Characteristics of the complete mitochondrial genome of the monotypic genus *Arctictis* (Family: Viverridae) and its phylogenetic implications

Siuli Mitra1,*, Vaishnavi Kunteepuram1,*, Klaus-Peter Koepfli2, Neha Mehra1, Wajeeda Tabasum1, Ara Sreenivas1 and Ajay Gaur1

1 Laboratory for Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology, Hyderabad, Telangana, India
2 Center for Species Survival, Smithsonian Conservation Biology Institute, Washington, D.C., USA
* These authors contributed equally to this work.

ABSTRACT

The binturong (*Arctictis binturong*) is classified as a member of the subfamily Paradoxurinae within the family Viverridae (Carnivora: Mammalia) and comprises nine subspecies spread across Southern and Southeast Asia. Here, we describe the complete mitochondrial genome of the Indian subspecies *A. b. albifrons* using next-generation sequencing methods. The total length of the *A. b. albifrons* mitogenome was 16,642 bp. Phylogenetic analyses based on 13 mitochondrial protein-coding genes placed the binturong as a sister taxon to *Paguma larvata* within the Paradoxurinae and supported the clustering of *Genettinae* and *Viverrinae* and the monophyly of Viverridae and six other families of feliforms, consistent with previous studies. Divergence time estimates suggest that the Viverridae diversified during the Miocene (22.62 Mya: 95% CI [20.78–24.54] Mya) and that *Arctictis* and *Paguma* split 12.57 Mya (95% CI [8.66–15.67] Mya). Further molecular studies are required to test the distinctiveness and diversity of the nine putative subspecies of binturong.

Subjects Conservation Biology, Genomics, Molecular Biology

Keywords Mitochondrial DNA, Time calibrated phylogeny, Arctictis binturong albifrons, Viverridae

INTRODUCTION

*Arctictis binturong* (*Raffles, 1822*), commonly called binturong or bearcat, is the largest known member of the Viverridae (Carnivora: Mammalia) and is characterized by coarse, black fur and a prehensile tail (*Pocock, 1933*). In forest ecosystems of Southeast Asia, the frugivorous binturong has co-evolved with fig trees to form a keystone relationship, wherein the animal facilitates and propagates seed germination while the fig tree provides a stable dietary source (*Kinnaird & O’Brien, 2007*). Binturongs are presently being poached for their meat, traditional medicines and the pet trade, and alongside habitat destruction, these factors have contributed to decreasing the numbers of binturong to a few geographical pockets across the species’ former range (*Willcox et al., 2016*). As a result of these increasing...
pressures, the binturong is listed as 'Vulnerable' on the IUCN Red List of Threatened Species (Wilcox et al., 2016).

Nine subspecies of *A. binturong* have been described primarily on the basis of region-specific variations in fur color (Pocock, 1933; Cosson et al., 2006). In addition, shared morphological similarities with other viverrids like perineal scent glands and syndactyly of the third and fourth digits of the hind foot, along with the unique features of completely naked soles of the hind feet and a prehensile tail, have helped determine the phylogenetic position of binturong within the viverrid subfamily Paradoxurinae (Pocock, 1933; Gregory & Hellman, 1939; Veron, 2007). More recent molecular phylogenetic studies indicate that this subfamily also includes *Paguma*, *Arctogalidia* and *Macrogalidia* (Albert, 2001; Cosson et al., 2006; Patou et al., 2008; Nyakatura & Bininda-Emonds, 2012; Zhou, Wang & Ma, 2017). However, the genetic structure of the binturong has not been studied in detail (but see Cosson et al., 2006), which will be essential to validate the evolutionary and conservation genetic implications of the existence of nine geographically and morphologically disparate subspecies.

*Mohd Salleh et al. (2017)* reported the first mitochondrial genome of a binturong as part of a larger study to generate an expanded reference mitogenome dataset of mammals from Southeast Asia that could be applied for monitoring mammalian biodiversity using environmental DNA approaches. However, the sequence reported in that study came from an animal at the Tier Park Berlin Zoo of unknown provenance. Moreover, the sequence contains many missing nucleotides (Ns) and is therefore incomplete. To rectify this, we generated a complete (gapless) mitochondrial genome sequence from a wild-caught binturong of known provenance belonging to the Indian subspecies, *Arctictis binturong albifrons*. The aims of our study were to: (a) characterize the *Arctictis* mitogenome in comparison with other viverrids and feliforms, and (b) provide the first molecular phylogenetic and divergence dating analysis of the *Arctictis* in the context of Viverridae and other feliform families based on whole mitochondrial genomes.

