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The Diversity and Characterization of Phytophagous Scarabaeids

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ABSTRACT

The diversity of phytophagous scarabaeid beetles from various geographical locations of India and crops (arecanut, coconut, groundnut, millets, mulberry, neem, soybean, sugarcane and vegetables) was explored. The beetles were morphologically identified and characterized for COI gene fragment using specific primers. Sequence analysis and divergence among the species was assessed. Genbank accession numbers were obtained for the species. Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. Phylogenetic tree based on Maximum-Likelihood method was drawn on the basis of multiple sequence alignment to assess the genetic relatedness, with those reported elsewhere in the world. The genus Anomala and Protaeta sp. formed distinct clades with high boot strap values. The studies indicate the relevance of DNA sequencing to match different forms of beetles and address ambiguities in morphological identification, while the information on species diversity and abundance would help plan strategies for pest management.

Keywords: Beetle, Characterization, *COI* gene, Diversity, Phylogeny scarabaeid, Sequence

INTRODUCTION

The family scarbaeidae is the second largest family with more than 30,000 species recorded worldwide [1]. About 2500 species are reported from India [2] and a majority of these are phytophagous (sub families Melonthinae, Rutelinae, Dynastinae and Cetoninae) [1,3]. The adult beetles and their grubs cause extensive damage to fruit crops, vegetables, ornamental plants, plantation crops pastures, turf and meadow grasses, lawns, golf courses and forest trees [4,5]. Adults of the sub-family Melolonthinae and Rutelinae are pre-dominantly leaf feeders [6,7] where as those of Cetoniinae feed on flowers and fruits, and are popularly referred to as flower beetles, prefer nectar, sap or juice of ripening fruits and vegetables. Members of Dynastinae usually attack stems or roots of plants. Grubs of Melolonthinae, Rutelinae and Dynastinae commonly referred to as white grubs are often soil dwelling and cause extensive damage to the roots of cereals, legumes, small fruit plants, shrubs and trees [8-10]. In India, the white grubs are pests of national importance [11,12].

The scarab fauna of India is very rich and diverse and has not been explored to a greater extent. Scattered information on the diversity of the beetles of Chhattisgarh [13], Madhya Pradesh [14-17], Himachal Pradesh [18], North Western Ghats [19,20], is documented. Integrated intensive farming systems and climate change had resulted in depleting biodiversity and habitat degradation, which had necessitated for an inventory of species richness of insect pests, their cataloguing and documentation. The occurrence and distribution of beetles in different geographic and ecosystems is imperative to understand their ecology to formulate effective strategies for their management.

An authentic classification of species is a pre-requisite for research in ecology and biodiversity. Lack of taxonomic understanding has been a major impediment to the study and management of scarabaeid beetles. Proper identification of the species and knowledge of their distribution, geographical variation, population dynamics, feeding and reproductive behavior are the first steps in developing environmentally compatible/sustainable integrated pest management strategies. Identification of scarabaeid species is a challenging task due to variable morphological differences among species and delineation among the immature forms, the grubs and adults. Morphological identification keys are often effective only for a particular life stage or gender [21]. The use of taxonomic keys often requires proficiency to avoid inaccuracy for those similarities which cannot be easily deciphered.

The indistinctness due to high similarity in morphology is overcome by application of molecular techniques. In the identification of scarabaeid beetles, although identification keys are available, uncertainties are still common especially for many unexplored species from the tropics [22]. Molecular markers for effective identification, linking the grubs and adults are currently available [23,24]. Efficient Mitochondrial DNA (mtDNA)-based methods in the delineation and identification of scarabaeid species have been reported [25-27].

Taxonomic database [28] and species richness of scarabaeid beetles needs to be ascertained due to the diminution of biodiversity. Hence, studies were contemplated to assess the diversity of scarabaeids from different crop ecosystems and geographic locations with their identity determined through morphological characters and molecular tools.

MATERIALS AND METHODS

Field survey and collection of scarabaeid beetles

The diversity of scarab beetles depends on the availability of food for larvae and adult, weather conditions and soil type. Collection of scarab beetles was made randomly by hand picking and light trapping. Grubs were collected from a soil depth of 0.25-0.5 nm in cultivated fields. The beetles were collected during May- June which is the major activity period to assess the diversity. The populations were collected from different states and geographical locations of the country from various trees and crop plants (arecanut, coconut, groundnut, mulberry, millets, neem, soybean, sugarcane and vegetables).

