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TWO NEW SPECIES AND A NEW SYNONYM OF *EUSTIGMAEUS* (ACARI: STIGMAEIDAE) FROM RUSSIA WITH COI BARCODE

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ABSTRACT: Two new species, *Eustigmaeus reticulatus* sp.n. and *E. pseudolacunus* sp.n. (Acari: Stigmaeidae) are described based on females and males collected from different parts of Russia. A fragment of the COI barcoding gene was sequenced to facilitate DNA-based identification. Based on the study of the holotype of *Eustigmaeus mineus* Barilo, it is considered a junior synonym of *Eustigmaeus collarti* Cooreman. An updated key to the *Eustigmaeus* species of Russia is provided.

KEY WORDS: Acarina, Raphignathoidea, morphology, systematics, key.

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INTRODUCTION

The mite family Stigmaeidae (Acari: Prostigmata) is the largest in the superfamily Raphignathoidea and currently includes about 640 species of 33 valid genera (Fan *et al.* 2016, 2019; Beron 2020; Khaustov 2022; Mohammad-Doustaresharaf *et al.* 2023). Most stigmaeid mites are free-living predators of various small arthropods. Some *Eustigmaeus* species feed on mosses, while several *Stigmaeus* and *Eustigmaeus* species are parasites of sand flies (Diptera: Psychodidae). Species of the genera *Zetzelia* and *Agistemus* are probably the second most important group of plant mite predators (after the Phytoseiidae) (Gerson *et al.* 2003).

The genus *Eustigmaeus* Berlese, 1910 is one of the largest in the family Stigmaeidae. It comprises 119 described species (Beron 2020; Khaustov 2021a, b, 2022), nineteen of which have been reported from Russia (Khaustov and Tolstikov 2014; Khaustov 2016, 2019, 2021a, b, 2022).

During the study of predatory mites of Asian Russia, we have collected two undescribed species of *Eustigmaeus*. The aim of this paper is to describe them. *Eustigmaeus mineus* Barilo, 1989 is considered a junior synonym of *Eustigmaeus collarti* (Cooreman, 1955). In addition, a fragment of the COI barcoding gene of two new species was sequenced in order to facilitate DNA-based identification. A key to the *Eustigmaeus* species of Russia is also provided.

MATERIALS AND METHODS

Mites were collected from soil samples using Berlese funnels and mounted in Hoyer's medium. In the description below, the palpal, idiosomal and leg setations follow Grandjean (1939, 1944, 1946). The nomenclature of prodorsal setae follows Kethley (1990). All measurements for the holotype and paratypes (in parentheses) are given in micrometres (μm). Mite morphology was studied using a Carl Zeiss AxioImager A2 compound microscope with a phase contrast and DIC illuminations. Photomicrographs were taken with an AxioCam ICc5 (Carl Zeiss, Germany) digital camera.

DNA extraction, oligonucleotide primers, amplification and sequencing have been done following Klimov and OConnor (2008), Matthews *et al*. (2018), Klimov *et al*. (2022). The sequences are deposited in the GenBank under the accession No. OP960175 for *E. reticulatus* sp.n., OP960176 for *E. pseudolacunus* sp.n. and OL863255, OP960162, OP960166 for *E. collarti*. The mite exoskeletons are deposited in Tyumen State University under voucher Nos. X-BIOTU St-56, X-BIOTU St-60 and X-BIOTU St-33, St-44, St-53, respectively. The obtained sequences were compared to those in public databases, available on the NCBI website,[1] using the Nucleotide Basic Local Alignment Search Tool, BLASTn and BLASTx. The sequences were aligned with published sequences taken from the GenBank using the CLUSTAL algorithm (Sievers *et al*. 2011). Maximum likelihood reconstruction was conducted in the IQTREE v. 1.6.12 software (Nguyen *et al*., 2015) with 1,000 bootstrap replicates to test topology stability. The ModelFinder method (Kalyaanamoorthy *et al.,* 2017) was used to select the optimum partitioning scheme and the best-fit substitution model under the BIC criterion. Ge-

[1] https://blast.ncbi.nlm.nih.gov/Blast.cgi

netic distances were estimated using the MEGA v. 11 software (Tamura *et al.*, 2021).

SYSTEMATICS

Family **Stigmaeidae Oudemans, 1931**

Genus ***Eustigmaeus*** Berlese, 1910
Type species: *Stigmaeus kermesinus* Koch, 1841, by original designation.

***Eustigmaeus reticulatus* sp. n.**
(Figs. 1–8)

Description. *Female.* Length of idiosoma 285 (279–285), width 215 (195–215).

Idiosomal dorsum (Figs. 1A, 5, 6A, B). Ocelli present. Idiosoma almost completely covered by two large shields. Shields with large round dimples and distinct subcuticular reticulation (Figs. 6A, B). All dorsal setae baculiform, weakly barbed and with well-developed hyaline sheaths distally better visible on posterior hysterosomal setae. Setae *h1* and *h2* situated ventrally. Prodorsal shield subtriangular with one pair of small apodemal marks near bases of setae *ve*. Lengths of dorsal setae: *vi* 39 (33–39), *ve* 41 (38–41), *sci* 29 (25–30), *sce* 34 (32–34), *c1* 32 (28–32), *c2* 34 (28–34), *d1* 31 (29–31), *d2* 31 (28–33), *e1* 31 (28–33), *e2* 31 (30–32), *f* 42 (38–42), *h1* 35 (31–36), *h2* 29 (22–29).

Idiosomal venter (Figs. 1B, 6C). Callosities absent. Suranal plate situated ventrally, with distinct large dimples. Endopodal plates separated medially. Humeral plate subtriangular, with distinct large dimples. All ventral setae smooth or weakly barbed and pointed; with two pairs of simple subequal aggenital and three pairs of pseudanal setae (Fig. 6C). Aggenital plate smooth. Coxisternal plates of legs I and II with weak subcuticular reticulation. Lengths of ventral setae: *1a* 20 (18–21), *1b* 23 (22–24), *1c* 21 (20–22), *2b* 21 (20–23), *2c* 20 (19–21), *3a* 22 (21–24), *3b* 20 (19–22), *3c* 21 (19–22), *4a* 20 (19–22), *4b* 19 (19–21), *4c* 20 (19–21), *ag1* 17 (16–18), *ag2* 14 (14–15), *ps1* 18 (15–18), *ps2* 16 (15–17), *ps3* 14 (14–15).

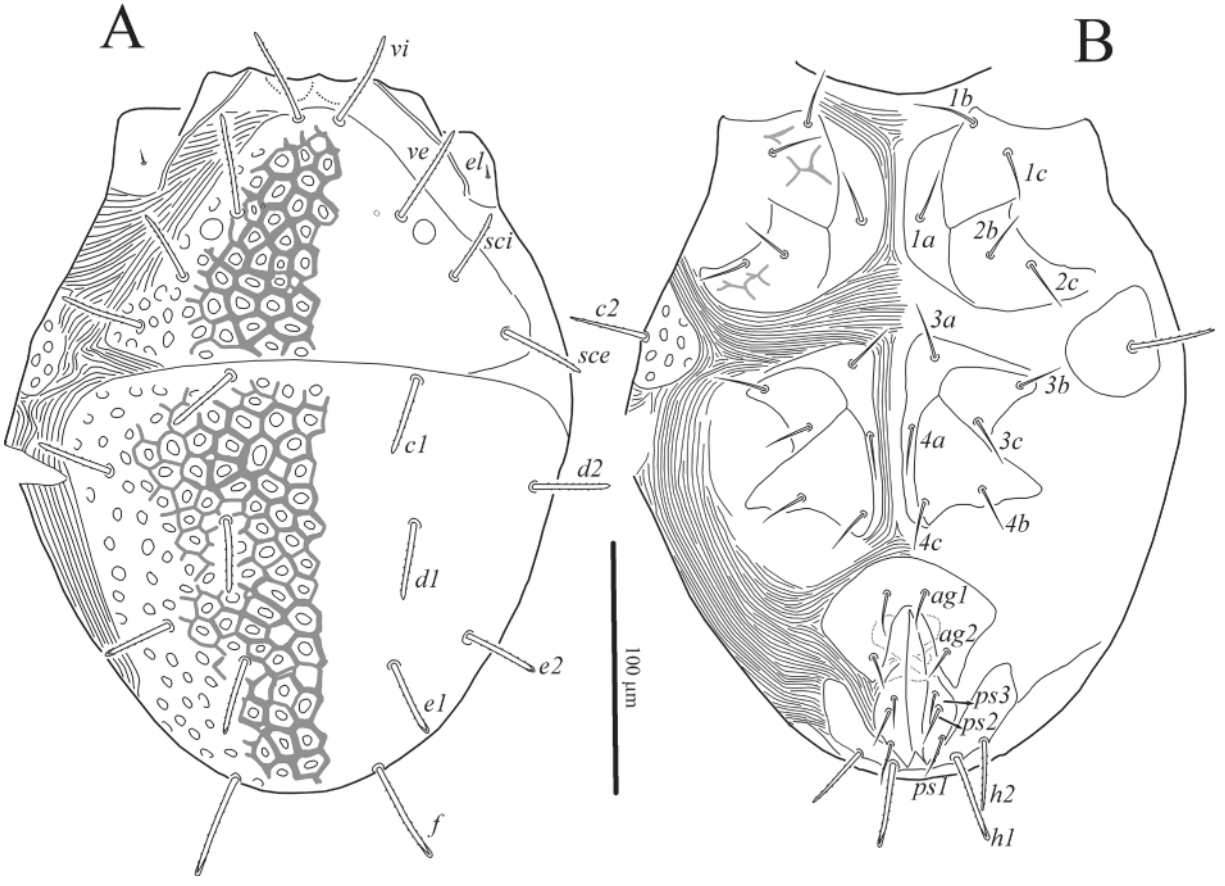

Fig. 1. *Eustigmaeus reticulatus* sp. n., female (holotype): A—dorsum of idiosoma, B—venter of idiosoma. Legs omitted.

Gnathosoma (Figs. 2, 6D). Tibial claw well-developed, slightly longer than palptarsus. Seta *l'* on palpal tibia short, spiniform. All palpal setae of femur, genu and tibia (except *l'Ti*) pointed and weakly barbed; all setae of palptarsus smooth. Number of setae on palpal segments: Tr 0, Fe 3 (*d*, *l'*, *v''*), Ge 2 (*d*, *l''*), Ti 3 (*d*, *l'*, *l''*), Ta 8(1) (fused eupathidia *ul'*, *ul''*, *sul*, eupathidion *acm*, *ba*, *bp*, *lp*, 1 solenidion *ω*). Palpal supracoxal setae (*ep*) needle-like, located dorsally. Rostrum of subcapitulum distinctly elongate. All subcapitular setae pointed; setae *or1* smooth, other setae with few weak barbs. Basal part of subcapitulum smooth (Fig. 6D). Length of subcapitular setae: *m* 23 (23–25), *n* 19 (19–21), *or1* 13 (13–14), *or2* 18 (18–19). Chelicerae dorsally smooth, length of stylets 48 (46–48); length of palps 87 (86–94); length of palpal solenidion *ω* 7 (7).

