ZR | Zoological Research

Article   **Open Access**

Diversifying on the Ark: multiple new endemic lineages of dwarf geckos from the Western Ghats provide insights into the systematics and biogeography of South Asian *Cnemaspis* (Reptilia: Squamata)

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ABSTRACT

The highly speciose gekkonid genus *Cnemaspis* Strauch, 1887 is polyphyletic, with three distantly related and geographically isolated clades from Africa, South Asia (SA), and Southeast Asia. At present, there are 85 known species within SA *Cnemaspis*, although the number continues to increase rapidly with focused surveys and rigorous taxonomic work. Recent studies have provided valuable insights into the diversity and evolutionary history of SA *Cnemaspis*; however, most of these studies lack sufficient sampling in the Western Ghats (WG), where the genus has its greatest diversity. We addressed this research gap by conducting extensive sampling across the WG and re-examining museum specimens, thus providing a systematic account of various extant *Cnemaspis* species along with their distribution and natural history. We described 12 new species and a southern WG endemic clade of SA *Cnemaspis.* Ten of the newly described species are endemic to the forests of the southern WG. We also identified 10 well-supported subclades that can be separated across morphological, geographic, and phylogenetic axes. A time-calibrated phylogeny and ancestral area reconstructions confirmed the Paleocene origin of SA *Cnemaspis* in the WG and provide insights into its evolutionary history and biogeography. The discovery of multiple endemic and deeply divergent lineages further highlights the evolutionary significance of the WG for lizards.

Keywords: Biogeography; *Cnemaspis*; Dwarf geckos; Molecular phylogeny; New species; Osteology; South Asia; Systematics; Western Ghats

INTRODUCTION

The tropical forests of the Western Ghats (WG) are considered as rainforest refugia for various biota (Prasad et al., 2009). During the Upper Jurassic, the Indo-Madagascar plate is said to have carried biota from Gondwana after their split ~150–160 million years ago (Mya) (Briggs, 2003; Datta-Roy & Karanth, 2009). The Madagascar plate separated from

the Indian plate ~84–96 Mya, with the isolated Indian plate then drifting northwards towards Asia (Ali & Aitchison, 2008; Briggs, 2003; Datta-Roy & Karanth, 2009). It has been hypothesized that some extant endemic WG fauna, e.g., members of the gekkonid genus *Dravidogecko*, likely dispersed onto the Indian plate through transmarine dispersal from the Palearctic before the India-Asia collision (Agarwal et al., 2014; Chaitanya et al., 2019). Despite the occurrence of widespread extinction on parts of the Indian plate due to Deccan Traps volcanism, several lineages survived in refugia and eventually diversified across India, with some dispersing into Asia (Joshi & Karanth, 2013; Samant & Mohabey 2009). Among the Indian saurian fauna, the genus *Cnemaspis* Strauch, 1887 from South Asia (SA), which originated in the Late Cretaceous-Paleocene, appears to have potential Gondwanan ancestry (Agarwal et al., 2020b).

The Afro-Asian gekkonid genus *Cnemaspis* Strauch, 1887 is one of the most diverse gecko genera in the Old World, with over 170 known species (Uetz et al., 2020). Multiple phylogenetic studies in the last decade have established that the genus is polyphyletic, with three distantly related and geographically isolated clades from Africa, SA, and Southeast Asia (Gamble et al., 2012; Grismer et al., 2014). Due to the highly conservative morphology of members across its range, all species are currently grouped under a single genus.

The diverse SA clade of *Cnemaspis* comprises some 85 known species with disjunct distributions across Peninsular India and Sri Lanka, as well as Myanmar, northeast India, and islands west of Sumatra (Agarwal et al., 2017; Grismer et al., 2014; Khandekar et al., 2020a; Lee et al., 2019; Uetz et al., 2020). In the past two decades, there has been a rapid increase in the discovery of new species of SA *Cnemaspis* in both Sri Lanka and India, primarily due to extensive taxonomic revisions and the use of molecular phylogenies to delimit species (Cyriac et al., 2018, 2020; Karunarathna et al., 2019a, 2019b; Khandekar et al., 2019a; Manamendra-Arachchi et al., 2007; Sayyed et al., 2018). Neverthless, the diversity of SA *Cnemaspis* is likely to be greatly underestimated.

Within Peninsular India, the WG harbor the highest diversity of *Cnemaspis* (32 known species), while 13 species are currently known from the Eastern Ghats (EG) and the Mysore plateau, and two species do not have proper type localities (Cyriac et al., 2020; Khandekar, 2019; Khandekar et al., 2019a, 2020a; Murthy et al., 2019; Sayyed et al., 2020). Recent studies from regions outside the WG in Peninsular India have also led to the discovery of many new species, with 10 in the last five years alone. In comparison, large parts of the WG remain unexplored in terms of the true diversity of this gekkonid genus (Khandekar et al., 2020a).

Based on museum specimens, Manamendra-Arachchi et al. (2007) revised the Sri Lankan *Cnemaspis*, provided detailed redescriptions for the Indian members described prior to 1984, described three new species from southern India, and synonymized *C. anaikattiensis* Mukherjee, Bhupathy & Nixon 2005 with *C. sisparensis* (Theobald, 1876). Their study and others helped stabilize the taxonomy of some Indian species and provided descriptions of many new ones (Cyriac & Umesh, 2013; Cyriac et al., 2019; Ganesh et al., 2011; Giri et al., 2009a; Khandekar, 2019; Murthy et al., 2019). However,

taxonomic confusion regarding most *Cnemaspis* species from Peninsular India remains due to a lack of information on type localities, distribution, and natural history, as well as incorrectly designated types. For example, a recent study showed that the series of *C. ornata* (Beddome, 1870) paralectotypes designated by Manamendra-Arachchi et al. (2007) actually consists of more than one species (Sayyed et al., 2019). In addition, barring recent preliminary phylogenetic analyses, few studies have reported on the relationships within the highly diverse gekkonid group from Peninsular India, or have failed to include most WG endemics (Cyriac et al., 2020; Khandekar et al., 2019a; Sayyed et al., 2018). It is important, therefore, to use multiple lines of evidence to address taxonomic uncertainty and better estimate species diversity, especially for groups like *Cnemaspis*, which show conserved morphology (Agarwal et al., 2017; Grismer et al., 2014).

The WG escarpment in Peninsular India supports a remarkable diversity of endemic fauna and large evolutionary radiations, primarily due to the eco-climatic and topographic heterogeneity of the mountain range (Chaitanya et al., 2019; Biju et al., 2014a; van Bocxlaer et al., 2012; Vijayakumar et al., 2016). In the last decade, intensive explorations of the WG have resulted in the discovery of many new and evolutionarily distinct lineages, signifying the importance of this biodiversity hotspot (Biju et al., 2014b; Deepak et al., 2018; Garg & Biju, 2019; Mallik et al., 2019; Pal et al., 2018; Robin et al., 2017; Vijayakumar et al., 2019).

Members of the genus *Cnemaspis* are small to medium-sized round-eyed geckos, predominantly restricted to forested habitats. Due to the presence of a circular eye pupil, *Cnemaspis* geckos are often thought to be diurnal and are commonly called "day geckos", although studies report that at least some are nocturnal (Grismer et al., 2014) and/or crepuscular. Similar to other endemic fauna of the WG, most *Cnemaspis* geckos have limited distribution ranges and microhabitats (Agarwal et al., 2020a; Chaitanya et al., 2019; Cyriac et al., 2018, 2019), and many are known only from the type locality and museum specimens (Manamendra-Arachchi et al., 2007; Murthy et al., 2019). It is important to assess and validate the status of these species by locating extant populations, and to address taxonomic uncertainties and systematic relationships. Understanding the diversity and relationships among these habitat-specialist geckos can also provide insight into the drivers of speciation in the WG.

In this study, we constructed an updated phylogeny of *Cnemaspis* from Peninsular India, including near complete taxon sampling of WG species. We used a multi-criteria approach including molecular phylogeny, genetic distance, morphological comparison, and geographical distribution to delimit species (Mallik et al., 2020; Pal et al., 2018; Vijayakumar et al., 2014). We identified multiple strongly supported clades in *Cnemaspis* from Peninsular India, supported by morphological evidence and geographical distribution. We identified 12 new lineages, which are described herein, and redescribe several *Cnemaspis* species from the WG, which were previously described before 2000, including information on their natural history and distribution. We also explored the phylogenetic relationships within WG

Cnemaspis and other SA species and examined their divergence within WG using a fossil-calibrated time-tree.

