

## DNA Barcoding of the two insectivorous Bats species from Punjab, Pakistan

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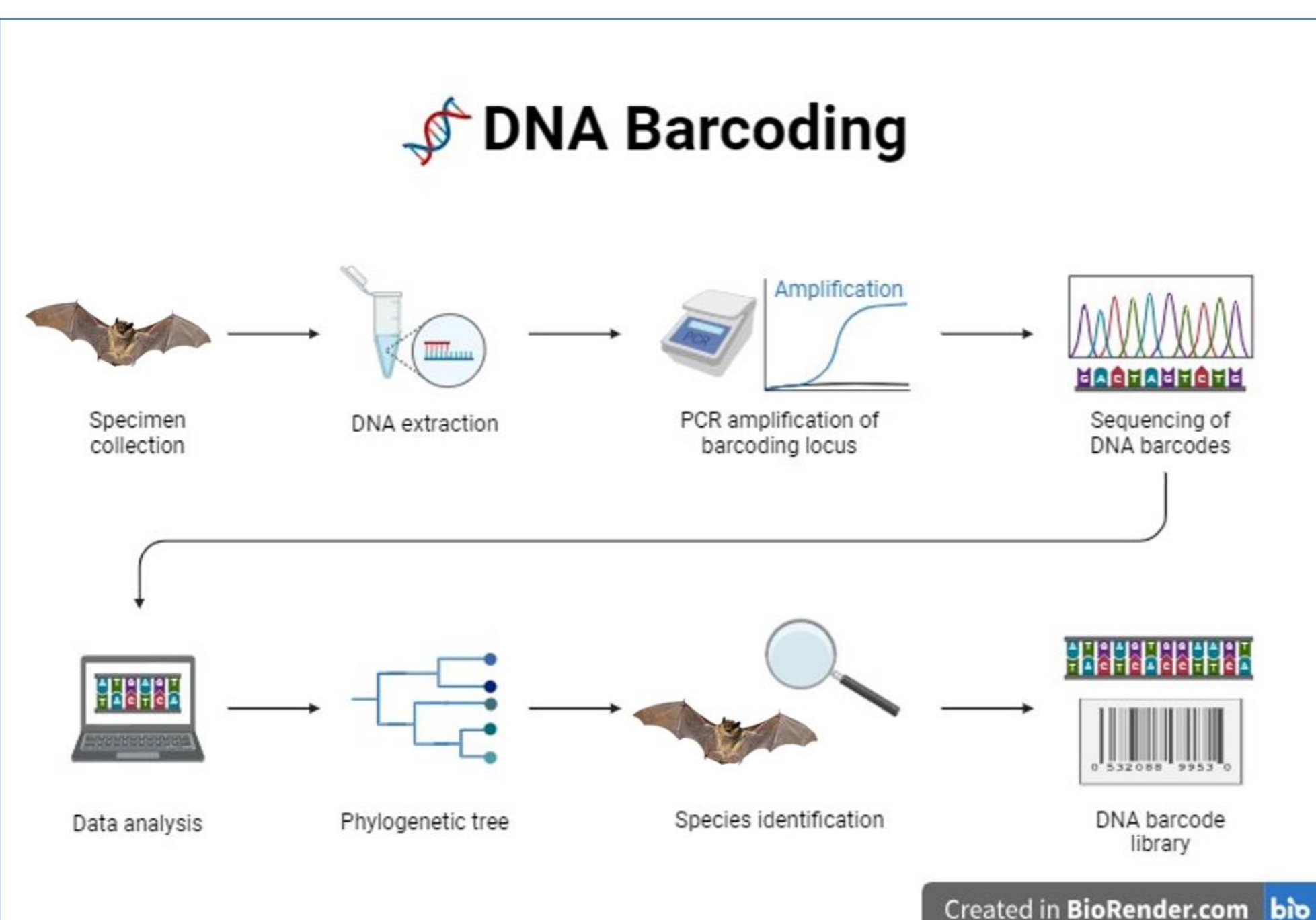
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### INTRODUCTION & AIM

The efficiency of the mitochondrial gene COI as a standard barcode gene has been set against many taxa. This was shown in studies by (Borisenko et al., 2008; Hebert et al., 2003). Identifying species by traditional morphological characters has been almost replaced by molecular taxonomy. This is due to the reliability and robustness of molecular markers. Mitochondrial DNA has a small genome. It has high mutation rates and sex-specific life histories. This makes it a powerful marker for population differentiation. The current study tested the COI gene's efficacy for identifying Chiroptera specimens from Punjab, Pakistan. Pakistan has huge diversity of bat species comprising 28% of mammalian species in the country. Much published data is available on the distribution and morphological taxonomy of bats species in Pakistan (Hamidullah et al., 2018; Javid et al., 2014; Mahmood-ul-Hassan et al., 2012). However, there is still little data on identifying species in this group using the power of COI gene.