Legs (Figs. 3, 4). Lengths of legs: I 170 (165–170), II 140 (135–140), III 140 (135–140), IV 170 (160–170). Empodial raylets not capitate. Leg I (Fig. 3A). Coxae I posterodorsally with needle-like leg supracoxal setae (*el*). Leg setation: Tr 1 (*v'*), Fe 6 (*d*, *l'*, *l''*, *v'*, *v''*, *bv''*), Ge 4 (*d*, *l'*, *l''*, *k*), Ti 5(2) (*dξ*, *l'*, *l''*, *v'*, *v''*, *φ*, *φp*), Ta 13(1) (*p'ξ*, *p'' ξ*, *tc'ξ*, *tc'' ξ*, *ft'ξ*, *ft'' ξ*, *u'*, *u''*, *a'*, *a''*, *pl'*, *pl''*, *vs*, *ω*). Setae *d* of tibia, (*p*), (*tc*) and (*ft*) of tarsus eupathid-like. Setae *d* of femur and genu barbed, with hyaline sheath; seta *k* 32 (31–35) smooth, blunt-tipped, subequal to seta *d* of genu; other setae pointed and barbed. Solenidion *ω* 23 (23–26) digitiform; solenidion *φ* 10 (9–10) baculiform, solenidion *φp* 19 (14–19) attenuate. Leg II (Fig. 3B). Leg setation: Tr 1 (*v'*), Fe 5 (*d*, *l'*, *l''*, *v'*, *bv''*), Ge 4 (*d*, *l'*, *l''*, *k*), Ti 5(1) (*dξ*, *l'*, *l''*, *v'*, *v''*, *φ*), Ta 9(1) (*p'ξ*, *tc'ξ*, *tc''*, *u'*, *u''*, *a'*, *a''*, *pl'*, *vs*, *ω*). Setae *d* of tibia, *p'* and *tc'* of tarsus eupathid-like. Setae *d* of femur and genu blunt-tipped and barbed; seta *k* 6 (6) of genu rod-like; other setae pointed and barbed. Solenidion *ω* 13 (13) digitiform; solenidion *φp* 17 (13–17) attenuate. Leg III (Fig. 4A). Leg setation: Tr 1 (*v'*), Fe 3 (*d*, *l'*, *ev'*), Ge 1 (*d*), Ti 5(1) (*d*, *l'*, *l''*, *v'*, *v''*,

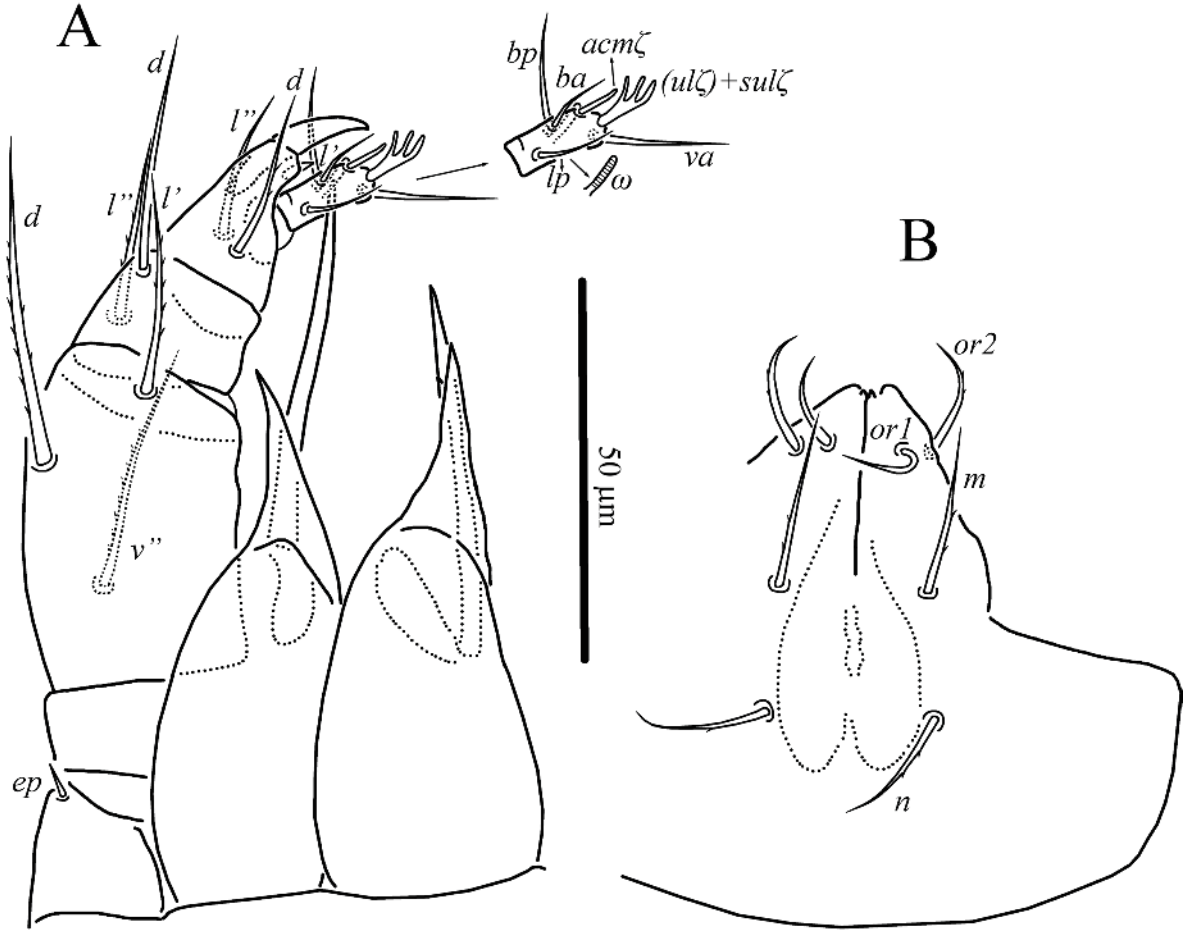

Fig. 2. *Eustigmaeus reticulatus* sp.n., female (holotype): A—gnathosoma, dorsal aspect; B—subcapitulum.

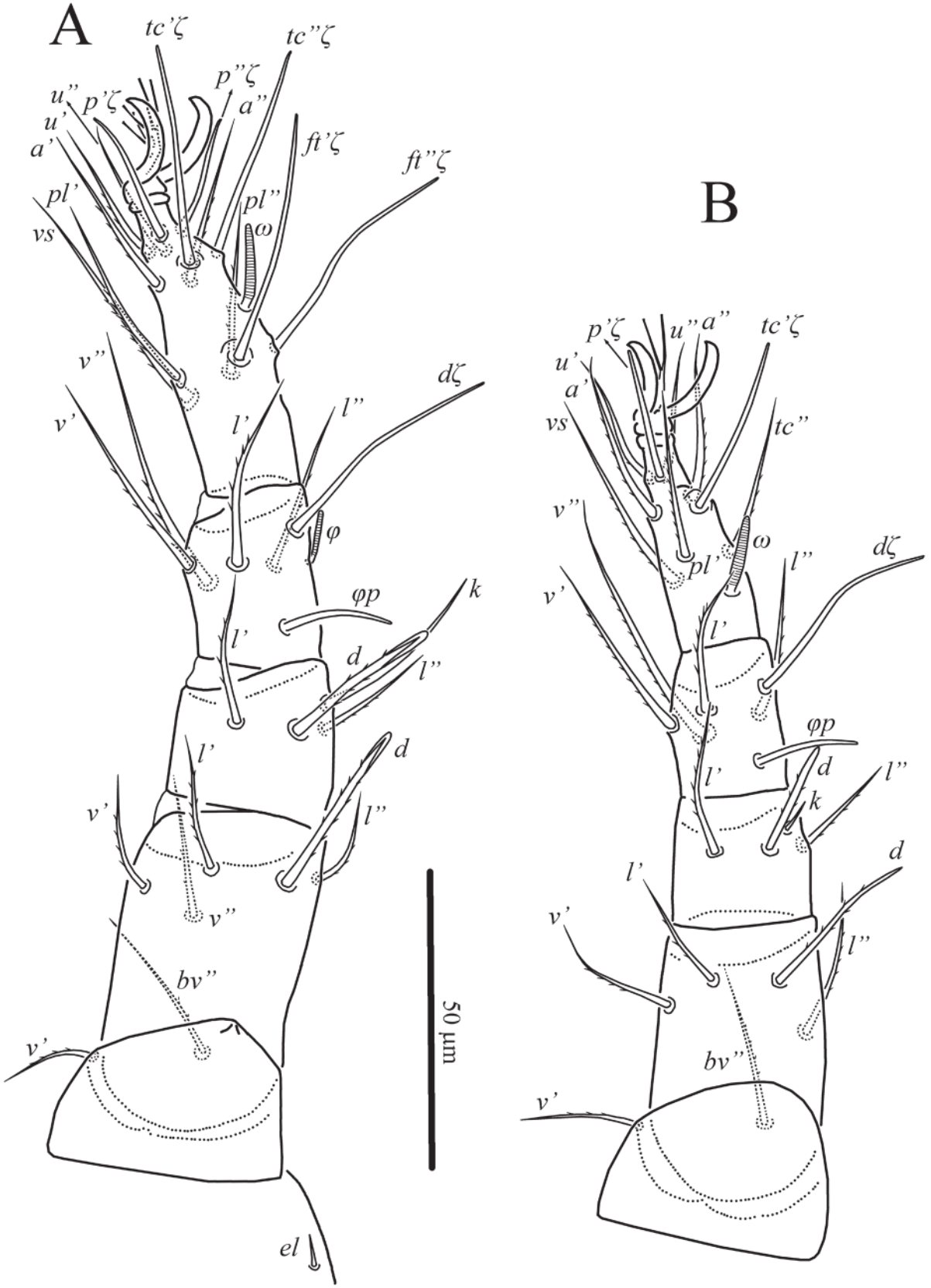

Fig. 3. *Eustigmaeus reticulatus* sp.n., female (holotype): A—right leg I, dorsal aspect; B—right leg II, dorsal aspect.