**MATERIALS AND METHODS**

**Sampling, Extraction and PCR amplification**

A blood sample of an individual identified as *A. b. albifrons* was collected and forwarded by the Veterinary Assistant Surgeon of the Sepahijala Zoo, Tripura (Vide Letter No. F5(D)VD/Sep/Sl No. 100-102, dated 19/07/2008) for DNA analysis, and deposited in the Genome Bank at the Laboratory for Conservation of Endangered Species, CCMB, Hyderabad, India. Genomic DNA was isolated from the blood sample by the phenol-chloroform-isoamyl alcohol method (Sambrook, Fritsch & Maniatis, 1989) and the DNA integrity was checked electrophoretically in a 0.8% agarose gel. The mitochondrial genome was amplified by long range PCR using the TaKaRa LA PCR kit v2 (Takara Bio Inc, USA) following the manufacturer’s recommendations. Three PCR products of 4.1 kbp, 8.8 kbp and 4.5 kbp were generated using three sets of primers (Table S1).
Genome sequencing, assembly and annotation

Next-generation sequencing libraries were constructed in three steps: enzymatic shearing, adapter ligation and fragment size selection. PCR product quality was first assessed using the Qubit® 2.0 Fluorometer (Life Technologies, USA). Amplified products were then subjected to enzymatic fragmentation to an average of 550 bp fragments using the Covaris M220 system (Covaris, USA). Libraries were prepared with the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer’s recommendations. The High Sensitivity DNA Analysis Kit (Agilent Technologies, USA) was used for quantification and size estimation of the libraries generated on a 2100 Bioanalyzer (Agilent Technologies, USA). The libraries were standardized to 1.5pM and sequenced using the NextSeq 500 sequencer (Illumina, USA). The paired end reads generated on Illumina were used for reference assembly. Raw sequences were extracted in the FASTQ format and checked for quality using the CLC Genomics Workbench v 9.0 software (https://www.qiagenbioinformatics.com/). Raw sequences filtering was performed by trimming adaptors. Nucleotides and sequence reads showing ambiguity or low quality scores (<Q20) were excluded from further analysis. High quality data obtained after filtering was assembled and annotated using CLC Genomic Workbench v 9.0. Mauve version 2.4.0 (Darling et al., 2004) was used for the comparative reference assembly. The binturong mitogenome sequence was analyzed using CLC Genomic Workbench v 9.0 to identify the mitochondrial gene locations, their order, and start and end points.

Genome analysis

The circular map of mitogenome was created using Geneious R 10.1 (Kearse et al., 2012). Sequences of the 13 protein-coding genes were translated into amino acid sequences using ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) to verify orthology with other feliform taxa and exclude the potential presence of NUMTs (nuclear-mitochondrial paralogues). The control region was extracted and scanned for the presence of palindromes and other repeats using the EMBOSS and REP FIND tools (Rice, Longden & Bleasby, 2000; Betley et al., 2002). We evaluated the base composition of the binturong mitogenome and those of 11 other feliform species using Geneious R 10.1 (Table S2).

Phylogenetic analysis and estimation of divergence times

The phylogenetic relationships of the binturong within Viverridae and Feliformia were reconstructed by aligning its 13 protein-coding gene sequences with those of 22 feliform species comprising five viverrids, 11 felids, two hyaenids, and one species each from Nandiniidae, Herpestidae, Eupleridae and Prionodontidae (Table S2). The Cuon alpinus mitogenome (Canidae, NCBI Accession No. NC_013445.1) was also included as outgroup to root the feliform tree. Sequences were aligned using MEGA 6.06 with the default parameters of CLUSTALW (Thompson, Higgins & Gibson, 1994) and then concatenated, resulting in an 11,313 bp alignment. Maximum likelihood phylogenetic analysis was conducted using raxmlGUI v1.3 (Silvestro & Michalak, 2012). Support for different nodes was estimated using 1,000 bootstrap replicates (ML + bootstrap option) under the GTR+I+G model, as estimated with jModeltest 2.1.5 (Darriba et al., 2012) and Bayesian Inference (BI) method using Mr. Bayes v.3.2.5.
We jointly estimated the phylogeny and divergence times between species within a Bayesian inference framework using the program BEAST v1.7.5 (Drummond & Rambaut, 2007). The 13 protein-coding genes were partitioned and the appropriate model of sequence evolution was determined by the BIC criteria in jModeltest 2.1.5 (Darriba et al., 2012). Substitution rates were estimated under a relaxed uncorrelated clock model for $1 \times 10^8$ million generations, sampling every 1,000th generation to allow for adequate mixing. Base frequencies were set to “All equal” and the number of gamma categories was set to 4. Four fossil priors with a uniform probability distribution were used for calibration: (a) Minimum and maximum ages of split between Caniformia and Feliformia were set at 43 Mya and 63.8 Mya, respectively (Benton & Donoghue, 2007) (b) Minimum age of origin of Viverridae was set at 23 Mya (Hunt, 1991; Hunt Jr, 1996) (c) Minimum age of the split between Crocuta and Hyaena was set 9.5 Mya (Wozencraft, 2005); and (d) Minimum and maximum ages of origin of Felidae were set at 5.3 Mya and 23 Mya (McKenna & Bell, 1997). The first 25% of MCMC iterations were removed from the posterior sample as burn-in. Convergence was monitored in Tracer (Ver. 1.4) to determine if effective sampling sizes (ESS) were adequate (>200). Trees were summarized with Tree Annotator and represented as the maximum clade credibility tree.

