BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2024. Vol. **21**(1), p. 25-36

Molecular Identification of the Diversity of Insects, Spiders, Lizards, Birds and Mammals of Tuensang District, Nagaland, India

Chaueichongla Phom[1] and Jeyaparvarthi Somasundaram[2]*

Department of Zoology, St. Joseph University, Dimapur, India.

https://dx.doi.org/10.13005/bbra/3199

(Received: 29 August 2023; accepted: 15 March 2024)

DNA barcoding is a method of species identification that revolutionized the way we study and understand biodiversity. With advancements in molecular genetics and DNA sequence archives, it has become possible to use short DNA sequences to identify species, even when the specimens are difficult to distinguish by traditional morphological methods. In this article, we explore the utility of DNA barcoding in the Tuensang ecosystem of Nagaland, India and evaluate its effectiveness for species identification, informing ongoing conservation of populations and species. A total of 62 species, which included insects, spiders, lizards, birds and mammals, were collected from Tuensang areas of northeast India and identified using DNA barcodes. DNA was extracted from muscle tissue and PCR was done with two pairs of primers targeting the mitochondrial COI gene. Sanger sequencing was employed and the obtained sequences were analysed to identify the species and reconstruct the evolutionary relationships amongst them. Our results provided molecular characterization of species from Tuensang areas of Nagaland for the first time.

Keywords: Biological resources; COI; DNA barcoding; Evolutionary relationship; Extinction; Sanger sequencing.

"Biodiversity" refers to the variety of living organisms from all sources, including terrestrial, marine, and other aquatic ecosystems as well as the ecological complexes to which they belong. At the same time, the presence of various faunal genetic resources located in any region or country is a component of animal biodiversity. Genetic diversity is the basis for a population to evolve and adapt to rapid changes in the environment[25]. Today's biodiversity provides the opportunity for sustaining vital environmental services that support life on Earth. Therefore, it is crucial to prioritise species protection and sustainable use. The identification of species paves the way to a deeper understanding of evolutionary processes among varied animal lineages by recording information on patterning and the range of variation.

Various methods have been developed over the decades for the characterization of wild animals which are largely categorized into phenotypic, biochemical and molecular markers.

*Corresponding author E-mail: jeya5001@gmail.com

The history of taxonomy and evolutionary research is rooted in the study of morphology. For many years, the classification of species has been most heavily influenced by morphology in its widest meaning, which includes all expressions of structure and form. Morphology was used as a fundamental organising principle to arrange an apparently chaotic variety of living forms into higher ("macrotaxonomic") levels initially[17]. Recently, the DNA barcoding approach based on the cytochrome c oxidase subunit I (COI) gene present in mitochondrial DNA has become adopted as a global biological identification method for all species due to its accuracy when compared to existing classical taxonomic methods[21]. This approach uses DNA extraction, sequencing and barcoding for genomic characterisation[8], and it has become as an efûcient and reliable tool for identifying, conûrming and resolving closely related taxa[19]. Molecular techniques are increasingly employed in conservation of biological diversity in response to the alarming extinction of different species. Therefore, many conservation geneticists study genetic markers to make decisions for conservation of endangered wild animals. Here, choosing of effective profiling technique is a very critical step, as incorrect data on molecular information may result in incorrect conservation actions[29]. In this regard, the application of both karyotypes and molecular genetic markers to conserve wild animals has been put into practice[24]. Identification and characterization of species are two important steps towards crafting sustainable conservation strategies for wild animals. Studying genetic diversity within and between populations, estimation of effective population size and assessment of possible bottlenecks are practices carried out for characterization of wild animal populations.

Molecular characterization is based on studies of both mitochondrial DNA (mtDNA) and nuclear DNA[1]. In recent years, advances in molecular techniques have encouraged the application of simple and precise DNA analysis in the taxonomic field. Among the existing genome-based approaches, DNA barcoding stands as a robust strategy to identify existing species and to discover unknown species through comparative analysis of sequence variation[2,18]. Extensive research has shown that DNA barcoding can identify a wide range of animal species, including mammals, reptiles, birds, fishes, amphibians and crustaceans[12,28,30]. The classification and identification of various life forms, particularly insects have been a major challenge to the scientific community especially with the dwindling interest and funding for taxonomy[16]. DNA barcoding provides an alternative approach, allowing expedited examination of species richness of all animal lineages at comparatively low cost. Its capacity to enhance progress depends on the fact that members of most species form a distinct barcode cluster[7,22,23]; this relationship has now been operationalized for large-scale surveys by allotting a barcode index number (BIN)[9,20].

