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Describing ten new species of *Eutyphoeus* (Clitellata: Acanthodrilidae) from Manipur, North-East India, together with one new record

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Abstract. A comprehensive investigation into the genus *Eutyphoeus* (Clitellata: Acanthodrilidae) in Manipur, North-East India, has led to the discovery of ten novel species and the identification of one previously unrecorded species in the region. The study utilized an integrative taxonomic approach, combining both morphological and genetic analyses. Nine of the newly described species – *Eutyphoeus morehensis* Tiwari & Yadav sp. nov., *E. lamdengensis* Tiwari & Yadav sp. nov., *E. shiroensis* Tiwari & Yadav sp. nov., *E. kashongensis* Tiwari & Yadav sp. nov., *E. ukhrulensis* Tiwari & Yadav sp. nov., *E. lokchaensis* Tiwari & Yadav sp. nov., *E. baronongini* Tiwari & Yadav sp. nov., *E. yangoupokpensis* Tiwari & Yadav sp. nov. and *E. tengnoupalensis* Tiwari & Yadav sp. nov. – belong to the *E. hastatus* group, characterized by holandric male organs, bidiverticulate spermathecae, and annular tumescence around the male porophore. However, *E. kwathensis* Tiwari & Yadav sp. nov. was found to diverge from *E. turaensis*, and is diagnosed by metandric male organs, avestibulate and apenile male genital orifices. The study is based on extensive field surveys and the use of DNA barcoding. Each species is accompanied with morphological descriptions that emphasize distinctive features and geographical distribution. In addition, DNA barcodes were analyzed to confirm species identities and determination of genetic distinctiveness. The provided microphotographs of important morphological features complement the taxonomic descriptions. The study significantly enriches the understanding of the diversity of species of *Eutyphoeus* in Manipur, northeastern region of India, and underscores the importance of integrating molecular techniques in the taxonomy of earthworms and surveys on biodiversity.

Keywords. Biodiversity, conservation, DNA barcoding, earthworm diversity, new record, new species, taxonomy.

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Introduction

Manipur is a state located in the northeastern region of India, bordered to the north by Nagaland, to the east and south by Myanmar (Burma), and to the west and southwest by Assam and Mizoram, respectively. The name, Manipur, evokes its essence as the ‘land of gems’. It encompasses 22 327 square km with a diverse terrain, embracing the Manipur River valley and encircling mountain ranges. The climate exhibits a range of variations, providing moderate temperate in the valley and colder climates in the hills. The temperature in the area varies from 1–2°C to about 32–34°C. The rainfall is plentiful and provides an average of over 1650 mm annually (Lodrick & Standley 2024). Despite its ecological significance, the earthworm diversity of Manipur remains largely unexplored. In 2020, we documented only 13 species from the designated area in the checklist of earthworms (Tiwari *et al.* 2020). By 2023, this number had increased to 17 for the state (Thounaojam *et al.* 2020; Hijam *et al.* 2022; Tiwari *et al.* 2022; Narayanan *et al.* 2023). Further research by Tiwari *et al.* (2024, 2025a, 2025b) led to the identification and addition of 8 more species to the region’s biodiversity, bringing the total to 25 species in the region. *Eutyphoeus* Michaelsen, 1900 is a native genus of earthworms that belongs to the Acanthodrilidae Claus, 1880 and is an endemic to India. The Acanthodrilidae is widely distributed across the Indian Peninsula, and the genus *Eutyphoeus* maintains a significant place within it (Gates 1972). *Eutyphoeus* is thought to have evolved near the Indo-Burmese Mountain range. From this location, it dispersed across the Gangetic plain, extending into the Himalayas, and reaching the western and central regions of Burma to the edge of the Shan Plateau (Gates 1972). Consequently, *Eutyphoeus* harbors endemic species within the Northeastern ranges, Myanmar, the Eastern Himalayas, and the Indo-Gangetic plains (Julka 1988). Members of this genus are dispersed throughout India, Bangladesh, Pakistan, Myanmar, and Nepal (Gates 1958, 1972; Julka 1988; Blakemore 2006; Csuzdi *et al.* 2015; Tiwari *et al.* 2021).

The key diagnostic characteristics of the genus *Eutyphoeus* include lumbricine setae, paired male pores situated just behind the prostate pores on the setal arc of segment 17, and a single gizzard in the esophagus located between septa 5/6 and 8/9. Additionally, it features one pair of distinct intramural calciferous glands in segment 12, a simple lamelliform typhlosole, the presence of supra-intestinal glands, and meronephric nephridia positioned posterior to the supra-intestinal glands, with paired stomates and exonephric megameronephridia in each segment (Julka 1988).

There are 53 recognized species worldwide in the genus (Csuzdi 2012; Csuzdi *et al.* 2015; Tiwari *et al.* 2021; Ahmed *et al.* 2025). Of all the species documented, India is home to 29 (Tiwari *et al.* 2021; Ahmed *et al.* 2023, 2025; Rodingpuia *et al.* 2024). Among these species, only two (Tiwari *et al.* 2020) have been recorded in Manipur, i.e., *Eutyphoeus gammiei* (Beddard, 1888) and *Eutyphoeus manipurensis manipurensis* Stephenson, 1921. The study provides detailed morpho-anatomical descriptions and genomic signatures of ten unknown species, one previously unsequenced species and one new record belonging to the genus *Eutyphoeus* from this state.

Material and methods

Sampling

Earthworm specimens were collected through excavation and manual separation from various locations in Manipur, in the year 2017. The aforementioned locations encompass the protected forests of Yangoupokpi-Lokchao Wildlife Sanctuary, Kwatha Range and Tengnoupal, forest range of Lamdeng Makhaleikai, Shiroi National Park, Shirui Kashong (Peak), and the residential area of Churachandpur

(Fig. 1). After being collected, the samples were preserved for morphoanatomical and molecular investigations, using the established methods (Tiwari *et al.* 2021). Holotypes of the newly identified species were deposited at the Central Zone Regional Centre of the Zoological Survey of India (ZSI CZRC), located in Jabalpur, Madhya Pradesh, India.

Abbreviations used in figures

amp	=	ampulla
div	=	diverticulum
fp	=	female pore
gm	=	genital marking
gm gland	=	genital marking gland
lic	=	lateral intestinal caeca
mp	=	male pore
sp	=	spermathecal pore
i-xxx	=	Roman numerals indicating segments

Morpho-anatomical characterization

The morpho-anatomical characterization was performed by dissecting the specimens fixed in a formalin solution of 4% from their dorsal side. This process entailed employing a stereoscopic zoom microscope (Leica Model No. M60) and consulting pertinent literature sources, including Stephenson (1923), Gates (1958, 1972), Julka (1988), Julka *et al.* (2005), Tiwari *et al.* (2021), Ahmed *et al.* (2023) and Rodinguia *et al.* (2024).

DNA Barcoding & phylogenetic analysis

The caudal tissue samples preserved in absolute alcohol were sent to the Biodiversity Institute of Ontario, University of Guelph, Canada, for COI sequencing, barcode generation, and BIN assignment (Barcode Index Number), the latter serving as a globally unique identifier for sequence clusters of

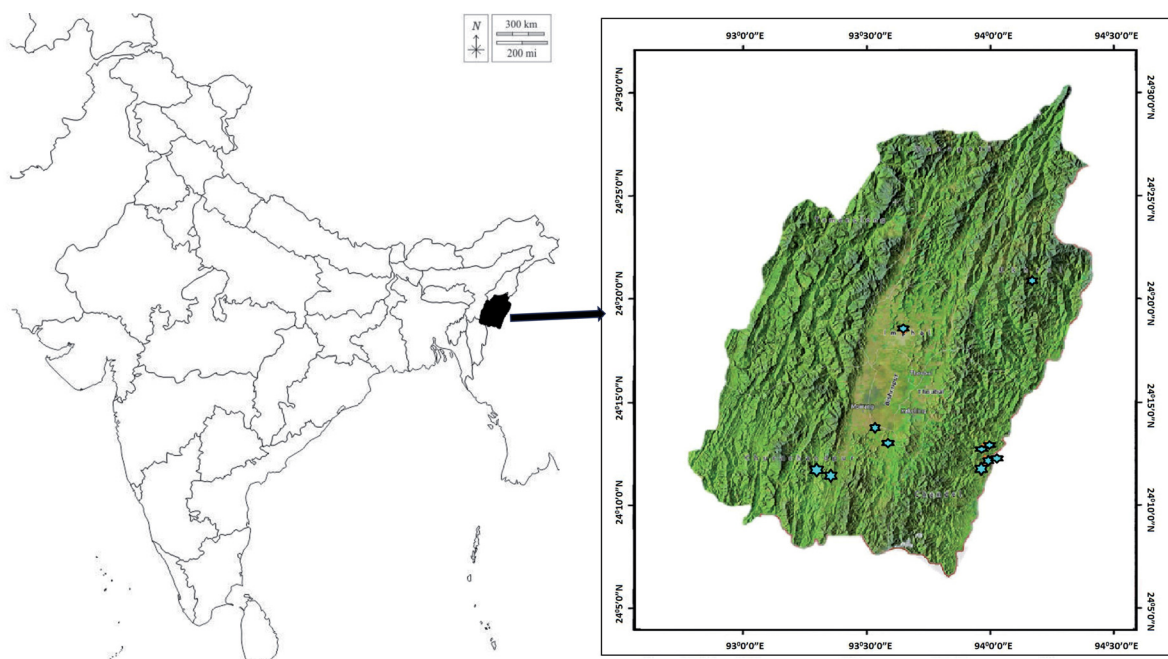


Fig. 1. Map showing sampling sites.

closely related species. Among the collected specimens, 68 were identified as *Eutyphoeus* spp. based on morphoanatomical characterization. For molecular characterization, sequences of *Eutyphoeus* spp. were retrieved from the Barcode of life Data System (BOLD; Ratnasingham & Hebert 2007), considering only published records with a detailed morphoanatomical characterization. The compiled dataset “DS-IEWMNPEU”, consisting of 139 COI sequences (from this study and retrieved data), is available on the BOLD website under the project “Diversity Studies in Earthworms of India” (IEW). Further molecular analysis was conducted using the “Barcode Gap Analysis” tools in BOLD, employing the K2P distance model to assess intra- and interspecific genetic distances, including the distance to the nearest neighbor species (Ratnasingham & Hebert 2007).

For the phylogenetic analysis, sequences were aligned using the MUSCLE algorithm (Edgar 2004) and subsequently trimmed to 584 bp in MEGA 11 (Tamura *et al.* 2021). The best-fit evolutionary model was determined using the “RUN MODELTEST” function in raxmlGUI 2.0, with the Bayesian Information Criterion (BIC) identifying the HKY+I+G4 model (BIC score: 13,658.2949, lnL: -5,934.1763). The Maximum Likelihood (ML) phylogenetic tree reconstruction was conducted with 1000 bootstrap replicates in raxmlGUI ver. 2.0 (Edler *et al.* 2021). The resulting phylogenetic trees were visualized and edited using iTOL ver. 5 (Interactive Tree of Life; Letunic & Bork 2021).

Results

Systematic account

Class Clitellata Michaelsen, 1919
 Subclass Oligochaeta Grube, 1850
 Order Crassiclitellata Jamieson, 1988
 Family Acanthodrilidae Claus, 1880
 Genus *Eutyphoeus* Michaelsen, 1900

Eutyphoeus morehensis Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:9E2C0A11-3E55-427D-B26D-0DD145BE44A9

Figs 2, 14; Tables 1–2

Diagnosis

Length 96–150 mm, diameter 3–4 mm, segments 190–210. Closed epilobitic prostomium. First dorsal pore in ix/x. Male genital orifices in xvii avestibulate, apenile. In intersegment 7/8, there is a single pair of spermathecal pores centred at *b*. Pair of presetal genital marking present ventrally in segments xiii at *a* on xvi paired, postsetal at *ab*. Septa 6/7/8 thickened. Lateral intestinal caeca in xxvii. Holandric, seminal vesicles in xii extends up to xiv. Three/four pairs of supra-intestinal glands. Prostate tubular, duct long muscular with one or two loops. Bidiverticulate spermathecae. Penial setae ornamented with circles of spine, shaft straight, tip bluntly rounded. Genital marking glands present.

Etymology

The epithet ‘*morehensis*’ refers to the name of Moreh, a small town located within the Tengnoupal district, which is significant as part of the species’ habitat. By incorporating ‘*morehensis*’ into its scientific name, this earthworm species pays homage to the geographical region where it was first discovered, highlighting the importance of local ecosystems in biodiversity conservation efforts.

Material examined

Holotype

INDIA – Manipur • clitellate (2–3 segments from the caudal region taken for DNA extraction); Kwatha Range, Yangoupokpi-Lokchao Wildlife Sanctuary; 24.3238° N, 94.2923° E; 9 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/6; MNP17-1065-43A5.

Paratypes

INDIA – **Manipur** • 2 clitellates; Tengnoupal, Yangoupokpi-Lokchao Wildlife Sanctuary; 24.3831° N, 94.1492° E; 10 Oct, 2017; Shweta Yadav leg.; MNP17-1061-43A1.

Other material

INDIA – **Manipur** • 6 specs; same data as for holotype; MNP17-1064-43A4, MNP17-1066-43A6, MNP17-1069-43A9, MNP17-1070-43A10, MNP17-1073-43A13, MNP17-1074-43A14 • 6 specs; same data as for holotype; MNP17-1097-44A23, MNP17-1098-44A24, MNP17-1099-44A25, MNP17-1101-44A27, MNP17-1102-44A28, MNP17-1103-44A29.

Description

Holotype

Length 150 mm, diameter 3.94 mm at preclitellar region. Total number of segments ca 205. Prostomium closed epilobic. Color chilean pink, uniformly pigmented. First dorsal pore starts from intersegmental furrow of 9/10. Setae start from segment ii, $aa:ab:bc:cd:dd = 2.62:1.05:2.34:1:10.63$ on xii, $aa:ab:bc:cd:dd = 3.03:1:1.93:1.29:9.47$ on xxiv and $dd < \text{half circumference of body}$. Clitellum annular, covering $\frac{1}{2}$ xiii– $\frac{1}{3}$ xvii. Spermathecal pore on intersegmental region of 7/8 centered at *b*, slit-like aperture, surrounded by swollen lips. Female pores in separate patch, paired, presetal at *a* on xiv. Male genital region appears as hemispherical, bowl-shaped structure covering segments 17 and 18, with narrow furrow separating them, avestibulate, apenile, on surface of body, male genital orifices discharge in transverse fissures on circular porophores surrounded with grooved rounded circle bounded with translucent margin, at *b*. Genital markings present on xvi paired, postsetal at *ab* with dark inner circle followed by whitish large swollen ring.

Septa 6/7/8 thickened present. Gizzard in viii. Pair of discrete intramural calciferous glands in xii. Intestine begins in xiv. Lateral intestinal caeca in xxvii. Typhlosole simple, lamelliform originated from xxviii. Ventral intestinal caeca 16–18, present in segments xxx–lvi. Supra-intestinal glands 4 pairs in segments lxxiv–lxxix. Last pair of hearts in xiii. Holandric, testis sac in x and xi, annular, seminal vesicles in ix and xii, posterior one extends up to xiv. Single pair of prostates extends from segments xvii to xix or xx. Each prostate has a long, muscular duct that opens into the body wall at segment xvii. Glandular part with one or two loops. One pair of spermathecae with two lateral diverticula, lobulated with 4–6 seminal chambers, which open separately into duct; ampulla oblate spheroid; duct slender, long, covered with bunch of nephridia. Penial setae two per battery, length 1.1 mm, width 0.036 mm, ornamented with circles of spine, shaft straight, tip bluntly rounded. Paired genital marking glands present on xvi, sessile.

Variation

Differences were noted in the number and position of the genital markings, especially in paratype MNP17-1061-43A1. The genital markings present in this paratype are on xiii, xiv, xv and xviii, paired, postsetal at *a* with 3 pairs of supra-intestinal glands in lxix–lxxiii.

Remarks

The novel species *Eutyphoeus morehensis* Tiwari & Yadav sp. nov. is classified within the *hastatus* group as proposed by Gates (1958). This group is characterized by holandric testes, a lumbricine arrangement of setae, a complete dorsal blood vessel, and paired female pores. Gates originally included four species in the *hastatus* group: *E. hastatus* Gates, 1929, *E. manipurensis manipurensis*, *E. marmoreus* Gates, 1933 and *E. bullatus* Gates, 1933. However, Gates (1958) limited this grouping to Burmese species of *Eutyphoeus*, while Indian species were placed in a separate category.