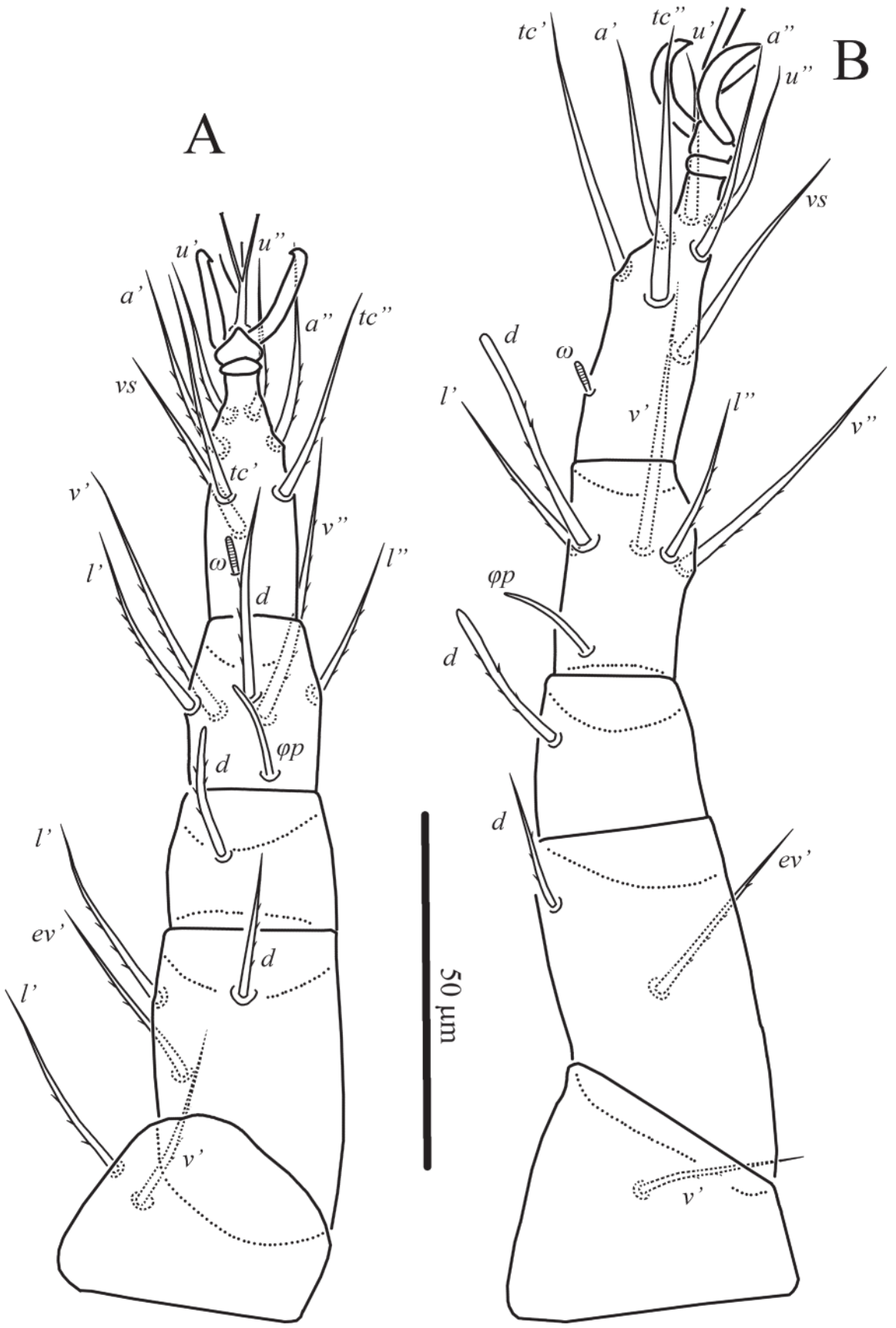

Fig. 4. *Eustigmaeus reticulatus* sp. n., female (holotype): A—right leg III, dorsal aspect; B—right leg IV, dorsal aspect.

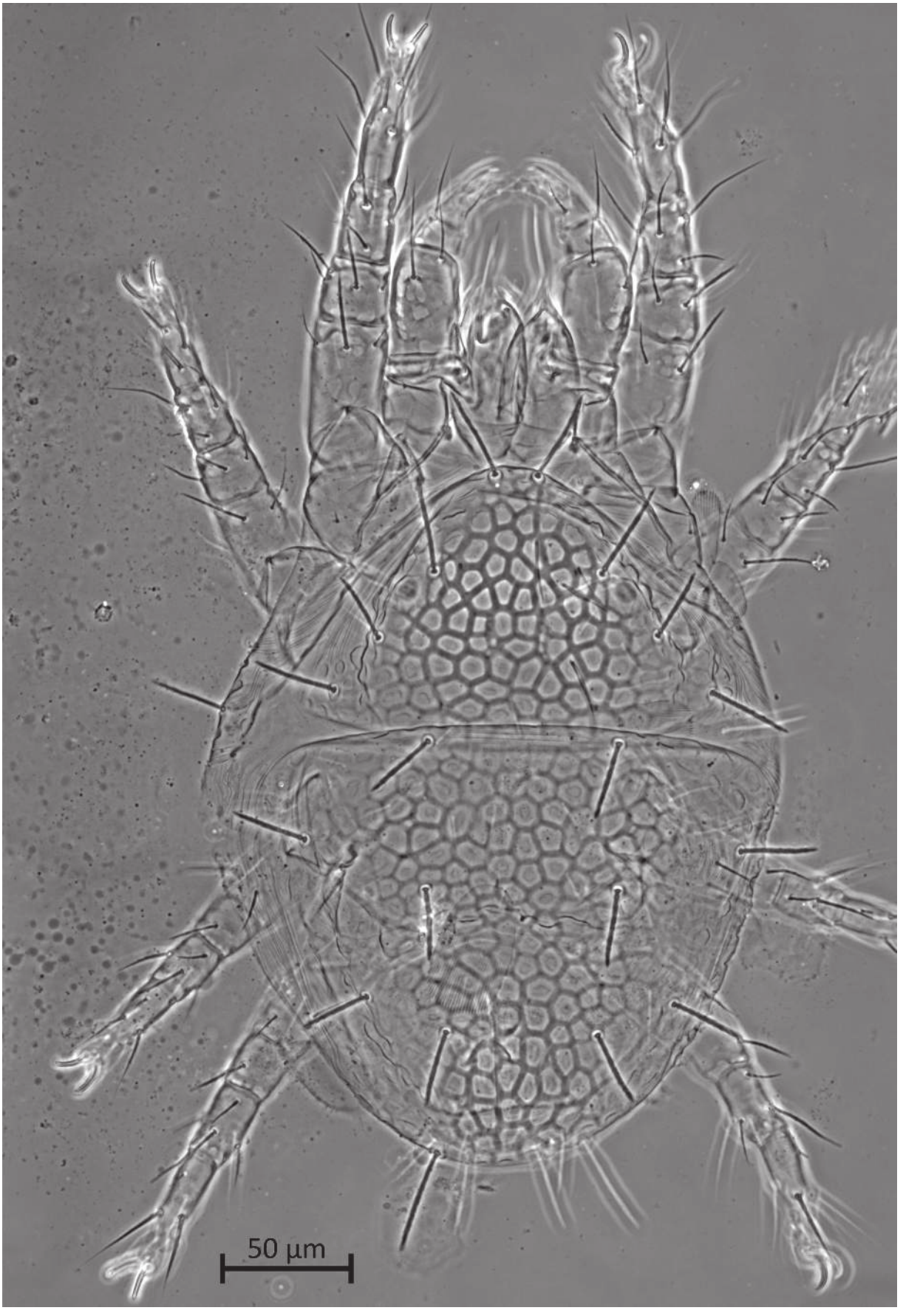

Fig. 5. Phase contrast micrograph of *Eustigmaeus reticulatus* sp.n., female (holotype): general view dorsally.

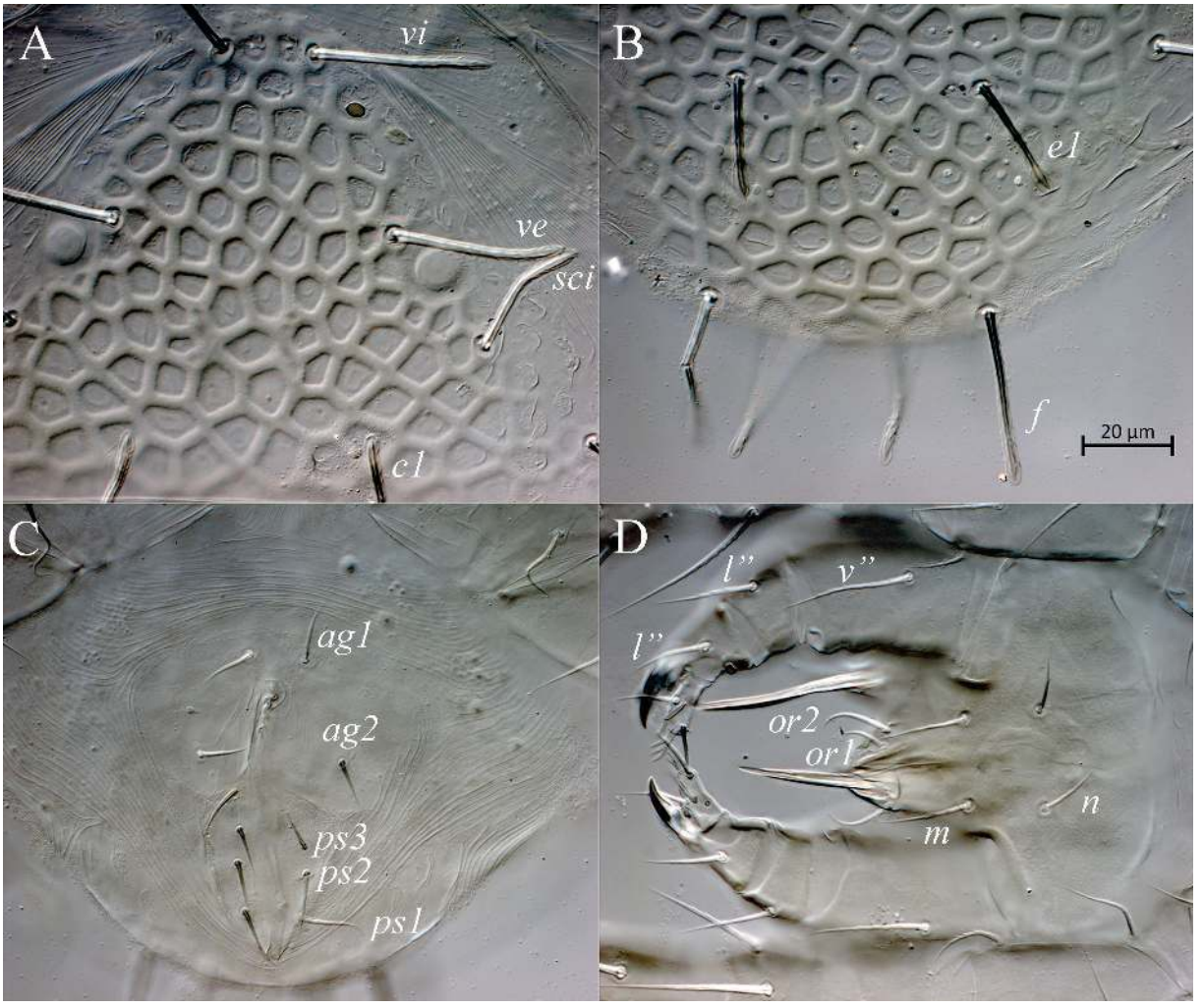

Fig. 6. DIC micrographs of *Eustigmaeus reticulatus* sp. n., female: A—prodorsum, B—opisthosomal dorsum, C—anogenital area, D—gnathosoma, ventral aspect.

