ORIGINAL ARTICLE

A phylogeny of open-habitat lizards (Squamata: Lacertidae: *Ophisops*) supports the antiquity of Indian grassy biomes

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ABSTRACT

Aim India is dominated by tropical grassy biomes (TGBs), traditionally considered seres or degraded forest, with low diversity relative to the restricted, ancestral wet zone. It is unclear if Indian grasslands and other open habitats are anthropogenically derived or native, old-growth habitats; without a clear timescale of grassland evolution. One way to understand grassland evolution is to study the diversification in taxa restricted to open habitats. We use a dated phylogeny of *Ophisops* to address questions related to the origin, diversification and inter-relationships of Indian and Saharo-Arabian *Ophisops*, and ultimately the origin of Indian grasslands and open habitats.

Location The Indian subcontinent; the Saharo-Arabian Realm.

Methods We generated up to 2736 base pairs of aligned sequence data (one mitochondrial, two nuclear genes) for Indian lacertids and reconstructed phylogenetic relationships using maximum likelihood and Bayesian inference. We use a fossil-calibrated timetree, diversification analyses and ancestral area reconstructions to test the hypotheses of origin and relationships with Saharo-Arabian *Ophisops*.

Results *Ophisops* is strongly supported as monophyletic, with a basal split into a large-bodied (LBC) and small-bodied clade (SBC). The Saharo-Arabian species are nested within the Indian species in the LBC. Species diversity in Indian *Ophisops* is grossly underestimated, with 26–47 candidate species. *Ophisops* began diversifying in the late Oligocene with significant rate shifts in the late Miocene-Pliocene and Pleistocene within the SBC.

Main conclusions Our results are consistent with an ancient origin of grassland taxa and TGBs in India. *Ophisops* is a dramatic example of overlooked cryptic diversity outside forests, with ~30 species where five were known. *Ophisops* dispersed into India from the Saharo-Arabian Realm in the Oligocene with a back dispersal in the Middle Miocene, a novel biogeographical pattern. Diversification in the SBC of *Ophisops* increased 8-fold during the global C4 grassland expansion. Indian TGBs are old-growth ecosystems that need urgent conservation attention.

Keywords

Asia, C4 grasslands, diversification rate shift, India, lacertids, out-of-India, tropical grassy biomes

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INTRODUCTION

Tropical grassy biomes (TGBs) include C4-dominated grasslands and savannas across a latitudinal belt ~30° N and S that encompass ~20% of terrestrial ecosystems (Scholes & Archer, 1997). TGBs often occur in areas with annual rainfall that can support trees (Staver *et al.*, 2011), with both top and bottom-down processes acting to keep the canopy open (Bond *et al.*, 2005; Sankaran *et al.*, 2005). Anthropogenically derived grassy habitats often resemble natural TGBs in

structure (Putz & Redford, 2010), and thus, the traditional view that most grasslands, savannas and open habitats represent disturbed systems or seral stages of degraded forest (e.g. Pandey & Singh, 1991; Banerjee, 1995). It is only recently that TGBs have been recognized as ancient ecosystems with high diversity and endemism (Sankaran, 2009; Putz & Redford, 2010; Veldman et al., 2015a).

Asian and Indian TGBs are particularly poorly known, even though recent estimates suggest that they cover over 40% of Indian terrestrial ecosystems (ICFRE 2013). A combination of biogeography and history has led to the long-standing view that TGBs in the region are anthropogenically derived (Pemadasa, 1990; Pandey & Singh, 1991; Banerjee, 1995), with a consequent forest-centric conservation bias (Ratnam et al., 2016). The existing paradigm for Indian biodiversity is high diversity and a deep history in the restricted, ancestral, forested wet zone; whilst the extensive dry zone, within which most TGBs are located, is considered relatively depauperate and recent in origin (e.g. Karanth, 2003, 2006). However, molecular data reveals high diversity and a pre-Miocene history in at least dry zone lizards (Agarwal et al., 2014; Deepak et al., 2016). The decline of forests and the subsequent rise of grasslands are linked with the development of aridification and seasonality. Thus, we review the evolution of grasses and grasslands, as well as past records of aridification and seasonality in the Indian subcontinent, to understand the possible timing of diversification of open, grassy habitats and their associated biota.

The first grasses (Prasad et al., 2005) and seasonally arid climates date back to the Cretaceous (Samant & Mohabey, 2009), when aseasonal megathermal rain forests dominated the Indian Plate (Morley, 2007). The India-Asia collision ~50 Ma (Ma; Ali and Aitchinson 2008; Meng et al., 2012) triggered Himalayan uplift and the onset of the Asian monsoon, the earliest evidence for which is from ~40 Ma (Licht et al., 2014). Three main phases of aridification and seasonality followed; a prominent cooling and drying event at the Eocene-Oligocene boundary (~34 Ma; Zachos et al., 2001; Licht et al., 2014), increased seasonality associated with accelerated Himalayan orogeny in the early Miocene (23 Ma; Clift et al., 2008), and finally, intensified aridification from the late Miocene (~10 Ma; Ganjoo & Shaker, 2007; Nelson, 2007; Molnar & Rajagopalan, 2012). Palynological data suggest that forests became more fragmented from the Late Eocene, apart from a brief expansion during the warm, ever-wet Middle Miocene Climatic Optimum (MMCO; Zachos et al., 2001), and late Miocene aridification caused severe forest fragmentation (Morley 2007; Pound et al., 2012). C4 grasses evolved ~ 35 Ma, but only rose to dominance in the Late Miocene, 8–6 Ma (Cerling et al., 1997; Edwards & Smith, 2010; Bouchenak-Khelladi et al., 2014). Records from the Indian subcontinent, based on multiple proxies from the Siwaliks, suggest coincident regional expansion of C4 grasslands, beginning with drier areas about 8 Ma, with the complete transition to homogeneous C4 vegetation 5–4 Ma (Quade & Cerling, 1995; Sanyal et al., 2010; Singh et al.,

