Pakistan J. Zool., pp 1-7, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230326110308

DNA Barcoding of Pest Rodents: An Approach in Integrated Rodent Pest Management

R.L. Rengarajan[1,2]*, G. Archunan[1,3], B. Balamuralikrishnan[4], I. Peatrise Geofferina[5] and A. Vijaya Anand[5]

[1]*Department of Animal Science, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India*
[2]*Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0), Madurai Kamaraj University, Tamil Nadu, Madurai-625021, Tamil Nadu, India*
[3]*Marudupandiyar College, Tamil Nadu, Thanjavur-613403, Tamil Nadu, India*
[4]*Department of Food Science and Biotechnology, Sejong University-05006, Seoul, South Korea*
[5]*Department of Human Genetics and Molecular Biology, Coimbatore-641046, Tamil Nadu, India*

Article Information
Received 26 March 2023
Revised 20 July 2023
Accepted 09 August 2023
Available online 18 December 2023 (early access)

Authors' Contribution
RLR and GA conceived and planned the experiments. RLR carried out the field work and lab experiments and preparation of manuscript. PG assisted in preparation of manuscript. BB and AVA contributed to the final version of the manuscript. Finally all authors helped to shape the manuscript.

Key words
Rodent pests, Population survey, *COI* gene, Rodent, Muridae, Species identification, Rodentia neighbour-joining phylogenetic tree

ABSTRACT

The most significant vertebrate pests in agriculture are rodents and their habitation, dispersal, mass, and productive importance differs depending upon the intake of crops, climatic seasons, and terrestrial areas across India. Molecular identification using DNA barcoding with the *COI* gene provides a high level of taxonomic differentiation (>95%) at the species level. An attempt was made to identify certain pest species of rodent namely *Bandicota indica, Rattus rattus, Millardia meltada,* and *Tatera indica*, from six different places through the *COI* gene in the context of rodent pest management program (RPM). The present study also covered the population survey, seasonal variation, and species of rodent pest morphology. The study revealed that the number of rodents seems to be equal in pre-monsoon and post-monsoon and there is a seasonal variation between the numbers of species in different localities. However, there were no morphological changes within the species. The present investigation showed that there were no intra-specific nucleotide alterations within *M. meltada* and *T. indica* and low intraspecific variation within *B. indica* and *R. rattus* at a greatest 0.3% and 0.5%, respectively. *T. indica* and *R. rattus* had the most elevated interspecific divergence, around 22%, and *M. meltada* and *B. indica* had the lowest, around 4%. The current results revealed that the *COI* gene's 650 bp is accurate for the finding of major species of rodents from other species. Consequently, molecular identification of individual rodent pest species would generally assist to develop rodent pest programs by using species-specific (i.e., Pheromone) compounds.

INTRODUCTION

Rodents are the most abundant and the most prosperous class of mammals on the planet. Most predominant rodent pests such as *Bandicota indica, Bandicota bengalensis, Mus booduga, Rattus rattus, Millardia meltada*, and *Tatera indica* are found in south India and affects agriculture and warehouse, etc. Some of them are widely distributed while others are restricted in their dispersal. Attempts have been made to develop methods for the rodent pest management (RPM) using different techniques but none was successful in management (Archunan and Achiraman, 2006; Capizzi *et al*., 2014). Pheromones involved in chemical communication may have a major part in RPM (Robert, 2003; Archunan, 2009). Earlier on urine and scent glands of rodents used as lone sources with bait showed partial success in RPM (Selvaraj and Archunan, 2002). Since pheromones are species-specific their influence makes an impact on reproduction and social behaviour. It is necessary to identify the different rodents in the field and then only the pheromone cues can be used to develop a technique for the RPM program. Conventional approaches for the identification of rodent pests have traditionally been based on external morphological features and morphometric analysis such as colour, fur, teeth, skull, etc. Unfortunately, due to the lack

* Corresponding author: adhibio2000@gmail.com
0030-9923/2023/0001-0001 $ 9.00/0

of taxonomic experts, the identification of these rodents is often difficult (Bacher, 2012). For implementing integrated pest management (IPM), accurate species identification is important to understand the distribution and life history of a particular species. For this reason, the identification of rodents based on molecular methods such as DNA barcoding is imperative (Dobigny *et al*., 2011).