### METHOD

The carcass bat samples were collected randomly from two areas in Punjab, Pakistan (District Pakpattan and District Bahawalpur) and further processed for DNA extraction in laboratory with DNA extraction kit. The barcode gene was amplified using the primer pair (PBCOIdF & PBCOIdR1) for bats collected from District Pakpattan and primer pair (PBCOIdF & PBCOIdR2) for bats collected from District Bahawalpur. The sequences generated in the study were clustered through Neighbour joining tree along with additional dataset from NCBI of similar and closely related species from other countries. The intra and interspecific genetic distances were also calculated.



### RESULTS & DISCUSSION

The barcode sequences of specimens collected from District Pakpattan (n=4) and District Bahawalpur (n=3) had revealed 100% identity match with species *Scotophilus heathii* and (97-100%) identity match with species *Taphozous nudiventris* on NCBI BLASTN respectively. Intraspecific and interspecific genetic distances of similar and closely related species from other countries had shown a definite relation with geographical distance (Table 1 and 2). The sequences generated in the study were clustered through Neighbour joining tree along with additional dataset from GenBank of similar and closely related species from other countries (Figure 1 and 2).

Table 1: Genetic Distances of Species *Scotophilus heathii* (current study) with sequences of similar and closely related Species from Public data.

Sr. No.	Congeneric Species	Number of Sequences (N)	Geographical Location	Genetic Distances (%) ± SE	Within Species Genetic Distances
1.	S. dinganii	8	Ethiopia	14.27 ± 2.12	1.245 ± 0.22
2.	Scotophilus sp.	42	Sudan	15.38 ± 1.73	10.51 ± 1.06
3.	S. khulii	2	India	20.12 ± 2.57	0
4.	S. viridis	6	South Africa	15.26 ± 2.16	0.163 ± 0.096
5.	S. leucogaster	4	South Africa	15.3 ± 2.16	0.303 ± 0.18
6.	S. dinganii	10	South Africa	16.75 ± 2.46	0.635 ± 0.21
7.	S. khulii	14	Vietnam	19.69 ± 2.48	3.59 ± 0.38
8.	S. dinganii	3	Kenya	13.73 ± 2.01	0
9.	S. khulii	2	Laos	20.77 ± 2.79	0.1785 ± 0.18
10.	S. heathii	4	Laos	6.38 ± 1.03	2.26 ± 0.42
11.	S. heathii	1	India	0.92 ± 0.35	NC
12.	S. heathii	15	Pakistan	0.61 ± 0.21	0.05 ± 0.03
13.	Scotophilus	1	Kenya	14.81 ± 2.03	NC
14.	S. nigrita	1	South Africa	20.31 ± 2.87	NC

Table 2: Genetic Distances of Species *Taphozous nudiventris* (current study) with sequences of similar and closely related Species from Public data

Sr. No.	Congeneric Species	Number of Sequences (N)	Geographical Location	Genetic Distances (%) ± SE	Within Species Genetic Distances
1.	Taphozous sp.	2	India	28.23 ± 4.01	1.74 ± 0.21
2.	Taphozous mauritanus	3	South Sudan	23.94 ± 3.19	0.408 ± 0.204
3.	Taphozous longimanus	1	India	22.32 ± 2.84	NC
4.	Taphozous mauritanus	3	South Africa	21.42 ± 3.24	0.35 ± 0.226
5.	Taphozous melanopogon	9	Vietnam	27.05 ± 3.57	0.03 ± 0.02
6.	Taphozous longimanus	2	Vietnam	22.61 ± 2.84	1.08 ± 0.4
7.	Taphozous hildegardeae	3	Kenya	21.45 ± 2.81	0
8.	Taphozous	2	Kenya	2.931 ± 0.76	0
9.	Taphozous nudiventris	1	Syria	3.41 ± 0.81	NC
10.	Taphozous nudiventris	2	Iran	0.19 ± 0.12	0.641 ± 0.278
11.	Taphozous nudiventris	1	India	0	NC