RESULTS AND DISCUSSION

Binturong mitogenome assembly and annotation
A total of 2,726,370 high-quality reads were obtained after filtering and trimming sequences. The assembly of the binturong mitogenome resulted in a total of 162 contigs with an N50 length of 1074 bp and the length of contigs ranging from 501 bp to 16,752 bp. After trimming ends, the A. b. albifrons mitogenome was 16,642 bp in length (Fig. 1). The assembly size of the binturong mitogenome reported by Mohd Salleh et al. (2017) is 17,067 bp, 425 bp longer than our assembly. However, this increased length is due to the presence of inserted Ns (missing nucleotides) within the sequence. The A. b. albifrons mitogenome is generally shorter when compared with those of nine other feliform species (Table S2). The A. b. albifrons mitogenome sequence was deposited in NCBI GenBank (accession number KX449332).

Annotation yielded a total of 37 genes: 13 protein-coding genes (PCGs), 22 tRNAs, 2 rRNAs and the non-coding control (D-loop) region (Table 1). No NUMTs were detected among the 13 PCGs when they were translated into amino acids (no insertion, deletions, frame shift mutations or premature stop codons). Analysis of base composition showed a bias towards higher adenine and thymine content in the binturong mitogenome (Table 2), amounting to 64.63% and individual base proportions amounting to 32.80% A, 31.83% T, 16.46% G and 18.91% C. This pattern is consistent with the base composition of twelve other feliform species, with AT content ranging from 60.28% in Hyaena to 64.64% in P. larvata while a higher average bias was maintained within Viverridae (65.02%). The control region was 1,234 bp long, located from 15,408–16,642 bp in the binturong mitogenome (Fig. 1). The region included five mononucleotide T stretches, two of (T)$_4$ and three of (T)$_5$. A 12 bp long palindrome sequence (5′-TATCTATAGATA- 3′) was located between
nucleotide positions 890-901 in the alignment of the control region sequences of the binturong and 11 other feliform species.