2011). It is unclear how Pleistocene glacial cycles affected Indian grassy biomes, but there is scattered evidence for grasslands being more extensive during the arid Last Glacial Maximum (e.g. Furley & Metcalfe, 2007; Sarkar et al., 2014), whilst periods with wetter conditions might have hampered dispersal of arid adapted groups.

The Indian subcontinent has a poor record of past vegetation cover, further confounded by the classification of many TGBs into 'forest', and tremendous anthropogenically induced land-cover change (Ratnam et al., 2011, 2016). We have neither a clear timescale for the spread of Indian grasslands and open habitats and associated biota, nor an understanding of what proportion of these habitats are natural or derived. One way to understand the evolution of TGBs is to study the diversification in taxa that are restricted to grasslands. Ophisops Ménétries, 1832 are small, diurnal lizards (snout to vent length, SVL 35–70 mm) of the Old World Lacertidae that are restricted to open, predominantly grassland habitats (Fig. 1); with five species known from the Indian subcontinent and three species in Saharo-Arabia (Uetz, 2016; Fig. 2). Global lacertid phylogenies recover a Miocene age for Ophisops, which is nested within a larger Saharo-Arabian clade that includes the genera Acanthodactylus, Eremias and Mesalina, all of which are adapted to xeric, open habitats (Mayer & Pavlicev, 2007; Hipsley et al., 2014). Limited sequence data from Saharo-Arabian Ophisops reveals cryptic species complexes, with the single sampled Indian species Ophisops jerdonii Blyth the divergent sister to this group (Kyriazi et al., 2008). Little is known of the other Indian Ophisops species, including how the species from these regions are related.

We use a timetree of Indian Ophisops to test two alternate hypotheses concerning the history of Indian grassy biomes and open habitats: (1) if Indian grasslands are solely of recent anthropogenic origin, Ophisops would have few, generalist, widely distributed species that have undergone range expansions with human-induced change; or (2) if Indian grasslands expanded with the global spread of C4 grassy biomes, Ophisops would have a deep history dating back to at least the Pliocene, with high diversity and endemism. In addition, we examine the tempo and timing of Ophisops diversification, as well as inter-relationships of Indian and Saharo-Arabian Ophisops.

MATERIALS AND METHODS

Taxon and gene sampling

Ophisops and other lacertids were collected opportunistically from across India during fieldwork on dry zone lizards from 2008 to 2015, and samples contributed by other researchers (see Acknowledgements). Our sampling of Ophisops spans the known range within India and over 16° each in latitude and longitude (Fig. 2). We made an effort to obtain topotypical material for all valid species. Lizards collected for this study were euthanized with halothane, tail-tips or liver
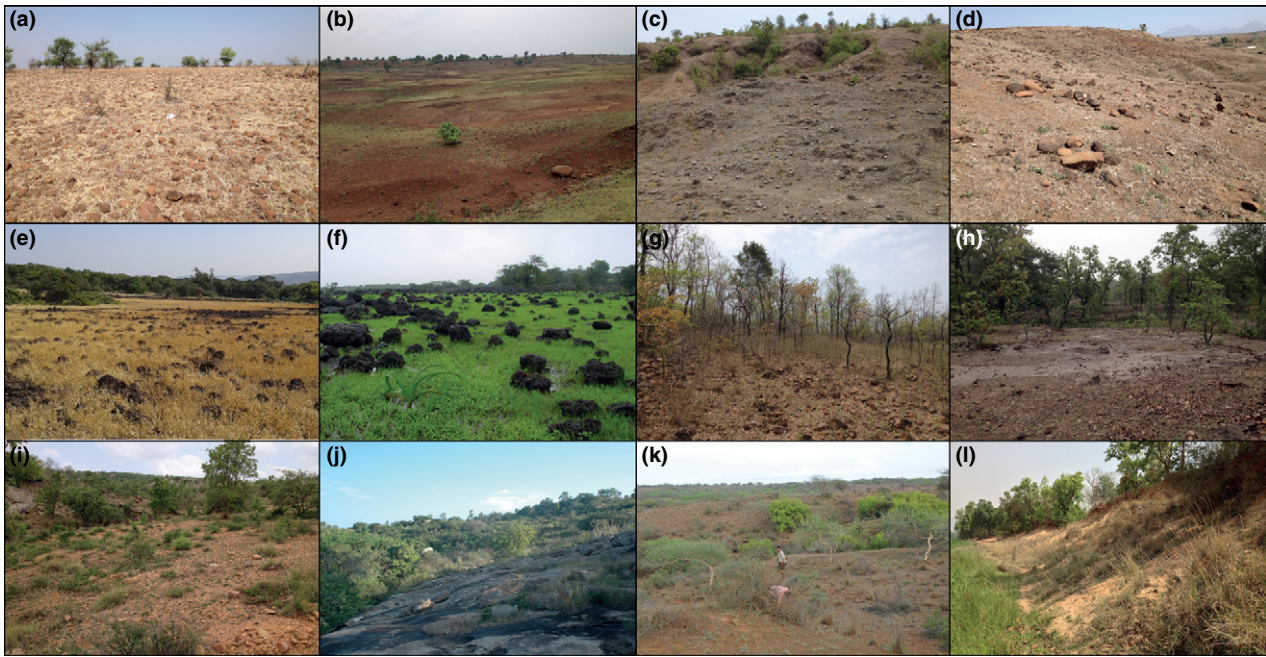
Figure 1 Habitats of *Ophisops* species complexes in India: *O. jerdonii*, (a) Osmanabad District, Maharashtra, (b) Betul District, Madhya Pradesh, (c) Chhindwara District, Madhya Pradesh, (d) Nashik District, Maharashtra); *O. beddomei* (Maharashtra) (e) Sangli District, (f) Satara District); *O. nictans*, (g) Sironcha District, Maharashtra, (h) Korba District, Chhattisgarh); *O. leschenaultii*, (i) Tumkur District, Karnataka, (j) Chittoor District, Andhra Pradesh); *O. microlepis*, (k) Kacchh District, Gujarat, (l) Korba District, Chhattisgarh). All photographs were taken in the dry season (March–May) except f), monsoon (August). Photographs, Ishan Agarwal, except e), Akshay Khandekar.

