

## Molecular Phylogenetics of Small Indian Civet (*Viverricula indica*)

Nandkishor Warghat<sup>1</sup>, Navin Sharma<sup>2</sup>, Sameera Farah<sup>3</sup>, Ashwin Atkulwar<sup>3</sup>, Ramesh Chondekar<sup>4</sup>, Mumtaz Baig<sup>3\*</sup>

<sup>1</sup> Department of Zoology, Arts and Science College, Pulgaon, Wardha Maharashtra India.

<sup>2</sup> Department of Zoology, Art, Commerce and Science College, Malegaon-Yavatmal, Maharashtra-India.

<sup>3</sup> Department of Zoology, Government Vidarbha Institute of Science and Humanities,  
Amravati, Maharashtra-India. 444604

<sup>4</sup> Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University,  
Aurangabad, Maharashtra-India. 431004

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### ABSTRACT

The genus *Viverricula* is represented by single species, *Viverricula indica*, commonly called as small Indian civet. Notably, in distribution, range *Viverricula indica* span from Malaysia in southeast Asia to Pakistan in south Asia. It appears surprising that a genus with such a large distribution consists of a single species only. To address the issue, we performed pilot study to access phylogeny and molecular divergence in small Indian civet, *Viverricula indica* using partial cytochrome *b* gene sequence information. Our Bayesian inference revealed that *Viverricula indica* is not strictly monophyletic and exhibits deep divergence in the form of at least two divergent clades. Using relaxed molecular clock assumption, we trace the divergence of this clades in *Viverricula indica* to 7.59 Million year before present with 95% HPD of 5.13-9.91 Million year before present, which correspondence to late Miocene. Vicariance was probably the driving force that shaped the divergence in *Viverricula indica* both in the Southeast Asia and in the Indian subcontinent. Based on this, we proposed that there is a need for a taxonomic revision for *Viverricula indica* and that this species should be split into at least two species/subspecies.

**Key words:** Civet, mitochondrial DNA, Phylogenetics, *Viverricula indica*

### INTRODUCTION

The small Indian civet belongs to the family Viverridae which also includes genets, binturong, and linsangs. Presently, viverrids are confined to old world tropics, all of Africa and to Iberian peninsula. Viverrids are the earliest known carnivore that appears in the fossil record about 60 million years ago (Fox & Youzwyshy 1994). Three subfamilies: Hemigalinae, Paradoxurinae, and Viverrinae have been recognized in the family Viverridae. Furthermore, subfamily status of civet is also debatable. However, based on molecular phylogenetic analyses, Gaubert and Cordeiro-Estrela (2006) suggested further splitting of Viverrinae into two subfamilies, the Viverrinae and the Genettinae. Even though in distribution, Viverrinae are distributed across Asia and Africa, but as per the list of IUCN, five species reached the status of endangered or vulnerable (www.iucnredlist.org). Loss of habitat due to deforestation is the main reason that has threatened this group (Brooks, et al., 1999; Sodhi, et al., 2004). Due to nocturnal and elusive nature of the civet very little is known about the ecology and systematics of civet. The most remarkable feature about the systematics of small Indian civet is that this viverrid species distribution range span from Malaysia in southeast Asia to Pakistan in south Asia. Despite its vast range of distribution, the species is put into a single genus consisting of single species only.