MATERIALS AND METHODS

Field sampling and specimen collection

We aimed to investigate the diversity of *Cnemaspis* species from the WG and their phylogenetic relationships with other members of the genus from Peninsular India and to address the lack of knowledge of their geographical distribution. To resolve the systematic and taxonomic issues, we used a multi-criteria approach integrating molecular evidence with morphological data and spatial distribution (Shanker et al., 2017; Vijayakumar et al., 2014). The sampling framework was designed to address the topographic, climatic, and habitat heterogeneity within the WG. We stratified elevational gradients across major hill ranges and selected sampling sites across various habitats in each hill range (Pal et al., 2018; Vijayakumar et al., 2014). Extensive surveys were carried out in each selected site over a four-year period to locate existing populations of *Cnemaspis*. We specifically targeted known type localities to sample individuals wherever possible (Figure 1). Specimens were hand-collected, photographed in life, euthanized using halothane following standard animal euthanasia guidelines (Leary et al., 2013), and finally fixed in
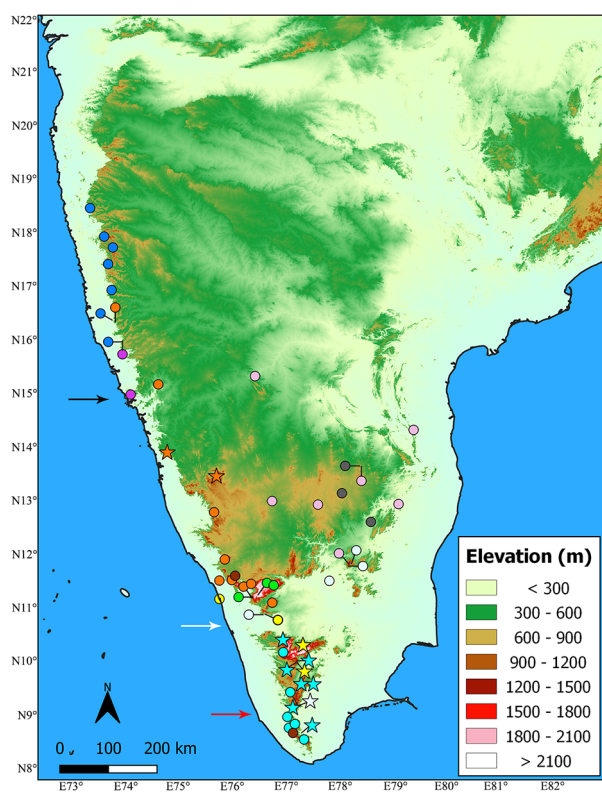

Figure 1 Map of Peninsular India showing type localities of extant species of *Cnemaspis*

Star denotes new species identified in this study; color corresponds to distinct clades (see details in Figure 2). Arrows indicate major geographical barriers in WG (black: Goa Gap, white: Palghat Gap, and red: Shencottah Gap).

4% formaldehyde for 24 h. Tissues (liver/tail tips) were extracted in 95% ethanol before fixing and subsequently stored at –20°C for genetic analysis. The fixed specimens were later stored in 70% ethanol and maintained in the herpetological collection at the Centre for Ecological Sciences (CES), Indian Institute of Science (IISc), Bangalore, India. The type specimens were deposited in the collection of the Bombay Natural History Society (BNHS), Mumbai, India, and CES, IISc. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All animal sample collection protocols complied with approved permits as per the current laws of India.

DNA isolation, sequencing, and phylogenetic analyses

Individuals that resembled extant species from type localities with known distribution, as well as additional individuals representing distinct populations from various geographical locations across the WG were selected. In total, 64 individuals belonging to *Cnemaspis* from across the WG and three individuals representing species distributed outside the WG were selected for phylogenetic analysis. Genomic DNA was isolated from the tail and liver tissues for all selected samples using the phenol-chloroform-isoamyl alcohol (Sambrook et al., 1989) and salt extraction method (Aljanabi & Martinez, 1997), then dissolved in 30 µL of 0.1% TE buffer (pH 7.6–8.0, 10 mmol/L TrisHCl, 0.1 mmol/L EDTA) and stored at 4 °C. To delimit lineages and estimate species diversity, we amplified the *16S* mitochondrial gene for sequencing, both the forward and reverse strands, using primers *16S*ar: (5'-CGCCTG TTTATCAAAAACAT-3') and *16S*br: (5'-CTCCGGTTTGAACT CAGATCA-3'), respectively (Simon et al., 1991). For a subset of individuals, we also generated partial sequences of the mitochondrial *NADH dehydrogenase 2* gene (*ND2*) using primers MetF1 (L4437 5'-AAGCTTTCGGGCCCATACC-3') and CO1R1 (H5934 5'-AGRGTGCCAATGTCTTTGTGRTT-3') for polymerase chain reaction (PCR) amplification and sequencing (Macey et al., 1997), carried out commercially at Barcode Biosciences and Medauxin, Bangalore, India. The PCR and sequencing protocols for *16S* followed Pal et al. (2018). We combined these with published sequences of *16S* and *ND2* representing extant SA *Cnemaspis* species from Peninsular India, Sri Lanka, and Southeast Asia.

The generated sequences were aligned using Muscle in MEGA v7.0 (Kumar et al., 2016). The final alignment of the *16S* gene sequences was 638 bp long and comprised a total of 87 individuals of most extant *Cnemaspis* species from the WG as well as three species from outside the WG. The *ND2* gene alignment was 1 041 bp long and comprised a total of 77 individuals, including representative species from the WG, Peninsular India outside the WG, and Sri Lanka. The final 1 679 bp concatenated dataset (*ND2*+*16S*) consisted of 117 *Cnemaspis* individuals from SA, including our collection and previously published sequences. All newly generated sequences were uploaded to GenBank under accession Nos. *16S*: MZ291569–MZ291621; *ND2*: MZ701801–MZ701834 (Supplementary Table S1 includes details of sequences used in this study).

Maximum-likelihood (ML) and Bayesian inference (BI) analyses of the final concatenated dataset of *16S* and codon-

partitioned *ND2* partial sequences were carried out using IQ-TREE (Nguyen et al., 2015) on the PhyloSuite (Zhang et al., 2020) platform and MrBayes v3.2 (Ronquist et al., 2012), respectively. ML analysis was carried out using the GTR+I+G model of sequence evolution, with support assessed through 10 000 ultra-fast bootstrap (UFBoot) replicates. Nodes with ML UFBoot values (UF) of 95 and above were considered strongly supported (Minh et al., 2013). PartitionFinder v2 (Lanfear et al., 2016) was used to determine the best-fit sequence substitution model for the dataset with the "greedy" search algorithm, with branch lengths as "linked" and AICc as model selection criteria (Lanfear et al., 2012). For BI analysis, the dataset was independently run twice for two million generations with a random starting tree, four Markov chains, and sampling every 1 000 generations. Convergence of the two runs was determined by ensuring that the standard deviation of split frequencies was less than 0.01, by confirming that the effective sample size (ESS values) was more than 200, and by checking the trace plots in Tracer v1.6 (Rambaut et al., 2014). Node values with Bayesian posterior probabilities (BPP) of 0.95 and above were considered strongly supported (Huelsenbeck et al., 2001; Wilcox et al., 2002). Uncorrected pairwise sequence divergences were calculated for the *16S* dataset in MEGA v7.0 for species delimitation (Table S2). *Lygodactylus miops* Günther, 1891, *Lygodactylus picturatus* (Peters, 1870), *Phelsuma lineata* Gray, 1842, and *Phelsuma ornata* Gray, 1825 were used as outgroups to root the tree for phylogenetic reconstructions (Cyriac et al., 2020; Sayyed et al., 2020).

Divergence dating

Divergence dating analysis was carried out in BEAST v2.4.8 implemented through the CIPRES portal (Miller et al., 2010) using an expanded gekkotan dataset with 122 additional taxa (Agarwal et al., 2020b; Chaitanya et al., 2019) (Table S3) along with a single lineage per putative SA *Cnemaspis* species for the *ND2* dataset (83 lineages). We partitioned the dataset by codon position based on the partition scheme selected by PartitionFinder v2 and applied the GTR+I+G model of sequence evolution in BEAST. We used BEAUti v2.4.8 to generate the input xml file with a relaxed lognormal clock model for each partition and a Yule speciation tree prior and constrained the Gekkotan families in the dataset to match the ML tree and previously published phylogenies. Three fossil calibrations with exponential distributions based on mean heights at the nodes and an arbitrary mean of 5, and one geological calibration, were used in line with previous studies (Agarwal et al., 2019, 2020b), and included amber fossils from Myanmar (crown Gekkota; offset 99); New Zealand diplodactylid material (most recent common ancestor (MRCA) New Zealand Diplodactylidae; offset 19); *Pygopus hortulanus* Hutchinson, 1997 (stem calibration for MRCA *Pygopus* Merrem; offset 23); and divergence of *Phelsuma inexpectata* Mertens, 1966 on Reunion from its closest relative in Mauritius *P. ornata* (uniform prior, 0.05–5). Final analyses were run for 100 million generations with sampling every 10 000 generations, and convergence (ESS>200) was determined by examining the log files in Tracer v1.6 (Rambaut et al., 2014). A maximum clade credibility tree (MCC) with median heights was generated using TreeAnnotator v2.4.8. Divergence times

are presented as 95% of the highest posterior densities (HPD) for corresponding nodes in Mya.

