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Insights into Himalayan biogeography from geckos: A molecular phylogeny of *Cyrtodactylus* (Squamata: Gekkonidae)

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ABSTRACT

The India–Asia collision profoundly influenced the climate, topography and biodiversity of Asia, causing the formation of the biodiverse Himalayas. The species-rich gekkonid genus *Cyrtodactylus* is an ideal clade for exploring the biological impacts of the India–Asia collision, as previous phylogenetic hypotheses suggest basal divergences occurred within the Himalayas and Indo-Burma during the Eocene. To this end, we sampled for *Cyrtodactylus* across Indian areas of the Himalayas and Indo-Burma Hotspots and used three genes to reconstruct relationships and estimate divergence times. Basal divergences in *Cyrtodactylus*, *Hemidactylus* and the Palaearctic naked-toed geckos were simultaneous with or just preceded the start of the India–Asia collision. Diversification within *Cyrtodactylus* tracks the India–Asia collision and subsequent geological events. A number of geographically concordant clades are resolved within Indo-Burmese *Cyrtodactylus*. Our study reveals 17 divergent lineages that may represent undescribed species, underscoring the previously undocumented diversity of the region. The importance of rocky habitats for *Cyrtodactylus* indicates the Indo-Gangetic flood plains and the Garo-Rajmahal Gap are likely to have been important historical barriers for this group.

1. Introduction

The most visible evidence of the India–Asia collision are the Himalayas, bounding the Indian subcontinent with the highest mountains in the world (Fig. 1). This colossal geological event profoundly affected Asian biodiversity by bringing together the relatively insular biota of the Indian Plate and mainland Asia (Conti et al., 2002; Gower et al., 2002; Datta-Roy and Karanth, 2009), with subsequent Himalayan uplift causing regional climate change (Patnaik et al., 2012) and creating mountain barriers to dispersal. Though the exact configuration and timing of the India–Asia collision is an active area of research, evidence indicates India and Asia began colliding about 50 million years ago (mya), preceded by the collision of Tethyan island arcs with India and/or Asia, and final India–Asia suturing was between 45 and 35 mya (Rowley, 1996; Ali and Aitchison, 2008; Hall, 2012; Bouilhol et al., 2013; Metcalfe, 2013; but see Van Hinsbergen et al., 2012 for an opposing model), culminating in the uplift of the Himalayas by ~23 mya (Clift et al., 2008). While the geology of the India–Asia collision is relatively well studied, its biological impacts are still being revealed (Che et al., 2010; Li et al., 2013). Large-scale faunal exchange between the drifting Indian plate and Southeast Asia initiated in the Eocene (Klaus et al., 2010; Li et al., 2013), and the appearance of Holarctic mammal fossils in northwest India suggests some connectivity to mainland Asia as early as 54 mya (Clementz et al., 2011). Molecular evidence supports Eocene–Oligocene biotic links between Peninsular India and mainland Asia, with dispersal both out of India (Gower et al., 2002; Datta-Roy and Karanth, 2009) and into India (Van Bocxlaer et al., 2009; Bansal and Karanth, 2013; Li et al., 2013). Dispersal of at least some groups ceased in the Middle to Late Miocene, coincident with the final uplift of the Himalayas and increasing seasonality (Li et al., 2013). However, vagile taxa such as mammals and birds have moved into the Himalayas in the last 10 million years (my), with the influence of recent glaciation and climate change implicated in diversification (Reddy, 2008; den Tex and Leonard, 2013; Srinivasan et al., 2013; Yuan et al., 2013).

The contemporary Himalayan region is climatically and topographically heterogeneous, the rugged mountains finely dissected by river valleys, with local environmental variation linked to altitude, slope, and aspect; and broad east–west gradients in rainfall, temperature and seasonality. Highly biodiverse, this area includes the Himalaya Hotspot and part of the Indo-Burma Hotspot (Mittermeier et al., 2005). A combination of location and geological history make the Himalayas biogeographically unique, the fauna a

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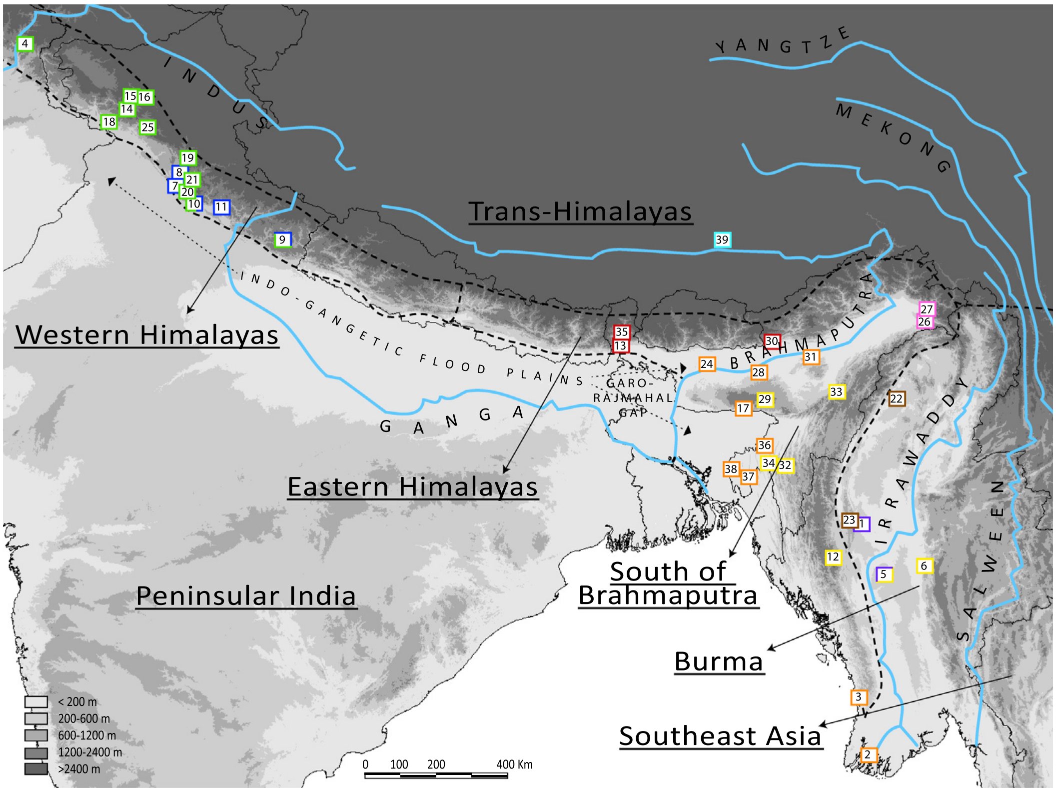

