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Unraveling the diversity, phylogeny, and ecological role of cryptic Coleopteran species of Vadodara district: a first comparative approach from India

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Abstract

Background: More than 350 K described species, Coleoptera (beetles) represent the most diverse order from Class Insecta in the entire animal kingdom. However, their phylogeny is highly controversial due to their morphological crypticness and multiple markers used previously for sequence homology. Although many studies suggest that their diversity currently relies majorly on morphological analysis, nevertheless DNA barcodes may provide a functional, standardized tool for their unique identification. In the present report, a fragment of the mitochondrial cytochrome c oxidase I (COI) gene has been proposed as standard DNA barcoding marker for the identification of organisms.

Result: To evaluate this hypothesis, a random sampling was conducted in and around Vadodara, Gujarat, where 2690 individual of 65 species belonging to 16 families were reported from different sites, and diversity indices were employed to unravel the species composition of that habitat. Further, 12 beetles from dominant families were selected for sequence analysis using various bioinformatics tools and were compared with the rest of the beetles to obtain a more robust phylogeny which is not reported earlier in previous studies.

Conclusion: Hence, the present study suggests that Scarabaeidae tends to be more diverse in and nearby of Vadodara compared to all other families of Coleoptera and while in contrast Chrysomelidae showed maximum diversity of pest species. DNA barcoding and nucleotide analysis resolves the phylogeny of controversial taxa; (Adephaga(Gyrinidae+ (Dytiscidae++ Carabidae)) (Polyphaga(Histeridae+Buprestidae+Lampyridae+Elateridae+(Scarabaeidae+(Coccinelidae+ Apionidae+Curculionidae+ (Meloidae+Tenebrionidae+(Cerambycidae+Chrysomelidae))))).

Keywords: Coleoptera, Phylogeny, DNA-barcoding, Sequence, Vadodara, Cryptic

Background

Insects comprise the most diverse and successful group of multicellular organisms on the planet, and they contribute significantly to vital ecological functions such as pollination, pest control, decomposition, and maintenance of wildlife species (Losey and Vaughan, 2006). The composition of this group reflects the dynamicity of the ecosystem (Fagundes et al. 2011) and is fundamental in the investigating of the landscape structure. An approximation of

350,000 species of beetles that is identified which comprise about 40% of all insects and 30% of all animals out of which 15,088 are reported from India (Choate, 2001; Kazmi and Ramamurthy, 2004). Beetles comprise of 25% of all described species (Hunt et al. 2007).

The Coleoptera can be found in nearly all natural habitats, that is, vegetative foliage, from trees and their bark to flowers, leaves, and underground near roots, even inside plant tissue like galls, tissue, including dead or decaying ones (Gullan and Cranston, 2010). About three fourth of beetle species are phytophagous in both the larval and adult stages, living in or on plants, wood, fungi, and a variety of stored products, including cereals, tobacco, and

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dried fruits (Gillott, 2005). Many of the beetles are considered as pest (Banerjee, 2014); they are also beneficial, acting as predators by controlling the populations of pests (Davis et al. 2001; Shearin et al. 2007; Brown et al. 2010). Some are bio-control agents for, e.g., dung beetles have been successfully used to reduce the populations of certain pestilent flies and parasitic worms that breed in cattle dung (Brown et al. 2010; Kakkar and Gupta, 2010), and they also prompt a series of ecosystem functions ranging from secondary seed dispersal to nutrient cycling and parasite suppression (Nichols et al. 2009), forest disruptions (Davis et al. 2001) and shaping the landscape structure. (Büchs, 2003).

Routine identification based on morphological characters are sometimes difficult and time-consuming if the specimen is damaged or is an immature stage of development, even specialist may be unable to make identification. However, barcoding solves these problems and serves a dual purpose as a new tool in the taxonomists' toolbox supplementing their traditional knowledge as well as being an innovative device. (Valentini et al. 2008). The DNA barcoding are of high value which helps in obtaining more detailed analysis of the species, broadening our understanding of both phylogenetic signal and population-level variation. (Hajibabaei et al. 2007). In this context, the use of DNA sequences represents a promising and effective tool for fast and accurate species identification. Further, DNA barcoding shares an emphasis on large-scale genetic data acquisition that offers new answers to questions previously beyond the reach of traditional disciplines. It consists of a standardized short sequence of DNA that can be easily generated and characterized for all species on the planet (Koch, 2010).

Globally, Coleoptera is the largest order among insects in terms of described species diversity (Foottit and Adler, 2009). Despite "the Creator's inordinate fondness for beetles" (Puspitasari, 2016), Coleoptera has not been favored to date by barcodes. For example, using the public data portal available through BOLD v.4, there were only 23,000 public barcode records for beetles forming 32,758 BIN'S from 159 countries. By comparison, the others of the top four most diverse insect orders are represented by approximately 2.5-fold (Hymenoptera), 3.5-fold (Diptera), and 8.5-fold (Lepidoptera). Moreover, several important studies on genetic variability within and between beetle species have largely employed genetic regions other than the standard animal barcode region (Bergsten et al. 2012). Thus, the DNA barcoding of Coleoptera is still in its infancy, especially when considering their known (Foottit and Adler, 2009) and projected (Odegaard, 2000) global diversity.

Diversity of Coleoptera based on their morphological characters in Gujarat has been reported earlier from Shoolpaneshwar sanctuary (Pilo et al. 1996); from Gir