Ancestral area reconstruction

The dated BEAST consensus tree was pruned to retain only ingroup taxa that represent SA *Cnemaspis* in RASP v4.2. (Yu et al., 2015). The species were coded with their current distribution as follows: Northern Western Ghats (NWG) region starting from southern Gujarat through Maharashtra to the north of Goa Gap; Central Western Ghats (CWG) region south of the Goa Gap to the north of the Palghat Gap; Southern Western Ghats (SWG) region south of the Palghat Gap; Peninsular India outside the WG (PIO) regions that include the Mysore plateau and EG; and Sri Lanka and Southeast Asia. Ancestral area reconstruction was implemented with the consensus tree using the Bayesian Binary MCMC model and was run with the following constraints: maximum number of areas allowed was 4, number of cycles for the MCMC run was 50 000, with 10 chains sampled every 100th iteration and 100 results discarded as burn-in, run with the fixed Jukes Cantor model to account for state frequencies.

Morphological and meristic data

In total, 240 *Cnemaspis* individuals were examined for morphological analyses, including type specimens of the new lineages described herein as well as extant species from Peninsular India, topotypical specimens, type specimens, and other museum specimens listed in the material examined section (Appendix II). Comparative data on the described *Cnemaspis* species from Peninsular India were also taken from published literature (Agarwal et al., 2020b; Cyriac & Umesh, 2013, 2014; Cyriac et al., 2018, 2019, 2020; Das & Bauer, 2000; Giri et al., 2009a, 2009b; Khandekar, 2019; Khandekar et al., 2019a, 2019b, 2020b; Manamendra-Arachchi et al., 2007; Mirza et al., 2014; Mukherjee et al., 2005; Murthy et al., 2019; Sayyed et al., 2016, 2018, 2019, 2020; Srinivasulu et al., 2015). Counts and external observations of morphology and meristic characters were made under a Leica stereo dissecting microscope, and on the left side of the body for symmetrical characters where possible. We considered three body-size classes for *Cnemaspis* from Peninsular India based on snout vent length data, i.e., small (<35 mm), medium (35–45 mm), and large bodied (>45 mm). The following measurements were recorded using a Mitutoyo dial caliper to the nearest 0.1 mm: snout vent length (SVL, from tip of snout to vent); axilla to groin length (AGL, from posterior margin of forelimb insertion to anterior margin of hindlimb insertion); body width (BW, maximum body width); forearm length (FL, from elbow to distal end of wrist); crus length (CL, from knee to heel); tail length (TL, from vent to tip of tail); tail width (TW, widest point of tail); head length (HL, distance between retroarticular process of jaw and snout tip); head width (HW, maximum width of head); head depth (HD, maximum head depth at occiput); eye diameter (ED, greatest horizontal diameter of eye); eye to nares distance (EN, distance between anterior margin of eye and posterior edge of nostril); eye to snout distance (ES, distance between anterior margin of eye and tip of snout); eye to ear distance (EE, distance from anterior edge of ear opening to posterior

margin of eye); ear length (EL, maximum length of ear opening); internarial distance (IN, distance between nares); and interorbital distance (IO, shortest distance between left and right supraciliary scale rows).

The meristic data recorded for all specimens included: number of supralabials (SL) and infralabials (IL) (from rostral and mental, respectively, to posterior-most enlarged scale at angle of jaw); supralabials at midorbital position (SLM) and infralabials at midorbital position (ILM) (from rostral and mental, respectively, to below middle of orbit); dorsal tubercle rows (DTR, number of longitudinal rows of enlarged tubercles around body counted at midbody); paravertebral tubercles (PVT, number of enlarged tubercles between limb insertions counted along straight line immediately left of vertebral column); ventral scales (VS, counted from posterior of mental to anterior border of cloaca); midbody scale rows across belly (MVSR, counted at midbody ventral between lowest rows of dorsal scales); femoral pores (FP); precloacal pores (PP); number of poreless scales between precloacal pores (SBPP); number of poreless scales between femoral pores (SBFP); and number of poreless scales between femoral and precloacal pores (SB, FP, & PP); transverse subdigital lamellae, counted from base of digits to claw, including claw sheath on finger 1 (LamF1), finger 4 (LamF4), toe 1 (LamT1), toe 4 (LamT4), and toe 5 (LamT5). Additional characters evaluated (modified from Grismer et al., 2014) were presence or absence of row of enlarged, widely spaced tubercles and spine-like tubercles between limb insertions along ventrolateral edge of body (flank); general arrangement (i.e., random or more or less linear) of dorsal body tubercles; orientation and shape of femoral and precloacal pores; degree and arrangement of body and tail tuberculation; relative size and morphology of subcaudal scales; presence or absence of postcloacal tubercles (if present then distinct or indistinct) on each side of tail base, number of postcloacal tubercles on each side, when present (PCT); presence or absence of row of enlarged scales under 1st toe till end of feet; and presence or absence of whorls of caudal tubercles. Color pattern was recorded from photographs taken in life.

Micro-CT scans were generated for one male and female of *Cnemaspis* cf. *monticola* and one male specimen of *Cnemaspis galaxia* **sp. nov.** from the *beddomei* clade using a Bruker® Skyscan 1272 (Bruker BioSpin Corporation, USA). Each specimen was placed in polypropylene tube packed with polystyrene to ensure that the specimen was stable during the scan. The head and sacral regions of the specimens were scanned for 90 min at a resolution of 3.5 μm and data were recorded for every 0.5° rotation for 360° with (AL) a 1 mm filter. The source voltage and current for the scan were 65 kV and 153 μA, respectively. Volume rendering was performed with CTVox (Bruker BioSpin Corporation, USA) and images were edited in Adobe Photoshop CS6. The osteological descriptions were based on volume renders retrieved from CTVox following the terminology of the skull described in Evans (2008) and Scherz et al. (2017). Additionally, one male and one female specimen of *Cnemaspis magnifica* were cleared and stained following the protocols of Hanken & Wassersug (1981).

Species delimitation

We followed a hierarchical approach towards delineating lineages using the concatenated mitochondrial tree to identify clusters and multiple lines of evidence, including phylogenetic position, genetic divergence, morphological difference, and geographic isolation, to delimit distinct species (Shanker et al., 2017; Vijayakumar et al., 2014). Morphological examination and comparison of collected specimens with museum type specimens and topotypical material helped in identifying extant species. First, we identified well-supported subclades and used genetic divergence within members of each subclade to identify distinct lineages. For lineage diagnosis, we used the uncorrected pairwise distance of the *16S* mitochondrial gene and classified genetic divergence in the following categories: shallow genetic divergence (1%–2%); moderate genetic divergence (2%–4%); high genetic divergence (4%–6%); and very high genetic divergence (>6%) (Mallik et al., 2020; Shanker et al., 2017; Vijayakumar et al., 2014). We designated lineages with high or very high genetic divergence as species, and further examined shallow and moderately divergent species along morphological and geographical axes. Those that were morphologically distinct and geographically separated (allopatric) were also designated as species. In addition, to confirm the putative lineages, we used the Bayesian implementation of the Poison Tree Process (bPTP) model for species delimitation (Zhang et al., 2013). The concatenated tree was used as the input tree where outgroup taxa were removed prior to the run. We used the online-based server for bPTP and ran 500 000 MCMC iterations with 100 thinning to obtain convergence (http://species.h-its.org/ptp/).

Institutional abbreviations used in this study are as follows: BNHS (Bombay Natural History Society Museum, Mumbai), BMNH (Natural History Museum, London, UK), CES (Centre for Ecological Sciences, Indian Institute of Science, Bangalore), NCBS (National Centre for Biological Sciences, Bangalore), ZSI (Zoological Survey of India, Kolkata), WGRC, ZSI (Western Ghats Regional Centre, Zoological Survey of India, Kozhikode), MNHN (National Museum of Natural History, Paris, France), NMNHI (National Museum of Natural History, New Delhi), and FMNH (Field Museum of Natural History, Chicago). Other abbreviations include meters above sea level (m a.s.l.).

