

A new species of *Euphaea* Selys, 1840 (Odonata, Euphaeidae) from Wayanad, Western Ghats, Kerala, India

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ABSTRACT: A new species of *Euphaea* Selys, 1840 (Odonata, Euphaeidae) is described from the Wayanad landscape of Western Ghats of Kerala State based on integrated taxonomy. *Euphaea wayanadensis* **sp. nov.** is differentiated morphologically from the closely similar *E. pseudodispar* Sadasivan & Bhakare, 2021, based on the longer black hind wing patch; broader humeral and antehumeral stripes of males, which are never interrupted and not significantly tapering towards the alar sinus; humeral stripes not terminating in a distinct spot; S10 distal margin wavy at mid-ventrum and apex of the dorsal keel on S10 stepped in lateral view; and the difference in length ratios of male aedeagus and structure of the male genital vesicle. Molecular analysis of the mitochondrial cytochrome oxidase subunit I (mt COI) region showed 12.9 per cent nucleotide divergence between the two species. Phylogenetic analysis with Maximum Likelihood method using MEGA 11 also revealed the distinctiveness of *E. wayanadensis* **sp. nov.** The habitat, distribution and ecology of the new species are discussed, and a key to *Euphaea* species of Western Ghats is also provided. © 2025 Association for Advancement of Entomology

KEY WORDS: Integrated taxonomy, mtCO1, molecular analysis, phylogeny, key to species, distribution, Coorg landscape

INTRODUCTION

The genus *Euphaea* Selys, 1840 (Odonata, Euphaeidae), in Western Ghats of Peninsular India

is characterized by males with hind wing apices broadly opaque black with metallic iridescence, rounded and shorter than the forewing; discoidal cell traversed; distinct keel on 10th abdominal

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segment, and the anal appendages with forcipate cerci and tiny paraprocts (Fraser, 1934). The genus ranges from Sri Lanka, Peninsular and Northeast India, Thailand, Malaysia, Borneo to Indonesia. Currently, five species of *Euphaea* are known from Western Ghats of Peninsular India, namely *E. cardinalis* (Fraser, 1924), *E. dispar* (Rambur, 1842), *E. fraseri* (Laidlaw, 1920), *E. pseudodispar* Sadasivan & Bhakare, 2021 and *E. thosegharensis* Sadasivan & Bhakare, 2021 (Subramanian and Babu., 2017; Bhakare *et al.*, 2021; Nair *et al.*, 2021). The genus *Euphaea* can be a good model for studying the allopatric speciation mediated by the biogeographical gaps of the Western Ghats. Of the three species of *Euphaea* occurring in the Southern and Central Western Ghats, *E. fraseri* has a wide distribution throughout the Western Ghats from Kanyakumari to Maharashtra at 100–1200m (Subramanian *et al.*, 2018; Fraser, 1934), whereas, *E. cardinalis* is restricted to the mountains south of Palghat Gap (Southern Western Ghats), and *E. dispar* is restricted to the north of the Palghat Gap, mainly in the mountains between the Palghat Gap and Coorg (Central WG) (Fraser, 1934). During our fieldwork we observed a morphologically distinct population of *Euphaea* from the Wayanad–Coorg landscape of Western Ghats, which is described here as new to science based on morphological and molecular differences from all other congeners.

MATERIALS AND METHODS

The authors (SSA & CS) came across the distinct morphotype at Kalindi River, Thirunelli, Wayanad district Kerala, during a survey in 2013, followed by observations along the same stretch till 2019. Later field surveys by KS & VPN from 2019 to 2023 revealed similar morphotypes from Thirunelli in Wayanad, Aaralam in Kannur, and the western slopes of Coorg in Karnataka. The specimens of new morphotypes were photographed in the field, collected using an insect net, and preserved in absolute ethanol. Morphometric studies were done using a digital vernier caliper (Mitutoyo CD-6" CS). The field photographs were captured using Canon 600D fitted with Canon EF 100 mm f/2.8L USM Macro lens. Specimen photographs captured in the laboratory were done using a Nikon Z 50 mirrorless camera fitted with a Nikkor Z DX 16-15 f/3.5-6.3

VR lens. Microphotography of specimens were carried out using a camera-mounted Leica M80 stereo zoom microscope and processed using Leica Application Suit V 4.8 Software. Taxonomic description follows Fraser (1934) and Bhakare *et al.* (2021). The terminology for morphological description follows Garrison *et al.* (2006). The distribution map was prepared using QGIS3 software and edited in Adobe Photoshop CS5.

DNA extraction and PCR amplification:

Genomic DNA was isolated from the muscle tissues of the thorax using a DNeasy animal tissue kit following the manufacturer's protocol (Qiagen, Valencia, CA) and was visualized using 0.8% agarose gel. The universal barcode primer described by Folmer *et al.* (1994) (LCO-50-GGT CAA CAA ATC ATAAAG ATA TTG G-30; HCO-50-TAA ACT TCA GGG TGA CCA AAA AAT CA-30) specific to mitochondrial cytochrome oxidase I (COI) was used.