**Phylogenetic relationships and divergence time estimates**

Maximum likelihood and Bayesian inference analyses (Fig. S1) of the concatenated sequences of the 13 PCGs (11,313 bp) support the monophyly of Viverridae among feliforms, the monophyly of Paradoxurinae among viverrids, the clustering of Paradoxurinae and Hemigalinae, as found by Veron et al. (2017), and the clustering of Genettinae with Viverrinae as proposed by Veron (2007) and Eizirik et al. (2010). The monophyly of Viverridae was strongly supported in both the analyses, with a Bayesian Posterior Probability (bP) of 1 and a bootstrap support (BS) of 100%. Viverrid monophyly was also observed by Patou et al. (2008) and finds morphological support as the species therein share a characteristic union of the third and fourth digits in the hind foot and a hypocarnivorous dentition (Patou et al., 2008; Veron, 2007). Within Viverridae, Paradoxurinae (A. binturong and P. larvata) and Paradoxurinae + Hemigalinae (C.
| Gene     | Strand | Start | End   | Size (bp) | Start codon | Stop codon | Base composition (%) | AT SKEW | GC SKEW |
|----------|--------|-------|-------|-----------|-------------|------------|----------------------|---------|---------|
|          |        |       |       |           | (A+T)%      | (G+C)%     |                      |         |         |
|          |        |       |       |           |             |            |                      |         |         |
| tRNAPhe  | H      | 1     | 69    | 68        | –           | –          | 60 39 0.26 –0.02    |         |         |
| 12S rRNA | H      | 70    | 1,034 | 964       | –           | –          | 59 39 0.25 –0.17    |         |         |
| tRNAVtyr | H      | 1,035 | 1,103 | 68        | –           | –          | 59 39 0.22 –0.28    |         |         |
| 16S rRNA | H      | 1,104 | 2,678 | 1,574     | –           | –          | 62 37 0.19 –0.08    |         |         |
| tRNA2    | H      | 2,679 | 2,754 | 75        | –           | –          | 58 40 0.0 –0.1      |         |         |
| nad1     | H      | 2,755 | 3,697 | 942       | ATG AGA     |            | 60 38 0.4 –0.25    |         |         |
| tRNAIap  | H      | 3,698 | 3,767 | 69        | –           | –          | 72 26 0.08 0.15     |         |         |
| tRNAQeu  | L      | 3,768 | 3,841 | 73        | –           | –          | 66 32 0.09 –0.4     |         |         |
| tRNAMhis | H      | 3,842 | 3,911 | 69        | –           | –          | 55 42 0.01 –0.25    |         |         |
| nad2     | H      | 3,912 | 4,944 | 1,032     | ATT AGA     |            | 65 33 0.13 –0.51    |         |         |
| tRNAWsr  | H      | 4,945 | 5,015 | 70        | –           | –          | 57 41 0.12 –0.17    |         |         |
| tRNAAsr  | L      | 5,016 | 5,085 | 69        | –           | –          | 64 34 0.15 –0.35    |         |         |
| tRNAYval | L      | 5,086 | 5,159 | 73        | –           | –          | 60 39 0.2 –0.2      |         |         |
| trNACala | L      | 5,160 | 5,228 | 68        | –           | –          | 56 42 –0.07 –0.19   |         |         |
| tRNAVal  | L      | 5,229 | 5,297 | 68        | –           | –          | 60 74 0.4            |         |         |
| cox1     | H      | 5,298 | 6,831 | 1,533     | ATG TAA     |            | 62 36 –0.1 –0.11    |         |         |
| tRNAs2   | L      | 6,832 | 6,901 | 69        | –           | –          | 63 35 0.17 –0.2     |         |         |
| tRNAIval | H      | 6,902 | 6,971 | 69        | –           | –          | 70 28 –0.05 0.2     |         |         |
| cox2     | H      | 6,972 | 7,653 | 681       | ATG TAA     |            | 64 34 0.03 –0.2     |         |         |
| tRNAPhe  | H      | 7,654 | 7,722 | 68        | –           | –          | 72 26 0.08 0        |         |         |
| atp8     | H      | 7,723 | 198    | 7,921     | ATG TAA     |            | 71 27 0.12 –0.5     |         |         |
| atp6     | H      | 7,922 | 8,597 | 675       | ATG TAA     |            | 63 34 –0.01 –0.5    |         |         |
| cox3     | H      | 8,598 | 9,381 | 783       | ATG TAG     |            | 61 37 –0.04 –0.4    |         |         |
| tRNAASr  | H      | 9,382 | 9,452 | 69        | –           | –          | 65 33 0.04 –0.09    |         |         |
| nad3     | H      | 9,453 | 9,798 | 345       | ATA TA      |            | 64 34 0 0.35        |         |         |
| tRNASr   | H      | 9,799 | 9,868 | 69        | –           | –          | 77 21 0.14 –0.04    |         |         |
| nad4l    | H      | 9,869 | 10,163| 294       | ATG TAA     |            | 64 33 –0.09 –0.33   |         |         |
| nad4     | H      | 10,164| 11,352| 1,158     | ATG TA      |            | 64 34 0.03 –0.41    |         |         |
| tRNAHval | H      | 11,353| 1,368 | 11,532    | ATG TA      |            | 64 34 0.03 –0.41    |         |         |
| tRNASIlu | H      | 11,533| 11,602| 69        | –           | –          | 76 22 0.02 0s       |         |         |
| tRNASIva | H      | 11,603| 11,662| 59        | –           | –          | 65 33 0.07 –0.09    |         |         |
| nad5     | H      | 11,663| 11,733| 70        | –           | –          | 68 31 0.2 0.09      |         |         |
| nad6     | L      | 11,734| 1,806 | 13,540    | ATT TAA     |            | 65 34 0.01 –0.4     |         |         |
| tRNAPhe  | L      | 13,541| 14,063| 522       | ATG TAA     |            | 62 35 0.32 –0.4     |         |         |
| Cob      | H      | 14,064| 14,133| 69        | –           | –          | 69 29 0.13 –0.24    |         |         |
| tRNAItyr | H      | 14,134| 15,268| 1,134     | ATG AGA     |            | 60 38 0 –0.31       |         |         |
| Control region | H | 15,269 | 15,340 | 71 | – | – | 63 34 0.04 –0.11 |         |         |
| Control region | L | 15,341 | 15,407 | 66 | – | – | 55 43 0.23 –0.39 |         |         |

Mitra et al. (2019), PeerJ, DOI 10.7717/peerj.8033
Table 2  Genome length, base composition, (A+T) percentage and AT and GC skewness in mitogenomes of binturong and nine other feliforms.