RESULTS

Phylogeny
The BI and ML analyses provided strong support for the monophyly of SA *Cnemaspis* and revealed a deep split separating two large well-supported clades (Figure 2). These included a well-supported *C. kolhapurensis* Giri, Bauer & Gaikwad, 2009+*C. wynadensis* (Beddome, 1870) clade (CL I) from NWG and CWG (north of the Palghat Gap) and another clade containing the remaining SA *Cnemaspis* species, similar to previous studies (Agarwal et al., 2020b; Sayyed et al., 2018). The latter clade could be further divided into multiple subclades, with a deep basal split separating a strongly supported *C. beddomei* (Theobald, 1876) clade (CL II)

endemic to SWG (south of the Palghat Gap) and another strongly supported clade, including multiple subclades of the remaining SA members.

Most of the identified subclades showed strong BI and ML support (see subclade composition below), except the split separating CL VII+VIII+IX+X from the other SA *Cnemaspis* species as well as between CL IX and CL X, which were moderately supported in the BI and ML analyses (BPP 0.78/UF 88 and 0.87/91, respectively). Based on our species delimitation protocol, we identified and described 12 new lineages belonging to various subclades (Figure 2), which varied in genetic and morphological divergences and patterns of geographical distribution. Overall, bPTP analysis supported our lineage delimitation, although it slightly overestimated the total number of lineages (Supplementary Figure S1).

Subclade composition: Based on phylogenetic analysis using the concatenated dataset, we identified 10 strongly supported clades, each composed of a minimum of two lineages (Figure 2). Most identified clades were supported by a combination of morphological characters and geographical distribution, unique to each clade (Table 1). We assigned names and numbers to these well-supported clades, with the component lineages listed below:

I. *wynadensis* clade: Comprising nine known lineages viz. *C. kolhapurensis*; *C. sisparensis* (Theobald, 1876); *C. kottiyoorensis* Cyriac & Umesh, 2014; *C. heteropholis* Bauer, 2002; *C. anaikattiensis* Mukherjee, Bhupathy & Nixon 2005; *C. wynadensis*; *C. chengodumalaensis* Cyriac, Palot, Deuti & Umesh, 2020; *C. zacharyi* Cyriac, Palot, Deuti & Umesh, 2020; and *C. magnifica* Khandekar, Thackeray, Pal & Agarwal, 2020. We identified two new well-supported, distantly related lineages with moderate support, described here as *Cnemaspis balerion* **sp. nov.** and *Cnemaspis lithophilis* **sp. nov.** (Figure 3).

II. *beddomei* clade: Comprising six known lineages viz. *C. beddomei*; *C. aaronbaueri* Sayyed, Grismer, Campbell & Dileepkumar, 2019; *C. anamudiensis* Cyriac, Johny, Umesh & Palot, 2018; *C. maculicollis* Cyriac, Johny, Umesh & Palot, 2018; *C. ornata* (Beddome, 1870); and *C. nairi* Inger, Marx & Koshy, 1984. Seven of the newly identified lineages belong to this clade (Figure 2), described here as *Cnemaspis rubraoculus* **sp. nov.**, *Cnemaspis wallaceii* **sp. nov.**, *Cnemaspis nimbus* **sp. nov.**, *Cnemaspis smaug* **sp. nov.**, *Cnemaspis nigriventris* **sp. nov.**, *Cnemaspis galaxia* **sp. nov.**, and *Cnemaspis regalis* **sp. nov.** (Figure 3).

III. *littoralis* clade: Comprising two known lineages from the WG, including *C. littoralis* and the recently described *C. palakkadensis* Sayyed, Cyriac & Dileepkumar, 2020. Two new lineages belong to this clade, described here as *Cnemaspis flavigularis* **sp. nov.**, and *Cnemaspis palanica* **sp. nov.** (Figure 3). This clade includes members of the *C. podihuna* clade from Sri Lanka, as observed in other recent studies (Agarwal et al., 2020b; Khandekar et al., 2019a).

IV. *indica* clade: Comprising three known lineages viz. *C. indica*; *C. nilagirica* Manamendra-Arachchi, Batuwita & Pethiyagoda, 2007; and *C. anandani* Murthy, Nitesh, Sengupta & Deepak, 2019.

**V. *bangara* clade:** Comprising three known lineages viz. *C. bangara*; *C. granticola* Agarwal, Thackeray, Pal & Khandekar, 2020; and *C. yelagiriensis* Agarwal, Thackeray, Pal & Khandekar, 2020.

VI. *girii* clade: Comprising seven known lineages viz. *C. girii*; *C. mahabali* Sayyed, Pyron & Dileepkumar, 2018; *C. limayei* Sayyed, Pyron & Dileepkumar, 2018; *C. ajijae* Sayyed, Pyron & Dileepkumar, 2018; *C. flaviventralis* Sayyed, Pyron & Dahanukar, 2016; *C. amba* Khandekar, Thackeray & Agarwal, 2019; and *C. koynaensis* Khandekar, Thackeray & Agarwal, 2019.

VII. *goaensis* clade: Comprising two known lineages viz. *C. amboliensis* Sayyed, Pyron & Dileepkumar, 2018 and *C. goaensis*.

VIII. *mysoriensis* clade: Comprising seven known lineages viz. *C. mysoriensis* (Jerdon, 1853); *C. otai* Das & Bauer, 2000; *C. yercaudensis* Das & Bauer, 2000; *C. adii* Srinivasulu, Kumar & Srinivasulu, 2015; *C. avasabinae* Agarwal, Bauer & Khandekar 2020; *C. rishivalleyensis* Agarwal, Thackeray & Khandekar 2020; and *C. stellapulvis* Khandekar, Thackeray & Agarwal, 2020.

IX. *gracilis* clade: Comprising four known lineages viz. *C. gracilis*; *C. agarwali* Khandekar, 2019; *C. shevaroyensis* Khandekar, Gaitonde & Agarwal, 2019; and *C. thackerayi* Khandekar, Gaitonde & Agarwal, 2019. A well-supported new lineage is described here as *Cnemaspis jackieii* **sp. nov.** (Figure 3).

X. *monticola* clade: Comprising two known lineages viz. *C. monticola* and *C. australis* from the WG. This clade also includes members of the *C. kandiana* clade from Sri Lanka and Southeast Asian representatives of SA *Cnemaspis*, as observed in other recent studies (Agarwal et al., 2020b; Khandekar et al., 2019a).

Divergence times and biogeography

We combined the newly created *ND2* dataset with earlier published datasets to estimate divergence times for the WG clades. The observed divergence dates overlapped closely with recent studies using overlapping taxon sets (Agarwal et al., 2020b; Chaitanya et al., 2019) (Supplementary Figure S2). We showed that SA *Cnemaspis* likely originated in the WG north of the Palghat Gap ~63 (74–53) Mya during the Paleocene, wherein the ancestors diverged into two clades, one comprising of members inhabiting the NWG and CWG (Clade I) and a second clade with a more complex biogeographic history (Figure 4). The initial diversification of SA *Cnemaspis* coincided with the K-T boundary, in particular the Deccan Traps volcanic eruption followed by the Paleocene-Eocene Thermal Maximum. The ancestors that gave rise to the present diversity of the group seem to have survived these events that otherwise led to mass extinctions (Ali & Aitchison, 2008; Cyriac & Kodandaramaiah, 2017; Datta-Roy & Karanth, 2009; Ivany et al., 2000; Joshi & Karanth, 2013). Clade I (*wynadensis* clade) began to diversify ~28 (35–21) Mya during the mid-Oligocene, leading to the single extant lineage *C. kolhapurensis* in the NWG, and to a clade distributed in the CWG, which began to diversify in the early Miocene ~21 (26–16) Mya.