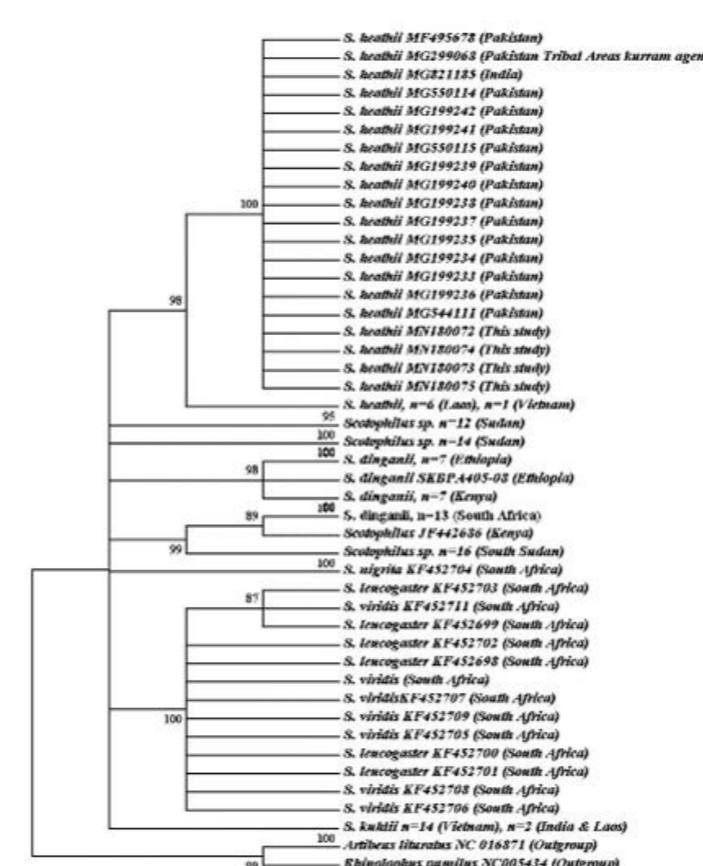


Figure 1. Neighbour-joining tree of Bats sequences (*Scotophilus heathii*) from current study and sequences of similar and closely related species from public data sources

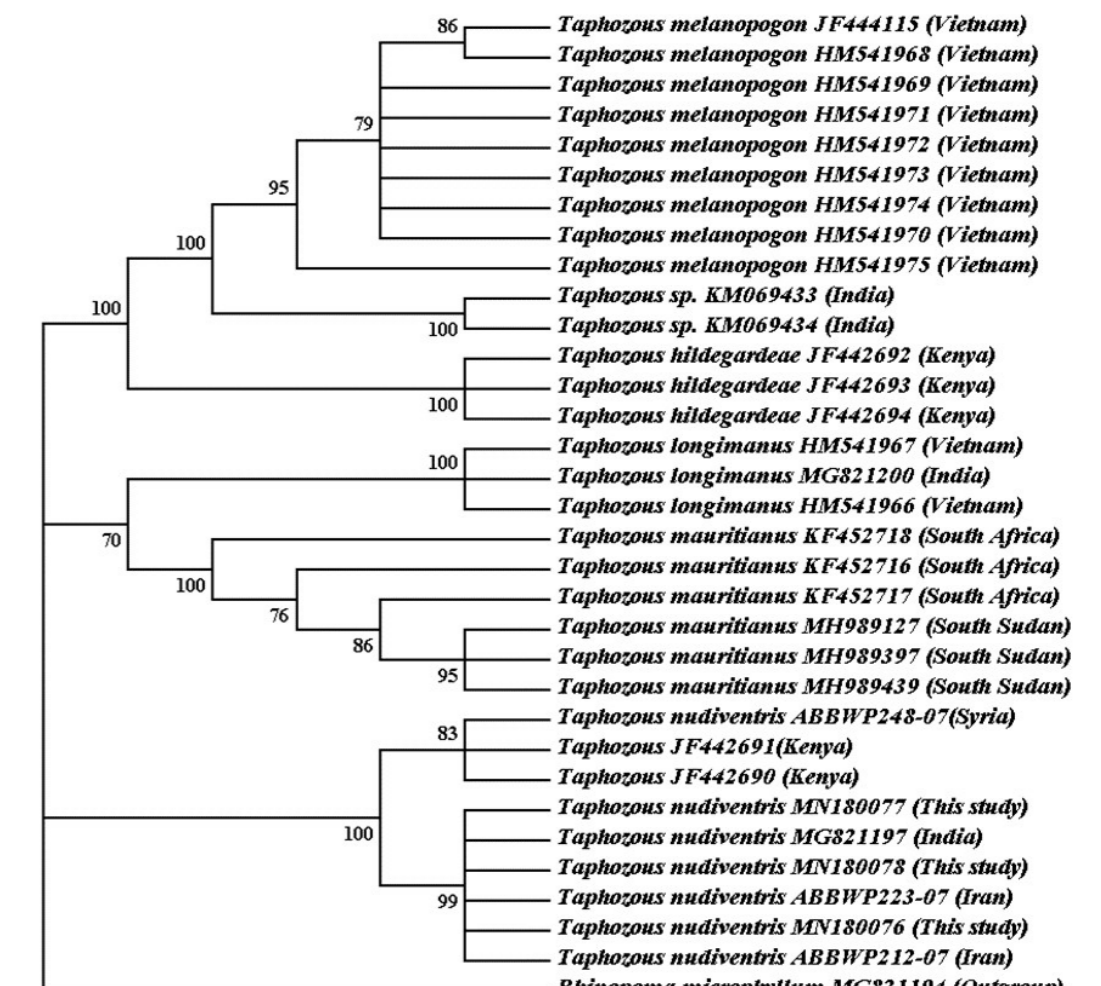


Figure 2. Neighbour-joining Tree of Bats sequences (*Taphozous nudiventris*) from current study and sequences of similar and closely related species from public data sources

### CONCLUSION

This study strongly supported the credibility of DNA barcoding for species identification and encourages the scientists in Pakistan to conduct more studies based on Molecular taxonomy.

### FUTURE WORK / REFERENCES

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