Collection of adult beetles using light traps

Light traps were used for four months (May–September) to collect the beetle populations. The light traps were placed in the centre of the fields at a height of about 3 m above the ground and operated between 7:00 PM to 5:00 AM to attract the scarabaeid beetles which are positively heliotactic in nature.

The light trap comprised of PVC plastic funnel of 25 cm in height, and 30 cm diameters. The bottom diameter of the funnel was 5 cm. The rain shed cone for protecting the bulb was fixed at 17 cm above the funnel with the help of three white metal sheets. The diameter of the rain shed cone was 20 cm. The light source consisted of a 125-watt incandescent light bulb with copper wire choke. The light trap had three baffles (30 cm × 10 cm), placed at a uniform distance of 10 cm around the circumference of funnel. The baffles were fixed to emit light uniformly in all directions without any interference, when the beetles are attracted to light they collide with baffles and fall into the trap. A nylon bag was attached to the bottom of this funnel for collection of beetles. The collected beetles were preserved in a vial containing 70% alcohol and taken to the laboratory for morphological identification.

Identification of the beetles

The scarab adults and grubs collected from larvae collected from different locations were identified up to the genus level at the Department of Entomology, University of Agricultural Sciences, Bangalore and the Division of Entomology, Indian Agricultural Research Institute, New Delhi, based on the keys and characters listed by [29-33].

Adult beetles were identified based on the morphological characters such as body size, colouration, surface sculpture and male genitalia, while the grubs were differentiated based on the color, size of the cephalic capsule, number and form of dorsal sensorial maculae of the last antennomere, distribution, stridulatory structures in the maxilla and mandible, raster pattern arrangement of bristles and hairs on the underside of the abdomen, shape of anal slit (crescent,Y shaped, strongly Y shaped), shape and size of the respiratory plates, proportions of each pair of legs and tarsungulus size [5,34,35].

MOLECULAR CHARACTERISATION OF BEETLES

Extraction of genomic DNA

Total genomic DNA was isolated using the method described by Gavarāne [36]. The insects were washed thoroughly in double distilled water. Total genomic DNA was isolated from the leg portion of the insect. The cleaned insect leg portion was homogenized in 1.5 ml Eppendorf tube in 500 µl of TE (Tris-EDTA-pH 8), with hand pestle and the homogenate was centrifuged at 10,000 rpm for 10 minutes in cooling centrifuge (-4°C). The supernatant was discarded and the pellet was dissolved in 500 µl of lysis buffer (400 µl of TE and 100 µl of 5% SDS), followed by the addition of 6 µl of Proteinase K, and the solution was incubated at 65°C for one and half hours. A mixture of 120 µl phenol chloroform isomyl alcohol (25:24:1) was added and the tubes vortexed for 30 seconds and then centrifuged for 10 minutes at 10,000 rpm in cooling centrifuge. The upper aqueous layer was carefully transferred in to fresh tube, without disturbing the protein layer at the interphase. Isopraponal (500 µl) was added to this aqueous layer and stored at -4°C overnight and then centrifuged at 7000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with 70% alcohol and later the alcohol was drained out, the pellet was dried and dissolved in 30 µl of TE-I was stored at -20°C after checking on 0.8% agarose gel and visualized after staining with ethidum bromide.

PCR amplification of COI gene fragment

The DNA obtained from beetle samples was used for amplifying a portion of mitochondrial *COI* gene fragment, using the primers CO1 forward (F: 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer (CO1 R: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The primers were procured from M/s Eurofins Limited, Bangalore. The protocol of Williams JG et al. [37] was followed. Each reaction mixture of 25 µl consisted of 2.5 µl of 10X PCR buffer, 2.0 µl $MgCl_2$ (2.5 mM), 0.2 µl dNTPs (200 µM), 1 µl of *Taq* Polymerase (1 U/µl), 1 µl of each of forward and reverse primer sequences, 1 µl of DNA, and 16.3 µl of distilled water.

