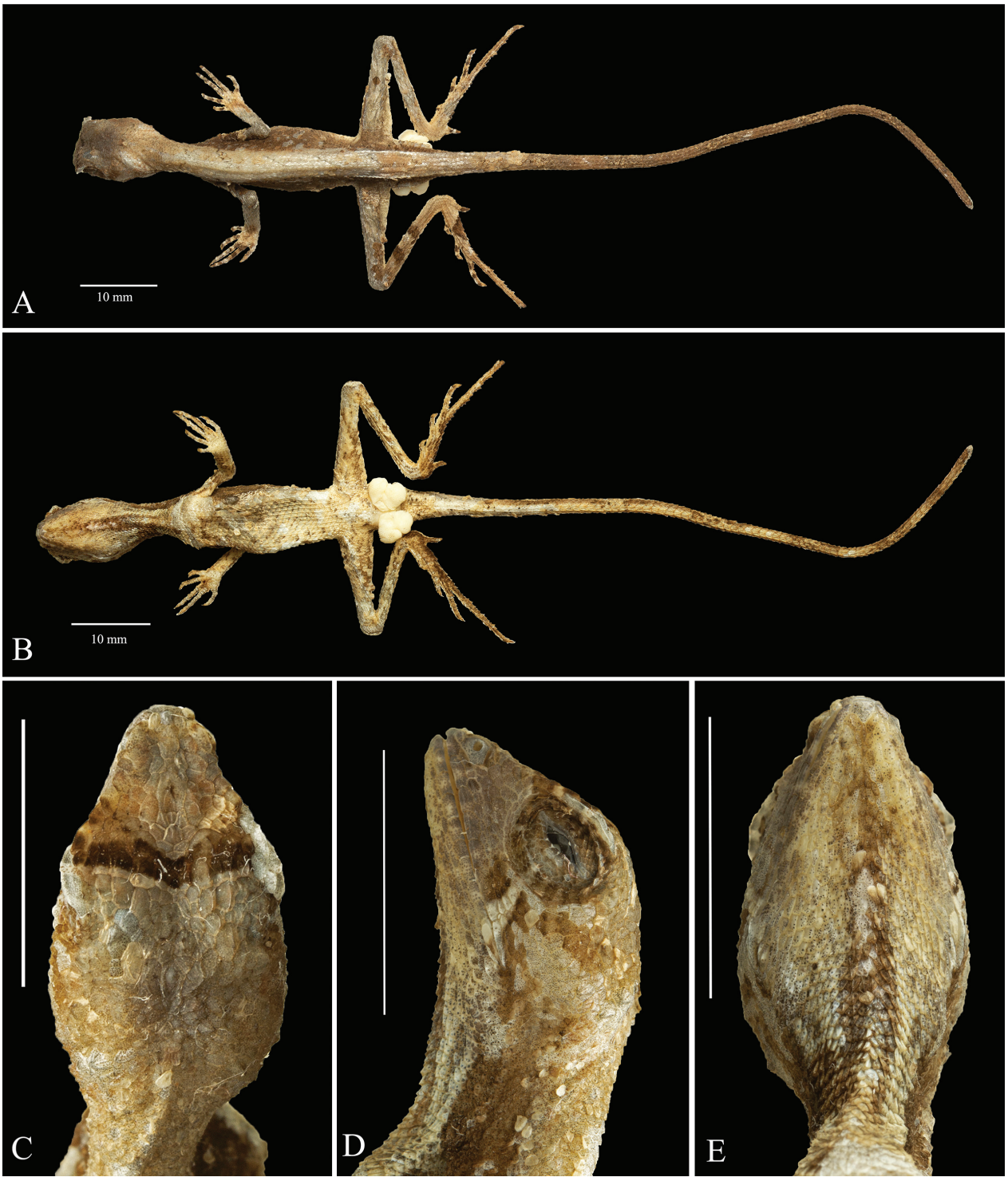

Figure 6. Holotype of *Agasthyagama edge* **sp. nov.** (ZSI/WRC/R/1165): **A** dorsal, **B** ventral view of body; **C** dorsal, **D** lateral, **E** ventral view of head. Scale bars 10 mm. Photographs by Saunak Pal.

way till anterior junction of forelimbs, roughly speckled with small brown spots.

