DNA barcoding reveals distinct population of *Dopasia gracilis* (Squamata: Anguidae) in Mizoram, Northeast India

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**ABSTRACT**

The DNA barcode data of Asian Glass Lizard, *Dopasia gracilis*, is limited in the global database, especially from India. The present study aimed to generate a barcode sequence of morphologically identified *D. gracilis* from the Mizoram state in northeast India and compared with other Anguidae species. The studied species showed monophyletic clustering in the Bayesian analysis (BA) phylogeny with strong posterior probability support and also discriminated sufficient Kimura 2 parameter genetic distances. The barcode data of *D. gracilis* revealed high intra-species genetic variability and formed two clusters in BA phylogeny. The Templeton, Crandall, and Sing network also depicted four different haplotypes within the barcode sequences of *D. gracilis*. The DNA sequences generated from northeast India showed 6.5–6.6% and 7.3% genetic distances with the sequences generated from Yunnan Province and Tibetan Plateau, respectively. Considering the high genetic distances, multiple clustering, and distinct haplotypes, the present study assumed the presence of possible cryptic diversity of *D. gracilis* in the Indochina sub-region and a distinct population in northeast India. We recommended the generation of more DNA information from different localities to elucidate the actual diversity of *D. gracilis* within the known range distribution.

**Introduction**

The members of family Anguidae are carnivorous reptiles having a unique conserved body plan and either ground-dwelling or arboreal habits in nature (Lin et al. 2003). The Anguidae comprises 102 species within 15 genera and has widespread distribution in North, Central, and South America, West Indies, Europe, Asia, and North Africa (McConkey 1954; Pough et al. 1998). Among them, the subfamily Anguinae consists of about 20 described species (six are distributed in Europe, one in North Africa, five in Asia, two in Indonesia, and six in North America) (Lavin and Girman 2019). Due to the complex taxonomic characters and lack of keys, the classification of Anguinae species has been repeatedly revised (Gvozdik et al. 2010; Nguyen et al. 2011). For example, the genus *Ophisaurus* was believed to include 12 species, widely distributed in North to Central America, East and Southeast Asia, and North Africa. Later on, based on a taxonomic revision and subsequent conformity, the generic name of East and Southeast Asian *Ophisaurus* species was erected to the genus *Dopasia* (Brygooé 1987; Macey et al. 1999; Conrad 2008; Nguyen et al. 2009; Conrad et al. 2011). Currently, the genus *Dopasia* consists of seven species; five are distributed in the Indochina sub-region, one is distributed in Malaysia (Sabah, Sarawak), and Indonesia (Borneo), and one is endemic to Indonesia (Sumatra) (Nguyen et al. 2011; Uetz et al. 2020).

The limbless Asian Glass Lizard or Burmese Glass Lizard, *Dopasia gracilis* Gray, 1845 is originally described from the Khasi Hills in the state of Meghalaya, northeast India. This species can be distinguished based on the number of vertebrae from atlas to remnants of hind leg bones (Brygooé 1987; Nguyen et al. 2011). *Dopasia gracilis* is widely distributed in India, China, Myanmar, Laos, Thailand, and Vietnam (Nguyen et al. 2009, 2011). Because of the deforestation produced by agriculture and plantations and the exploitation of biological resources, this lizard group confronts threats of habitat destruction throughout their known distribution range including northeast India (IUCN 2020). Due to insufficient systematics studies, the conservation status of *D. gracilis* has not yet been assessed by the International Union for the Conservation of Nature (IUCN). Apart from taxonomic studies, few integrated approaches have been induced to understand this charismatic reptile group (Gvozdik et al. 2010, 2013; Pan et al. 2015; Song et al. 2015; Lavin and Girman 2019; Cai et al. 2020). The advancement of molecular tools is not only helpful to identify species, but is valuable information to reconstruct phylogenetic relationship (Wiens and Slingluff 2020).
Materials and methods

The *D. gracilis* specimen was collected from the Mizoram state (23.78N 92.72E) in northeast India (Figure 1(A)) and was morphologically identified based on the original description and previous literature, and with the help of naturalist’s guides (Gray 1845; Lalremsanga et al. 2010; Das and Das 2017). Field work and sampling were performed after obtaining prior permission (No.A.33011/2/99-CWLL/225) from the Chief Wildlife Warden of Environment, Forests and Climate Change, Govt. of Mizoram, India. Tissue sample was collected and preserved in 70% ethanol until downstream analysis. The entire specimen was preserved and vouchered (MZMU1329) in the Department of Zoology, Mizoram University, India. The molecular analyses were executed as per previously published protocols (Kundu et al. 2018). The genomic DNA was stored at the Center for DNA Taxonomy Laboratory, Molecular Systematics Division, Zoological Survey of India, Kolkata. The low-quality reads and gaps within the generated sequence were checked by SeqScanner V1.0 (Applied Biosystems Inc., CA, USA), nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/), and ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). The generated sequence was submitted to the GenBank database to acquire the accession number. The dataset was constructed by using 27 publicly available database sequences of nine Anguidae species. Two database sequences of *Anniella geronimensis* (EU445975) and *Anniella pulchra* (EU445968) under family Anniellidae were used as an out-group. The dataset was aligned by ClustalX (Thompson et al. 1997) and Kimura 2 parameter (K2P) genetic distances were calculated by MEGAX (Kumar et al. 2018). The suitable model for Bayesian analysis (BA) was estimated through MrModeltest v2 with lowest BIC value (Nylander 2004). The BA phylogeny was constructed in Mr. Bayes 3.1.2 by selecting nst = 6 and rates = invgamma for GTR + G + I model. The MCMC (one cold and three hot chains) was run for 1,000,000 generations with 25% burn-in and trees saving at each 100 generation (Ronquist and Huelsenbeck 2003). The phylogeny was further illustrated in web-based iTOL tool (https://itol.embl.de/) (Letunic and Bork 2007). The haplotype network was further constructed to infer the genealogical link within the different population of *D. gracilis*. The numbers of haplotypes, haplotype diversity (Hd), and number of polymorphic sites were calculated using DnaSP v6 (Rozas et al. 2017). The haplotype network was constructed using PopART (http://popart.otago.ac.nz) (Leigh and Bryant 2015) with standard Templeton, Crandall, and Sing (TCS) method (Clement et al. 2000).