Fig. 1. Map of the circum-Himalayan region showing sampling locations of Himalayan and Indo-Burmese *Cyrtodactylus*. Numbered localities are referenced in Table 1; multiple samples of a species are numbered serially; colors correspond to clades marked in Fig. 2: Blue = Clade F, brown = Clade E, cyan = Clade A, green = Clade B, orange = Clade N, pink = Clade L, purple = Clade G, red = Clade H, yellow = Clade M. Major rivers are marked by a bold line and capitalized text, areas used in ancestral area reconstruction delineated by dotted lines and river courses, labelled with underlined text (refer to methods for definitions). The Indo-Gangetic Flood Plains (dotted arrows indicate the extent) lie south of the Himalayas and the Garo-Rajmahal Gap separates the Shillong Plateau and Rajmahal Hills. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mix of peninsular Indian, Oriental and Palaearctic elements (Mani, 1974; Das, 1996). The recent discoveries of numerous morphologically distinct vertebrates from the Himalayas and northeast India (Bauer, 2002, 2003; Sinha et al., 2005; Athreya, 2006; Das et al., 2010; Sondhi and Ohler, 2011; Kamei et al., 2012; Datta-Roy et al., 2013) are indicative of gross underestimation of biodiversity. Well sampled phylogenies are needed to reveal patterns of diversity and assembly in the Himalayas, and to further understanding of the processes that shaped this biodiversity, with the potential utility to predict impacts of future change (Richardson and Whittaker, 2010; Sinervo et al., 2010).

Cyrtodactylus is the most speciose gekkonid genus globally, with over 175 described species, distributed from the Western Himalayas through Southeast Asia to the western Pacific (Wood et al., 2012; Uetz, 2014). With a probable Laurasian origin in the Palaeocene, global phylogenies of *Cyrtodactylus* reveal a west to east pattern of diversification, and basal divergences in the Eocene separate clades in the Trans-Himalayas, Western Himalayas, Indo-Burma and Southeast Asia (Wood et al., 2012; Bauer et al., 2013). The onset of the India–Asia collision is coincident with early diversification within this group (Wood et al., 2012; Bauer et al., 2013), though it is unclear how this geological event has impacted diversification within circum-Himalayan *Cyrtodactylus* from the middle Eocene to present, as few Himalayan species have been sampled. *Cyrtodactylus* is the most speciose lizard genus in the Himalayan and Indo-Burma region, with 17 species known from relatively well surveyed Burma, three from Nepal, and two from Pakistan (Bauer, 2002, 2003; Schleich and Kästle, 2002; Zug et al., 2003; Khan, 2006; Venugopal, 2010; Wood et al., 2012; Bauer et al., 2013; Masroor pers. comm.). A probable artefact of inadequate sampling, just five species are known from India — the catchall species *C. khasiensis* is reported from across northeast India, *C. gubernatoris* and *C. himalayanus* are known only from their type descriptions from the Eastern and Western Himalayas respectively, and *C. fasciolatus* and *C. lawderanus* are known from the Western Himalayas (Sharma, 2002). The distribution of *Cyrtodactylus* species and the timing of diversification, with basal divergences seemingly correlated with the India–Asia collision, make the genus an ideal candidate to explore the faunal impacts of the collision. To this end, we sampled for *Cyrtodactylus* across Indian regions of the Indo-Burma and Himalayan hotspots (Fig. 1) and used molecular data to build phylogenies and estimate divergence times.

2. Materials and methods

2.1. Taxon sampling, DNA data, and sequence alignment

We generated sequence data for 41 individuals of *Cyrtodactylus* from 32 localities across the Himalayas, northeast India and the Andaman and Nicobar Islands (Table 1 and Fig. 1). We targeted type localities and other likely habitats and spotted geckos at night by eyeshine on fieldtrips from 2009 to 2011. Tissue samples were collected in the field from subsequently vouchered specimens and preserved in 95–100% ethanol, and a few tissue samples were contributed by other researchers (see acknowledgements). Collections from protected areas were made with forest department permits. Genomic DNA was extracted using Qiagen DNeasy™ tissue kits.

The final sequence alignment contained 2477 aligned base pairs (bp) of data including one mitochondrial gene, NADH