PA, (Parikh, 2001) from Vadodara district, (Naidu and Kumar, 2011), and from Gujarat (Thakkar and Parikh, 2016), if the identification of beetles with DNA barcodes are concerned, no studies have been reported for Vadodara district till date. Moreover, studies linking the diversity and ecological status (pest and beneficial) are scarce. Hence, the present study aims to find the diversity and classify beetles with the help of DNA barcode approach in agriculture field of Vadodara district.
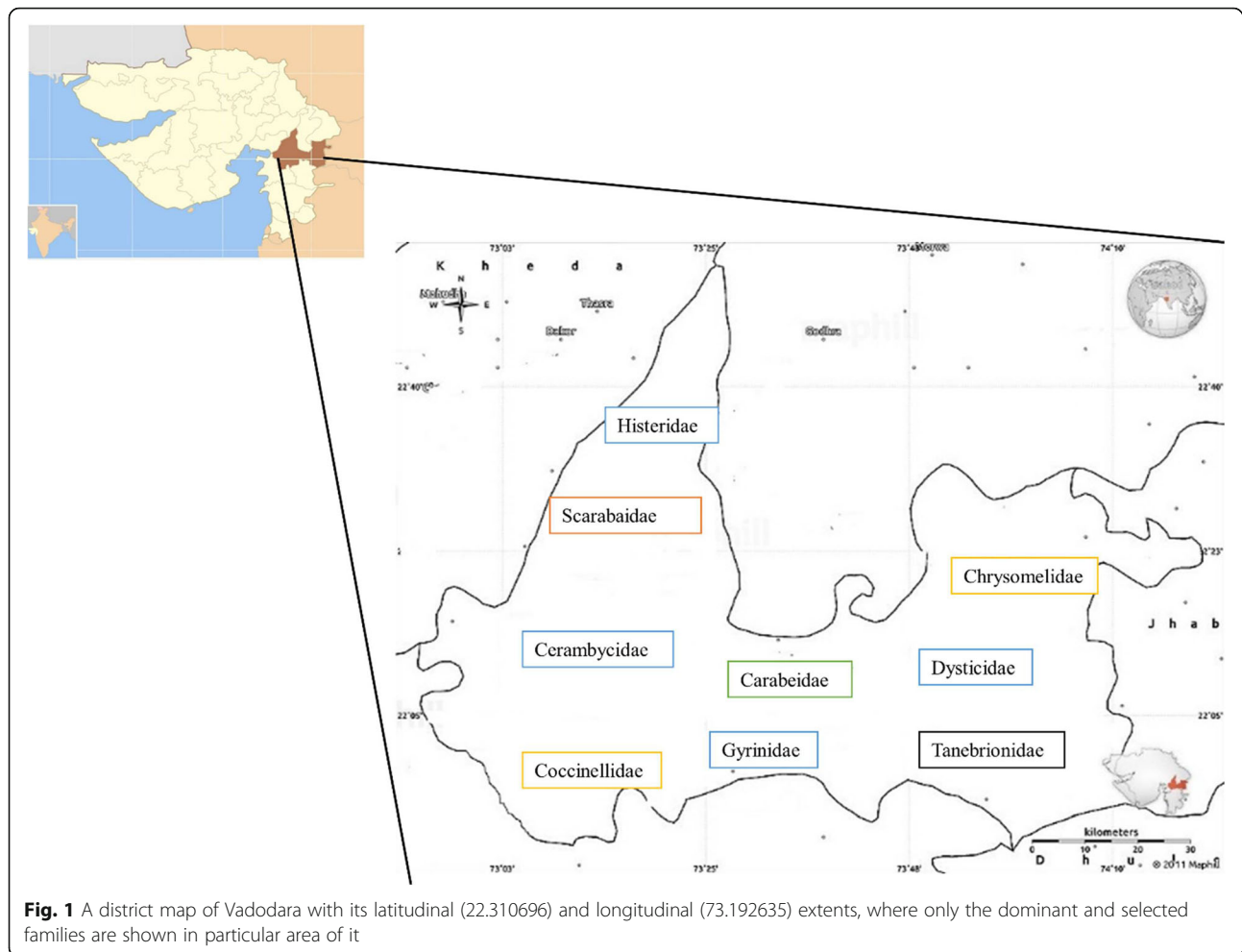
Materials and methods
Site selection and insect collection
Vadodara was surveyed and a total of 20 sites were visited monthly without any habitat bias keeping agricultural fields common in each (Fig. 1). They were collected by standard collecting methods, viz. hand-picking, light trap, net-sweeping, and pitfall trap. In addition, visual sighting and photo documentation were also carried out. Following the standard protocols of pinning each specimen was pinned for further identification. Identification was done by using standard reference books and published articles according to their morphological traits till species level or morphospecies. The species identified were confirmed by comparing with the authentic specimens from the repository at Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda.

Isolation of DNA, PCR, and sequencing
For DNA isolation, the protocol from Silveira et al. (1998) was followed by required modification. The procedure used was as follows: muscles were removed from the thoracic and abdominal region and were homogenized with lysis buffer (0.4 M NaCl, 10 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, containing proteinase k, and 2% SLS). Then, the homogenate was incubated at 60 °C for 1 h, later 6 M NaCl was added to the suspension and vortexed for 1 min, followed by centrifugation at 14,000 rpm for 20 min. The supernatant was removed, and an equal amount of isopropanol was added (1:1) and was further incubated at − 20 °C for 1 h followed by next cycle of centrifugation at 14,000 rpm for 20 min. The obtained pellet was washed with 70% ethanol and kept in 25 μl of milliQ water at − 20 °C condition for further process. The integrity of DNA was checked by using 0.8% Agarose using gel electrophoresis. Quantification of DNA was done by calculating the ratio of optical density obtained at 260/280 nm. The DNA product was then amplified in PCR at 94 °C denaturation for 1 min, 45 °C annealing for 1:30 min and 50 °C for 1:30 min, and extension was carried out 72 °C for 7 min. A total of 35 cycles were performed using primers LCO-1490 5′GTCAACAAATCATAAAGATATTGG-3′, HCO2198–5′TAAACTTCAGGGTGACCAAAAAATCA

Fig. 1 A district map of Vadodara with its latitudinal (22.310696) and longitudinal (73.192635) extents, where only the dominant and selected families are shown in particular area of it

-3′ (Folmer et al. 1994). The amplified product was purified using the thermos-Exosap kit (cat. no. 78200.200.UL.), and sequencing steps were performed according to Yuan et al. (2015a, b).

Data analysis
The diversity indices like Shannon diversity indices, Pielou's evenness, and Margalef's richness were calculated by PAST 3.X software.

Sequence validation and bioinformatics analysis
The sequence was validated and specific contigs of 12 beetles were obtained using BioEdit 7.0.5.3.

Further, the sequence was submitted to BOLD v.4 having process Id as given in Additional file 1: Table S1 and barcode gap analysis (Additional file 2: Table S2) was performed to find out the distance from the nearest neighbor. To have a holistic approach, COI sequences of 43 beetles were downloaded from NCBI as given in (Additional file 2: Table S2). Nucleotide composition and codon usage of all the beetles was determined using the MEGA7 software. The sequence was subjected to strand asymmetric analysis which was calculated using the formula(s)

AT-skew = (A−T)/(A + T), GC skew = (G−C)/(G + C).

Taxa selection and phylogenetic analysis
Among all the families reported Scarabaeidae, Carabidae, and Tenebrionidae species were selected due to their abundance. Chrysomelidae and Cerambycidae, due to their pest status, on the other hand, Lampyridae and Gyrinidae were selected due to its sparse availability and only representative of water beetle respectively. Hence, a total 65 individuals belonging to 16 families found in the present study, from which 56 species were selected for phylogenetic tree analysis. Furthermore, it was subjected to tree construction using statistical neighborhood joining distance where the test of phylogeny was performed using the bootstrap method with 500 replicates and was subjected to nucleotide type substitution. The maximum composite likelihood method and Gamma rate (G) were used to assess the diversity, and complete deletion was applied to obtain the complete sequence. This was accomplished through phyloT and was viewed in

interactive Tree of life (iTOL) and re-confirmed in MEGA7 software.

Results

Diversity, pest-status, and indices

During the present survey, 2690 individuals representing 65 species, belonging to 16 families of Coleoptera were recorded. Annotated checklist and pest statuses of recorded species are represented in Table 1. Of the total 16 families, Scarabaeidae was found to be the most dominant with 18 representatives, followed by Carabidae with 9 species; Chrysomelidae with 8 species, followed by Cerambycidae having 5 species. Tenebrionidae, Meloidae, and Coccinellidae were represented by four species and Elateridae with three species. Only two representatives were from Dytiscidae and Curculionidae. Families Gyrinidae, Buprestidae, Histeridae, Apionidae, Cicindelidae, and Lampyridae were represented by only one species each.