collected in ethanol, and whole specimens vouchered in formalin. We generated sequence data for 125 *Ophisops* from 108 localities (see Appendix S1 in supporting information, Fig. 2). DNA was extracted using the Qiagen® DNEasy kit and we generated up to 2736 aligned base pairs (bp) of sequence data, including one mitochondrial gene (cyt *b*, 1143 bp; or a 307 bp fragment in case the entire gene did not amplify) and partial fragments of two nuclear genes (RAG1, 1012 bp; c-mos, 581 bp) using previously published protocols and primers (Mayer & Pavlicev, 2007; Červenka et al., 2008; Pavlicev & Mayer, 2009; Engleder et al., 2013). We sequenced cyt *b* for all individuals, RAG1 for a subset of taxa that were collected more than 200 km apart or whose cyt *b* sequences were > 2% divergent, and c-mos for a few members of each species complex (Appendix S1). Sequences were aligned using ClustalW (Thompson et al., 1994) and translated to amino acids and checked for stop codons in MEGA 5.2 (Tamura et al., 2011).

Species delimitation

MtDNA sequence data indicated high cryptic diversity, and we needed to delimit species both to understand the true diversity within this group and to select a cut-off for diversification analyses (a large number of conspecific terminal lineages could bias divergence dating and diversification analyses). Recent methods allow automated detection of candidate species based on within-species and between-species branching patterns on a single-locus phylogenetic tree. The cyt *b* alignment for this analysis included only *Ophisops*, with one *Acanthodactylus* sequence as the outgroup. We used a RaxML tree partitioned by codon position with the GTR+G substitution model, and the bPTP model (Zhang et al., 2013) as implemented on the web server (http://species.h-its.org/ptp/), dropping the outgroup, running the final analysis for 500,000 generations with 25% burn-in and thinning set to 100.

Phylogeny & divergence dating

PARTITIONFINDER 1.2 (Lanfear et al., 2012) was used to pick the best models of sequence evolution and partitions using the greedy algorithm with linked branch lengths and Bayesian information criteria for the concatenated dataset and individual gene alignments (Appendix S1). The maximum likelihood (ML) phylogeny was reconstructed with the concatenated dataset using RAxML HPC 8.1.2 (Stamatakis, 2014) implemented in RAXMLGUI 1.5 (Silvestro & Michalak, 2012) with the GTR + G model (as recommended by Stamatakis, 2016), and 10 independent ML runs with support assessed through 1000 rapid bootstraps. The Amphisbaenidae were used to root the tree.

