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Molecular identification of mimetic Mock Viper, *Psammodynastes pulverulentus* (Boie, 1827) (Reptilia: Squamata: Lamprophiidae) from Northeast India

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Abstract

The genetic information (mtCytb) of wide-spread Mock Viper, *Psammodynastes pulverulentus* is restricted to China and Myanmar. We collected the live individual of *P. pulverulentus* from Mizoram state in northeast India and generate the partial mtCytb data to affirm the morphology-based species identification. The generated DNA data showed 94.67% similarity with the sequences generated from Myanmar; however, 92.59% to 92.98% similarity with the sequences generated from China through BLAST results. In comparison with other recognized families and subfamilies of alethinophidian and scolecophidians snakes, the studied species depicted discrete clade in the Bayesian Inference (BI) analysis and closely related with the sister species *Psammodynastes pictus*. The haplotype network revealed distinct haplotype of *P. pulverulentus* collected from northeast India with 6.6% and 8.9% to 9.6% Kimura 2 parameter (K2P) genetic distance with the Burmese and Chinese collections respectively. The study elucidates the possible cryptic diversity of *P. pulverulentus* within its wide range distribution, which requires further large-scale attempts with more genetic information to adjudicate the actual diversity.

Keywords: Mimicry, Mitochondrial DNA, Ophidian, Phylogeny, Taxonomy

Introduction

The Mock Viper, *Psammodynastes pulverulentus* was first described under the family Dendrelaphidae from its type locality Java, Indonesia (Boie, 1827). It is a pretty common snake in tropical Asia and widely distributed from peninsular India to Taiwan through Nepal, Bhutan, Bangladesh, Myanmar, China, and Thailand, Malaysia, Laos, Indonesia and up to Philippines (Koch, 2012; Miller & Zug, 2016; Uetz *et al.*, 2020). The species have unique enigmatic characters, dentition patterns with distinct enlarged anterior maxillary teeth, and grooved and pointed posterior fangs (Smith, 1943; Jackson & Fritts, 1996). Although, the species is mildly venomous, their envenomation sign often creates confusion with other venomous snakes. Consequently, the rural peoples are often bewildering to identify this species due to mimetic behavior and overlapping distribution with other venomous viper species. Moreover, the medical persons were sometimes confounded to treat the victims and recommend the effective antivenom. The species is not yet evaluated by the specialist group of International Union for Conservation of Nature (IUCN), but considered as Schedule IV species in Indian Wildlife (Protection) Act, 1972. The habitat destruction and road-kill are the major threat to the species in northeast India.

The identification of *P. pulverulentus* is challenging due to the high amount of phenotypic plasticity exhibited by the species (Miller & Zug, 2016). Nevertheless, the taxonomy and systematics position of *P. pulverulentus* is debatable from long back and even not clear by adopting molecular data (Rasmussen, 1975; Pyron *et al.*, 2011). The species was thought to be closely related with colubroid species group, and subsequently accommodates under

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the subfamily Pseudaspidinae of family Lamprophiidae (Pyron *et al*., 2011; Pyron *et al*., 2013; Li *et al*., 2020). Despite the wide range distribution, the molecular information (mitochondrial Cytochrome b gene) of *P. pulverulentus* is restricted to China and Myanmar (Lawson *et al*., 2005; Li *et al*., 2020). Both mitochondrial and nuclear DNA data are predominately used in ophidian's systematics and resolved several biological questions; such as species identification, diversity estimation, cryptic species detection, and predicts the phylogenetic and evolutionary relationship (Slowinski & Lawson, 2002; Nagy *et al*., 2012; Chambers *et al*., 2016; Figueroa *et al*., 2016; Ratnarathorn *et al*., 2019; Slowinski & Keogh, 2000). Besides the sexual dimorphism, both Batesian and Acoustic mimicry often creates uncertainty in ophidian species identification, which were also resolved through molecular analyses (Pyron & Burbrink, 2009; Aubret & Mangin, 2014; Valkonen & Mappes, 2014; Rabosky *et al*., 2016; Vaughan *et al*., 2019). Notably, the recent studies evidenced the unique genetic diversity of northeast Indian snakes (*Bungarus fasciatus*, *Naja kaouthia*, *Naja naja*, and *Ophiophagus hannah*) through molecular investigation (Kundu *et al*., 2020b; 2000c). Hence, the present study collected the *P. pulverulentus* specimen from northeast India and generated molecular data (mtCytb) for the first time. The present study also acquired the genetic information of all representatives of alethinophidian and scolecophidians snakes from global database and estimate genetic distance and topology to check the efficacy of mtCytb gene to discriminate the targeted species. The study further adopted haplotype network analysis to detect the genetic distinctiveness of Indian population. This preliminary data from Indian zoogeography will help to elucidate the genetic diversity of Mock Viper and encouraged further large-scale effort to illustrate the exact diversity of this species within its range distribution.