| Species       | Size (bp) | A%  | G%  | T%  | C%  | (A+T)% | AT skew | GC skew |
|---------------|-----------|-----|-----|-----|-----|--------|---------|---------|
| **WHOLE MITOCHONDRIAL GENOME** |           |     |     |     |     |        |         |         |
| A. binturong  | 16,642    | 32.75 | 16.45 | 31.81 | 18.99 | 63.64 | 0.07    | −0.19  |
| A. binturong  | 17,067    | 33.1  | 12.9 | 29.2 | 23.5 | 62.3 | 0.06    | −0.29  |
| C. bennettii  | 15,785    | 34.2  | 12.3 | 31.0 | 22.6 | 65.2 | 0.04    | −0.29  |
| P. larvata    | 16,710    | 33.43 | 16.11 | 30.97 | 19.49 | 64.64 | 0.04    | −0.09  |
| G. servalina  | 16,938    | 32.90 | 16.72 | 30.08 | 20.30 | 62.98 | 0.04    | −0.09  |
| V. indica     | 16,583    | 32.99 | 16.41 | 30.66 | 19.94 | 63.65 | 0.04    | −0.09  |
| H. javanicus  | 16,758    | 32.38 | 17.00 | 29.96 | 20.66 | 62.34 | 0.04    | −0.09  |
| M. decemlineata | 16,905   | 31.74 | 12.48 | 32.41 | 23.22 | 64.30 | 0.03    | −0.30  |
| G. servalina  | 11,410    | 31.02 | 13.24 | 30.41 | 16.14 | 64.09 | 0.08    | 0.07   |
| V. indica     | 11,410    | 31.45 | 12.86 | 31.30 | 23.94 | 62.75 | 0.04    | −0.30  |
| H. javanicus  | 11,301    | 32.77 | 19.07 | 31.09 | 17.07 | 63.86 | 0.03    | 0.05   |
| M. decemlineata | 11,512   | 32.41 | 19.75 | 30.38 | 17.46 | 62.79 | 0.03    | 0.06   |
| H. hyaena     | 11,292    | 30.94 | 13.74 | 28.79 | 26.52 | 65.94 | 0.03    | −0.32  |
| F. catus      | 11,286    | 30.61 | 13.66 | 30.79 | 24.94 | 61.40 | 0.00    | −0.29  |
| N. binotata   | 11,303    | 31.74 | 12.64 | 28.23 | 27.39 | 59.97 | 0.06    | −0.37  |

| Protein Coding Genes (PCGs) | |     |     |     |     |        |         |         |
|-----------------------------|-----------|-----|-----|-----|-----|--------|---------|---------|
| A. binturong                | 11,313    | 30.99 | 12.71 | 33.15 | 23.15 | 63.46 | 0.03    | −0.37  |
| A. binturong                | 11,444    | 31.8  | 12.4 | 31.5 | 24.1 | 63.3 | 0.004   | −0.31  |
| C. bennettii                | 11,332    | 32    | 11.9 | 33.3 | 22.6 | 65.3 | −0.01   | −0.31  |
| P. larvata                  | 11,316    | 31.89 | 12.48 | 32.41 | 23.22 | 64.30 | −0.01   | −0.30  |
| G. servalina                | 11,410    | 31.02 | 13.24 | 30.41 | 25.33 | 61.44 | 0.01    | −0.32  |
| V. indica                   | 11,410    | 31.45 | 12.86 | 31.30 | 24.39 | 62.75 | 0.00    | −0.31  |
| H. javanicus                | 11,301    | 31.12 | 13.60 | 29.44 | 25.84 | 60.56 | 0.03    | −0.31  |
| M. decemlineata             | 11,295    | 29.83 | 14.71 | 27.33 | 28.13 | 57.16 | 0.04    | −0.31  |
| H. hyaena                   | 11,310    | 30.22 | 14.50 | 27.54 | 27.74 | 57.77 | 0.05    | −0.31  |
| F. catus                    | 11,292    | 30.94 | 13.74 | 28.79 | 26.52 | 59.74 | 0.03    | −0.32  |
| P. pardicolor               | 11,286    | 30.61 | 13.66 | 30.79 | 24.94 | 61.40 | 0.00    | −0.29  |
| N. binotata                 | 11,303    | 31.74 | 12.64 | 28.23 | 27.39 | 59.97 | 0.06    | −0.37  |

| tRNA                       |           |     |     |     |     |        |         |         |
|-----------------------------|-----------|-----|-----|-----|-----|--------|---------|---------|
| A. binturong                | 1,519     | 33.45 | 18.63 | 31.77 | 16.14 | 64.09 | 0.08    | 0.07   |
| A. binturong                | 1,582     | 33.5  | 18.83 | 30.7  | 16.87 | 64.28 | 0.04    | 0.05   |
| C. bennettii                | 1,516     | 33.9  | 18.46 | 31.26 | 16.2  | 65.16 | 0.04    | 0.06   |
| P. larvata                  | 1,517     | 34.14 | 18.20 | 31.09 | 16.58 | 65.22 | 0.05    | 0.05   |
| G. servalina                | 1,512     | 33.71 | 18.79 | 30.63 | 16.86 | 64.35 | 0.05    | 0.05   |
| V. indica                   | 1,515     | 33.61 | 18.50 | 31.06 | 16.82 | 64.67 | 0.04    | 0.05   |
| H. javanicus                | 1,514     | 32.77 | 19.07 | 31.09 | 17.07 | 63.86 | 0.03    | 0.05   |
| M. decemlineata             | 1,512     | 32.41 | 19.75 | 30.38 | 17.46 | 62.79 | 0.03    | 0.06   |
| H. hyaena                   | 1,512     | 31.91 | 20.07 | 30.07 | 17.95 | 61.98 | 0.03    | 0.06   |
| F. catus                    | 1,515     | 33.62 | 18.80 | 30.77 | 16.81 | 64.39 | 0.04    | 0.05   |
| P. pardicolor               | 1,514     | 33.39 | 18.54 | 31.55 | 16.51 | 64.94 | 0.03    | 0.05   |
| N. binotata                 | 1,513     | 33.60 | 18.83 | 31.17 | 16.40 | 64.77 | 0.04    | 0.07   |

