



Molecular and Morphological identification of the genus *Dineutus* MacLeay, 1825 (Coleoptera, Gyrinidae) from Eastern Ghats, India

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ABSTRACT. The present study is on the morphology and molecular data of the genus *Dineutus* MacLeay, were collected from the Eastern Ghats mountains in Odisha, Andhra Pradesh, Telangana, Tamil Nadu states located in the eastern Ghats of India. The morphological identification was based on elytral spines, setation on paramere and structure of the median lobe. Molecular characterization was based on the mitochondrial cytochrome oxidase subunit I (COI) and 16s ribosomal RNA gene. Species identified as being *D. indicus*, *D. spinosus*, and *D. unidentatus*. Phylogenetic trees were constructed for both genes, and the COI fragment shows raw genetic distance between 10–12% among the three species of *D. indicus*, *D. spinosus* and *D. unidentatus*, whereas 16s gene shows a divergence of 4–5%. The present study contributes five novel mitochondrial COI and five ribosomal RNA sequences for *D. spinosus* and *D. unidentatus* for the first time from India.

Key words: Aquatic beetles, DNA, barcodes, mtCOI, ribosomal 16s, taxonomy

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INTRODUCTION

The surface inhabiting aquatic beetle genus *Dineutus* MacLeay, 1825 belongs to family Gyrinidae which is characterized by having divided eyes allowing them to see in and outside of the water. They are commonly

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found in slowly moving streams, reservoir, ponds and shows skating in circle on the surface of the aquatic environment. The world diversity of Gyrinidae is rich including about 1000 species under 25 genera, of which 73 species are reported from India (Chandra et al., 2017). The genus *Dineutus* further have four subgenera in which, *Cyclous* Gustafson & Miller, 2017 had 67 species. 92 species of the genus *Dineutus*, are recorded worldwide, making it the most diverse genus of the Dineutini (Gustafson & Miller, 2015, 2017). Three species *Dineutus indicus*, *D. spinosus* and *D. unidentatus* are reported from India so far (Vazirani, 1984). The DNA barcoding data is a secondary tool for species identification and confirmation of taxonomy, and is also an efficient tool to assess the evolution of species (Sheth et al., 2021). It has been a widely accepted method in different animal groups throughout the world (Akhilesh & Sebastian, 2015; Somayeh & Incekara, 2016; Gustafson & Miller, 2017, Michat et al., 2017, Sheth et al., 2021). DNA barcoding studies on the genus *Dineutus* in India are limited. Hence the present study aims to study genetic identification along with morphological characters of the genus *Dineutus* in India.

MATERIAL AND METHODS

The *Dineutus* specimens were collected from the different parts of Eastern Ghats, India (Fig. 1, Table 1). Collected specimens were preserved in 95% ethanol and studied using Leica M205A Stereo Zoom microscope. Morphological identification done by using relevant literature (Vazirani, 1984; Miller & Bergsten, 2012; Gustafson & Miller, 2015, 2017). The identified specimens were deposited in the National Zoological Collection of Zoological Survey of India, Freshwater Biological Regional Centre, Hyderabad. Fore legs of the chosen specimens were used for DNA extraction by using Dneasy® Blood & Tissue kit (Qiagen, Germany). Amplification done through primer LCO-1490 5'-GGTCAACAAATCATAAAGATATTGG-3', HCO-2198 5'-TAAACTTCAGGGTGACCAAAAATCA-3' for the mitochondrial Cytochrome Oxidase Subunit I (COI) gene (~620bp) (Folmer et al., 1994) and 16Sa 5'-CGCCTGTTTATCAAAAACAT-3', 16Sb 5'-CCGGTCTGAACTCAGATCATGT-3' for the ribosomal RNA gene (~450bp) (Palumbi et al., 1991). Polymerase chain reaction prepared for 25µl which contains 12.5µl of 2x PCR Taq mixture, 0.5µl (10pmol Con) of each primer, 10.5µl of molecular grade water and 1µl of template DNA used for both genes. The thermal cycle profile was set as 95°C for 1 min, 94°C for 30sec, 48°C for 30sec 35 cycles, 72°C for 1 min and subsequently hold at 10°C for mtCOI and 95°C for 3 min, 94°C for 30sec, 47°C for 30sec 35 cycles, 72°C for 40sec and subsequently hold at 10°C for 16s RNA gene. 0.8% and 1% Agarose gel was used to check the DNA which also contains Ethidium bromide (10mg/ml). The PCR product were purified using NucleoSpin® Gel and PCR clean up kit protocol were followed. Forward and reverse sequences were generated using the Sanger method by Eurofins Genomics (Ebersberg, Germany). Generated chromatogram files of three species have been handled in MEGA X (Kumar et al., 2018). The generated sequences were searched in NCBI (<https://www.ncbi.nlm.nih.gov>) to prepare final dataset. A total of 14 sequences (Both COI and 16S) were acquired from NCBI database to compare and prepare final database for the study (Appendix 1). MEGA X software was used to align the generated sequences. The genetic divergent were calculated by using Kimura-2-Parameter (K2P) model in MEGA X (Kumar et al., 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980) in MEGA X. The tree with the highest log likelihood (-3157.52) is shown. Branch supports was estimated using the Bootstrap value analysis with 1000 replicates. *Cybister tripunctatus* Sharp, 1899, *Copelatus deccanensis* Sayali, Hemant, & Jiří, 2018, was used as an out-group.

RESULTS

Taxonomic hierarchy

Class Insecta Linnaeus, 1758

Order Coleoptera Linnaeus, 1758

Family Gyrinidae Latreille, 1802

Tribe Dineutini Desmarest, 1851

Genus *Dineutus* Macleay, 1825

Subgenus *Cyclous* Gustafson & Miller, 2017

The genus *Dineutus* characterized by the divided eyes, gular suture complete, frons without lateral bead, antenna with 6 segments hind legs paddle-like, protarsus of male broad with protarsal discus, elytra without spines, with pointed spines and hairs on paramere, shape of the median lobe blunt or pointed.

Table 1. The sampling localities, where the specimens were collected.

