



## Full Length Article

DNA barcoding and description of new species of *Paradorydium* Kirkaldy (Cicadellidae: Deltocephalinae: Eupelicini) from India

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## ABSTRACT

A new species of leafhopper *Paradorydium kirkaldyi* sp. nov. (from Himachal Pradesh: Kinnaur: Powri) from India, is described. Detailed photographic illustration, annotated checklist and modified key to the species of India is also provided. This species closely resembles *P. khasianum* Viraktamath but differs in male genitalia characters and molecular analysis using *COI* gene confirmed the difference and also the taxonomic position of species in the tribe is established with Histone *H3* gene.

<http://www.zoobank.org/urn:lsid:zoobank.org:act:066F6658-ABB4-4585-A4DF-D8735A0230B5>

## Introduction

The tribe Eupelicini is considerably small with two subtribes Eupelicina and Paradorydiina. This group exclusively feeds on grasses having wide distribution around Palearctic, Afrotropical, Oriental and Australian regions with 7 genera and 64 valid species (Zahniser and Dietrich, 2013). The tribe is easily diagnosed by the strongly produced or spatulate head, gena without fine erect seta beside laterofrontal suture, genal margins strongly emarginate (~90° angle), forewing veins raised or carinate, metafemur apical setae 2 + 0, metatarsomere I expanded apically, male pygofer macrosetae absent or reduced, subgenital plates without macrosetae, and aedeagus not hinged basally. Paradorydiina comprises most of tribe Eupelicini, with 6 genera and 62 species worldwide. These are medium to large sized, elongate, produced leafhoppers and usually whitish, greenish, or brownish in color (Zahniser and Dietrich, 2013). They can be distinguished from Eupelicina by the crown not extending over the eyes anteriorly, ocelli on the anterior margin of the head, lateral margin of the pronotum not carinate, frontoclypeus without a median anterior carina, forewing appendix reduced or absent, forewing and hind wing marginal vein incomplete, male valve fused to the pygofer, style gracile, ovipositor extending far beyond the pygofer apex, first valvula dorsal sculpturing pattern maculose or granulose, second valvula without a dorsal median tooth and without dorsal teeth on apical 1/3 (Zahniser and Dietrich, 2013).

*Paradorydium* is largest among six genera of the Paradorydiina having 34 species worldwide and species can be compared to the seeds of the grasses (Demir, 2005). This genus is distinguished in having

elongated sculptured head, male plate usually fused with valve (Viraktamath and Viraktamath, 1989). This genus was erected by Kirkaldy 1901 with type species *Dorydium lanceolatum* Burmeister, 1839. Our recent exploration from North West Himalayan region yielded many specimens of leafhoppers with further study leads to discovery of *P. kirkaldyi* sp. nov. complemented with molecular data from India.

## Material and methods

Specimens were collected through sweep net collection from Powri: Kinnaur (India: Himachal Pradesh), were processed by series of steps like sorting, cleaning and mounting. Photographs were taken with a Leica DFC 425C digital camera on the Leica M205FA stereozoom autotomontage microscope. Male genitalia dissections were carried out as described by Oman (1949) and Knight (1965). Type material is deposited in the National Pusa Collection, Division of Entomology, Indian Council of Agricultural Research-Indian Agricultural Research Institute, New Delhi, India (NPC).

## Molecular study

## DNA extraction and PCR amplification

For mitochondrial cytochrome oxidase subunit I (*mtCOI*) and Histone *H3* analysis, the DNA was extracted from one leg of holotype specimen according to the manufacturer protocols, QIAGEN QIAamp1 DNA Investigator Kit. The isolated DNA was stored at -20 °C until required (Shashank et al., 2014). The DNA extractions were amplified for PCR products, *mtCOI* primers are LCO1490: 5'-GGTCAACAAATCATA