The polymerase chain reaction (PCR) steps were carried out using sterile PCR tubes. The primers were diluted to 10µM using DNAase and RNAase-free molecular water. The PCR master mix used was the Emerald Amp GT PCR Master mix (TAKARA). The master mix included an optimised buffer, PCR enzyme, dNTP mixture, gel loading dye (emerald green), and a density reagent in a 2x premix format Gradient PCR was carried out for standardising the annealing temperature. The best annealing temperature for this reaction was recorded as 51°C. For PCR amplification of mitochondrial DNA, Cytochrome oxidase subunit I locus, a reaction mixture of 40µL was prepared. The composition of the reaction mixture was as follows: 20 µL PCR master mix, 2 pl template DNA, 1µL forward, 1 µL reverse primer, and 16 µl molecular water.

Thermal conditions for PCR amplification were as follows; A. initial denaturation: 94°C for 4 minutes, B. denaturation: 94°C for 30 seconds, C. annealing: 51°C for 1 minute, D. extension: 72°C for 2 minutes, steps B-D were repeated for 30 cycles, E. Final extension: 72°C for 10 minutes. The PCR product obtained was stored at 4°C. For electrophoresis, 1.2 per cent agarose gel was prepared with 100ml

of 1x TAE buffer, and 3 µL of Ethidium bromide was added. The prepared gel was cast in a horizontal gel casting tray. After solidifying, the comb was removed, and the tray was placed in the electrophoresis chamber and filled with 1x TAE buffer. The PCR product (6 µL) was directly loaded into the wells since the dye was already present in the PCR master mix. Three µL of 1kb DNA ladder (Invitrogen TrackIt) was also loaded in a separate well. 1x TAE buffer was used as the electrophoresis buffer, and the run was carried out at 70 V cm. The electrophorised gels were visualised, and images were documented with UV light using the Bio-Rad gel documentation system.

DNA sequencing: Sanger sequencing of PCR product was done by sending PCR product to Eurofins India Pvt. Ltd., Bangalore. For each sample, bidirectional sequencing was done.

Bioinformatic analysis: The forward and reverse sequences obtained were trimmed and aligned using Bioedit software. Multiple sequence alignment was done using MUSCLE algorithm in MEGA 11 software. For phylogenetic classification of the identified species by DNA barcoding, sequences belonging to the two *Euphaea* species which were generated in this study and the relevant mtCOI-5P sequences available in the NCBI database were retrieved and analysed by constructing a phylogenetic tree in MEGA11 software (Tamura *et al.*, 2021). The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. All positions containing gaps and missing data were eliminated. The ML Heuristic method applied was the Nearest-Neighbour-Interchange method (Robinson, 1971). A total of 1000 bootstrap replications (Felsenstein, 1985) were followed in the construction of the phylogeny tree. Nucleotide sequence similarity count matrix, was prepared to confirm the species distinctiveness using Ape package in R studio software and a heat map showing percentage similarity was plotted using ggplot2 package.

The following abbreviations/notations are used in the description — Fw: Forewing, Hw: Hind wing, TL: Total length including appendages, AL: abdominal length, FL: forewing length, HL:

hindwing length, BHL: length of black apices of the hindwing, Ax: antenodal veins, Px: postnodal veins, Pt: pterothorax, S1-S10: corresponding abdominal segments, SSA: S S Anooj, CS: C Susanth, KS: Kalesh Sadasivan, MB: Milind Bhakare, VPN: Vinayan P Nair.

RESULTS

Euphaea wayanadensis Anooj, Susanth, & Sadasivan sp. nov. (Figs. 1, 2, 3, 4)

LSIDurn:lsid:zoobank.org:act:3CD9CAF7-EFAD-4A2E-B7F9-CF6CE312E301

Holotype: Male, Thirunelli, Wayanad district, Kerala, India, (910 m ASL), private land on banks of River Kalindi, Thirunelli, coll. S.S. Anooj on 28.ix.2019, wet specimen in ethanol, to be deposited in ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru.

Paratypes: Four males and three females (including one allotype in ethanol), the same information as the holotype, TORG 1035 male and TORG 1036 male, Aaralam, Kannur, Kerala, wet specimens in ethanol, coll. Kalesh Sadasivan on 19.ix.2022; TORG 1037 male and TORG 1038 male, Thirunelli, Wayanad, coll. Vinayan P.Nair on 18.ix.2022, TORG 1035 and TORG 1037 Paratype specimens to be deposited in ZSI, Calicut, Kerala.

Description of the holotype – Male (Fig. 2): Measurements (in mm): TL (including appendages) 52.2, AL 42.0, FL 36.6, HL 34.1 and BHL 10.4.