Material and methods

The live specimen of *P. pulverulentus* were collected from the Mizoram state (23.73N 92.66E) in northeast India (Figure 1 A, B). The morphological characters were acquired as per standard protocol (Dowling, 1951). The sampling permit was incurred from the Chief Wildlife Warden of Environment, Forests and Climate Change, Govt. of Mizoram, India. The muscle tissue was collected and preserved in 70% ethanol; however, the whole-body specimens were stored in 10% formalin as a voucher (MZMU946) in the Department of Zoology, Mizoram University, India.

The genomic DNA extraction, PCR, and Sanger sequencing were carried out as per previous protocols (Kundu *et al*., 2020a; 2020c). The generated sequence was submitted to the GenBank database to acquire the accession number (MT585642). Further, 55 database sequences of same and related species were downloaded from GenBank representatives of all recognized families and subfamilies of alethinophidian and scolecophidians snakes (Deepak *et al*., 2018). Both generated and database sequences were further aligned together by using ClustalX to build a final dataset (Thompson *et al*., 1997). The Kimura 2 Parameter (K2P) genetic distance was estimated by using MEGAX (Kumar *et al*., 2018). The best model for Bayesian Inference (BI) analysis was computed through Mr. Modeltest v2 with the lowest BIC value (Nylander, 2004). The Bayesian phylogeny was constructed in Mr. Bayes 3.1.2 by choosing nst=6 and rates=invgamma for GTR+G+I model (Ronquist & Huelsenbeck, 2003). The Bayesian phylogeny was further illustrated in the web-based iTOL tool (https://itol.embl. de/) (Letunic & Bork, 2007). The sequences of scolecophidians snakes were used as an out-group in the present analysis. To realize the genealogical links, the haplotype networks were built within the different population of *P. pulverulentus*. The numbers of haplotypes were generated by using DnaSP v6 (Rozas *et al*., 2017). The haplotype diversity (Hd) and the number of polymorphic sites were estimated through DnaSP v6. The haplotype network was constructed by PopART (http://popart.otago.ac.nz) (Leigh & Bryant, 2015) with standard parsimonious network by the Templeton, Crandall and Sing (TCS) method (Clement *et al*., 2000).

Results and discussion

The collected specimen was morphologically identified following the previous literatures and field guides (David & Vogel, 1996; Whitaker & Captain, 2004; Grismer, 2011; Das, 2012; Wallach *et al*., 2014). The hemipenis was examined (Figure 1 B), the organ with a length of ca.5 mm reaches up to the fifth subcaudals, vaguely bilobed, sulcus spermaticus bifurcated just below the crotch, and distal surfaces sparsely covered with spines that diminished in size at the apical region. Due to adjacent zoogeography,