	Sampling station	District	State	Latitude	Longitude
1	Baghalati Dam	Ganjam	Odisha	19°08'16.80"N	84°23'56.76"E
2	Ghodahada Dam	Ganjam	Odisha	19°17'27.96"N	84°27'35.64"E
3	Pharuasahi	Ganjam	Odisha	19°10'31.08"N	84°22'22.80"E
4	Dudum waterfall	Jaypore	Odisha	18°31'40.08"N	82°25'30.72"E
5	Yellow Dam	Jaypore	Odisha	18°50'05.28"N	82°34'57.00"E
6	Shakti Waterfall	Jaypore	Odisha	18°49'51.96"N	82°36'00.72"E
7	Dharamattam Waterfall	Visakhapatnam	Andhra Pradesh	17°42'47.52"N	82°28'41.88"E
8	Thatiguda Waterfall	Visakhapatnam	Andhra Pradesh	18°13'33.96"N	83°00'05.76"E
9	Vishakapatnam	Visakhapatnam	Andhra Pradesh	17°50'30.84"N	83°19'16.68"E
10	Moddisalava Dam	Visakhapatnam	Andhra Pradesh	17°46'00.84"N	83°17'50.28"E
11	Relli lake	Visakhapatnam	Andhra Pradesh	17°48'35.28"N	83°19'45.84"E
12	Thatipudi reservoir	Visakhapatnam	Andhra Pradesh	18°10'42.96"N	83°12'15.48"E
13	Amruthadara Waterfall	Alluri Sitaramaraju	Andhra Pradesh	17°38'20.04"N	81°36'45.00"E
14	Kintukuru	Alluri Sitaramaraju	Andhra Pradesh	17°30'17.28"N	81°41'51.36"E
15	Marredmalli coffe plantation	Alluri Sitaramaraju	Andhra Pradesh	17°36'05.04"N	81°41'16.44"E
16	Talakona Waterfall	Chittoor	Andhra Pradesh	13°48'44.64"N	79°12'58.32"E
17	Rollapadu Wildlife Sanctuary	Kurnool	Andhra Pradesh	15°27'03.60"N	78°07'17.40"E
18	Rollapadu Wildlife Sanctuary	Kurnool	Andhra Pradesh	15°27'05.04"N	78°12'43.92"E
19	Kandeleru reservoir	Nallore	Andhra Pradesh	14°38'33.72"N	80°01'04.80"E
20	Jonnavada	Nellore	Andhra Pradesh	14°27'13.32"N	79°51'34.92"E
21	Loddi Mallanna Temple	Nagarkarnool	Telangana	16°18'27.72"N	78°43'12.72"E
22	Saleshwaram Waterfall	Nagarkarnool	Telangana	16°10'11.64"N	78°38'21.48"E
23	Mallelathiertham Waterfall	Nagarkarnool	Telangana	16°16'01.92"N	78°51'21.96"E
24	Billakal	Nagarkarnool	Telangana	16°15'51.84"N	78°38'11.76"E
25	Vattavarlapally	Nagarkurnool	Telangana	16°15'04.68"N	78°45'40.68"E
26	Mannanur	Nagarkurnool	Telangana	16°19'12.00"N	78°44'43.80"E
27	Marredmadine	Nagarkurnool	Telangana	16°10'01.56"N	78°26'57.84"E
28	Nadimpally	Nagarkurnool	Telangana	16°24'25.20"N	78°40'55.92"E
29	Beemarapatti Waterfall	Dharmapuri	Tamil Nadu	12°00'49.68"N	78°43'46.92"E
31	Masila waterfall	Namakal	Tamil Nadu	11°18'08.64"N	78°23'37.32"E
32	Govindapuram Waterfall	Krishnagiri	Tamil Nadu	12°18'53.28"N	78°35'27.96"E
33	Muttal Waterfall	Salem	Tamil Nadu	11°38'41.64"N	78°38'50.28"E
34	Aagaya Gangai Waterfall	Salem	Tamil Nadu	11°16'01.92"N	78°23'37.68"E
35	Kottachedu Kari	Salem	Tamil Nadu	11°49'09.84"N	78°16'09.12"E