Head: Labium pale brown medially and darker laterally; labrum pale bluish with a median black ‘tongue’ shaped mark; mandibles concolourous with labrum with an upper transverse black streak; anteclypeus shiny dark amber-brown to black with an upper azure blue pruinose stripe visible in live specimens; postclypeus matt black; antefrons and postfrons black; genae pale bluish-white; eyes black superiorly, medially and laterally dark amber to brownish-black, inferolateral area brown; antennae shiny black; vertex, occiput, occipital bar, post-ocular area, all matt black (Figs. 2B, E).

Prothorax: Ground colour matt black; anterior lobe completely black; middle lobe black with triangular

yellowish spots laterally; notopleural suture black; propleuron matt black with a pair of indistinct reddish spots; posterior lobe black without any ornamentations or spines (Figs. 2 B, D, F).

Pterothorax: Ground colour orange-red with yellow dorsally, orange hue inferiorly; triangular mesothoracic acrotergite black, dorsal carina black; mesepisternum matt black; humeral and antehumeral stripes broad, never interrupted, does not taper conspicuously towards alar sinus; mesostigmal plate matt black. Orange posterior lateral and black antero-superior border region of mesinfraepisternum distinctly separated diagonally; mesepimeron superiorly yellow and orange inferiorly, centrally black; a reddish-black patch of interpleural suture extends to about middle of mesepimeron; dorsal third of second lateral suture marked black; metepisternum, metepimeron yellowish-orange; metinfraepisternum brownish-orange; metathoracic spiracle pale yellowish-white (Figs. 2 B, D, F).

Legs: Fore-coxae anteriorly brown, posteriorly grey; trochanter of similarly coloured as former; mid and hind coxae orangish to brownish red; femur, tibia, tarsus red with cyan hue; superior 1/3rd of extensor surface of forelegs black; knees and claws black (Figs. 2A, B, D).

Wings: Fw hyaline. Nodal formula: left Fw Ax-22 & Px-35, right Fw Ax 22 & Px 34, left Hw Ax 20 & Px 35, right Hw Ax 21 & Px 35; Pt black, 10 cells wide in Fw, 11 cells wide in Hw. Hw hyaline, distal 1/3 black with a metallic coppery-lilac shine when viewed in some angles (Figs. 2A, C); anterior margin of this black patch convex, extends to 11 cells proximal to Pt (Fig. 2M); cubital space with three cross veins in all wings.

Abdomen: Proximal segments reddish-orange, distal segments black; S6, dorsally red, black ventrally; distal 1/4th black (Figs. 2A, C); segmental joints black; proximal margin of segments lustrous black; S1 yellowish with posterior part having orange tint; S2 orange anteriorly, reddish-orange distally; pseudoauricles on S2 not flanged, with four teeth interspaced with few erect hairs (Fig. 2H); hamule distal end hooked, blunt, black at distal tip

(Fig. 2I); S3, S4 reddish orange with indistinct orange lateral patch; S5 reddish orange with distal 1/5th brownish-black which merges with ground colour; proximal part of S6 orange red which gradually transits to black at its distal 1/3rd; S7–10 fully black; broad dorsal carina, distal dorsal keel on S10; ventrally base of S8 sternite and base of lateral border of S8 tergum with tuft of black hairs; ventral side of S9 with tuft of long hairs on proximal part of its lateral tergite, small tuft on distal ventral border of S9 tergite, and a small tuft of hairs on the S9 sternite proximal to its ventral beak before gonopore on either side (Figs. 2L, N, O); S9 sternite ventral beak bordered on either side with a prominent node; gonopore margin oval in ventral view; gonocoxae with blunt apices; S10 distal margin wavy at mid-ventrum, apex of dorsal keel on S10 stepped on lateral view (Fig. 2L); medial part of distal margin of S9 extending ventrally, its tip extends over gonopore as a short tooth (Fig. 2L); length of S9 1.5 times that of S10. In lateral view, S10 shows comparatively lesser curvature i.e., comparatively broader on ventrolateral area.

Genitalia: S2 with lustrous brownish black, rhomboidal genital vesicle with distal end angulated, transverse surface rugosities absent, vesicle longer than broad and its distal margin not oval (Figs. 2G, J); genital ligula illustrated; penis with a single seta on either side; length of penile structure after setae comparatively more in dorsal view (Fig. 2K).

Anal appendages: Cerci forcipate, black; paraprocts black, in ventral view with tips medially directed and hence converge at rest. Paraprocts with tip hooked, directed dorsally. Paraproct longer than gonocoxae, of similar size as that of dorsal prominence of S10 (Fig. 2L, N, O).

Description of Female (Fig. 3): Measurements (in mm): TL (including appendages) 45.9, AL 35.6, FL 35.2 and HL 33.2.