Subsequently, additional Indian species were discovered and incorporated into the *hastatus* group, including *E. mizoramensis* Julka, Ramanujam & Lalthanzara, 2005; *E. phawngpuiensis* Tiwari, Lone, Thakur, James & Yadav, 2021 and *E. tawi* Tiwari, Lone, Thakur, James & Yadav, 2021. Given this expanded classification, we propose that Indian species such as *E. incommodus* (Beddard, 1901),

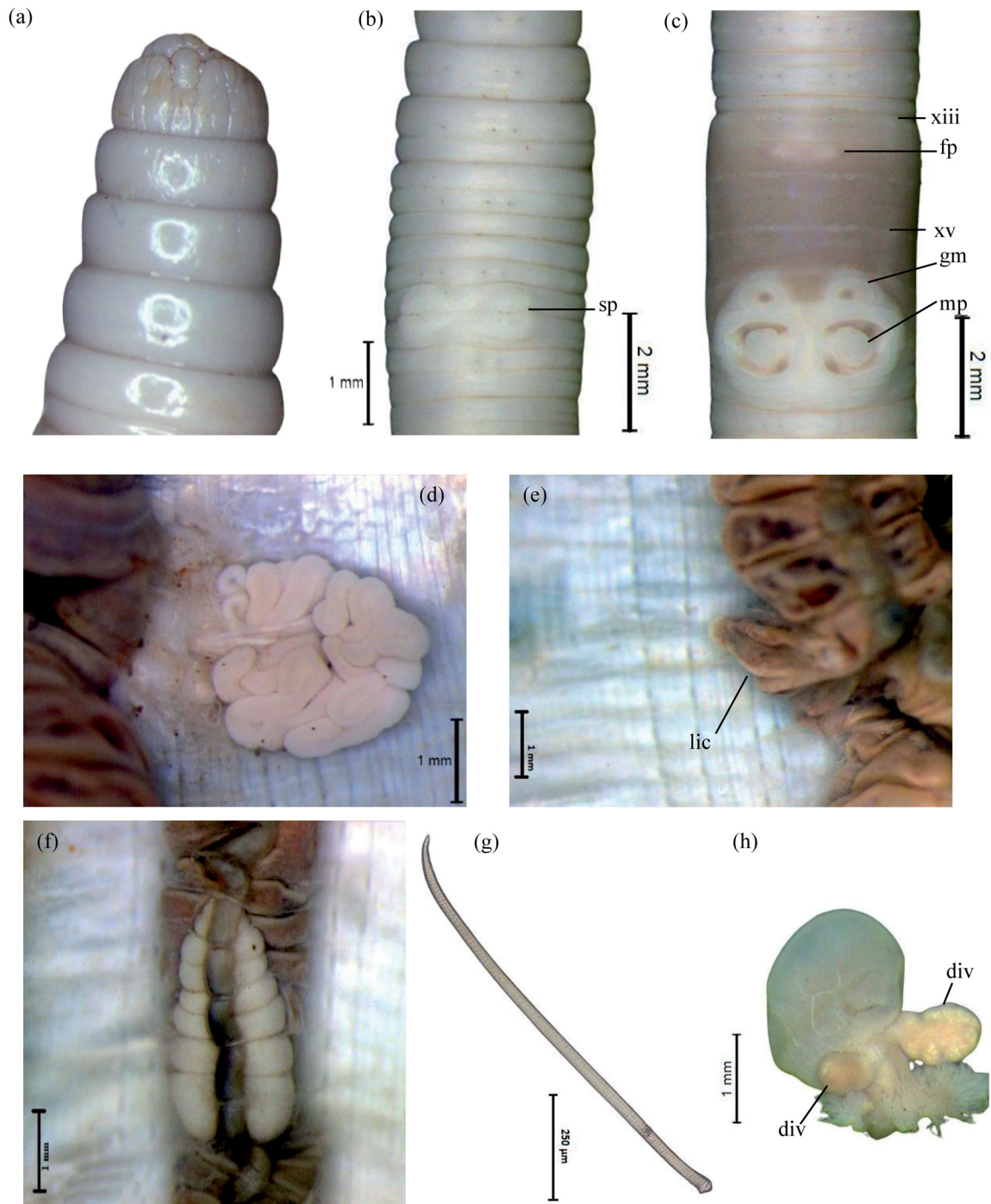


Fig. 2. *Eutyphoeus morehensis* Tiwari & Yadav sp. nov., holotype (MNP17-1065-43A5). **a.** Prostomium. **b.** Spermathecal pores. **c.** Clitellar region. **d.** Prostate. **e.** Lateral intestinal caeca. **f.** Supra-intestinal glands. **g.** Penial setae. **h.** Spermathecae. Abbreviations: see Material and methods.

E. annandalei Michaelsen, 1907, and *E. quadripapilatus* Michaelsen, 1907 should also be included in the *E. hastatus* group, as they share the characteristic holandric male organs and paired female pores.

The novel species is distinct from other species in the group by the position of its spermathecal pores, which are centered at *b*, a trait shared only with *E. tawi* and *E. marmoreus*. In contrast, the spermathecal pores of *E. manipurensis manipurensis*, *E. mizoramensis*, *E. quadripapilatus*, and *E. phawngpuiensis* are located at *a* or *ab*, while those of *E. annandalei* and *E. incommodus* are positioned along the setal line of *bc*, and *E. hastatus* has at *c*.

Additionally, *E. tawi* and *E. marmoreus* are characterized by vestibulate male genital orifices, whereas the novel species is distinguished by its avestibulate male genital orifices (Table 1).

DNA barcode

The COI barcode data for the holotype, with process ID IEW1019-17, has been assigned the BIN number BOLD: ADL1325. For the species *Eutyphoeus morehensis* sp. nov, the mean intraspecific divergence is 1.17%, with a maximum intraspecific divergence of 2.23%. Its nearest neighbor species is *Eutyphoeus lamdengensis* sp. nov. with a nearest neighbor distance of 10.6% (Table 2). All paratypes and syntypes of the novel species share the same BIN number, BOLD: ADL1325, without any BIN discordance.

Eutyphoeus lamdengensis Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:885223D9-C855-4A6C-9768-892D6CA9B113

Figs 3, 14; Tables 1–2

Diagnosis

Length 67–80 mm, diameter 3.6–4 mm, segments 134–150. Prostomium tanylobic. First dorsal pore in 10/11. Male genital orifices in xvii avestibulate, apenile. Single pair of spermathecal pores in intersegment 7/8 large at *ab*. Female pores paired, presetal, slightly median to setae *a*. Pairs of genital markings usually on xvi, postsetal at *ab* sometimes on xi, on xviii single on left side, postsetal at *ab*; on xii paired, postsetal at *ab*. Septa 6/7/8 and 11/12 absent. Lateral intestinal caeca small in xxvii, ventrally directed. Holandric. 3–4 pairs of supra-intestinal glands. Prostate extends from xvii–xxi, duct looped twice. Bidiverticulate spermathecae. Penial setae ornamented on anterior end; tip pointed. Sessile genital marking gland present.

Etymology

The etymology of *Eutyphoeus lamdengensis* sp. nov. is intricately tied to the discovery of the species within the Lamdeng Makhaleikai forest range in Manipur, India. The specific epithet ‘*lamdengensis*’ is derived from ‘Lamdeng’, referring to the geographical location where the species was originally found. The inclusion of ‘*lamdengensis*’ into its scientific name is a tribute to the forest range where this earthworm species naturally resides.

Material examined

Holotype

INDIA – **Manipur** • clitellate (2–3 segments from the caudal region was taken for DNA extraction); Lamdeng Makhaleikai forest range; 24.8376° N, 93.874° E; elev. 844.306 m; 12 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/18; MNP17-1167-47A17.

Paratypes

INDIA – **Manipur** • 3 clitellates; same data as for holotype; MNP17-1158-47A7, MNP17-1166-47A16, MNP17-1168-47A18.

Description

Holotype

Length 67 mm, diameter 3.6 mm. Total number of segments 134. Prostomium tanylobic. Color wood brown uniformly pigmented. First dorsal pore starts from intersegmental furrow of 10/11. Setae small,

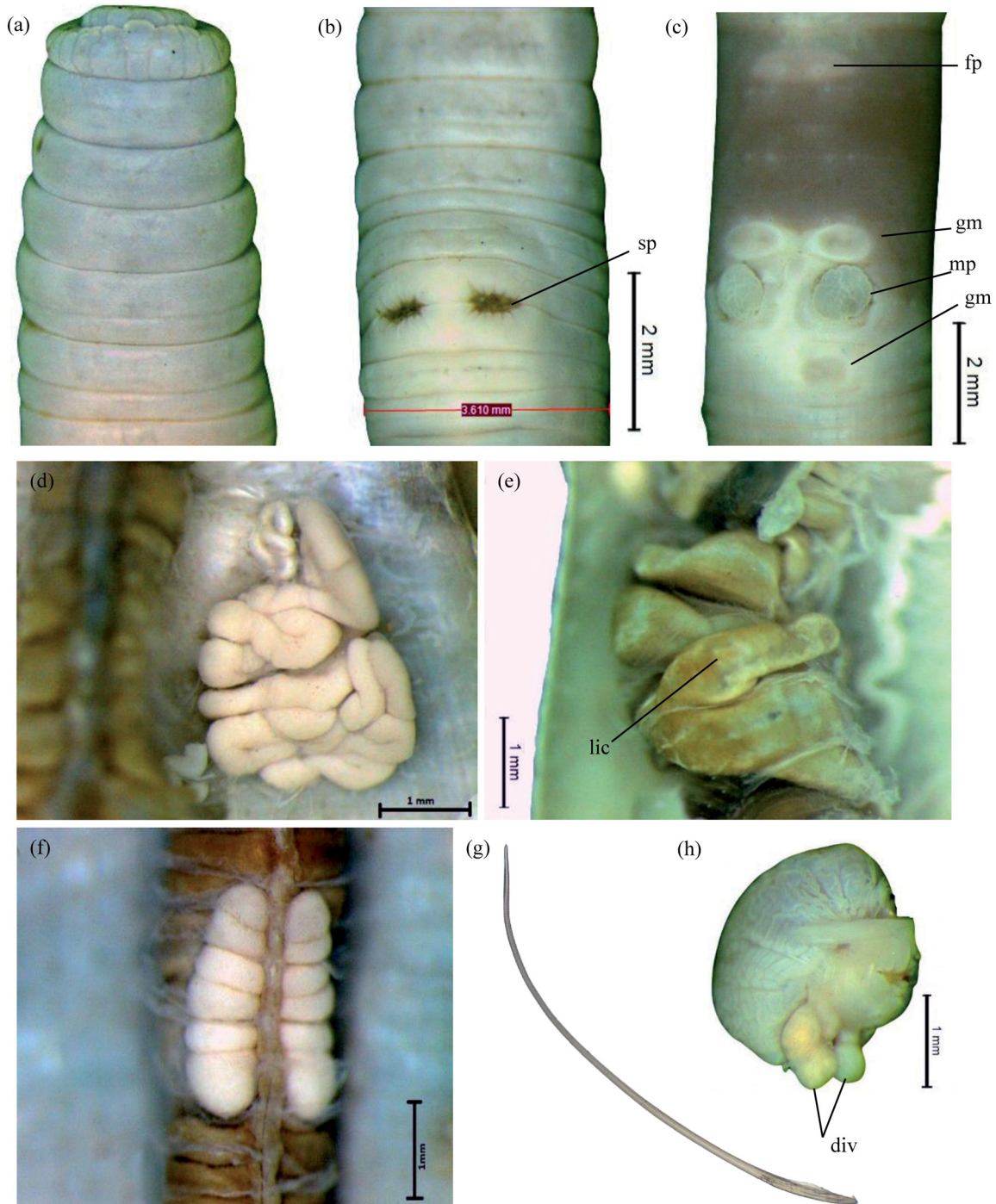


Fig. 3. *Eutyphoeus lamdengensis* Tiwari & Yadav sp. nov., holotype (MNP17-1167-47A17). **a.** Prostomium. **b.** Spermathecal pores. **c.** Clitellar region. **d.** Prostate. **e.** Lateral intestinal caeca. **f.** Supra-intestinal gland. **g.** Penial setae. **h.** Spermathecae. Abbreviations: see Material and methods.

$aa:ab:bc:cd:dd = 2.03:1:1.69:1.04:8.34$ on xii, $aa:ab:bc:cd:dd = 2.93:1:1.90:1.10:10.93$ on xxiv and $dd =$ half circumference. The spermathecal pores in intersegmental region of vii/viii, large at *ab*, the body wall around the pore fissured. Clitellum covers $\frac{2}{3}$ xiii– $\frac{1}{2}$ xvii. Female pores paired, presetal, on xiv, slightly median to setae *a* easily visualized in oval patch which extends between *bb*. Male genital orifices avestibulate and apenile, on anterior wall below the papillae on segment xvii at *b*, the paired male papillae oval, and slightly slantwise so that the anterior ends directed away from each other. Each papilla extends from just internal to *a*, nearly to *c*, posterior part of the marking without elevation. Genital markings transversely elliptical with fine whitish outer margin, on xvi, paired, postsetal at *ab*, on xviii single, circular marking on rightside, postsetal, at *ab*.

Septa 6/7/8 and 11/12 absent, 9/10 thickened. Gizzard in viii extends up to ix. One pair of intramural calciferous glands in xii. A large lymph gland present in xiii–xiv. Intestine begins from xv. Lateral intestinal caeca small in xxvii, ventrally directed. Typhlosole simple lamelliform, begins from xxvii. Nine to twelve ventral intestinal caeca begins from segment xxxiii–lvi. 3–4 pairs of supra-intestinal gland present in segments lxix–lxxiii. Last pair of hearts in xiii. Holandric male organ, seminal vesicles in ix and xii, posterior vesicles extend from xi to xii/xiii, testis sacs in x and xi, anterior one ventral while posterior one annular. Prostate extends from xvii–xxi, duct looped twice. Spermathecae bidiverticulate, lateral, diverticulum stout, club-shaped with 4–5 seminal chambers, ampulla dome-shaped, duct short and wide. Penial setae length 1.45–1.55 mm, width 0.030 mm, ornamented on anterior end with a series of transverse striations, tip pointed, 2 per battery. Paired genital marking glands present in xvi and xvii, sessile.

Variation

Variations were observed in the number and position of the genital marks. In paratype MNP 17-1166-47A16, a single genital marking present on xi, on the right side, between *ab*, on xii, paired, postsetal between *ab* and genital marking absent on segment xviii. In paratype MNP 17-1168-47A18, paired genital marking present on xi; in paratype MNP17-1158-47A7, paired genital markings present on xviii and only one in xix on right side, between *ab*. In paratype MNP17-1158-47A7, first dorsal pore in 9/10.

Remarks

The novel species *Eutyphoeus lamdengensis* sp. nov., characterized by avestibulate male genital orifices, holandric testes, paired spermathecal pores in segments vii/viii at *ab*, and a complete dorsal blood vessel, closely resembles *E. phawngpuiensis* and is accordingly placed within the *hastatus* group (Gates 1958). However, it is distinguished by its unique arrangement of genital markings, ventrally oriented lateral intestinal caeca, and the presence of genital marking glands (Table 1).

DNA barcode

The COI barcode data for the holotype (process ID IEW1121-17) and one paratype (MNP17-1158-47A7, process ID IEW1112-17) has been assigned the BIN number BOLD: ADL1721. In contrast, the other two paratypes, MNP17-1166-47A16 (process ID IEW1120-17) and MNP17-1168-47A18 (process ID IEW1122-17), have been assigned a different BIN number, BOLD: ADL1570. This BIN discordance within the newly discovered species indicates a mean intraspecific divergence of 2.44% and a maximum intraspecific divergence of 3.28% for *Eutyphoeus lamdengensis* sp. nov. Its nearest neighbor species is *Eutyphoeus manipurensis manipurensis*, with a nearest neighbor distance of 10.01% (Table 2).

Eutyphoeus shiroensis Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:D7ACB23D-6818-4585-BD53-EF7C7DA1EFD6

Figs 4, 14; Tables 1–2

Diagnosis

Length 130 mm, diameter 4.7 mm, segments 125. First dorsal pore in 11/12. Male genital orifices in xvii avestibulate, apenile. One pair of spermathecal pores in intersegment 7/8 large at *ab*. Genital markings on xvi paired, postsetal at *ab*, also in xviii paired, postsetal at *a*. Septa 6/7/8/9 absent. Lateral intestinal caeca in xxviii. Holandric, seminal vesicles in xii segment extend up to xvii. 4 pairs of supra-intestinal glands. Prostate extends up to xxi, duct with one short coiled loop. Bidiverticulate spermathecae. Penial setae absent.

Etymology

The etymology of *Eutyphoeus shiroensis* Tiwari & Yadav sp. nov. is derived from its discovery in the Shiroi National Park, located in the northeastern state of Manipur, India. The specific epithet '*shiroensis*' is derived from 'Shiroi', the name of the national park. This terminology intends to emphasize the significance of protecting the distinct ecosystems present in the park and to emphasize the necessity of preserving biodiversity in Manipur's natural landscapes.

Material examined

Holotype

INDIA – Manipur • clitellate (2–3 segments from the tail region cut for DNA extraction); Shirui Kashong (Peak), Shiroi National Park; 25.1302° N, 94.4202° E; elev. 2106.19 m; 11 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/15; MNP17-1104-45A1.

Description

Holotype

Length 130 mm, diameter 4.7 mm. The total number of segments 125 (excluding tail region aborted for molecular analysis). Prostomium (?). Color coffee brown, uniformly pigmented. First dorsal pore starts from intersegmental furrow of 11/12. $aa:ab:bc:cd:dd = 2.04:1:2.04:1.07:10.24$ on xii, $aa:ab:bc:cd:dd = 3.51:1:1.74:1.83:12.64$ on xxiv. Clitellum pithos-shaped (terminals narrow and middle part enlarged in segment xv), covers four segments $\frac{1}{2}$ xiii– $\frac{1}{3}$ xvii. Spermathecal pores in 7/8, at *ab*, large slit-like, body wall around pore swollen, fissured and swelling of pores merged mid ventrally without any demarcation, spermatophore present. Female pore paired, presetal on xiv slightly median to *a*. Male genital region beyond clitellum, male genital orifices avestibulate and apenile, on xvii under a hoof-like callus papillae at *ab*, each papilla extends laterally beyond *ab*. Paired and postsetal genital markings on xvi (at *ab*) and xviii (at *a*).

Septa 6/7/8/9 absent, 9/10 thickened. Gizzard in viii extends up to ix. Intramural calciferous gland in xii. Intestine begins in xv. Lateral intestinal caeca in xxviii. Typhlosole begins in xxviii. Ventral intestinal caeca 8 in number begins in xxxi. Four supra-intestinal glands pairs in 74–79. Last pair of hearts in xiv. Holandric, testis sacs in x and xi, annular; seminal vesicles in ix and xii with distinct hexagonal chambers, anterior one large petal-like in shape, posterior one extended up to xvii. Prostate in xvii extends up to xxi, duct short with one coiled loop. Spermathecae bidiverticulate, median and lateral overlapping each other, each diverticulum with two seminal chambers, ampulla large, oval-shaped with bulbous outgrowth on latero-anterior end, while ventrally no demarcation of bulging, ductless. Penial setae absent. Paired genital marking glands present in xvii, sessile.