As far as diversity indices are concerned, Scarabaeidae, Carabidae, and Chrysomelidae families were found to be most diverse and rich among all families with Shannon diversity index above 2.0 and Margalef's Index in the range of 1.1–2.5 (Fig. 2). Tenebrionidae, Elateridae, Meloidae, Coccinellidae, and Cerambycidae had Shannon index ranged between 1.08 and 1.56, and the Margalef's richness index was in the range of 0.23–0.56 indicating moderately rich families. Buprestidae, Gyrinidae, Apionidae, Histeridae, Cicindelidae, and Lampyridae had just one representative species making the least diverse families (Fig. 2). Pest and beneficial status was observed in different species as an ecological evaluation parameter. Among all the species reported, the highest numbers of Phytophagus pest species were from Chrysomelidae (six) trailed by Scarabaeidae (five), Carabidae (four), and Cerambycidae (four). However, the beneficial insects were represented by Scarabaeidae (five) as decomposers, followed by Elateridae, Meloidae, and Buprestidae as pollinators having one species each.

Universal primers were used in this study, which perfectly amplified a 710 bp fragment of the *mitochondrial COI* gene when applied to template DNA (Fig. 3) from the specimens as shown in Fig. 4. Twelve species barcode sequences were obtained from the seven selected families. Table 2 represents the processID and sampleID of the species submitted on BOLD v.4.

Sequence annotation

To find the sequence composition, GC% analysis was performed which resulted in decreasing order as follows; *O. nasicora* (39.4%), *B. rufomacula* (35.9%), *C. maderae* (35.3%), *Luciola* sp. (34.9%), *Pherophophus* sp. (34.4%), *Mesostena* sp. (33.4%), *Oides* sp. (31.9%), *G. miliaris* (31.6%), *X. jamaicensis* (30.9%), *G. natator* (29.9%), *P.*

albipes (29.3%). Further, the sequence was analyzed for GC and AT skews, where GC skew was found the maximum in *Luciola* sp. (0.1), whereas the lowest was found in *O. nasicora* (– 0.2) among the selected species. However, overall comparison with other species, the maximum GC skew was shared between *Chlaenius* sp. and *Luciola* sp. of Carabidae and Lampyridae respectively (Fig. 5).

However, the AT% was ranging from 70.7% to 60.6%, where *P. albipes* was more AT-biased (70.7%), while *O. nasicora* had the lowest AT% (60.4%). Additionally, AT skew analysis revealed that *Luciola* sp. (0.10) has the highest AT value, whereas the lowest one was *C. indicus* (– 0.13). To map the AT richness of selected species among the other species, we found that *P. strennus* had the highest value (+ 0.13), while *Trachysida* sp. was recorded with the lowest value of (– 0.2) (Fig. 6).

Phylogenetic analysis

Coleoptera is considered to be one of the most controversial orders of Class Insecta, as it is the dominant order representing the highest level of species among other insect orders. Hence, the neighbor-joining statistical method for phylogeny reconstruction was performed which yielded maximum likelihood among the selected species. The sequence analysis also revealed that the computed overall mean distance was 833.28 ± 83.3 among the species; however, the pairwise mean distance showed a remarkable range of 2254.84 (*Trachysida* sp.) to 0.024 (*O. taurus*) of individual species comparison as depicted in Table 3 and Additional file 2: Table S2.

Nucleotide substitution with maximum composite likelihood method with Gamma distribution (G) yielded the sequence similarity suggesting that among all the species selected for the study, *G. natator*, *P. albipes*, and *Pheropsophus* sp. belong to suborder Adephaga, while other nine species belong to Polyphaga. The present study also reported that scarabaeids were closest to Elateridae and Coccinellidae compared to other Polyphagans. On the other hand, Carabidae was found to be closer to Dytiscidae (Fig. 7).

Further, we tried to look into the phylogeny of individual species (Additional file 1: Table S1) taking into consideration only barcoded species, the study showed that in family Scarabaeidae, *O. nasicora*, and *X. jamaicensis* were phylogenetically more close belonging to subfamily of Dynastini. On the other hand, *C. indicus* and *G. miliaris* were closest belonging to subfamily Scarabaeinae. In case of Carabidae, the obtained phylogeny showed that *P. albipes*, *C. maderae*, *Pheropsophus* sp. belong to Platininae, Carabinae, and Brachininae respectively (Fig. 8). The least distance was obtained between *P. albipes* and *C. maderae* suggesting they are more phylogenetically close. Moreover, as in the present study, individual species reported from Gyrinidae, Tenebrionidae, Chrysomelidae, Lampyridae, and
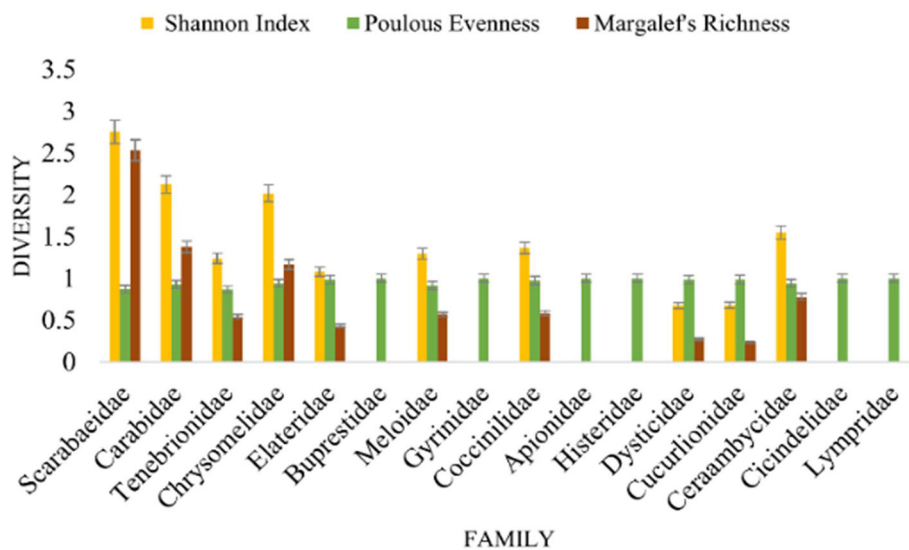
Table 1 Annotated checklist and pest status of Coleoptera of Vadodara, Gujarat, India