φ), Ta 7(1) (*tc'*, *tc''*, *u'*, *u''*, *a'*, *a''*, *vs*, *ω*). Solenidion *ω* 5 (5–7) baculiform; solenidion *φp* 14 (12–14) attenuate. All leg setae barbed. Setae *d* of genu with weak hyaline sheath, other setae pointed. Leg IV (Fig. 4B). Leg setation: Tr 1 (*v'*), Fe 2 (*d*, *ev'*), Ge 1 (*d*), Ti 5(1) (*d*, *l'*, *l''*, *v'*, *v''*, *φ*), Ta 7(1) (*tc'*, *tc''*, *u'*, *u''*, *a'*, *a''*, *vs*, *ω*). Solenidion *ω* 4 (4–6) baculiform; solenidion *φp* 14 (12–14) attenuate. Setae *d* of genu and tibia with hyaline sheaths; other setae pointed; all tarsal setae smooth or with few weak barbs, other setae barbed.

Male (Figs. 7–8). Idiosoma ovoid, but opisthosoma narrower than in female. Length of idiosoma 230–255, width 145–155.

Idiosomal dorsum (Fig. 7A). In general similar to female, but hysterosomal shield transversely divided into two shields; both shield clearly reticulated; anterior shield with three pairs of setae *c1*, *d1*, *d2*; posterior shield with three pairs of setae *e1*, *e2*, *f*. Suranal shield located dorsally. Setae *ps1–2* short, smooth, needle-like, setae *h1* and *ps3* smooth and pointed; setae *e1* smooth and blunt-tipped; other dorsal setae weakly barbed, baculiform, setae *f* with weak hyaline sheath. Lengths of dorsal setae: *vi* 25–27, *ve* 29–32, *sci* 18–22, *sce* 23–24, *c1* 21–25, *c2* 22–24, *d1* 22–25, *d2* 22–24, *e1* 11–12, *e2* 21–23, *f* 25–29, *h1* 6–7, *h2* 18– 19, *ps1* 3, *ps2* 4, *ps3* 6.

Idiosomal venter (Fig. 7B). Podosoma as in female. Opisthosoma with smooth, weakly sclerotized aggenital plate. Aggenital plate with two pairs of smooth or weakly barbed aggenital setae. Aedeagus weakly sclerotized (Fig. 8A). Lengths of ventral setae: *1a* 16–18, *1b* 18–19, *1c* 17–18, *2b* 17–18, *2c* 15–16, *3a* 16–17, *3b* 15–16, *3c* 15–17, *4a* 15–16, *4b* 14–15, *4c* 15–16, *ag1* 12–13, *ag2* 10–12.

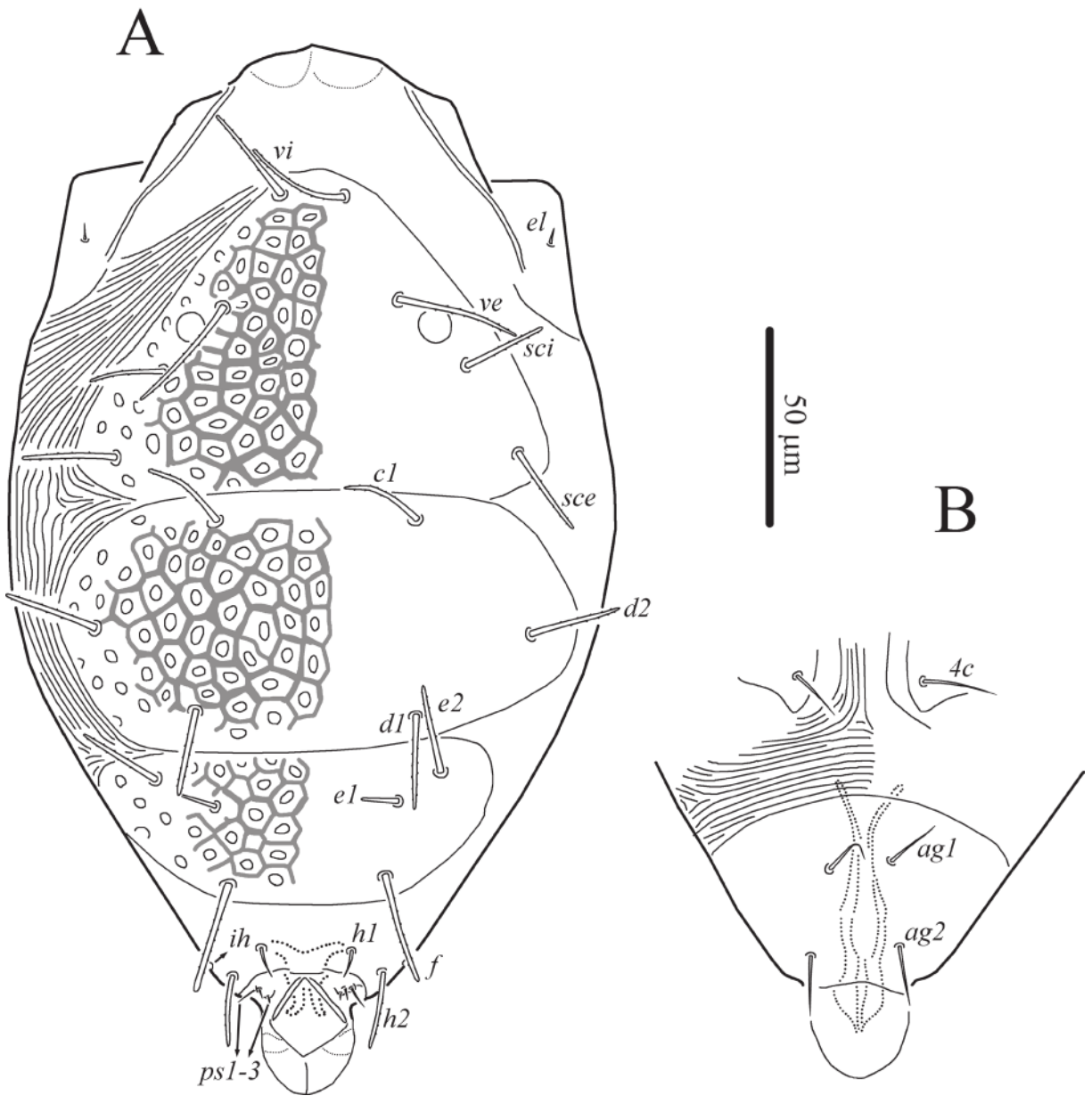

Fig. 7. *Eustigmaeus reticulatus* sp.n., male: A—dorsum of idiosoma, B—venter of opisthosoma.

Gnathosoma as in female. Length of palp 77–80; length of stylets 35–38; length of palpal solenidion ω *8*; length of subcapitular setae: *m* 19, *n* 16, *or1* 10, *or2* 16.

Legs (Figs. 8B–D) in general similar to those of female except presence of male solenidia on tarsi I–IV (Figs. 8B–D). Lengths of legs: I 145, II 110–120, III 110–115, IV 115–125. Lengths of solenidia and setae *k*: *ω*I 13–15, *ω*♂I 38 –39, *φ* 7, *φp*I 13, *k*I 25–27, *ω*II 13–14, *ω*♂II 26–37, *φp*II 11–12, *k*II 5, *ω*III 6, *ω*♂III 32 –35, *φp*III 10–11, *ω*IV 5–6, *ω*♂IV 33 –36, *φp*IV 11.

Larva and *nymphs* unknown.

Type material. Female holotype, slide № ZISP T-St-010, Russia, Tyumen Oblast, vicinity of Lake Kuchak, 57°22′07.0″ N 66°03′04.5″ E, in rotting stump, 26 September 2018, collected by A.A. Khaustov; paratypes: 2 females, same data; 5 females, 1 male, same locality, 5 September 2018; 8 females, 3 males, Russia, Primorsky Krai, Shotovsky District, 43°08′04.5″ N 132°43′36.6″ E, in forest litter, 14 May 2022, collected by S.V. Kravchenko; 10 females, 2 males, Russia, city of Cheboksary, 56°05′ N 47°13′ E, in rotting stump, collected by I.V. Badanin.

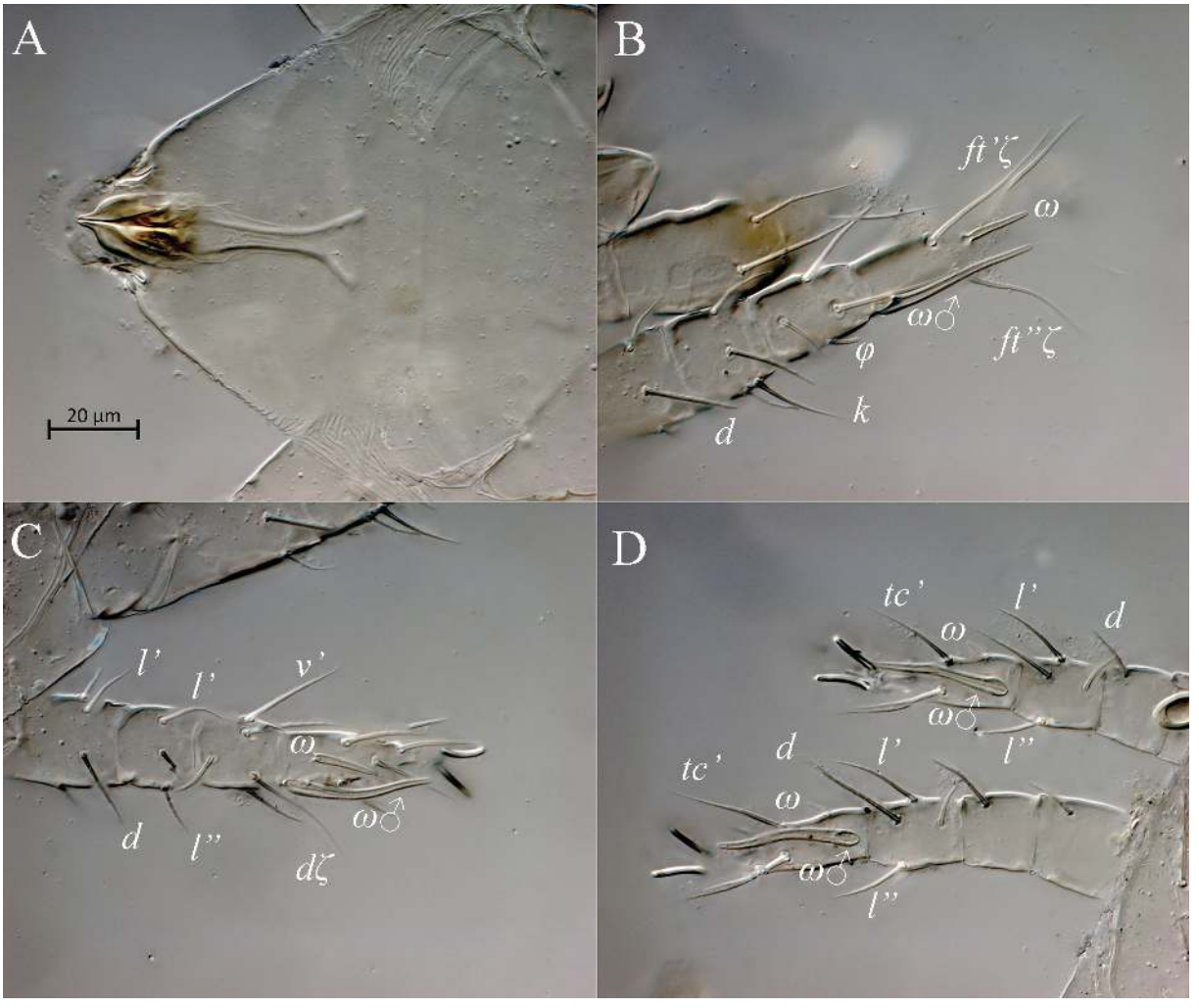

Fig. 8. DIC micrographs of *Eustigmaeus reticulatus* sp.n., male: A—aedeagus, B—right leg I, dorsal aspect, C—right leg II, dorsal aspect, D—left legs III and IV, dorsal aspect.