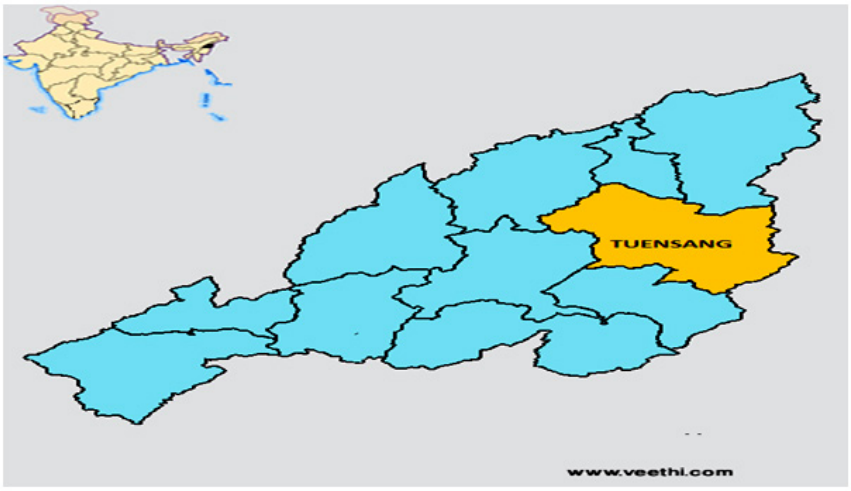

Fig. 1. Map of Tuensang District, Nagaland, India

Table 1. Details of accession numbers and amplicon sequence length of insects, spiders, lizards, birds and mammals taken for molecular study

No	Common name	Scientific name	Family	Accession numbers obtained for each samples	Sequence length	*IUCN 3.1
1	Red-whiskered bulbul bird	*Pycnonotus jocosus*	Pycnonotidae	OQ874330	623 bp	Least concern
2	Indian scops owl	*Otus bakkamoena*	Strigidae	OQ821222	642 bp	Least concern
3	Streak-throated barwing bird	*Actinodura waldeni*	Leiothrichidae	OQ874334	608 bp	Least concern
4	Scaly thrush bird	*Zoothera dauma*	Turdidae	OQ821223	639 bp	Least concern
5	Blyth's tragopan	*Tragopan blythii*	Phasianidae	OQ874335	655 bp	Vulnerable
6	Mountain bamboo partridge bird	*Bambusicola fytchii*	Phasianidae	OQ874336	616 bp	Least concern
7	Red-faced liocichla bird	*Liocichla phoenicea*	Leiothrichidae	OQ874338	609 bp	Least concern
8	Mrs. Gould's sunbird	*Aethopyga gouldiae*	Nectariniidae	OQ874339	632 bp	Least concern
9	Red-vented bulbul bird	*Pycnonotus cafer*	Pycnonotidae	OQ821233	652 bp	Least concern
10	Beautiful nuthatch bird	*Sitta formosa*	Sittidae	OQ920213	624 bp	Vulnerable
11	Great barbet bird	*Psilopogon virens*	Megalaimidae	OQ920214	648 bp	Least concern
12	kalij pheasant bird	*Lophura leucomelanos*	Phasianidae	OQ920215	640 bp	Least concern
13	Grey-sided bush warbler bird	*Cittia brunnifrons*	Cettiidae	OQ920217	621 bp	Least concern
14	Golden-throated Barbet bird	*Magalaima franklinii*	Megalaimidae	OQ920218	645 bp	Least concern
15	Grey-winged blackbird	*Turdus boulboul*	Turdidae	OQ920219	624 bp	Least concern
16	Maroon oriole	*Oriolus traillii*	Oriolidae	OQ920220	659 Bp	Least concern
17	Black bulbul bird	*Hypsipetes leucocephalus*	Pycnonotidae	OQ780819	632 bp	Least concern
18	White-headed bulbul bird	*Hypsipetes thompsoni*	Pycnonotidae	OQ780820	624 bp	Least concern
19	Orange-bellied leafbird	*Chloropsis hardwickii*	Chloropseidae	OQ780821	654 bp	Least concern
20	Rufous-breasted antthrush bird	*Formicarius rufipectus*	Formicariidae	OQ780843	620 bp	Least concern
21	Spotted linsang	*Prionodon pardicolor*	Prionodontidae	OQ874331	646 bp	Least concern
22	Masked palm civet	*Paguma larvata*	Viverridae	OQ874332	626 bp	Least concern
23	Southern red muntjac	*Muntiacus muntjak*	Cervidae	OQ874333	623bp	Least concern
24	Northern flying squirrel	*Glaucomys sabrinus*	Sciuridae	OQ821224	652 bp	Least concern
25	Swinhoe's striped squirrel	*Tamiops swinhoei*	Sciuridae	OQ821225	657 bp	Least conncern
26	Sambar deer	*Rusa unicolor*	Cervidae	OQ874337	624 bp	Vulnerable
27	Himalayan striped squirrel	*Tamiops mcclellandii*	Sciuridae	OQ920212	624 bp	Least concern
28	Orange-bellied Himalayan squirrel	*Dremomys lokriah*	Siuridae	OQ920216	624 bp	Least concern
29	Tanezumi rat	*Rattus tanezumi*	Muridae	OQ780822	623bp	Least concern