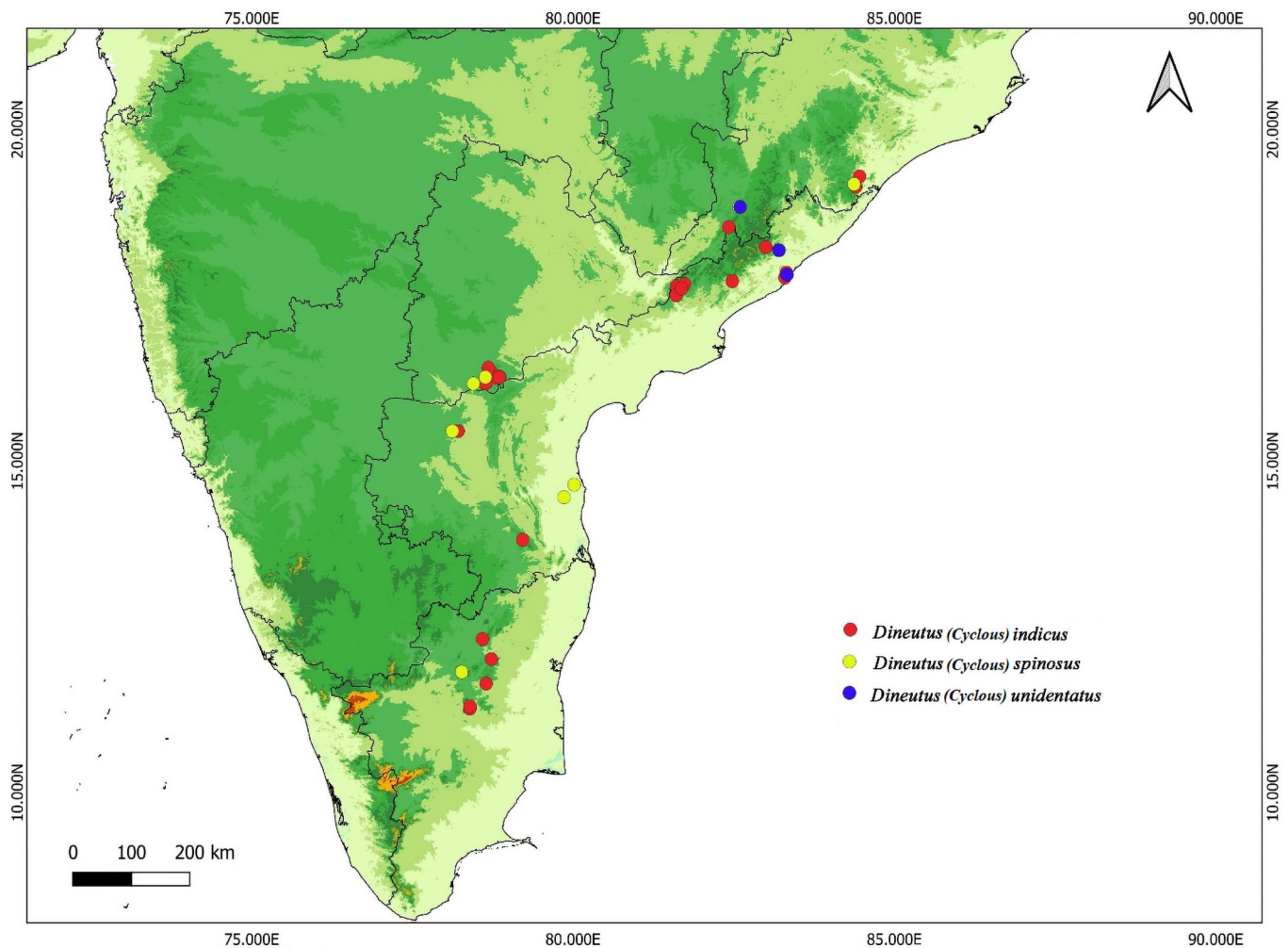


Figure 1. Map of the collection localities from different state of Eastern Ghats, India, where the *Dineutus* species were collected.

Keys to Indian species of *Dineutus* MacLeay, 1825

- 1 Body shape somewhat round, elytra lateral margin black, posterior without spine; median lobe and paramere almost equal in length (Figs 2A, 2D, 2G) [Vazirani (1984)], length 13.5–15.5 mm. *D. indicus*
- Body broadly oval, elongated, elytra lateral margin yellow, pale marginal band, posteriorly with spine; median lobe and paramere are not in same length, length 5.5–8.5 mm. 2
- 2 Body shape oval, medium size, elytra lateral margin black, with yellow marginal band, lateral margin posteriorly with a pair of spines; median lobe shorter than paramere (Figs 2B, 2E, 2H) [Vazirani (1984)], length 6.5–8.5 mm. *D. spinosus*
- Body somewhat flattened, elytra medially black, slightly pale marginal band, lateral margin posteriorly with spine; median lobe slightly longer than paramere (Figs 2C, 2F, 2J) [Vazirani (1984)], length 5.5–7.0 mm. *D. unidentatus*

***Dineutus (Cyclous) indicus* Aube, 1938 (Figs 2A, 2D, 2G)**

Dineutus indicus Aube, 1938, 6:772; *Dineutus (Protodineutus), indicus* Ochs, 1928, 40:61–74; *Dineutus (P.) indicus*, Vazirani, 1984:22–24.

Material examined. **India, Odisha**, 16♂♂, 24♀♀, Dudum waterfall (18°31'40.08"N, 82°25'30.72"E), 29.xii.2019, Reg. No. FBRC/ZSI/INS/1813; 1♀, Ghodahada Dam (19°17'27.96"N, 84°27'35.64"E), 21.i.2022, Reg. No. FBRC/ZSI/INS/3344; 2♀♀, Baghalati Dam (19°8'16.8"N, 84°23'56.76"E), 21.i.2022, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/3345; **Andhra Pradesh**, 6♂♂, 16♀♀, Dharamattam waterfall (17°42'47.52"N, 82°28'41.88"E), 3.ix.2018, Reg. No. FBRC/ZSI/INS/1127; 1♂, Thatiguda waterfall (18°13'33.96"N, 83°0'5.76"E), 27.i.2020, Reg. No. FBRC/ZSI/INS/1853; 21♂♂, 49♀♀, Amruthadara waterfall (17°38'20.04"N, 81°36'45"E), 14.xi.2019, Reg. No. FBRC/ZSI/INS/2071; 2♂♂, 5♀♀, Talakona waterfall (13°48'44.64"N, 79°12'58.32"E), 22.xii.2018, Reg. No. FBRC/ZSI/INS/2072; 34♂♂, 56♀♀, Visakhapatnam (17°50'30.84"N, 83°19'16.68"E) 25.xii.2020, Reg. No. FBRC/ZSI/INS/2232; 1♀, Kintukuru, (17°30'17.28"N, 81°41'51.36"E), 15.vii.2021, Reg. No. FBRC/ZSI/INS/2461; 8♂♂, 20♀♀, Rollapadu Wildlife Sanctuary (15°27'5.04"N, 78°12'43.92"E), 16.viii.2021 Reg. No. FBRC/ZSI/INS/2531; 4♀♀, Moddisalava Dam (17°46'0.84"N, 83°17'50.28"E), 21.vii.2022 Reg. No. FBRC/ZSI/INS/3284; 2♂♂, Marredmalli coffee plantation (17°36'5.04"N, 81°41'16.44"E), 16.vii.2021, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/3307; **Telangana**, 3♂♂, 5♀♀, Mallelatheertham waterfall (16°16'1.92"N, 78°51'21.96"E), 14.xii.2020, Reg. No. FBRC/ZSI/INS/1518; 2♂♂, Billakal (16°15'51.84"N, 78°38'11.76"E), 17.ix.2019, Reg. No. FBRC/ZSI/INS/1597; 10♂♂, 23♀♀, Saleshwaram waterfall (16°10'11.64"N, 78°38'21.48"E), 12.ii.2020, Reg. No. FBRC/ZSI/INS/1812; 2♂♂, 8♀♀, Vattavarlapally (16°15'4.68"N, 78°45'40.68"E), 1.x.2020, Reg. No. FBRC/ZSI/INS/2192; 5♀♀, Mannanur (16°19'12"N, 78°44'43.8"E), 3.x.2020, Reg. No. FBRC/ZSI/INS/2273; 4♂♂, Marredmadine (16°10'1.56"N, 78°26'57.84"E), 27.xii.2018, Reg. No. FBRC/ZSI/INS/2395; 1♂, 2♀♀, Nadimpally (16°24'25.2"N, 78°40'55.92"E), 21.xii.2021 Reg. No. FBRC/ZSI/INS/2740; 6♂♂, 2♀♀, Loddi Mallanna Waterfall (16°18'27.72"N, 78°43'12.72"E) 24.xii.2018, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/1322; **Tamil Nadu**, 3♂♂, 2♀♀, Aagaya Gangai waterfall (11°16'1.92"N, 78°23'37.68"E), 9.iv.2019, Reg. No. FBRC/ZSI/INS/2350; 1♂, 3♀♀, Muttal waterfall (11°38'41.64"N, 78°38'50.28"E), 5.i.2019, Reg. No. FBRC/ZSI/INS/1519; 15♂♂, 35♀♀, Beemarapatti waterfall (12°0'49.68"N, 78°43'46.92"E), 9.i.2019, Reg. No. FBRC/ZSI/INS/1444; 2♀♀, Masila waterfall (11°18'8.64"N, 78°23'37.32"E), 6.i.2019, Reg. No. FBRC/ZSI/INS/1446; 1♂, 5♀♀, Kottachedu Kari (11°49'9.84"N, 78°16'9.12"E), 7.i.2019, Reg. No. FBRC/ZSI/INS/1447; 4♀♀, Govindapuram Waterfall (12°18'53.28"N, 78°35'27.96"E), 10.i.2019, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/1448.