Colour in preservative. Entire dorsal surface of the body from the back of the head extending onto the tail, between the dorsolateral keels is pale creamy white, dorsal surface of the head pale brown, supraorbital stripe distinct. Lateral surface of head pale brown followed by darker brown marking below eye, narrow stripe from posterior end of eye distinct, pale creamy white. Body laterally uniform

Figure 7. Scales on trunk of holotype of *Agasthyagama edge* **sp. nov.** (ZSI/WRC/R/1165): **A** dorsal view, **B** lateral view, **C** ventral view. Scale bars 10 mm. Photographs by Saunak Pal.

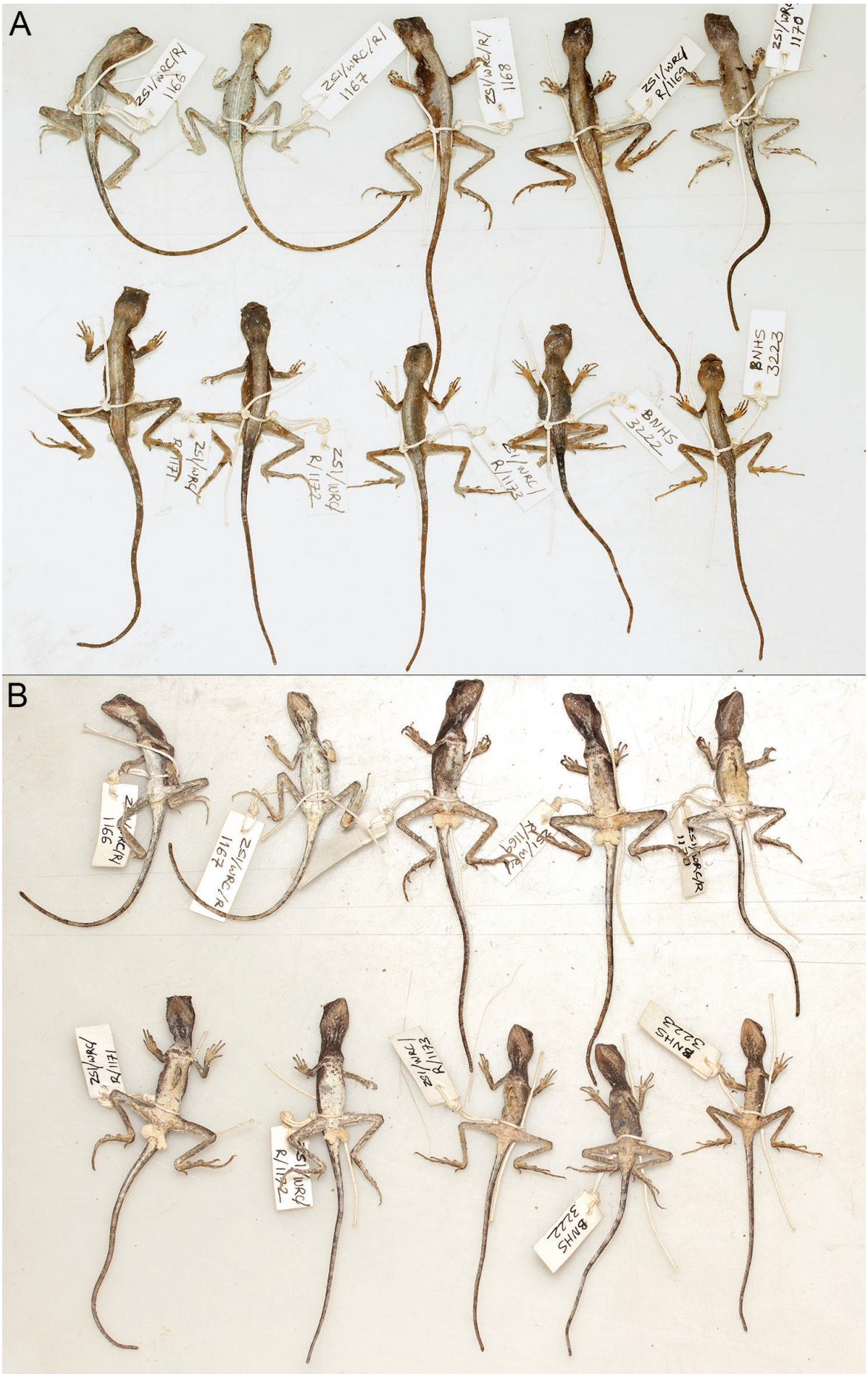

Figure 8. Paratype series of *Agasthyagama edge* **sp. nov.**: **A** dorsal view, **B** ventral view. Image not to scale. Photographs by Saunak Pal.

dark brown, speckled with few light grey to white scales. Limbs pale grey with darker blotched towards the digits. Abdomen, underside of limbs and tail dirty white intermixed with darker scales. Throat stripe brown with pale creamy white scales at the centre. Scales adjacent to the stripe buff to creamy white.