Remarks

The novel species *Eutyphoeus shiroensis* is characterized by the paired spermathecal pores in segments vii/viii at *ab*, avestibulate male genital orifices, and holandric testes and thus classified within the *hastatus* group (Gates 1958). It closely resembles *E. phawngpuiensis*, but is distinguished by the male genital orifices on xvii under hoof-like callus papillae at *ab*, each papilla extends laterally beyond *ab*, pithos-shaped clitellum, seminal vesicles with distinct hexagonal chambers in segments ix and xii, with the posterior vesicle extending to segment xvii, and the absence of penial setae (Table 1).

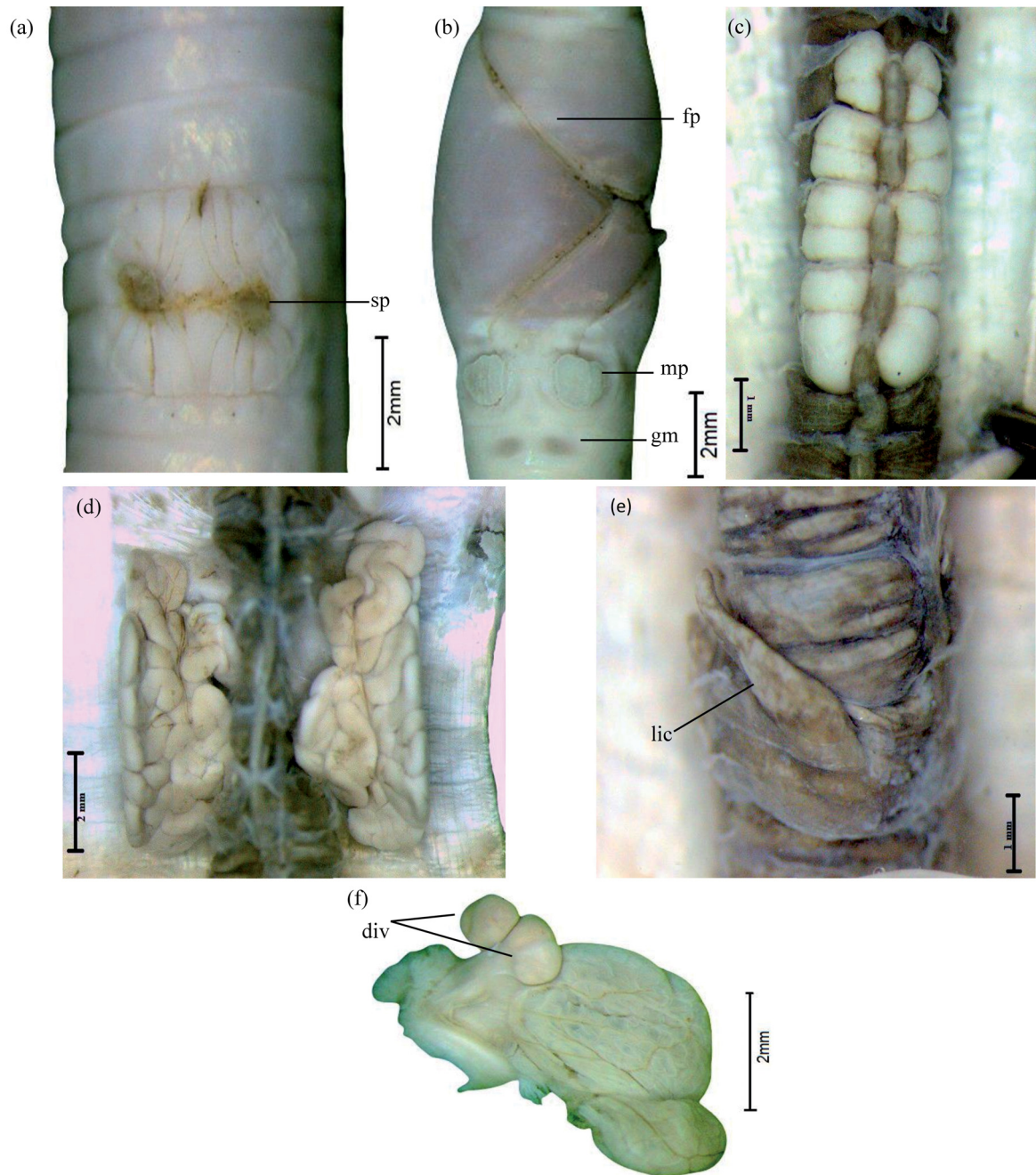


Fig. 4. *Eutyphoeus shiroensis* Tiwari & Yadav sp. nov., holotype (MNP17-1104-45A1). **a.** Spermathecal pore. **b.** Genital region. **c.** Supra-intestinal glands. **d.** Prostate. **e.** Lateral intestinal caeca. **f.** Spermathecae. Abbreviations: see Material and methods.

DNA barcode

The COI barcode data for the holotype MNP17-1104-45A1, with process ID IEW1058-17, has been assigned the BIN number BOLD: ADL1719. For the newly described species, *Eutyphoeus shiroensis* sp. nov., the nearest neighbor species is *Eutyphoeus tengnoupalensis* Tiwari & Yadav sp. nov., with a nearest neighbor genetic distance of 10.94% (Table 2).

Eutyphoeus kashongensis Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:32D476D3-EBB1-4691-B4AA-931469B01046

Figs 5, 14; Tables 1–2

Diagnosis

Length 75–124 mm, diameter 3–4 mm, segments 75–140. Prostomium tanylobic. First dorsal pore in 11/12. Male genital orifices avestibulate and apenile discharges under cuneate wedge-shaped papillae at *b*. One pair of spermathecal pores in intersegment 7/8 at *ab*. Genital marking paired, postsetal, annular at *b*, the inner circle dark pigmented, outer circle light in color extends up to *a* on xv and xvi. Septa 7/8 and 8/9 missing. Intestinal caeca small, in xxvii. Holandric. Three pairs of supra-intestinal glands. Prostate tubular, prostatic duct shining white, looped three times. Bidiverticulate spermathecae. Penial setae ornamented with longitudinal series of triangular spine on anterior end, tip blunt. Genital marking glands present.

Etymology

The etymology of *Eutyphoeus kashongensis* Tiwari & Yadav sp. nov. refers to the fact that it was discovered near the Kashong Peak, specifically in the neighborhood of Shirui Kashong (Peak) in Manipur, India. Its scientific name indicates the specific geographic origin in the area surrounding Kashong Peak, in order to highlight the necessity of identifying and protecting the biodiversity in the area surrounding Shirui Kashong (Peak). It also emphasizes the significance of conservation efforts in safeguarding the natural habitats of Manipur's diverse landscapes.

Material examined

Holotype

INDIA – **Manipur** • clitellate (few segments from caudal region taken for DNA extraction); Shirui Kashong (Peak), Shiroy National Park; 25.1302° N, 94.4202° E; elev. 2106.19 m; 11 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/14; MNP17-1110-45A7.

Paratypes

INDIA – **Manipur** • 3 clitellates; same data as for holotype; MNP17-1112-45A9, MNP17-1114-45A11, MNP17-1119-45A16.

Other material

INDIA – **Manipur** • 3 specs; same data as for holotype; MNP17-1113-45A10, MNP17-1129-45A27, MNP17-1134-45A32.

Description

Holotype

Length 115 mm, diameter 3.58 mm. Total number of segments 135. Prostomium tanylobic. Body coloration is non-uniform, with anterior 16 segments being dark wood brown, while posterior region is light cedar brown. First dorsal pore in 11/12. Setae begins from segment ii, $aa:ab:bc:cd:dd = 2:1:2.66:1.49:12.31$ on xii and $aa:ab:bc:cd:dd = 2.94:1:2.35:1.44:12.54$ on xxiv. Clitellum narrow with rounded ends at xvii somewhat alabastron pot-like, occupying $\frac{2}{3}$ xiii– $\frac{1}{2}$ xvii. Spermathecal pores at *ab* on intersegmental region of vii/viii, opens with a slit-like aperture on raised area amid fissured area.

Female pores paired on xiv, presetal, at *a* slightly towards median. Male genital region in hemispherical cauldron shape shallow vent, in $\frac{1}{2}$ xvi– $\frac{1}{2}$ xviii, male genital orifices avestibulate and apenile discharge under cuneate (wedge-shaped) papillae with fimbriated margin at the anterior wall on segment xvii at *b*,

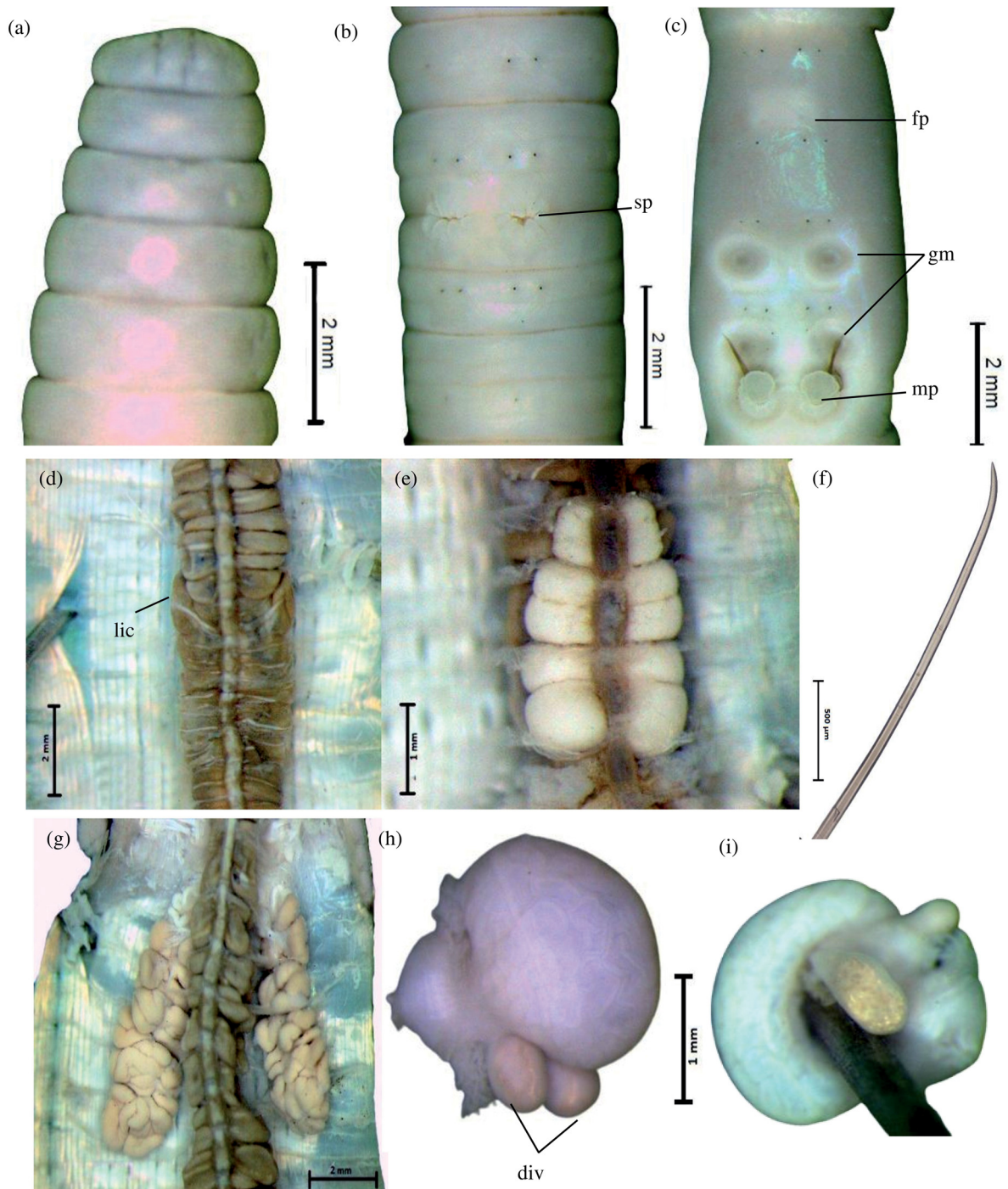


Fig. 5. *Eutyphoeus kashongensis* Tiwari & Yadav sp. nov., holotype (MNP17-1110-45A7). **a.** Prostomium. **b.** Spermathecal pore. **c.** Clitellar region. **d.** Lateral intestinal caeca. **e.** Supra-intestinal glands. **f.** Penial setae. **g.** Prostate. **h.** Spermathecae. **i.** Spermathecae highlighting seminal chambers in diverticulum. Abbreviations: see Material and methods.

each papilla extends from *a* to *b*. A pair of long (2.38 mm) penial setae emerge out from male genital orifices. Genital markings paired, postsetal, at *b*, the inner circle dark pigmented, outer circle light in color, extends up to *a* on xv and xvi.

Septa 4/5/6/7 thickened, 7/8 and 8/9 missing, 9/10 and 10/11 thickened and displaced backwards, 11/12 missing or represented by a ventral vestige. Gizzard in viii, dorsal blood vessel terminates anterior to gizzard in vi. Intramural calciferous gland present in xii, intestine begins in xv, last pair of hearts in xiii. Supra-intestinal gland 3 pairs in lxv–lxvii. Lateral intestinal caeca small in xxvii. Tufts of nephridia present in segment iii. Holandric, testis sacs in x and xi, male funnels usually rounded iridescent, seminal vesicles on ix and xii, second one extends up to xiv. Prostate extends from xvii–xxii. The prostatic duct shining white, looped three times. Spermathecae are bidiverticulate, with sausage-shaped diverticula. These diverticula exhibit a spermatozoal iridescence, confined to the terminal seminal chambers, duct short and wide, ampulla ellipsoid. Penial setae deeply embedded in the body wall, length 2.38 mm, width 0.031 mm, ornamentation with longitudinal series of triangular spine on anterior end, tip blunt, two per battery. Genital marking glands paired, sessile, spherical, swollen in xv and xvi.

Variations

The paratype MNP17-1114-45A11 exhibits a single, post-setal genital marking on xviii. Also in paratype MNP17-1112-45A9 in one spermatheca, one of the diverticula has two lobes which apprehends the presence of three diverticula.

Remarks

The species was categorized as part of the *hastatus* group (Gates 1958), which is characterized by a pair of spermathecal pores in vii/viii at *ab*, avestibulate male genital orifices and holandric testes. It seems to be close to *Eutyphoeus shiroensis* sp. nov. and distinguished from other species of the group by the presence of an alabastron pot clitellum. In addition, the male genital orifices discharges under cuneate wedge-shaped papillae with a fimbriated margin at *b* (Table 1).

DNA barcode

The COI barcode data for the holotype MNP17-1110-45A7 (process ID IEW1064-17) is assigned BIN BOLD: ADL1717. *Eutyphoeus kashongensis* sp. nov. has a mean intraspecific divergence of 0.07%, with a maximum of 0.15%. Its closest relative, *Eutyphoeus tengnoupalensis* sp. nov. has a nearest neighbor distance of 10.35% (Table 2). All specimens of this species share the same BIN.

Eutyphoeus ukhrulensis Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:A1F1C323-1F9A-43E2-B73F-ECA919C5F616

Figs 6, 14; Tables 1–2

Diagnosis

Length 82–120 mm, diameter 2.6–3.3 mm, segments 86–110. Prostomium combined pro-tanylobic. First dorsal pore in 10/11. Male genital orifices avestibulate, apenile, each male genital orifice distantly located on circular porophores centered at *b*, one pair of spermathecal pores in intersegment 7/8 at *ab*. Genital marking paired on xvi and xviii postsetal at *b*. Septa 6/7/8 thick. Intestinal caeca in xxvii, conical shape, ventrally directed. Holandric, seminal vesicle both present in ix and xii, posterior one extends up to xv. Three pairs of supra-intestinal glands. Prostate extends from xvii–xxi, prostatic duct long tubular with two loops. Bidiverticulate spermathecae. Penial setae straight except for little curvature to neck, ornamented with irregularly scattered large spines in anterior region, tip curved, pointed. Genital marking glands absent.

Etymology

Eutyphoeus ukhrulensis Tiwari & Yadav sp. nov. named after the Ukhrul district in the Shirui Kashong Peak area of Shirui National Park, Manipur, India.