The amplification was carried out in thermal-cycler (BioRad, USA), following PCR conditions of denaturation at 94°C for 60 sec; annealing at 54°C for 90 sec and extension at 72°C for 90 sec (30 cycles, plus an initial denaturation at 94°C for 1 min and a final extension at 72°C for 8 min). PCR-amplified products were purified using Bioneer's PCR purification Kit (www.Bioneer.com). The amplified products were run on 2% agarose gel (stained with ethidium bromide) with DNA ladder (100 bp). Gels were visualized in a gel doc system.

Sequence analysis and data interpretation

The most commonly used method of DNA sequencing is the dideoxy method or chain termination method. The amplified products of *COI gene* were got sequenced at M/s. Eurofin Pvt Ltd, Bangalore. The *COI gene* sequence data was retrieved in the form of chromatograms. Several individuals from each species were sequenced and chromatograms were subjected to VSQual [38] to evaluate the reliability of the data, and good quality fragments were used to construct a consensus sequence for each sample. Chromatograms were edited to discard ambiguous bases, and edited sequences were aligned by using the Basic Local Alignment Search Tool (BLAST), with the sequences of same or related genera retrieved from the nucleotide database (PUBMED) of National Centre for Biotechnology Information (NCBI). The sequence data's was submitted to NCBI and accession numbers were obtained. Consensus sequences of COI partial gene were multiple aligned using Clustal W (ver. 1.83) Thompson JD et al. [39]. Neighbor joining phylogenetic tree was drawn by using 'Meg Align' program of 'Lasergene' software package (DNASTAR Inc., USA).

Phylogenetic analysis

The Blast search analysis was done to compare all the sequences of *COI* gene sequences available in the Gene bank data base. Phylogenetic tree was constructed using character based maximum-likelihood method based on the Tamura and Nei model [40]. MEGA-5 bioinformatics tool was used to construct phylogenetic tree and the genetic relatedness between the isolates was analyzed [41]. The bootstrap analysis using 1000 iterations was done to test the accuracy of phylogeny. Constructed phylogenetic tree was visualized using tree viewer program.

RESULTS AND DISCUSSION

Collection of scarabaeid beetles

The collection of the beetles was restricted to the phytophagous group, belonging to the subfamilies Melolonthinae, Rutelinae, Cetoniinae and Dynastinae. The population of scarabaeids was collected from different geographical locations (Table 1) in the country from the following states:

Table 1: Geographical locations

Andhra Pradesh	Tirupathi
Arunachal Pradesh	Pasighat
Himachal Pradesh	Shimla
Karnataka	Agumbe, Bagalkot, Bangalore, Chintamani, Chikkaballapur, Gudalur, Hubli, Mangalore, Shimoga and Sringeri
Meghalaya	Shillong, Tondon
Tamilnadu	Dindigul, Dharmapuri, Hosur, Nagercpoil, Ooty, Valparai and Yercaud
Uttar Pradesh	Aligarh, Kushgal and Kapatganj

Identification of the beetles

The collected adult beetles were identified based on the morphological characters up to the genus level. The beetles were distinguished based on antennae, mandibles, maxillae, size, colouration of surface, male genitalia and size of cephalic capsule. Identity of the grubs was based on the shape of the anal slit, raster pattern, arrangement of bristles and hairs (palida), spiracles and legs. The identity of the grubs from the collections made from the various crops belonged to sub families Melolonthidae, Rutelinae, Cetoniinae and Dynastinae. These were primarily differentiated by the raster pattern and anal slit. The grubs of melolonthinae had a Y shaped anal slit with varying raster pattern (inverted V, lemon shaped and circular fashion) (Figure 1). In the dynastids, rutelinids and cetoniids the anal slit is transverse (crescent shaped) with a triangular raster (Figure 2). The adult rutelinidae were prominently differentiated based on the tibial claws with two setae (Figure 3).