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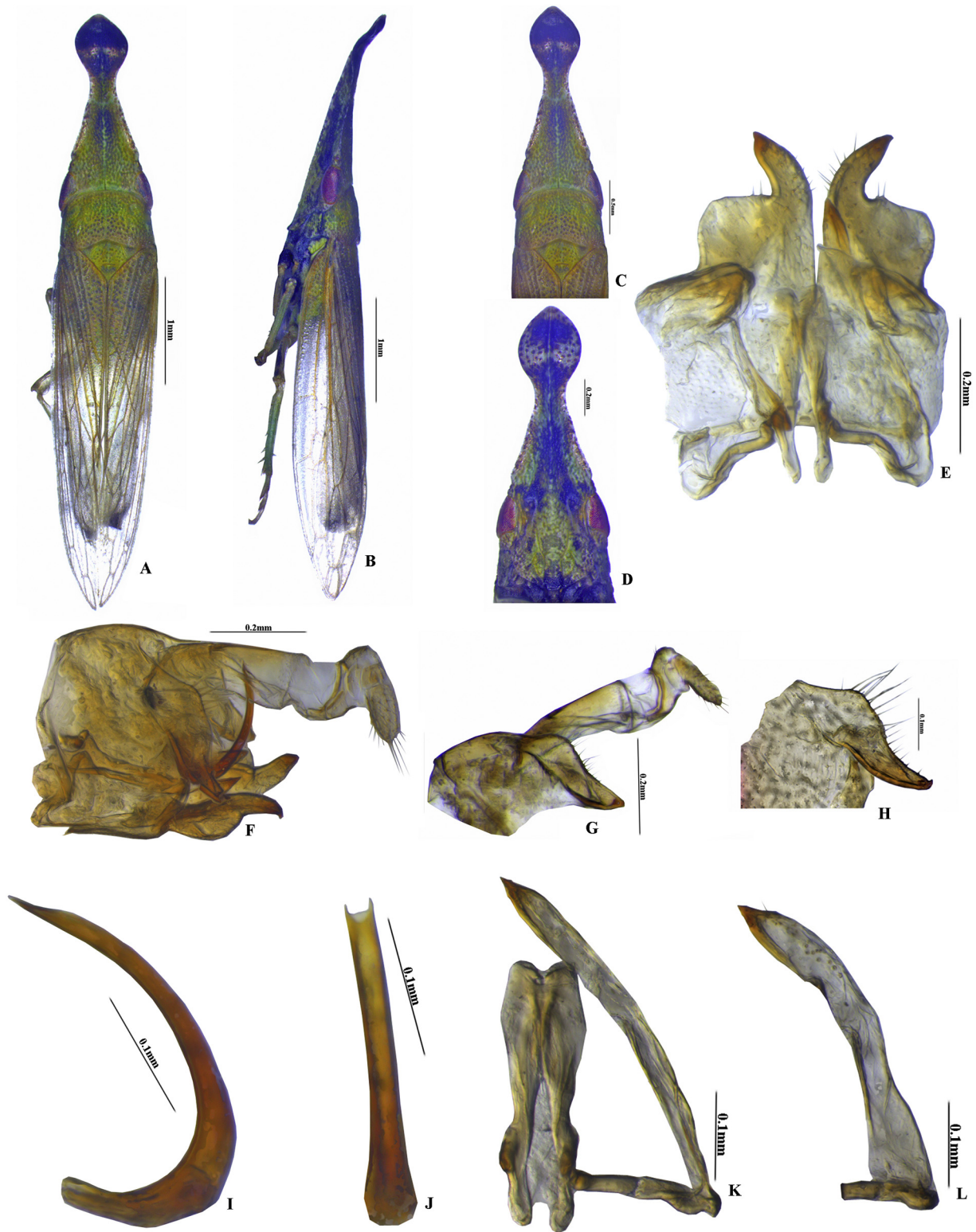


Fig. 1. *Paradorydium kirkaldyi* sp. nov. A. Habitus dorsal; B. Habitus lateral; C. Head, pronotum, mesonotum, and forewing base; D. Face; E. Subgenital plate; F. Pygofer; G. X segment; H. Pygofer process; I. Aedeagus lateral; J. Aedeagus ventral; K. Style and connective; L. Style.