Variation. Morphometric and meristic data for the type specimens is presented in Table 1. The adult paratypes include nine male specimens ranging from 30–41.1 mm in SVL and one female specimen (ZSI/WRC/R/1170) with an SVL of 33.7 mm (Fig. 8). The paratypes agree with the holotype (ZSI/WRC/R/1165) in general morphology and scalation except for the following characters: 76–82 rows of scales around midbody; 49–58 rows of dorsal scales from behind occiput to above cloacal ending; male paratypes with 32–35 dewlap ventral scales; 13–15 subdigital lamellae under fourth finger, 17–22 subdigital lamellae under fourth toe; 9 or 10 supralabial and 8–10 infralabial scales. The female paratype, ZSI/WRC/R/1170 agrees with the holotype in overall scalation except that it lacks the small dewlap, has 78 ventral scales from mentum to cloaca and tail base not swollen. In colouration overall darker reddish brown, vertebral band paler, not as prominent as in males; head overall darker with a distinct creamy white stripe from below the eye till posterior end of labials; supraorbital band distinct, flanked by a narrow creamy white stripe; five distinct black chevron shaped transverse band, pointed backwards; abdomen pale buff, throat reddish brown with medial stripe; tail darker greyish brown. The male paratypes generally similar to the holotype in colouration. Subadults (ZSI/WRC/R/1173, BNHS 3222 and BNHS 3223) overall paler with darker markings on vertebral region, stripes on head lighter cream to buff.

Natural History and Distribution. *Agasthyagama edge* **sp. nov.** is currently known only from the type locality

Figure 9. *Agasthyagama edge* **sp. nov.** in life: **A** uncollected adult male from Kulamavu, Idukki; **B** uncollected female from the same locality. Photographs by Sandeep Das.

Table 2. Morphometric and meristic characters for *Agasthyagama edge* **sp. nov.** examined during this study. "_" missing data, "na" character not available, "M" male and "F" female. Samples (voucher no.) All specimens collected from Idukki district, Kerala.