The remaining clades diverged at a similar time as the initial diversification of SA *Cnemaspis* ~61 (72–51) Mya.
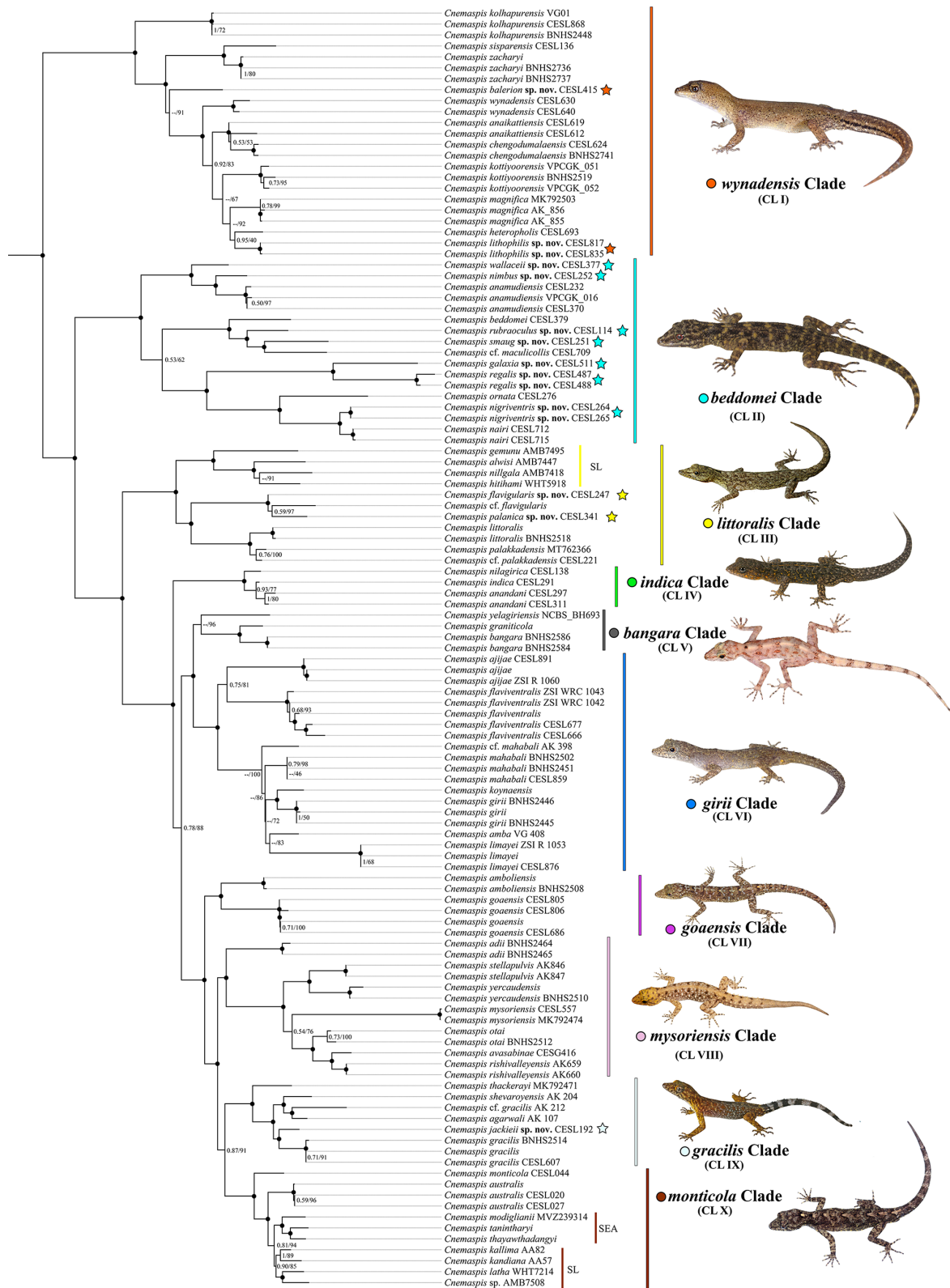
Figure 2 ML phylogeny illustrating relationships within SA *Cnemaspis* based on concatenated mitochondrial *16S* and *ND2* dataset
Values along nodes are posterior probabilities (BPP) based on Bayesian analysis followed by ultra-fast bootstraps (UF) of ML analysis. Solid circles represent nodes supported by BPP and UF values >0.95 and >95, respectively; "- -" indicates unresolved relationships. Lineages described in this study are marked by stars. Clades are named, represented by vertical specific-colored bars, and denoted with Roman numerals (CL I–X). Photos: representative species of each clade from Peninsular India; Photos by Saunak Pal.

Table 1 Major diagnostic characters of *Cnemaspis* clades from Peninsular India

	wynadensis Clade (CL I)	*beddomei* Clade (CL II)	*littoralis* Clade (CL III)	*indica* Clade (CL IV)	*bangara* Clade (CL V)
Distribution	NWGs, CWGs	SWGs	CWGs, SWGs	high elv of CWGs	EGs, Mysore plateau
Body size	Medium to large; stout	Medium to large; stout	Small to medium; slender	Small to medium	Medium; slender
Dorsal pholidosis	Homogenous or heterogenous	Heterogenous	Homogenous	Homogenous	Heterogenous
Spine-like tubercles on flank	Absent	Absent	Few, often present, scattered	Few, small, subconical; absent in *C. indica*	Absent
Femoral Pores	Present (except *C. kolhapurensis* which has series of precloacal-femoral pores)	Absent	Present; more than 10 on each side	Present	Present
Precloacal Pores	Absent	Present	Absent	Absent	Present
Tubercles forming whorls on tail	Absent	Present or absent	Present or absent; when present reduced	Absent	Present; only on few anterior rows
Postcloacal tubercle	Mostly absent; if present, small, reduced	Present, distinct	Often present; small, reduced	Present, distinct	Present
Sub-caudals	Smooth	Smooth	Smooth	Smooth	Smooth
Median sub-caudal scale row	Prominently enlarged	Prominently enlarged	Prominently enlarged	Prominently enlarged	Prominently enlarged
	girii Clade (CL VI)	*goaensis* Clade (CL VII)	*gracilis* Clade (CL VIII)	*mysorensis* Clade (CL IX)	*monticola* Clade (CL X)
Distribution	NWGs	NWGs	CWGs, SWGs, EGs, Mysore plateau	Mysore plateau	CWGs, SWGs
Body size	Small to medium; slender	Small to medium; slender	Small to medium; slender	Small to medium	Small to medium; slender
Dorsal pholidosis	Heterogenous	Heterogenous	Heterogenous	Heterogenous	Heterogenous
Spine-like tubercles on flank	Absent	Present	Absent	Absent	Present
Femoral Pores	Present	Present	Present	Present (except *C. avasabinae*)	Present
Precloacal Pores	Absent	Present	Present	Present	Present
Tubercles forming whorls on tail	Absent	Present	Present	Present	Present
Postcloacal tubercle	Mostly present, small	Present	Present	Present	Present
Sub-caudals	Smooth	Smooth or weakly keeled	Smooth	Smooth	Smooth or keeled
Median sub-caudal scale row	Not enlarged	Slightly enlarged	Prominently enlarged	Not to slightly enlarged	Enlarged

NWGs: Northern Western Ghats; CWGs: Southern Western Ghats; EGs: Eastern Ghats.

Diversification within Clade II began ~46 (56–37) Mya, during the Eocene to early Oligocene. Members of this clade are endemic to the SWG, and display diversification based on hill ranges and climatic regimes. The Shencottah Gap may have played a crucial role in the diversification of at least some members of this clade with lineages diverging across this gap during the early Miocene, as seen in *Cnemaspis galaxia* **sp. nov.** and *Cnemaspis regalis* **sp. nov.**, as well as *Cnemaspis smaug* **sp. nov.** and *C.* cf. *maculicollis* (Figure 4).

Clades III (*littoralis* clade) and IV–X diverged ~48 (57–39) Mya during the mid-Eocene. Clade III comprises wet zone species that dispersed into Sri Lanka during the early to mid-Oligocene (40–26 Mya), potentially facilitated by lower sea levels (Bossuyt et al., 2004), while diversification within the Indian members began ~27 (34–19) Mya. The western part of the WG receives much higher annual rainfall, which may have influenced an east-west diversification, as seen in *Cnemaspis palanica* **sp. nov.** and *Cnemaspis* cf. *flavigularis*. Clades IV–X started to diversify during the early Oligocene, with multiple dry and wet zone lineages. Clade IV (*indica* clade) is a unique clade endemic to the Nilgiri Hills in the CWG which diverged from the other clades in the early Oligocene ~34 (41–28) Mya, with recent diversification giving rise to closely related species. Clade X (*monticola* clade) began to diversify ~11 (14–9) Mya, with a single lineage dispersing out of India during the Late Miocene and colonizing Sri Lanka and Southeast Asia. The Southeast Asian representatives of SA *Cnemaspis*, i.e., *C. modiglianii* Das, 2005; *C. tanintharyi* Lee, Miller, Zug & Mulcahy, 2019, and *C. thayawthadangyi* Lee, Miller, Zug & Mulcahy 2019, split from the Sri Lankan members of Clade X (*kandiana* clade) ~7 (9–6) Mya.

The lineage-through-time plot suggests that since the Late Pliocene Thermal Maximum and the Deccan Traps volcanism ~50 Mya, *Cnemaspis* has been diversifying gradually, with two distinct plateaus and a slight increase post the Oi-1 glaciation event and a further rise during the Miocene. There is no evidence for a rapid burst in diversification at any point (Supplementary Figure S4).