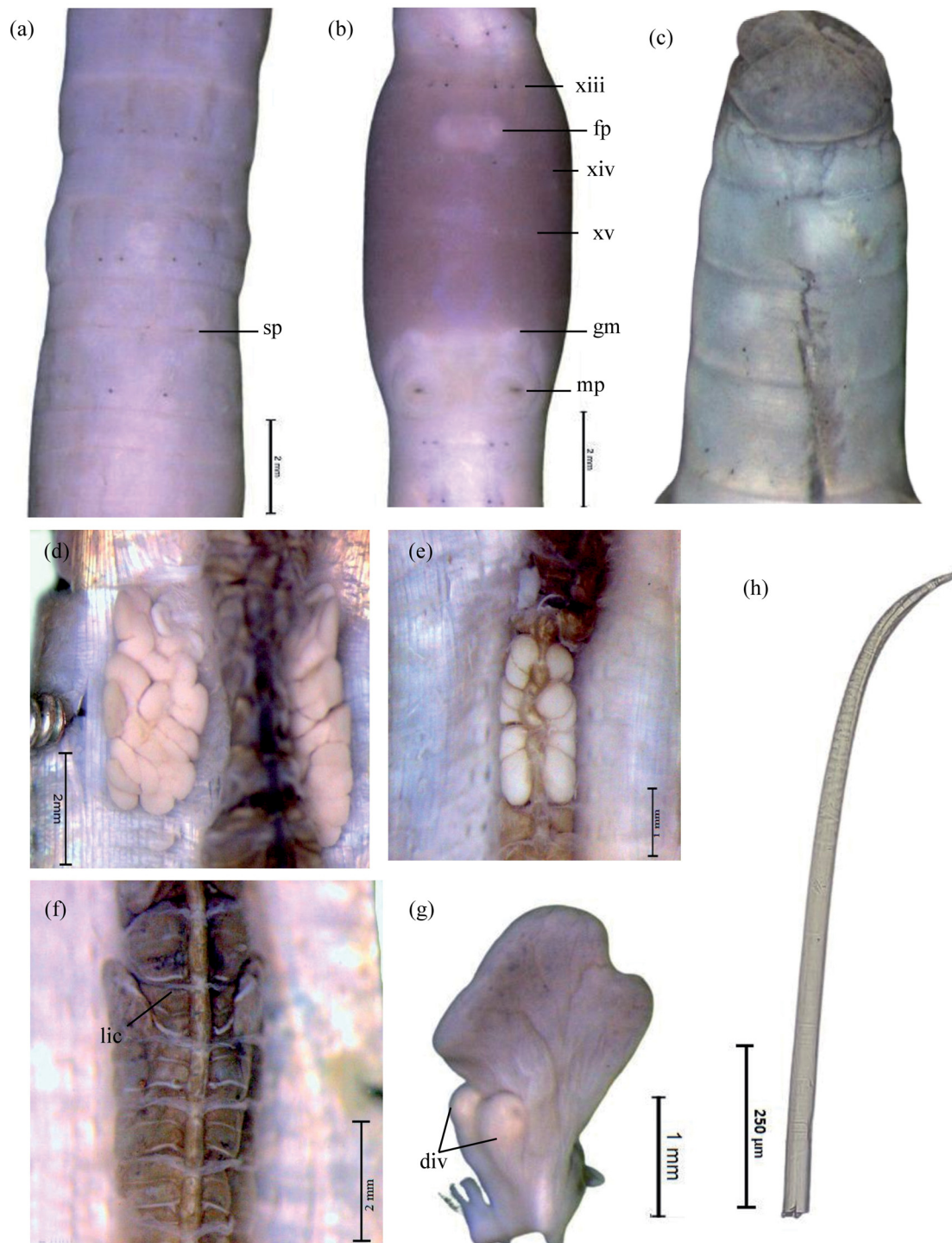


Fig. 6. *Eutyphoeus ukhrulensis* Tiwari & Yadav sp. nov., holotype (MNP17-1116-45A13). **a.** Spermathecal pore. **b.** Clitellar region. **c.** Prostomium. **d.** Prostate. **e.** Supra-intestinal glands. **f.** Lateral intestinal caeca. **g.** Spermathecae. **h.** Penial setae. Abbreviations: see Material and methods.

Material examined

Holotype

INDIA – **Manipur** • clitellate (2–3 segments from the tail region cut for DNA extraction); Shirui Kashong (Peak), Shiroy National Park; 25.1302° N, 94.4202° E; elev. 2106.19 m; 11 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/16; MNP17-1116-45A13.

Paratypes

INDIA – **Manipur** • 2 clitellates; same data as for holotype; MNP17-1120-45A17, MNP17-1131-45A29.

Description

Holotype

Length 82 mm, diameter 2.6 mm. Total number of segments ca 86. Prostomium combined protanylobic. Color is non-uniform, with anterior 16 segments being dark wood brown, while posterior region is pale brown. First dorsal pore starts from intersegmental furrow of 10/11. Setae closely paired, $aa:ab:bc:cd:dd = \text{on xii}$, $aa:ab:bc:cd:dd = \text{on xxiv}$. Clitellum olpe-shaped with narrow anterior and broad middle body, ranges from $\frac{1}{2}$ 13/17. Spermathecal pore slit-like aperture bounded with a little swollen lip at ab on 7/8. Female pore paired slightly median to a surrounded by separate circles, on segment xiv. Male genital region inverted hemispherical with flat base, avestibulate, apenile, each male genital orifice distantly located on circular porophores centered at b , bounded with thick circular swelling with fissured inner margin on segment xvii. Genital markings paired on xvi and xviii postsetal at b .

Septa 6/7/8 thick. Gizzard in segment viii–ix. Intestine starts from xv. Lateral intestinal caeca in xxvii, conical shape, ventrally directed. Typhlosole starts from xxvii. Ventral intestinal caeca 4–5 in xxxi–xxxvi. Three pairs of supra-intestinal glands in segment lxii–lxvi. Last pair of heart in xiii. Holandric male organ, testis sac in x and xi, annular, seminal vesicle both present in ix and xii, posterior one extends up to xv. Prostates occupy xvii–xxi, duct long tubular with two loops. Spermathecae bidiverticulate and club-shaped diverticula, one median and one lateral, each opening separately into the lumen of the duct, ampulla is relatively large and sac-like, while the duct is long and slender. Penial setae two per battery, length 1.2 mm, width 0.024 mm, straight except for little curvature to neck, ornamented with irregularly scattered large spines in anterior region, tip curved, pointed. Genital marking glands absent.

Remarks

Eutyphoeus ukhrulensis sp. nov. with diagnostic traits of the holandric male organ, spermathecal pores at ab and penial setae present closely resembles *Eutyphoeus kashongensis* sp. nov. However, it can be easily distinguished from *E. kashongensis* and other species of the group by the presence of male genital orifices on the circular porophores centered at b , genital marking glands absent and a club-shaped diverticulum (Table 1).

DNA barcode

The holotype MNP17-1116-45A13, with process ID IEW1070-17, has been assigned the BIN number BOLD: ADL1324 based on COI barcode data. *Eutyphoeus ukhrulensis* sp. nov. has a mean intraspecific divergence of 1.14 % and a maximum intraspecific divergence of 1.47%. Its nearest neighbor species is *Eutyphoeus tengnoupalensis* sp. nov. with a nearest neighbor distance of 9.64 (Table 2). All specimens of the novel species share the same BIN number.

Eutyphoeus lokchaensis Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:D33C4A10-69FD-4AEB-9CB9-3232DAE02B73

Figs 7, 14; Tables 1–2

Diagnosis

Length 125–185 mm, diameter 4.2–5 mm, segments 115–138. Prostomium tanylobic. First dorsal pore in 10/11. Male genital orifices bivestibulate, apenile, at *ab* left gonopore at *a* below the triangular, multi-fissured papillae. One pair of spermathecal pores in intersegment 7/8 at *b* or lateral to *b*. Genital markings post setal, median to *aa*, single in vii, viii, ix, x, xvi, presetal on xviii; postsetal, paired, at *ab* in vii, xvi; presetal, paired, at *ab* on viii, xviii; paired, postsetal, lateral to *b*. Septa all present. Intestinal caeca in xxvii, originated extremely lateral, ventrally directed. Holandric, seminal vesicle both present in ix and xii, posterior one extended up to xvi. Four-five pairs of supra-intestinal glands. Prostate extended up to xxii, duct very long, coiled, thick. Bidiverticulate spermathecae. Penial setae closely crowded circles of small fine triangular tooth-like ornamentation, slightly bent on anterior end, with blunt tip.

Etymology

The etymology of *Eutyphoeus lokchaensis* Tiwari & Yadav sp. nov. refers to its discovery location in the Yangoupokpi-Lokchao Wildlife Sanctuary, situated in Tengenoupal district, Manipur, India.

Material examined

Holotype

INDIA – **Manipur** • clitellate (few segments from caudal region taken for DNA extraction); Tengenoupal, Yangoupokpi-Lokchao Wildlife Sanctuary; 24.3831° N, 94.1492° E; 10 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/9; MNP17-1075-44A1.

Paratypes

INDIA – **Manipur** • 3 clitellates; same data as for holotype; MNP17-1075-44A, MNP17-1076-44A2, MNP17-1077-44A3.

Other material

INDIA – **Manipur** • 1 spec.; same data as for holotype; MNP17-1096-44A2.

Description

Holotype

Length 150 mm, diameter 4.2 mm. Total number of segments 136. Color cedar brown, uniformly pigmented. First dorsal pore starts from intersegmental furrow of 10/11. Prostomium tanylobic. Setae small, closely paired *aa:ab:bc:cd:dd* = 2.2 : 1 : 2.65 : 1.42 : 12.37 on xii, *aa:ab:bc:cd:dd* = 3.3 : 1 : 2.29 : 1.49 : 12.89 on xxiv. Clitellum annular, covering $\frac{1}{3}$ xiii to xvi segment. Spermathecal pore on intersegmental region of 7/8 at *b* or lateral to *b*, surrounded by fissured swollen lips with genital marking at lateral ends. Female pore on xiv, paired, presetal, slightly median to *a*, independent with or without common circle. Male genital region pentagonal in shape, male genital orifices bivestibulate, apenile, at *ab* left gonopore at *a* below triangular, multi-fissured papillae with narrow anterior end. Muscular composition of papilla compact. Genital markings post setal, median to *aa*, single in vii, viii, ix, x, xvi, presetal on xviii; postsetal, paired, at *ab* in vii, xvi; presetal, paired, at *ab* just below spermathecal pore on viii, xviii; paired, postsetal, lateral to *b* on surrounding surface of spermathecal pore. Median genital marking of xvi and xviii triangular in shape.

All septa present. Gizzard in viii segment. Intramural calciferous gland in xii. Intestine begins in xv. Lateral intestinal caeca in xxvii, originating extremely lateral, ventrally directed, conical with broad base

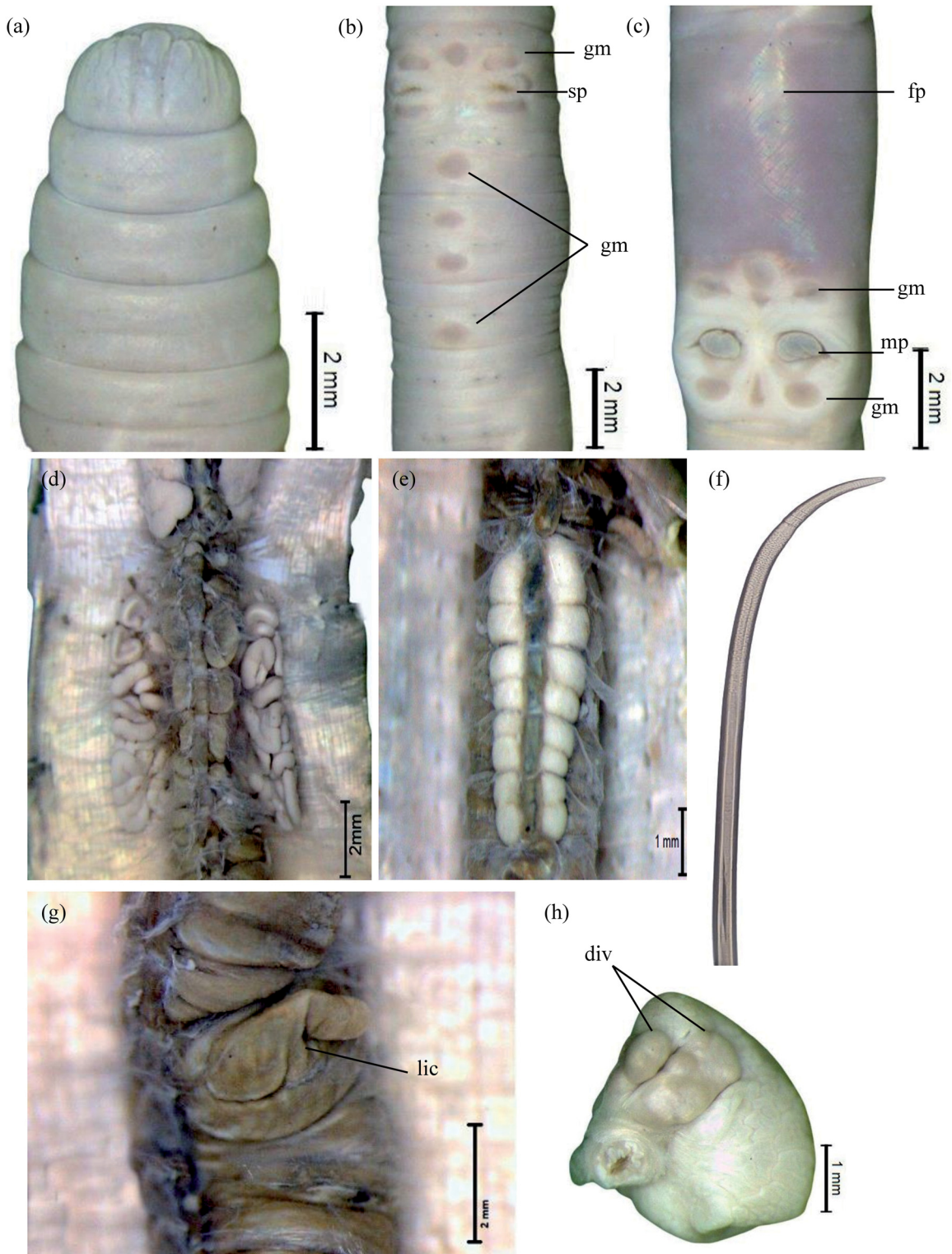


Fig. 7. *Eutyphoeus lokchaensis* Tiwari & Yadav sp. nov., holotype (MNP17-1075-44A1). **a.** Prostomium. **b.** Pre-clitellar region. **c.** Clitellar region. **d.** Prostate. **e.** Supra-intestinal glands. **f.** Penial setae. **g.** Lateral intestinal caeca. **h.** Spermathecae. Abbreviations: see Material and methods.

and rounded tip. Last pair of hearts in xiii. Ventral intestinal caeca 7 in number, begins from xxxi–xxxviii. Typhlosole begins from xxvii. Supra-intestinal glands 4–5 pairs in segment lxxiv–lxxix. Holandric, testis sac annular in x and xi, seminal vesicles in ix and xii, posterior one extends up to xvi. Prostate in xvii, extended up to xxii, duct very long, coiled, thick. Spermathecae bidiverticulate, lateral, upwardly directed, multilobulated with 4–5 seminal chambers, duct very short or ductless, sac-like large ampulla. Penial setae two per battery, length 1.75–1.86 mm, width 0.039 mm, with closely crowded circles of small fine triangular tooth-like ornamentation, slightly bent on anterior end with blunt tip.

Variation

Overall, no substantial variations were detected.

Remarks

The species is classified within the *hastatus* group (Gates 1958), and characterized by traits such as spermathecal pores positioned at *b* or lateral to *b*, bidiverticulate spermathecae, paired female pores, and holandric testes. It is distinguished by its vestibulated male genital orifices, a feature shared with *E. tawi* and *E. marmoreus*. However, the presence of lateral intestinal caeca sets it apart from *E. tawi*, while the presence of genital markings differentiates it from *E. marmoreus* (Table 1).

DNA barcode

The holotype MNP17-1075-44A1 (process ID IEW1029-17) has been assigned the BIN number BOLD: ADL0070 based on COI barcode data. *Eutyphoeus lokchaensis* sp. nov. has a mean intraspecific divergence of 0.08% and a maximum intraspecific divergence of 0.15%. Its nearest neighbor species is *Eutyphoeus lamdengensis* sp. nov. with a nearest neighbor distance of 10.37 (Table 2). All specimens of the novel species share the same BIN number.

Eutyphoeus barongini Tiwari & Yadav sp. nov.

urn:lsid:zoobank.org:act:8FFA26BA-5EAE-48F5-86F6-C169D6CE2D7C

Figs 8, 14; Tables 1–2

Diagnosis

Length 75–90 mm, diameter 4.2–5 mm, segments 110–156. Prostomium combined pro-tanylobic. First dorsal pore in 10/11. Male genital orifices bivestibulate, apenile, at *b* under the knob-like rounded papillae. One pair of spermathecal pores in intersegment 7/8 at *b* extends laterally. Female pore paired, presetal, median to *a*. Genital marking single median in xvii and xviii triangular in shape. Also, on xviii a single circular marking on right side, post setal, at *a*. Septa 6/7/8 absent. Intestinal caeca dorsally directed. Holandric, paired seminal vesicles in ix and xii, posterior one extends up to xvi. Four pairs of supra-intestinal glands. Prostate tubular, duct narrow, twisted takes one round before opening into the vestibule. Bidiverticulate spermathecae. Penial setae irregular triangular tooth-like ornamentation, slightly bent on anterior end, with blunt tip.

Etymology

Combining ‘baro’ (from ‘bar-tailed’) and ‘nongini’ to highlight the local name associated with the bird, tying the species to the regional identity. The etymology acknowledges that the Nongin Humes Bar-tailed Pheasant, the state bird, resides in the Shiroy National Park, which is the original location where this species was first identified.