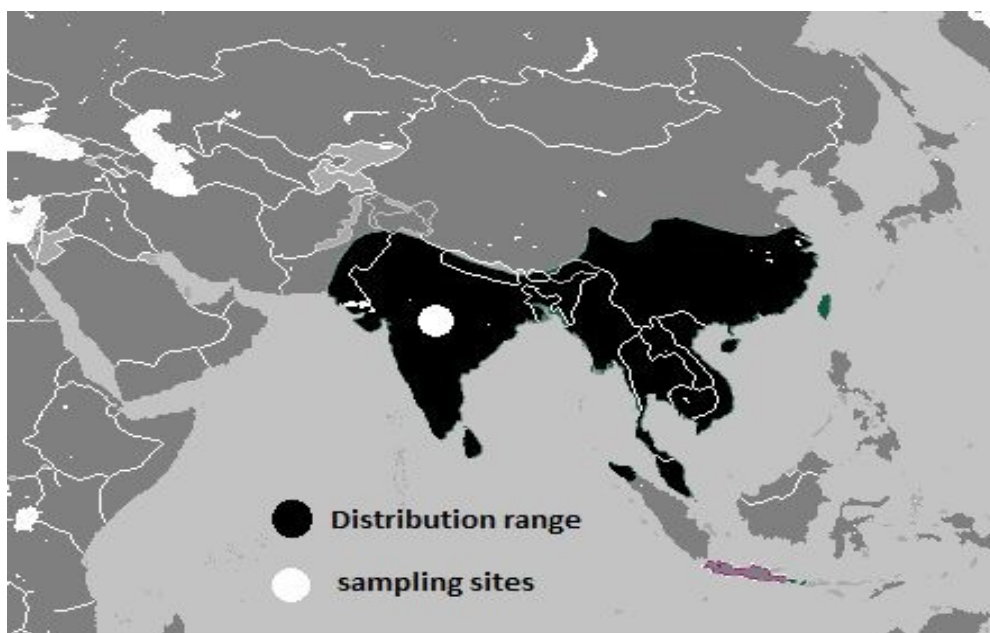
In India, small Indian civet is found throughout the country except Jammu and Kashmir (Figure 1). This shy and nocturnal animal is mainly confined to agricultural fields, territorial forest ranges of many states in India. Due to its widespread distribution, IUCN declared the status of small Indian civet as least concern (Duckworth, et al., 2008). Because of the proximity of their habitat with the human settlement, the small Indian civets are often killed by moving vehicles while crossing the roads.

In the present study, we have analyzed two road killed small Indian civets samples from Central India, with a objective to revisit the systematic of poorly studied small Indian civet using molecular phylogenetics.

### MATERIALS AND METHODS

Tissue samples were obtained from two small Indian civets from 20°38'59.88" N to 77°50'46.05" E and 20°11'15.52" N to 79°59'17.17" E locations in Central India (Figure1). DNA was extracted using DNA Sure mini Prep kit™ of Genetix Biotech Asia pvt ltd. In both samples, 280bp of cytochrome *b* gene was amplified by using primers and PCR protocol described by Kocher et al. (1989). The PCR amplifications were carried out in a 50 µl of volume containing 2.5 mM MgCl<sub>2</sub>, 200 µM dNTP, 1 µM of each primer and 0.50 U Taq polymerase. Initial denaturation of 2 minutes at 94°C was

\*Corresponding Author's E-mail: mumtazbaig@gmail.com



**Figure 1.** A map showing the distribution range and sampling sites of *Viverricula indica* (Figure modified from IUCN Red List).

followed by 35 cycles. Each cycle was consist of denaturation of 45 seconds at 94°C, primer hybridization of 1 minute at 50°C and extension of 45 seconds at 72°C. Products were finally extended for 5 minutes at 72°C. Amplicons were purified by QIAGEN, QIAquick PCR Purification kit and directly sequenced for both the strands on automated ABI 3730 DNA sequencer. Resulting raw sequences were edited manually as well as by DNASTAR 6.0 (DNASTAR Inc., Madison, W. I.), DnaSP version 5.0 (Librado and Rozas 2009) and deposited in the GenBank wide accession number KF139344 - KF139345. All available eight (08) cytochrome *b* sequences of *Viverricula indica* available in the database were retrieved. Nucleotide sequences of other viverrid species belonging to the subfamily paradoxurinae found in India were also retrieved to be used as outgroup (Table 1).

The newly generated sequences by us were aligned with the retrieved sequence information using the program Clustal X (Thompson, et al., 1997). Sequence EF662299 from India was omitted as it falls outside the sequence coverage range when compared with rest of the samples. We used Bayesian MCMC method to obtain the Bayesian Inferred (BI) tree with the use of program BEAST v 2.3.1 (Drummond, et al., 2012). The same program was used to measure the divergence time between clades under the relaxed molecular clock assumption (Drummond, et al., 2006). Clades comprising of *Viverricula indica* were calibrated using multiple calibration points within the family viverridae (Table 2). For node calibration, time estimates were drawn from mtDNA as well as from mtDNA plus nuclear DNA information described by Patou et al. (2008).