DNA barcoding plays a key role in the anticipation of well-known pests to the database from all over the world. In DNA barcoding accurate identification is made at the species level based on the sequence similarity of Cytochrome C oxidase 1 (*COI*) gene rather than on morphological characters of (Hebert *et al*., 2003). The combined use of both DNA barcoding and traditional taxonomy species identification can be put together as an effective tool for identifying species identification across a wide variety of taxa. The main objective of this study is to identify the selected four rodent pest species by morphometric analysis and DNA barcoding. In addition, population status and seasonal variation of selected four rodent pest species in and around the selected six study areas were done. The identified four rodent pest species were compared with the other species for similarities and divergence by phylogenetic analysis. This attempt has been made as support of developing a pheromone trap controlling for rodent pests as part of IPM.

MATERIALS AND METHODS

Study animals

Bandicota indica (Bechstein, 1800), commonly known as greater bandicoot rat, is found throughout most parts of India, Sri Lanka, Bangladesh, the lowlands of Nepal, Myanmar, southern China, Taiwan, Thailand, Cambodia, Vietnam and the island of Java. *Millardia meltada* (Gray, 1837) also called a South Asian field rat is commonly known as a metad and soft-furred field rat. *M. meltada* is. It is widely distributed from India, Nepal, Pakistan and Sri Lanka. *Rattus rattus* (Linnaeus, 1758) is commonly known as house rat, black rat, roof rat and ship rat. It is originally an Indohimalayan species. A native of the Indian sub-continent, this rat has now spread throughout the world as a result of human activities. *Tatera indica* (Hardwicke, 1807), commonly called Indian Gerbil has been recorded from southeastern Turkey, eastern Syria, Kuwait and Iraq, ranging through much of central and southern Iran to Pakistan and Afghanistan, Furthermore, it ranges throughout India and Sri Lanka.

Study area

The present investigation was carried out in six areas. (1) Lalgudi (10.8733N 78.8194 E), (2) Nidur (11.1018N 79.6522E), (3) Keeranur (10.5734 N-78.7823E), (4) Bhudhalor (10.7849N 79.1404E), (5) Perumbandi (10.5734N78.7823E), (6) Ottangadu (10.4728N 79.3353E) of Tiruchirappalli, Nagapttanam, Pudukkottai and Tanjore districts, close to delta basins of the River Cauvery, Tamil Nadu, South India (Fig. 1). The cauvery delta zone (CDZ) is located in eastern Tamil Nadu between 10.00-11.30, North latitude and 78.15-79.45 longitudes. Other rodent species *Mus booduga*, *Bandicota bengalensis* and *Funambulus palmarum* are also found in the study area.

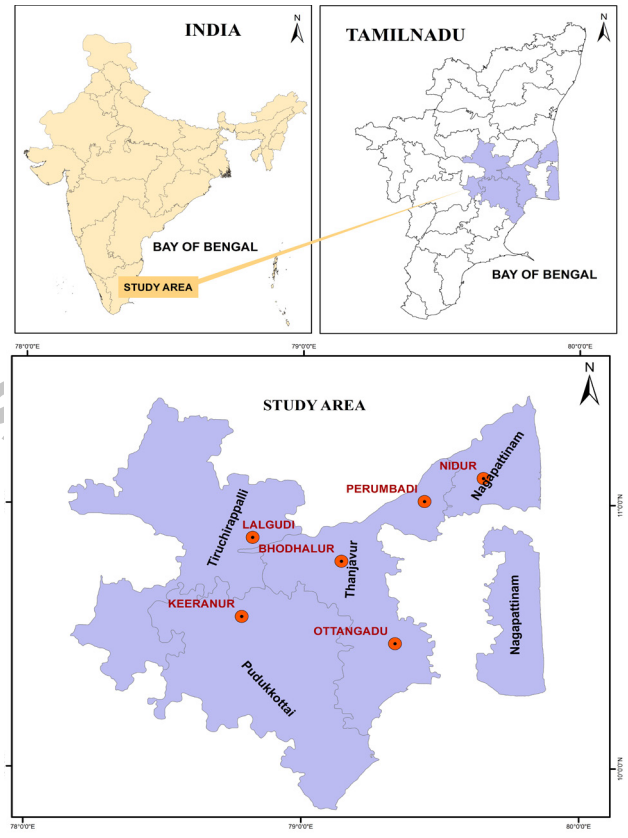

Fig. 1. The geographical location of the study areas in Tamilnadu, India.

