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First record and DNA barcode of a scarab beetle, *Adoretus kanarensis* Arrow, 1917 (Coleoptera: Scarabaeidae: Rutelinae), from Maharashtra, India

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Abstract: *Adoretus kanarensis* Arrow, 1917, composed within the subfamily Rutelinae of family Scarabaeidae, was described from India about 107 years ago. These conspicuous, attractive, and tiny leaf chafers are recorded as predators of several agricultural crops. The mt DNA Barcodes of many *Adoretus* Dejean, 1833 species are available in global databases. In this work, first DNA barcode of *A. kanarensis* has been reported. Morphological details with photographs of the species along with a checklist of the genus *Adoretus* from Maharashtra is provided. The mt COI DNA sequences of *Adoretus* species recorded until now for the genus that are listed in GenBank are analyzed.

Keywords: Chafers, dung beetle, Nashik, new record, polyphagous pest, survey.

The genus *Adoretus* Dejean, 1833 is distributed in Africa, Asia, and Europe (Anonymous 2023). It is a polyphagous and economically important genus, due to its potential damage to a wide variety of host plants including important agricultural cash crops like sugarcane, tobacco and ornamental plants (McQuate & Jameson

2011). There are about 460 species (Krajcik 2007) reported in the world, of which 47 are reported in India (Arrow 1917), few literature is available on this genus from India (Chandra 2009; Chandra et al. 2012; Ghosh et al. 2020). Some of the species of this genus are of biosecurity concerns (McQuate & Jameson 2011). The accurate identification based on the morphological characters are important for undertaking proper control measures. Beetles of the subfamily Rutelinae are not dung beetles in the true sense. They are phytophagous and commonly known as May or June beetles or shining leaf chafers (Sreedevi et al. 2017). The pioneering work on this group in India was undertaken by Arrow (1917) and Balthasar (1963, 1974). Since the fauna has not been revised, few scattered publications are available on this particular group from India, without detailed distribution records (Kumar & Pandey 2023).

The present study was undertaken to document the

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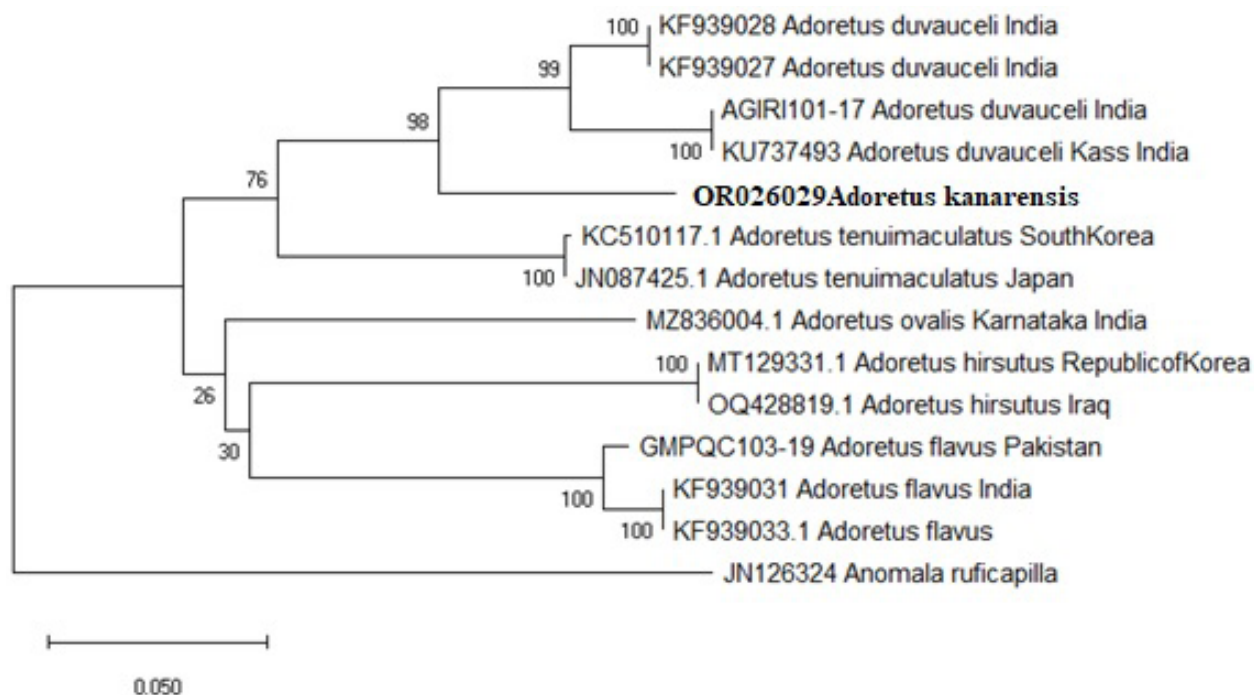