Voucher	**ZSI/ WRC/R/1165**	**ZSI/ WRC/R/1170**	**ZSI/ WRC/R/1166**	**ZSI/ WRC/R/1167**	**ZSI/ WRC/R/1168**	**ZSI/ WRC/R/1169**	**ZSI/ WRC/R/1171**	**ZSI/ WRC/R/1172**	**ZSI/ WRC/R/1173**	**BNHS3222**	**BNHS 3223**
Sex	M	F	M	M	M	M	M	M	M	M	M
Type status	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
HL	12.8	11.3	11.8	11.4	12	12.5	12.1	11.5	10.6	10.7	10.4
HW	8.1	7.3	7.0	7.3	7.4	7.4	7.5	7.4	6.9	7.0	6.6
HH	7.4	6.4	6.7	6.8	6.9	7.1	6.9	6.4	5.9	5.8	5.6
IO	5.4	4.3	4.2	4.5	4.9	4.8	5,5	4.4	3.8	4.0	3.7
JL	9.6	8	8.5	8.6	8.5	8.9	9.3	8.5	7.3	8.1	7.4
NE	4.2	3	2.9	2.7	3.0	3.2	3.3	2.8	2.6	2.9	3.0
SO	5.5	3.9	4.2	4.4	4.4	4.2	4.6	4.2	3.2	3.5	3.7
SW	2.9	2.4	2.4	2.4	2.6	2.8	2.7	2.6	1.9	2.2	1.8
OD	4.0	3.3	2.8	3.4	3.4	3.7	3.6	3.4	2.9	3.0	2.5
3FL	5.3	3.7	3.8	3.7	4.1	4.3	4.3	3.8	3.0	4.2	2.9
4FL	6.5	3.9	4.4	4.3	4.9	4.7	4.8	4.1	3.6	4.5	3.2
4TL	10.9	9.3	10.2	9.9	9.8	9.7	10	9.8	9.0	10.1	7.9
5TL	2.3	1.8	1.5	1.6	1.8	2.0	2.1	1.9	1.5	1.9	1.4
FL	13.6	11.1	11.5	11.3	13.0	13.4	12.9	11.8	10.8	10.4	9.9
CL	14.1	11.6	12.8	12.4	12.8	14.0	13.6	12.6	11.0	10.8	10.5
HeL	19.6	15.3	16.6	16.5	17.8	18.4	18.4	15.9	14.8	15.2	14.3
LAL	7.6	7.0	5.8	6.2	7.0	7.7	7.5	6.9	6.0	6.3	5.7
UAL	7.1	6.1	5.2	6	6.3	6.8	6.9	5.8	5.7	5.9	5.6
PL	7.5	5.3	5.6	6.4	5.9	6.7	6.6	5.8	5.8	5.8	5.0
SVL	42.5	33.7	34.8	36.2	38.2	40.3	41.1	35.4	31	33	30
TL	78.9	59.8	64.2	68.4	70.6	70.4	72.2	62.9	59.7	58.3	54.5
TW	5.3	2.6	3.5	3.4	4.1	4.3	4.3	3.7	3.1	4.2	3.0
TrL	16.8	14.8	13.9	15.8	15.8	16.1	16.6	14.5	12.6	15.0	11.7
MBS	79	76	79	78	77	80	76	79	82	78	77
DS	53	57	49	55	51	58	51	56	51	54	56
AVEN	40	na	41	45	43	45	44	48	43	41	46
DVEN	34	na	32	33	35	34	32	32	35	35	34
VS	74	78	73	78	78	79	76	80	78	76	80
SL (left,right)	8,9	10,10	10, 10	10,10	9,9	10,9	9,9	10,10	9,10	9,9	10,10
IL (left,right)	10,10	10,10	9, 8	9,10	10,9	9,10	10,10	10,9	9,9	10,9	9,10

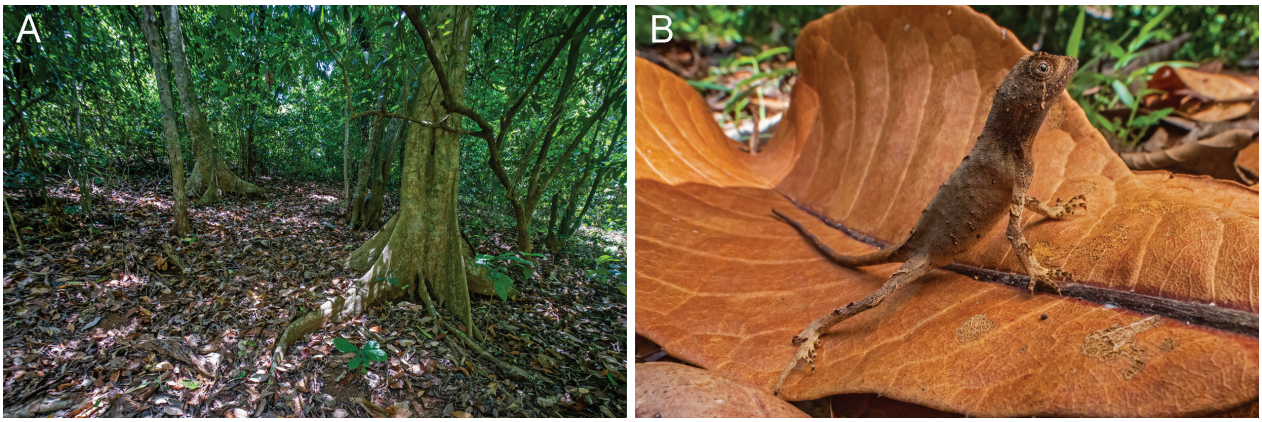

Figure 10. A Habitat at the type locality of *Agasthyagama edge* **sp. nov.** in Kulamavu, Kerala, India. **B** In-situ image of an uncollected female in its habitat at the type locality. Photographs by Sandeep Das.

and its vicinity and appears to be relatively common in the type locality (Fig. 10A). The habitat type at the type locality is evergreen forests. All records of this species are between 636–835 m elevation. In most of our surveys between 2016–2022 a minimum of 20 and a maximum of 50 individuals were sighted within a three-kilometre stretch within two hours of search. A greater number of individuals were encountered during March–May months. In disturbed habitats like roadside vegetation, and plantation areas, the numbers were smaller compared to undisturbed vegetation types. Males had a creamy throat patch during the breeding months of March to May (Fig. 3). Young ones were observed during June and July.