Type deposition. The holotype and two female paratypes are deposited in the mite collection of the Zoological Institute of RAS, St. Petersburg, Russia; other paratypes are deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

Differential diagnosis. Female of the new species is most similar to those of *Eustigmaeus arcticus* (Wood, 1972) and *E. collarti* (Cooreman, 1955) in having strongly reticulated dorsal idiosomal shields and baculiform dorsal setae, in the presence of two pairs of aggenital setae, in the absence of callosities and in having the same leg setation. Female of the new species differs from both of the above species in having shorter dorsal idiosomal setae *vi* 33–39, *ve* 38–41, *sci* 25–30, *sce* 32–34, *c1* 28–32, *c2* 28–34, *d1* 29–31, *d2* 28–33, *e1* 28–33, *e2* 30–32, *f* 38–42, *h1* 31–36, h_2 22–29 (vs. *vi* 44–50, *ve* 46–53, *sci* 34–42, *sce* 42–48, *c1* 40–48, *c2* 42–48, *d1* 40–48, *d2* 40–48, *e1* 44–48, *e2* 42–46, *f* 50–55, *h1* 40–50, *h2* 32–40 in *E. arcticus* after Stathakis *et al*. (2016) and *vi* 45–51, *ve* 46–49, *sci* 36–38, *sce* 38–41, *c1* 40–44, *c2* 41–44, *d1* 41–43, *d2* 39–41, *e1* 42–44, *e2* 40–43, *f* 50–52, *h1* 42–45, *h2* 37–40 in *E. collarti* after Khaustov and Tolstikov (2014)); in suranal shield located ventrally (vs. suranal shield located dorsally in both species); and in having smooth aggenital shield (vs. clearly reticulated in both species). Female of the new species differs from that of *E. arcticus* in having dorsal idiosomal setae completely covered with hyaline sheaths (vs. tip of setal shaft protruding through hyaline sheaths in *E. arcticus*). Female of the new species differs from that of *E. collarti* in having dorsal idiosomal setae with distinct hyaline sheaths (vs. dorsal idiosomal setae without hyaline sheaths in *E. collarti*). Male of the new species differs from males of *E. arcticus* and *E. collarti* in having

smooth setae *e1* that are two times shorter than *e2* (vs. setae *e1* weakly barbed and subequal to *e2* in the other two species). The new species also differs from *E. collarti* with respect to habitats; *E. reticulatus* sp.n. inhabits rotting wood and forest litter, while *E. collarti* inhabits extremely wet habitats such as moss on bogs, banks of lakes, etc. The new species also significantly differs from *E. collarti* in COI barcodes (see below).

Etymology. The name of the new species is derived from Latin *reticulatus* meaning *reticulate* and refers to strongly reticulated dorsal idiosomal shields.

Eustigmaeus pseudolacunus sp.n.
(Figs. 9–16)

Description. *Female* (Figs. 9–14). Idiosoma broadly-ovate. Length of idiosoma 370 (360–415, maximum width 310 (305–320).

Idiosomal dorsum (Figs. 9A, 13, 14A). Prodorsal and hysterosomal shields almost completely cover dorsal idiosoma. Dorsal idiosomal setae baculiform, weakly barbed, with rounded tips; their tips with distinct hyaline sheaths. Dorsal shields with large round dimples and tiny puncta; lateral parts of dorsal shields less sclerotized than central parts; lateral parts without reticulation. Ocelli present. Suranal shield located ventrally and clearly reticulated. Two pairs of callosities present; major callosity slightly larger than minor one and located on prodorsal shield anterolaterad setae *sce*; minor callosity located on hysterosomal shield posterolaterad setae *d2* (Fig. 14A). Cupules not evident. Female holotype without left seta *e1* (Fig. 13). Lengths of dorsal setae: *vi* 46 (43–52), *ve* 70 (70–82), *sci* 40 (38–45), *sce* 49(47–51), *c1* 49 (49–57), *c2* 35 (33–37), *d1* 50 (50–57), *d2* 46 (40–46), *e1* 53 (53–54), *e2* 40 (38–40), *f* 50 (50–53), *h1* 44 (48–50), *h2* 39 (39–45).

Idiosomal venter (Figs. 9B, 14B, C). Three pairs of aggenital setae. Aggenital, humeral and endopodal plates with distinct subcuticular reticulation; coxal fields I–IV punctate (Fig. 14C). All ventral setae pointed and smooth or with few weak barbs; setae *ps2* about three times shorter than *ps1* and *ps3* (Fig. 14B). Lengths of ventral setae: *1a* 31 (31–35), *1b* 31 (30–33), *1c* 26 (26–30), *2b* 28 (27–29), *2c* 25 (23–25), *3a* 35 (32–36), *3b* 26 (26–28), *3c* 25 (25–27), *4a* 20 (29–32), *4b* 27 (26–28), *4c* 26 (26–29), *ag1* 22 (21–24), *ag2* 26

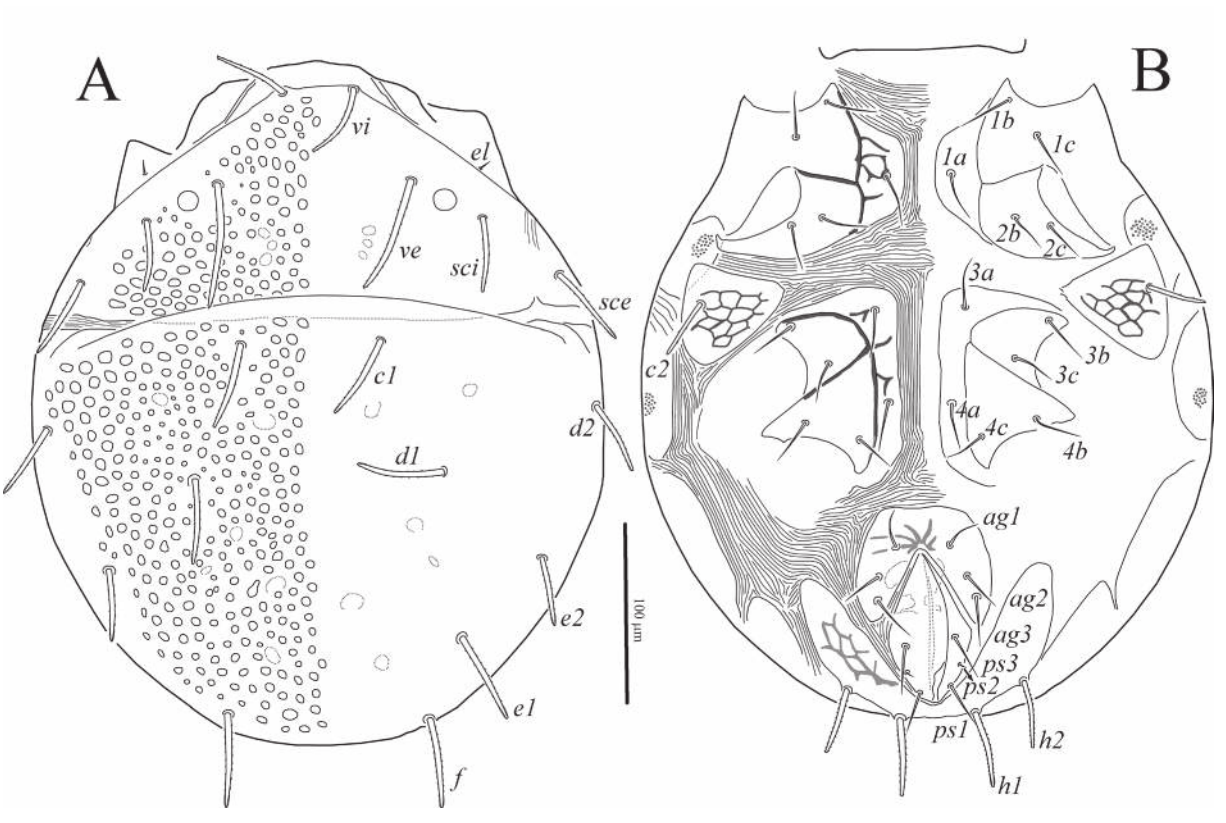

Fig. 9. *Eustigmaeus pseudolacunus* sp.n., female (holotype): A—dorsum of idiosoma, B—venter of idiosoma. Legs omitted.

