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RESEARCH ARTICLE

First Report on Molecular Characterisation of Asiatic Rhinoceros Beetle, *Oryctes rhinoceros* (Linnaeus, 1758) (Coleoptera: Scarabaeidae: Dynastinae) from the Deccan Part of Maharashtra

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ABSTRACT

The rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus, 1758), is a significant pest of palms, including coconut, causing direct and indirect losses in the palm-growing countries. This study presents the first mitochondrial DNA barcode data of the rhinoceros beetle reported from the Deccan part of Maharashtra (Pune). Additionally, the detailed descriptions and illustrations of the external morphological features and male genitalia are provided. Integrating molecular data with conventional taxonomic methods significantly enhances species identification and clarifies taxonomic uncertainties, offering a more robust approach for pest management and conservation initiatives.

Key words: *Beetle; Cytochrome COI; Taxonomy; Molecular study; Maharashtra; India*.

INTRODUCTION

Dynastinae MacLeay, 1819, is a large group of beetles, known for their horns and large size. One such beetle, the Asiatic rhinoceros beetle from the family Dynastinae, is a phytophagous insect named for the horn-like projection of males. The initial study on the Dynastinae worldwide was carried out by Hermann Burmeister in 1847, documenting 350 species from this subfamily. Later, Arrow (1910) revised the Dynastinae fauna of the Indian subregion, which includes India, Ceylon (now Sri Lanka), and Burma (now Myanmar), and recorded 17 genera representing 46 species, of which 30 species belonging to 14 genera were reported explicitly from India.

The Asiatic rhinoceros beetle is referred to as the coconut rhinoceros beetle due to its breeding in coconut, *Cocos nucifera* L. (Aceraceae). The decaying wood at the top of a dead palm trunk is a preferred breeding site for these beetles (Bedford, 1976, 1980, 1981, 2013a, 2013b). It is a significant pest of palms and coconuts globally, and the adult stage of the Asian rhinoceros beetle is considered the most destructive. They feed on the sap, causing damage to developing fronds. On the other hand, because its grubs mostly consume decaying wood, they are advantageous as natural decomposers (Giblin-Davis, 2001). There are 50 host plants registered for the Asiatic rhinoceros beetle (CAPS, 2014). Severe infestations decrease coconut and oil palm yields by 10% to 50% (Bedford, 1980). Repeated or severe infestations can destroy the growing point of young palms, leading to

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their death. The attacked fronds, when fully opened, show characteristic triangular cuts. As per TNAU (2025) the damaging symptoms includes: holes in central spindle; holes with chewed fibre sticking out in central spindle; triangular cuts on leaves; central spindle appears cut or toppled; fully opened fronds showing characteristic diamond shaped cuttings; holes with chewed fibre sticking out at the base of central spindle. In Indian conditions, it occurs throughout the year, but maximum damage or its population is observed during June to September, coinciding with the onset of monsoon (TNAU, 2025).

It is an economic pest, and hence, proper and quick identification is essential before undertaking any control measures. This is possible with the help of traditional taxonomy and appended with DNA barcode. This study attempted to generate a first DNA barcode from Maharashtra for Asiatic rhinoceros' beetle.

MATERIALS AND METHODS

The specimen was collected in a collection jar from a shop near Ravet, Pune. Then it was transported to the laboratory for further analysis. In the laboratory, the beetle was stretched, pinned, labelled, and dry-preserved in fumigated entomological boxes filled with preservatives. For morphological studies, the specimens were studied under a Leica EZ4E stereomicroscope. The map of the collection locality was prepared using open, free QGIS software. The details of the collection locality are given under the material examined. Identification of the specimens was done as per Arrow (1910). Male genitalia were studied following Mathur *et al*. (1959). The details of the collection locality of the present study are given under the material examined and are also provided in Figure 1 with the distribution of *Oryctes rhinoceros* in India. The identified specimens are deposited at the National Zoological Collections of the Zoological Survey of India, Western Regional Centre, Pune, Maharashtra, India (ZSI/WRC).