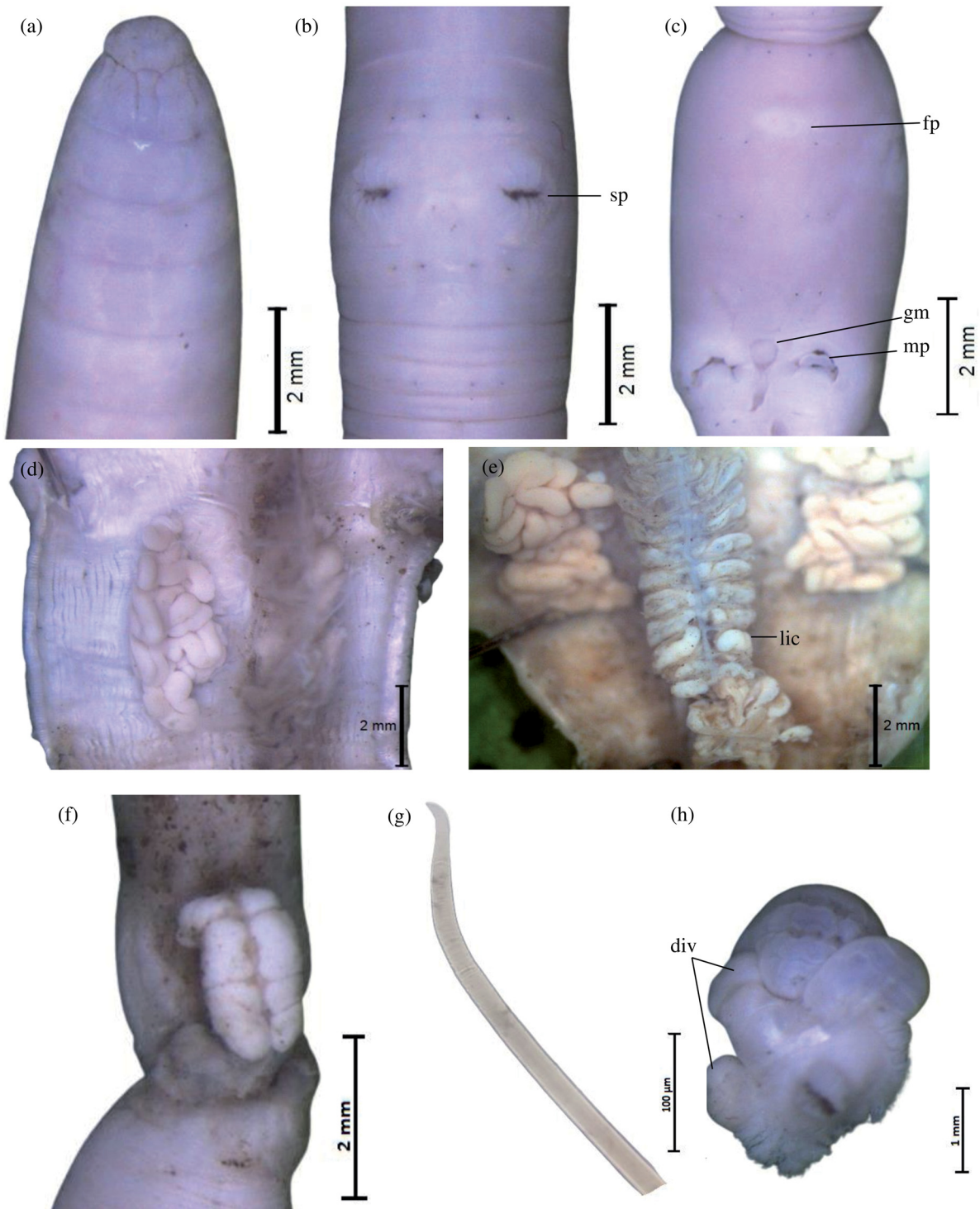


Fig. 8. *Eutyphoeus baronongini* Tiwari & Yadav sp. nov., holotype (MNP17-1105-45A2). **a.** Prostomium. **b.** Spermathecal pore. **c.** Clitellar region. **d.** Prostate. **e.** Lateral intestinal caeca. **f.** Supra-intestinal glands. **g.** Penial setae. **h.** Spermathecae. Abbreviations: see Material and methods.

Material examined

Holotype

INDIA – **Manipur** • clitellate (few segments from caudal region taken for DNA extraction); Shirui Kashong (Peak), Shiroi National Park; 25.1302° N, 94.4202° E; elev. 2106.19 m; 11 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/13; MNP17-1105-45A2.

Paratypes

INDIA – **Manipur** • 2 clitellates; same data as for holotype; MNP17-1106-45A3, MNP17-1115-45A12.

Other material

INDIA – **Manipur** • 7 specs; same data as for holotype; MNP17-1124-45A21, MNP17-1126-45A23, MNP17-1127-45A24, MNP17-1128-45A26, MNP17-1132-45A30, MNP17-1133-45A31, MNP17-1136-45A34.

Description

Holotype

Length 75 mm, diameter 4.2 mm. Total number of segments 110. Color is uniformly pigmented woody cedar brown, with prostomium being slightly darker. First dorsal pore starts from intersegmental furrow of 11/12. Prostomium combined pro-tanylobic. Setae small, $aa:ab:bc:cd:dd = 2.29:1.20:2.24:1:11.19$ on xii. Clitellum barrel-shaped posteriorly widened, occupying $\frac{2}{3}$ xiii to xvii segment. Spermathecal pore on intersegmental region of $\frac{7}{8}$ at *b* extends laterally, surrounded by fissured thick swollen lips. Female pore in xiv, paired, presetal, median to *a*, in common elliptical white circle. Male genital region bowl-shaped, male genital orifices bivestibulate, apenile, at *b* under knob-like rounded papillae. In one paratype, porophores protruded during preservation and hid the vestibule, gave impression of being avestibulate. Muscular composition of papillae tough. Genital marking single median in xvii and xviii triangular in shape. Also, on xviii single circular marking present on right side, post setal, at *a*.

Septa 6/7/8 absent. Gizzard in space between septa 5/6–8/9. Intramural calciferous gland in xii. Intestine begins in xv. Lateral intestinal caeca present on segment xxvii dorsally directed. Ventral intestinal caeca 13 in number, begins from xxxii–xlvi. Typhlosole (?). Supra-intestinal glands 4 pairs in segment lx–lxv. Last pair of hearts in xiii. Holandric, testis sacs annular in x and xi, seminal vesicles in ix and xii, posterior one extends up to xvi. Prostates in xvii, extends up to xx–xxi, duct narrow, twisted takes one round before opening into vestibulae. Spermathecae bidiverticulate, stout shape diverticulum with bud-like terminals with 4–5 seminal chamber, opens with common aperture in short and wide spermathecal duct, ampullae large sac-like ventrally lobulated, dorsally appears in one capsule. Penial setae two per battery, length 1.03 mm, width 0.029 mm, irregular triangular tooth-like ornamentation, slightly bent on anterior end, with blunt tip.

Variation

In paratype MNP17-1106-45A3, the porophores extended externally during preservation, concealing the vestibule, thus creating the impression of being avestibulate.

Remarks

The novel species *Eutyphoeus baronongini* sp. nov. is classified within the *hastatus* group (Gates 1958). It has similar traits as *Eutyphoeus lokchaensis* sp. nov., such as the spermathecal pores positioned at *b* or lateral to *b*, bidiverticulate spermathecae, paired female pores, and holandric testes. However, it could be differentiated by the absence of pre-clitellar genital markings (Table 1).

DNA barcode

The holotype MNP17-1105-45A2, designated process ID EW1059-17, has been assigned the BIN number BOLD: ADL1716 based on the COI barcode data. *Eutyphoeus baronongini* sp. nov. has a mean intraspecific divergence of 0.75 % and a maximum intraspecific divergence of 1.28%. Its nearest neighbor species is *Eutyphoeus tengnoupalensis* sp. nov. with a nearest neighbor distance of 10.58 (Table 2). All specimens of the novel species share the same BIN number.

Eutyphoeus yangoupokpensis Tiwari & Yadav sp. nov.
urn:lsid:zoobank.org:act:F689F1CE-6188-4B23-B5B6-4DEE427678DF
Figs 9, 14; Tables 1–2

Diagnosis

Length 60–80 mm, diameter 3.7–4 mm, segments 84–140. Prostomium tanylobic. First dorsal pore in 10/11. Male genital orifices bivestibulate, pentagonal vestibula, apenile, between *ab* just beneath the pear-shaped papillae. One pair of spermathecal pores in intersegment 7/8 at *b* encircled with 4–5 small circular genital markings. Genital markings paired on xvi post setal, at *b*, single median on xvii. Sometimes paired on xviii post setal, at *b* and absent on xvii. Septa 6/7/8/9 absent. Intestinal caeca in xxvi, small, ventrally directed. Holandric, seminal vesicles in ix and xii, anterior one with distinct hexagonal chambers, posterior one extends up to xvi. Three pairs of supra-intestinal glands. Prostate tubular, duct elongated, wide at posterior end. Bidiverticulate spermathecae. Penial setae ornamented with triangular teeth in transverse rows, tip blunt, slightly bent on anterior region. Genital marking glands present.

Etymology

The species epithet ‘*yangoupokpensis*’ is after the Yangoupokpi-Lokchao Wildlife Sanctuary in Tengnoupal, Manipur, where it was discovered.

Material examined

Holotype

INDIA – **Manipur** • clitellate (2–3 segments from caudal region taken for DNA extraction); Tengnoupal, Yangoupokpi-Lokchao Wildlife Sanctuary; 24.3831° N, 94.1492° E; 10 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/8; MNP17-1086-44A12.

Paratypes

INDIA – **Manipur** • 2 clitellates; same data as for holotype; MNP17-1085-44A11, MNP17-1089-44A15.

Description

Holotype

Length 80 mm, diameter 3.9 mm. Total number of segments 100. Color desert brown, uniformly pigmented. First dorsal pore starts from intersegmental furrow of 10/11. Prostomium tanylobic. $aa:ab:bc:cd:dd = 1.89:1:1.96:1.07:9.97$ on xii, $aa:ab:bc:cd:dd = 3.14:1:2.57:1.57:13.43$ on xxiv. Clitellum barrel-shaped, swelling of clitellum commenced from $\frac{1}{2}$ xiii and extends up to xvi. Spermathecal pores on intersegmental region of 7/8, at *b*, slit-like aperture, surrounded by 4–5 small circular genital markings arranged in ring. Female pores paired on segment xiv, presetal at *a*. Male genital region hemispherical bowl-like, male genital orifices bivestibulate, pentagonal vestibula, apenile, between *ab* just beneath pear-shaped papillae. Genital markings paired on xvi post setal, at *b*, single median on xvii and 4–5 circular marking encircles spermathecal pore in vii–viii.

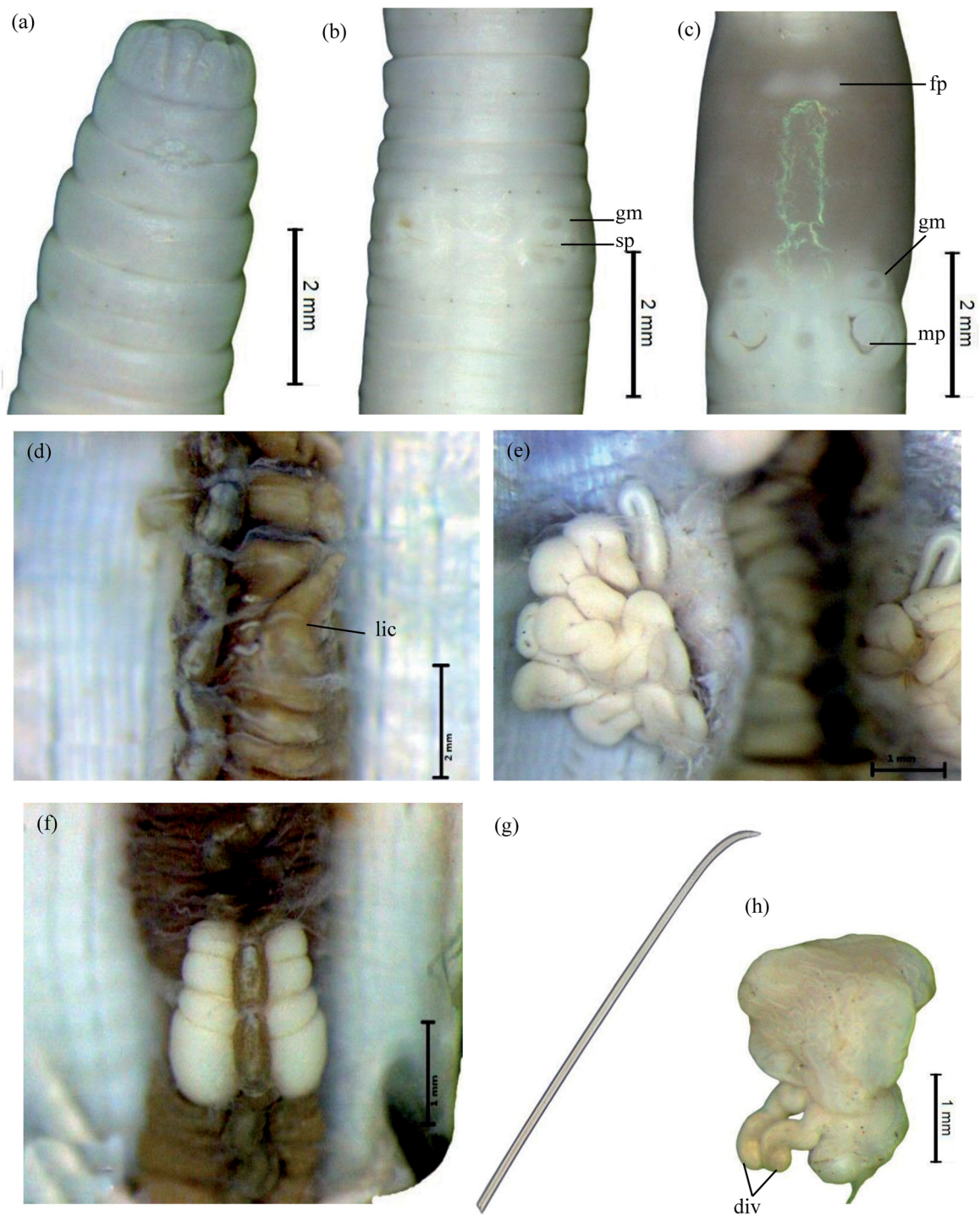


Fig. 9. *Eutyphoeus yangoupokpensis* Tiwari & Yadav sp. nov., holotype (MNP17-1086-44A12). **a.** Prostomium. **b.** Spermathecal pore. **c.** Clitellar region. **d.** Lateral intestinal caeca. **e.** prostate. **f.** Supra-intestinal glands. **g.** Penial setae. **h.** Spermathecae. Abbreviations: see Material and methods.

Table 1 (continued on next three pages). List of comparative characters of novel species and its closely related species.

Characters	<i>E. tawi</i>	<i>E. morehensis</i> sp. nov.	<i>E. lokchaensis</i> sp. nov.	<i>E. baronongini</i> sp. nov.	<i>E. yangoupokpensis</i> sp. nov.	<i>E. tengnoupalensis</i> sp. nov.	<i>E. phawngpuiensis</i>
Length (mm)	130–150	96–125	125–185	75–90	60–72	60–92	119–187
Diameter (mm)	5	3.94	4.2	4.2–5	3.7–4	3.7–4	4.9
Segments	100–110	190–210	115–138	110–156	84–140	121–156	130–160
Prostomium	Tanylobic	Closed epilobic	Tanylobic	Combined pro-tanylobic	Tanylobic	Tanylobic	Tanylobic
First dorsal pore	xi/xii	ix/x	x/xi	xi/xii	x/xi	x/xi	xi/xii
Clitellum	Annular xvii.	Annular, ½ xiii–½ xvii.	Annular, ½ xiii–xvi.	Barrel-shaped ⅔ xiii–xvii.	Barrel-shaped, ½ xiii–xvi	Annular, ½ xiii–½ xvii.	Annular, ½ xiii–½ xvii.
Spermathecal pore	vii/viii with transverse slit-like opening lateral to <i>b</i> .	vii/viii, centered at <i>b</i> , slit-like aperture, surrounded by swollen lips.	vii/viii at <i>b</i> or lateral to <i>b</i> , surrounded by fissured swollen lips.	vii/viii at <i>b</i> extends laterally, with fissured swollen lips.	vii–viii, at <i>b</i> , slit-like aperture, encircled with 4–5 small genital markings.	vii/viii, lateral to <i>b</i> , slit-like aperture with transverse slit-like aperture	vii/viii, at <i>ab</i> with transverse slit-like aperture
Female pore (on xiv)	Paired, minute, pre-setal at <i>a</i> .	In a separate patch, paired, pre-setal at <i>a</i> .	Paired, pre-setal, slightly median to <i>a</i> .	Paired, pre-setal, median to <i>a</i> .	Pre-setal at <i>a</i> .	Slightly median to <i>a</i> , paired, pre-setal.	Paired, pre-setal, slightly median to <i>a</i> .
Male genital region	Bivestibulate, male genital orifices present in a deep quadrangular depression/fissure.	Avestibulate, apenile, male genital orifices discharge on body surface in transverse fissures on circular porophores, at <i>b</i> .	Bivestibulate, apenile, at <i>ab</i> with multi- fissured papillae.	Bivestibulate, apenile, at <i>b</i> under the knob- like papillae.	Bivestibulate, pen- tagonal vestibula, apenile, between <i>ab</i> just beneath the pear- shaped papillae.	Bivestibulate, apenile, on xvii, lateral to <i>b</i> , under bilobed genital papillae.	Avestibulate, apenile, male genital orifices discharges through transverse aperture on porophores in xvii on setal arc of <i>b</i> or lateral to <i>b</i> .
Genital markings	Absent	Present	Present	Present	Present	Present	Present
Seminal vesicles	In ix and xii, those of xii extends up to xiv.	In ix and xii, those of xii extends up to xiv.	In ix and xii, those of xii extends up to xiv.	In ix and xii, those of xii extends up to xiv.	In ix and xii, those of xii extends up to xvi.	In ix and xii, those of xii extends up to xiv.	In ix and xii, those of xii extends up to xiv.
Prostate	xvii–xix.	xvii–xix or xx	xvii–xxii.	xvii–xx/xxi.	xvii–xxi.	xvii–xxi/xxii.	xvii–xxi.
LJC	Absent	In xxvii	In xxvii	Present (?)	In xxvi	In xxvi	In xxvii
VIC	6–7, xxxiii–li.	16–18, xxx–lvi.	7 xxxi–xxxxviii.	?	5 xxx–xxxv.	4–5 (?) from xxx	18 xxxiii–l

Table 1 (continued). List of comparative characters of novel species and its closely related species.