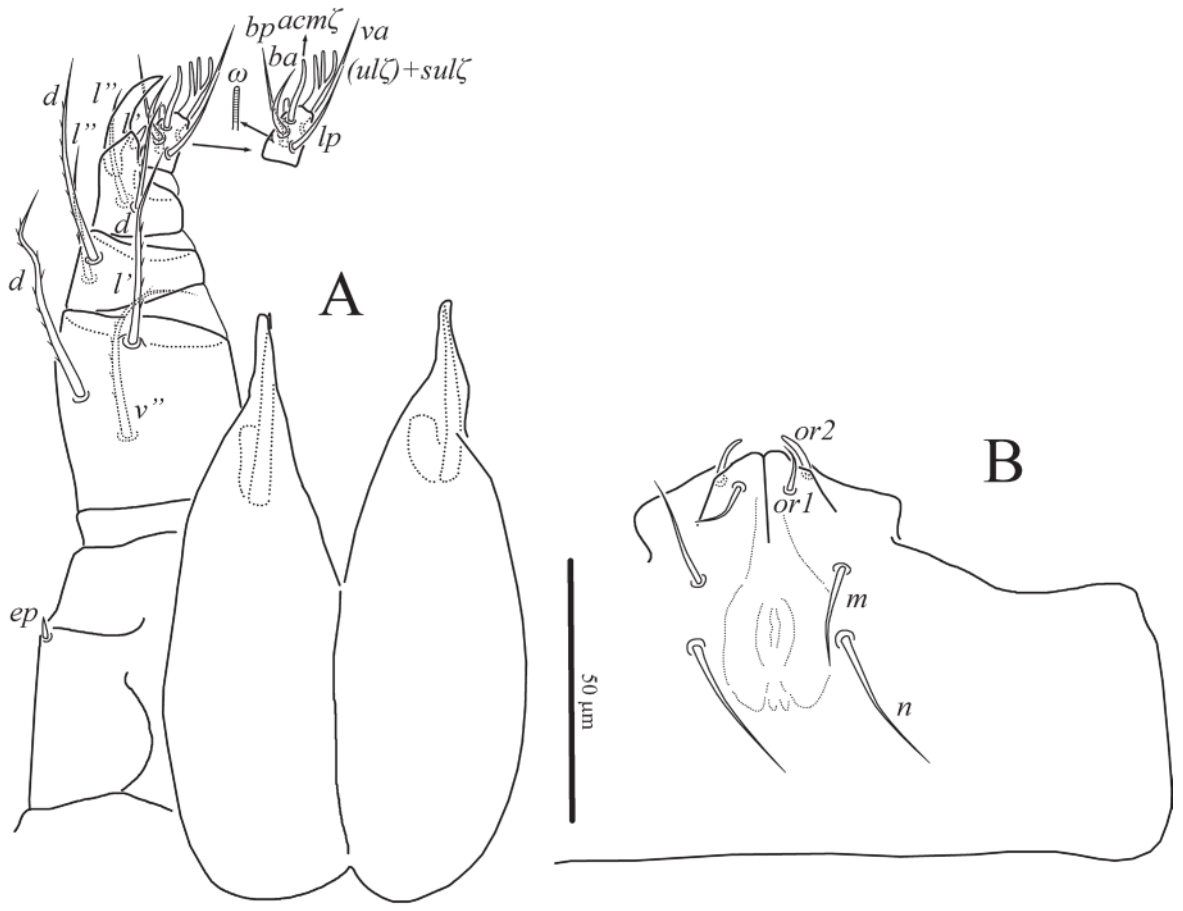

Fig. 10. *Eustigmaeus pseudolacunus* sp.n., female (holotype): A—gnathosoma, dorsal aspect; B—subcapitulum.

(25–27), *ag3* 26 (25–30), *ps1* 25 (23–26), *ps2* 8 (6–9), *ps3* 26 (25–27).

Gnathosoma (Figs. 10, 14D) not covered by anterior part of prodorsal shield. Tibial claw large, distinctly longer than palptarsus. Seta *l'* on palpal tibia short, spiniform, with rounded tip. All setae of femur, genu and tibia barbed; other setae of palptarsus smooth; all palpal setae (except tarsal eupathidia) pointed. Number of setae on palpal segments: Tr 0, Fe 3 (*d*, *l'*, *v''*), Ge 2 (*d*, *l''*), Ti 3 (*d*, *l'*, *l''*), Ta 8(1) (fused eupathidia *ul'*, *ul''*, *sul*, eupathidion *acm*, *ba*, *bp*, *lp*, 1 solenidion *ω*). Palpal supracoxal setae (*ep*) slightly thickened, with rounded tip. Rostrum of subcapitulum very short and wide. Subcapitular setae *or1* distinctly thickened and blunt-tipped, other setae pointed; all subcapitular setae smooth. Basal part of subcapitulum with densely distributed puncta (Fig. 14D). Lengths of subcapitular setae: *m* 21 (21–22), *n* 25 (25–29), *or1* 9 (9–11), *or2* 10 (9–10); length of palps 89 (81–92); length of cheliceral stylets 36 (34–37); length of tarsal solenidion *ω* 7 (7–8).

Legs (Figs. 11, 12). Lengths of legs: I 215 (215–220), II 190 (185–195, III 190 (190–195), IV 225 (220–225). Leg segments without distinct reticulation. Empodial raylets with distinctly widened tips. Leg I (Fig. 11A). Coxae I posterodorsally with short leg supracoxal setae (*el*). Leg setation: Tr 1 (*v'*), Fe 6 (*d*, *l'*, *l''*, *v'*, *v''*, *bv''*), Ge 4 (*d*, *l'*, *l''*, *k*), Ti 5(2) (*dζ*, *l'*, *l''*, *v'*, *v''*, *φ*, *φp*), Ta 13(1) (*p'ζ*, *p''ζ*, *tc'ζ*, *tc''ζ*, *ft'ζ*, *ft''ζ*, *u'*, *u''*, *a'*, *a''*, *pl'*, *pl''*, *vs*, *ω*). Setae *k* of genu, *d* of tibia and (*p*), (*tc*), (*ft*) of tarsus smooth, blunt-tipped, eupathid-like; other setae sparsely barbed; setae *d* of femur and genu blunt-tipped, other setae pointed. Seta *k* 19 (19–22). Solenidion *ω* 28 (26–28) digitiform; solenidion *φ* 11 (10–11) baculiform, *φp* 34 (32–34) attenuate, with rounded tip. Leg II (Fig. 11B). Leg setation: Tr 1 (*v'*), Fe 5 (*d*, *l'*, *l''*, *v'*, *bv''*), Ge 4 (*d*, *l'*, *l''*, *k*), Ti 5(1) (*dζ*, *l'*, *l''*, *v'*, *v''*, *φp*), Ta 9(1) (*p'ζ*, *tc'ζ*, *tc''*, *u'*, *u''*, *a'*, *a''*, *pl'*, *vs*, *ω*). Setae *k* of genu, *d* of tibia, *p'* and *tc'* of tarsus smooth, blunt-tipped, eupathid-like, other setae barbed; setae *d* of femur and genu blunt-tipped, other setae pointed. Sole-

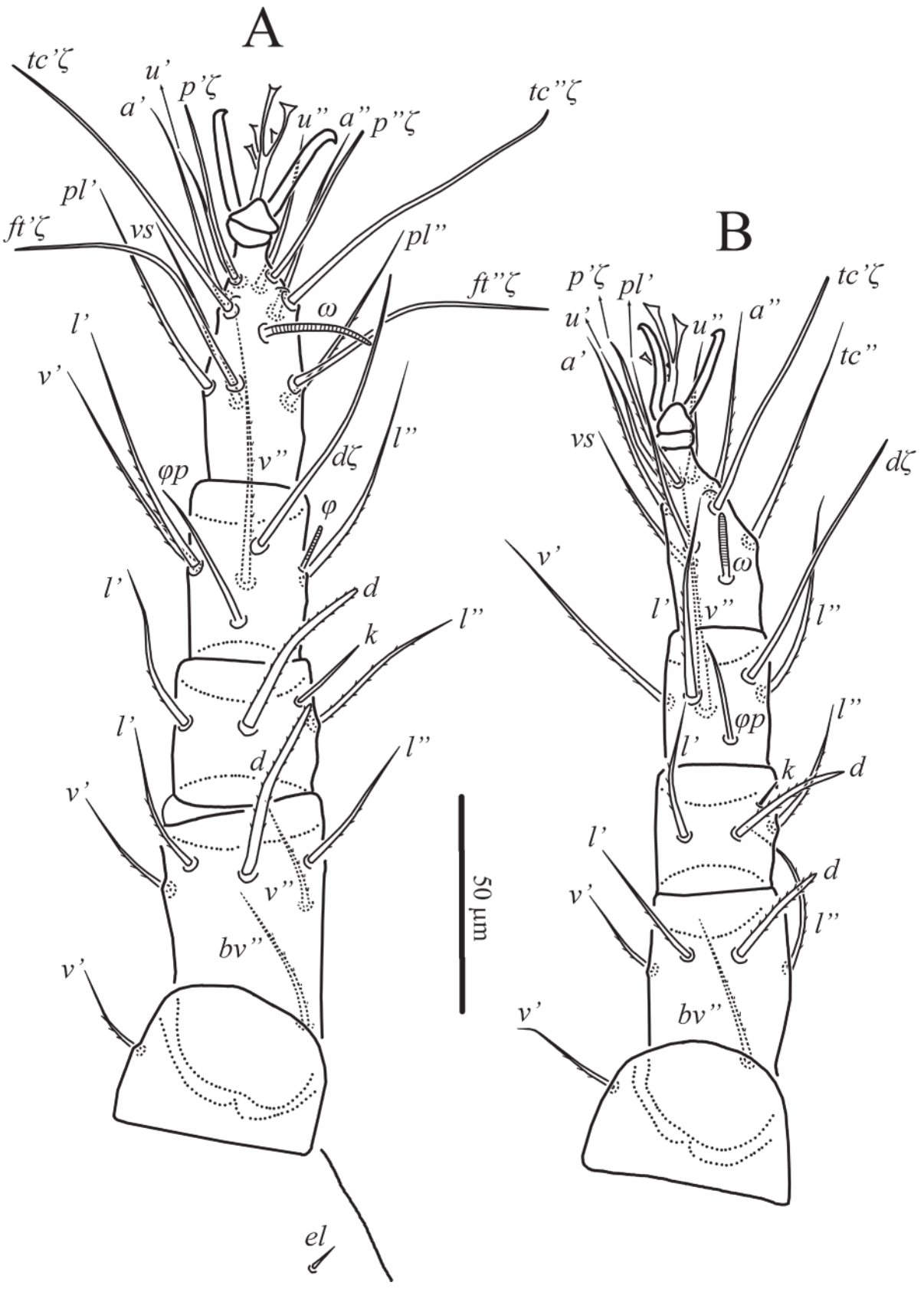

Fig. 11. *Eustigmaeus pseudolacunus* sp.n., female (holotype): A—right leg I, dorsal aspect; B—right leg II, dorsal aspect.