AAGATATTGG-3'; HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAA TCA-3' (Folmer et al., 1994), standard PCR protocol for mtCOI was followed from Folmer et al., 1994 and Histone H3 primers are: HEXAF (forward) 5'-ATGGCTCGTACCAAGCAGACGGC-3' and HEX- AR (reverse) 5'-ATATCCTTGGGCATGATGGTGAC-3' (Ogden and Whiting, 2003). The PCR protocol for Histone H3, Product was amplified in 25 µl reactions using DNA polymerase (Fermentas GmbH, St. Leon- Rot, Germany) under the following cycling protocol: 4 min. Hot start at 94 °C, 35 cycles of denaturation for 30 s at 94 °C, annealing for 60 s at 48 °C, elongation for 50 s at 72 °C and a final extension 72 °C for 8 min in a C1000™ Thermal cycler. The reactions were combined (as described by KOD FX puregene™ manufacturer protocol) of DNA template 4 µl, 2× PCR buffer 12.5 µl, 2 mM dNTP 10 µl, TAQ (KODFX) enzyme 1 unit, and forward and reverse primers were 0.3 µM each at final concentration. The products were checked on 2% agarose gel and visualized under UV using Alphaview1 software version 1.2.0.1. The amplified products were sequenced at SciGenome Pvt. Ltd. (Cochin, India). The quality sequences were assembled with BioEdit version 7.0.0 and deposited in NCBI GenBank.

The availability of mtCOI sequence information is very meager for species of Eupelicini. Due to this reason we have selected *Paradorydium*

*kirkaldyi* sp. nov., *Paradorydium khasianum*, *Eupelicinae* sp., *Nephotettix virescens*, *Doratura stylata* and *Aphrodes diminuta* (Table 2) for phylogenetic analysis.

Annotated checklist to the species of genus *Paradorydium* from India.

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*Paradorydium* Kirkaldy, 1901f: 339 n. nov. Type: *Dorydium lanceolatum* Burmeister, 1839

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1	<i>deccani</i> Viraktamath and Viraktamath, 1989: 20	India (Karnataka, Gujarat and Rajasthan)
2	<i>dharwarensis</i> Viraktamath, 1976: 81	India (Karnataka)
3	<i>elongatum</i> Distant, 1918: 32	India (Uttar Pradesh)
4	<i>kirkaldyi</i> sp. nov.	India (Himachal Pradesh)
5	<i>khasianum</i> Viraktamath and Viraktamath, 1989:22	India (Meghalaya)
6	<i>omani</i> Viraktamath, 1976: 82	India (Karnataka)
7	<i>ruberosum</i> Pruthi, 1930: 20	India (Madhya Pradesh)

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1. Vertex without pronounced notch in margin anterior to ocelli; Costal margin of forewing hyaline; male plates with strongly laterally directed projection ..... ***Paradorydium omani* Viraktamath**
- Vertex with a pronounced notch in margin anterior to ocelli (Viraktamath and Viraktamath, 1989: Figs. 1, 3, 15, 17); male plates without a strong lateral projection.....2
2. Vertex gradually narrowing to interocular width in apical 0.33; male plate 1.5 times as wide at base as at apex in ventral aspect, rather truncate apically..... ***Paradorydium elongatum* Distant**
- Vertex rapidly narrowing to interocular width slightly beyond ocelli; male plate more than twice as wide at base as at caudal apex..... 3
3. Vertex not expanded before apex (Viraktamath and Viraktamath, 1989: Fig. 1, 3), predominantly green or yellowish green; small species (male 3.90 to 4.34 mm long, female 5.04 to 6.05 mm long); male pygofer without a distinct caudal finger-like projection (Viraktamath and Viraktamath, 1989: Fig. 8)..... ***Paradorydium deccani* Viraktamath and Viraktamath**
- Vertex after narrowing at about apical 0.33, expanded before apex (Viraktamath and Viraktamath, 1989: Figs. 15, 16); larger species (males more than 6.00 mm long, females more than 7.00 mm long); pygofer with a finger-like projection.....4
4. Male plates more than twice as long as width at base, slender, finger-like.....***Paradorydium ruberosum* Pruthi**
- Male plates less than twice as long as width at base, robust, rather triangular..... 5
5. Apex of aedeagus shaft deeply bifid (Viraktamath and Viraktamath, 1989: Fig. 26)..... 6
- Apex of aedeagus shaft shallowly bifid.....***Paradorydium dharwarensis* Viraktamath**
6. Subapical region of vertex more narrower (Fig. 1 A-C), apex of plates digitate, preapical lobe well developed with small hair like setae (Fig. 1E) ..... ***Paradorydium kirkaldyi* sp. nov.**
- Subapical region of vertex not narrower, apex of plates short not digitate, preapical lobe not developed (Viraktamath and Viraktamath, 1989: Fig. 15, 21).....***Paradorydium khasianum* Viraktamath and Viraktamath**