Figure 1. Maximum likelihood tree for the species of *Adoretus kanarensis* based on the 578 bp of mitochondrial COI DNA gene sequences.

first record of *A. kanarensis* from Maharashtra since Arrow (1917) noted the distribution as Bombay: North Kanara, indicating that North Kanara was included at that time when Bombay was the Bombay presidency during British India. Considering this, the present report forms the first report of *A. kanarensis* from Maharashtra, India based on voucher specimen with first DNA barcode for the country.

Though *Adoretus* species are widely distributed, only 12–13 mitochondrial cytochrome oxidase subunit (COI) sequences are currently available in global database. Hence, during one of our experiments to generate mt DNA barcodes for the coleopteran species, we report the first mt DNA barcode of *A. kanarensis* from Maharashtra, India.

MATERIAL AND METHODS

Sampling of dung beetles

Specimen for the present study was collected at night using light trap. The map of the collection locality was prepared using open free QGIS software. The details of collection locality are given under material examined and also shown in Figure 1.

Preservation and Identification

The collected specimen was euthanized in the vapours of ethyl acetate and brought to the laboratory for further studies. For morphological identification, the specimen

was studied under Leica EZ4E stereomicroscope. The identification was done following the keys of Arrow (1917). Further, the voucher specimen was deposited in the National Repository of Zoological Survey of India, Western Regional Centre, Pune, Maharashtra (India).

DNA isolation, PCR and Sequencing

The ethanol preserved tissue was used for DNA isolation. DNA from the tissues of the beetle was extracted from metathoracic leg using DNeasy kit (Qiagen), according to the manufacturer's protocol. The obtained DNA was amplified using polymerase chain reaction (PCR) using ABI thermocycler. Following primers (Meyer et al. 2005) were used for amplification of COI gene: dgLCO F1 5'GGTCAACAAATCATAAAGAYATYGG 3' and dgHCO R1 5'TAAACTTCAGGGTGACCAAARAAYCA 3'. PCR reaction was carried out in total volume of 25 μ l containing 2 μ l DNA template, 10 pmol of each primer and 2 μ l of dNTP and 0.2 μ l of Taq polymerase (Bangalore GeNei). Thermo-cycling conditions were as follows:

One initial cycle of 1 min at 95°C followed by five cycles of 95°C for 1 min, then denaturation 35 cycles of 95°C for 1 min, annealing at 52°C for 40 s, extension at 72°C for 1 min 15 s, with final extension of 72°C for 5 min.