Animal collection

Animals were collected from the residential houses, agriculture fields, and storage godowns with intensive trapping procedures for twelve months. Live trapping methods in which single and multiple traps were used to capture *R. rattus* and *B. indica* (Flowerdew *et al*., 2004). The traps were placed in places where the signs of regular rodent activity showed. Net trapping and burrow identification method were used for capturing *T. indica* and *M. meltada* (Neelanarayanan *et al*., 1994; Palanivelu, 2004). The seasonal variation of these four rodent species

was calculated. To determine the trap success for *B. indica* and *R. rattus*, trapping was carried out bimonthly with 10 and 20 traps, respectively. Trap success (abundance) was calculated for *R rattus and B. indica* by using the formula described by (Lathiya *et al*., 2003). The animals that have been captured were incarcerated in cages made of steel and polypropylene, before being transported to the animal house of Bharathidasan University. The collected and cage animals were identified to the species class using morphological features (Aplin *et al*., 2003).

Population survey and morphometric analysis

As a preliminary study, we analyzed a population study of four species of rodents in diverse localities to know the variations among the species. The number of animals captured was recorded and surveys of rodent species were done according to the procedure of Aplin *et al*. (2003). As many as 586 adult rodent pests of four different species from six different localities were taken for morphometric analysis. The external morphometric characters were measured and analyzed by one-way ANOVA using SPSS (Ver. 16.0) Software. The p-value <0.01 is considered significant in the present investigation.

Sequence and phylogenetic analysis

DNA was extracted from liver samples, amplified, and sequenced (Lakshminarayanan *et al*., 2015) which was checked, aligned, and manually edited by using CLUSTAL W (Thompson *et al*., 1994). The *COI* sequences were blasted in the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) for similarity searches. All the appropriate sequence information was documented in the barcode of life database (BOLD, www.barcodinglife.org) (Ratnasingham and Hebert, 2007). The exact specimen record and sequence data for each rodent pest species are available on the BOLD in the project file (Rodents in India). Every sequence has been presented to Genbank and accession numbers were obtained. Thirteen rodent species available in the Genbank database along with the four study species were used for phylogenetic analysis. All the seventeen species included in the study belonged to Murinae, Arvicolinae, Cricetomyinae, Sigmodontinae, Gerbillinae subfamilies. Interspecific and intraspecific nucleotide sequence divergences were calculated for the rodent species founded on the Kimura-2 parameter (Kimura, 1980) for distinctive *COI* sequences. The neighbour-joining (NJ) tree was also created by utilizing the MEGA X version software (Kumar *et al*., 2018) with a bootstrap value of 1500 replicates. All the sequences were aligned and trimmed evenly for balanced weight, for the appropriate species assessment phylogenetic tree analysis.

RESULTS

Population survey and seasonal variation

Among the rodents collected, *R. rattus* was highest in number and *B. indica* was found to be lowest in number. By considering the predominance of individual species populations and the twelve-month field survey among the six sites Bhudhalur showed the highest population of *B. indica* (n=46), *M. meltada* (n=45), and *R. rattus* (n=63). Similarly, Keeranur had the highest population of *T. indica* (n=61) and the lowest number of *B. indica* (n=23) was recorded in both Lalgudi and Nidur, however, the number of species is equal in both study areas. Astonishingly, the lower population of *M. meltada* (n=29) and *R. rattus* (n=34) was found only in Lalgudi. Similarly, the lowest population of *T. indica* (n=47) was identified in Ootangadu when compared to that of other respective localities. Invariably in all three seasons *R. rattus* and *T. indica* were found in maximum number when compared with other species of *B. indica* and *M. meltada* irrespective of localities. However, *R. rattus* and *T. indica* were more predominant in the pre-monsoon than in the monsoon and post-monsoon periods. Among the subordinate groups, the *B. indica* and *M. meltada* were less abundant in pre-monsoon and monsoon respectively when compared to post-monsoon season.