Figure 1: Anal slit and raster pattern in Melolonthinae

Figure 2: Anal slit and raster pattern in cetoniinae, dynastinae and rutelinae

Figure 3: Tarsal claw with two seate in Rutelinid

Table 2: Scarabaeid beetles (family: Scarabaeidae) collected from various geographical locations

S.No.	State	Location	Code	Source	Identity	Sub family
1	Uttar Pradesh	Aligarh	Aliig-SC-1	Millets	Apogonia clypeata	Melolonthinae
2			Alig-Sc-2	Millets	Orphnus sp	Orphninae
3			Alig-Sc-3		Apogonia sp	Melolonthinae
4			Alig-Sc-4		Apogonia sp.	Melolonthinae
5		Kapatganj	Kpt-Sc-3		Adoretus sp,	Rutelinae
6			Kpt-Sc-4		Apogonia	Melolonthinae

7			Kpt-Sc-8		Trox sp.	Trogidae
8		Kushinagar	Kshn-SC-1	Light trap	Alissonotum piceum	Dynastinae
9	Arunachal Pradesh	Pasighat	Phas-Sc-1	Light trap	Protaetia affinis	Cetoniinae
10			Phas-Sc-3	Light trap	Protaetia affinis	Cetoniinae
11			Phas-Sc-8		Oxycetonia versicolor	Cetoniinae
12			Phas-Sc-9		Oxycetonia versicolor	Cetoniinae
13			Phas-Sc-10		Oxycetonia versicolor	Cetoniinae
14			Phas-Sc-11		Anomala sp.	Rutelinae
15			Phas-Sc-12		Oxycetonia versicolor	Cetoniinae
16			Phas-Sc-13		Adoretus sp.	Rutelinae
17			Phas-Sc-25		Omthophagus sp.	Scarabaeinae.
18	Himachal Pradesh	Shimla	Smla-Sc-1	Potato	Anomala dimidata	Rutelinae
19			Smla-Sc-2		Malodera	Melolonthinae
20			Smla-Sc-3		Malodera sp.	Melolonthinae
21	Meghalaya	Shillong	Shil-Sc-1		Anomala sp.	Rutelinae
22			Shil-Sc-2		Anomala sp.,	Rutelinae
23			Shil-Sc-3		Apogonia sp.,	Rutelinae
24			Shil-Sc-4		Adoretus sp,	Rutelinae
25			MGH-SC-1	Light trap	Anomola albopilosa	Rutelinae
26			MGH-SC-2		Protaetia brevitarsis	Cetoniinae
27	Karnataka	Bangalore	Das-Sc-5		Schizonycha sp.	Melolonthinae
28			Das-Sc-4		Copris sp.	Scarabaeinae
29			Das-Sc-6		Anomala sp.	Rutelinae
30			Das-Sc-7		Malodera sp.	Melolonthinae
31			Das-Sc—8		Anomala sp.	Rutelinae
32			Das-Sc-9		Schizonycha sp.	Melolonthinae
33			Das-Sc-10		Anomala sp.	Rutelinae
34			Das-Sc-11		Anomala sp.	Rutelinae
35			Das-Sc-12		Anomala sp.	Rutelinae
36			Das-Sc-13		Anomala sp.	Rutelinae
37			Das-Sc-14		Anomola	Rutelinae
38			Das-Sc-15		Leucopholis	Melolonthinae
39			Das-Sc-16		Adoretus sp.	Rutelinae
40			Das-Sc-17		Orphinus sp.	Orphninae
41			Das-Sc-18		Adoretus sp,	Rutelinae
42			Das-Sc-29		Leucopholis	Melolonthinae
43			Slv-Sc-2		Amolala sp.	Rutelinae