Family	Species	Pest status	Importance
Scarabaeidae	*Oxycetonia versicolor*	Yes	Pest of pigeon pea, mung beans, and rose
	Phyllophaga sp.	Yes	Pest of grasses and cereal roots
	Onthophagus gazella	No	Pest predator
	Oryctes nasicora	Yes	Pest of coconut
	Xyloryctes jamaicensis	Yes	Pest of coconut
	Aphodius fossor	No	Decomposers, enricher
	Onthophagus sp.	No	
	Onthophagus taurus	No	Decomposer, enricher
	Copris incertus	No	Enricher
	Cremastocheilus sp.	Yes	Pest of banana
	Heliocopris gigas	No	
	Onthophagus dama	No	
	Phyllophaga nebulosa	No	Pest predator
	Ateuchus sp.	No	
	Gymnopleurus miliaris	No	
	Canthon viridis	No	Decomposer and enricher
	Garreta sp.	No	Decomposer and enricher
	Cheironitis indicus	No	
	Catharsius molossus	No	Decomposer and enricher
Carabidae	*Calosoma maderae*	No	Pest predator
	Pterostichus strenuus	No	
	Pheropsophus verticalis	No	
	Pterostichus aethiops	No	Pest predator
	Scaritus quadriceps	No	Pest predator
	Paranchus albipes	No	
	Anthia sexguttata	No	Pest predator
	Chlaenius bimaculatus	No	
	Chlaenius sp.	No	
	Cicindela sp.	No	Pest predator
Tenebrionidae	*Tribolium castenium*	Yes	Pest of flour and stored grains
	Mesostena sp.	No	
	Gonocephalum sp.	Yes	Pest of groundnut
	Tenebrio molitor	Yes	Pest of stored grains
Chrysomelidae	*Oides bipunctata*	Yes	Pest of grapes and grape vine
	Oulema melanopus	Yes	Pest of oat crop
	Aspidomorpha sp.	No	
	Podagrica fuscicornis	Yes	Pest of okra leaves
	Clytra sp.	Yes	Pest of soalu leaves, *Vateria indica* L.
	Lilioceris sp.	No	biological control
	Metriona bicolor	Yes	Pest of sweet potato
	Charidotella sp.	Yes	Pest of Ipomoea, sunflower, and cabbage
Elateridae	*Athous haemorrhoidalis*	No	Pollinator of *Coeloglossum viride*
	Hemicrepidius sp.	Yes	Pest of cereals, crops, and grasses
	Lanelater fuscipes	Yes	Pest of cereals and vegetable crops

Table 1 Annotated checklist and pest status of Coleoptera of Vadodara, Gujarat, India *(Continued)*

Family	Species	Pest status	Importance
Buprestidae	*Acmaeodera* sp.	No	Pollinator of lily
Meloidae	*Mylabris cichorii*	No	Pollinators and Phytophagus
	Phodaga alticeps	No	
	Mylabris postulata	No	Pollinators and Phytophagus
	Alosimus syriacus	Yes	Pest of cotton fields
Gyrinidae	*Gyrinus natator*	No	
Coccinilidae	*Cheilomenes sexmaculata*	No	Pest predator
	Coccinella transversalis	No	Pest predator
	Harmonia sedecimnotata	Yes	Pests of eggplant and cauliflower
	Cheilomenes sp.	No	
Apionidae	*Apion clavipes*	Yes	Pests of pigeon pea and soya bean
Histeridae	*Euspilotus nigrita*	No	
Dytiscidae	*Eretes* sp.	No	
	Cybister fimbriolatus	No	
Cucurlionidae	*Polydrusus sericeus*	Yes	Pests of oak and hazel
	Cleonus sp.	Yes	Pest of lack plant
Cerambycidae	*Trachysida* sp.	No	
	Batocera rufomaculata	Yes	Major pest of fig trees
	Macrotoma palmate	Yes	Pests of fruits, woods, and ornamental plants
	Celosterna scabratoea	Yes	Minor pests of mango, pomegranate, and casuarina
	Xystrocera globosa	Yes	Pest of cocoa
Lampyridae	*Luciola* sp.	No	

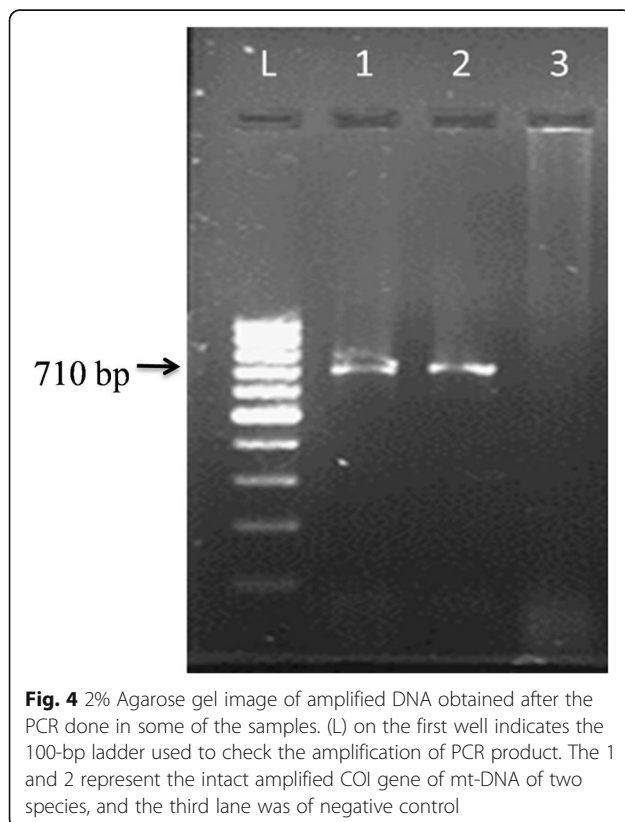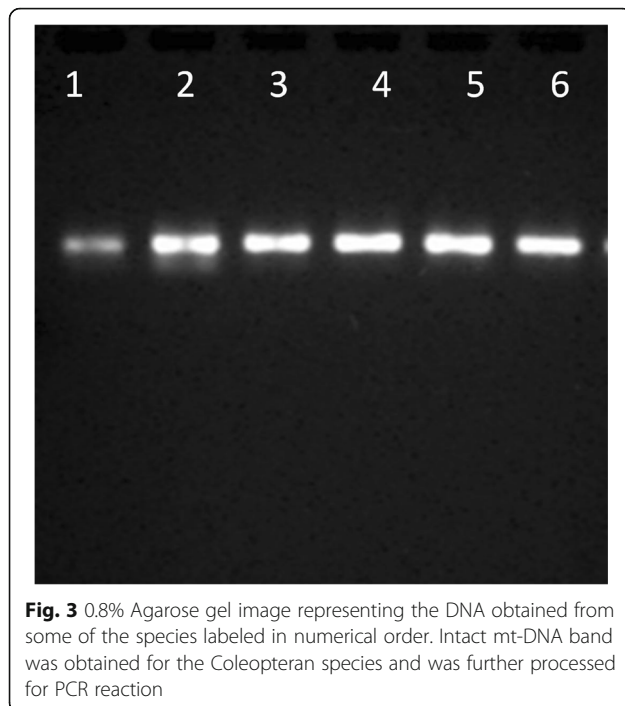**Fig. 2** Graph depicting the diversity indices, calculated for the obtained families in Vadodara

Fig. 3 0.8% Agarose gel image representing the DNA obtained from some of the species labeled in numerical order. Intact mt-DNA band was obtained for the Coleopteran species and was further processed for PCR reaction

710 bp →

Fig. 4 2% Agarose gel image of amplified DNA obtained after the PCR done in some of the samples. (L) on the first well indicates the 100-bp ladder used to check the amplification of PCR product. The 1 and 2 represent the intact amplified COI gene of mt-DNA of two species, and the third lane was of negative control