Characters	<i>E. lamdengensis</i> sp. nov.	<i>E. shiroensis</i> sp. nov.	<i>E. kashongensis</i> sp. nov.	<i>E. ukhrulensis</i> sp. nov.	<i>E. turaensis</i> sp. nov.	<i>E. kwathensis</i> sp. nov.
Length (mm)	50–94	130	83–114	82–120	100–177	63–80
Diameter (mm)	3.6	4.7	3.58	2.6–3.3	3.5	2.5–3
Segments	96–198	125	135–140	86–110	171–174	142–170
Prostomium	Tany lobic	?	tanylobic	combined pro-tanylobic	Pro or pro/tanylobic	Tanylobic
First dorsal pore	x/xi	xi/xii	x/xi, xi/xii	x/xi	xi/xii	xi/xii
Clitellum	Annular, $\frac{2}{3}$ xii– $\frac{1}{2}$ xvii.	Pithos-shaped $\frac{1}{2}$ xii– $\frac{1}{3}$ xvii.	Narrow body with a rounded end $\frac{2}{3}$ xiii– $\frac{1}{2}$ xvii.	Olpe-shaped from $\frac{1}{2}$ xii–xvii.	?	Annular $\frac{1}{2}$ xii– $\frac{1}{4}$ of xvii.
Spermathecal pore	vii/viii, at <i>ab</i> with fissured body wall around pore.	vii/viii at <i>ab</i> , large slit- like.	vii/viii, at <i>ab</i> with a slit- like aperture.	vii/viii, slit-like aperture bounded with a little swollen lip at <i>ab</i> on vii/ viii.	vii/viii, small, transverse slit, slightly lateral to <i>b</i>	vii/viii, lateral to <i>b</i>
Female pore (on xiv)	Paired, presetal, median to <i>a</i> .	Paired	Paired, presetal, median to <i>a</i> .	Paired median to <i>a</i> .	Paired, presetal at <i>a</i>	Paired, presetal slightly median to <i>a</i> .
Male genital region	Avestibulate and apenile, below the papilla on xvii at <i>b</i> .	Paired, to setae <i>a</i> . Avestibulate and apenile, on xvii under the hoof-like callus papillae at <i>ab</i> .	Avestibulate and apenile discharges under cuneate wedge-shaped papillae with fimbriated margin at the anterior wall on segment xvii at <i>b</i> .	Avestibulat, apenile, each male pore distantly located on circular porophores centered at <i>b</i> .	Avestibulate male genital orifices disch- arges on the body surface in slightly depression, penes annular.	Avestibulate, apenile, slit-like aperture at <i>ab</i> on segment xvii.
Genital markings	Present	Present	Present	Present	Present	Present
Seminal vesicles	In ix and xii, those of xii extends up to xi.	In ix and xii, those of xii extends up to xvii.	In ix and xii, those of xii extends up to xiv.	In ix and xii, those of xii extends up to xv.	One pair in xii, extends up to xiv.	One pair in segment xii.
Prostate	xvii–xxi.	xvii–xxi.	xvii–xxii.	xvii–xxi.	xviii–xix,	xvi–xx
LIC	In xxvii	In xxviii	In xxviii.	In xxvii	In xxviii	In xxvi
VIC	9 from xxxi	8 from xxxi	8 from xxxi–xxxix	4–5 xxxi–xxxvi	14–18 in segment xxxii–xlx	4–5 from segment xxxi

Table 1 (continued). List of comparative characters of novel species and its closely related species.

Characters	<i>E. tawi</i> sp. nov.	<i>E. morehensis</i> sp. nov.	<i>E. lokchaensis</i> sp. nov.	<i>E. baronongini</i> sp. nov.	<i>E. yangoupokpensis</i> sp. nov.	<i>E. tengnoupalensis</i> sp. nov.	<i>E. phawngpuiensis</i>
Typhlosole	lxxvii	xxviii	xxvii	?	xxvi	xxvi	xxvii
Supraintestinal glands (pairs)	4, lxiv–lxix	4, lxxiv–lxxix	4–5, lxxiv–lxxix	4, lx–lxv	3, xvii–lxx	3, lxv–lxx	4, lxxi–lxxx
Spermathecae (bidiverticulate)	Diverticula with bulbous terminal end, circular ampulla.	Diverticula lobulated with 4–6 seminal chambers, ampulla oblate spheroid, duct slender, long.	Diverticulum lateral with 4–5 seminal duct very short or ductless, ampulla large sac-like.	Diverticulum stout shape with 4–5 seminal chambers, ampullae large sac-like.	Diverticulum long tubular, with 5–6 seminal chambers, ampulla sac-like, duct separately into duct. short and wide	Diverticulum digitiform, ampulla sac-like flat, opens separately into duct.	Diverticulum multiloculate, ampulla sac-like, duct short and stout.
Penial setae (mm)	3.84 tip curved.	1.1 tip bluntly rounded.	1.75–1.86 tip blunt.	1.03 tip blunt.	1.2–1.4, tip blunt.	1.54 tip pointed.	1.30–2.16 tip pointed. 3–4 penial setae per battery.
Gm gland	Absent	Paired in xvi, sessile.	Absent	Absent	4–5 small spheroidal paired sessile in xvi.	5 spheroidal paired	Absent

Table 1 (continued). List of comparative characters of novel species and its closely related species.

Characters	<i>E. lamdengensis</i> sp. nov.	<i>E. shiroensis</i> sp. nov.	<i>E. kashongensis</i> sp. nov.	<i>E. ukhrulensis</i> sp. nov.	<i>E. turaensis</i>	<i>E. kwathensis</i> sp. nov.
Typhlosole	xxvii	xxviii	xxvi	xxvii	xxvii–xxviii	?
Supra-intestinal glands (pairs)	3–4, lxxix–lxxxiii	4, lxxiv–lxxxii	3, lxxv–lxxviii	3, lxxii–lxxvi	3–7, lxxv–lxxi	3, lxxii–lxxv
Spermathecae (bidiverticulate)	Diverticulum stout, ampulla dome-shaped, duct short.	Diverticulum with two seminal chambers, ampulla large, oval-shaped, ductless.	Diverticulum multi lobulate with 3–4 seminal chambers, ampulla ellipsoid, duct short and wide.	Diverticulum club-shaped, ampulla large sac-like.	Diverticulum short stalked ental, ampullae ovoid, duct long.	Diverticulum tubular with two seminal chambers, ampulla oval-shaped, duct short.
Penial setae (mm)	1.45–1.55 tip pointed, 2 per battery.	Absent	2 tip blunt, 2 per battery.	1.2 tip pointed.	1.5–1.8 tip bluntly pointed.	2.5–2.7 tip spoon-shaped with a small bulbous end.
Gm gland	Paired in xvi and xvii, sessile	Paired present in xvii, sessile	Paired, sessile, swollen in xv and xvi.	Absent	Absent	Absent

Septa 6/7/8/9 absent. Gizzard in viii. Intramural calciferous glands in xii. Intestine starts from xv. Lateral intestinal caeca in segment xxvi, small, ventrally directed. Typhlosole begins from xxvi. Ventral intestinal caeca 5 in number in segments xxx–xxxv. Supra-intestinal glands 3 pairs (first pair hidden underneath second one) in segment lxvii–lxx. Last pair of hearts in xiii. Holandric, testis sacs in x and xi, annular, seminal vesicles present in ix and xii, anterior one with distinct hexagonal chambers, posterior one extends up to xvi. Prostates in xvii–xxi, duct elongated, wide at posterior end. Penial setae two per battery, length 1.2–1.4 mm, width 0.037 mm, ornamented with triangular teeth in transverse rows, tip blunt, slightly bent on anterior region. Spermathecae bidiverticulate, one median and one lateral, diverticulum long tubular, helical, each diverticulum opens separately into duct with 5–6 seminal chambers, ampulla sac-like, duct short and wide. 4–5 small spheroidal genital marking glands present around spermathecal duct with one pair of sessile genital markings glands in xvi.

Variation

In paratype MNP17-1085-44A11, a paired genital marking present on xviii post setal, at *b* and absent on xvii.

Remarks

Eutyphoeus yangoupokpensis sp. nov., classified within the *hastatus* group (Gates 1958), is characterized by holandric testes, bidiverticulate spermathecae, and spermathecal pores positioned at *b*. The species is grouped with other vestibulated members of the *hastatus* group, including *E. tawi*, *E. marmoreus*, *E. lokchaensis* sp. nov. and *E. baronongini* sp. nov. However, it is distinguished by the presence of lateral intestinal caeca, genital markings, and genital marking glands surrounding the spermathecal duct in segment vii (Table 1).

DNA barcode

The holotype MNP17-1086-44A12 (process ID IEW1040-17) has been assigned the BIN number BOLD:ADL1713 based on COI barcode data. *Eutyphoeus yangoupokpensis* sp. nov. has a mean intraspecific divergence of 0.1% and a maximum intraspecific divergence of 0.15%. Its nearest neighbor species is *Eutyphoeus tengnoupalensis* sp. nov. with a nearest neighbor distance of 8.22 (Table 2). All specimens of the novel species share the same BIN number.

Eutyphoeus tengnoupalensis Tiwari & Yadav sp. nov.
 urn:lsid:zoobank.org:act:A2FB72E5-54DF-4C5B-92E6-FA997446C14E
 Figs 10, 14; Tables 1–2

Diagnosis

Length 60–92 mm, diameter 3.7–4 mm, segments 121–156. Prostomium tanylobic. First dorsal pore in 10/11. Male genital orifices bivestibulate, apenile, on xvii, lateral to *b*, under bilobed genital papillae. One pair of spermathecal pores in intersegment 7/8 lateral to *b* surrounded by 4–5 small circular genital markings. Genital markings on xvi and xvii paired, presetal at *b*, on xvii single, median, circular. On vii segment all postsetal, one median and 2 lateral to *b*. On viii segment presetal, one at *b* and 2 lateral to *b*. Septa 6/7/8 present. Intestinal caeca in xxvi, small oar-shaped, ventrally directed. Holandric, seminal vesicles present in ix and xii, those of xii extend up to xiii or xiv. Three pairs of supra-intestinal glands. Prostate tubular, duct small, twisted loop-like. Bidiverticulate spermathecae. Penial setae ornamented, hook-like, shaft with slight curvature of anterior region, pointed tip.

Etymology

The species name ‘*tengnoupalensis*’ is a Latinized adjective derived from ‘Tegnoupal’, the location where this species was discovered, specifically the Yangoupokpi-Lokchao Wildlife Sanctuary in Tegnoupal, Manipur.

Material examined

Holotype

INDIA – Manipur • clitellate (few segments from caudal region taken for DNA extraction); Tengnoupal, Yangoupokpi-Lokchao Wildlife Sanctuary; 24.3831° N, 94.1492° E; 10 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/12; MNP17-1079-44A5.

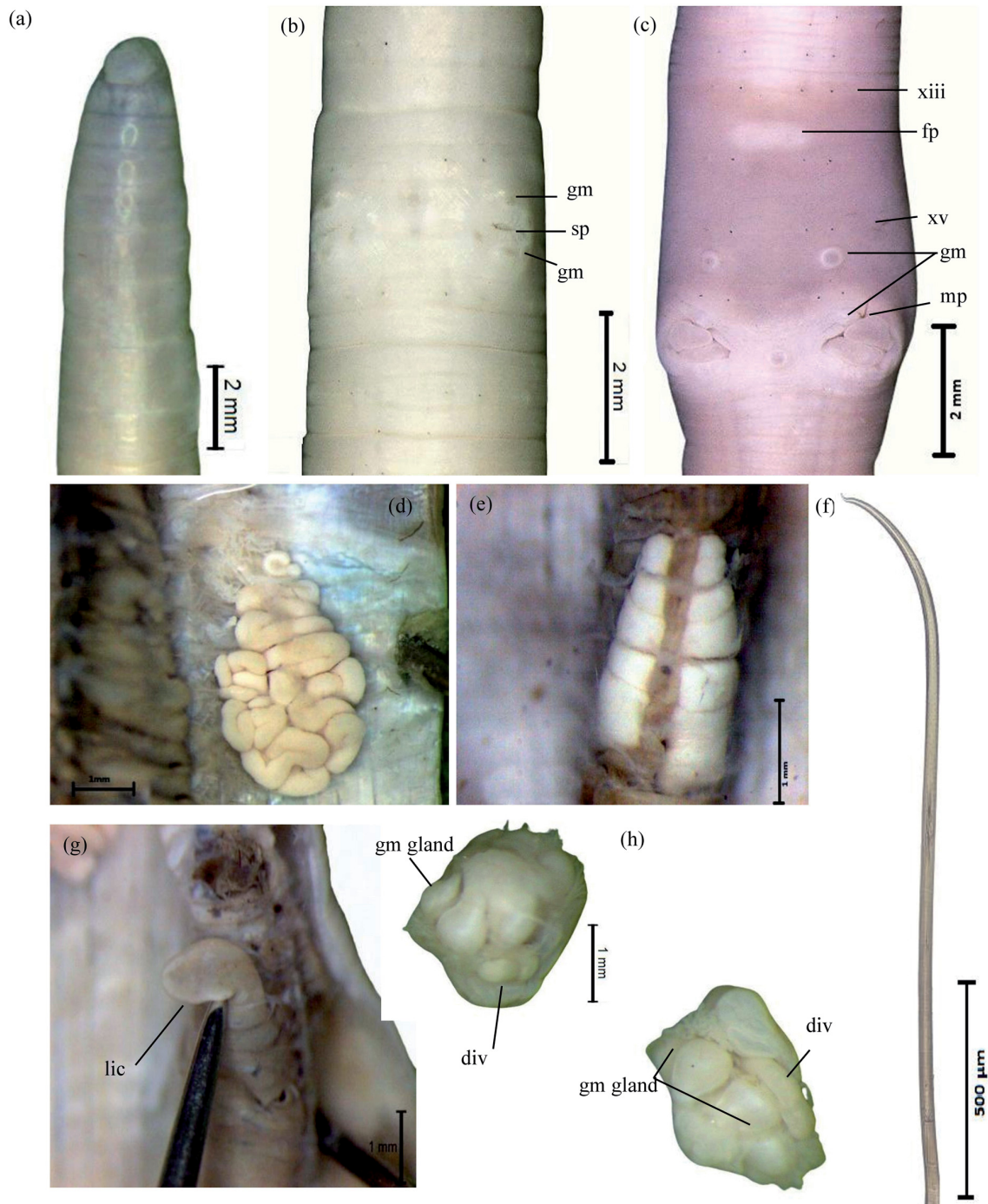


Fig. 10. *Eutyphoeus tengnoupalensis* Tiwari & Yadav sp. nov., holotype (MNP17-1079-44A5). a. Prostomium. b. Spermathecal pore. c. Clitellar region. d. Prostate. e. Supra-intestinal gland. f. Penial setae. g. Lateral intestinal caeca. h. Spermathecae. Abbreviations: see Material and methods.

Paratypes

INDIA – Manipur • 2 clitellates; same data as for holotype; MNP17-1079-44A5, MNP17-1080-44A6.

Other material

INDIA – Manipur • 2 specs; same data as for holotype; MNP17-1078-44A4, MNP17-1090-44A16.

Description

Holotype

Length 80 mm, diameter 3.77 mm. Total number of segments 128. Prostomium tanylobic. Color dark beige brown, uniformly pigmented throughout body, with only openings having a slightly lighter lining. First dorsal pore starts from intersegmental furrow of 10/11. $aa:ab:bc:cd:dd = 3.21:1.39:2.66:1:13.33$ on xii and $aa:ab:bc:cd:dd =$ on xxiv. Clitellum annular, slightly broadened in xvi–xvii, covers $\frac{1}{2}$ xiii to $\frac{1}{2}$ xvii. Spermathecal pores on intersegmental region of 7/8, lateral to *b*, slit-like aperture surrounded by 4–5 small circular genital markings. Female pore slightly median to *a*, paired, presetal on xiv in distinct white elongated patch with slight demarcation in centre. Male genital region dumbbell-shaped, male genital orifices bivestibulate, apenile, on xvii, lateral to *b*, under bilobed genital papillae (sharp demarcation present between two lobes of papillae). Genital markings on xvi and xvii paired, presetal at *b*, on xvii single, median, circular. On vii segment all postsetal, one median and two lateral to *b*. On viii segment presetal, one at *b* and two lateral to *b*.

Septa 6/7/8 present. Gizzard in segment viii. Intramural calciferous gland present in segment xii. Lateral intestinal caeca present in xxvi, small oar-shaped, ventrally directed. Ventral intestinal caeca 4–5 (?) in number from segment xxx, intestine broken. Typhlosole start from xxvi. Three pairs of supra-intestinal glands present in segments lxx–lxx. Last pair of hearts in xiii. Holandric, testis sacs in x and xi, annular, seminal vesicles in ix and xii those of xii extends up to xiii or xiv. Intestine begins from xiv. Prostates tubular, on segment xvii and extend up to xxi–xxii, duct small, twisted loop-like. Spermathecae bidiverticulate, lateral, 5 spheroidal genital markings attached in a ring-like manner directly to short wide duct, ampulla sac somewhat flat, diverticula digitiform, distantly situated in opposite direction, opens separately into duct. Penial setae two per battery, length 1.54 mm, width 0.036 mm, ornamented, hook-like, shaft with slight curvature of anterior region, pointed tip.

Variation

In paratype MNP17-1080-44A6, an additional pair of genital marking present on xv, postsetal at *b*.