Morphometric analysis and species abundance

Table I shows the comparative analysis of eight external morphometric characteristics, which differed statistically (P<0.01) among the four rodent pest species. Present results revealed that there is no significant statistical difference in morphological observation within the species of the different localities. The current investigation showed that an increased abundance of *B. indica* was recorded in Nidur, while Ootangadu had a lower abundance. The percentage of dissimilarity in the mass of *B. indica* was statistically significant (P<0.01). Likewise, a high abundance of *R. rattus* was observed in Nidur whereas its abundance was low in Keeranur. The percentage of difference in abundance of *R. rattus* was statistically significant (P<0.01).

Genetic identification

In the present study, the *COI* proteins were found to be conserved in which no insertion, deletion, or stop codons were observed in the sequence. The BLAST result of the *COI* sequence for all four species showed more than 97% identity with the same species found in the Genbank. The intraspecific nucleotide divergences observed within *B. indica* and *R. rattus* were 0.3% and 0.5%, respectively. By distinction, there were no interspecific nucleotide variations within the species of *M. meltada* and *T. indica* (Table II).

Table I. Morphometric characteristics of *B. indica, M. meltada, R. rattus, T. indica* collected from six different localities.

Location	Total body length (mm)	Tail length (mm)	Body length (mm)	Ear length (mm)	Pes length (mm)	Upper tooth length (mm)	Lower tooth length (mm)	Body weight (g)
***B. indica* (n=105)**								
Lalgudi	629.59±16.65	307.41±5.85	321.76±15.79	34.05±0.89	65.11±2.08	7.05±0.96	23.29±1.21	794.35±63.84
Bhudhalur	629.89±16.68	308.17±5.89	322.67±15.82	33.94±0.87	65.27±2.24	7.16±0.92	23.27±1.17	797.78±63.61
Nidur	630.88±16.63	308.06±6.05	322.06±15.37	34.00±0.86	65.05±2.10	7.17±0.95	23.29±1.21	800.59±64.41
Keeranur	632.22±17.11	308.72±6.51	322.94±15.82	34.00±0.84	65.22±2.15	7.11±0.96	23.27±1.17	797.78±63.61
Perumbandi	630.94±16.67	307.29±5.94	323.24±16.28	34.05±0.89	65.00±2.15	7.17±0.95	23.17±1.23	794.35±6.38
Ootangadu	629.89±16.68	308.17±5.89	322.67±15.82	33.94±0.87	65.27±2.24	7.16±0.92	23.27±1.17	797.78±63.61
***M. meltada* (n=138)**								
Lalgudi	372.05±23.80	160.55±18.61	213.35±8.96	25.20±1.32	35.70±4.69	7.05±0.75	14.90±1.11	275.00±79.47
Bhudhalur	374.15±21.52	164.04±17.89	210.12±7.78	24.96±1.31	36.15±4.25	6.88±0.81	14.61±1.32	279.04±75.61
Nidur	371.23±19.78	162.00±18.28	208.54±6.85	24.76±1.17	36.03±4.34	6.84±0.83	14.57±1.27	275.38±75.60
Keeranur	362.59±20.85	154.82±17.78	207.76±7.06	24.76±1.30	34.29±4.85	7.11±0.92	14.82±1.50	241.76±81.14
Perumbandi	371.48±24.04	160.16±18.03	211.52±8.36	24.96±1.33	35.96±4.58	7.08±0.81	14.80±1.15	267.80±87.58
Ootangadu	374.75±21.84	164.29±17.78	210.46±8.24	24.91±1.28	36.04±4.41	6.95±0.80	14.66±1.20	282.92±7.45
***R. rattus* (n=133)**								
Lalgudi	402.25±15.30	212.65±7.05	189.60±13.37	24.00±2.02	34.00±2.17	5.65±0.98	11.70±1.34	179.35±31.14
Bhudhalur	398.08±25.46	213.29±7.86	191.25±13.13	23.66±2.18	33.75±2.11	5.66±1.04	11.95±1.39	173.00±23.76
Nidur	402.85±14.87	214.73±6.58	188.88±13.86	24.03±2.029	33.76±2.45	5.73±1.07	11.73±1.28	170.00±20.88
Keeranur	401.95±14.52	213.36±7.41	188.64±12.28	24.09±1.94	33.68±2.35	5.72±1.03	11.72±1.42	172.27±22.61
Perumbandi	400.65±15.02	212.95±7.49	187.75±12.24	23.60±2.11	33.65±2.27	5.70±1.12	11.65±1.30	174.10±25.20
Ootangadu	402.19±13.68	212.38±6.91	189.48±11.69	23.66±2.03	33.95±2.26	5.57±1.07	11.76±1.22	174.76±25.37
***T. indica* (n=210)**								
Lalgudi	418.51±14.34	215.05±9.72	203.46±8.53	23.97±1.06	46.24±1.78	5.75±0.83	8.81±0.65	309.08±27.32
Bhudhalur	419.26±15.44	214.41±9.46	204.85±9.35	23.91±0.83	46.67±2.08	5.76±0.78	8.67±0.68	300.50±28.75
Nidur	419.69±15.83	215.00±8.50	203.81±9.72	23.93±0.84	46.53±1.98	5.75±0.80	8.78±0.65	293.59±33.41
Keeranur	418.14±13.71	212.69±10.03	202.95±8.35	23.61±0.88	46.45±2.02	5.92±0.77	8.85±0.71	312.02±25.27
Perumbandi	419.56±16.03	215.28±9.61	204.28±9.69	23.84±0.84	46.65±2.04	5.75±0.76	8.78±0.70	299.66±32.52
Ootangadu	418.21±14.76	217.64±15.91	203.30±8.65	24.09±1.04	46.21±1.76	5.78±0.85	8.81±0.63	302.64±29.80