Head: labium black, labrum pale bluish-white with a median black 'tongue' shaped mark; mandibles pale bluish-white having an upper transverse black streak similar to male; anteclypeus, postclypeus, antefrons, postfrons, vertex and occiput matt black; genae pale bluish-white; antennae black; eyes as



Fig 1. *Euphaea wayanadensis* **sp.nov** adult male in field © Vinayan P Nair



Fig. 2 *Euphaea wayanadensis* **sp. nov.** A, B: field photo, C: holotype, D: thorax lateral view, E: facial view, F: thorax dorsal view, G: Secondary genitalia and vesicles lateral view, H: Pseudoauricles lateral view showing teeth, I: hamule lateral view, J: secondary genitalia and vesicles ventral view, K: penile structure dorsal view, L: terminal abdominal segments and appendages lateral view, M: hind wing portion showing the extension of black patch with respect to Pt, N: terminal abdominal segments and appendages ventral view, O: terminal abdominal segments and appendages dorsal view © S.S. Anooj and C. Susanth

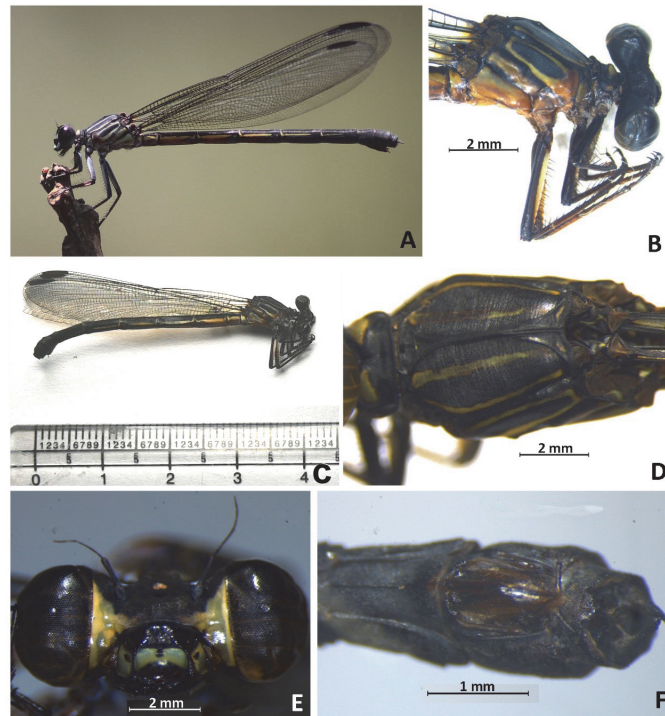


Fig. 3 *Euphaea wayanadensis* **sp. nov.** female, A: field photo, B: thorax lateral view, C: allotype, D: thorax dorsal view, E: facial view, F: terminal abdominal segment dorsal view showing vulvar scale
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Fig. 4 *Euphaea wayanadensis* **sp. nov.** paratypes, A: paratype males, B: paratype females, C-D: habitat
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in males (Fig. 3E).

Prothorax: as in males; spots on second lobe pale yellowish-white (Fig. 3B, D).

Pterothorax: Pale greenish-white replaces the red colour in males; humeral and antehumeral stripes pale yellowish blue, latter not interrupted; reddish markings of mesepisternum, mesepimeron and metepisternum in males replaced with pale bluish-yellow; mesepisternum and metepisternum with central black area (Figs. 3B, D).

Legs: Coxae posterolaterally pale dirty white and pruinose; trochanters grey; femur, tibia, tarsus, claws black; basal 1/4th of flexor region of forelegs, whole of mid and hind tibia creamy white (Figs. 3A, B).

Wings: Hyaline. Left Fw Ax-24 & Px-31; right Fw Ax21 & Px28; left Hw Ax 7 & Px27; right Hw Ax 18 & Px28. Pt black, 10 cells wide in Fw and Hw (Figs. 3A, C).

Abdomen: All segments black with a dorsal yellow carina that disappears at S6; laterally inferior half of segments with yellow transverse streaks narrowing distally and disappearing on S7; remaining areas, including whole of S8 to S10, black (Fig. 3A, C); vulvar scales longer compared to that

of *E. pseudodispar* and without central sulcus, postero-lateral angle forms blunt tooth (Fig. 3F); appendages and ovipositor black.

Variation: *Males:* In the paratype males (n= 4); TL, AL, FL, HL and BHL (in mm) ranged from 50.3–52.2, 39.2–42.0, 35.5–36.6, 32.1–34.1 and 9.9–10.7 respectively; humeral stripes appeared to be more or less stable (Table 1).

Females: The paratype females (n= 2); TL, AL, FL, HL and BHL (in mm) ranged from 45.1–45.3, 34.6–35.1, 34.2–35.5 and 32.2–33.1 respectively (Table 1).

Etymology: The species name '*wayanadensis*' is derived from the name of the district Wayanad, in which the type locality Thirunelli is located.