DNA Barcoding

The genomic DNA was extracted from an entire foreleg dissected from the coxa region of the beetle specimen, using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's protocol. The DNA was eluted in AE Buffer in a 100µL volume, and quantification was done using the dsDNA HS Assay Kit (Invitrogen) on a Qubit 2.0 fluorometer. The mitochondrial COI gene was amplified using the primers LCO1490 (5'- GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5- TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). The PCR reactions were carried out in a 25 µL volume containing 12.5 µL of 2X Hot start PCR master mix (Promega), 1.0 µL of each primer forward and reverse (10 pmol), 2.0 µL of gDNA (>100), and filled the final volume up to 25 µL with nuclease-free water. The PCR thermal cycle consisted of an initial denaturation at 95° for 5 minutes, followed by 30 cycles at 95° for 30 seconds, 47°-51° for 40 seconds, and 72° for 60 seconds, and followed by a final extension at 72° for 5 min. Amplified PCR products were checked by gel electrophoresis on a 1.2% agarose gel stained with 6.0µL EtBr and viewed under UV light via the Gel documentation system. Successfully amplified PCR products were purified using the Invitrogen Pure Link PCR Purification Kit. Sanger's sequencing was outsourced to Barcode Biosciences Pvt Ltd., Bengaluru, India.

The generated Sequences were manually checked and edited in Chromas v.2.6.5 software (Technelysium Pty. Ltd. 2018). 34 mt COI sequences of *Oryctes* were downloaded from GenBank and BOLD, including outgroup (Appendix I). Sequence alignment and editing were done using the MUSCLE algorithm in MEGA X software (Kumar *et al.*, 2018). Maximum likelihood analysis was performed with sequences of 628 bp, including one sequence generated in this study on the IQ-TREE multicore version 1.6.12 (Trifinopoulos *et al.*, 2016) web server (Figure 3). Analysis was set for 1000 Ultrafast bootstrap with default parameters and GTR+F+I+G4 substitution model auto-selected according to BIC (Bayesian Information Criterion). The final consensus tree was visualised in Fig Tree v 1.4.0 (Figure 3) treating *Eophileurus chinensis* (Faldermann, 1835) as outgroups (Ayivi *et al*., 2021). Sequences generated in the studies are submitted to GenBank (PQ340520.1).

RESULTS AND DISCUSSION

Family: Scarabaeidae Latreille, 1802
Subfamily: Dynastinae MacLeay, 1819
Tribe: Oryctini Mulsant, 1842
Genus*:* *Oryctes* Hellwig, 1798

Species: *Oryctes rhinoceros* (Linnaeus, 1785)

(Figure 1, 2a-d,3)

Scarabaeus rhinoceros Linnaeus, 1785. *Syst. Nat.,* I: 346.