(continued on next page)
Table 2 (continued)

| Species        | rRNA  | CONTROL REGION |
|----------------|-------|----------------|
|                | Size (bp) | A%  | G%  | T%  | C%  | (A+T)% | AT skew | GC skew |
| A. binturong   | 2,538  | 37.31 | 16.97 | 23.93 | 21.80 | 60.5 | 0.22 | −0.13 |
| A. binturong   | 2,537  | 37.24 | 18.8  | 24   | 21.75 | 61.2 | 0.21 | −0.12 |
| C. bennettii   | 2,537  | 37.24 | 17.06 | 25.10 | 20.57 | 62.34 | 0.19 | −0.09 |
| P. larvata     | 2,537  | 36.68 | 17.39 | 23.73 | 22.19 | 60.41 | 0.22 | −0.12 |
| G. servalina   | 2,532  | 37.24 | 19.56 | 24.84 | 21.03 | 60.89 | 0.22 | −0.12 |
| V. indica      | 2,530  | 37.05 | 17.25 | 23.84 | 21.86 | 60.89 | 0.22 | −0.12 |
| H. javanicus   | 2,534  | 35.96 | 21.95 | 23.63 | 23.63 | 58.41 | 0.25 | −0.14 |
| M. decemlineata| 2,535  | 36.46 | 17.96 | 21.95 | 23.63 | 58.41 | 0.25 | −0.14 |
| H. hyaena      | 2,533  | 35.96 | 18.33 | 26.84 | 22.04 | 58.00 | 0.24 | −0.13 |
| F. catus       | 2,538  | 36.52 | 18.05 | 22.86 | 22.57 | 59.38 | 0.23 | −0.11 |
| P. pardicolor  | 2,544  | 35.35 | 18.22 | 24.21 | 22.22 | 59.55 | 0.19 | −0.10 |
| N. binotata    | 2,538  | 37.35 | 17.13 | 21.86 | 23.66 | 59.21 | 0.26 | −0.16 |

Notes.
*Sequence generated in this study.

bennettii) were both monophyletic, each with bpP of 1 and BS = 100%. Finally, Genettinae and Viverrinae were found to have a sister relationship with maximum bpP support and BS = 94%. Inferred relationships among other feliform families were well supported and congruent with previous studies (Veron & Heard, 2000; Gaubert & Veron, 2003; Yoder et al., 2003; Flynn et al., 2005; Koepfli et al., 2006; Eizirik et al., 2010).

Divergence time estimation (Fig. 2) showed that Viverridae began diversifying around 22.65 Mya (95% Credibility Interval (CI) [20.78 Mya –24.54 Mya]), close to the dates estimated in earlier studies (Gaubert & Veron, 2003; Koepfli et al., 2006; Patou et al., 2008; Zhou, Wang & Ma, 2017) but more recent than that suggested in other studies (Gaubert & Cordeiro Estrela, 2006; Eizirik et al., 2010). The estimates obtained from this study and most others agree with the temporal position of the earliest evidence for Viverridae, represented by the late Oligocene-early Miocene fossil Herpestides, which has been dated at 23 Mya (Johnson et al., 2006; Morlo, Miller & El-Barkooky, 2007). The split between Arctictis and Paguma in Paradoxurinae was estimated at 12.57 Mya (95% CI [8.66 Mya –16.51 Mya]),
Figure 2  Mean divergence date estimates among 24 feliform species. The 24 feliform species (listed in Table S2) includes five from Viverridae showing divergence of Viverridae and sub-families Paradoxurinae (Arctictis binturong and Paguma larvata), Genettinae (Genetta servalina), Hemigalinae (Cynogale bennetti), and Viverrinae (Viverricula indica). Blue bars spanning nodes show 95% highest posterior density (HPD) for divergence times. Timescale in millions of years ago (Mya) is shown at the bottom. The Asiatic dhole (Cuon alpinus) was used as the outgroup to root the feliform tree.

which agrees with the date of origin of Paradoxurinae suggested to be during the Miocene (Gaubert & Cordeiro Estrela, 2006; Veron, 2007). Divergence times estimated for the origin of Feliformia (41.80 Mya, CI [26.82–63.98]) and the feliform families are consistent with those reported in previous studies (Gaubert & Cordeiro Estrela, 2006; Koepfli et al., 2006; Eizirik et al., 2010; Zhou, Wang & Ma, 2017).