30	Big brown bat	*Eptesicus fuscus*	Vespertilionidae	OQ780827	613 bp	Least concern
31	Cave myotis bat	*Myotis velifer*	Vespertilionidae	OQ780828	620 bp	Least concern
32	Spotted forest skink	*Sphenomorphus maculatus*	Scincidae	OQ780823	619 bp	Not evaluated
33	Farooq's garden lizard	*Calotes farooqi*	Agamidae	OQ780824	626 bp	Not evaluated
34	Giant Golden Orbweaver spider	*Nephila pilipes*	Araneidae	OQ780848	631 bp	Least concern
35	Cape Rainspider	*Palystes castaneus*	Sparassidae	OQ821217	648 bp	Not evaluated
36	Joro spider	*Trichonephila clavata*	Araneidae	OQ821219	658 bp	Least concern
37	Kogane-gumo spider	*Argiope amoena*	Araneidae	OQ821220	658 bp	Not evaluated
38	Brown widow spider	*Latrodectus geometricus*	Theridiidae	OQ780838	617 bp	Not evaluated
39	Ant	*Platythyrea punctata*	Formicidae	OQ780845	619 bp	Not evaluated
40	Ant	*Messor ebeninus*	Formicidae	OQ780846	612bp	Not evaluated
41	Common sailor butterfly	*Neptis hylas*	Nymphalidae	OQ821228	658 bp	Not evaluated
42	Gypsy moth	*Lymantria dispar*	Erebidae	OQ821229	654 bp	Not evaluated
43	Small ant	*Tapinoma sessile*	Formicidae	OQ780847	624 bp	Not evaluated
44	Common batwing butterfly	*Atrophaneura varuna*	Papilionidae	OQ821232	658 bp	Least concern
45	Tropical swallowtail moth	*Lyssa zampa*	Uraniidae	OQ825991	658 bp	Not evaluated
46	Giant peacock moth	*Saturnia pyri*	Saturniidae	OQ825992	658 bp	Not evaluated
47	Brown tussock moth	*Olene mendosa*	Erebidae	OQ825993	658 bp	Not evaluated
48	Thief ant	*Solenopsis molesta*	Formicidae	OQ825994	576 bp	Not evaluated
49	Northern warrior wasp	*Synoeca septentrionalis*	Vespidae	OQ780849	626 bp	Least concern
50	Giant Asian mantis	*Hierodula patellifera*	Mantidae	OQ825995	658 bp	Not evaluated
51	Common hairy caterpillar	*Spilarctia obliqua*	Erebidae	OQ825989	658 bp	Least concern
52	Kaempfer cicada	*Platypleura kaempferi*	Cicadidae	OQ825986	685 bp	Not evaluated
53	Giant shield bugs	*Pycanum ochraceum*	Tessaratomidae	OQ825985	630 bp	Not evaluated
54	Shield bugs	*Pentatoma metallifera*	Pentatomidae	OQ825983	641 bp	Not evaluated
55	Leopard lacewing butterfly	*Cethosia cyane*	Nymphalidae	OQ825990	642 bp	Not evaluated
56	Black soldier fly	*Hermetia illucens*	Stratiomyidae	OQ825987	658 bp	Not evaluated
57	Silver- spotted skipper butterfly	*Epargyreus clarus*	Hesperiidae	OQ825988	658 bp	Least concern
58	Cicada	*Okanagana rimosa*	Cicadidae	OQ780833	624 bp	Not evaluated
59	Grasshopper	*Dociostaurus maroccanus*	Acrididae	OQ780836	590 bp	Not evaluated
60	Tobacco grasshopper	*Atractomorpha crenulata*	Pyrgomorphidae	OQ780837	597 bp	Not evaluated
61	Southern green stink bug	*Nezara viridula*	Pentatomidae	OQ825984	658 bp	Least concern
62	Florida woods cockroach	*Eurycotis floridana*	Blattidae	OQ780841	630 bp	Not evaluated

*Consevation status

There has never been a molecular marker-based study of native faunal species of Tuensang areas of northeast India. Hence, our study aimed to characterize the diversity of animals from these areas using the DNA barcoding technique. Further, the inherent phylogenetic relationships present among those organisms were analyzed. The main objective of this study was to develop a reference library with generated barcodes.