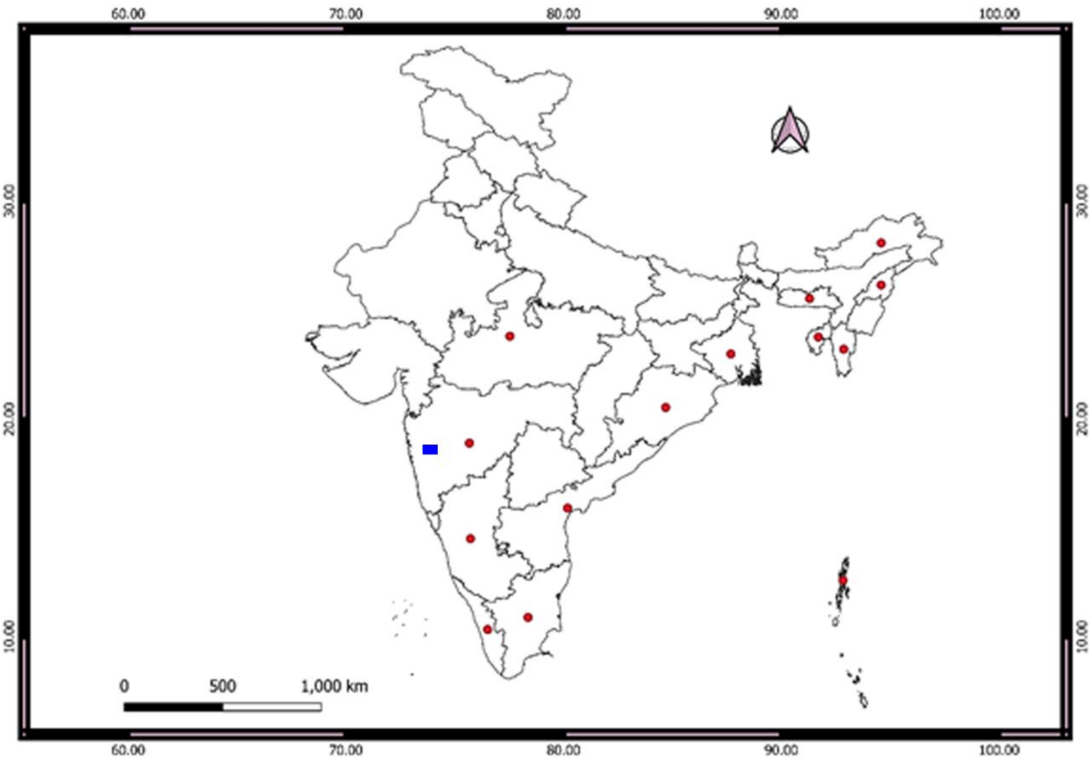

Figure 1. The present collection locality is shown in a blue rectangle, and the other Distribution of the Asiatic rhinoceros beetle from India is shown in a red circle

Oryctes rhinoceros, Burmeister, 1847. *Handb. Ent.,* V: 202; Arrow, 1910, *Fauna Brit. India*, p. 278. Endrödi, 1985, *The Dynastinae of the World*, p. 520.

Specimen examined. 1ex., Maharashtra, Pune, Ravet, Near Mahalaxmi Shop; 12.v.2024, A.S. Kalawate (ZSI, WRC, Ent-1/5057).

Diagnostic characters. Adult male, 45.78 mm long and 20.46 mm wide. Body blackish, clypeus forked with long horn; foretibiae armed with four teeth. Pronotum longer than width, convergent at anterior; elytra strong, punctured; ventral side reddish, clothed with fine hairs; propygidium large, pygidium rounded and rugose.

Male genitalia. It is highly chitinised; phallus elongated; phallobase rounded and slightly spherical; the aedeagus cylindrical and elongated.

Distribution. India (Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, West Bengal, Mizoram, Andhra Pradesh, Arunachal Pradesh, Andaman and Nicobar Islands, Meghalaya, Nagaland, Tripura (Ghosh *et al.*, 2020; Ghosh *et al.*, 2023); Global: Pakistan, Bangladesh, Sri Lanka, Burma (=Myanmar), Thailand, Malaysia, Indonesia (Java, Sumatra), Cambodia, South Korea, Laos, Philippines, Taiwan, Vietnam, American Samoa, Fiji, Palau, Papua New Guinea, Samoa, Tokelau, Tonga, Wallis, Hong Kong, Japan, Singapore, Annam, Futuna (Nishida & Evenhuis, 2000; Ghosh *et al.*, 2020; Ghosh *et al.*, 2023).

In the single gene-based mt COI phylogenetic tree, *O. rhinoceros* is recovered as a deeply divergent member of the larger *Oryctes* clade, exhibiting *O. agamemnon, O. nasicornis, O. elegans, O. borbonicus,* and *O. gnu.* Interestingly, within the populations of *Oryctes,* genetic distance was varying from 0.3% to 1.7% (for 622 bp of mt COI data), exhibiting a phylogeographic structuring. Within the Indian population, sequence homology was seen between the sequences of Maharashtra (present study, PQ340520.1), Kerala (KY313855.1), and Karnataka (KP898260.1). Still, the genetic distance between one of the sequences from Maharashtra (present study, PQ340520.1) and the sequence from Kerala (KY313837.1) was 0.6%, and another from Kerala (KY313834.1) was 0.9%. At the larger scale, *O. rhinoceros* exhibited 21.7% to 26.1% genetic distance among the congeners. The preliminary single-gene-based mt COI phylogenetic tree presented here represents six species among the