Table 2 List of species with their families submitted to BOLD v.4 along with their processID and sampleID

Sr. no	Family	Species name	processID	sampleID
1	Scarabaeidae	*Gymnopleurus miliaris*	PBSL002-17	GMA1
2	Carabidae	*Calosoma maderae*	PBSL006-17	CMS1
3	Cerambycidae	*Batocera rufomaculata*	PBSL001-17	BRC1
4	Chrysomelidae	*Oides bipunctata*	PBSL004-17	OBF1
5	Tenebrionidae	*Mesosterna* sp.	PBSL003-17	MSE1
6	Lampyridae	*Luciola* sp.	PBSL005-17	LSG1
7	Scarabaeidae	*Orcytes nasicora*	PBSL008-17	ONcol2
8	Scarabaeidae	*Xyloryctes jamaicensis*	PBSL010-17	XJCol4
9	Scarabaeidae	*Chironitis indicus*	PBSL012-17	CICol6
10	Carabidae	*Paranchus abipes*	PBSL009-17	PACol3
11	Carabidae	*Pherophosphus verticalis*	PBSL011-17	PVCol5
12	Gyrinidae	*Gyrinus natator*	PBSL007-17	GNCol1

Cerambycidae were less, it is difficult to comment on its phylogeny.

Discussion

In the present study, an attempt has been made to correlate the identification and assess the diversity of Coleoptera of Vadodara morphologically and genetically. An annotated list of the Coleoptera is represented in Table 1. Of all the collected species, the Scarabaeidae was the most dominant which is not a surprise as it is the largest family of the order Coleoptera. Unlike other groups of insects, the members of the family Scarabaeidae contain both coprophagous (beneficial) and phytophagous (harmful) beetles. The coprophagous represented by 14 species have an important ecological role through their habitat of burrowing and burying of dung. This performs a series of ecological functions such as nutrient cycling, soil aeration, and secondary seed dispersal. (Larsen 2004; Chandra et al. 2012a, b; Chandra and Gupta, 2013). On the other hand, phytophagous (leaf chafers) represented by four species were the agricultural pests of various commercial crops, feeding on leaves, flowers, fruits, roots, and other parts of the plant. A good assemblage of Scarabaeidae is thus self-explanatory. (Chatterjee, 2009; Thakkar and Parikh, 2016). Due to the presence of diverse vegetation, five Phytophagus pest beetles were recorded, two pest-feeding species, and five species of decomposers suggest that they were evenly distributed in different habitats.

The second most diverse family was Carabidae, representing a total of nine species. These beetles are increasingly used as a taxonomic study group in biodiversity and as bio-indicators in monitoring or site assessment studies for nature conservation purposes (Ghahari et al. 2010). Four predator species were found in the study that were feeding on small insects and larvae suggesting
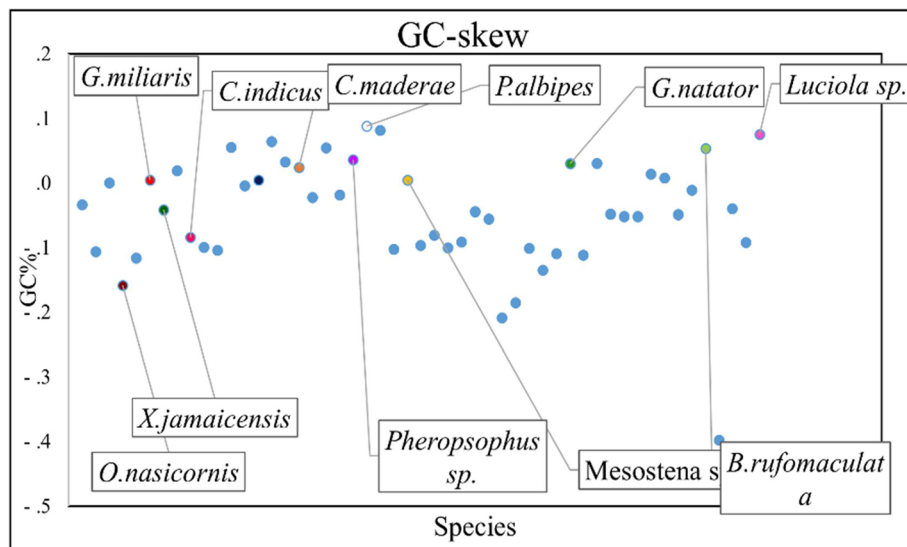
Fig. 5 GC skew of all the species in study is calculated with predefined formula as mentioned in materials and method. The species sequenced are highlighted in the graph. *Luciola* sp. had the maximum value of + 0, while the lowest was of *O.nasicornis*. *G.miliaris*, *C.maderae*, *P.albipes*, *G.natator*, *B.rufomaculata*, *Pherpsophus* sp., and *Luciola* sp. had values between 0 and + 1

population of pest species are high due to which Carabids were found more. So an appreciable number of ground beetles in Vadodara and its predator habit thus suggest a positive ecological role of these insects. (Koivula, 2011; Thakkar and Parikh, 2016).

Leaf beetles or Chrysomelidae are considered to be one of important pest family due to their phytophagous feeding habits (Ding et al. 2007; Meissle et al. 2009). In the present study, eight species were reported making it the third most diverse family. Among all, the highest number of Phytophagus pest species was recorded (six) in this family. Furthermore, the remaining data obtained

suggests it biological control activity. Hence, feeding characteristic and their high abundance makes them an important component of the food webs and a major component of herbivore guilds as well as an important food component for higher trophic organisms (Sánchez-Reyes et al. 2014).

Tenebrionidae is one more family having the highest number of stored grain pest. Stored product insects have a large economic impact on stored bulk grains and processed commodities. These insects can survive on small amounts of food that accumulate in inaccessible places. (Campbell and Arbogast, 2004). It is estimated that
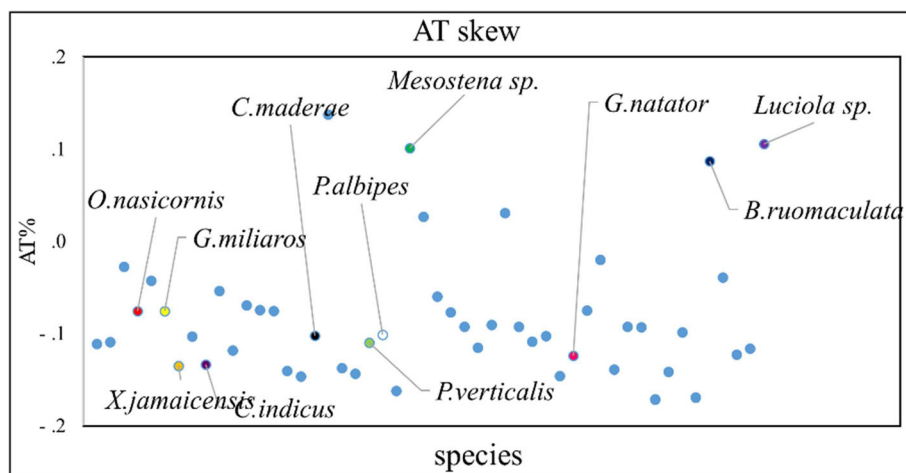