Diagnostic characters (Figs 2A, 2D, 2G). Body size 13.5–15.5 mm, somewhat round, elongated, slender, black in color, punctuated, clypeus bronze, lateral sides green iridescence, reticulation distinct, antennae black with 6 segments, pronotum black, lateral margin oblique, elytra black, striate, reticulation clear, the surface somewhat like mat appearance, punctuation double, ventral surface black, legs reddish to black, median lobe and paramere equal in length, median lobe point, paramere with dense sitation, paramere subparallel rounded towards apex.

Distribution. *Oriental* (India [Andhra Pradesh, Bihar, Gujarat, Himachal Pradesh, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Odisha, Puducherry, Tamil Nadu, Telangana, Utter Pradesh, West Bengal]); *Palaeartic* (Pakistan) (Vazirani, 1984; Deepa et al., 2022).

***Dineutus (Cyclous) spinosus* Fabricius, 1781 (Figs 2B, 2E, 2H)**

Gyrinus spinosus Fabricius, 1781, 1:298; *Dineutus (Spinodineutus) spinosus* Vanirani, 1984:17–20.

Material examined. **India, Odisha**, 1♀, Pharuasahi (19°10'31.08"N, 84°22'22.8"E), 21.i.2022, Reg. No. FBRC/ZSI/INS/3342; 2♂♂, 1♀, Yellow Dam (18°50'5.28"N, 82°34'57"E), 18.i.2022, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/3343; **Andhra Pradesh**, 2♂♂, 1♀, Kaneleru reservoir (14°38'33.72"N, 80°1'4.8"E), 14.x.2018, Reg. No. FBRC/ZSI/INS/1549; 1♂, 5♀♀, Jonnavada (14°27'13.32"N, 79°51'34.92"E),