Appendix I

S. No.	GenBank No.	Species	Location	Reference
1	PQ340520.1	*Oryctes rhinoceros*	Maharashtra, India	Present study
2	KY313834.1	*Oryctes rhinoceros*	Kerala, India	Marshall *et al*., 2017
3	KY313837.1	*Oryctes rhinoceros*	Kerala, India	Marshall *et al*., 2017
4	KY313838.1	*Oryctes rhinoceros*	Indonesia	Marshall *et al*., 2017
5	KY313857.1	*Oryctes rhinoceros*	Malaysia	Marshall *et al*., 2017
6	MH800555.1	*Oryctes rhinoceros*	Palau	Reil *et al*., 2018
7	KY313848.1	*Oryctes rhinoceros*	Papua New Guinea	Marshall *et al*., 2017
8	MN809517.1	*Oryctes rhinoceros*	Philippines	Etebari *et al*., 2021
9	MN809511.1	*Oryctes rhinoceros*	(Guadalcanal) Solomon Islands	Etebari *et al*., 2021
10	MN809510.1	*Oryctes rhinoceros*	(Guadalcanal) Solomon Islands	Etebari *et al*., 2021
11	OL546416.1	*Oryctes rhinoceros*	Solomon Islands	Marshall *et al*., 2023
12	KY198095.1	*Oryctes rhinoceros*	–	Reil *et al*., 2016
13	KY313836.1	*Oryctes rhinoceros*	Samoa	Marshall *et al*., 2017
14	KY313835.1	*Oryctes rhinoceros*	Malaysia	Marshall *et al*., 2017
15	KY313855.1	*Oryctes rhinoceros*	Kerala, India	Marshall *et al*., 2017
16	MN809519.1	*Oryctes rhinoceros*	Fiji	Etebari *et al*., 2021
17	MN809518.1	*Oryctes rhinoceros*	Fiji	Etebari *et al*., 2021
18	KP898260.1	*Oryctes rhinoceros*	Bengaluru, Karnataka, India	Unpublished
19	KY198028.1	*Oryctes rhinoceros*	–	Reil *et al*., 2016
20	MN809513.1	*Oryctes rhinoceros*	Solomon Islands (Guadalcanal)	Etebari *et al*., 2021
21	OL546438.1	*Oryctes rhinoceros*	Solomon Islands	Marshall *et al*., 2023
22	OL546448.1	*Oryctes rhinoceros*	Solomon Islands	Marshall *et al*., 2023
23	MN840591.1	*Oryctes rhinoceros*	Bangladesh	Unpublished
24	KY313833.1	*Oryctes rhinoceros*	Malaysia	Marshall *et al*., 2017
25	KY313828.1	*Oryctes rhinoceros*	Malaysia	Marshall *et al*., 2017
26	KC576780.1	*Oryctes agamemnon*	Tunisia	Abdallah *et al*., 2013
27	KC576775.1	*Oryctes agamemnon*	Tunisia	Abdallah *et al*., 2013
28	JF889672.1	*Oryctes nasicornis*	Germany	Unpublished
29	MF059280.1	*Oryctes nasicornis*	–	Unpublished
30	PP472477.1	*Oryctes elegans*	Saudi Arabia	Unpublished
31	PP472648.1	*Oryctes elegans*	Saudi Arabia	Unpublished
32	KJ915148.1	*Oryctes borbonicus*	–	Meyer *et al*., 2016
33	KJ915078.1	*Oryctes borbonicus*	–	Meyer *et al*., 2016
34	MN078206.1	*Oryctes gnu*	Long Island Aquarium, Riverhead, New York, USA	Unpublished
35	LC199297.1	*Eophileurus chinensis*	Japan	Outgroup