Fig. 6 AT skew of the species in study is calculated with predefined formula. The selected species sequenced are highlighted in the graph. *Luciola* sp. had the maximum value of + 0.1, while the lowest was of *C.indicus* (− 0.13). *Mesostena* sp., *Luciola* sp., and *B.rufomaculata* are the only three species having values > 0. All the rest species are less AT biased

Table 3 Families of Coleoptera with their species submitted to NCBI along with its extension numbers by different authors used in the present report

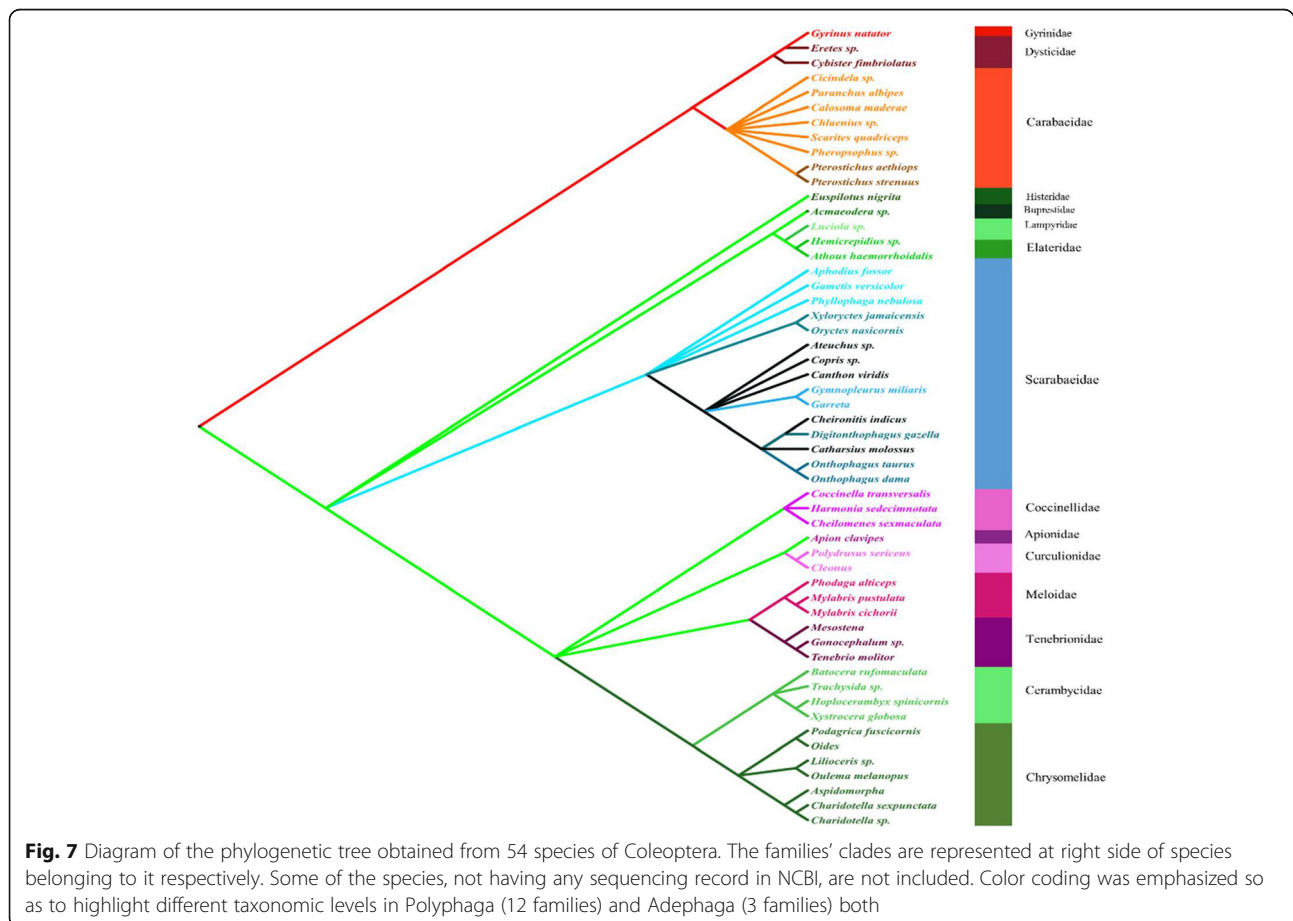| Family	Species	NCBI ID/BOLD ID	References	bp length
Scarabaeidae	*Gametis versicolor*	KJ559408.1	Karthika, P. and Krishnaveni, N. (unpublished)	695 bp
	Phyllophaga sp.	JX963332.1	García-López, et al. 2011	693 bp
	Onthophagus gazella	EU162450.1	Emlen, D.J., et al. 2005	612 bp
	Oryctes nasicornis	AAP9162	Direct submission	657 bp
	Xyloryctes jamaicensis	PBSL010-17	Direct submission	776 bp
	Aphodius fossor	AY132615.1	Mate, J.F. and Vogler, A.P. (unpublished)	795 bp
	Onthophagus sp.	KR486381.1	Hebert, P.D., et al. 2003	573 bp
	Onthophagus taurus	EU162476.1	Emlen, D.J., et al. 2005	612 bp
	Copris sp.	MF804615.1	Blaimer, B. and Mulcahy, D.G. (unpublished)	658 bp
	Onthophagus dama	*Taxonomy ID:* 1675891		
	Phyllophaga nebulosa	KX686390.1	Richmond, M.P. et.al. 2016	1191 bp
	Ateuchus sp.	EU162435.1	Emlen, D.J., et al. 2005	584 bp
	chirontits indicus	PBSL012-17	Direct submission	517 bp
	Gympleurus miliaris	PBSL002-17	Direct submission	718 bp
	Canthon viridis	AY131817.1	Monaghan, M.T et al. 2005	792 bp
	Garreta sp.			
	Catharsius molossus	JQ855895.1	Wang, C.Y., et al. 2005	658 bp
Carabidae	*Calosoma maderae*	ADJ6357	Direct submission	696 bp
	pterostichus strenuus	EU142597.1	Will, K.W. and Gill, A.S. 2008	817 bp
	Pheropsophus verticalis	AAZ0127	Direct submission	555 bp
	Pterostichus aethiops	KM444318.1	Hendrich, L., et al. 2015	658 bp
	Scarites quadriceps	DQ063222.1	Greenstone, M.H., et al. 2005	1237 bp
	Paranchus albipes	AAL7246	Direct submission	658 bp
	Chlaenius sp.			
Tenebrionidae	*Tribolium castaneum*	KU494281.1	Fohrer, F. and Meusnier, I. (unpublished)	658 bp
	Mesostena sp.	PBSL003-17	Direct submission	668 bp
	Gonocephalum sp.	JQ753368.1	Gunter, N.L., et al. (unpublished)	723 bp
	Tenebrio molitor	KR915361.1	Hebert, P.D., et al. 2003	615 bp
Chrysomelidae	*Oides bipunctata*	PBSL005-17	Direct submission	733 bp
	Oulema melanopus	KR490936.1	Hebert, P.D., et al. 2003	658 bp
	Podagrica fuscicornis	KF656267.1	Baselga, A., et al. 2015	655 bp
	Lilioceris sp.	AM283150.1	Vogler, A.P. (unpublished)	826 bp
	charidotella sexpunctata	AM283219.1	Vogler, A.P. (unpublished)	823 bp
Elateridae	*Athous haemorrhoidalis*	JF781273.1	Wysocka, A et al. 2011	588 bp
	Hemicrepidius sp.	KF442245.1	Han, T., et.al. (unpublished)	669 bp
	Lanelater fuscipes	MF804547.1	Blaimer, B. and Mulcahy, D.G. (unpublished)	658 bp
Buprestidae	*Acmaeodera* sp.	KM364403.1	Evans, A.M., et al. 2015	1206 bp
Meloidae	*Mylabris cichorii*	FJ462783.2	Park, H.C.(unpublished)	658 bp
	Mylabris pustulata	KX013089.1	Ajish, S.S. and Balaji, S. (unpublished)	682 bp
Gyrinidae	*Gyrinus natator*	PBSL007-17	Direct submission	655 bp
Coccinellidae	*Cheilomenes sexmaculata*	FJ154102.1	Khushboo, S et al. (unpublished)	517 bp
	Coccinella transversalis	KX758112.1	Poolprasert, P. (unpublished)	675 bp
	Harmonia sedecimnotata	EU392410.1	Aruggoda, A.G.B., (unpublished)	583 bp