Distribution: As far as known, the species is distributed in the central WG from River Kalindi, Thirunelli, Wayanad district, in Kerala, India extending further north in Aaralam WLS, and foothills on western slopes of central Western Ghats south of Someshwara (Fig. 7).

Habitat and Ecology: Fast-flowing streams with rocks/boulders and aquatic vegetation with luxuriant evergreen and Semi-evergreen tree growth on the

Table 1. Morphometrics of *Euphaea wayanadensis* sp. nov.

No	Specimen	Total length (mm)	Abdominal length (mm)	Forewing length (mm)	Hindwing length (mm)	Length of black hindwing patch (mm)	Ratio of the hind wing length to the length of black hindwing patch
1	Holotype ♂	52.2	42.0	36.6	34.1	10.4	3.3
2	Paratype ♂	52.6	41.5	35.7	33.7	9.9	3.4
3	Paratype ♂	52.9	42.6	35.5	33.5	10.1	3.3
4	Paratype ♂	51.0	41.2	36.6	33.4	10.7	3.1
5	Paratype ♂	50.3	39.2	36.0	32.1	9.9	3.2
6	Paratype ♀	45.9	35.6	35.2	33.2	—	—
7	Paratype ♀	45.3	34.6	34.2	32.2	—	—
8	Paratype ♀	45.1	35.1	35.5	33.1	—	—

*Black hind wing patch absent in females

banks, were the most preferred by the species (Fig. 4C–D). The shrubs and rocks on the riverine habitats were preferred by the adults for perching. Males prefer to perch on branches at a height of 1–2m while females prefer higher perches. The emergent rocks and boulders are preferred by males for display. The elevational distribution is from 100–1000m ASL. The species is observed throughout the year, except during the dry seasons of March–April. Monsoon and post-monsoon season from May to October is the flight season based on the field observations.

Differential diagnosis

The species is close to *E. pseudodispar*; however, can be differentiated by the black patch on hind wing apices in males which covers up to 1/3 of the hind wing, whereas in *E. pseudodispar* it is only 1/5th the length of the hind wing; also, the anterior margin of this black hind wing patch extends to 11 cells proximal to the Pt in *E. wayanadensis* **sp. nov.** whereas in *E. pseudodispar* it extends to only 4 cells proximal to the Pt. In males, unlike in *E. pseudodispar*, the humeral and antehumeral stripes are broader, never interrupted and not significantly taper towards the alar sinus. Unlike its congener, the humeral stripes are not terminating in a distinct spot. Also, S9 of *E. wayanadensis* **sp. nov.** in lateral view shows lesser curvature; broader on the lower area in comparison with *E. pseudodispar*. S10 distal margin wavy at mid-ventrum and apex of the dorsal keel on S10 stepped in lateral view. When compared to *E. pseudodispar*, the length of penile structure of *E. wayanadensis* **sp. nov.** after the setae was more, in dorsal view. Male genital vesicle longer than that of *E. pseudodispar* and its distal margin not oval in *E. wayanadensis* **sp. nov.** The *E. wayanadensis* **sp. nov.** is comparatively larger. The TL, AL, FL, & HL of the males are more in *E. wayanadensis* **sp. nov.** i.e., average (n=5), TL, AL, FL, HL of males is 51.8, 41.3, 36.1 and 33.4. In male *E. wayanadensis* **sp. nov.**, there is a pale azure blue pruinose stripe on anteclypeus in fresh specimens. In the case of females, the vulvar scale is longer when compared to *E. pseudodispar* and the TL, AL, FL and HL of the female is more

compared to *E. pseudodispar* i.e., average (n=3) TL, AL, FL and HL of female is 45.4, 35.1, 35.0 and 32.8.

In the field, the new taxon can be easily confused with the rarely co-occurring *E. dispar*; however, can be differentiated by the extensor surface of the fore-femora which is black and red, hind and mid femora red as in *E. fraseri*, however, with no yellow patch on legs as in *E. dispar* (in males). The most commonly co-occurring species *E. fraseri* males can be very easily differentiated in the field by the pale sky blue humeral and antehumeral stripes (yellowish-orange in *E. wayanadensis* **sp. nov.**) and the azure blue labrum (pale blue with a median black knob like marking in *E. wayanadensis* **sp. nov.**). *Euphaea wayanadensis* **sp. nov.** can be easily differentiated from *E. cardinalis* males, seen south of Palghat Gap, which also has an extended black hind wing patch (more extended than *E. wayanadensis* **sp. nov.**) by its pale blue labrum (reddish-ochreous in *E. cardinalis*) and shorter S9 in the former.