44		Hessaraghatta	Hessar-Sc-2		Apogonia sp.	Melolonthinae
45		Yellahanka	Yella-Sc-1		Adoretus sp	Rutelinae
46		Sringeri	Sring-SC-1	Light trap	Anomola singularis	Rutelinae
47		Shimoga	Shim-SC-1	Millets	Schizonycha ruficollis	Melolonthinae
48	Tamilnadu	Ooty	Ooty-Sc-2		Anomala	Rutelinae
49		Ooty	Ooty-Sc-10		Anomalchola sp.	Rutelinae
50		Ooty	Ooty-Sc-11	Light trap	Heterorrhina elegans	Cetoniinae
51		Ooty	Ooty-Sc-13		Anomala	Rutelinae
52		Ooty	Ooty-SC-14	Light trap	Protaetia brevitarsis	Cetoniinae
53		Gudalur	G-Sc-1	Light trap	Protaetia brevitarsis	Cetoniinae
54		Gudalur	G-Sc-2		Protaetia brevitarsis	Cetoniinae
55	Andhra Pradesh	Tirupathi	Tpt-Sc-2		Holotrichia sp.	Melolonthinae
56			Tpt-Sc-3		Holotrichia floflava	Melolonthinae
57			TPT-SC-5	Pigeon pea	Protaetia brevitarsis	Cetoniinae
58			Tpt-Sc-6		Alissonotum	Dynastinae
59			Tpt-Sc-7		Onthophagus	Scarabaeinae
60			Tpt-Sc-8		Schizonycha sp.	Melolonthinae

Persusal of the data on the collection of scarabaeid beetles from the different geographical locations and crops and their identification had revealed the diversity of beetles in the country. An array of phytophagous beetles (Table 2) belonging to the subfamilies (cetoniinae, dynastinae, melolonthinae and rutelinae) were recorded. Such diversity was earlier reported from various locations of the country. Chandra [15] reported the diversity and relative abundance of pleurostrict scarabaeidae in the Achanakmar-Amkarkantak biosphere reserve in Chattisgarh state. About 22 species belonging to 11 genera and 6 subfamilies were reported from the region, while in Madhya Pradesh, 47 species were reported [4,13,14]. The genus *Anomala* predominated over among all the scarabeids in both the states. In Maharashtra, the occurrence of different species of *Holotrichia* was widespread on sugarcane, sorghum, groundnut and soybean crops in South Konkan and Vidharba regions [5] and in Pune [19,20]. *Holotrichia serrata* was predominant among all the species recorded. In Himachal Pradesh, *Anomala* sp. followed by *Brahmina* sp. were dominant in Chamba, Kanra, Kullu and Shimla areas [1,2,42,43].

Congenial habitat, natural vegetation, food availability and appropriate soil type contribute to the diversity of scarabaeids and the species richness [44-46]. In addition, climatological factors rainfall, humidity, temperature and wind velocity play a decisive role on the emergence, movement, distribution and bioecology of scarabaeids [3]. Knowledge on species diversity, abundance, richness and dominance through surveys would be helpful in planning strategies for conservation of natural enemies, habitat management, design and develop pest management strategies.

Molecular characterisation

DNA was extracted from alcohol preserved specimens using DNeasy Qiagen Kit. DNA samples were stored in the Qiagen elution buffer at 4˚C until subjected to PCR. The hind leg portion of the beetle was taken for DNA isolation, The genomic DNA was isolated from the populations of scarabaeids collected from Aligarh (Uttar Pradesh), Dasarahalli , Gudalur, (Karnataka), Ooty (Tamilnadu), Phasighat (Arunachal Pradesh), Tirupathi (Andhra pradesh), Shimla (Himachal Pradesh) and Shillong (Meghalaya). The isolated genomic DNA was confirmed by checking the quality using agarose gel electrophoresis (0.8%) (Figure 4).

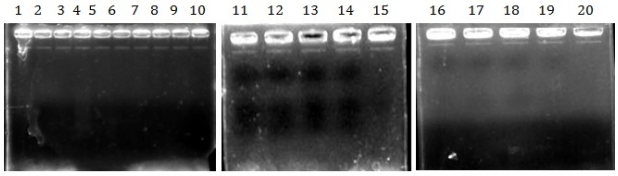

Figure 4: Isolation of genomic DNA of some scarbaeid beetles

Note: 1-G-SC-1; 2- G-SC-2; 3-DAS-SC-11; 4-DAS-SC-12; 5- Phas-SC-1; 6- Phas-SC-2; 7- Phas-SC-3; 8- Phas-SC-4; 9- Phas-SC-5; 10-Phas-SC-6; 11- Ooty-SC-10; 12- Ooty-SC-11; 13- Alig--SC-1; 14- Ooty-SC-13; 15- Shim-SC-14 , 16- KPT-SC--1 17- TPT-SC-6; 18- TPT-SC-7; 19- Sringeri -SC-1; 20- MGH-SC) (GSC- Gudalur; DAS-Dasarahalli; Phas-Phasighat; Ali-Aligarh; Shim-Shimla; KPT-Kapatganj; TPT-Tirupathi; MGH-Meghalaya).