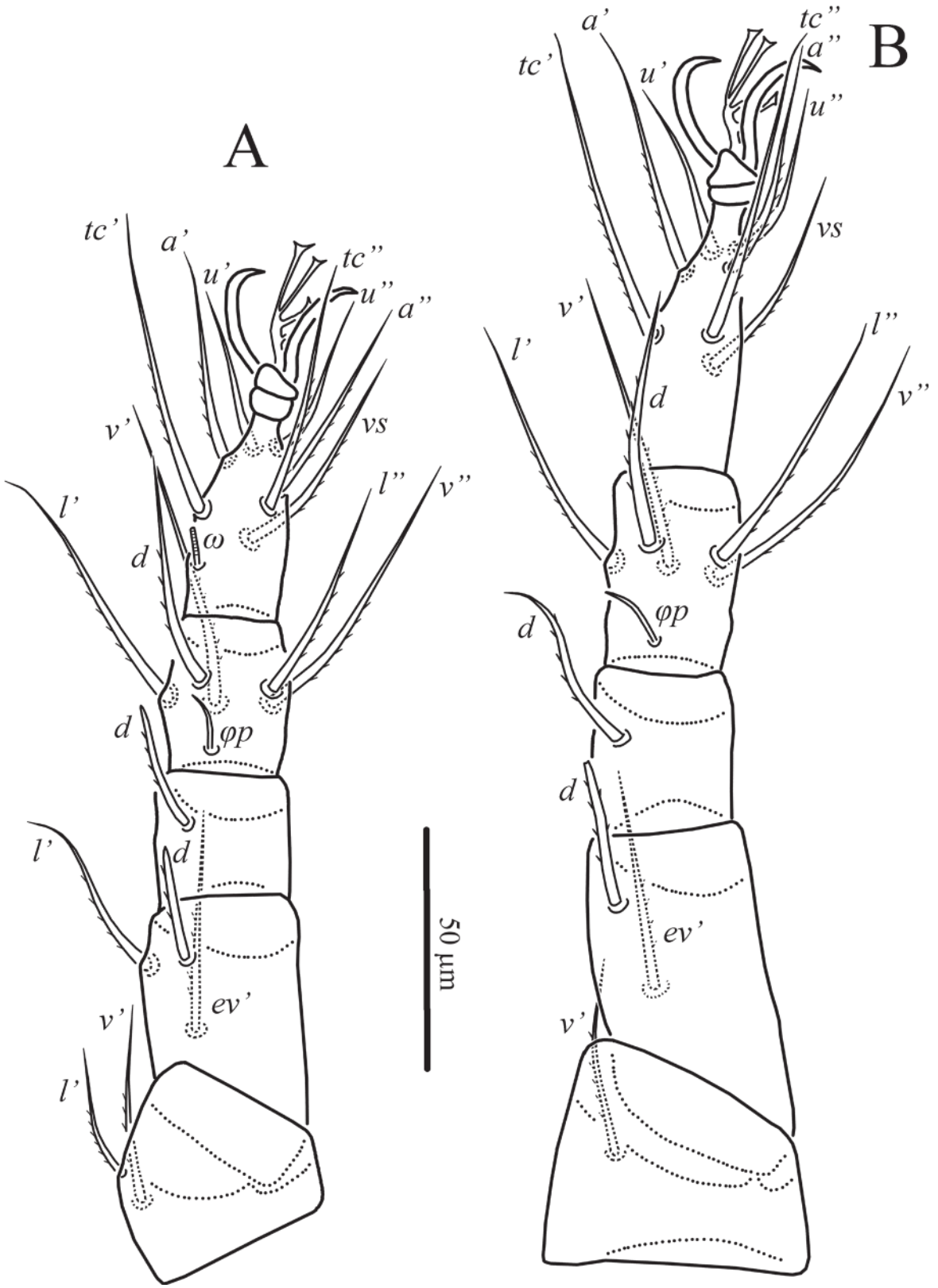

Fig. 12. *Eustigmaeus pseudolacunus* sp.n., female (holotype): A—right leg III, dorsal aspect; B—right leg IV, dorsal aspect.

Fig. 13. Phase contrast micrograph of *Eustigmaeus pseudolacunus* sp.n., female (holotype): general view dorsally.

nidion *ω* 15 (15–17) digitiform; solenidion *φp* 25 (23–25) attenuate, with rounded tip. Seta *k* 8 (8–9).

Leg III (Fig. 12A). Leg setation: Tr 2 (*v'*, *l'*), Fe 3 (*d*, *l'*, *ev'*), Ge 1 (*d*), Ti 5(1) (*d*, *l'*, *l''*, *v'*, *v''*, *φp*),

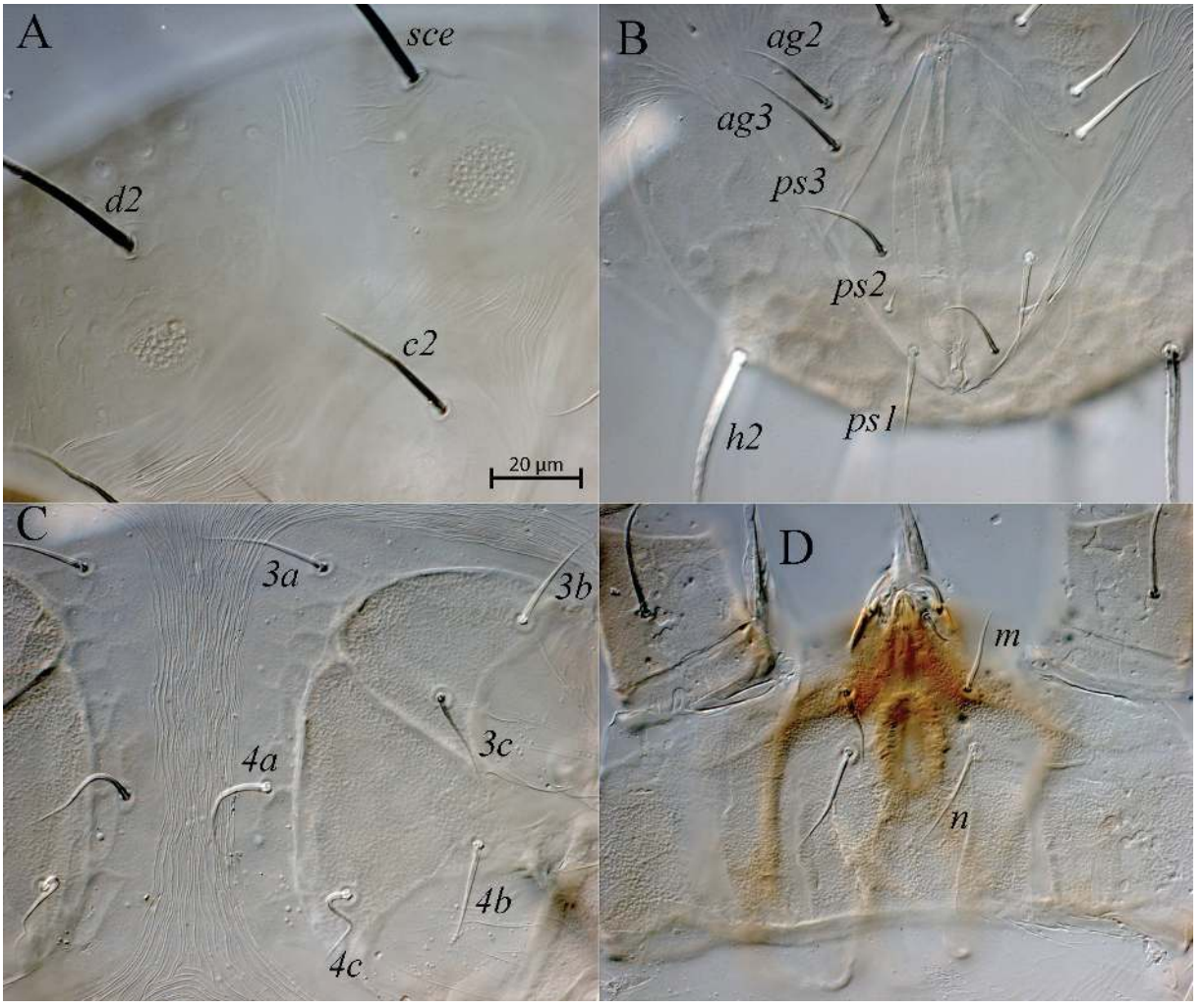

Fig. 14. DIC micrographs of *Eustigmaeus pseudolacunus* sp.n., female: A—major and minor callosities, B—ano-genital area, C—metapodosomal venter, D—subcapitulum.

Ta 7(1) (*tc'*, *tc"*, *u'*, *u"*, *a'*, *a"*, *vs*, *ω*). Solenidion *ω* 9 (8–9) digitiform; solenidion *φp* 15 (15–20) attenuate, with rounded tip. All setae barbed; setae *d* of femur and genu blunt-tipped, other setae pointed. Leg IV (Fig. 12B). Leg setation: Tr 1 (*v'*), Fe 2 (*d*, *ev'*), Ge 1 (*d*), Ti 5(1) (*d*, *l'*, *l"*, *v'*, *v"*, *φp*), Ta 7 (*tc'*, *tc"*, *u'*, *u"*, *a'*, *a"*, *vs*). Solenidion *ω* absent; solenidion *φp* 17 (17–19) attenuate, with rounded tip. All setae weakly barbed; setae *d* of femur blunt-tipped, other setae pointed.

Male (Figs. 15, 16). Length of idiosoma 260–290, width 185–200.

Idiosomal dorsum (Figs. 15A, 16). Podosoma as in female. All dorsal shields with distinct dimples and tiny puncta (Fig. 16). Setae *ps1–3* located dorsally; setae *ps1–2* small, spiniform, setae *ps3* and *h1* pointed and smooth; setae *h2* densely barbed and pointed; setae *e1* weakly barbed and pointed, sometimes setae *e2* also pointed; other dorsal setae blunt-tipped and barbed. Aedeagus well sclerotized, long, narrow and curved. Lengths of dorsal setae: *vi* 56–66, *ve* 80–105, *sci* 43–48, *sce* 51–55, *c1* 57–64, *c2* 33–36, *d1* 61–64, *d2* 43–45, *e1* 39–48, *e2* 28–31, *f* 44–52, *h1* 14–16, *h2* 50–51.

Idiosomal venter (Fig. 15B). Opisthosomal venter with two pairs of smooth and pointed aggenital setae located on large, weakly reticulated aggenital plate; podosomal venter as in female. Lengths of ventral setae: *1a* 29–30, *1b* 23–29, *1c* 22–23, *2b* 23–24, *2c* 22–23, *3a* 20–33, *3b* 26–27, *3c* 22–23, *4a* 23–25, *4b* 22–24, *4c* 23–24, *ag1* 21–25, *ag2* 18–21, *ps1* 4, *ps2* 6, *ps3* 15–16.

Gnathosoma as in female. Lengths of subcapitular setae: *m* 18–19, *n* 23–24, *or1* 8–9, *or2* 8–9; length of palps 70–74; length of palpal solenidion *ω* 7; length of cheliceral stylets 27–29.