From each PCR reaction, 2 μ l of the PCR product was visualized on a 2% agarose gel stained with ethidium bromide, together with a GeneRuler 100 bp Plus DNA

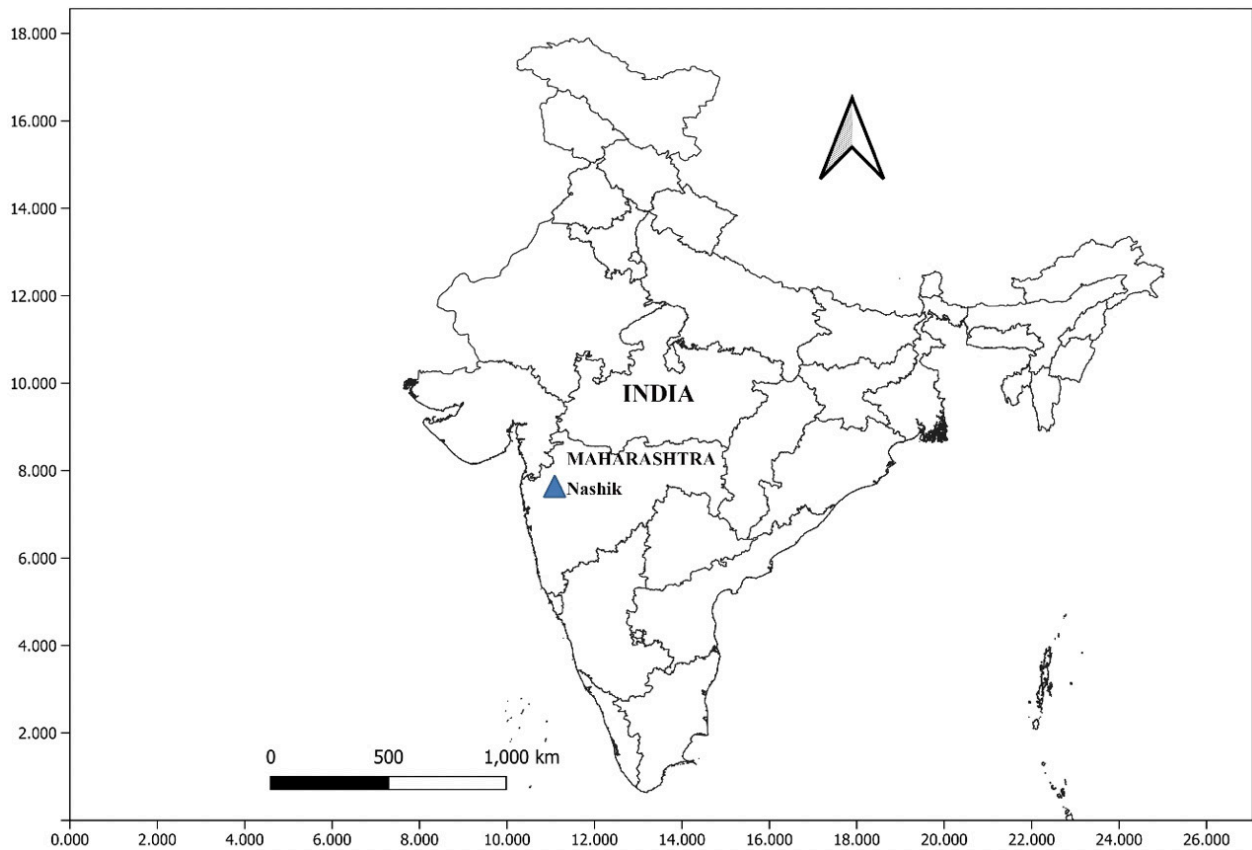


Figure 2. Collection locality of *Adoretus Kanarensis* from Maharashtra, India.

Ladder (Thermo Scientific). The obtained PCR products were sequenced with both, the forward and reverse, primers using an automated sequencer (3730 DNA analyzer, ABI, Hitachi).

Data analysis

Sequence was edited to remove ambiguous base calls and the forward and the reverse sequences were assembled using Chromas Pro version 1.34 (Technelysium Pty Ltd., Tewantin, Queensland, Australia). FASTA format of *Adoretus kanarensis* sequences was used for performed BLAST search at NCBI and species identification tool at Barcode of Life Data System (BOLD). All the obtained sequences were aligned and manually edited using BioEdit version 7.2.6. The Maximum Likelihood method and General Time Reversible model (GTR) model of base substitution was used to calculate pairwise genetic distance in MEGA X version 10.0.5. Additionally, to check the performance of DNA barcoding, sequences were downloaded from NCBI and BOLD (Table 2, Supplementary data) for some species of same genus submitted from different geographical areas. Only sequences which formed

monophyletic clades with the sequences studied here were selected, to avoid use of sequences from wrongly identified species. These sequences along with our data were used to generate trees using MEGA X (Nei & Kumar 2000; Kumar et al. 2018).