The COI region was amplified and was analyzed using 1.5% agarose gel electrophoresis and the product size was obtained by using low range ladder of 100 bp from Genei, and these were got sequenced at M/s Eurofin Pvt. Ltd, Bangalore. The sequence data was submitted to the NCBI Genbank and access numbers were obtained for a few scarabaeid beetles (Table 3).

Table 3: Characterization of certain scarabeied beetles based on *COI* gene and accession numbers

Code	Organism	Sub family	Place of collection	Source	Genbank/Bank it accession
Shil-Sc-1	*Anomola albopilosa*	Rutelinae	Shillong (Meghalaya)	Light trap	KM657491
Shil-Sc-2	*Protaetia brevitarsis*	Cetoninae	Shillong	Potato	KM657489
TPT-Sc-5	*Protaetia brevitarsis*	Cetoninae	Tirupathi (Andhra)	Pigeon pea	KM657490
DAS-Sc-1	*Anomola albopilosa*	Rutelinae	Dasarahalli (Karnataka)	Light trap	KM657492
OOTY-Sc-11	*Heterorrhina elegans*	Cetoninae	Ooty(Tamilnadu)	Light trap	KM657485
Ooty-Sc-14	*Protaetia brevitarsis*	Cetoninae	Ooty (Tamilnadu)	Light trap	KM657486
PHAS-Sc-1	*Protaetia affinis*	Cetoninae	Pasighat	Light trap	1762766
PHAS-Sc-3	*Protaetia affinis*	Cetoninae	Pasighat (Arunachal)	Light trap	1762769
G-Sc-1	*Protaetia brevitarsis*	Cetoninae	Gudalur (Karnataka)	Potato	1762776
G-Sc-2	*Protaetia brevitarsis*	Cetoninae	Gudalur	Light trap	1762777
Smla-Sc-1	*Anomala dimidata*	Rutelinae	Shimla (Himachal)	Potato	1762765
Alig-Sc-1	*Apogonia clypeata*	Melonthinae	Aligarh (Uttarpradesh)	Millets	1762764
Shim-Sc-1	*Schizonycha ruficollis*	Melolonthinae	Shimoga (Karnataka)	Millets	1762749
Kshn-Sc-1	*Alissonotum piceum*	Dynastinae	Kushinagar (Uttarpradesh)	Light trap	1762754
Sring-Sc-1	*Anomola singularis*	Rutelinae	Sringeri (Karnataka)	Light trap	1762765

The utility of DNA data in taxonomy and species diagnosis in the scarabaeid beetles was reported by [24,47] based on the sequence variation in DNA based groups which was highly structured. The population of scarabaeids from various locations were characterized using Cytochrome C oxidase subunit I (*COI*) gene, which has been recognized as an effective marker not only for species identification but also for phylogenetic relationship [21,48-50]. In the present studies, the isolated genomic DNA of scarab beetles from various locations was characterized through *COI gene* fragment (648-656 bp size). The amplified gene was sequenced and the Blast done with NCBI database to decipher the identity of the scarabaeids from various locations and crops (Table 3). Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. Our observations, corroborate with the reports of [23,51-54].

Michael et al. [55], suggested that where sequence information is available in Genbank for morphologically defined species, which can be matched with some DNA based clusters, close relationship can be identified readily in

sequence variation in field collected field samples and these clusters are likely to correspond to previously described unknown species. Sipek et al. [22,56] reported that the sequence information based on mitochondrial markers can be utilized for species delineation of adults and grubs of scarabaeids inferring larval taxonomy. Our studies indicate the relevance of DNA sequencing to match different forms of scarabs and address the issues of having to depend exclusively on morphological features and avoid misdiagnosis.

Phylogenetic analysis

A phylogenetic tree of the species using Maximum-Likelihood method was drawn on the basis of multiple sequence alignment of *COI gene* (Figure 5).