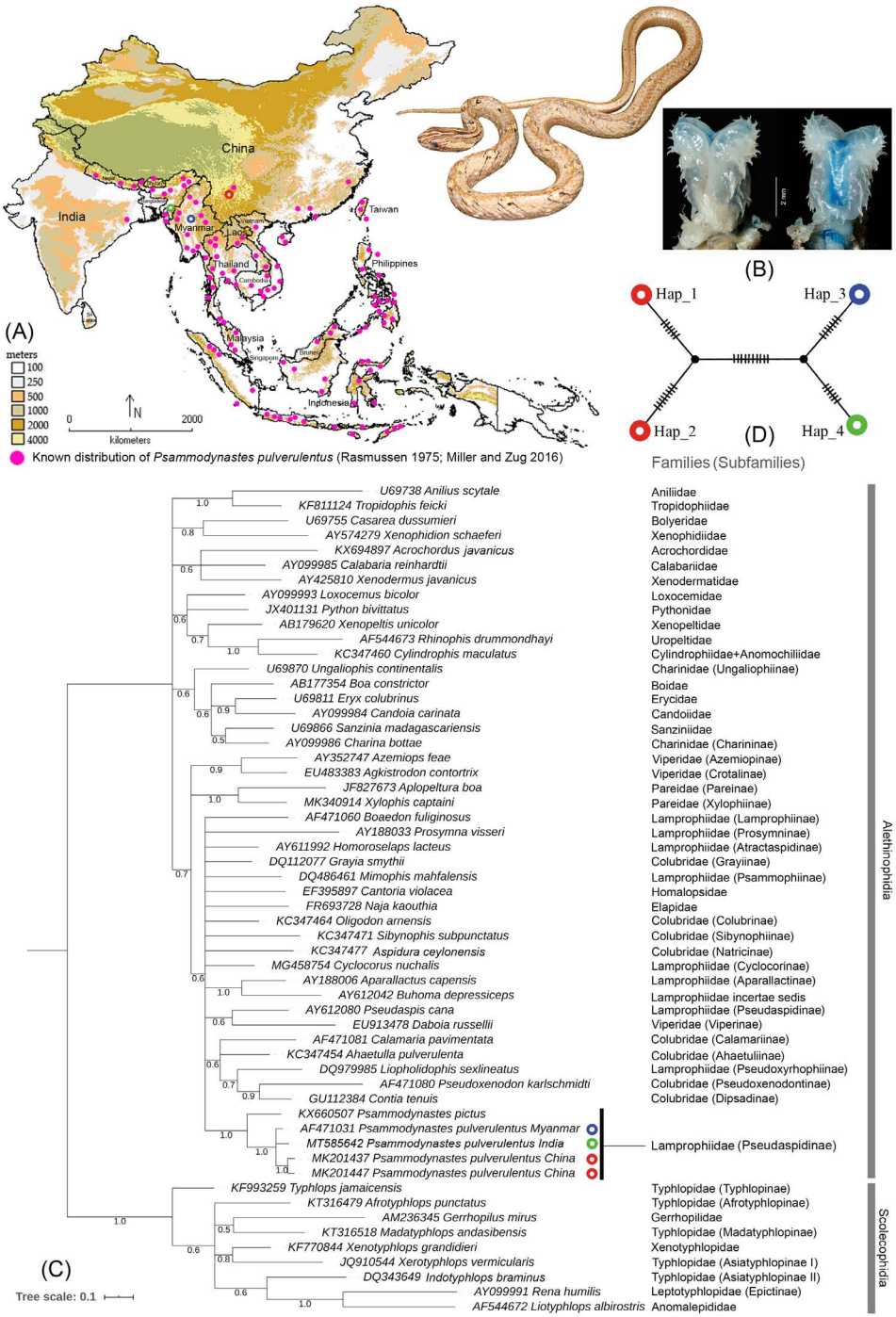

Figure 1. **(A)** Map showing the distribution of *P. pulverulentus* in South and Southeast Asian countries. Green circle indicates the collection locality of *P. pulverulentus* from Mizoram state in northeast India. **(B)** Live photographs of *P. pulverulentus* collected from northeast India andthe processed hemipenis showing its sulcal (left) and asulcal (right) surfaces. **(C)** Bayesian phylogeny based on partial mtCytb gene inferred the phylogenetic relationship of *P. pulverulentus* with all extant Ophidian families and subfamilies. Numbers at internal branches indicate posterior probabilities support. **(D)** TCS networks showed distinct haplotype of *P. pulverulentus* collected from northeast India, compared with other collection localities (China and Myanmar). The estimated haplotypes are shown in different colors as represent by collection sites marked in the phylogeny.

the morphology and scalation patterns of *P. pulverulentus* collected from Mizoram, northeast India showed similarity with the specimens examined from Myanmar (Miller & Zug, 2016). The interpretation of molecular analysis by mitochondrial Cytb gene is described below. Considering the valid families of alethinophidian and scolecophidians snakes in the studied dataset, the overall mean genetic distance was 32.9% with ranging from 19.9% (Charinidae) to 27.1% (Pareidae). The targeted species, *P. pulverulentus* revealed 16.2% to 17.3% genetic distance with their sister species, *P. pictus*. All the studied species showed distinct clade in the present topology (Figure 1 C). The evolutionary relationship of the extant snake species was not tested in the present analysis with multiple gene and other phylogenetic methods. Hence, we restricted the analysis with genetic distance and Bayesian topology to discriminate the targeted taxa, *P. pulverulentus*. The topology clearly distinguished the *P. pulverulentus* from their sister species *P. pictus* with high posterior probability support. Further, the four mtCytb DNA sequences of *P. pulverulentus* revealed four different haplotypes with 35 polymorphic sites and haplotype diversity= 1.00. The TCS network depicted a distinct haplotype of *P. pulverulentus* collected from Mizoram state in northeast India; however, the sequences generated from China and Myanmar showed three different haplotypes in the present dataset (Figure 1 D). The northeast Indian *P. pulverulentus* showed 6.6% genetic distance with the Myanmar specimen (accession no. AF471031), while 8.9% to 9.6% genetic distances with two Chinese specimens (accession nos. MK201437, MK201447). Hence, the present study elucidates the distinct population of *P. pulverulentus* in northeast India and presumed the possible cryptic diversity within India, Myanmar, and China. The present study recommended further scope to generate more genetic information, especially microsatellite and nuclear DNA sequences from different geographical regions to confirm the exact diversity and population structure of this wide-spread Mock Viper in the near future.

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