**Table 1.** List of sequences used in the study

Sub family	Species	Accession number	Location
Viverrinae	<i>Viverricula indica</i>	EF462467	India
Viverrinae	<i>Viverricula indica</i>	AF125145	Vietnam
Viverrinae	<i>Viverricula indica</i>	JN709946.	Vietnam
Viverrinae	<i>Viverricula indica</i>	AF511044	Thailand
Viverrinae	<i>Viverricula indica</i>	DQ267556	Not known
Viverrinae	<i>Viverricula indica</i>	DQ286777	China
Viverrinae	<i>Viverricula indica</i>	AY241890	Not known
Viverrinae	<i>Viverricula indica</i>	This study KF139344	Central India
Viverrinae	<i>Viverricula indica</i>	This study KF139345	Central India
Paradoxurinae	<i>Paguma larvata</i>	AF125151	Not known
Paradoxurinae	<i>Paradoxurus hermaphroditus</i>	AF511056	Vietnam
Paradoxurinae	<i>Paradoxurus jerdoni</i>	DQ683994	India
Paradoxurinae	<i>Arctictis binturong</i>	AY048793	Not known
Paradoxurinae	<i>Arctogalidia trivirgata</i>	AF125140	Not known

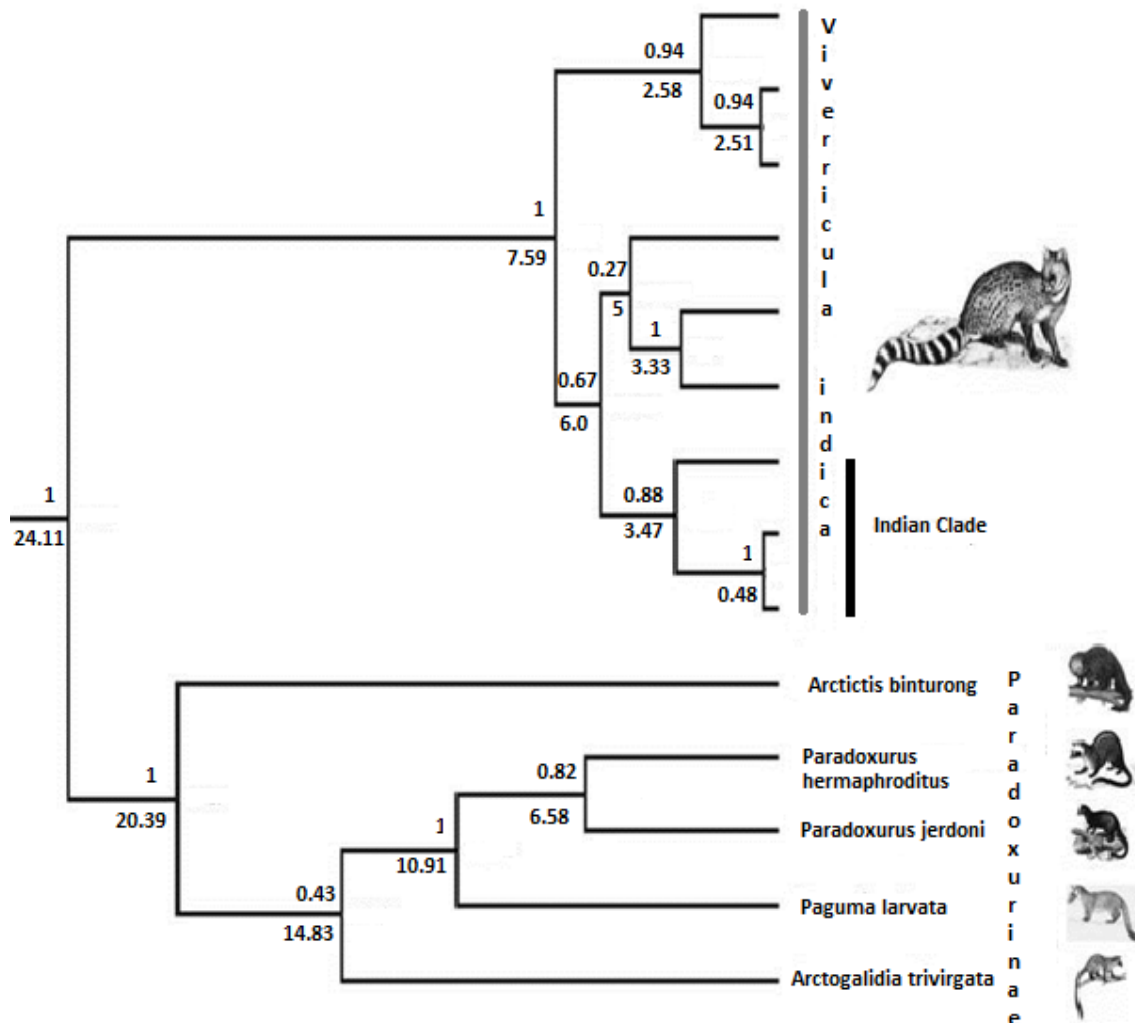
## RESULTS AND DISCUSSION

In terms of topological relationship, the BI tree was in agreement with the tree described earlier for viverrids (Gaubert and Cordeiro-Estrela 2006; Patou, et al., 2008). Both subfamilies and generic nodes yielded high posterior probabilities (Figure 2). Importantly, BI tree reconstruction shows that all nine *Viverricula* sequences are not strictly monophyletic. On the contrary, a deep divergence resulting splitting of sequences into two major diverged clades were found. BI tree shows three distinct clades of which two were supported by a high posterior

values (Figure 2). Two samples sequenced by us tightly clustered together and outgroup by a sole sequence of *Viverricula indica* from India (Sahajpal and Goyal 2010). The observed deep divergence might have resulted due to vicariance eventually leading to allopatric speciation in the Indian subcontinent and in Southeast countries. Based on the characteristic of these sequences, we suggest that taking into account the vast distribution range of small Indian civet, from South China to greater part of Indian subcontinent, from Malaysia, Indonesia to Myanmar, cannot be fitted into a single species i.e. the *Viverricula indica*. Based on morphology, five sub

**Table 2.** Divergence time estimation of nodes in million years (Modified from Patou et al., 2008). The 95% HPD levels are shown in parentheses.

Nodes	mtDNA	allDNA(nuclear+mtDNA)
<b>Viverricula</b>	13.77(9.10-19.00)	7.59(5.13-9.91)
<b>Paradoxurinae</b>	31.34(21.64-40.68)	20.39(16.16-24.70)



