Polymorphism of Randomly Amplified Polymorphic DNA (RAPD) fragment is confirmed by presence or absence of bands resulting from deletion, insertion or differences in the nucleotide sequence in or between priming regions (Clark and Lanigan, 1993). Some highly conserved regions on the mitochondrial (mt) cytochrome b gene are suitable for species identification in vertebrates. The PCR technique is quite reliable, accurate and authentic, but requires species-specific or conserved oligonucleotide primers for identification of species. The mt DNA PCR technique was used in the present study to identify leopard, wolf and tiger.

For the present work, blood samples of leopard and wolf were collected from Maharaj Bagh Zoo, Nagpur and Government Polyclinic, Chandarpur for their identification by using mt DNA PCR technique. The DNA was isolated from blood samples of tiger, leopard and wolf by standard methods of Proteinase K digestion, phenol-chloroform extraction and DNA precipitation in ethanol. The DNA yield ranged from 100 μg to 400 μg from 1 ml of blood samples. Isolated DNA was subjected to PCR assay to amplify gene fragment of different size in different species by using established species-specific forward and reverse primers. The amplified PCR products from the animals were found to range from 110 to 243 bps. The band pattern for tiger, leopard and wolf was produced at 217, 110 and 278 bps, respectively indicating a clear-cut demarcation among the animals. There was no cross species amplification in any of the reaction. The results of the present study indicated that the species specific primer would be useful in identification of carnivore animal species.

Key words: Carnivore, DNA, Identification, PCR
All the PCRs prominently amplified the target sequences. There was no cross species amplification in any of the reaction i.e. tiger specific PCR gave amplified product only in tiger DNA and not in any of the other DNA.

**iv) Primer sequence:** The primer sequences were derived from the gene bank by using primer BLAST. The sequences were as follows:

**a) PCR Reaction:** PCR was carried out in a final reaction volume of 25 μl. Each reaction volume contained 10X PCR buffer (with MgCl₂) 2.50 μl, dNTPs (10 mM each) 1.50 μl, Primer mix (10 pmole/μl) 2.00 μl, Taq DNA polymerase (5 U/μl) 1.50 μl, DNA Template 5.00 μl and DNase free water 11 μl.

**b) PCR protocol:** Initial denaturation (95°C for 10 min), then denaturation (95°C for 30 sec), annealing (60°C for 30 sec for tiger and leopard and 58°C for wolf for 30 sec), extension (72°C for 30 sec) and final extension (72°C for 10 min) with 35 cycles were done. For loading the sample, 5 μl diluted DNA was taken and after mixing it with 2 μl of 6 X gel loading dye in the electrophoresis tank containing 1X TBE buffer. A DNA marker was also run in one of the well.

The blood samples collected from tiger, leopard and wolf were subjected for isolation of mt DNA. For each of the PCR, amplicon of expected size was obtained. The specific PCR amplified fragments to each species produced a characteristic band pattern on agarose gel electrophoresis. Amplified PCR products from three species were found to range between 110 to 243 bps. The present work confirmed that the band pattern for tiger, leopard and wolf was 217, 110 and 278 bps, respectively indicating species specific differences. The observations recorded for tiger were in agreement with the earlier findings of Zhang and Shi (2009). In contrast, with the present findings Bhagavatula and Singh (2006) reported a band pattern at 636 bps and 759 bps in the Bengal tiger *Panthera tigris tigris*.

The blood samples collected from tiger, leopard and wolf were subjected for isolation of mt DNA. For each of the PCR, amplicon of expected size was obtained. The specific PCR amplified fragments to each species produced a characteristic band pattern on agarose gel electrophoresis. Amplified PCR products from three species were found to range between 110 to 243 bps. The present work confirmed that the band pattern for tiger, leopard and wolf was 217, 110 and 278 bps, respectively indicating species specific differences. The observations recorded for tiger were in agreement with the earlier findings of Zhang and Shi (2009). In contrast, with the present findings Bhagavatula and Singh (2006) reported a band pattern at 636 bps and 759 bps in the Bengal tiger *Panthera tigris tigris*.

The leopard showed band pattern at 110 bps as reported by Mukherjee *et al.* (2010). The species specific mt DNA primer produced a PCR product at 278 bps for wolf species in the present study as reported by Vila *et al.* (1999).

### ACKNOWLEDGEMENTS

The authors are deeply grateful to the Dean, Nagpur Veterinary College, Nagpur, Officers in Incharge of Maharaj Bagh Zoo, Nagpur and Government Polyclinic, Chandarpur for their co-operation and support in providing necessary facilities for the successful completion of this research work in time.

### REFERENCES