Revised key to males of the genus *Euphaea* Selys, 1840 of Western Ghats, Peninsular India

1. Ventral hair tufts present on male terminal abdominal segments; antehumeral stripes on thorax not azure blue; lateral aspect of middle lobe of prothorax with orange spots.....2
 - Ventral hair tufts absent on male terminal abdominal segments; antehumeral stripes bright azure blue and lateral aspect of the middle lobe of prothorax with an azure blue or pale blue spot (male genital vesicle red, longitudinally elongated, apple-shaped. TL 45.7± 2.10 mm).....*E. fraseri* (Laidlaw, 1920)
2. Labrum pale turquoise blue; antehumeral stripe yellowish.....3
 - Labrum orange-ochre to vermilion red; antehumeral stripe reddish-orange.....4
3. Extensor surface of all femora black, flexor surface of proximal 2/3rd, all femora with bright yellow patch; Fw tipped blackish-brown, apical fourth of Hw black; genae black. Male genital

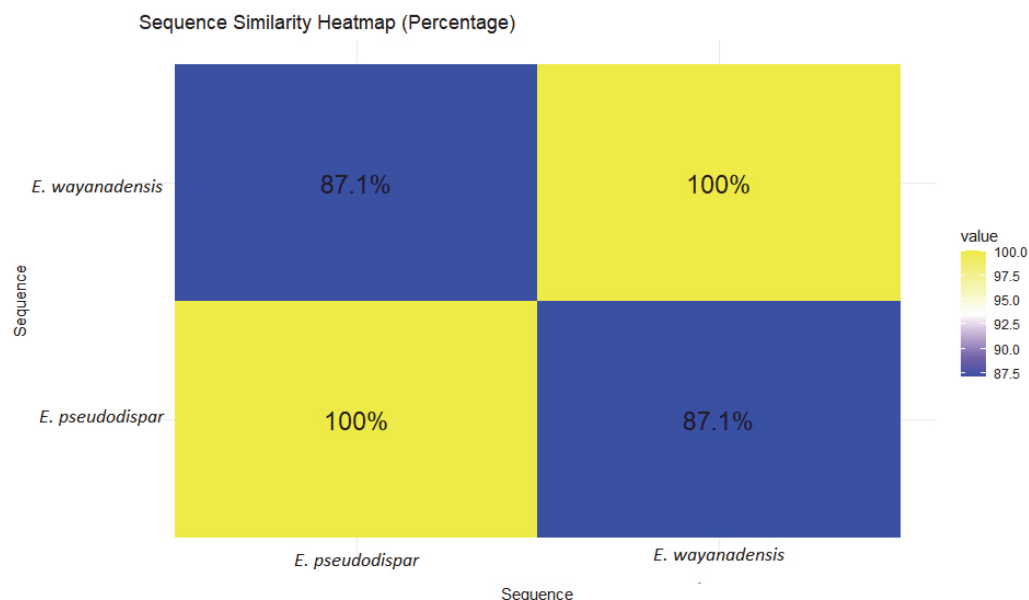


Fig. 5 Nucleotide sequence similarity heat map (percentage) for *Euphaea wayanadensis* sp. nov. and *E. pseudodispar* prepared using Ape package in R studio software and plotted using ggplot 2 package

vesicle black with distal border rounded and with fine transverse rugosities; penis with three pairs of setae; sternite of S9 not prominently extending ventrally. TL 53.25 ± 1.06 mm.....
.....*E. dispar* (Rambur, 1842)

– Extensor surface of all femora black, no yellow patch on legs.....5

4. Extensor and flexor surface of all femora bright red; apical half of Hw black; S8 and S9 with tufts of long ventral hairs; male genital vesicle black, hexagonal with rounded angles; S9 and S10 of equal length. TL 52.5 ± 0.70 mm.....
.....*E. cardinalis* (Fraser, 1924)

– Extensor and flexor surface of all femora black; apical fourth of Hw black; a tuft of sparse stub black hair on either side of tergite of S9; male genital vesicle matt black, with distal border rounded angles; S9 twice the length of S10. TL 50.6 ± 2.10 mm.....
.....*E. thosegharensis* Sadasivan & Bhakare, 2021

5. Humeral stripes narrow, starting well beyond the suture between the episternum-2 and infraepisternum-2 and thus close to the origin of

antehumeral stripe; antehumeral stripes narrow and significantly tapering towards the alar sinus; upper part of infraepisternum black with a small pale yellow spot, just below the origin of the humeral stripes in the episternum 2; HW black patch extends to 4–5 cells proximal to the Pt; node on either side of the midline keel on ventral beak of S9 sternite inconspicuous; apex of the dorsal keel on S10 not stepped in lateral view; penile structure short after the setae; male genital vesicle shorter with oval distal margin. TL 47.0 ± 0.70 mm
.....*E. pseudodispar* Sadasivan & Bhakare, 2021

– Humeral stripes broader starting at the suture between the episternum-2 and infraepisternum-2, well away from the origin of antehumeral stripe; antehumeral stripes broader and not tapering towards the alar sinus; upper part of infraepisternum-2 fully black and no pale yellow spot; HW black patch which extends to 10–11 cells proximal to the Pt; ventral beak of S9 sternite with a prominent node on either side of the midline keel; apex of the dorsal keel on S10 stepped in lateral view; penile structure longer after the setae; male genital vesicle longer with its distal margin not oval but nearly angular. TL 51.0 ± 0.70 mm.....
.....*E. wayanadensis* **sp. nov.**