CONCLUSIONS

We have reported the first complete (gapless) mitogenome of the binturong, representing the Indian subspecies Arctictis binturong albifrons. The binturong mitogenome, along with the one previously reported by (Mohd Salleh et al., 2017), provides a starting point for further testing the distinctiveness and diversity of the nine putative subspecies of binturong and thereby provide critical information for designing conservation management plans for this vulnerable species.
ACKNOWLEDGEMENTS
The authors wish to thank Dr. Rakesh Mishra, Director, CSIR-CCMB and Dr. Karthikeyan Vasudevan, Scientist In-Charge LaCONES, CSIR-CCMB for their incessant support. Thanks are also due to Dr. Sajal Das, Veterinary Assistant Surgeon, Sepahijala Zoological Park, Agartala, for providing the samples used in this study. The authors thank the editor and the three anonymous reviewers whose comments have helped in significant improvement of the manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
The work was funded by Council for Scientific and Industrial Research (BSC0207), India. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
Council for Scientific and Industrial Research: BSC0207.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Siuli Mitra conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
• Vaishnavi Kunteepuram performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
• Klaus-Peter Koepfli, Wajeeda Tabasum and Ara Sreenivas analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
• Neha Mehra performed the experiments, authored or reviewed drafts of the paper, approved the final draft.
• Ajay Gaur conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):
The blood sample was collected and forwarded by the Veterinary Assistant Surgeon, Sepahijala Zoo, Tripura. The authors did not handle any animal.

Data Availability
The following information was supplied regarding data availability:
The raw data is available at NCBI GenBank: KX449332.
Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8033#supplemental-information.

REFERENCES

Albert R. 2001. Gene structure and gene flow in selected populations of spotted hyaena (Crocuta crocuta). Diss. Thesis, Freie Universitaet Berlin.

Benton MJ, Donoghue PC. 2007. Paleontological evidence to date the tree of life. *Molecular Biology and Evolution* 24(1):26–53 DOI 10.1093/molbev/msl150.

Betley JN, Frith MC, Graber JH, Choo S, Deshler JO. 2002. A ubiquitous and conserved signal for RNA localization in chordates. *Current Biology* 12:1756–1761 DOI 10.1016/S0960-9822(02)01220-4.

Cosson L, Grassman Jr LL, Zubaid A, Vellayan S, Tillier A, Veron G. 2006. Genetic diversity of captive binturongs (Arctictis binturong, Viverridae, Carnivora): implications for conservation. *Journal of Zoology* 271:386–395.

Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Research* 14:1394–1403 DOI 10.1101/gr.2289704.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModeltest 2 more models, new heuristics and parallel computing. *Nature Methods* 9(8):772.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7:214 DOI 10.1186/1471-2148-7-214.

Eizirik E, Murphy WJ, Koepfli KP, Johnson WE, Dragoo JW, Wayne RK, O’Brien SJ. 2010. Pattern and timing of diversification of the mammalian order Carnivora inferred from multiple nuclear gene sequences. *Molecular Phylogenetics and Evolution* 56:49–63 DOI 10.1016/j.ympev.2010.01.033.

Flynn JJ, Finarelli JA, Zehr S, Hsu J, Nedball MA. 2005. Molecular phylogeny of the Carnivora (Mammalia): assessing the impact of increased sampling on resolving enigmatic relationships. *Systematic Biology* 54(2):317–337 DOI 10.1080/10635150590923326.

Gaubert P, Cordeiro Estrela P. 2006. Phylogenetic systematics and tempo of evolution of the Viverrinae (Mammalia, Carnivora, Viverridae) within feliformians: implications for faunal exchanges between Asia and Africa. *Molecular Phylogenetics and Evolution* 41:266–278 DOI 10.1016/j.ympev.2006.05.034.

Gaubert P, Veron G. 2003. Exhaustive sample set among Viverridae reveals the sister-group of felids: the linsangs as a case of extreme morphological convergence within Feliformia. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:2523–2530 DOI 10.1098/rspb.2003.2521.

Gregory WK, Hellman H. 1939. On the evolution and major classification of the civets (Viverridae) and allied fossil and recent Carnivora; a phylogenetic study of the skull and dentition. *Proceedings of the American Philosophical Society* 81:309–392.
Hunt RM. 1991. Evolution of the aeluroid Carnivora: viverrid affinities of the Miocene carnivoran: Herpestides. American Museum Novitates 3023:1–34.