Divergence times were estimated on the concatenated dataset with a Bayesian relaxed clock model implemented in BEAST 1.8.2 (Drummond et al., 2012). The dataset for divergence dating included one sequence for each candidate *Ophisops* species and a few geographically widespread lineages,
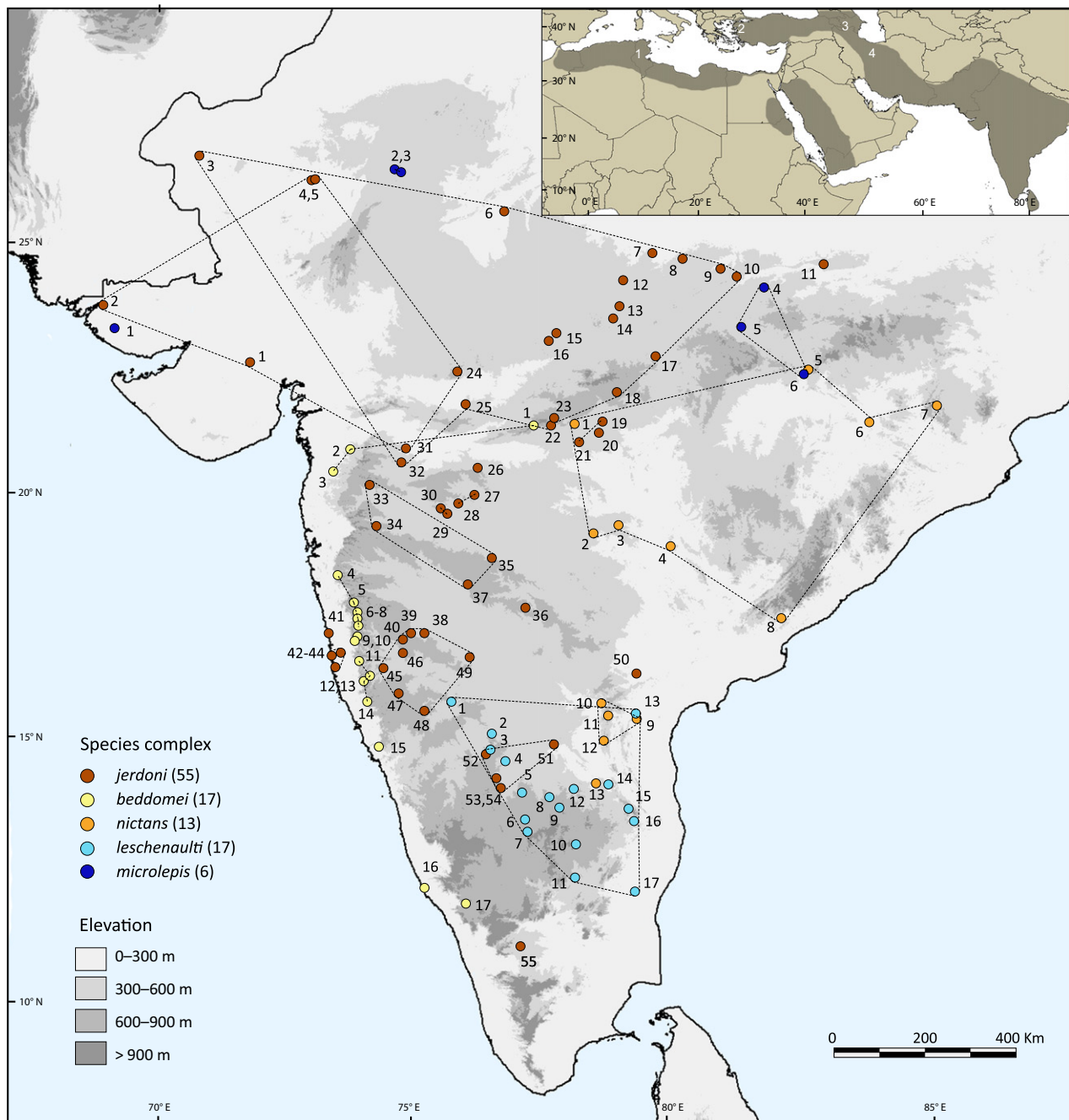
Figure 2 Relief map of the Indian subcontinent showing *Ophisops* sample localities, numbered for each species complex (total localities in parentheses). Known distributional ranges of putative species recorded from more than one locality indicated by dotted lines (forming minimum convex polygons or connecting 2–3 localities linearly). Inset map shows global range of *Ophisops* (shaded) (modified from http://lacerta.de/), numbers indicate Saharo-Arabian *Ophisops* sample localities. Localities referenced in Appendix S1.

and representative genera from the Lacertinae, Eremiadinae and Gallotinae, with Amphisbaenidae as the outgroup (Appendix S1). Though a number of Oligocene-Pleistocene fossils have been assigned to the Lacertidae and even extant genera/species groups (e.g. Hipsley *et al.*, 2009, 2014; http://fossilworks.org/), we only used fossils that have been included in phylogenies. We used exponential priors (mean = 2) for three fossil calibrations:

1. Crown Amphisbaenidae (offset 66 Ma), based on the oldest amphisbaenid fossil, the Palaeocene *Chthonophis subterraneus*, which is nested within the extant amphisbaenians (Longrich *et al.*, 2015);

2. Crown Lacertidae (offset 47.8), *Cryptolacerta*, originally described as a stem amphisbaenian (Müller *et al.*, 2011), the Lutetian *Cryptolacerta*, clusters with the Lacertidae in a recent comprehensive analysis (Longrich *et al.*, 2015);

3. Crown Gallotinae (offset 24.5), based on *Dracaenosaurus* which clusters with the Gallotinae (Müller *et al.*, 2011) and ranges as far back as MP28 (Mertz *et al.*, 2007; Augé & Hervet, 2009).

We used unlinked lognormal relaxed clock models across partitions, a Yule speciation tree prior, and an uninformative prior for all partition rates (ucld.mean: $10^{\wedge-6}$–1). We ran two independent analyses for 100,000,000 generations, sampling every 10,000 generations, with convergence determined based on examination of trace files in Tracer 1.6 (Rambaut *et al.*, 2014). The Maximum Clade Credibility (MCC) tree for each analysis was summarized using median heights in TreeAnnotator 1.8.2 (Rambaut & Drummond, 2015) with a burn-in of 20% based on Tracer outputs. Trees were viewed and manipulated in FigTree 1.4.1 (Rambaut, 2014).