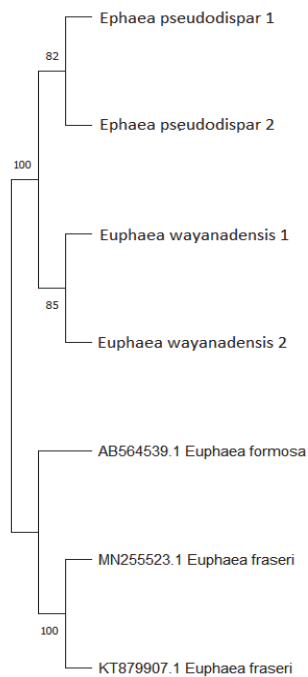


Fig. 6 Phylogenetic tree constructed using Maximum Likelihood method (MEGA 11) with 1000 bootstrap value

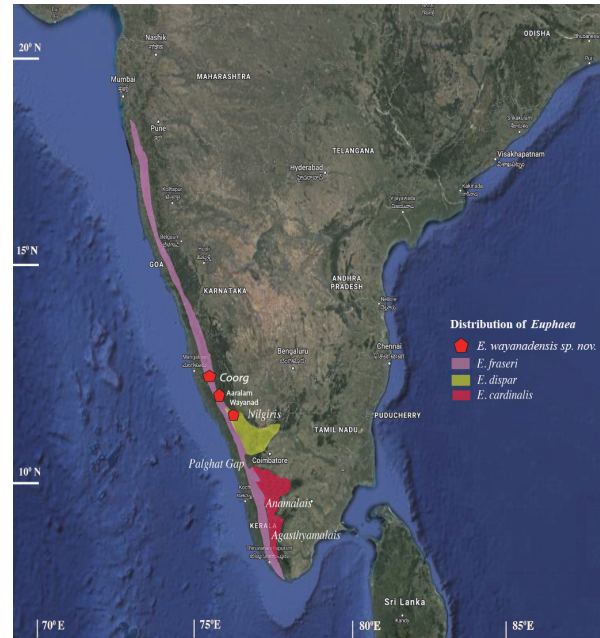


Fig. 7 Distribution map of *E. wayanadensis* sp. nov., *E. dispar*, *E. cardinalis* and *E. fraseri* in the Western Ghats

Molecular analysis

The sequences generated for *Euphaea wayanadensis* sp. nov. and *Euphaea*

pseudodispar and used in the nucleotide similarity study are presented below —

Euphaea wayanadensis sp. nov.:

TCTCCACCTCCTGCAGGGTCGAAGAATGAAGTGTTAATGTTTCGGTCTGTTAATAGTATT
GTAATTGCACCTGCTAATACAGGTAGTGATAAGAGTAAGAGTAGTGCTGTGATTACTACT
GCTCATACAAATAATGGTATTTGATCTATTTTTATTCTCGCGTTTTTATATTAATTGTT
GTTCTAATAAAGTTAATTGCTCCTAAAATTGACGATACACCTGCTAGGTGTAGTGAAAAA
ATAGTTAGATCTACTGATCCCCCGCATGTGCAATAGCTCCTGCTAATGGGGGGTATACT
GTTTCATCCTGTTCCCTGCTCCTCTTTCTACAAGTCTTCTTGTTAGGAGAAGTGTAATGGAA
GGGGGTAGTAATCAAAATCTTATATTATTAGTCGTGGGAATGCCATATCTGGGGCTCCT
AGTATTAATGGTACTAATCAGTTTCCGAATCCTCCAATTATAATAGGTATAACTATAAAG
AAAATCATTACGAATGCGTGTGCTGTGACAATCACATTATAAATTTGGTCATCTCCAATT
AATGATCCTGGTTGACCAATTCAATTGCAATTAATATTCTTAGGGCTGTTCTACTATTCTCTG

Euphaea pseudodispar:

TGAAGTGTTAATGTTTCGGTCTGTTAATAGTATTGTAATTGCACCTGCTAATACAGGTAG
TGATAAGAGTAAGAGTAGTGCTGTGATTACTACTGCTCATACAAATAATGGTATTTGATC
TATTTTTATTCTCGCGTTTTATATTAATTGTTGTTCTAATAAAGTTAATTGCTCCTAA
AATTGACGATACACCTGCTAGGTGTAGTGAAAAAATAGTTAGATCTACTGACCCCCCAGC
ATGTGCAATAGCTCCTGCTAGTGGGGGGTATACTGTTTCATCCTGTTCCCTGCTCCTCTTTC
TACAAGTCTTCTTGTTAGGAGAAGTGTAATGGAAGGGGGTAATAATCAAAATCTTATATT
ATTTAGTCGTGGGAATGCCATATCTGGGGCCCCCTAGTATTAATGGTACTAATCAGTTTCC
GAATCCTCCAATTATAATAGGTATAACTATAAAGAAAATCATTACGAAGGCGTGTGCTGT
GACAATCACATTATAAATTTGGTCATCTCCAATTAATGATCCTGGTTGACCC

