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

**Abstract**

The rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus, 1758), is a major 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 rhinoceros beetle reported from Deccan part of Maharashtra (Pune). Additionally, the detailed descriptions and illustrations of the external morphological features and male genitalia were provided. The integration of 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, 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 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 specifically reported from India.

Asiatic rhinoceros beetle is referred as coconut rhinoceros beetle due to its breeding in coconut, *Cocos nucifera* L. (Aceraceae). The decaying wood at the top of dead palm trunk serves as a preferred breeding site for these beetles (Bedford, 1976, 1980, 1981, 2013a, 2013b). It is a major pest of palms and coconut globally, and the adult stage of asiatic rhinoceros beetle is considered to be 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 hosts plants registered for Asiatic rhinoceros beetle (CAPS, 2014). Severe infestations decrease yields of coconut and oil palm by 10% to 50% (Bedford, 1980). Repeated or severe infestations can destroy the growing point of young palms, leading to their death. The attacked fronds when fully opened shows 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 condition the it occurs round 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 important before undertaking any control measures. This is possible with the help of traditional taxonomy and appended with DNA barcode. In this study an attempt was made 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 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 present study are given under the material examined and is also provided in Figure-1 with 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 fore leg dissected from coxa region of the beetle specimen, using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturers protocol. The DNA was eluted in AE Buffer in 100µL volume and Quantitation was done using dsDNA HS Assay Kit (Invitrogen) on Qubit 2.0 fluorometer. 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), 1.0µL of each primer forward and reverse (10pmol), 2.0 µL of gDNA (>100) and filled the final volume up to 25µL 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’s Pure Link PCR Purification Kit. Sanger’s sequencing was outsourced to Barcode Biosciences Pvt Ltd., Bengaluru, India.

The generated Sequences were manually checked, edited in Chromas v.2.6.5 software (Technelysium Pty. Ltd. 2018). A total of 34 mt COI sequences of *Oryctes* were downloaded from GenBank and BOLD including outgroup (Appendix I). Sequence alignment and editing was done using MUSCLE algorithm in MEGA X software (Kumar *et al.*, 2018). Maximum likelihood analysis was performed with sequences of 628bp including one sequence generated in this study on 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). Final consensus tree was visualised in Figure 3 Tree v 1.4.0 treating *Eophileurus chinensis* (Faldermann, 1835) as outgroups (Ayivi *et al*., 2021). Sequences generated in the studies are submitted to the 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)

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

*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 teeths. 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; the phallus is elongated; the phallobase has rounded and slightly spherical shaped; the aedeagus is 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), but genetic distance between one of the of 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 45 extant species, considering the boot strap 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 important, 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 important economic pest can be well monitored by proper identification using the fast and reliable tool like, DNA barcode. DNA barcoding may along with the traditional taxonomy is an effective tool to correctly identify the pest and the beneficial insects. The use of DNA barcode along with the traditional taxonomy to identify an organism is commonly called as Integrated Taxonomic Approach (ITA). So, till today farmers and the agricultural scientists were dependant on the Integrated Pest Management (IPM), to control the insect-pests. Now onwards one need to look for ITA and IPM for successfully controlling the pests. With the use of the advance technology, the Integrated Pest Management decision-making, can be improved which solely dependent on the ability to correctly identify pest 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 important crops. This library should also comprise of taxonomic characters along with the barcode of the species for error free identification. Following the same path an attempt has been made in this study to present here the first COI barcode of Asiatic rhinoceros beetle along with its morphological characters from Maharashtra, India.

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**Ethics statement**

No specific permits were required for the studies.

**Originality and Plagiarism**

This manuscript is an original work and not 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 done genitalia dissection, specimen photograph, DNA barcoding and analysis done by S.S and K.P.D.

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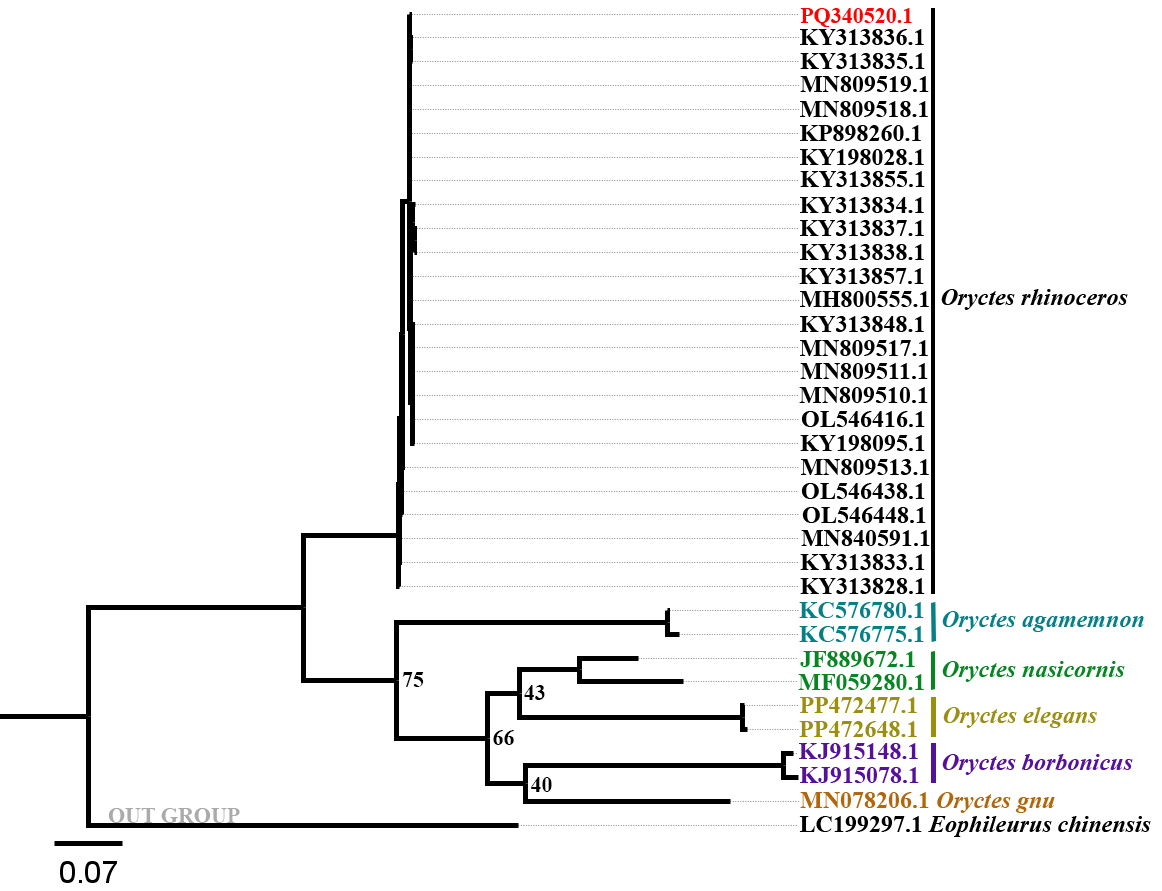
**Figure 1.** Present collection locality shown in blue rectangle and otherDistribution of Asiatic rhinoceros beetle from India shown in red circle.



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

**Appendix I**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. no.** | **Gen bank no.** | **Species** | **Location** | **Ref.** |
| 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*., 2015 |
| 33 | KJ915078.1 | *Oryctes borbonicus* | **̶** | Meyer *et al*., 2015 |
| 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 mt COI gene for the members of the genus *Oryctes*.