Remarks

The novel species is classified within the *hastatus* group (Gates 1958) and appears to be diverged from *E. yangoupokpensis* sp. nov. due to its comparable spermathecal pore and arrangement of genital markings. It is characterized by its non-pentagonal shape of vestibula and penial setae with a pointed tip (Table 1).

DNA barcode

The COI barcode data of the holotype MNP17-1079-44A5 (process ID IEW1033-17), has been assigned the BIN number BOLD: ADL1715. *Eutyphoeus tengnoupalensis* sp. nov. has a mean intraspecific divergence of 0.38% and a maximum intraspecific divergence of 0.76%. Its nearest neighbor species is *Eutyphoeus yangoupokpensis* sp. nov. with a nearest neighbor distance of 8.22 (Table 2). All specimens of the novel species share the same BIN number.

Eutyphoeus kwathensis Tiwari & Yadav sp. nov

urn:lsid:zoobank.org:act:68895F78-62C2-4FF0-87B4-390D8548815E

Figs 11, 14; Tables 1–2

Diagnosis

Length 63–80 mm, diameter 2.5–3 mm, segments 142–170. Prostomium tanylobic. First dorsal pore in 11/12. Male genital orifices avestibulate, apenile, slit-like aperture at *ab*. One pair of spermathecal pores in intersegment 7/8 lateral to *b*. Genital markings with dark inner circle followed by fine translucent ring which is further encircled with whitish large swollen ring, all postsetal, on xi paired at *b* projected towards lateral, on xiv single on one side at *ab*, on xv, xvi paired at *ab*. Septa 5/6/7/8 present. Intestinal caeca simple, ventrally directed, on segment xxvi. Metandric male organs, testis sac annular on segment xi, seminal vesicle one pair in segment xii. Three pairs of supra-intestinal glands. Prostate extends from segment xvi to xx, duct looped twice or thrice. Bidiverticulate spermathecae. Penial setae serpentine, ornamented anteriorly with circle of strips, tip spoon-shaped with small bulbous end.

Etymology

The species epithet '*kwathensis*' denotes a species that is associated with the Kwatha range of Yangoupokpi-Lokchao Wildlife Sanctuary in Manipur, which is its type locality. This nomenclature not only aids in taxonomic classification but also acknowledges the sanctuary's role in preserving the wide range of biodiversity and facilitates scientific exploration.

Material examined

Holotype

INDIA – **Manipur** • clitellate (few caudal segments excised for DNA extraction); Yangoupokpi-Lokchao Wildlife Sanctuary; 24.2542° N, 94.2992° E; elev. 418.8 m; 8 Oct. 2017; Shweta Yadav leg.; registration number – ZSI CZRC T/5; MNP17-1044-42A3.

Paratypes

INDIA – **Manipur** • 2 clitellates; same data as for holotype; MNP17-1045-42A4, MNP17-1046-42A5.

Other material

INDIA – **Manipur** • 1 spec.; same data as for holotype; MNP17-1042-42A1 • 1 spec.; Kwatha range, Yangoupokpi-Lokchao Wildlife Sanctuary; 24.3238° N, 94.2923° E; 9 Oct. 2017; Shweta Yadav leg.; MNP17-1072-43A12.

Description

Holotype

Length 63 mm, diameter 2.6 mm. Total number of segments 142. Prostomium tanylobic. Color non uniform, anterior 4–5 segments rosy brown while, rest of body cinnamon brown. First dorsal pore starts from intersegmental furrow of 11/12. Setae small and setae *c* and *d* not easily observable, not viable to measure all of setal distance on segment xii and xxiv, thus distance *aa* : *ab* = 2.48 : 1 at xii and 2.81 : 1 at xxiv. Clitellum annular, extends from segment ½ xiii to ¼ xvii. Paired spermathecal pores on intersegmental region of 7/8, lateral to *b*. Female pores paired, presetal slightly median to *a* in separate patches on segment xiv. Male genital orifices avestibulate, apenile, slit-like aperture at *ab* on segment xvii. Genital markings with dark inner circle followed by fine translucent ring, further encircled with whitish large swollen ring, all postsetal, on xi paired at *b* projected towards lateral, on xiv single on one side at *ab*, on xv, xvi paired at *ab*.

Septa 5/6/7/8 present. Gizzard in segment viii occupies little vii and extends up to ix. One pair of intramural calciferous glands on segment xii. Intestine begins from segment xiv. Lateral intestinal caeca simple, ventrally directed, on segment xxvi. Ventral intestinal caeca 4–5 in number begins from segment

xxxi. Three pairs of supra-intestinal glands present in segments lxii–lxv. Last pair of hearts in xiii. Metandric, testis sacs annular on segment xi, seminal vesicle one pair in xii. Prostates extend from segment xvi to xx, duct looped twice or thrice. Spermathecae bidiverticulate, lateral, with two seminal chambers, tubular outwardly directed diverticula opens through common aperture into duct, ampulla

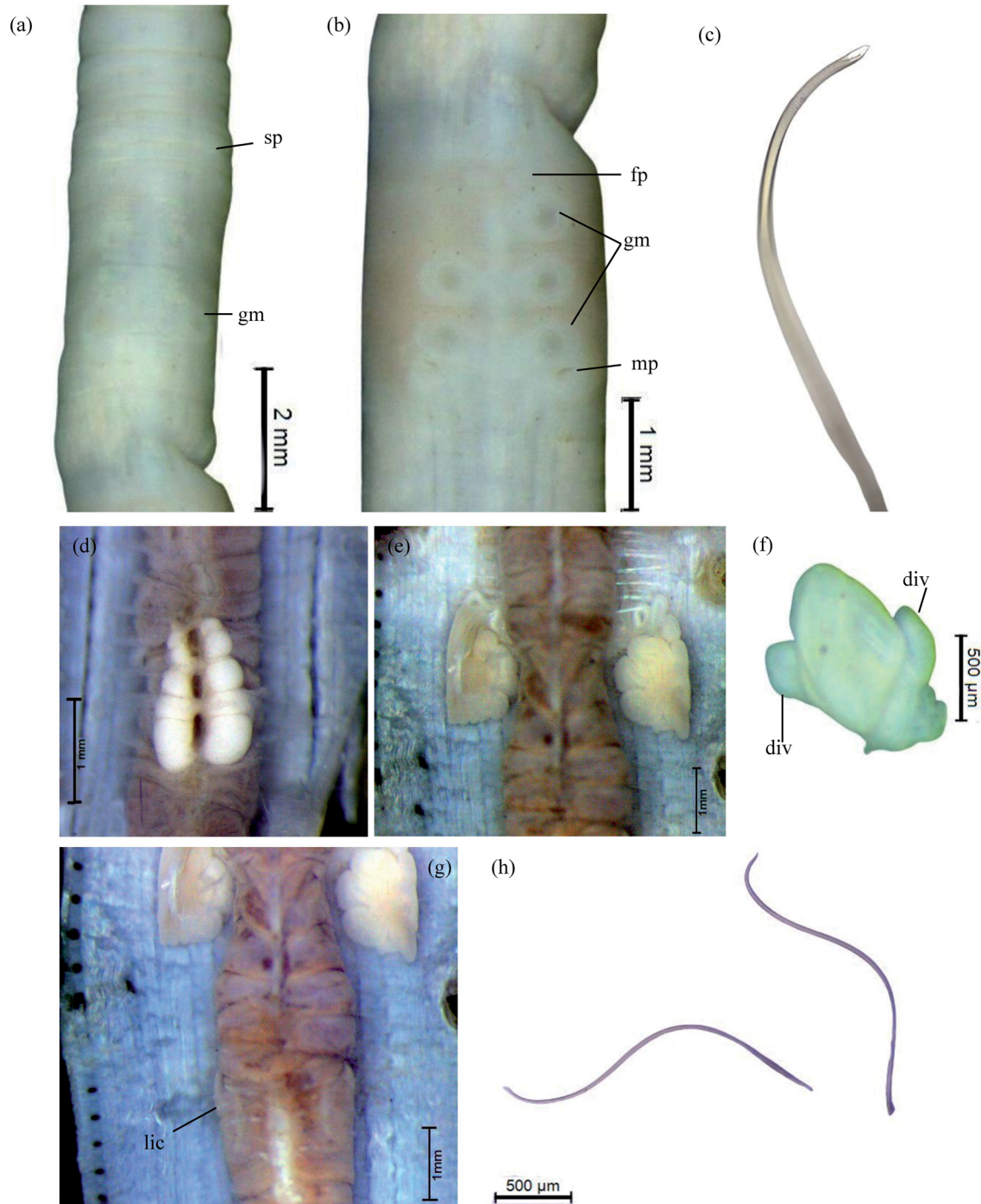


Fig. 11. *Eutyphoeus kwathensis* Tiwari & Yadav sp. nov., holotype (MNP17-1044-42A3). **a.** Preclitellar region. **b.** Clitellar region. **c.** Penial setae. **d.** Supra-intestinal glands. **e.** Prostate. **f.** Spermathecae. **g.** Lateral intestinal caeca. **h.** Penial setae. Abbreviations: see Material and methods.

Table 2. Comparison of mean and maximum intra-specific genetic distances with the nearest neighbor distances for each species. Detailed pairwise genetic distance values are provided in Supp. file 1.

Species	Mean intra-sp	Max intra-sp	Nearest neighbor species	Distance to NN
<i>Eutyphoeus assamensis</i>	0	0	<i>Eutyphoeus tawi</i>	12.49
<i>Eutyphoeus kashongensis</i> sp. nov.	0.07	0.15	<i>Eutyphoeus tengnoupalensis</i> sp. nov.	10.35
<i>Eutyphoeus kwathensis</i> sp. nov.	0.15	0.31	<i>Eutyphoeus assamensis</i>	18.82
<i>Eutyphoeus lamdengensis</i> sp. nov.	2.44	3.28	<i>Eutyphoeus manipurensis manipurensis</i>	10.01
<i>Eutyphoeus lokchaensis</i> sp. nov.	0.08	0.15	<i>Eutyphoeus lamdengensis</i> sp. nov.	10.37
<i>Eutyphoeus manipurensis manipurensis</i>	0.23	0.46	<i>Eutyphoeus lamdengensis</i> sp. nov.	10.01
<i>Eutyphoeus morehensis</i> sp. nov.	1.17	2.23	<i>Eutyphoeus lamdengensis</i> sp. nov.	10.6
<i>Eutyphoeus shiroensis</i> sp. nov.	N/A	0	<i>Eutyphoeus tengnoupalensis</i> sp. nov.	10.94
<i>Eutyphoeus tengnoupalensis</i> sp. nov.	0.38	0.76	<i>Eutyphoeus yangoupokpensis</i> sp. nov.	8.22
<i>Eutyphoeus ukhrulensis</i> sp. nov.	1.14	1.47	<i>Eutyphoeus tengnoupalensis</i> sp. nov.	9.64
<i>Eutyphoeus yangoupokpensis</i> sp. nov.	0.1	0.15	<i>Eutyphoeus tengnoupalensis</i> sp. nov.	8.22
<i>Eutyphoeus baronongini</i> sp. nov.	0.75	1.28	<i>Eutyphoeus tengnoupalensis</i> sp. nov.	10.58

oval-shaped, duct short. Penial setae 3–4 per battery, serpentine, length 2.5–2.7 mm, width 0.036 mm, ornamented anteriorly with circle of strips, tip spoon-shaped with small bulbous end.

Variation

The paratype MNP17-1045-42A4 exhibits variation in number and position of genital markings. These markings are paired in x, xiv and xv, postsetal at *b*, on xvi single on one side. In contrast, paratype MNP17-1046-42A5 lacks genital markings on segments xiv and xvi, but has a single marking on xv.

Remarks

The novel species *Eutyphoeus kwathensis* sp. nov. having traits such as metandric male organs, paired female pores, lateral intestinal caeca ventrally directed and bidiverticulate spermathecae resembles the characteristics of *E. turaensis* Stephenson, 1920 and can be differentiated by the presence of serpentine penial setae with a small bulbous end and a spoon-shaped tip (Table 1).

DNA barcode

The holotype MNP17-1044-42A3 (process ID IEW998-17) has been assigned the BIN number BOLD: ADL1718 based on the COI barcode data. *Eutyphoeus kwathensis* sp. nov. has a mean intraspecific divergence of 0.15% and a maximum intraspecific divergence of 0.31%. Its nearest neighbor species is *Eutyphoeus assamensis* with a nearest neighbor distance of 18.82 (Table 2). All specimens of the novel species share the same BIN number.

Eutyphoeus assamensis Stephenson, 1926

Figs 12, 14; Table 2

Material examined

INDIA – **Manipur** • 2 clitellates; Churachandpur, residential area; 24.3372° N, 93.7117° E; 6 Oct. 2017; Shweta Yadav leg.; MNP17-1036-41A11, MNP17-1038-41A13.

Description

Length 80 mm, diameter 4.8 mm. Total number of segments 153. First dorsal pore (?). Prostomium tanylobic. Setae visible from ii. Spermathecal pore on the intersegmental region of 7/8 at *b*, slit extends from *a*–*c*. Clitellum annular, covering $\frac{1}{2}$ of xiii to xvi without thickening of the body wall. Female pore paired at *a* in a single elliptical patch, presetal on segment xiv. Male genital region oblong shape, avestibulate and apenile, male genital orifices on xvii at *b*, at the centre of papillae composed of posteriorly united two cotyledons, each papilla in a conspicuous protuberant u-shaped ridge, the limbs of the ‘u’ directed in the middle, terminating abruptly at line *a*. A midventral region (*aa*) appears to be flat continuous (contrary to the observation of Gates 1938). Genital markings on xvi elliptical transversely placed, postsetal, paired at *b*, while margins extend laterally towards *a* and the crest of the marking exist on *b*.

Septa thickened 6/7/8. Gizzard in viii. One pair of intramural calciferous glands in xii. Intestine begins in xv. Lateral intestinal caeca? Ventral intestinal caeca? Typhlosole? Supra-intestinal gland? Last pair of hearts in xiv. Metandric, testis sac annular in xi, seminal vesicles in xii extends up to xvii. Prostates in xvii extends up to xxi, duct long tubular with single elongated loop. Spermathecae bidiverticulate, tubular, lateral with 4–5 seminal chambers, attached to the junction of duct and ampulla, one on outer side of organ, another hidden slightly towards inner side, ampulla large bladder-like, ductless. Penial setae 6–7 per battery, 2.3–3.5 mm long, highly ornamented with broken circle of irregular-sized jagged teeth, freely hanging in cavity, distal $\frac{2}{3}$ of length forming little bow-like curve with slightly curved pointed tip.

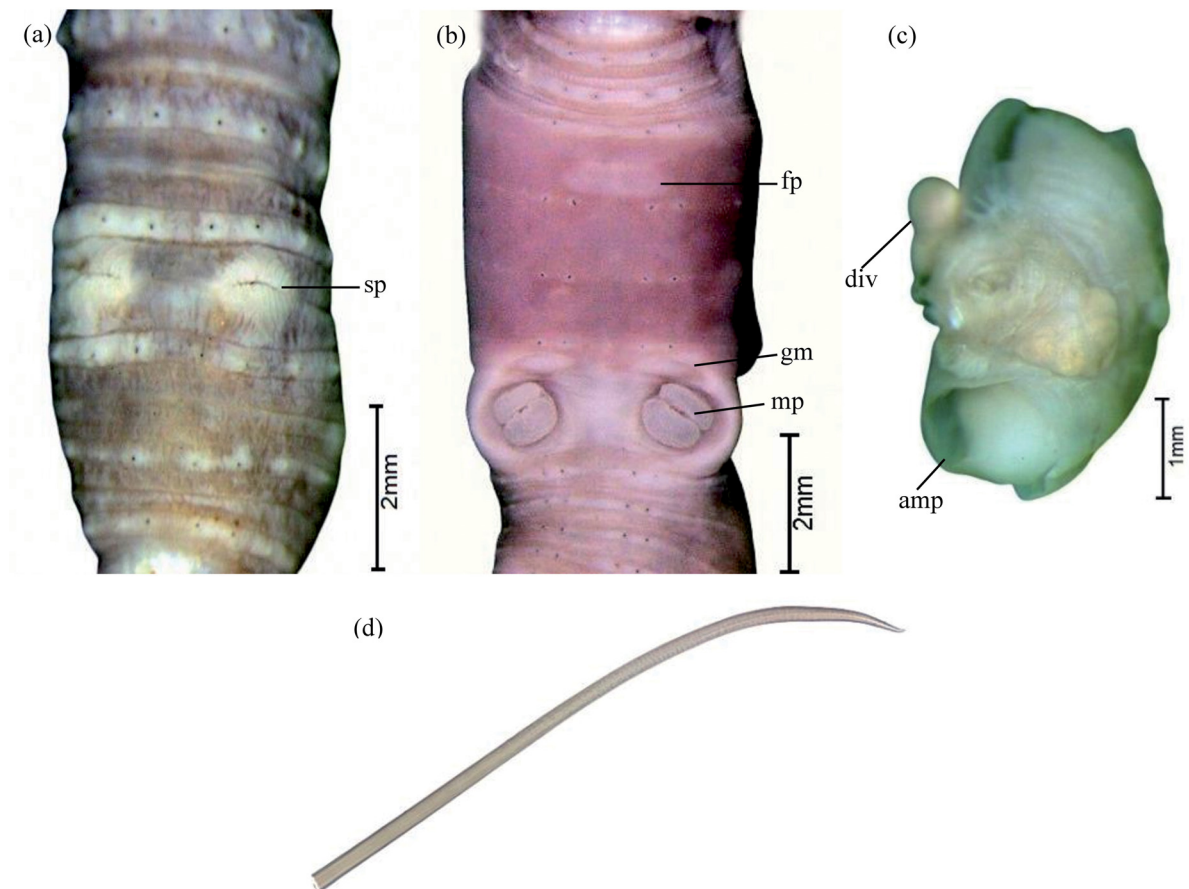


Fig. 12. *Eutyphoeus assamensis* Stephenson, 1926. **a.** Spermathecal pore. **b.** Genital regions. **c.** Spermathecae. **d.** Penial setae. Abbreviations: see Material and methods.