Figure 3 Coloration in life of all 12 holotypes

A: *Cnemaspis balerion* **sp. nov.**; B: *Cnemaspis lithophilis* **sp. nov.**; C: *Cnemaspis rubraoculus* **sp. nov.**; D: *Cnemaspis nimbus* **sp. nov.**; E: *Cnemaspis wallaceii* **sp. nov.**; F: *Cnemaspis smaug* **sp. nov.**; G: *Cnemaspis regalis* **sp. nov.**; H: *Cnemaspis galaxia* **sp. nov.**; I: *Cnemaspis nigriventris* **sp. nov.**; J: *Cnemaspis flavigularis* **sp. nov.**; K: *Cnemaspis palanica* **sp. nov.**; L: *Cnemaspis jackieii* **sp. nov.**. Photos by Saunak Pal.

Osteology

The skull in *Cnemaspis* is depressed dorsoventrally and wedged shaped in the lateral view (Figure 5). The skull is lightly built and not robust like *Hemidactylus* (Mirza et al., 2018). Calcification near the orbit is poor and details are lost when scanned under high-energy CT, especially in males (Figures 5; Supplementary Figure S5). The short subtriangular ascending nasal process of the premaxilla and the premaxillae on either side border the nasal anteriorly; the nasal has a 'v'-shaped indentation on its anterior border, in which the ascending nasal process of the premaxilla resides; the nasal bears a longitudinal suture along its length in some species (Figure 5); the frontal is elongated with a broad posterior width, and appears fused anteriorly with the nasal, prefrontal, and premaxilla, with a distinct suture between it and the parietal; the parietal is broad with a median suture; the maxilla bears sharply pointed isodont teeth ranging from 20 to 25 in number; the prefrontal is strongly curved and bound to the posterior border of the maxilla until it contacts the frontal on the dorsum; the jugal is elongated and slender and extends from the posterior process of the maxilla outwards as a

process clearly evident in some species of the genus in dorsal view (*beddomei* clade) to barely visible (*monticola* clade). The postorbitofrontal is thin, in broad contact with the frontal along its posterolateral border and extends to the end of the parietal along its lateral edge. The epipterygoid is oriented diagonally backwards, hinged at its base to the pterygoid, which meets the jugal anteriorly. The quadrate is robust, lacking any fossa in the *beddomei* clade, but bears a large fossa at its basal end, more pronounced in females, as seen in the *monticola* clade (Supplementary Figure S5). The squamosal is thin and highly curved, running from the posterior border of the parietal to the paroccipital process. Twenty-five presacral and two sacral vertebrae are present (Supplementary Figure S6). The phalangeal formula for both manus and pes is 2–3–4–5–3. A single pair of postcloacal bones is present only in males (Supplementary Figure S7).

Systematics and taxonomy

In this study, we included most known *Cnemaspis* species from Peninsular India, except for *C. boiei* (Gray, 1842) and *C. jerdonii* (Theobald, 1868), for which a precise type locality is
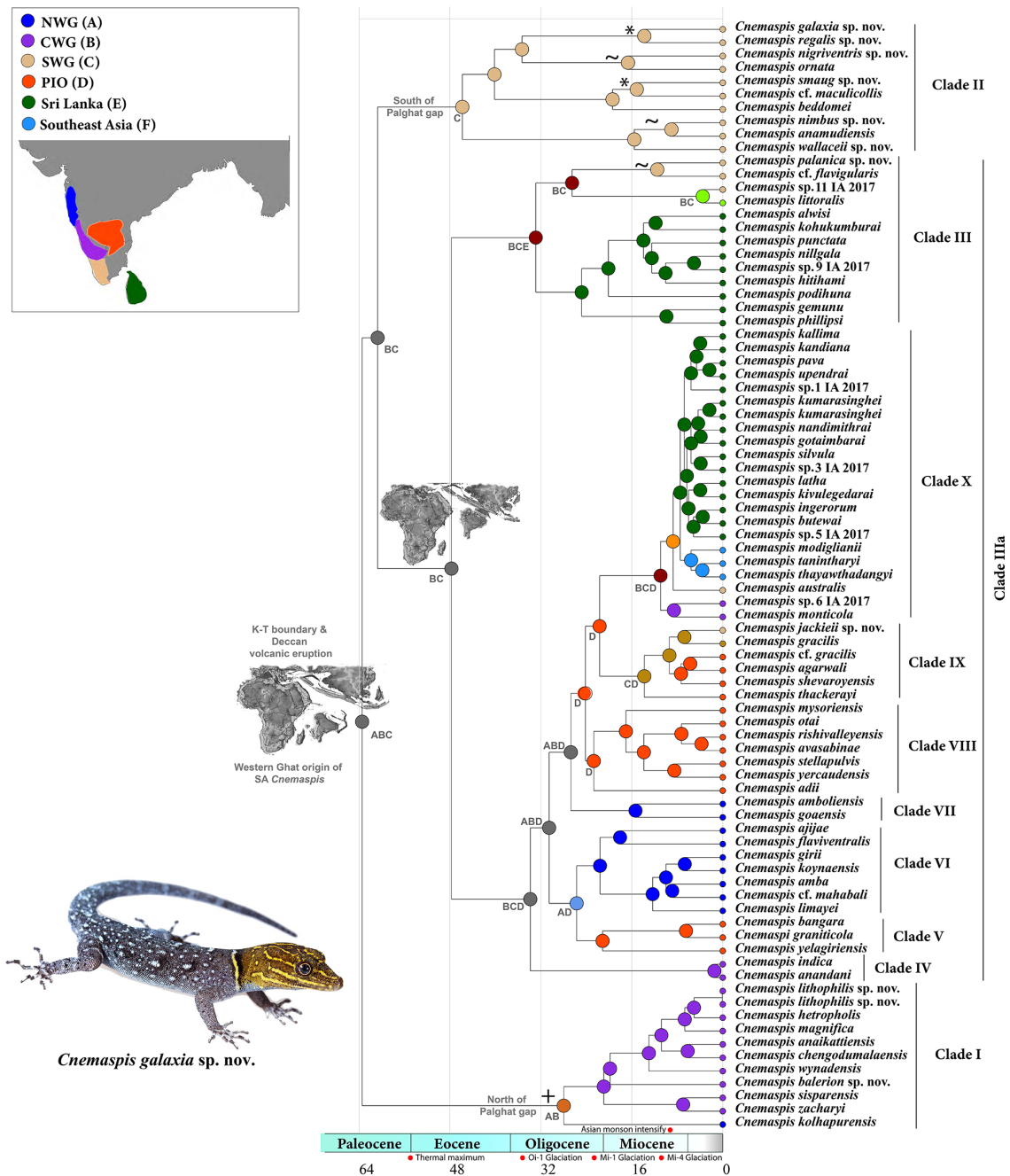
Figure 4 Geographical ancestral area reconstruction of time-calibrated phylogeny of SA _Cnemaspis_ produced using RASP, showing most likely ancestral areas

Maps at nodes show relative position of Indian plate at that time. Inset on top left shows color codes for ranges of species. See Supporting Material (Figures S3, S4) for RASP phylogeny tree with all possible ancestral ranges at nodes and lineage-through-time plot. "*" denotes separation by Shencottah Gap, "~" for east-west separation, and "+" for separation by Goa Gap). Photo by Saunak Pal.

currently unknown. The type specimens of _C. jerdonii_ deposited in ZSI are damaged and cannot be used for morphological observations (Manamendra-Arachchi et al., 2007, pers. obs.). Despite considerable effort during fieldwork, we were unable to discover any _Cnemaspis_ population that matched the descriptions of _C. jerdonii_ and _C. boiei_. Thus, for now, the status of these two species and their relationship with other members from Peninsular India remain unknown.

For species diagnosis and description, we compared individual lineages identified through our delimitation protocol with all other members of their respective clades from Peninsular India (Supplementary Tables S7–S10). We were unable to establish the phylogenetic position of the newly described _C. aaronbaueri_ Sayyed, Grismer, Campbell & Dileepkumar, 2019, but assigned the species to Clade II based on distinct morphological characters and geographical

distribution. We provide a morphological description, distribution, and taxonomic history for each identified subclade with a combination of defining characters. We also describe the newly identified lineages and redescribe poorly known species from each clade (See Appendix I in the Supplementary Material for clade descriptions, new species accounts, and species redescriptions).