Hunt Jr RM. 1996. Biogeography of the order Carnivora. In: Gittleman JL, ed. Carnivore behavior, ecology, and evolution. Vol. 2. Cornell University Press, 485–541.

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O'Brien SJ. 2006. The late Miocene radiation of modern Felidae: a genetic assessment. Science 311:73–77 DOI 10.1126/science.1122277.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649 DOI 10.1093/bioinformatics/bts199.

Kinnaird MF, O’Brien TG. 2007. Ecology and conservation of Asian hornbills. Farmer of the forest. In: Chapter feeding ecology: how to survive on fruits. Chicago and London: Chicago Press.

Koepfli KP, Jenks SM, Eizirik E, Zahirpour T, Valkenburgh BV, Wayne RK, Molecular systematics of the Hyaenidae. 2006. Relationships of a relictual lineage resolved by a molecular supermatrix. Molecular Phylogenetics and Evolution 38:603–620 DOI 10.1016/j.ympev.2005.10.017.

McKenna MC, Bell SK. 1997. Classification of mammals above the species level. New York: Columbia University Press.

Mohd Salleh F, Ramos-Madrigal J, Peñaloza F, Liu S, Mikkel-Holger SS, Riddhi PP, Martins R, Lenz D, Fickel J, Roos C, Shamsir MS, Azman MS, Lim BK, Stephen JR, Wilting A, Gilbert MTP. 2017. An expanded mammal mitogenome dataset from Southeast Asia. GigaScience 6:1–8.

Morlo M, Miller ER, El-Barkooky AN. 2007. Creodonta and Carnivora from Wadi Moughra, Egypt. Journal of Vertebrate Paleontology 27(1):145–159 DOI 10.1671/0272-4634(2007)27[145:CAFWM]2.0.CO;2.

Nyakatura K, Bininda-Emonds OR. 2012. Updating the evolutionary history of Carnivora (Mammalia): a new species-level super tree complete with divergence time estimates. BMC Biology 10:12.

Patou ML, Debruyne R, Jennings AP, Zubaid A, Rovie-Ryan JJ, Veron G. 2008. Phylogenetic relationships of the Asian palm civets (Hemigalinae & Paradoxurinae, Viverridae, Carnivora). Molecular Phylogenetics and Evolution 47(3):883–892 DOI 10.1016/j.ympev.2008.03.026.

Pocock RI. 1933. The rarer genera of oriental Viverridae. Journal of Zoology 103(4) 969–1035 DOI 10.1111/j.1096-3642.1933.tb01638.x.

Raffles TS. 1822. In Sir T. S. Raffles’s descriptive catalogue of a zoological collection made in Sumatra. The Transactions of the Linnean Society of London XIII:253–254.

Rice P, Longden I, Bleasby A. 2000. EMBoss: the European molecular biology open software suite. Trends in Genetics 16(6):276–277 DOI 10.1016/S0168-9525(00)02024-2.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press.
Silvestro D, Michalak I. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12(4):335–337 DOI 10.1007/s13127-011-0056-0.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680 DOI 10.1093/nar/22.22.4673.

Veron G. 2007. Phylogeny of the viverridae and ‘viverrid-like’ feliforms. *Journal of Vertebrate Paleontology* 3:162A–162A.

Veron G, Bonillo C, Hassanin A, Jennings AP. 2017. Molecular systematics and biogeography of a Hemigalinae civets. *European Journal of Taxonomy* 285:1–20 DOI 10.5852/ejt.2017.285.

Veron G, Heard S. 2000. Molecular systematics of the Asiatic Viverridae (Carnivora) inferred from mitochondrial Cytochrome b sequence analysis. *Journal of Zoological Systematics and Evolutionary Research* 38(4):209–217 DOI 10.1046/j.1439-0469.2000.384132.x.

Willcox DH, Chutipong W, Gray TN, Cheyne S, Semiadi G, Rahman H, Coudrat CN, Jennings A, Ghimirey Y, Ross J, Fredriksson G. 2016. Arctictis binturong. The IUCN Red List of Threatened Species. Available at https://www.iucnredlist.org/species/41690/45217088.

Wozencraft WC. 2005. Order Carnivora. In: Wilson DE, Reeder DM, eds. *Mammal species of the world—a taxonomic and geographic reference*. Baltimore: Johns Hopkins University Press, 532–628.

Yoder AD, Burns MM, Zehr S, Delefosse T, Veron G, Goodman SM, Flynn JJ. 2003. Single origin of Malagasy Carnivora from an African ancestor. *Nature* 421:734–737 DOI 10.1038/nature01303.

Zhou Y, Wang SR, Ma JZ. 2017. Comprehensive species set revealing the phylogeny and biogeography of Feliformia (Mammalia, Carnivora) based on mitochondrial DNA. *PLOS ONE* 12:e0174902 DOI 10.1371/journal.pone.0174902.