Values are expressed as mean ± SD for eight morphometric parameters for four rodent pest species. Statistical comparisons were made with one-way analysis of variance (ANOVA). No statisticaldifferences ($p < 0.01$) were observed within the each morphometric characters.

Table II. Interspecific nucleotide divergence between the four rodent pest species (P<0.01).

Nucleotide divergence	*B. indica*	*M. meltada*	*R. rattus*	*T. indica*
B. indica				
M. meltada	0.04			
R. rattus	0.15	0.16		
T. indica	0.19	0.19	0.22	

The interspecific nucleotide divergence among rodents is as follows: *B. indica* and *R. rattus* were 15%, *B. indica* and *T. indica* were 19%, *M. meltada, T. indica* were 19%, *R. rattus and M. meltada* were 16%. The most elevated nucleotide variation was found between *T. indica* and *R. rattus* at 22% and the lower between *M. meltada* and *B. indica* at 4 % (Table III). The phylogenetic tree analysis using *COI* fragments clearly showed that *T. indica* was grouped under the Gerbillinae subfamily whereas the other three species (i.e.,) *B. indica, M. meltada,* and *R. rattus* were grouped under murinae subfamily (Fig. 2). Furthermore, all four pest species of rodents (i.e.) *B. indica, M. meltada, R. rattus,* and *T. indica* are grouped under a single separate clade individually. All the species described additionally than one sample formed 99% - 100% supported clades. The phylogenetic tree further indicated a vital species distinct from other species. The subfamily and genera relationship was also extremely endorsed with 100% bootstrap values within 1500 replicates. All the *COI* sequences from the four rodent species were further analyzed in BOLD. Among the four different species, three species, (*B. indica, M. meltada,*

and R. rattus) belonged to the murinae subfamily and one, (*T. indica*) belonged to the Gerbillinae subfamily. The BOLD database showed 100% sequence similarity within the species for all four rodent species. The NJ phylogenetic tree revealed a straightforward identification and showed a single cluster of individuals for the four rodent pest species (Fig. 3). Further, an illustrative barcode was generated by BOLD based on the *COI* sequence obtained from the four rodent species.