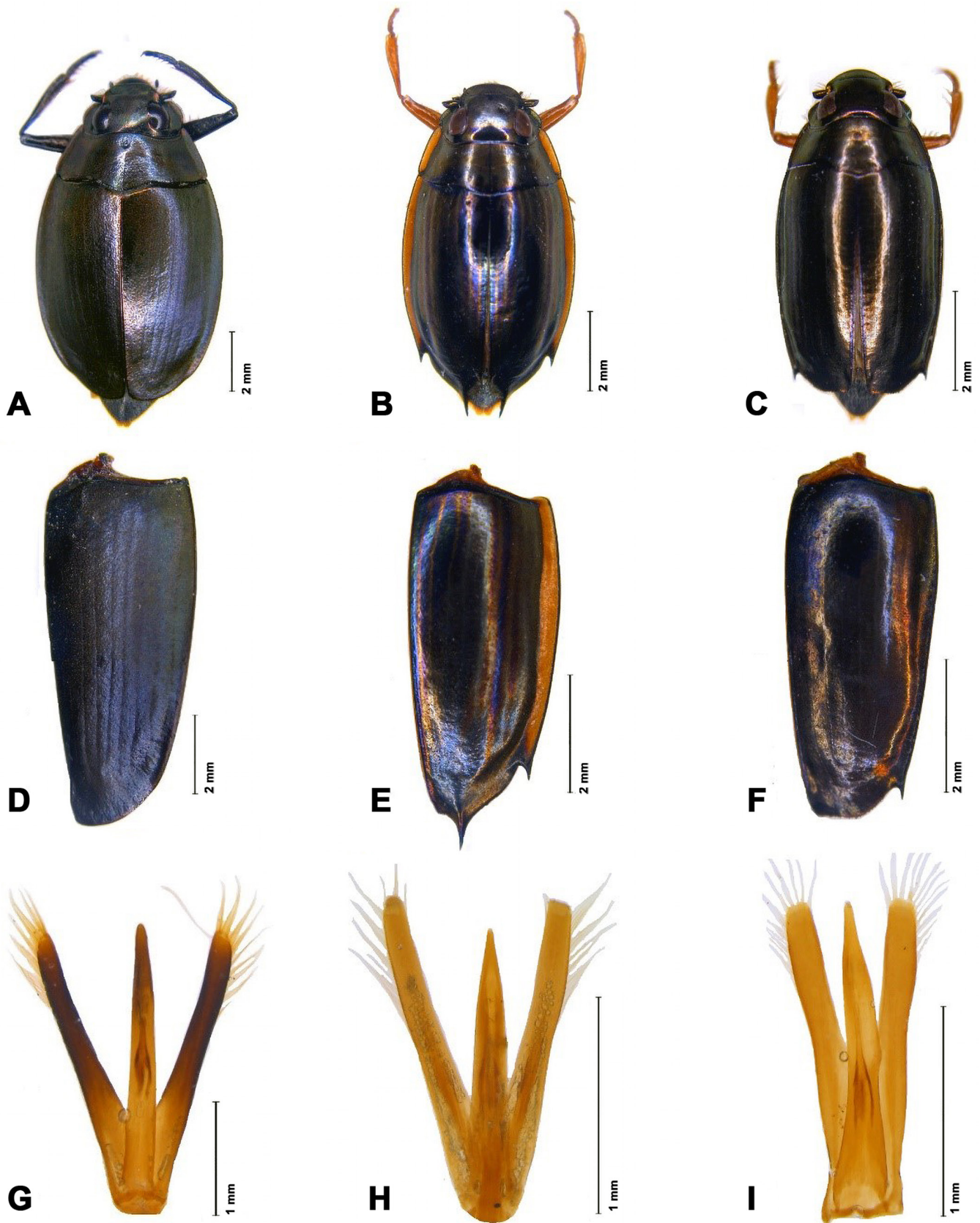


Figure 2. *Dineutus* species from India. **A-C.** General habitus, dorsal view; **D-F.** Elytra; **G-I.** Male genitalia. **A., D. & G.** *Dineutus indicus*; **B., E. & H.** *D. spinosus*; **C., F. & I.** *D. unidentatus*.

17.xii.2018, Reg. No. FBRC/ZSI/INS/2137; 1♀, Relli lake (17°48'35.28"N, 83°19'45.84"E), 21.xii.2020, Reg. No. FBRC/ZSI/INS/2230; 5♂♂, 4♀♀, Thatipudi reservoir (18°10'42.96"N, 83°12'15.48"E), 26.xii.2019, Reg. No. FBRC/ZSI/INS/2272; 1♂, Rollapadu Wildlife Sanctuary (15°27'3.6"N, 78°7'17.4"E), 18.xi.2021, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/2780; *Telangana*, 4♀♀, Marredmadine (16°10'1.56"N, 78°26'57.84"E), 27.xii.2018 Reg. No. FBRC/ZSI/INS/2407; 1♂, 1♀, Billakal (16°15'51.84"N, 78°38'11.76"E), 17.ix.2019, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/1599; *Tamil Nadu*, 2♂♂, 5♀♀, Kottachedu Kari (11°49'9.84"N, 78°16'9.12"E), 7.i.2019, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/2699.

Diagnostic characters (Figs 2B, 2E, 2H). Body size 6.5–8.5 mm, body oval, slender, elongate, depressed posteriorly, head black, bronze sides, clypeus reflecting pale, densely punctated, reticulated, antennae six segmented, pronotum black or bronze, margins yellow, scutellum small, elytra black or bronze, variable, 7 striae discernible, punctures clear, apex with acute double truncature and parasutural spines present, Sutural angle obtuse, straight, ventral side of body testaceous to ferruginous, legs brownish, metacoxal process truncate, median lobe shorter than parameres, somewhat narrowed to apex. Paramere with dense setation.

Distribution. *Oriental* (Bangladesh, India [Andhra Pradesh, Assam, Bihar, Manipur, Meghalaya, Odisha, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Malaysia, Myanmar, Thailand, Vietnam); *Palaeartic* (Pakistan [Sindh]) (Vazirani, 1984; Mustafa & Zubair, 2015; Deepa et al., 2022).

Dineutus (Cyclous) unidentatus Aube, 1838 (Figs 2C, 2F, 2J)

Dineutus unidentatus Aube, 1838, 6:788; *Dineutus unidentatus*, Regimbart, 1882, 2:424; *Dineutus unidentatus* Severin, 1890, 34:194.

Material examined. *India, Odisha*, 2♂♂, Shakti Waterfall (18°49'51.96"N, 82°36'0.72"E), 18.i.2022, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/3341; *Andhra Pradesh*, 7♂♂, 15♀♀, Relli Lake (17°48'35.28"N, 83°19'45.84"E), 21.xii.2020, Reg. No. FBRC/ZSI/INS/2228; 6♂♂, 12♀♀, Thatipudi reservoir (18°10'42.96"N, 83°12'15.48"E), 26.xii.2019, Leg.: J. Deepa & S. Shankar, Reg. No. FBRC/ZSI/INS/2271.

Diagnostic characters (Figs 2C, 2F, 2J). Body size 5.5–7.0 mm, oval, slender, depressed anteriorly, head black, copper or green in color, punctuated, clypeus bronze, reticulation distinct, antennae black with 6 segments, pronotum black, greenish on sides, lateral margin distinctly raised, elytra black, margins slightly pale, dense, visible punctuation on elytra, epipleural angle extended into straight spine, ventral side ferruginous, metacoxal process truncate, pointed, median lobe and paramere subequal, paramere with dense punctuation.

Distribution. *Oriental* (Burma, India [Andhra Pradesh, Bihar, Madhya Pradesh, Kerala, Meghalaya, Odisha, Rajasthan, Tamil Nadu], Indonesia, Malaysia, Sri Lanka, Thailand) (Vazirani, 1984).

Molecular identification. DNA barcoding is considered accurate for species identification (Hebert et al. 2003; Iftikhar et al., 2016). A total of 22 sequences were generated, including mitochondrial COI and 16S RNA for the genus *Dineutus* and resulted in the identification of three species namely *D. indicus*, *D. unidentatus* and *D. spinosus*. Mitochondrial COI genes shows notable genetic distance between the three species *Dineutus indicus* and *D. spinosus* 12%, *D. indicus* and *unidentatus* 10%, *D. spinosus* and *D. unidentatus* 12% (Table 2). 16S RNA genes shows genetic distance between *Dineutus indicus* and *D. spinosus* 4%, *D. indicus* and *unidentatus* 5%, *D. spinosus* and *D. unidentatus* 5% (Table 3). Phylogenetic trees constructed for both genes found three lineages corresponding to the three nominal species (Figs 3A, 3B). Phylogenetic tree of COI gene show two separate clades in which *D. indicus* in one, and both *D. spinosus* and *D. unidentatus* in other clade and also shows *Dineutus unidentatus* and *D. spinosus* are closely related when compared with three species.