**Figure 2.** Bayesian Inferred (BI) tree comprising of subfamily Viverrinae that includes small Indian civet, *Viverricula indica* and members of subfamily Paradoxurinae as outgroup. The posterior probability values are shown above line and divergence time estimates below line on nodes, while 95% HPD levels are illustrated in Table 2.

species of *Viverricula indica* has been reported in India alone. They are *Viverricula indica indica* in southern India ranging from Western to Eastern Ghat, *Viverricula indica bengalensis* in plains of Northern India, south of the Ganges and from Kolkata to Gujarat, *Viverricula indica deserti* from Rajasthan, *Viverricula indica wellsi*, from Uttar Pradesh and Uttarakhand states and *Viverricula indica baptistee* ranging from Upper Bengal to Assam (Pocock, 1939; Srinivasulu and Srinivasulu 2012). The application of relaxed molecular clock illustrates two major divergence events in the evolution of *viverricula indica* (Figure 2).

The first major divergence event is supported by a high posterior of one, while rest have posterior less than one. Divergence estimate shows that the first cladogenesis occurs 7.59 million years ago, a period that corresponds to late Miocene (Table 2). Genetic evidences of faunal exchanges of several mammalian species including viverrid between Asia and Africa during Miocene has been well established and documented (Gaubert and Cordeiro-Estrela 2006, Woodruff and Turner 2009). Also, role of Isthmus of Kra as significant biogeographical barrier in the diversification of palm civet, *Paradoxurus* in Asia was described previously (Patou, *et al.*, 2010). Apart from Isthmus of Kra, other vicariance factors like mountains and rivers might have deterred the gene flow between this small nocturnal carnivore mammal in South Asia and Southeast Asia. Role of north western mountain ranges like Patkai, Chin, Arakan and rivers like Brahmaputra and Salween as potential biogeographical barriers between Indian subcontinent and south east Asian countries were confirmed before (Meijaard and Groves 2006; Su, *et al.*, 2006; Veron, *et al.*, 2007). In the context of biogeographical and molecular evidences, we hypothesize that in line with the palm civet, *Paradoxurus hermaphrodites*, the *Viverricula indica* had also undergone independent divergence in south and Southeast Asia. Large genetic distances between the inferred *Viverricula indica* clades, point towards occurrence of more than one species/subspecies. In the light of this phylogenetics reconstruction, we propose that *Viverricula indica* should be split into at least two separate species/subspecies. Thus, considering the limitations of single locus, small sample size and uncertainty of the geographical origin of the samples, we should not read too much, but our pilot study highlight the need to revisit the systematics of small Indian civet. In future sampling of more *Viverricula indica* from major parts of the South Asia and South East Asia and typing them using multiple mtDNA and nuclear markers would provide deeper insight into the systematics and biogeography of small Indian civet.

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