RESULTS AND DISCUSSIONS

Morphologically, the collected sample was identified as *Adoretus kanarensis* Arrow, 1917 (Figure 3).

Systematic account

Order **Coleoptera** Linnaeus, 1758

Suborder **Polyphaga** Emery, 1886

Super family **Scarabaeoidea** Latreille, 1802

Family **Scarabaeidae** Latreille, 1802

Subfamily **Rutelinae** MacLeay, 1819

Genus ***Adoretus*** Dejean, 1833

Adoretus kanarensis Arrow, 1917

Material examined: ZSI-WRC-ENT-1/2856, 27.i.2017, 01 Female, Sinner, Nashik district (19.8543 N, 73.9922 E), Maharashtra, coll. Pranil Jagdale.



Image 1. Adult female of *Adoretus kanarensis* Arrow, 1917. © Pranil Jagdale.

Table 1. Checklist of the genus *Adoretus* Laporte, 1840 from Maharashtra, India.

| | Species | Distribution | References |
|----|--|---|--|
| 1 | <i>Adoretus caliginosus</i> Burmeister, 1844 | India (Karnataka, Haryana, Himachal Pradesh, Maharashtra, West Bengal, Punjab, Sikkim, Tamil Nadu); Myanmar; Pakistan. | Arrow 1917; Mittal 1981; Chandra 1986; Ghosh et al. 2020; Schoolmeester 2023 |
| 2 | <i>Adoretus excisus</i> Ohaus, 1914 | India (Maharashtra, Madhya Pradesh, West Bengal). | Arrow 1917; Schoolmeester 2023 |
| 3 | <i>Adoretus incurvatus</i> Ohaus, 1914 | India (Bihar, Haryana, Himachal Pradesh, Maharashtra, Tamil Nadu). | Arrow 1917; Mittal 1981; Chandra 1986; Ghosh et al. 2020; Schoolmeester 2023 |
| 4 | <i>Adoretus kanarensis</i> Arrow, 1917 | India (Karnataka, Maharashtra (present study), Madhya Pradesh). | Arrow 1917 |
| 5 | <i>Adoretus lasiopygus</i> Burmeister, 1855 | India (Assam, Haryana, Bihar, Chhattisgarh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal); Bangladesh; Bhutan; Nepal; Sri Lanka. | Arrow 1917; Mittal 1981; Chandra 2000; Ghosh et al. 2020 |
| 6 | <i>Adoretus limbatus</i> Blanchard, 1850 | India (Bihar, Chhattisgarh, Haryana, Jammu & Kashmir, Madhya Pradesh, Maharashtra, Odisha, Uttar Pradesh, West Bengal); Bangladesh; Myanmar; Thailand. | Arrow 1917; Mittal 1981; Chandra 1986; Ghosh et al. 2020 |
| 7 | <i>Adoretus lobiceps</i> Arrow, 1917 | India (Gujarat, Madhya Pradesh, Maharashtra). | Arrow 1917; Chandra 2000. |
| 8 | <i>Adoretus pusillus</i> Arrow, 1917 | India (Maharashtra) | Arrow 1917; Schoolmeester 2023 |
| 9 | <i>Adoretus stoliczkae</i> Ohaus, 1914 | India (Maharashtra, Madhya Pradesh); Pakistan. | Arrow 1917; Chandra 2000 |
| 10 | <i>Adoretus sorex</i> Arrow, 1917 | India (Maharashtra & Tamil Nadu), Sri Lanka | Arrow 1917; Schoolmeester 2023 |
| 11 | <i>Adoretus versutus</i> Harold, 1869 | India (Andaman & Nicobar Islands, Bihar, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttarakhand, West Bengal); Bangladesh; Fiji; Indonesia; Malaysia; Mauritius; Sri Lanka. | Arrow 1917; Mittal 1981; Chandra 1986; Chandra 2000; Ghosh et al. 2020 |