Remarks

The specimen was identified based on the literature of Stephenson (1926), Gates (1938) and Julka (1988), with a large number of variations. The study enumerated features of a single clitellate specimen and a single acitellate specimen. Specimens were delicate, therefore the lateral intestinal caeca, typhlosole, supra-intestinal gland, ventral caeca were not included in the study. Some observations differed from the original description. For example, the setae were visible from the second segment, there were 6–7 penial setae per battery, diverticula were lateral, penial setae had a slightly curved pointed tip, they were relatively shorter in length, the last pair of hearts were in xiv, and the penial setae were not projected out from male genital orifices.

Eutyphoeus manipurensis manipurensis Stephenson, 1921

Figs 13–14; Table 2

Material examined

INDIA – **Manipur** • 8 clitellates; Churachandpur; 24.3728° N, 93.7097° E; 6 Oct. 2017; Shweta Yadav leg.; MNP17-993-39A10, MNP17-994-39A11, MNP17-995-39A12, MNP17-996-39A13, MNP17-997-39A14, MNP17-999-39A17, MNP17-1001-39A19, MNP17-1003-39A21.

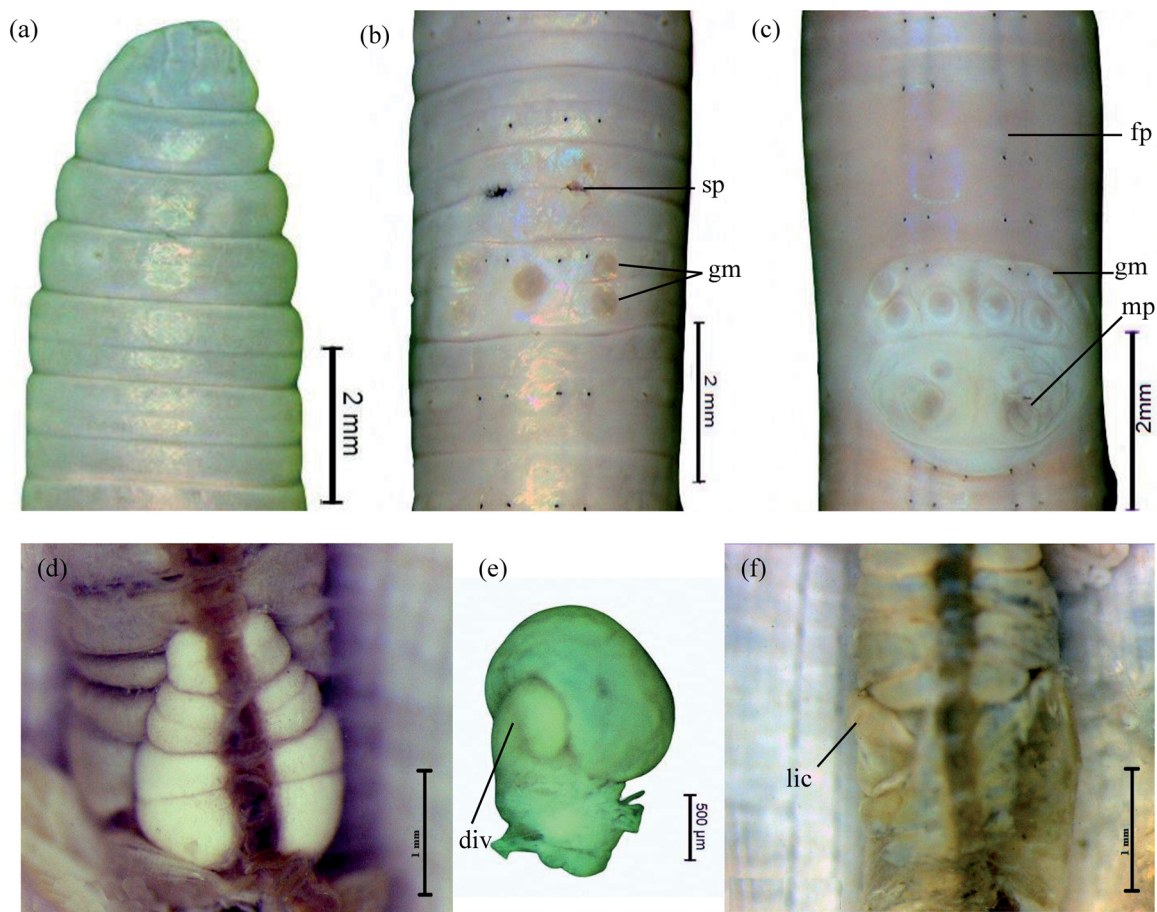


Fig. 13. *Eutyphoeus manipurensis manipurensis* Stephenson, 1921. **a.** Prostomium. **b.** Spermathecal pore. **c.** Genital region. **d.** Supra-intestinal glands. **e.** Spermathecae. **f.** Lateral intestinal caeca. Abbreviations: see Material and methods.

Description

Length 110–135 mm, diameter 3.9–4.4 mm. Total number of segments 150–189. Body color greyish brown, little paler ventrally. Prostomium tanylobic. First dorsal pore starts from intersegmental furrow of 10/11 or 11/12. Clitellum annular extends from $\frac{2}{3}$ xiii–xvii. Setal arrangement lumbricine, $aa:ab:bc:cd:dd = 1.72:1:1.82:1.39:10.15$ on segment xii and $aa:ab:bc:cd:dd = 2.96:1:2.24:1.23:11.91$ on segment xxiv. Male genital region reddish in live specimen. Male genital orifices on prominent round papillae between *ab* on xvii, bounded with deep trench, outer margin of trench little swollen. Female pores paired, presetal, little inside *a*. Spermathecal pore in intersegmental furrow of 7/8, at *ab*, opens with transverse slit. Median region of segment viii slightly raised and glandular with several small papillae. In some specimens, paired small papillae in setal zone of viii, also post setal on viii, lateral to *b*. Single median on vii and viii, sometimes just beside spermathecal aperture on right side. On xvi, small paired papillae, postsetal, lateral to *b* with transverse row of four spherical papillae closely placed, postsetal. On xvii, paired small circular presetal papillae at *a*.

Single large gizzard between septa 5/6 and 8/9. Intestine originates from xv. Last pair of hearts in xiii. Lateral intestinal caeca in xvii, ventrally directed. Ventral intestinal caeca 20–24 in xxxi–lv. Supra-intestinal glands three pairs in lxxii–lxxvi. Holandric, testis sac in x and xi, seminal vesicles in ix and xii. Latter one extends up to xiv. Prostate tubular in xvii–xix. Spermathecae unidiverticulate, ampulla ovoid sac-like with small stalked hook-shaped diverticulum, spermathecal duct short and wide. Penial setae 4 per battery, 1–1.8 mm long, fine triangular teeth ornamentation with blunt tip and slightly bent anterior shaft.

Updated key to the species of Indian *Eutyphoeus* Michaelsen, 1900 (revised after Julka 1988 and Tiwari *et al.* 2021)

1. Holandric; seminal vesicles in ix and xii 2
 – Metandric; seminal vesicles in xii 16
2. Spermathecal pores at *a* or *ab* 3
 – Spermathecal pores at *b* or lateral to *b* 5
 – Spermathecal pores at *bc* 8
3. Spermathecae unidiverticulate *E. manipurensis manipurensis* Stephenson, 1921
 – Spermathecae bidiverticulate 4
 – Spermathecae polydiverticulate *E. quadripapillatus* Michaelsen, 1907
4. Spermathecal pore at *a* *E. mizoramensis* Julka, Ramanujam & Lalthanzara, 2005
 – Spermathecal pore at *ab* 9
5. Vestibulate 6
 – Avestibulate *E. morehensis* Tiwari & Yadav sp. nov.
6. Lateral intestinal caecae present 7
 – Lateral intestinal caecae absent *E. tawi* Tiwari, Lone, Thakur, James & Yadav, 2021
7. Genital markings present 13
 – Genital markings absent *E. marmoreus* Gates, 1933
8. Spermathecae bidiverticulate *E. annandalei* Michaelsen, 1907
 – Spermathecae polydiverticulate *E. incommodus* (Beddard, 1901)

9. Clitellum annular	10
– Clitellum not annular	11
10. Genital marking gland absent, lateral intestinal caeca dorsally directed	
..... <i>E. phawngpuiensis</i> Tiwari, Lone, Thakur, James & Yadav, 2021	
– Genital marking gland present, lateral intestinal caeca ventrally directed	
..... <i>E. lamdengensis</i> Tiwari & Yadav sp. nov.	
11. Penial setae present	12
– Penial setae absent	<i>E. shiroensis</i> Tiwari & Yadav sp. nov.
12. Male pore under cuneate wedge-shaped papillae with fimbriated margin at <i>b</i>	
..... <i>E. kashongensis</i> Tiwari & Yadav sp. nov.	
– Male pore on circular porophores centered at <i>b</i>	<i>E. ukhrulensis</i> Tiwari & Yadav sp. nov.
13. Genital marking gland around spermathecal duct in vii present	14
– Genital marking gland around spermathecal duct in vii absent	15
14. Vestibula pentagonal shape, penial setae with blunt tip	
..... <i>E. yangoupokpensis</i> Tiwari & Yadav sp. nov.	
– Vestibula not-pentagonal shape, penial setae with pointed tip	
..... <i>E. tengnoupalensis</i> Tiwari & Yadav sp. nov.	
15. Preclitellar genital marking present	<i>E. lokchaensis</i> Tiwari & Yadav sp. nov.
– Preclitellar genital marking absent	<i>E. baronongini</i> Tiwari & Yadav sp. nov.
16. Avestibulate	17
– Vestibulate	30
17. Spermathecae bidiverticulate	18
– Spermathecae polydiverticulate	28
18. Lateral intestinal caecae present	19
– Lateral intestinal caecae absent	24
19. Lateral intestinal caecae dorsally directed	20
– Lateral intestinal caecae ventrally directed	21
20. Genital marking glands present	<i>E. lippus</i> Gates, 1934
– Genital marking glands absent..... <i>E. rihnimensis</i> Rodingpuia, Lalthanzara, Julka, Chawngthu, Mathipi, Lalfelpuii, Kumar, Gurusubramaniam, Lalchandama, Vanlalrovi & Chhakchhuak, 2024	
21. Female pore single, on left side	22
– Female pore paired	23
22. Penial setae ornamented, with tapering tip	<i>E. gigas</i> Stephenson, 1917
– Penial setae without ornamentation, with spoon shaped tip	<i>E. serei</i> Tiwari, Lone, Thakur, James & Yadav, 2021
23. Penial setae shaft slightly curved, tip thickened, pointed and claw-shaped	
..... <i>E. turaensis</i> Stephenson, 1920	
– Penial setae serpentine, tip spoon-shaped with a small bulbous end	
..... <i>E. kwathensis</i> Tiwari & Yadav sp. nov.	

24. Spermathecal pores at <i>ab</i>	25
– Spermathecal pores lateral to <i>b</i>	26
25. Genital markings preclitellar, segmental	<i>E. comillahnus</i> Michaelsen, 1907
– Genital markings postclitellar, intersegmental	<i>E. festivus</i> Gates, 1938
26. Genital markings paired, segmental on xvi	27
– Genital markings unpaired, intersegmental on xv/xvi	<i>E. scutarius</i> Michaelsen, 1907
27. Sessile genital marking glands present	
.....	<i>E. dhubriensis</i> Ahmed, Julka, Banerjee & Marimuthu, 2023
– Sessile genital marking glands absent	<i>E. assamensis</i> Stephenson, 1926*
28. Seminal vesicles in xii very long, extending posteriorly into xxxiii	
.....	<i>E. pharpius</i> Michaelsen, 1907
– Seminal vesicles in xii shorter, at the most extending into xx	29
29. Genital marking intraclitellar, on xvi	<i>E. nainianus</i> Michaelsen, 1907
– Genital marking intra and post-clitellar, on xiv–xv, xviii/xix–xxi/xxii	
.....	<i>E. nepalensis</i> Michaelsen, 1907
30. Univestibulate	31
– Bivestibulate	32
31. Spermathecal pores at <i>ab</i> ; penes conical	<i>E. kherai</i> Julka, 1978
– Spermathecal pores at median half of <i>bc</i> ; penes annular	<i>E. gammiei</i> (Beddard, 1888)
32. Penes annular	33
– Penes elongate	35
33. Genital markings present on xv, usually also on xvi	<i>E. orientalis</i> (Beddard, 1883)
– Genital markings present on xv–xvi absent	34
34. Genital markings preclitellar on ix/x	<i>E. aborianus</i> Stephenson, 1914
– Genital markings pre- and post-clitellar on x/xi–xii/xiii, xviii/xix–xxi/xxii	
.....	<i>E. callosus</i> Gates, 1938
35. Spermathecal pores at <i>ab</i>	<i>E. nicholsoni</i> (Beddard, 1901)
– Spermathecal pores at <i>bc</i>	36
36. Spermathecae bidiverticulate; genital markings pre-, intra- and post-clitellar	
.....	<i>E. waltoni</i> Michaelsen, 1907
– Spermathecae polydiverticulate; genital markings post clitellar or absent	
.....	<i>E. kempfi</i> Stephenson, 1914

* This study confirms that *Eutyphoeus assamensis* is an avestibulate and apenile species showing features that were previously unrecorded or unclear in earlier taxonomic descriptions (Gates 1972; Julka 1988). While Julka (1988) provided a valuable categorization of the species based on the presence or absence of a vestibule, our examination of a mature, clitellate specimen of *E. assamensis* from our collection unequivocally confirms its avestibulate and apenile nature. This observation directly contrasts with potential ambiguities arising from Julka's (1988) description, which may have been influenced by his reliance on aclitellate specimens where the full development of male genitalia, including the

vestibule and penis, was incomplete. Consequently, our findings provide a crucial refinement of the existing taxonomic framework for the species, highlighting the importance of examining fully mature specimens for accurate species characterization, particularly concerning key reproductive structures like the vestibule and penis.

Discussion

The discovery of ten novel species and one new record of *Eutyphoeus* from Manipur underscores the region's biodiversity significance, particularly in the context of earthworm taxonomy. This study highlights the effectiveness of integrating morphological and molecular approaches to identify species, and addressing challenges associated with cryptic diversity in earthworms. The detailed morphological descriptions and DNA barcode not only enhance the robustness of species delineation but also set a precedent for future taxonomic research.

The Maximum Likelihood (ML) tree consistently delimits all *Eutyphoeus* species, including the ten new ones, with strong statistical support (Fig. 14). The rooted ML phylogeny reveals distinct clades, corresponding to different earthworm species, clearly separating each of the newly described species. The phylogram further categorizes the species into two major clades based on reproductive anatomy: the metandric group (comprising *E. serei*, *E. rihnimensis*, *E. turaensis*, *E. kempi*, *E. callosus*, *E. nepalensis*, *E. gammiei*, and the novel species *E. kwathensis*), and the holandric group (including *E. phawngpuiensis*, *E. tawi*, *E. ukhrulensis* sp. nov., *E. shiroensis* sp. nov., *E. yangoupokpensis* sp. nov., *E. tengnoupalensis* sp. nov., *E. lokchaensis* sp. nov., *E. lamdengensis* sp. nov., *E. kashongensis* sp. nov., *E. baronongini* sp. nov., *E. manipurensis manipurensis*, and *E. morehensis* sp. nov.). Notably, *E. assamensis*, though metandric, forms a sister clade with the holandric species *E. tawi*, presenting an exception to this pattern.

The diversity of *Eutyphoeus* species in Manipur can be attributed to the region's complex topography and varied microclimatic conditions, which provide an array of ecological niches. The newly identified taxa predominantly belong to the *E. hastatus* group, reaffirming Gates' (1958) hypothesis regarding the group's evolutionary radiations in the Indo-Burmese region. However, distinct features such as variations in the arrangement of the male genital orifices, spermathecal morphology, and genital markings emphasize the random changes in isolation over millions of years within this genus, likely driven by microhabitat specialization.

The identification of *Eutyphoeus kwathensis* as metandric and distinct from other holandric species in the study reflects potential evolutionary transitions within the genus.

Moreover, the findings have important implications for conservation biology. Many of the newly discovered species are confined to specific habitats, such as wildlife sanctuaries and forested regions (Yangoupokpi-Lokchao Wildlife Sanctuary or Shirui National Park), making them vulnerable to habitat loss and anthropogenic disturbances. The documentation of such endemic taxa calls for targeted conservation strategies to preserve their habitats, emphasizing the role of molecular data in supporting biodiversity assessments and management plans.

The study highlights several areas for future research. The ecological roles of species of *Eutyphoeus* in soil health and nutrient cycling remain largely unexplored. Additionally, the phylogenetic relationships within the genus require further investigation to understand their evolutionary history and biogeographical patterns. Expanding the scope of similar studies to adjacent regions could uncover further biodiversity and clarify the distributional limits of species of *Eutyphoeus*.

This study not only enriches our knowledge of the genus *Eutyphoeus* but also emphasizes the importance of integrative taxonomy in documenting and conserving biodiversity. The findings serve as a reminder

of the vast yet fragile natural heritage of North-East India, urging continued scientific exploration and conservation efforts in the region.

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Conflict of interest

The authors declare no conflict of interest.

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Data availability

The sequences generated in this study are available on Barcode of Life Data System (BOLD) under project “IEW-Diversity studies in earthworms of India using DNA Barcode approach”

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Supplementary file

Supp. file 1. Table S1. Pairwise genetic distances among all studied sequences based on *COI* gene alignment. Distances were calculated using the uncorrected p-distance.

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