Table 3 Families of Coleoptera with their species submitted to NCBI along with its extension numbers by different authors used in the present report *(Continued)*
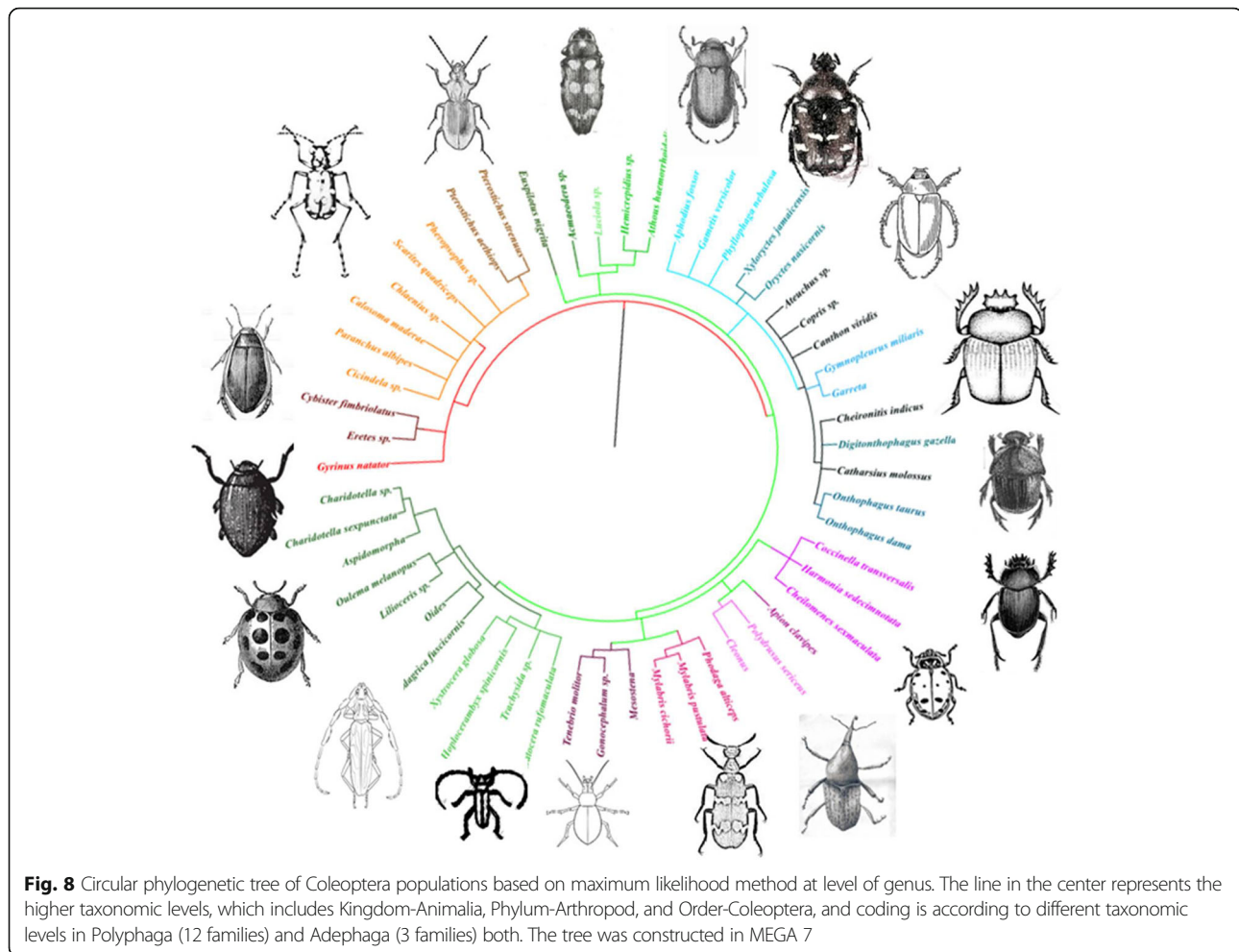
Family	Species	NCBI ID/BOLD ID	References	bp length
Apionidae	*Apion* sp.	HQ883612.1	Jordal, B.H., et al. 2011	690 bp
Histeridae	*Euspilotus nigrita*			
Dytiscidae	*Eretes* sp.	LT615895.1	Lam, A., (unpublished)	744 bp
	Cybister fimbriolatus	DQ813676.1	Miller, K.B., et al. 2007	1294 bp
Cucurlionidae	*Polydrusus sericeus*	KJ166400.1	Dewaard, J.R., et al. (unpublished)	621 bp
	Cleonus sp.			
Cerambycidae	*Trachysida* sp.	KR126483.1	Dewaard, J.R., et al. (unpublished)	564 bp
	Batocera rufomaculata	PBSL001-17	Direct submission	679 bp
	hoplocerambyx spinicornis	KJ159160.1	Zheng, S., et.al. (unpublished)	500 bp
	xystrocera globosa	KY357523.1	Wu, Y., et al. 2017	658 bp
Cicindelidae	*Cicindela* sp.	KC963753.1	Lopez-Lopez, A. and Vogler, A.P. (direct submission)	657 bp
Lampyridae	*Luciola* sp.	PBSL005-17	Direct submission	683 bp

over-all damage caused by stored grain pest account for 10–40% loss annually (Mishra and Tripathi, 2011). The beetles found in this family showed more or less Phytophagus pest habit. Example of tenebrionid species includes *Tribolium castaneum*, feeds on flour, cereals, meals, crackles', beans, spices, pasta, etc. (Weston and

Rattingourd, 2000). Due to their nocturnal habit, small size, and their burrowing tendency that may have led to less number of Tenebrionidae in this study.