Table 2. Details for the mt DNA COI sequences utilized in the construction of the phylogenetic tree.

| | GenBank Accession No./ BOLD ID | Locality | Species name as per NCBI | Publication details as per NCBI |
|----|--------------------------------|-------------------|---|---------------------------------|
| 1 | JN087425 | Japan | <i>Adoretus tenuimaculatus</i> Waterhouse, 1875 | Unpublished |
| 2 | OQ428819 | Iraq | <i>Adoretus hirsutus</i> Ohaus, 1914 | Unpublished |
| 3 | KC510117 | South Korea | <i>Adoretus tenuimaculatus</i> Waterhouse, 1875 | Unpublished |
| 4 | KF939031 | South Korea | <i>Adoretus hirsutus</i> Ohaus, 1914 | Unpublished |
| 5 | MT129331 | Republic of Korea | <i>Adoretus hirsutus</i> Ohaus, 1914 | Kang et al. 2021 |
| 6 | KU737493 | India | <i>Adoretus duvauceli</i> Blanchard, 1851 | Unpublished |
| 7 | AGIRI101-17 | India | <i>Adoretus duvauceli</i> Blanchard, 1851 | Unpublished |
| 8 | GMPQC103-19 | Pakistan | <i>Adoretus flavus</i> Arrow, 1917 | Unpublished |
| 9 | KF939033 | India | <i>Adoretus flavus</i> Arrow, 1917 | Unpublished |
| 10 | MZ836004 | India | <i>Adoretus ovalis</i> Blanchard, 1851 | Unpublished |
| 11 | KF939027 | India | <i>Adoretus duvauceli</i> Blanchard, 1851 | Unpublished |
| 12 | KU939028 | India | <i>Adoretus duvauceli</i> Blanchard, 1851 | Unpublished |
| 13 | OR026029 | India | <i>Adoretus kanarensis</i> Arrow, 1917 | Present study |

Diagnosis

Female (Image 1): Length, 10 mm; width, 5 mm. Bright brownish-yellow, moderately shining. The lateral margins of head, pronotum, broad sutural line reaching anteriorly till the humeral callus and posteriorly not reaching the margins and extremities of tibia and complete tarsus are dark reddish-brown. Head transversely rugose, small, with the clypeus broadly rounded. The pygidium has a bare apical area. Legs are slender, the front tibia is armed with three strong teeth, the larger claw of the front and middle feet is cleft, and the shorter hind claw is more than half the length of the longer.

DNA Barcode diagnosis

In this research study, *A. kanarensis* was identified using available literature and sequence of COI gene isolated from an adult female. No matches were found among the already-existing entries in the BOLD database after analysis with the BOLD Identification tool. The genetic difference between the two samples was over 10%, indicating that the examined species has not yet been recorded in BOLD. GenBank's BLAST analysis yielded the same outcome.

The preliminary molecular analysis was carried out using available material from NCBI GenBank (Table 2). As expected, *A. kanarensis* nested within the genus *Adoretus* with the outgroup taxon *Anomla ruficapilla* Burmeister, 1855 using Maximum Likelihood method with General Time Reversible model. In the current study, *A. kanarensis* formed a monophyletic clade with *A. duvauceli* Blanchard, 1851 species.

Since the species *A. kanarensis* is of economic significance, the present mt DNA barcode data generated is expected to be helpful in building a reliable DNA barcode library for the country intimated with a voucher specimen and helpful in addressing the taxonomic problems as the morphological characters are cryptic.

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