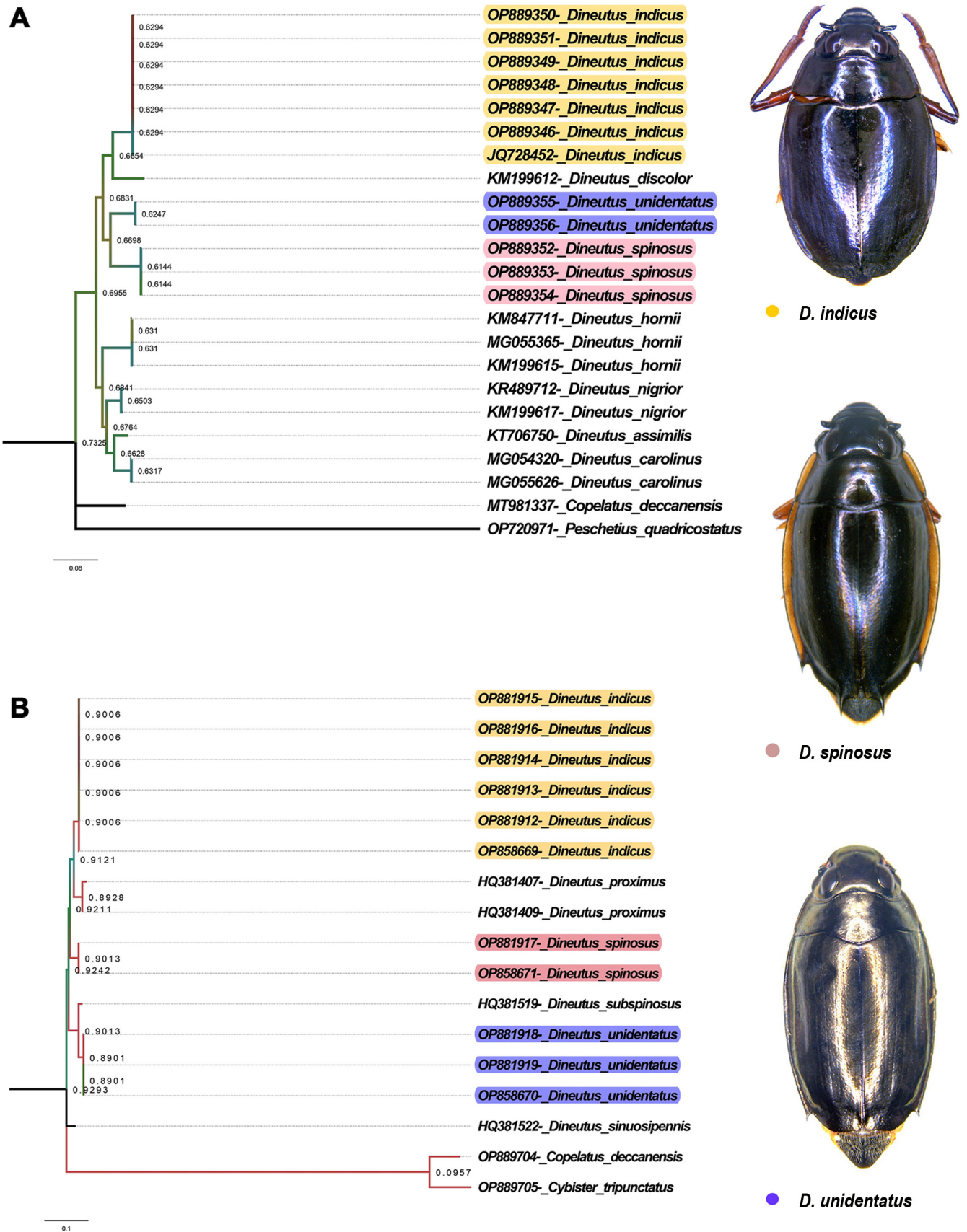


Figure 3. Maximum likelihood phylogenetic tree for *Dineutus* species, generated using sequence of two different genes: **A.** Cytochrome c oxidase I; **B.** Ribosomal RNA.

Table 2. Sequence divergence of COI gene (K2P) for *Dineutus* species and out groups.

	NCBI accession- Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	JQ728452- <i>Dineutus indicus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	OP889346- <i>Dineutus indicus</i>	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	OP889352- <i>Dineutus spinosus</i>	0.12	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	OP889353- <i>Dineutus spinosus</i>	0.12	0.12	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-
5	KT706750- <i>Dineutus assimilis</i>	0.10	0.10	0.10	0.10	-	-	-	-	-	-	-	-	-	-	-	-
6	OP889355- <i>Dineutus unidentatus</i>	0.10	0.10	0.10	0.10	0.12	-	-	-	-	-	-	-	-	-	-	-
7	OP889356- <i>Dineutus unidentatus</i>	0.10	0.10	0.10	0.10	0.12	0.00	-	-	-	-	-	-	-	-	-	-
8	KM199612- <i>Dineutus discolor</i>	0.09	0.09	0.12	0.13	0.10	0.11	0.11	-	-	-	-	-	-	-	-	-
9	KM199617- <i>Dineutus nigrrior</i>	0.09	0.09	0.11	0.10	0.06	0.11	0.11	0.09	-	-	-	-	-	-	-	-
10	KR489712- <i>Dineutus nigrrior</i>	0.10	0.10	0.10	0.10	0.06	0.11	0.11	0.10	0.01	-	-	-	-	-	-	-
11	KM847711- <i>Dineutus hornii</i>	0.11	0.11	0.10	0.10	0.08	0.10	0.10	0.12	0.08	0.09	-	-	-	-	-	-
12	MG055365- <i>Dineutus hornii</i>	0.11	0.11	0.10	0.11	0.09	0.10	0.10	0.11	0.08	0.09	0.00	-	-	-	-	-
13	KM199615- <i>Dineutus hornii</i>	0.12	0.12	0.10	0.11	0.09	0.10	0.10	0.12	0.09	0.09	0.00	0.00	-	-	-	-
14	MG054320- <i>Dineutus carolinus</i>	0.12	0.12	0.11	0.11	0.06	0.12	0.12	0.11	0.07	0.07	0.10	0.10	0.10	-	-	-
15	MG055626- <i>Dineutus carolinus</i>	0.12	0.12	0.12	0.11	0.05	0.11	0.11	0.12	0.07	0.07	0.09	0.09	0.10	0.00	-	-
16	MT981337- <i>Copelatus deccanensis</i>	0.18	0.18	0.18	0.18	0.16	0.15	0.15	0.16	0.16	0.16	0.16	0.15	0.16	0.15	0.15	
17	OP720971- <i>Peschetius quadricostatus</i>	0.81	0.81	0.85	0.84	0.74	0.84	0.84	0.78	0.77	0.78	0.86	0.82	0.85	0.78	0.78	0.82

Table 3. Sequence divergence of 16s gene (K2P) for *Dineutus* species and out groups.