Table III. Intraspecific nucleotide distance for the four rodent pest species from BOLD (P<0.01).

Order	Family	Species	Nearest species	Distance to nearest neighbor in %
Rodentia	Muridae	*B. indica*	*M. meltada*	3.99
Rodentia	Muridae	*M. meltada*	*B. indica*	3.99
Rodentia	Muridae	*R. rattus*	*B. indica*	16.17
Rodentia	Muridae	*T. indica*	*B. indica*	19.06

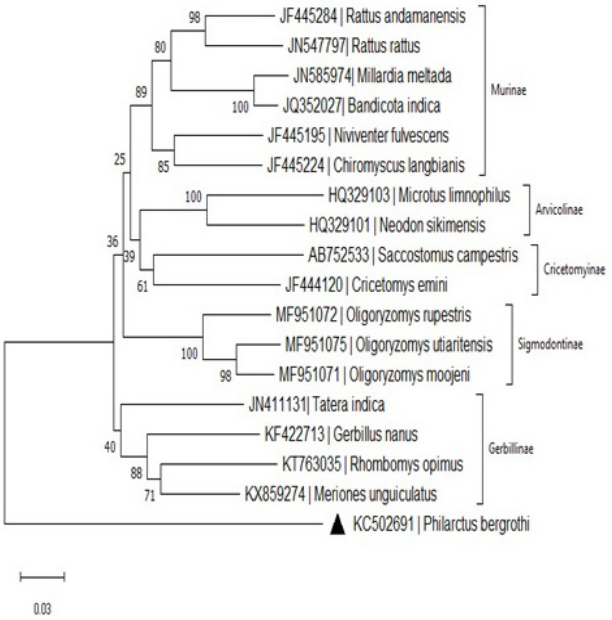

Fig. 2. NJ evolutionary phylogenetic tree constructed based on (K2P) inferred from 604 bp sequence of *COI* sequence. The analysis involved 18 nucleotide sequences. *Philarctus bergrothi* were utilized as an outgroup to implant the tree. NJ, Neighbor Joining

DISCUSSION

It is critical for efficient biosecurity and biosurveillance programmes to intercept potential invasive species at ports of entry. However, taxonomic assessment of immature stages of most arthropods is difficult; identifying features are frequently dependent on adult morphology and reproductive systems (Madden *et al*., 2019). The fundamental purpose of DNA barcoding is to distinguish all species from one another and to build a reference library for all organisms. There have been numerous studies in different animals using DNA barcoding and a portion of the *COI* gene (Waugh, 2007). There are recent reports