MATERIALS AND METHODS

Study area

One of Nagaland's largest and most eastern districts is Tuensang, Northeast India. The district of Tuensang covers 4,228 square kilometres and can be found at latitude of 26º 14¹ 8.67ºN and longitude of 94º 48¹ 47.47ºE, with elevations ranging from 800 to 3500m above mean sea level

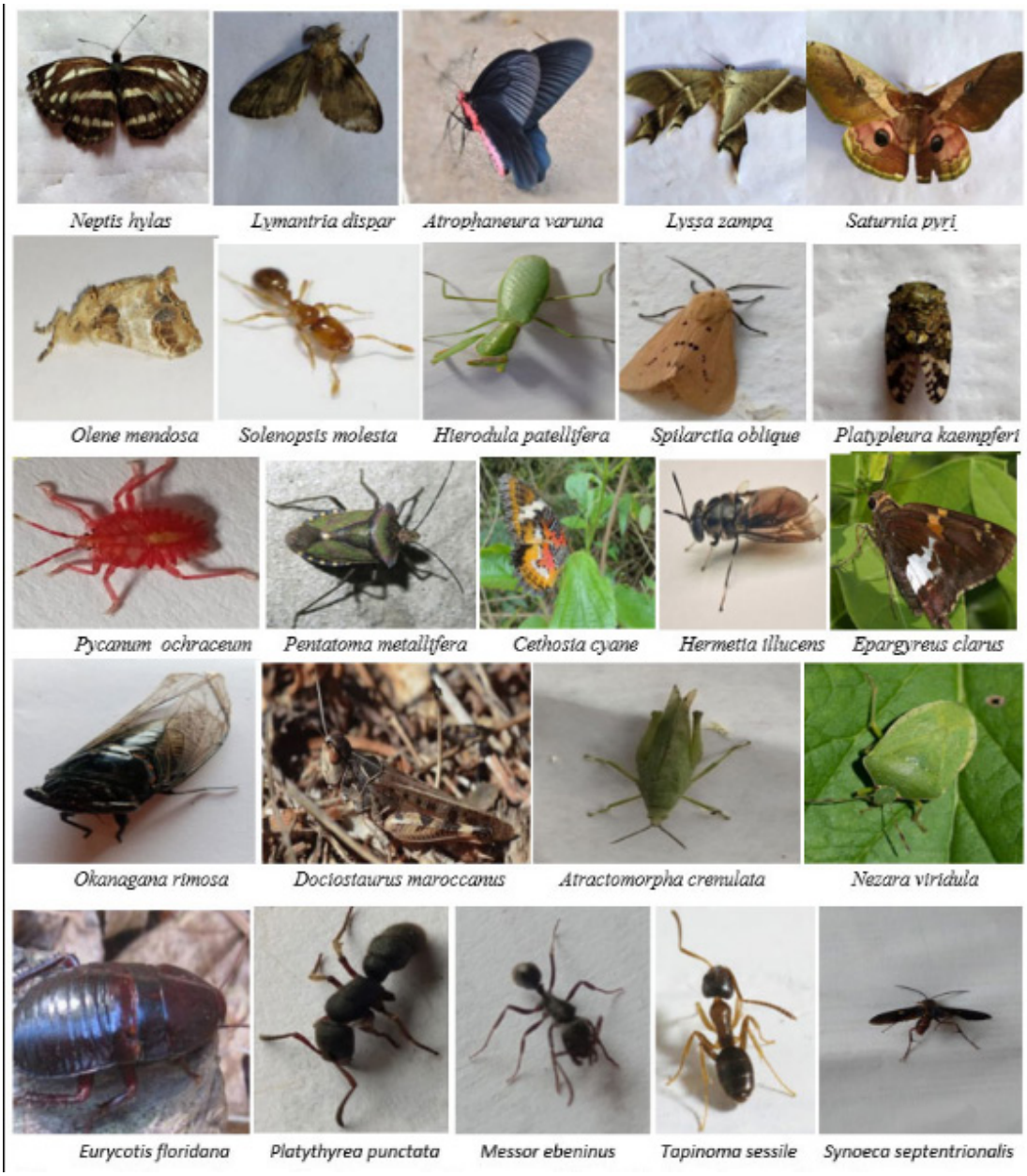

Fig. 2. Insects species from study areas identified using molecular markers

Species collection

For molecular characterization, animal species were collected from selected areas of Tuensang district, Nagaland, viz., Tuensang town, Tuensang village, Hakchang village, Helipong village, Ngangpong village, Sangchen compound village, Chendang village, Chingmei village and Momching village. Field research was conducted in this study area to collect the species from forest and agricultural field habitats using different techniques, such as hand collection, pitfall trap, sweep netting, snares, catapults, and air guns. The collected animal tissues were immediately stored in 100% ethanol. For carrying out this research work, a consent letter was obtained from the community leader of the district. The study was conducted from 2019 to 2022.