	NCBI accession- Species name	1	2	3	4	5	6	7	8	9	10	11
1	OP881912- <i>Dineutus indicus</i>	-	-	-	-	-	-	-	-	-	-	-
2	OP881913- <i>Dineutus indicus</i>	0.00	-	-	-	-	-	-	-	-	-	-
3	OP881917- <i>Dineutus spinosus</i>	0.04	0.04	-	-	-	-	-	-	-	-	-
4	OP858671- <i>Dineutus spinosus</i>	0.04	0.04	0.00	-	-	-	-	-	-	-	-
5	OP881918- <i>Dineutus unidentatus</i>	0.05	0.05	0.05	0.05	-	-	-	-	-	-	-
6	OP881919- <i>Dineutus unidentatus</i>	0.05	0.05	0.05	0.05	0.00	-	-	-	-	-	-
7	HQ381409- <i>Dineutus proximus</i>	0.03	0.03	0.04	0.04	0.06	0.06	-	-	-	-	-
8	HQ381407- <i>Dineutus proximus</i>	0.04	0.04	0.05	0.05	0.08	0.08	0.01	-	-	-	-
9	HQ381522- <i>Dineutus sinuosipennis</i>	0.04	0.04	0.05	0.05	0.06	0.06	0.04	0.06	-	-	-
10	HQ381519- <i>Dineutus subspinosus</i>	0.05	0.05	0.06	0.06	0.02	0.02	0.06	0.07	0.05	-	-
11	OP889704- <i>Copelatus deccanensis</i>	0.93	0.93	0.94	0.94	0.96	0.96	0.92	0.94	0.87	0.91	-
12	OP889705- <i>Cybister tripunctatus</i>	0.95	0.95	0.97	0.97	0.95	0.95	0.99	1.02	0.97	0.96	0.17

DISCUSSION

Studies on the genus *Dineutus* from oriental region scattered, and studied by Mazzoldi (1995), Gustafson et al. (2016), Vazirani (1984), Thapa (2000), Mustafa and Ahmed (2015), Hájek & Fery (2017). The Indian aquatic beetle family Gyrinidae was studied by Vazirani (1984), who documented more than 56 species based on morphological features. He also discussed the distributional status and presence of species in different parts of the country. Subsequently, many authors provided molecular data for morphologically different *Dineutus* species from different parts of the country (Ghosh & Hegde, 2013; Ghosh & Das, 2016; Sheth et al., 2019; Deepa et al., 2022; Chandra et al., 2021). The new world species of the genus *Dineutus* studied and reported a total of 18 species, 6 subspecies in two subgenera, *Cyclinus* Kirby, 1837, and *Dineutus* Hatch, 1930 (Gustafson & Miller, 2015). The species *D. unidentatus* and *D. spinosus* can be morphologically differentiated by their elytral spines. *Dineutus spinosus* has two pairs of elytral spines, while *D. unidentatus* has one, suggesting they are two distinct

species, as confirmed through DNA barcoding as well. *Dineutus indicus* does not have any elytral spines and having a larger body size when compared to *D. unidentatus* and *D. spinosus* suggests it is unique among the three species. The molecular data also suggests it belongs to a different group.

Studies on molecular systematics of the genus *Dineutus* has suggested *D. indicus* as the sister group to *D. aereus*, which is widely distributed in Africa; the subgenus *Cyclous* within had two clades, which are African and North American. The subgenus *Cyclous* was identified as the common ancestor of Dineutini and *Dineutus*, with ancestors from the Ethiopian bio-geographic region for the African clade and the Nearctic biogeographic region for the North American clade. Furthermore, it revealed their origin in the late Eocene, distributed by means of a dispersal event (Gustafson & Miller, 2017). This study was a preliminary attempt to use DNA barcoding as baseline data for better understanding Indian *Dineutus* species in combination with morphological data.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution in the present study as follows: J. Deepa & S. Shankar: Collecting and identification of specimens, writing the manuscript; D. Kumar: Preparing the map of the study area and photographing the specimens; K. Madasamy & S. Jadhav: Handled the molecular analysis and interpreting the data. All authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are openly available in NCBI GenBank database at (<https://www.ncbi.nlm.nih.gov>) with the accession numbers (OP889346–OP889356, OP881912–OP881919 and OP858670–OP858671, respectively) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The specimens listed in this study are deposited in the National Zoological Collection of Zoological Survey of India, Freshwater Biological Regional Centre, Hyderabad and are available from the curator, upon a reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The used study materials in these study does not require ethical standards and permission but permission should be taken from where insect specimens collected (Forest department).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Appendix 1. Specimen details for sequences generated in present study, and additional sequences obtained from GenBank.