Families like Buprestidae, Apionidae, Lampyridae, and Gyrinidae were very less diverse as beetles of these families are very habitat specific. Hence, in the present

Fig. 7 Diagram of the phylogenetic tree obtained from 54 species of Coleoptera. The families' clades are represented at right side of species belonging to it respectively. Some of the species, not having any sequencing record in NCBI, are not included. Color coding was emphasized so as to highlight different taxonomic levels in Polyphaga (12 families) and Adephaga (3 families) both

Fig. 8 Circular phylogenetic tree of Coleoptera populations based on maximum likelihood method at level of genus. The line in the center represents the higher taxonomic levels, which includes Kingdom-Animalia, Phylum-Arthropod, and Order-Coleoptera, and coding is according to different taxonomic levels in Polyphaga (12 families) and Adephaga (3 families) both. The tree was constructed in MEGA 7

study, the less number sighted could be because habitat species studies were not carried out. Buprestidae (jewel beetles) and Meloidae beetles are found nearby flowering plants suggesting their ecological role as pollinators. Gyrinids are water beetle so availability of water determines their presence. A single species of Apionidae family which was collected is *Apion clavipes* and is considered to be a pest of Pigeon pea (Bandyopadhyay et al. 2009). In case of Elateridae, they were commonly sighted under the bark of the trees. Phytophagus individuals were found on feeding large variety of crops, resulting in damage to seeds, roots, stems, and harvestable plant parts, which can facilitate secondary crop damage by pathogens.

Coccinellidae, Cerambycidae, and Curculionidae families were represented by total of 11 species in the study area. However, as these groups of coleopterans are usually considered to be the harmful one and at no time or place, they were reported to be in aggregation that can lead to the serious problem. Hence, the reported harmful groups of coleopterans are comparatively less. Although records of beetles as habitat indicator are

common, however, most of the species collected in this work hold up huge alteration in the environmental factors and there is no substitution of species along with the diversification of the habitat (Spector & Ayzama, 2005; Scheffler 2005; Da Silva et al. 2010). Thus, at this point, it is difficult to comment on their habitat distribution and ecophysiological nature of the studied beetles.

The vast number of insect species are often exceedingly difficult to recognize using only morphological approach (Witt et al. 2006) and thus creates an insurmountable barrier for cataloging total biodiversity by only traditional taxonomy (Blaxter 2004; Pentinsaari et al. 2014), for which morphological identification have fallen short and the DNA barcoding has filled the gap (Bourke et al. 2013; Laurito et al. 2013). Several scientists are now using DNA-barcoding to understand the biodiversity of insects (Hebert et al. 2003; Hajibabaei et al. 2007). In line with this, the present study was focused on barcoding of 12 species on the basis of its habit, number, previously known literature, and its controversial status (Yuan et al. 2016), where barcoding and sequence analysis were totally based on mitochondrial COI. The selection of this marker is

based on its low rate of mutation caused by habitat-specific adaptive radiation (Raupach et al. 2010) and so that a concrete phylogenetic tree is constructed of the selected species using standard models (lanfear et al. 2014).

Scarabaeidae considered to be one of the important family in Coleoptera as discussed above was found to be monophyletic to Elateridae, Buprestidae, Lampyridae, and Histeridae. The barcoding of *X. jamaicensis* and *O. nasicora* illustrated very less paired-wise distance indicating its phylogenetic closeness. Both the species had almost the same morphological characteristics, i.e., both have horns, similar size, thoracic, and abdominal hairs making it controversial for identification (Dechambre and Lachaume, 2001). However, in the present report, barcoding of these species revealed that both of them belong to individual tribe and genus. Similarly, Carabidae was found to be monophyletic to Gyrinidae; however, it shared homology with Dytiscidae. Species-level phylogeny showed *P. albipes* and *C. maderae* showed least paired-wise distance compared to *Pheropsophus* sp. This is the first study which shows the phylogenetic distance among these species of this family.

Moreover, Gyrinidae was found to be monophyletic to Dytiscidae and polyphyletic to Carabidae. During the present investigation, only *G. natator* was found as a representative of Gyrinidae and was closest to *Eretes* sp. belonging from Dytiscidae, suggesting that both shares ancestral characteristics of the clade (Yuan et al. 2016). Similarly, Tenebrionidae was polyphyletic to Cerambycidae and Chrysomelidae. However, it was found to be monophyletic to Meloidae (lanfear et al. 2014). The sister clade of *B. rufomaculata*, *O. bipunctata*, and *Mesostena* sp. was showing lowest pairwise distance to *Trachysida* sp., *P. fuscicornis*, and *Gonocephalum* sp. respectively. Our study is parallel with previous established molecular and morphological studies. (Caterino et al. 2002; Hunt et al. 2007; Pons et al. 2010; Lawrence et al. 2011; Bocak et al. 2014).

GC and AT% provided the evidence of two suborders, Adephaga and Polyphaga. Our results account for the fact that Scarabaeidae forms sister clade with Elateridae, Lampyridae, Buprestidae, and Histeridae. Correspondingly, Carabidae with Dytiscidae and Gyrinidae, Chrysomelidae with Cerambycidae, Tenebrionidae with Meloidae, Curculionidae, and Coccinellidae. Our studies were incongruent with earlier mitochondrial genomes studies of Yuan et al. (2015a, b, 2016).

Conclusion

Despite optimistic views, the taxonomic impediment remains the main concern and thus demands an urgent need for comprehensive biodiversity assessments due to biodiversity crises: the risk of human activity causing mass extinction. Thus, barcoding can accelerate the process of taxonomic inventory. In conclusion, the present study incorporates genetical, morphological, and ecological data which specify its species distribution, richness, and diversity in different sites of Vadodara, Gujarat, and the phylogeny derived from present study unravels the status of unknown species of Coleoptera:

1. (Adephaga(Gyrinidae+ (Dytiscidae++ Carabidae))
2. (Polyphaga(Histeridae+Buprestidae+Lampyridae +Elateridae+(Scarabaeidae+(Coccinelidae+ Apionidae+Cucurlionodae+ (Meloidae +Tenebrionidae+(Cerambycidae +Chrysomelidae))))))

Thus, from this study, it can be derived that Scarabidae are the most diverse group of beetles in and around Vadodara, and pest status of beetles are high due to several adaptations. Thus, we suggest further studies are needed to understand the ecophysiological role of different beetle families.

Additional files

Additional file 1: Table S1. Pairwise computation of distance of individual species using MEGA7 software (DOCX 44 kb)

Additional file 2: Table S2. The sequence obtained was subjected to barcode gap analysis that represented the distance to nearest neighbor (NN). The barcode gap analysis was performed in BOLD v.4to find the nearest neighbor distance using K2P parameter. The overall mean distance was reported to be 35.95% in the present study (DOCX 11 kb)

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