**Table 1**  
Showing GenBank Accession Numbers for Histone H3.

Species	GenBank sequence ID
<i>Paradorydium kirkaldyi</i> sp. nov.	MG547710
<i>Paradorydium lanceolatum</i>	GU123877*
<i>Eupelix cuspidata</i>	GU123828*
<i>Leofa dispar</i>	KR230303*
<i>Stirellus picinus</i>	KR230302*
<i>Chiasmus undulatus</i>	KR230251*
<i>Xestocephalus desertorum</i>	GU123892*

\* Downloaded from Genbank.

**Table 2**  
Showing GenBank Accession Numbers for mtCOI.

Species	GenBank sequence ID
<i>Paradorydium kirkaldyi</i> sp. nov.	MG489870
<i>Paradorydium khasianum</i>	JQ755805*
<i>Eupelicinae</i> sp.	KF226901*
<i>Nephotettix virescens</i>	KF371523*
<i>Doratura stylata</i>	KR580534*
<i>Aphrodes diminuta</i>	KF321754*

\* Downloaded from Genbank.

Key to the species of *Paradorydium* from India (modified from Viraktamath and Viraktamath, 1989)

*Taxonomic accounts*

*Paradorydium kirkaldyi* sp. nov. Meshram

*Description*

*Colouration.* Head, pronotum and scutellum greenish yellow. Anterior margin of vertex with 2 black transverse bands, medially with black maculation. Compound eyes and ocelli red. Forewing hyaline (Fig.1A-D).

*Morphology.* Head and thorax deeply punctate (Fig. 1C). Median length of vertex 1.69 mm, vertex about 2.5× longer than inter-ocular distance, medially carinate, lateral margins foliaceous, vertex with a notch in front of ocelli on lateral margins, progressively narrowing beyond middle, then widened and strongly upturned, forming a spoon like expansion (Fig. 1A-C). Face with a strong prominent mid-ventral ridge sharply edged beyond antennal insertion; broadened gradually posteriorly into fronto-clypeus (Fig. 1D). Pronotum half as long as wide, with a median and two lateral faint carinae, anterior margin convex,

the posterior margin concave in middle. Scutellum triangular, with faint median carinae extending upto scuto-scutellar suture. Stramineous, hyaline forewings, deeply punctated on anal region, venation prominent, with acute apex (Fig. 1A-B).

*Male genitalia.* Pygofer in lateral view 1.7× longer than broad, caudo-dorsal margin with stout setae, 10th segment 1× longer than length of pygofer (Fig. 1F-H). Plates are not fused with valve, apices acute, curved laterally, 1.6× longer than wide, preapical lobe well developed with small hair like setae (Fig.1E). Connective arms 1.2× longer than stem (Fig. 1K). Aedeagus bifurcated apically, shaft subequal in width throughout except broaden at base and pointed at apex (Fig.1I-J).

*Measurement.* Male 5.72 mm long, 0.9 mm wide across eyes.

*Etymology.* The species is named after Prof. G.W. Kirkaldy for his monumental contributions towards classification and leafhopper taxonomy.

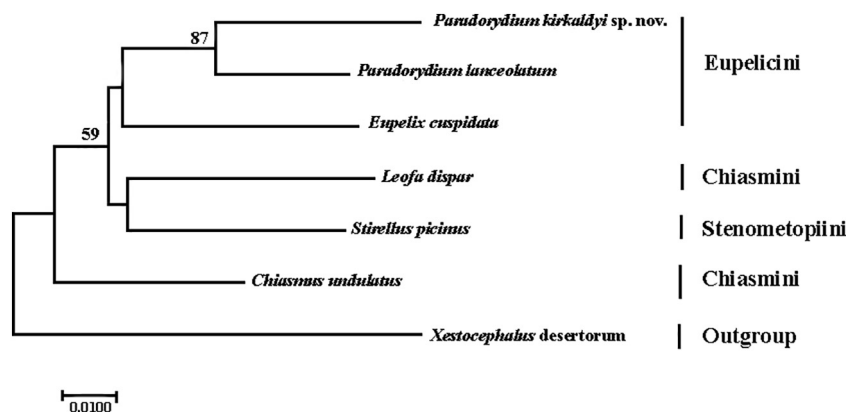
*Type material.* Holotype ♂, INDIA: Himachal Pradesh: Kinnaur: Powri (31°31'42.14"N 78° 16'19.18"E), 14.vii.2017, Sweep net collection. Rajgopal, N. N. (NPC).