Results and discussion

Evolutionary diversity is associated with specific topography, climates, soil, and vegetation that stimulate an unparalleled distribution of higher level taxon around the world (Antão et al. 2020). Although the morphology of an organism from different geographical locations sometimes looks similar due to their static phenotypic characters and selection regime, their genetic constitution might have changed corresponding to their habitat, history, and environmental interaction. To explore this scenario, the interventions of molecular tools are a fruitful tool for taxonomy. Considering the abrupt losses of biodiversity due to climate and habitat changes, connect the earth observation through DNA sequences offering an efficient monitoring of biodiversity, their functions, and services (Bush et al. 2017). Considering the abrupt losses of biodiversity in recent past, the DNA sequences offers an efficient platform for illuminating the extant earth’s biota quickly and reliably (Bush et al. 2017). Since the inception of DNA barcoding, genetic traits are more widely used in evolutionary studies, an enormous nucleotide database with multiple species delimitation methods have proliferated rapidly for answering several biological questions throughout the globe (Nagy et al. 2012; Chambers and Hebert 2016). Noteworthy, the evolutionary relationships between and within species is crucial for biodiversity conservation planning, which has been recently illustrated for amphibian and reptile species occurring in a biodiversity hotspot region (Jablonski et al. 2016; Carvalho et al. 2017). Focusing on the widespread geographical distribution of the Asian Glass Lizard, the intra-species genetic information is still lacking from India. Hence, the present study compared the DNA barcode data of *D. gracilis* from northeast India and other geographical location to elucidate their intra-species genetic variability. The overall mean genetic distance of the Anguidae species was 19.4% in the present dataset. Excluding the singleton species, the mean intra-species genetic distance ranged from 0% (*Anquis cephalonica, Abronia gramineae*, and *Dopasia harti*) to 5.1% (*D. gracilis*). The inter-species genetic distance ranged from 13.5% (*A. cephalonica and Anquis fragilis*) to 30.8% (*A. gramineae and D. harti*). The BA phylogeny showed cohesive clustering of all studied species with high posterior probabilities support (Figure 1(B)). *Dopasia gracilis* showed sister relationship with
D. hartii (named as Ophisaurus hartii in the database). Considering the high mean intra-species genetic distances (5.1%), we primarily depicted three clades (one clade from northeast India, one clade from Tibetan Plateau, China, and one clade from Yunnan Province, China). Further, due to the low posterior probability support (0.6) between two Chinese clades, we recognized two distinct clades (Clade-1 from India and Clade-2 from China) and assumed possible cryptic diversity of D. gracilis in Indochina sub-region. Altogether, the DNA barcode data of D. gracilis revealed four distinct combinations with 0.866 haplotype diversity and 61 polymorphic sites. For better illustration, the TCS haplotype network was also drawn and merged into the collection locality map (Figure 1(C)). The three database sequences of D. gracilis (collected from Mile and Gejiu City of Yunnan Province, China) showed 0.5% genetic distances with each other. However, the sequence generated from Tibetan Plateau, China, showed 5.5–5.8% genetic distance with the sequences generated from Yunnan Province, China. The barcode data generated from northeast India showed 6.5–6.6% and 7.3% genetic distances with the sequences generated from Yunnan Province and Tibetan Plateau, respectively. Based on the estimated high genetic variability, distinct clustering in BA phylogeny, and distinct haplotypes, the genetic information suggested the distinctness of the D. gracilis population in northeast India. The present study encourages more extensive sampling of D. gracilis from different geographical locations and the generation of multiple genetic marker data or high-throughput sequence information to elucidate the actual diversity of this reptile species. The current genetic information not only evidenced the possible cryptic diversity of D. gracilis from the Indochina sub-region, but also invites to begin other biological and ecological studies to facilitate the evolutionary understanding of this species, and the holistic conservation action plans needed to protect them in nature.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Data availability statement**

The data that support the findings of this study are openly available in NCBI GenBank database at (https://www.ncbi.nlm.nih.gov) with the accession number (MT450474) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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