DISCUSSION

Evolution and biogeography of SA *Cnemaspis*

Several recent studies have explored the phylogenetic relationships within SA *Cnemaspis* (Agarwal et al., 2017, Cyriac et al., 2018, 2020, Khandekar et al., 2019a, 2019b) as well as the evolutionary origin of lineages within the group (Agarwal et al., 2020b). However, these previous studies did not adequately sample the WG, where the genus is most diverse. Therefore, we addressed this considerable gap in *Cnemaspis* phylogeny with dedicated sampling across the WG, resulting in the discovery of several species and a deeply divergent clade endemic to the SWG. The discovery of this highly diverse clade, which dates to the Paleocene and contains multiple new endemic species, signifies the importance of the SWG in the diversification of SA *Cnemaspis*. These results provide important insights into the origin and *in situ* diversification of SA *Cnemaspis* in the WG.

As demonstrated earlier and confirmed here, SA *Cnemaspis* is one of the oldest extant vertebrate groups in Peninsular India, which began to diversify when the Indian plate was an island, before its collision with Asia. Notably, the *wynadensis*

clade split from the other clades ~63 Mya, thus suggesting a putative Gondwanan origin or trans-oceanic dispersal into India. The ancestors of SA *Cnemaspis* survived the K-T mass extinction event as well as the Deccan Traps volcanic eruptions.

The SWG (south of the Palghat Gap) region has long been considered as a refugium for wet-zone species during the Late Cretaceous (Bansal & Karanth, 2013; Datta-Roy & Karanth, 2009; Joshi & Karanth, 2013; Mani, 1974). However, the basal SA *Cnemaspis* Clade I hints at a potential origin north of the Palghat Gap. This finding is noteworthy as it adds to our growing knowledge of the biogeography of the WG as well as *Cnemaspis*. Lineages within Clade I only started to diversify in the mid-Oligocene, while those in Clade II began to diversify in the mid-Eocene, with deeply divergent lineages. The independent diversification of Clades I & II (*wynadensis* & *beddomei*) on either side of the Palghat Gap highlights the significance of the gap as a biogeographic barrier for SA *Cnemaspis*. Late Paleocene diversification of these clades suggests that the Palghat Gap served as an ancient barrier in the WG, predating the Indo-Asian plate collision.

In addition to the Palghat Gap, the lineages are closely associated with major hill ranges and their elevational gradients, as well as climatic regimes. The Shencottah Gap potentially drove allopatric speciation in certain *Cnemaspis* species (e.g., *Cnemaspis regalis* **sp. nov.** vs. *Cnemaspis galaxia* **sp. nov.**, *C. nairi* vs. *Cnemaspis nigriventris* **sp. nov.**, and *Cnemaspis smaug* **sp. nov.** and *C.* cf. *maculicollis*), while rainfall drove east-west diversification (e.g., *Cnemaspis wallaceii* **sp. nov.**, *Cnemaspis nimbus* **sp. nov.**, and *C.*
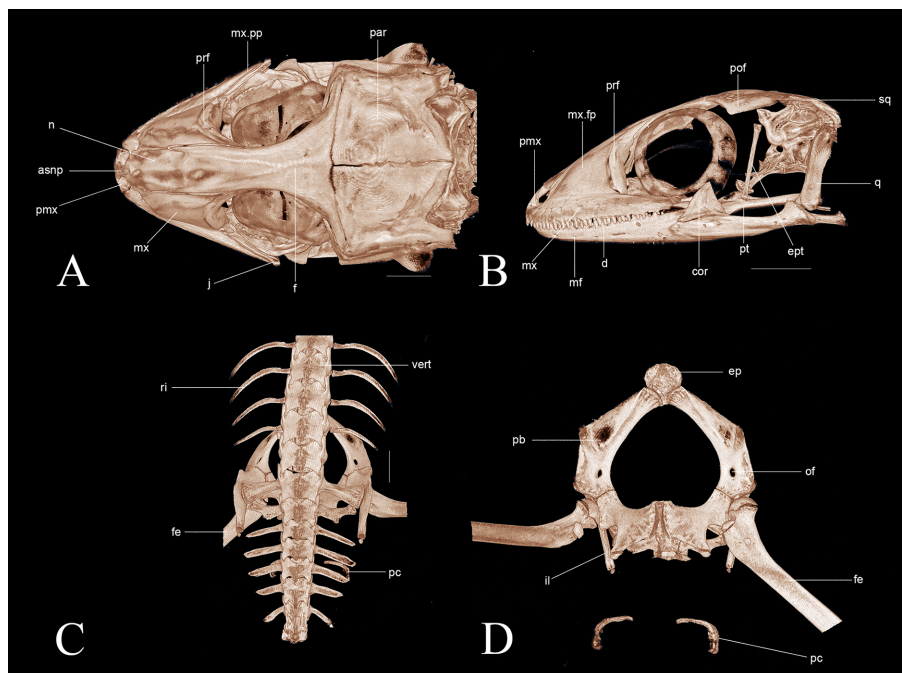
Figure 5 Micro-CT images of male paratype CESL 513 *Cnemaspis galaxia* sp. nov.

A: Dorsal view of skull; B: Lateral view of skull; C: Dorsal view of pelvic girdle; D: Ventral close up of pelvic girdle showing pair of cloacal bones. asnp: ascending nasal process of premaxilla, cor: coronoid, d: dentary, ept: epipterygoid, ep: epipubis, f: frontal, fe: femur, il: ilium, j: jugal, mf: mental foramen, mx: maxilla, mx.fp: facial process of maxilla, mx.pp: posterior process of maxilla, n: nasal, of: obturator foramen, par: parietal, pb: pubis, pc: post cloacal bone, pmx: premaxilla, pof: postorbitofrontal, prf: prefrontal, pt: pterygoid, q: quadrate, ri: ribs, sq: squamosal, vert: vertebrae.

anamudiensis across the Anamalai landscape). The existence of locally endemic, deeply divergent lineages across different massifs suggests the possibility of multiple micro-refugia across the WG. Two independent colonization events from the WG into drier parts of Peninsular India in the late Oligocene followed by range restriction during the Miocene aridification may explain the occurrence of the dry zone granite-associated clades in Peninsular India outside the WG.

Other gekkonid genera such as *Hemidactylus*, *Hemiphyllodactylus*, and *Cyrtodactylus* (*Geckoella*), which exhibit high diversity in regions outside the WG (e.g., EG and Mysore plateau), show much lower diversity in the WG. Studies have suggested that these geckos entered Peninsular India much more recently than *Cnemaspis*, dating back to the Eocene-Oligocene (Agarwal & Karanth, 2015; Agarwal et al., 2019, 2020b; Lajmi & Karanth, 2020). It is possible that the high *Cnemaspis* gecko diversity in the WG played a role in the lower diversity of these geckos in the region. The WG endemic genus *Dravidogecko* is the only other gekkonid genus known to have a late Paleocene origin; however, in contrast to *Cnemaspis*, these geckos started diversifying much later in the Miocene (Chaitanya et al., 2019).

Of the major clades identified in this study, Clades I and II, which started diversifying in the Oligocene and Eocene, show strong geographic separation, restricted to north and south of the Palghat Gap, respectively. Within members of these clades, there is apparent separation across elevational and rainfall gradients, as well as microhabitats and habits, with some species restricted to the forest floor and other scansorial species found on rocks and boulders in moist forests.

Clades III and X have a much more widespread distribution, with both found across the Palghat Gap and showing independent dispersal into Sri Lanka. Members of these two clades are distributed across different elevational gradients and forest types, ranging from low-elevation deciduous forests to high-elevation evergreen forests. Tolerance to different habitats and elevations may have helped them to diversify across the Palghat Gap and into Sri Lanka.

Clade IV (*indica* clade), which originated in the early Oligocene, is endemic to the Nilgiri Hills and contains three closely related species. Clade members show an east-west divide, with *C. anandani* and *C. nilagirica* occurring on either side of the Nilgiri Hills (Cyriac et al., 2019; Murthy et al., 2019). Clades V and VIII are both restricted to the drier parts of Peninsular India outside the WG, while clade IX is the only clade distributed across both the drier parts of Peninsular India as well as the WG. Most members of these clades display scansorial habits and frequent rocks and boulders in forested habitats. Clades VI and VII are restricted to the NWG, with members of Clade VI (*girii* clade) known mainly from moist semi-evergreen forest patches and those in Clade VII more widespread across different habitats and commensal at least in parts of their distribution (Khandekar et al., 2019b; Sayyed et al., 2018). Hence, multiple factors, such as the existence of ancient barriers, complex topography, and climatic gradients, coupled with highly specific habitat requirements, may have led to geographic isolation and the immense diversity of this ancient group of lizards in Peninsular India.