Diversification analyses

We used 'ape' (Paradis *et al.*, 2004), 'laser' (Rabosky, 2006) and 'TreePar' (Stadler, 2011) in R 3.1.2 (R-Development Core Team 2015) to reconstruct the tempo of diversification within *Ophisops*. We pruned chronograms from BEAST analyses in Mesquite 3.0.1 (Maddison and Maddison 2016) to include only *Ophisops*, with additional trees representing clade-specific comparisons. We used the MCC tree (for *Ophisops* and clade-specific comparisons) and a subset of 80 post burn-in trees (Indian *Ophisops*) from BEAST for 'laser' analyses and compared a single rate birth-death model to models with up to four rate shifts by comparing ΔAIC. We used the 'bd.shifts.optim' command in 'TreePar', which can detect rate shifts in an incompletely sampled phylogeny (Stadler, 2011), on the MCC trees (Indian *Ophisops*, separate clades) with a grid setting of 0.5 million years (Myr), testing up to three potential rate shifts (based on a visual inspection of lineage through time plots, LTT). The start time was set at 0, end time as the estimated crown age, and the fraction of sampled species was varied as it is unclear how much of Indian *Ophisops* diversity our sampling captured. We used a threshold of ΔAIC > 2 to choose between models.

Ancestral state reconstructions

Ancestral areas were treated as discrete characters and reconstructed using maximum likelihood with the mk1 model in Mesquite 3.0.1 (Maddison and Maddison 2010). We designated up to four ancestral areas (modified from Holt *et al.*, 2013): India, the Saharo-Arabian Realm, Africa and the Palaearctic. We used different subsets of taxa for analysis – the Eremiadinae; the clade including *Acanthodactylus*, *Eremias*, *Mesalina* and *Ophisops*; and finally within *Ophisops*. For *Ophisops*, we additionally reconstructed ancestral states, coding species as tropical or temperate. Analyses used MCC trees from BEAST analyses with a decision threshold of 2. Coded states for ancestral reconstructions are listed in Appendix S1.

RESULTS

Ophisops phylogeny and diversity

ML analyses using the concatenated dataset (Fig. 3; discussed in the text) were similar to individual gene analyses and BEAST outputs (Appendix S2), apart from poorly supported nodes, and recovered the same broad clades, though only cyt *b* separates all candidate lineages. The monophyly of *Ophisops* is strongly supported, with a basal split into a small-bodied clade (SBC, SVL< 45 mm) and a large-bodied clade (LBC, SVL > 50 mm). The LBC includes the species groups *Ophisops microlepis* Blanford, *O. leschenaultii* (Milne-Edwards) and the Saharo-Arabian species *O. elegans* Menetries and *O. occidentalis* (Boulenger). The SBC includes three species complexes; *O. beddomei* (Jerdon) sister to *O. jerdonii*, the two sister to *O. nictans* Arnold. *Ophisops leschenaultii* and *O. nictans*, formerly assigned to *Cabrita* Gray 1838, are not monophyletic. The two Saharo-Arabian *Ophisops* are well-supported sisters, nested within the Indian species in the LBC, though sister relationships vary between nuclear and mitochondrial data, neither with high support (Appendix S2). The LBC has a north-south split within the Indian species, whilest the SBC has a basal east-west split, with the subsequent divergence of the Western Ghats species group.

Species delimitation based on bPTP suggested between 26 and 47 candidate species (Appendix S3). Based on a qualitative assessment of the results, we chose to consider only candidate species that had posterior delimitation probabilities > 0.5, resulting in 30 candidate Indian *Ophisops* species. The LBC includes four candidate species, with the lowest uncorrected mtDNA distance between the species is 5.9%, whilst the SBC includes 26 candidate species with the minimum divergence between species is 3.3%. The *Ophisops leschenaultii* clade has uncorrected mtDNA distances up to 4%, exceeding distances between candidate species in the SBC, though the branching patterns are not geographically or phylogenetically concordant (Fig. 3), and species delimitation suggested a single widely distributed species. Species delimitation using all published cyt *b* sequences of Saharo-Arabian *Ophisops* suggested as many as 18 species, though the short length of these mtDNA sequences precluded inclusion in our larger phylogeny (http://www.ncbi.nlm.nih.gov/nuccore/).