Accession no.	Species	Collection locality details as per NCBI GenBank	Source
OP889346	<i>Dineutus indicus</i>	India: Mallelatheertham Waterfall, Nagarkurnool, Telangana	Current study
OP889347	<i>Dineutus indicus</i>	India: Dharamattam Waterfall, Visakhapatnam, Andhra Pradesh	Current study
OP889348	<i>Dineutus indicus</i>	India: Amruthadara Waterfall, Alluri Sitaramaraju, Andhra Pradesh	Current study
OP889349	<i>Dineutus indicus</i>	India: Dudum Waterfall, Jaypore, Odisha	Current study
OP889350	<i>Dineutus indicus</i>	India: Aagaya Gangai Waterfall, Salem, Tamil Nadu	Current study
OP889351	<i>Dineutus indicus</i>	India: Muttal Waterfall, Salem, Tamil Nadu	Current study
OP889355	<i>Dineutus unidentatus</i>	India: Shakti Waterfall, Jaypore, Odisha	Current study
OP889356	<i>Dineutus unidentatus</i>	India: Relli Lake, Kambalakonda Wildlife Sanctuary, Visakhapatnam, Andhra Pradesh	Current study
OP889352	<i>Dineutus spinosus</i>	India: Yellow Dam, Jaypore, Odisha	Current study
OP889353	<i>Dineutus spinosus</i>	India: Thatipudi Reservoir, Visakhapatnam, Andhra Pradesh	Current study
OP889354	<i>Dineutus spinosus</i>	India: Kottachedu kari, Salem, Tamil Nadu	Current study
OP881912	<i>Dineutus indicus</i>	India: Mallelatheertham Waterfall, Nagarkurnool, Telangana	Current study
OP881913	<i>Dineutus indicus</i>	India: Dharamattam Waterfall, Visakhapatnam, Andhra Pradesh	Current study
OP881914	<i>Dineutus indicus</i>	India: Amruthadara Waterfall, Alluri Sitaramaraju, Andhra Pradesh	Current study
OP881915	<i>Dineutus indicus</i>	India: Dudum Waterfall, Jaypore, Odisha	Current study
OP881916	<i>Dineutus indicus</i>	India: Aagaya Gangai Waterfall, Salem, Tamil Nadu	Current study
OP858669	<i>Dineutus indicus</i>	India: Pachnai, Harishchandraghat, Ahmednagar, Maharashtra	Current study
OP881917	<i>Dineutus spinosus</i>	India: Shakti Waterfall, Jaypore, Odisha	Current study
OP858671	<i>Dineutus spinosus</i>	India: Relli Lake, Kambalakonda Wildlife Sanctuaty, Visakhapatnam, Andhra Pradesh	Current study
OP881918	<i>Dineutus unidentatus</i>	India: Yellow Dam, Jaypore, Odisha	Current study
OP881919	<i>Dineutus unidentatus</i>	India: Thatipudi Reservoir, Visakhapatnam, Andhra Pradesh	Current study
OP858670	<i>Dineutus unidentatus</i>	India: Relli Lake, Kambalakonda Wildlife Sanctuaty, Visakhapatnam, Andhra Pradesh	Current study
OP889704	<i>Copelatus deccanensis</i>	India: Mula River, Pachnai wadi, Ahmadnagar, Maharashtra	Current study
OP889705	<i>Cybister tripunctatus</i>	India: Veerannapet, Ranga Reddy, Telangana	Current study
OP720971	<i>Peschetus quadricostatus</i>	India: Purna River, Purna, Parbhani, Maharashtra	Current study
KM199612	<i>Dineutus discolor</i>	USA	GenBank
KM199615	<i>Dineutus hornii</i>	USA	GenBank
KM199617	<i>Dineutus nigrior</i>	USA	GenBank
KI706750	<i>Dineutus assimilis</i>	Canada: Ontario, Cambridge, rare Charitable, Research Reserve, Grand River	Telfer et al. (2015)
MG054320	<i>Dineutus carolinus</i>	Canada: Ontario, Charleston Lake PP, Running's Bay	GenBank
MG055626	<i>Dineutus carolinus</i>	Canada: Ontario, Charleston Lake PP, Boat Launch	GenBank
KM847711	<i>Dineutus hornii</i>	Canada: Ontario, Point Pelee NP, Point Pelee National Park	GenBank
MG055365	<i>Dineutus hornii</i>	Canada: Ontario, Charleston Lake PP, Boat Launch	GenBank
KR489712	<i>Dineutus nigrior</i>	Canada: Ontario, Oliver Ecological Centre, ca. 5 km NE of Bobcayeon, lake front meadow	Hebert et al. (2016)
JQ728452	<i>Dineutus indicus</i>	India	GenBank
HQ381407	<i>Dineutus proximus</i>	Madagascar	Isambert et al. (2011)
HQ381409	<i>Dineutus proximus</i>	Madagascar	Isambert et al. (2011)
HQ381522	<i>Dineutus sinuosipennis</i>	Madagascar	Isambert et al. (2011)
HQ381519	<i>Dineutus subspinosus</i>	Madagascar	Isambert et al. (2011)
MT981337	<i>Copelatus deccanensis</i>	India: Mula River, Pachnai wadi, Ahmadnagar, Maharashtra	GenBank

شناسایی گونه‌های جنس *Dineutus* MacLeay, 1825 (Coleoptera, Gyrinidae) گهات شرقی (هند) به روش ریخت‌شناسی و اطلاعات مولکولی

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چکیده: این تحقیق شامل شناسایی گونه‌های جنس *Dineutus* MacLeay جمع‌آوری شده مناطق کوهستانی گهات شرقی واقع در ایالات اودیشا، آندرا پرادش، تلنگانا و تامیل‌نادو در هند بر اساس اطلاعات ریخت‌شناسی و مولکولی است. اطلاعات ریخت‌شناسی شامل خارهای بال‌پوش، موهای روی پارامر و ساختار لوب میانی برای شناسایی استفاده شدند. توالی دو ژن شامل زیرواحد I سیتوکروم اکسیداز (COI) و ژن ریبوزومی 16S نیز برای تفکیک گونه‌ها به روش مولکولی به کار برده شدند. گونه‌های شناسایی شده شامل *D. spinosus*، *D. indicus* و *D. unidentatus* و بودند. تبارنامه‌های فیلوژنتیک بر اساس هر دو ژن بازسازی شدند که بر اساس توالی COI فاصله ژنتیکی ناخالص به میزان ۱۰-۱۲ درصد بین سه‌گونه مشاهده شد. در حالی که این واگرایی بر اساس ژن 16S به میزان ۴-۵ درصد بود. طی این مطالعه برای اولین بار ۵ توالی جدید از هر دو ژن COI و 16S برای دو گونه *D. spinosus* و *D. unidentatus* از هند ثبت شد.

واژگان کلیدی: سخت‌بال‌پوشان آب‌زی، DNA، بارکد، سیتوکروم اکسیداز I، 16S ریبوزومی، تاکسونومی