DNA isolation

The extraction of DNA from collected animal tissue was performed using the Qiagen Tissue Kit following the manufacturer's protocol. 100 mg of tissue sample was mixed with 180ìl of ATL buffer, 20ìl of Proteinase K, vortexed, and incubated at 60°C in a dry bath for 4 hours. 200ìl of AL buffer was added and mixed in, followed by a 20-minute incubation at 60°C. 200ìl of ethanol was poured and put to a DNA silica column for 1 minute of spinning at 12000 rpm. 700 ìl of AW1 washed twice, with the AW2 buffer spinning at 12000 rpm for 1 minute. By adding elution buffer to 20 ìl of DNA, DNA was eluted and measured using the 1% agarose gel electrophoresis technique.

PCR Amplification and Gel purification

PCR amplification of the isolated DNA samples was done using universal COI primers[26]. The amplification reaction was performed in 25 ìl reaction mixtures with DNA template of 40-80ng. The reaction mixture included 1ìl of both forward and reverse primers and 25ìl of the master mix containing 1ìl dNTPs, 0.5ìl *Taq* DNA polymerase (Barcode Biosciences), 5ìl 10x buffer and 14.5ìl distilled water. The primers were standard primers available for COI gene amplification, as below:
COIF- GGTCAACAAATCATAAAGATATTGG- Tm 51˚C and
COIR- TAAACTTCAGGGTGACCAAAAAATCA- Tm 53˚C.

The PCR amplification conditions included an initial denaturation step at 94ºC for 3.5 minutes, 60ºC for 30 seconds, and 72ºC for 1 minute for 35 cycles. On a 1% agarose gel electrophoresis stained with ethidium bromide, PCR product quality was examined.

Sanger Sequencing

An Applied Biosystems 3130xl Genetic Analyzer was used to carry out bidirectional sequencing of PCR products at BioEdge Solutions, Bangalore, India. The data from the sequencing machine was collected and processed in Finch TV (https://finchtv.software.informer.com/1.4/). The obtained electropherogram files were analysed for base-calling peaks in ABI format, which was further converted to pdf and fasta files using Sequence Scanner 2 Software (https://sequence-scanner-software.software.informer.com/2.0/). The sequence data obtained during this study were subjected to NCBI-BLAST in the nucleotide database of GenBank (http://blast.ncbi.nlm.nih.gov/) to identify closely related species.

Construction of phylogenetic tree

To comprehend the link between unknown sequences and relevant top species found by NCBI-BLAST, all the sequences were aligned using the Clustal Omega software ((https://www.ebi.ac.uk/Tools/msa/clustalo/). In this manner, phylogenetic trees were created informing us of the evolutionary relationships among closely related species.

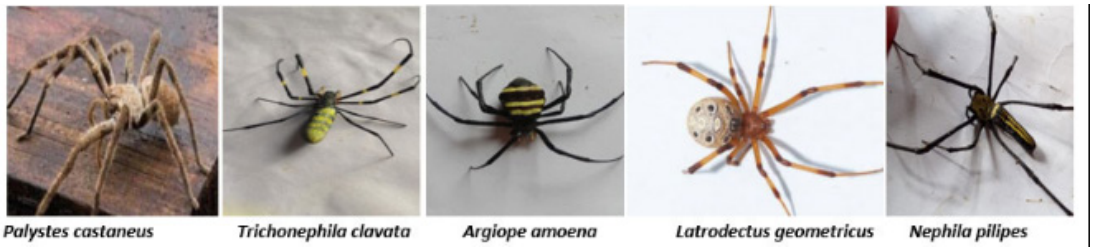

Fig. 3. Spiders species from study areas identified using molecular markers

Fig. 4. Lizards and birds species from study areas identified using molecular markers

RESULTS AND DISCUSSION

DNA barcodes provided a quick, accurate and affordable way to identify species, even those that are challenging to differentiate based on morphology or life stage. A total of 62 species, which included animals belonging to different classes, such as Insecta, Arachnida, Reptilia, Aves and Mammalia, were identified using DNA barcoding technique for the first time from the study areas of Tuensang, Nagaland. The sequences obtained from samples were submitted to GenBank, and their accession numbers and sequence lengths are reported in Table 1. Among 62 sequences, 8 COI sequences that were previously unknown were added to the GenBank database. These sequences species were *Cettia brunnifrons* (Hodgson, 1845), *Psilopogon virens* (Boddaert, 1789), *Megalaima franklinii* (Blyth, 1842), *Turdus boulboul* (Latham, 1790), *Hypsipetes leucocephalus* (Gmelin, JF, 1789), *Sitta formosa* (Blyth, 1843), *Oriolus traillii* (Vigors, 1832) and *Hypsipetes thompsoni* (Bingham, 1900). These accession numbers include: OQ920217, OQ920214, OQ920218, OQ920219, OQ780819, OQ920213, OQ920220 and OQ780820, respectively.