Discussion

The discovery of a second species of *Agasthyagama* adds to the ever-increasing reptile diversity in the Western Ghats. In the past decade, four species (*Monilesaurus acanthocephalus* Pal et al., 2018, *M. montanus* Pal et al., 2018, *Sarada superba* Deepak, Zambre, Bhosale & Giri, 2016, *Sitana marudhamneydhal* Deepak et al., 2016) of agamids were described from the Western Ghats (Deepak et al. 2016; Pal et al. 2018). Three out of these four species are restricted to high elevations >1000 m a.s.l. (Deepak et al. 2016, 2022). In the Western Ghats, the role of the Shencottah gap (SG) as a biogeographic barrier in speciation has been recognized in few high-elevation restricted amphibians (Van Bocxlaer et al. 2012; Vijayakumar et al. 2016), reptiles (Mallick et al. 2020; Deepak et al. 2022), fishes (Anoop et al. 2018) and birds (Robin et al. 2015). On the other hand, this gap appears to have played a small role in the genetic structuring of some of the low-elevation species' like *Monilesaurus rouxii* (Duméril & Bibron, 1837), *Raorchestes akroparallagi* (Biju & Bossuyt, 2009) to name a few (Vijayakumar et al. 2016; Pal et al. 2018). Given the limited sampling in the intervening area in our study, we cannot conclusively infer that the Shencottah gap is an actual barrier for *Agasthyagama*, and this needs to be verified in the future.

Among the agamids in the Western Ghats, *Agasthyagama* and *Microauris* Pal et al., 2018 are the only two genera which are endemic to southern Western Ghats. While *Microauris* is restricted to the high elevations (> 1000 m), *Agasthyagama* is found from low to high elevations (126–1379 m). *Agasthyagama beddomii* inhabit various habitat types in its range including myristica swamps (Jose et al. 2007), southern hilltop tropical evergreen forests, west coast tropical evergreen forests and west coast tropical semi-evergreen forests and southern secondary moist mixed deciduous forests whereas *Agasthyagama edge* **sp. nov.** is so far recorded only from tropical semi-evergreen and evergreen forests (Champion and Seth 1968). The present study shows that the two species are also geographically separated by approximately 80 km straight line distance.

It's also intriguing that the newly described population of *Agasthyagama edge* **sp. nov.** has never been reported from this region before, especially considering that these areas were relatively well explored during the colonial era (Mason 1888). This underscores the absence of comprehensive and focused sampling in some areas of the Western Ghats that have already been thoroughly explored. Although seemingly common at the type locality, *Agasthyagama edge* **sp. nov.** is relatively uncommon compared to *A. beddomii*. Additional surveys are required to understand the true latitudinal and elevational distribution of the two species of *Agasthyagama* in the southern Western Ghats.

We follow the evolutionary species concept to delimit the monophyletic lineage of *Agasthyagama* from Idukki district, Kerala as a distinct species (Simpson 1951; Wiley 1978; de Queiroz 2007). Out of the external morphological characters used to distinguish the two new species, one (dewlap ventral scale number) is a male-specific character while the other (dorsal scale number) is for both the sexes. The breeding colour variation, although distinct for the two species, is erased in preserved condition. However, the number of dorsal scale character used to distinguish the two species irrespective of the sexes, is consistent after examining specimens of *A. beddomii* (n = 19) from five different museum collections with various geographic origins. Additionally, we have provided

data for all the specimens we studied during this study (Tables 1 and 2) and they are available in international museum collections including NHMUK and FMNH, and national collections in India (BNHS and ZSI) for future comparative studies. The aforementioned evidence and the high genetic divergence between the two species show that they are independently evolving evolutionary lineages for which we designate a full species status.

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Supplementary Material 1

Tables S1, S2

Authors: Das S, Pal S, Narayanan S, Subin K, Palot MJ, Rajkumar KP, Deepak V (2024)
Data type: .zip
Explanation notes: Table S1. Gazetteer of confirmed locality records for *Agasthyagama beddomii* and *Agasthyagama edge* **sp. nov.** — **Table S2.** GenBank voucher numbers for the samples used in the study.

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