Divergence dating

Each independent BEAST run had converged (ESS > 200), with almost identical divergence estimates. Estimates of divergence times within the Lacertidae overlapped broadly with published dates (Hipsley *et al.*, 2009, 2014; Appendix S2 Fig. S2.5). We report median ages (95% HPD) rounded off to the closest Myr in the text. Crown Lacertidae began diversifying 49 Ma (53–48) and Eremiadinae 39 Ma (43–35). The basal divergence within *Ophisops* was 30 Ma (34–26); with the basal split within the LBC, between the Saharo-Arabian clade and Indian species, and within the SBC, all within a few
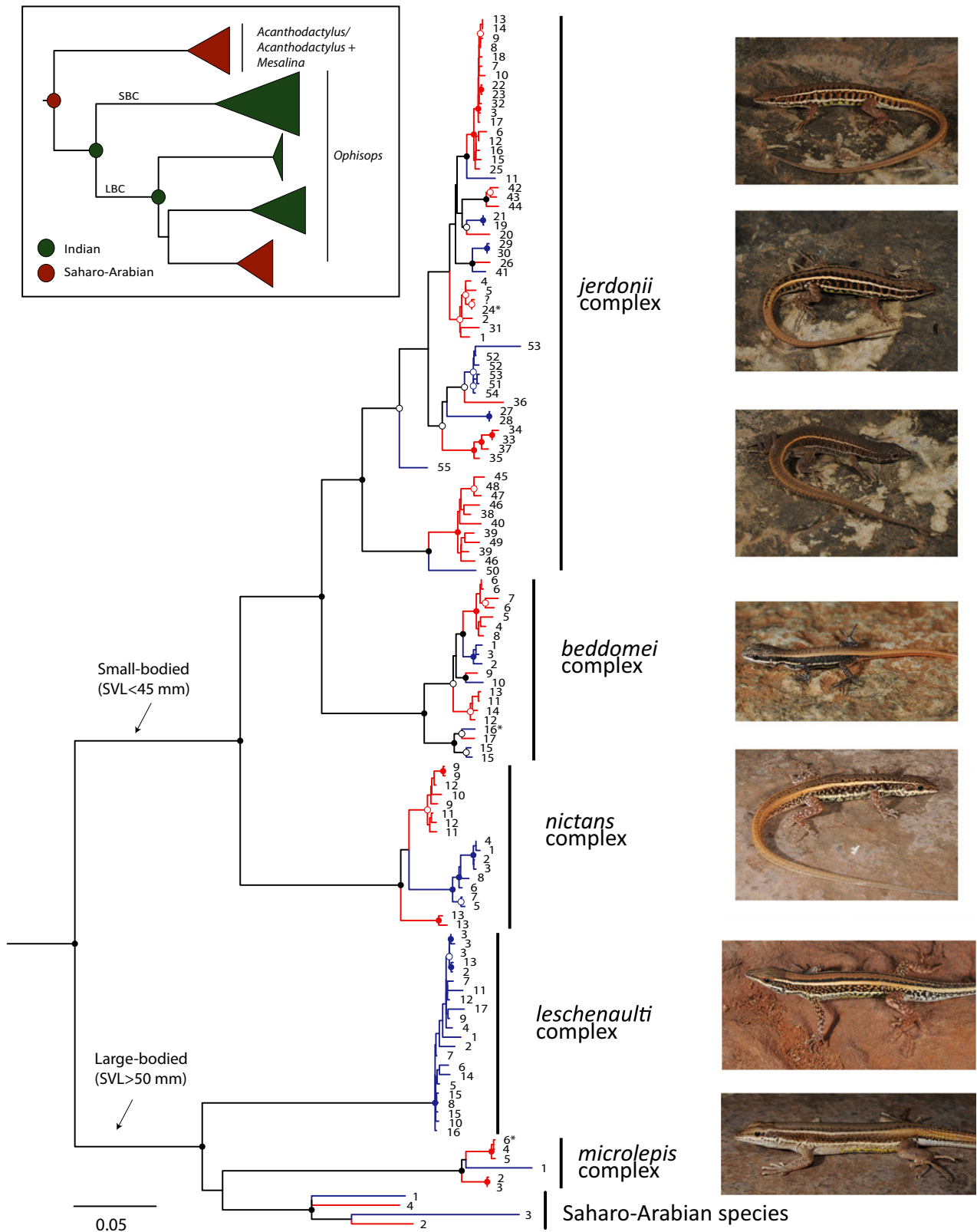
Figure 3 Maximum likelihood phylogeny (concatenated data, 2736 bp) of *Ophisops* with representative photographs of each species group. Candidate species identified by bPTP depicted by alternating red and blue, circles at nodes indicate bootstrap support > 70% (coloured fill > 90%, white fill < 90%). Sample localities referenced in Appendix S1, Fig. 2. Photographs approximately scaled by snout-vent length. Inset shows ancestral area reconstructions for *Ophisops* (SBC, small-bodied clade; LBC, large-bodied clade) and allied genera.

Myr (median 21–19, 95% HPD 25–15 Ma; Appendix S2). The most recent common ancestor (mrca) of *Ophisops beddomei* + *Ophisops jerdonii* diverged 14 Ma (17–12), with the basal split within *O. jerdonii* 11 Ma (13–9). All subsequent cladogenesis within Indian *Ophisops* is from 6 Ma (9–5) onward, except in *O. leschenaultii* (1, 2–1 Ma).

Diversification analyses

A visual examination of LTT plots suggested shifts in diversification rate within *Ophisops* (Fig. 4). Laser analyses picked a 3-rate model as the best model, with an increased rate from ~6.9 Ma followed by a decrease of ~1.7 Ma. The same model was picked on the sample of 80 post burn-in trees (74/80, 3-rate model with minimum AIC). Speciation rates were 3–4 X higher between the two rate shifts (rate 1, r1 0.069, r2 0.278, r3 0.092; Appendix S3). Separate analyses of the LBC and SBC MCC trees picked a single rate model and a 3-rate model, respectively (with rate shifts for the latter at 6.5 and 1.7 Ma, r1 = r2/8 = r2/4). TreePar analyses on the MCC trees detected a significant rate shift 1 Ma for *Ophisops*, two shifts at 1 and 6.5 Ma for the SBC (but a single shift at 1 Ma was also supported, ΔAIC 0.8), and a birth-death model for the LBC (but a single shift at 1 Ma was also supported, ΔAIC 0.9). Varying the sampling fraction did not change detected shift times. The split in the SBC at 6.5 Ma corresponding to the beginning of the rate shift has a 95% HPD of 9–5 Ma.