The species identified included, 20 birds, 11 mammals, 5 spiders, 2 lizards and 24 insects. From the present studied locations, *Tragopan blythii* (Jerdon, 1870), *Sitta formosa* (Blyth, 1843) and *Rusa unicolor* (Kerr, 1792) were reported and declared as Vulnerable species by IUCN.3.1 (Red List, 1964). These species are declining due to overexploitation, traditional Jhum shifting cultivation and hunting activities.

All the DNA barcoding sequences were aligned and used to create phylogenetic trees informing us of the evolutionary relationships among closely related species, as shown in Figure 5. Extensive research has shown that DNA barcoding can identify a wide range of animal species such as mammals, reptiles, birds, fishes, amphibians and crustaceans[12,28,30]. One of the most effective and advanced methods for identifying insects is DNA barcoding, which uses the mitochondrial gene cytochrome c oxidase I (COI) sequence as a DNA identifier[10]. The sequences discovered will help to clarify the evolutionary history, for example,

Fig. 5. Mammals species from study areas identified using molecular markers

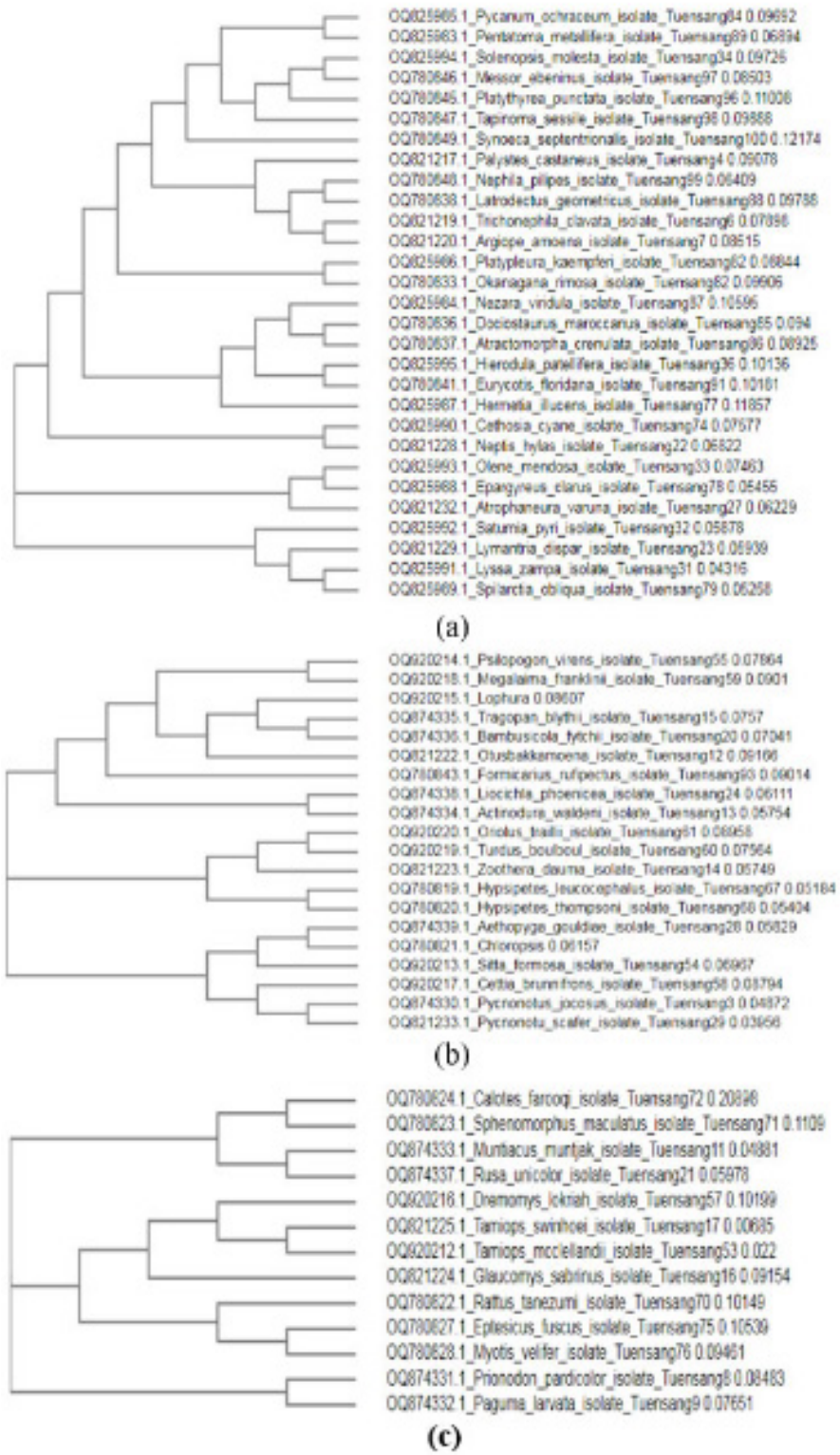

Fig. 6. The evolutionary relationships of: a, insects and spiders; b, birds; and c, mammals and lizards taken for molecular study

of spider mites and will enable quick species identification using molecular methods[11]. DNA barcodes cannot replace the need for taxonomists, but DNA barcodes provide an additional suite of characters that can be used in taxonomy, assisting identification of species by non-specialists with accurate identification of their specimens. When it comes to saving animals under difficult conditions, genetic information is extremely valuable[13].