Taxonomy and systematics of SA *Cnemaspis*

Based on multiple criteria, our study shows a significant increase in the number of new lineages of *Cnemaspis* in the WG. We describe 12 of these newly identified lineages, which can be diagnosed as species on the basis of strong phylogenetic support, genetic distance, and geographical and morphological separation (Figures 2, 4). Seven belong to the SWG endemic *beddomei* clade. Amongst these, *Cnemaspis regalis* **sp. nov.** and *Cnemaspis galaxia* **sp. nov.** are sister species that show high genetic divergence, distinct morphology, and geographic separation. These species are from the eastern slopes of the Agasthyamalai and Megamalai hills in the SWG, respectively, and are separated by the Shencottah Gap. *Cnemaspis nigriventris* **sp. nov.** is the only newly identified lineage that shows shallow divergence from its sister species *C. nairi* but strong morphological differentiation. These species are also separated by the Shencottah Gap, with *C. nairi* restricted to south of the gap and *Cnemaspis nigriventris* **sp. nov.** restricted to the north (Supplementary Figure S14). Allopatric species showing shallow divergence with strong morphological and geographical separation have also been reported in other WG taxa (Mallik et al., 2020; Vijayakumar et al., 2014, 2016). The other four newly identified species from the *beddomei* clade show moderate to high divergence from the other clade members and morphological separation. These species are also geographically separated from close relatives, being restricted to high elevation areas on isolated hills in the SWG.

We also describe two new species from the *wynadensis* clade, distributed north of the Palghat Gap (Supplementary Figure S8). *Cnemaspis balerion* **sp. nov.**, although superficially similar to *C. wynadensis*, shows high genetic divergence from all members of its clade and is geographically isolated in the high-elevation montane forests of the Baba Budan Hills in the CWG. *Cnemaspis lithophilis* **sp. nov.**, from mid-elevation forests of the Sharavati valley in the CWG, is closely related to *C. heteropholis*, *C. magnifica*, and *C. kottiyoorensis*, but differs from these species on the basis of moderate genetic divergence, distinct morphology, and wide geographic separation. *Cnemaspis palanica* **sp. nov.** and *Cnemaspis flavigularis* **sp. nov.** from the *littoralis* clade are both endemic to the high-elevation forests of the SWG (Palani and Cardamom hills, respectively) (Supplementary Figure S14). Rainfall gradients may have influenced the east-west diversification of these two closely related species.

Cnemaspis jackieii **sp. nov.**, from mid-elevation regions of the SWG, is the second member of the *gracilis* clade to be described from the WG. Other than *C. gracilis* and *Cnemaspis jackieii* **sp. nov.**, all other members of this clade are known from outside the WG. *Cnemaspis jackieii* **sp. nov.** shows moderate divergence, distinct morphological differences, and wide geographic separation from the closely related *C. gracilis*.

Most extant species from Peninsular India were included in this study due to wide-scale sampling across the WG, studying existing type specimens from various museums, and including results from multiple new studies. We also recovered extant populations of certain species, such as *C. australis* and

C. monticola, which were known only from their type specimens collected over 130 years ago. Based on fresh collections obtained near the type locality, as well as morphological distinction, phylogenetic position, and genetic distance, we established *C. anaikattiensis* as a valid species, not a synonym of *C. sisparensis* as considered earlier (Cyriac et al., 2020; Manamendra-Arachchi et al., 2007). However, the taxonomic status and phylogenetic position of *C. jerdonii* and *C. boiei* await further research. As the precise type localities for these species are not known, and the type specimens of *C. jerdonii* are badly damaged, accurate species assessment is not currently possible. Although the type specimens of *C. boiei* exist in the BMNH collections, we were unable to find populations in the WG that matched those specimens. Given the advancement of genetic studies of museum specimens, DNA extraction from these specimens may help in identifying their phylogenetic status.

There has long been taxonomic ambiguity regarding the identification of most *Cnemaspis* species from the WG. Based on our multi-criteria approach, we identified 10 distinct clades of *Cnemaspis* in Peninsular India, which can be separated on the basis of morphological characters and geographic distribution. By defining these clades, we provide insights that will enable further taxonomic studies as well as field-based identification and documentation of *Cnemaspis* in Peninsular India. The redescription of extant species, including information on diagnostic characters, phylogenetic position, ecology, and distribution, will assist in future studies on the evolution and biogeography of this highly diverse and evolutionarily distinct group of geckos.

We also present preliminary results regarding the osteology of this group of geckos. Das & Bauer (2000) reported the presence of two pairs of postcloacal bones, one crescentic cloacal bone, and a pair of bony nodules near the base of the postcloacal spur in *C. yercaudensis*, *C. otai*, and *C. indica*. However, only a single pair of crescentic cloacal bones was observed via Micro-CT scanning and clear staining of related species in the current study. A more detailed sampling of SA *Cnemaspis* is necessary to establish the osteological basis of group separation within *Cnemaspis sensu lato* as suggested by Das & Bauer (2000).

CONCLUSIONS

Despite being one of the world's "hottest biodiversity hotspots", the WG have undergone substantial habitat loss and degradation due to changes in land-use patterns (Jha et al., 2000; Myers et al., 2000; Reddy et al., 2013). It has been suggested that significant parts of the WG will be urbanized by 2030 due to population expansion and development (Seto et al., 2012). The recent discoveries of new evolutionarily distinct lineages from the WG highlight the biogeographic and evolutionary significance of this region as well as the importance of sampling strategies to detect endemic lineages while conducting broad-level phylogenetic studies (Britz et al., 2020; Chaitanya et al., 2019; Dinesh et al., 2020; Mallik et al., 2019; Pal et al., 2018; Robin et al., 2017; Vijayakumar et al., 2019). The recognition of 10 new morphologically distinct and geographically isolated species of *Cnemaspis* from the SWG

also highlights our lack of knowledge regarding the immense diversity of this region and its potential to harbor more unknown species. Recent discoveries of species that are morphologically similar to extant species within their respective clades (e.g., *C. palakkadensis* and *C. littoralis*; *C. nilagirica* and *C. anandani*, *C. nairi* and *Cnemaspis nigriventris* **sp. nov.**) further emphasize the potential for unexplored cryptic diversity in the WG. Most of the recently described evolutionarily distinct lineages, as well as those identified in this study, are range-restricted and have very specific microhabitat requirements. It is therefore important to identify these unique habitats within the landscape and focus efforts on the conservation of unique lineages with diverse evolutionary histories.

In the last decade, there has been a surge in the discovery of new species of *Cnemaspis* from Peninsular India, especially from regions not known to harbor high *Cnemaspis* diversity, such as the southern edge of the Mysore Plateau and the NWG. The molecular data generated in this study not only include new species, but also species previously known only from type localities or specimens, and thus provide a baseline for future taxonomic research. The discovery of a highly diverse SWG endemic clade (Clade II) and a dry zone granite-associated clade (Agarwal et al., 2020b) suggest that more effort is required to elucidate the diversity within SA *Cnemaspis*. Additionally, our findings stress the importance of spatial sampling and geographical distribution of species, along with molecular phylogenetic, and morphological methods, in uncovering deeply divergent lineages.

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/.

Publication LSID:
urn:lsid:zoobank.org:pub:6C6F83BA-1CEA-4E4A-A6BC-1B00 5957E856

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***Cnemaspis wallaceii* sp. nov.**
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***Cnemaspis regalis* sp. nov.**
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***Cnemaspis galaxia* sp. nov.**
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***Cnemaspis nigriventris* sp. nov.**
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***Cnemaspis flavigularis* sp. nov.**
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***Cnemaspis palanica* sp. nov.**
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***Cnemaspis jackieii* sp. nov.**
urn:lsid:zoobank.org:act:A7DB6E6A-4F98-4044-B0B4-3CA6A119C71C

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Fieldwork, including the collection of animals in the field, was conducted with permissions from, and following guidelines of, the responsible authorities in the State Forest Departments (Fieldwork research permits: No. WL 12-6574/2006, No. PS.CWL.CR-60/2006-07, C. No. WL5/74223/2010, No. 2/21/GEN/WL & ET (S)/2012-2013/591; D-22 (8)/Research/4520/2012-13 Permit No. 127, No. 1/16/WL & ET 12-13/184; Permit No. 67/2018).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

S.P. conceived and designed the study, carried out data analysis and fieldwork, participated in laboratory work, prepared the manuscript, authored and reviewed drafts of the paper, and approved the final draft; Z.A.M. carried out data analysis and fieldwork, participated in laboratory work, assisted in writing the manuscript, authored and reviewed drafts of the paper, and approved the final draft; P.D. carried out molecular analyses, participated in laboratory work, assisted in writing the manuscript, authored and reviewed drafts of the paper, and approved the final draft; K.S. helped design and coordinate the study, acquired funding, contributed reagents/materials/analysis tools, assisted in writing the manuscript, authored and reviewed drafts of the paper, and approved the final draft. All authors read and approved the final version of the manuscript.

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