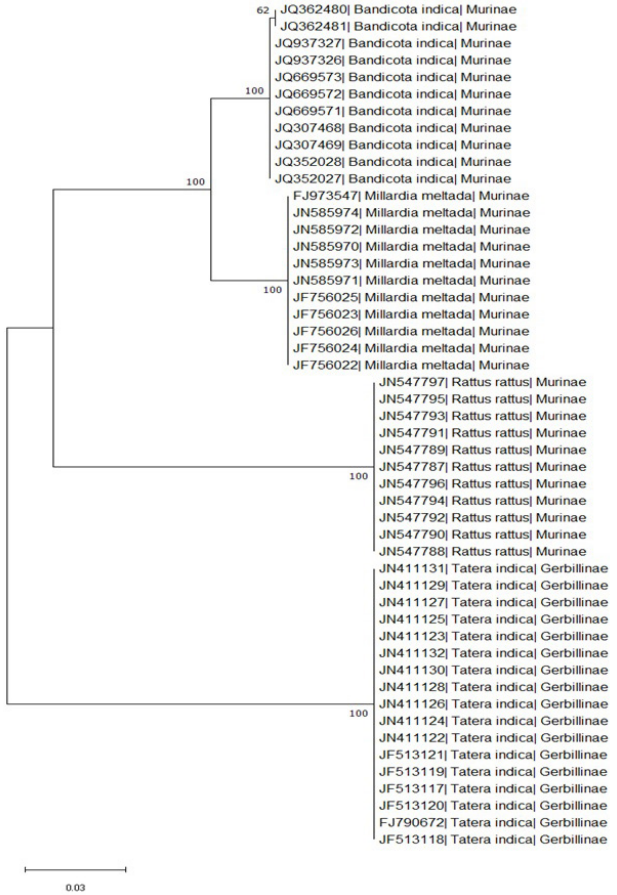

Fig. 3. NJ phylogenetic tree constructed based on (K2P) inferred from *COI* of *B. indica, M. meltada, R. rattus,* and *T. indica*. The bootstrap values are displayed adjacent to the branches. The study included 51 nucleotide sequences. All positions with gaps and missing data were removed. The final dataset contained 614 positions in total. NJ, Neighbor Joining.

that genome skimming can be used to widen the process of DNA barcoding to develop the standard barcode (Eric *et al*., 2016). Each of the four species studied provided a distinct *COI* sequence, differentiating them from other

species through the DNA barcode method. The genetic distance between these two species *T. indica and R. rattus* is found to be high (22%) and low between *M. meltada* and *B. indicia* (4%), while the genetic distance between *B. indica and R. rattus* is low (0.3% and 0.5%, respectively). The present study clearly showed that the phylogenetic analysis of all four different species comes under the same cluster. Sequences from identical species were grouped with lower divergences and exhibited 100% bootstrap supports in phylogenetic analysis. It is noted that near-interconnected species from the identical genus and subfamilies were also found separated in the analysis. This NJ phylogenetic tree analysis further shows a strong species distinct from other species.

Based on the results obtained in the population survey, it is found the fluctuations in the population size of rodents related to the sample sites, showed that there is a variation in population size from place to place. Further, the species abundance is varying due to their habitat in different geographical regions. The size of a population is likely to be based on the seasonal model of reproduction, like other *Didelphis marsupialis* (Cerqueira, 1988). In the present study, a total of eight morphologic characteristics are considered and found that all the characteristics do not vary significantly ($P<0.01$) within the rodent species in different localities, by contrast, it differs statistically, among the four different pest species of rodents irrespective of the study area.

The outcomes of the current analysis inferred that though the population density of rodent species was altered by the geographical regions, the *COI* gene provides strong help to the downward taxonomy up to the level of subfamily than to that of upward taxonomy. Our findings showed that 650 bp of *COI* gene sequence has been generally accurate and can be used as a novel technique for the identification of rodent pest species.

CONCLUSION

The identification of species among rodents through the *COI* gene provides strong support to choose a particular species in a particular region. Therefore, it could be a possible way to identify the species and control the use of pheromone compounds against the conspecific rodent. The present investigation does not cover all the species available in the study area and the same approach may be adapted and implemented for identifying other pests in future studies.

ACKNOWLEDGEMENT

RLR wishes to thank Bharathidasan University, Tiruchirappalli, for the award of University Research Fellowship, Project Fellow through UGC and CSIR for Research Associate (09/475/ (0199) 2016 EMR-1). GA is grateful to UGC, New Delhi, for the award of UGC-BSR Faculty Fellowship (F.18-1/2011(BSR) 2016). The facility availed through UGC-SAP and DST-FIST is gratefully acknowledged.

Funding

The study received no funding.

Ethical statement and IRB approval

The animals were sexed and sacrificed by following the procedures for animal maintenance by the Institutional Animal Ethics Committee (IAEC), India. The study has been approved by IRB with Institutional Ethical Clearance.

Statement of conflict of interest

All authors disclose no conflict of interest.

REFERENCES

Aplin, K.P., Brown, P.R., Jacob, J., Krebs, C.J. and Singleton, G.R., 2003. Field methods for rodent studies in Asia and the Indo-Pacific. *ACIAR Monograph*, **1**: 223.

Archunan, G., 2009. Vertebrate pheromones and their biological importance, *J. Exp. Zool., India*, **12**: 227-239.

Archunan, G. and Achiraman, S., 2006. Pheromones in rodent pest management, vertebrate pests in Agriculture. *Indian Scenario*, **15**: 365-386.