Ancestral state reconstructions

The Eremiadinae was reconstructed to have an African origin (proportional likelihood 0.91), whilst reconstructions within the clade containing *Acanthodactylus*, *Eremias*, *Mesalina* and *Ophisops* suggested a Saharo-Arabian origin for the mrca of *Ophisops* and its sister clade (0.91, 0.71; for alternate ML or BEAST topologies; Fig 2). *Ophisops* has an Indian origin (0.99), as do the SBC (1.00) and LBC (0.99), and Saharo-Arabian *Ophisops* have a Saharo-Arabian ancestor (0.96). The SBC has a tropical origin (0.94), with two transitions to temperate latitudes, as does the ancestor of *O. leschenaultii*, whilst the ancestor of *Ophisops* and the LBC was equivocally reconstructed, and only the mrca of *O. microlepis* and the Saharo-Arabian species within the LBC were reconstructed as temperate in origin (1.00, 0.95 respectively).

DISCUSSION

An ancient history for Indian grassy biomes

This is the first work on diversification in grassland/open-habitat restricted taxa across India, and reveals a history spanning millions of years and cryptic diversity associated with this biome. With Oligocene origins and rapid Miocene-Pliocene cladogenesis, the timing and tempo of diversification in *Ophisops* mirrors what we know of the emergence of global and Indian C4 grasslands. Assuming that ancestral *Ophisops* were restricted to grasslands and open habitats, as extant *Ophisops* are (Fig. 1), our data suggest that at least some Indian grasslands and open habitats are native, old-growth ecosystems (e.g. Sankaran, 2009). Further studies that integrate palaeoclimatic records and phylogenies across a range of grassland taxa are critical to better understand the history of Indian arid biomes and their associated biodiversity.

We surveyed many localities where we expected lacertids based on the habitat structure and time of sampling, but did not find any lacertids, and it is unclear if these are derived or natural habitats. Fine scale sampling is required to understand the proportion of native and derived grassy biomes in India, of pressing concern as increasing amounts of land are diverted to large-scale developmental activities. Native, old-growth grassy biomes must be recognized as unique, biodiversity rich habitats that require urgent conservation attention.
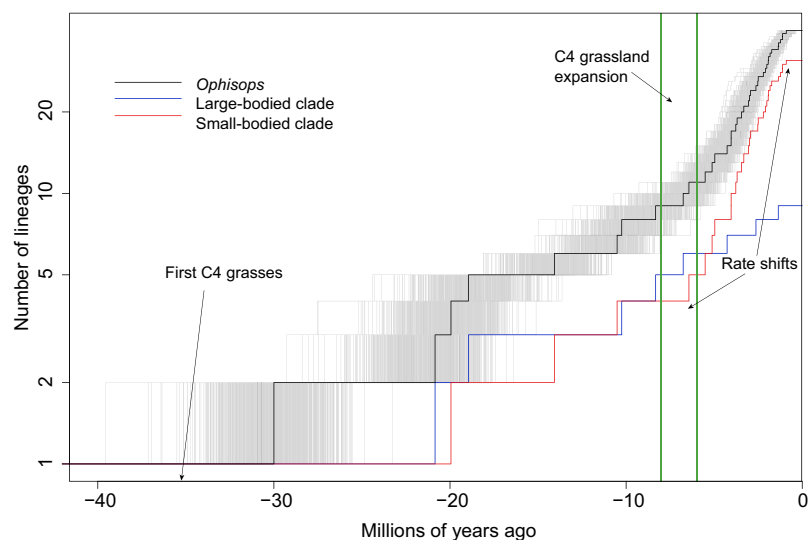

Figure 4 Lineages-through-time (LTT) plot for *Ophisops*, the small-bodied, and large-bodied clades from the maximum clade credibility tree of concatenated data (cyt *b*, c-mos, RAG1; 2.7 kb). Faded lines represent LTT plots from 80 post burn-in trees for *Ophisops*. A timeline for C4 grassland evolution is indicated (Strömberg, 2011).

Cryptic diversity

Based on our limited sampling, we estimate ~30 species of Indian *Ophisops* where five species were known, with the *Ophisops jerdonii* complex alone including 16 candidate species. *Ophisops* is a dramatic example of overlooked cryptic diversity outside forests, at least in part a casualty of forest-centric biodiversity research (Ratnam *et al.*, 2016). Why has this diversity gone hitherto undetected? Phylogenetically, such cryptic diversity is not unexpected, given the deep cryptic divergence within Saharo-Arabian *Ophisops* (Kyriazi *et al.*, 2008) and allied lacertid genera (Rastegar-Pouyani *et al.*, 2010; Smid & Frynta, 2012; Kapli *et al.*, 2014; Tamar *et al.*, 2014), and the deep history of the Indian dry zone (e.g. Agarwal & Karanth, 2015; Deepak *et al.*, 2016). However, candidate species within each species group are superficially similar in morphology, and though they have existed in museum collections for over a century (e.g. Blanford, 1870), no molecular or substantial morphological analyses have been carried out. Our data indicate a major taxonomic revision is clearly needed. The small-bodied clade of *Ophisops* adds to the growing list of endemic radiations in India (Karanth, 2015) and the Pleistocene rate shift is likely to represent unsampled diversity or possibly recent extinctions – thorough sampling across India is needed to understand the true diversity of this group.