Sequences compared using Ape package in R studio software showed 12.9 per cent difference between the two closely resembling species (Fig. 5). The phylogenetic analysis using sequence-based DNA barcode showed a significant correlation between morphological identification and molecular analysis. The phylogenetic analysis revealed that *E. wayanensis* **sp. nov.** formed a separate clade (Fig. 6). *Euphaea pseudodispar* was found to be the nearest associated species. *Euphaea fraseri* formed a separate clade, thus validating the distinct identity of the new species described here.

DISCUSSION

The new species *E. wayanadensis* **sp. nov.**, described here, superficially resembles *E. pseudodispar*, however, they are not sympatric, as per the observations. In the field, *E. wayanadensis* **sp. nov.** can be confused with the occasionally co-occurring *E. dispar*, but the former can be differentiated by the extensor surface of fore femora which is black and red hind and mid femora red as in *E. fraseri*, but with no yellow patch on legs as in *E. dispar* (in case of males). The commonly co-occurring *E. fraseri* males can be very easily differentiated in the field by the pale sky blue humeral and antehumeral stripes (yellowish-orange in *E. wayanadensis* **sp. nov.**) and the azure blue labrum (pale blue with median black knob like marking in *E. wayanadensis* **sp. nov.**). *Euphaea wayanadensis* **sp. nov.** can be easily differentiated from *E. cardinalis* males (seen south of Palghat Gap) which also has an extended black hind wing patch (more extended than *E. wayanadensis* **sp. nov.**), by its pale blue labrum (reddish-ochreous in *E. cardinalis*) and shorter S9 in the former.

Euphaea wayanadensis **sp. nov.** can be differentiated by the black patch on hind wing apices in males which covers up to 1/3rd of the hind wing, whereas in *E. pseudodispar* it is only 1/5th the length of the hind wing; also, the anterior margin of this black hind wing patch extends to 11 cells proximal to Pt in *E. wayanadensis* **sp. nov.** whereas in *E. pseudodispar*, it extends to only 4 cells proximal to the Pt. In males, unlike *E. pseudodispar*, the humeral and antihumeral stripes

are broader, never interrupted and do not significantly taper towards the alar sinus. Unlike its congener, the humeral stripes do not terminate within a distinct spot. Males of *E. wayanadensis* **sp. nov.** have a tuft of hairs on the base of the lateral border of the tergum of S8 and the proximal part of S9 unlike in *E. pseudodispar*. Also, S9 of *E. wayanadensis* **sp. nov.** in lateral view shows lesser curvature; broader on the lower area in comparison with *E. pseudodispar*. When compared to *E. pseudodispar*, the length of penile structure of *E. wayanadensis* **sp. nov.** after the setae is more in dorsal view. Male genital vesicle is longer than that of *E. pseudodispar* and its distal margin is not oval in *E. wayanadensis* **sp. nov.** and is comparatively a large species. In male *E. wayanadensis* **sp. nov.**, there is a pale azure blue pruinose stripe on anteclypeus in fresh specimens. In the case of females, the vulvar scale is longer when compared to *E. pseudodispar*. The molecular analysis reinforced the distinctiveness of *E. wayanadensis* **sp. nov.**

Muneer *et al.* (2022) reported the occurrence of *E. pseudodispar* at the banks of Kalindi River, from Thirunelli, Wayanad. The authors (CS, SSA, KS and VPN) had been studying odonates of Thirunelli and Wayanad since 2013 and had not come across *E. pseudodispar*, and all the '*pseudodispar*' morphotypes caught and examined turned out to be this new species, hence this is a case of misidentification. The mt CO1 gene had been used as a very reliable marker in DNA barcoding of Odonates. The analysis of nucleotide similarity and the phylogenetic tree clearly supports the distinctiveness of the new species discovered. *E. wayanadensis* **sp. nov.** has a very restricted distribution in the Wayanad region, though not uncommon further north on west-flowing rivers of Coorg landscape. Observations at other locations in Wayanad, namely Kalpetta (780m ASL), Kuruva Island (700m ASL) from 2013 to 2017 as a part of a different study by the same authors (Susanth and Anooj, 2020), failed to find this taxon. This could be attributed to the stream ecology and the riparian forest habitat of the new species. The closely resembling *E. pseudodispar* is reported to have similar habitat requirements as for the new species,

except for the slower speed of flow of the streams. The highly restricted distribution and habitat requirement of the new species highlights the need for targeted surveys and prioritizing the conservation of aquatic invertebrates of ecologically vulnerable landscapes like Wayanad.

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