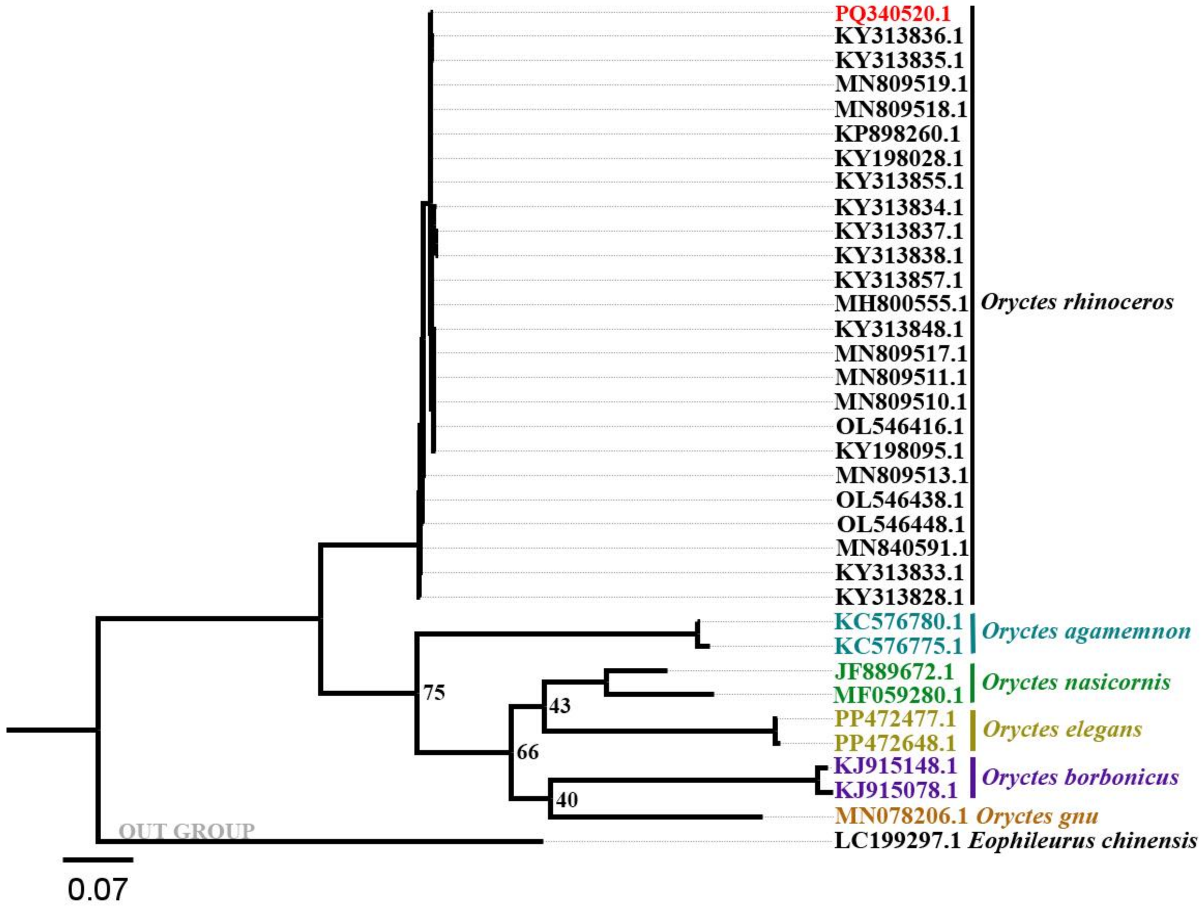

Figure 3. Maximum likelihood tree based on 628 bp of the mt COI gene for the members of the genus *Oryctes*.

45 extant species, considering the bootstrap support and high genetic distance phylogenetic position of the *Oryctes rhinoceros* within the *Oryctes* genus in the larger Dynastinae (Scarabaeidae) needs re-evaluation. The species being economically significant, the present DNA barcode data generated from Maharashtra with a verifiable voucher specimen is expected to be of greater utility for the future systematic position of the species within Dynastinae (Scarabaeidae) and as a baseline data for the DNA barcode library.

Such an essential economic pest can be well monitored by proper identification using a fast and reliable tool like a DNA barcode. DNA barcoding, along with traditional taxonomy, is an effective tool to identify the pest and the beneficial insects correctly. The use of DNA barcode and the traditional taxonomy to identify an organism is called Integrated Taxonomic Approach (ITA). So, till today, farmers and the agricultural scientists were dependent on the Integrated Pest Management (IPM) to control the insect pests. Now, one needs to look for ITA and IPM for successfully controlling the pests. With the use of advanced technology, the Integrated Pest Management decision-making can be improved, which is solely dependent on the ability to correctly identify pests and beneficial organisms (Quandahor *et al.*, 2024). This is the time to act and develop the DNA barcode library of all the major and minor pests of the essential crops. This library should also comprise taxonomic characters along with the barcode of the species for error-free identification. Following the same path, this study has attempted to present the first COI barcode of the Asiatic rhinoceros beetle along with its morphological characters from Maharashtra, India.

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Figure 2. *Oryctes rhinoceros* (Linnaeus, 1785) male a. Lateral view, b. Dorsal view; male genitalia c. Ventral view, d. Lateral view. a and b. not to scale.

Ethics statement

No specific permits were required for the studies.

Originality and Plagiarism

This manuscript is an original work has not been previously published, and proper citations are provided.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflicts of interest in the publication of this content.

Data Availability

Author contributions

Idea conceptualization, writing original draft, collection of specimens by A. S. K.; P. K. M., and T. K., undertaken conceptualisation of this work, identification, genitalia dissection, Plate preparation; DNA barcoding, and analysis performed by S. S. and K. P. D.

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