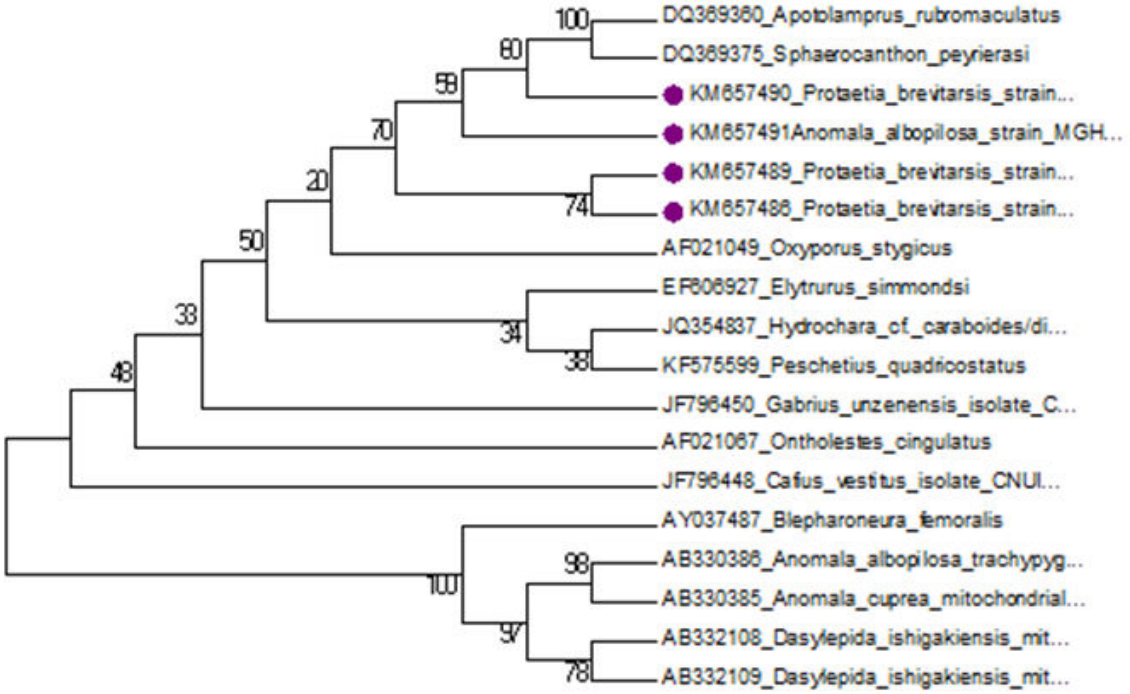

Figure 5: Neighbour joining tree showing the relationship of *COI* sequences of beetles with other sequences in genbank (Numbers at nodes indicate percentage bootstrap values)

The constructed phylogeny revealed the formation distinct clusters. Among these, the genus *Anomala* and *Protaeta* sp. formed distinct clades with high boot strap values. The phylogeny however did not infer conclusive evolutionary relationship among the scarabaeid beetles considered, due to low divergence obtained with those reported elsewhere in the world. Our findings, concur with the observations made by Javad et al. [57], on the significance of phylogeny based on DNA data. Weak phylogenetic foundation is related to issues like rare information about scarabaied taxa at the family level. The classification of the world dynastinae is fairly well established, while in melolonthinae, rutelinae and cetoniinae that are poorly known taxonomically, new genera cannot be reliably identified. A larger study of many populations and different genes may help reconstruction of the phylogeny and understand the evolutionary relationship. Further, phylogenetically, closely related species are likely to have a comparable physiology [58] which would facilitate precision management of the pest with insecticides. The present studies do not lead to exclusive inference, since a large number of scarabaeids are yet to be analyzed.

CONCLUSION

The diversity of phytophagous scarabaeid beetles from various geographical locations of India occurring in crops were morphologically identified and characterised using molecular tools, Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. Phylogenetic tree revealed the genetic relatedness among the beetles and understand the evolutionary relationship. The relevance of DNA sequencing to match different forms of beetles and address limitations in morphological identification is indicated. Knowledge on species diversity, abundance, richness and dominance through surveys

would be helpful in planning strategies for conservation of natural enemies, habitat management, design and develop pest management strategies.

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