A novel biogeographic pattern

The unique geological and climatic histories of the Indian Plate are reflected in its biota, with few relict taxa that predate Deccan Trap volcanism and the K/T mass extinction, most groups having subsequently moved into India (Datta-Roy & Karanth, 2009). *Ophisops* exhibits a novel biogeographic pattern, into India dispersal from Saharo-Arabia followed by a back dispersal out-of-India into Saharo-Arabia. Whilst several groups have moved out-of-India into forested Southeast Asia, these all have wet zone/forest origins (Datta-Roy & Karanth, 2009). It remains to be seen if westward dispersal is a unique pattern, or merely representative of the research bias towards forest and wet zone taxa. A focus on speciose, forest dwelling groups in the Indian subcontinent will be biased toward reconstructing origins from wet, forest regions such as Southeast Asia.

The timing of *Ophisops* dispersal into and out-of-India corroborates the antiquity of dry zone biota, and by extension, of the dry zone itself (Agarwal *et al.*, 2014); suggesting that at least some parts of India were dry and open by the Oligocene-Miocene, allowing connectivity with more arid western regions.

Climatic shifts and diversification of *Ophisops*

Ophisops are the only lacertids and only terrestrial lizards besides *Sitana* spp. found widely across arid and semi-arid open habitats of India. The ancestor of *Ophisops* moved into India in the Oligocene and diverged into the LBC and SBC, soon after the evolution of C4 grasses and early indications of seasonality, suggesting at least some areas of India had open/grassland habitats by then. Speciation rates are similar in the two body-size clades for ~ 20 Myr followed by a circa 8-fold increase in speciation rate within the SBC. The timing of the rate shift (~9–5 Ma) is coincident with both C4 grassland expansion (8–4 Ma) and intensified late Miocene aridification in the region (~11–5 Ma). This was after the divergence into different body-size clades and broad species groups, suggesting that these traits did not directly contribute to the rate shift seen in the SBC. The strong mismatch in diversification between the two body-size clades may be partly influenced by the microhabitat preference. Members of the LBC are relatively broadly distributed, *Ophisops leschenaultii* occupies a range of open habitats and members of the *O. microlepis* group are distributed in sandy or rocky grasslands. Members of the SBC, however, are patchy in distribution and have strong microhabitat associations. The *Ophisops beddomei* group is restricted to open lateritic plateaus above 800 m in the northern Western Ghats and at least the western Satpudas, besides coastal Kerala; *O. jerdonii* is found in open, rocky habitats across India excluding eastern India and elevations above 1500 m and *O. nictans* in central, southern and eastern India, associated with open, rocky areas within forests and grasslands (Fig. 1).

Climate appears important in structuring *Ophisops* diversity. With a temperate origin, the ancestor of *Ophisops* may have been tropical or temperate adapted, though poor support within the LBC precludes character reconstructions. The large-bodied and small-bodied clades overlap broadly in distribution, though we have not seen more than one species of *Ophisops* at any locality, whilst each of the candidate species within each complex *Ophisops* are allopatric, and only the widely distributed northern species within the *Ophisops jerdonii* complex have overlapping ranges (Fig. 2).

The time of divergence between *Ophisops jerdonii* and *O. beddomei* overlaps with a strong cooling phase (95% HPD spans the MMCO and subsequent cooling) and may reflect adaptation to cool temperatures in *O. beddomei*, which has translated into the current largely high-elevation, coastal distribution of this species complex. The presence of *Ophisops beddomei* outside the Western Ghats at high elevations in the Satpudas might be explained by recent climatic fluctuations, with dispersal during a cooling phase and subsequent restriction to higher elevations. The Satpuda individual is closely related to the most proximate sampled localities, in northern Gujarat (Figs 2 & 3).

Ophisops jerdonii does spill into temperate latitudes in north India, Pakistan and Afghanistan (Uetz, 2016), our data suggesting a relatively recent history outside the tropics, though we expect the subtropical Siwaliks and lower Himalayas may have endemic lineages. Individuals of *Ophisops jerdonii* from 26 of 30 localities north of 20.8° N belong to two widely distributed species with overlapping ranges, with an additional three species from the other four localities.

Much of this area is north of the Tropic of Cancer and the Indian peninsula, with a more seasonally variable climate than areas closer to the coast or further south. This is consistent with the general relationship between climatic niche breadth and latitude, and climatic niche breadth and within-locality seasonal variation (Janzen, 1967; Quintero & Wiens, 2013).

CONCLUSIONS

Our extensive geographical sampling of *Ophisops* from across their range in India reveals a history spanning millions of years with hugely underestimated diversity in this open-habitat restricted group. The timing of *Ophisops* entry into India closely follows the emergence of C4 grasses, with diversification in the SBC of *Ophisops* increasing 8-fold during global C4 grassland expansion. *Ophisops* is a striking example of overlooked cryptic diversity outside forests with ~30 species where five were known. It is likely that many more Indian grassland-restricted taxa have similar undervalued diversity. *Ophisops* also presents a novel biogeographical pattern, dispersal into India from the Saharo-Arabian Realm in the Oligocene, with a back dispersal in the Middle Miocene. Indian TGBs and other open habitats remain poorly understood and undervalued, and many are at equal or greater conservation risk than forests (Putz & Redford, 2010; Parr *et al.*, 2014; Veldman *et al.*, 2015a,b).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary materials and methods (including GenBank accession numbers).
Appendix S2 Supplementary figures: individual gene trees and timetree.
Appendix S3 Species delimitation output and diversification model summary.

BIOSKETCHE

Ishan Agarwal is an evolutionary biologist with a focus on biogeography of the Indian subcontinent and Old World squamates.

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Author contributions: IA and UR conceived the study; IA collected and analysed the data, led the writing; both authors contributed to revisions and interpretation.

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