*Remarks.* This species is closely resemble *Paradorydium khasianum*, but differs in having following characters: 1. Subapical region of vertex more narrower as compared to *P. khasianum* 2. Plates are not fused with valve and apex digitate, preapical lobe well developed with small hair like setae 3. Aedeagus abruptly narrowed at apex with shallow notch.

*Molecular analysis.* The phylogenetic position of the new species is ascertained with genera and species of Eupelicini by preliminary molecular analysis using available material of a series of taxa within Deltocephalinae from NCBI GenBank (Table 1&2). Analysis of H3 sequence data recovered Eupelicini as monophyletic with *Eupelix* sister to the clade comprising the two species of *Paradorydium*. Subtending this clade was a grade comprising the included Chiasmini and Stenometopiini. The final data matrix of our preliminary phylogenetic analysis (Table 1) included 7 terminals (2 ingroup taxa belonging to 2 genera of Eupelicini and 1 outgroup taxon). Analysis shows that this species belong to genus *Paradorydium* of tribe Eupelicini as this species grouped with *P. lanceolatum* and near to *Eupelix cuspidata*.

The molecular data are given in Figs. 2 and 3, MEGA V6.0 (Tamura et al., 2013) was used to calculate the Kimura 2- parameter model (Kimura, 1980) for mtCOI sequence. This revealed that the percent of sequence variation between *P. kirkaldyi* sp. nov., and *P. khasianum* is at least 9.8% which was confirmed from the mtCOI sequences. The neighbor joining tree was constructed including most closely related available species. Out groups were taken from NCBI Genbank, denoted with 1000 bootstraps and the node length depicted in the tree (Figs. 2 and 3).

*Distribution.* Himachal Pradesh: India.



**Fig. 2.** Phylogram of the analysed genera of Deltocephalinae showing relationships of *Paradorydium kirkaldyi* Meshram sp. nov. with related genera of tribe Eupelicini inferred using neighbor-joining (NJ) tree method and the kimura 2-parameter distances of Histone H3 sequences.

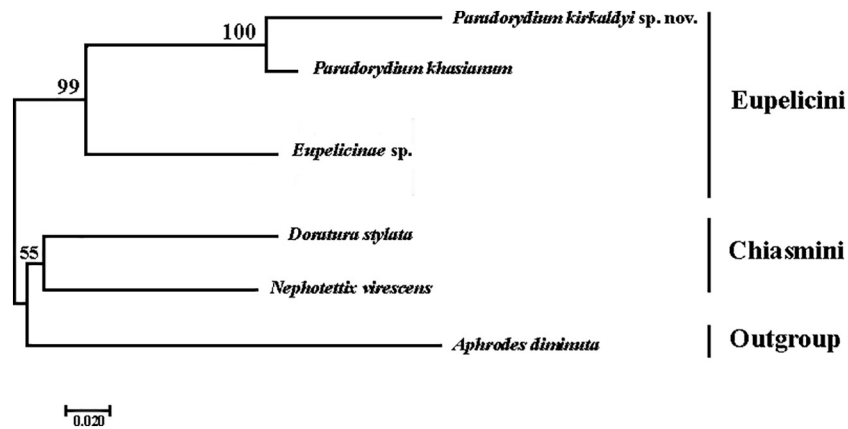


Fig. 3. Phylogram of the analysed genera of Deltoccephalinae showing relationships of *Paradorydium kirkaldyi* Meshram sp. nov. with related species of tribe Eupelicini inferred using neighbor-joining (NJ) tree method and the kimura 2-parameter distances mitochondrial *COI* sequences.

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