Bacher, S., 2012. Still not enough taxonomists: Reply to Joppa *et al*., *Trends Ecol. Evol.*, **27**: 65-66. https://doi.org/10.1016/j.tree.2011.11.003

Capizzi, D., Bertolino, S. and Mortelliti, A., 2014. Rating the rat: Global patterns and research priorities in impacts and management of rodent pests. *Mamm. Rev.*, **44**: 148-162. https://doi.org/10.1111/mam.12019

Cerqueira, R., 1988. *Models of breeding seasons in mammals in minutes of the meeting of interdisciplinary ecology mathematics*, pp. 49-58.

Dobigny, G., Tatard, C., Kane, M., Gauthier, P., Brouat, C., Ba, K. and Duplantier, J.M., 2011. A cytotaxonomic and DNA-based survey of rodents from Northern Cameroon and Western Chad. *Mamm. Biol.*, **76**: 417-427. https://doi.org/10.1016/j.mambio.2010.10.002

Eric, C., Peter, H., Sebastien, L. and Pierre, T., 2016. From barcodes to genomes: Extending the concept of DNA barcoding. *Mol. Ecol.*, **25**: 1423–1428.

https://doi.org/10.1111/mec.13549

Flowerdew, J.R., Shore, R.F., Poulton, S.M.C. and Sparks, T.H., 2004. Live trapping to monitor small mammals in Britain. *Mamm. Rev.*, **34**: 31-50. https://doi.org/10.1046/j.0305-1838.2003.00025.x

Hebert, P.D.N., Cywinska, A., Ball, S.L. and DeWaard, J.R., 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B.*, **270**: 313-321. https://doi.org/10.1098/rspb.2002.2218

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**: 111-120. https://doi.org/10.1007/BF01731581

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.*, **35**: 1547-1549. https://doi.org/10.1093/molbev/msy096

Lakshminarayanan, R.R., Shanmugam, A. and Govindaraju, A., 2015. Sequencing of *COI* gene in four rodent pests for species identification. *DNA Barcodes*, **3**: 1-4. https://doi.org/10.1515/dna-2015-0001

Lathiya, S.B., Khokhar, A.R. and Ahmed, S.M., 2003. Population dynamics of soft-furred rat *Millardia meltada*, in rice and wheat fields in central Punjab, Pakistan. *Turk. J. Zool.*, **27**: 155-161.

Madden, M.J.L., Young, R.G., Brown, J.W., Miller, S.E., Frewin, A.J. and Hanner, R.H., 2019. Using DNA barcoding to improve invasive pest identification at U.S. ports-of-entry. *PLoS One*, **14**: e0222291. https://doi.org/10.1371/journal.pone.0222291

Neelanarayanan, P., Nagarajan, R. and Kanakasabai, R., 1994. Burrow morphology of field rodents of Cauvery delta. *Rodent News Lett.*, **18**: 3-4.

Palanivelu, K., 2004. *Investigation of volatile components in urine, faeces and major scent glands of Indian Gebril (*Tatera indica *H.) with special reference to rodent pest management*. PhD. thesis, Bharathidasan University, Tiruchirappalli, India.

Ratnasingham, S. and Hebert, P.D.N., 2007. BOLD: The barcode of life data system (www.barcodinglife.org). *Mol. Ecol. Notes*, **7**: 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Robert, E.J., 2003. Chemical communication in rodents from pheromones to individual recognition. *J. Mamm.*, **84**:1141-1162. https://doi.org/10.1644/BLe-010

Selvaraj, R. and Archunan, G., 2002. Role of male scent glands in improving poison bait acceptance in female rats, *Rattus norvegicus*. *Indian J. Exp. Biol.,* **40**: 53-57.

Thompson, J.D., Higgins, D.G., Gibson, T.J. and Clustal, W., 1994. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choices. *Nucl. Acids Res.*, **22**: 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Waugh, J., 2007. DNA barcoding in animal species: Progress, potential and pitfalls. *Bio. Essays*, **29**: 188-197. https://doi.org/10.1002/bies.20529