A newly detailed understanding of species diversity can illuminate processes important in speciation, as suggested by the discovery that the most diverse lineages of animals. By using various molecular markers, it is possible to detect unique genetic variation in endangered population or species[4]. Knowledge of genetic diversity directs us to develop breeding programs minimizing inbreeding and safeguarding the loss of genetic variation. It is evident that the combination of morphological and molecular data sets permits a clearer recognition of evolutionary diversity among organisms. Further study is necessary to reveal the diversity among family members[14]. Applications of the mitochondrial gene-based species identification techniques cover a wide spectrum, and offer a fascinating perspective for future animal study[6,15].

The mitochondrial gene cytochrome c oxidase I (COI) barcode reference library for animals has become a standard resource for DNA metabarcoding applications recommending as standard metabarcode for metazoans[3]. These advances have opened up numerous new applications in aquatic sciences and ecological monitoring[27]. Still, further work is needed to optimize use of COI barcodes for these applications[5].

CONCLUSION

Molecular markers are very useful, providing information on genetic variability among and within animal populations, helping us to develop appropriate conservation strategies. Choosing an efficient profiling technique is important, as selection of an inappropriate technique may lead to incorrect conservation actions. It is important to highlight, however, that DNA barcoding should not replace traditional taxonomy, as integrating both methods can provide a more thorough knowledge of species diversity. The current study improved our knowledge of the faunal diversity of selected areas of Tuensang district, Nagaland. Non-taxonomists, researchers, biodiversity managers and policymakers will use the information provided to strengthen their efforts to develop efficient protective measures for this faunal biodiversity.

ACKNOWLEDGMENT

The author would like to thank the Head, Department of Zoology, St.Joseph University, Dimapur, Nagaland for her guidance and support.

Conflict of Interest

The authors declare no conflict of interest.

Funding Source

There are no funding sources.

Authors' Contribution

Chaueichongla Phom (Research scholar) and Dr. Jeyaparvarthi Somasundaram (Supervisor)

Data Availability Statement

Not applicable.

Ethics Approval Statement

The study does not involves an experiment on humans and animals. No Ethical approval was conducted.

REFERENCES

1. Abobakr Y., Al-Sarar A.S, Alzabib A.A and Saleh A.A. Morphological and molecular characterization of the invasive pestiferous land snail *Macrochlamys indica* Godwin-Austen, 1883 (Gastropoda: Ariophantidae) from Saudi Arabia. *Agriculture*. 2022; 12(11): 1756.
2. Abubakar B.M., Salleh F. M, Omar M S and Wagiran A. DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products. *Evidence-Based Complementary and Alternative Medicine*. 2017; 2017:1352948.
3. Andújar C., Arribas P., Yu D.W.,Vogler A.P and Emerson B.C. Why the COI barcode should be the community DNA metabarcode for the metazoa. *Mol. Ecol*. 2018; 27: 3968–3975.
4. Asmelash B., Diriba S and Pal S. K. Molecular markers based characterization and conservation of wild animals. *Research Journal of Recent Sciences.* 2017; 6(7):53-62.
5. Collins R.A., Bakker J., Wangensteen O.S., Soto A.Z., Corrigan L., Sims D.W., Genner M.J., Mariani S. Non-specific amplification compromises environmental DNA metabarcoding

with COI. *Methods Ecol. Evol.* 2019; 10:1985–2001.

6. Elyasigorji Z., Izadpanah M., Hadi F and Zare M. Mitochondrial genes as strong molecular markers for species identification. *Oxidative Stress in Applied Basic Researh and Clinical Practice*, 2023; 66:81-93.
7. Hebert P.D.N., Cywinska A., Ball S.L and deWaard J.R. Biological identifications through DNA barcodes. *Proc Biol Sci*. 2003; 270(1512): 313–321.
8. Hebert P.D.N., Ratnasingha S., Zakharov E.V., Telfer A., Levesque-Beaudin V., Milton M.A., Pedersen S., Jannetta P., deWaard J.R. Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society B: Bilolgical Sciences*. 2015; 371(1702): 0150333.
9. Hendrich L, Moriniere J, Haszprunar G, Hebert PDN, Hausmann A, Kohler F, Balke M. A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species in BOLD. *Mol. Ecol. Res*. 2015; 15: 795–818.
10. Hussain, K., Rashid, K., Hafeez, F, Amad I and Ashraf M. Molecular identification of sugarcane black bug (*Cavelarius excavates*) from Pakistan using cytochrome C oxidase I (COI) gene as DNA barcode. *Int J Trop Insect Sci*., 2020; 40: 1119–1124.
11. Ýnak E., Çobanoðlu S., Auger P and Migeon A. Molecular identification and phylogenetic analysis of spider mites (Prostigmata: Tetranychidae) of Turkey. *Exp Appl Acarol.,* 2022*;* 87:195–205.
12. Ivanova N.V., Clare E.L and Borisenko A.V. DNA barcoding in mammals. *Methods in Molecular Biology*. 2012; 858: 153–182.
13. Jayasundara S L, Algewatta H R, Jayawardana S, Perera M and Peiris L D C. Molecular identiûcation and evolutionary divergence of the Sri Lankan sambar deer, *Rusa unicolor* (Kerr 1792). *Animals,* 2023; 13: 2877.
14. Johansen J.R., Bohunická M., Lukešová A., Hrèková K., Vaccarino M.A and Chesarino N.M. Morpgological and molecular characterization within 26 strains of the Genus *Cylindrospermum* (Nostocaceae, Cynobacteria),with descriptions of three new species. *J.Phycol.,* 2014; 50(1):187-202.
15. Malewski T, Dzikowski A and Soltyszewski I. Molecular methods of animal species identification. *Polish Journal of Natural Science*, 2021; 36(1):79-94.
16. Mani M.,Venkatesan T., and Chethan B.R. Molecular identifications of insects pests of horticultural crops. *Trends in Horticulture Entomology,* 2022; 978-981-19-0343-4.
17. Mayr E. Speciation and macroevolution. *International Journal of Organic Evolution*. 1982; 36(6):1119-1132.
18. Mishra P., Kumar A., Nagireddy A., Mani D. N., Shukla A. K.,Tiwari R and Sundaresan V. DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market. *Plant Biotechnology Journal.* 2015; 14(1): 8–21.
19. Mushtaq H.M., Selah A.A., Kamran M and Alatawi F.J. Molecular-based taxonomic inferences of some spider mite species of the genus *Oligonchus* Berlese (Acari, Prostigmata, Tetranychidae. *Insects*. 2023;14:192.
20. Ratnasingham S and Hebert P.D.N. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE*. 2013; 8(7): e66213.
21. Sara M. F, Cristina S.L. , Moreira I and Felah B.S.A.B. DNA barcoding of commercially relevant marine fish species in Tunisian waters. *Journal of the Marine Biological Association of the United Kingdom*. 2022; 102(3-4): 178 – 185.
22. Schmidt S., Schmidt-Egger C., Moriniere J., Haszprunar G., Hebert P.D.N. DNA barcoding largely supports 250 years of classical taxonomy: Identifications for Central European bees (Hymenoptera: Apoideapartim). *Mol. Ecol. Res.* 2015; l5: 985–1000.
23. Shashank P.R., Naveena N.L., Rajgopal N.N., Elliott T.A, Sreedevi K, Sunil S and Meshram N M. DNA barcoding of insects from India: Current status and future perspectives. *Mol Biol Rep.,* 2022; 49:10617– 10626.
24. Smith T.B and Wayne R.K. *Molecular Genetic Approaches in Conservation.* Oxford University Press, New York. 1996; 101:504.
25. Szaro R.C and Johnston D.W. Biodiversity in mangrove landscapes, theory and practice. *Biodiversity Conservation*. 1996; 72(1):778 .
26. Tzudir L and Markandan M. Identification and documentation of edible insects of *Vespa affinis* in Nagaland by using DNA barcode techniques. *International Journal of Pharmaceutical Sciences and Research,* 2021; 12(3): 1529-1532.
27. Valentini A., Taberlet P., Miaud C., Civade R., Herder J., Thomsen P. F., Bellemain E., Besnard A., Coissac E., Boyer F., et al. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol*. 2016; 25:929–942.
28. Vences M., Nagy Z. T., Sonet G and Verheyen E. DNA barcoding amphibians and reptiles. *Methods in Molecular Biology*. 2012; 858:

79–107.

29. Wan Q.H., Wu H., Fang S.G and Fujihara T. Which genetic marker for which conservation genetics issue? *Electrophoresis*. 2004;25(14):2165-2176.

30. Yang F., Ding F., Chen H., He M., Zhu S., Ma X., Jiang L and Li H. DNA Barcoding for the identification and authentication of animal species in traditional medicine. *Evidence-Based Complementary and Alternative Medicine*. 2018; 5160254: 18.