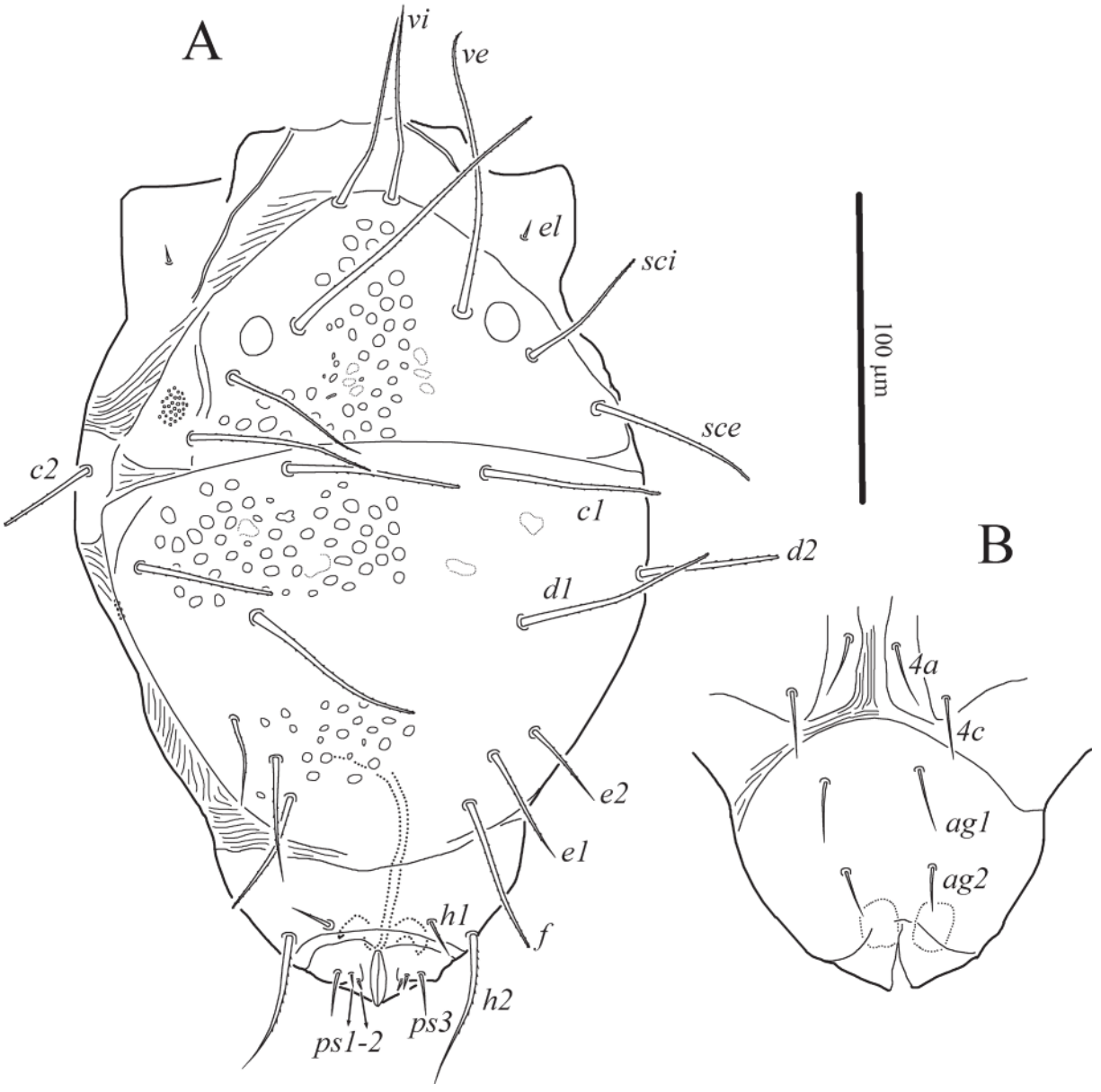

Fig. 15. *Eustigmaeus pseudolacunus* sp.n., male: A—dorsum of idiosoma, B—venter of opisthosoma.

Legs. Legs as in female, except presence of well-developed male solenidia on tarsi I–IV. Lengths of legs: I 180–195, II 150–165, III 160–165, IV 200–205. Lengths of solenidia and setae *k*: *ω*I 25–26, ω♂I 37–39, *φ*I 9–10, *φp*I 29–32, *k*I 16–17, *ω*II 15–16, ω♂II 35–38, *φp*II 21–22, *k*II 8–9, *ω*III 7, ω♂III 35–38, *φp*III 16–17, ω♂IV 33–34, *φp*IV 17–18.

Larva and *nymphs* unknown.

Type material. Female holotype, slide № ZISP T-St-011, Russia, Primorsky Krai, Shotovsky District, 43°08′04.5″ N 132°43′36.6″ E, in wet moss, 14 May 2022, collected by S.V. Kravchenko; paratypes: 17 females, 6 males, same data.

Differential diagnosis. Female of the new species is very similar to *Eustigmaeus lacunus* (Summers, 1957) in having two pairs of callosities, lacking solenidion on tarsus IV, same leg and palpal setations and similar shape of dorsal idiosomal setae. Female of the new species differs from that of *E. lacunus* in smaller body size: width of idiosoma 305–315 (vs. 355–395 in *E. lacunus* after Khaustov (2022)); dorsal idiosomal setae with clearly visible hyaline sheaths (vs. hyaline sheaths not visible in *E. lacunus*); distinctly larger dimples on dorsal idiosomal shields, especially in the central part of prodorsal shield (vs. dimples distinctly smaller in *E. lacunus* as illustrated in Figs. 15A and

Fig. 16. Phase contrast micrograph of *Eustigmaeus pseudolacunus* sp.n., male: idiosoma in dorsal view.

21 A, B in Khaustov (2022)); and shorter setae *ps2* 6–9 (vs. setae *ps2* 11–15 in *E. lacunus*). Compared to females, male of the new species is easier separable from that of *E. lacunus* in having distinctly

longer dorsal idiosomal setae *vi* 56–66, *ve* 80–105, *sce* 51–55, *c1* 57–64, *d1* 61–64 (vs. *vi* 45–49, *ve* 64–73, *sce* 42–46, *c1* 39–43, *d1* 44–48 in *E. lacunus*).

Etymology. The name of the new species is a combination of two words: Greek *ψευδής* meaning *false* and *lacunus*, the specific epithet of the closest species. This name refers to the similarity between the species.

Synonymy of *Eustigmaeus mineus* Barilo, 1989 and *Eustigmaeus collarti* (Cooreman, 1955)

During this study we have examined the holotype of *Eustigmaeus mineus* Barilo, 1989 for the purposes of comparing it with *Eustigmaeus reticulatus* sp.n. The holotype of *E. mineus* is in rather bad condition (Fig. 17), but still comparable with other species, and its main morphological charac-

Fig. 17. DIC micrograph of *Eustigmaeus mineus* Barilo, 1989, female (holotype): general view dorsally.

ters (the length and the shape of setae, leg setation, etc.) are clearly visible. As a result of our study, we have revealed that the female holotype of *E. mineus* is not separable from *E. collarti*. Therefore, we consider *E. mineus* a junior synonym of *E. collarti* (new synonymy).

Key to the species of the genus *Eustigmaeus* of Russia (females)

1. With one or two pairs of callosities …… 2
— Callosities absent, or unpaired callosity present between legs IV ……11
2. Minor callosity present; setae *4a* present ……3
— Minor callosity absent; setae *4a* absent … …… *E. parvisetus* (Chaudhri, 1965)
3. Setae *e2* present …… 4
— Setae *e2* absent …… …… *E. extremiorientalis* Khaustov, 2016
4. Solenidion on tarsus IV absent ……..… 5
— Solenidion on tarsus IV present …… 6
5. Width of idiosoma 305–320; dimples on dorsal idiosomal shields large; setae *ps2* 6–9 long …… …… *E. pseudolacunus* sp.n.
— Width of idiosoma 355–395; dimples on dorsal idiosomal shields smaller, especially in central part of prodorsum; setae *ps2* 11–15 long …… …… *E. lacunus* (Summers, 1957)
6. Setae *e1* and *e2* subequal in length …… 7
— setae *e2* very short, spiniform, much shorter than *e1* …… *E. acidophilus* (Wood, 1972)
7. Three pairs of aggenital setae ……8
— Two pairs of aggenital setae …… …… *E. grandis* Khaustov, 2019
8. Minor callosity situated very close to major callosity; distance between minor and major callosities less than diameter of minor callosity …… 9
— Minor callosity situated very far from major callosity; distance between minor and major callosities much larger than diameter of minor callosity …… 10
9. Dorsal idiosomal setae longer: *ve* 77–82, *sce* 63–66, *c1* 56–65, *d1* 64–69, *d2* 59–65, *e1* 71–73, *e2* 59–61 …… *E. summersi* Khaustov, 2021
— Dorsal idiosomal setae shorter: *ve* 55–61, *sce* 46–51, *c1* 40–46, *d1* 42–48, *d2* 44–53, *e1* 51–59, *e2* 44–50 …… *E. ottavii* (Berlese, 1910)
10. Distance between bases of setae *d1–d1* only slightly longer than distance *c1–c1*; dorsal idiosomal plates almost smooth …… …… *E. rhodomelus* (C.L. Koch, 1841)
— Distance between bases of setae *d1–d1* more than two times longer than distance *c1–c1*; dorsal idiosomal plates with large lateral and small median dimples …… … *E. tjumeniensis* Khaustov and Tolstikov, 2014
11. Ocelli present …… 12
— Ocelli absent … *E. coronaria* (Kuznetsov, 1977)
12. Endopodal plates I–II and III–IV separated medially …… 13
— Endopodal plates I–II and III–IV fused medially …… 16
13. Two pairs of aggenital setae; an unpaired callosity absent between legs IV … ……14
— Three pairs of aggenital setae; an unpaired callosity present between legs IV …… …… *E. bochkovi* Khaustov, 2019
14. Dorsal idiosomal setae rod-like; setae *4a* present; solenidion on tarsus IV present …… 15
— Dorsal idiosomal setae clavate; setae *4a* absent; solenidion on tarsus IV absent …… …… *E. jiangxiensis* Hu, Chen and Huang, 1996
15. Idiosomal setae *h1* and *h2* located ventrally; dorsal hysterosomal setae with distinct hyaline sheaths …… *E. reticulatus* sp.n.
— Idiosomal setae *h1* and *h2* located dorsally; dorsal hysterosomal setae without hyaline sheaths …… *E. collarti* (Cooreman, 1955)
16. Three pairs of aggenital setae; tarsus II with 9 setae and solenidion; seta *k* on genu II present … …… 17
— One pair of aggenital setae; tarsus II with 8 setae and solenidion; seta *k* on genu II absent … …… *E. segnis* (C.L. Koch, 1836)
17. Femur II with four setae; dorsal idiosomal setae variable in shape …… 18
— Femur II with five setae; dorsal idiosomal setae plumose …… *E. plumifer* (Halbert, 1923)
18. All dorsal setae similar in shape; dorsal hysterosomal shield without deep lateral incisions … …… 19
— Most of dorsal setae needle-like and smooth, setae *vi*, *f*, *h1* and *h2* distinctly barbed and slightly widened distally; dorsal hysterosomal shield with deep lateral incisions laterad setae *e2* …… …… *E. bisetalis* (Doğan, 2004)
19. Dorsal idiosomal setae fan- or rod-shaped … ……20
— Dorsal idiosomal setae lanceolate and curved …… *E. pinnatus* (Kuznetsov, 1977)
20. Dorsal idiosomal setae rod-shaped …… …… *E. anauniensis* (Canestrini, 1889)
— Dorsal idiosomal setae fan-shaped …… …… *E. vacuus* Doğan, 2005

COI-barcoding

Taxa that are closest to *E. reticulatus* sp.n. and *E. pseudolacunus* sp.n. based on COI sequences are shown in Table 1. *Eustigmaeus reticulatus* sp.n. from Siberia is closest to the unidentified stigmaeid mites from Canada (*p*-distance 6.01-9.20%) and *E. collarti* from Siberia (*p*-distance 9.24-9.52%). Some of the unidentified stigmaeid mites from Canada clustered with *E. collarti* according to the phylogenetic analysis (a clade with high bootstrap support 76% and within-group mean *p*-distance 1.76%).

Eustigmaeus pseudolacunus sp.n. from the Far East and the unidentified stigmaeid mites from Canada formed a single clade (bootstrap support 100%) that split into two lineages, *A* and *B* (Fig. 18). Thus, they may represent a single species with deep intraspecific divergence or separate species (*p*-distance between *A* lineage and *B* lineage is 5.37%). At this point, it is necessary to study the morphology of the Canadian specimens.

Table 1. Best matches to stigmaeid sequences from the GenBank

BLASTn			BLASTx		
Closest matches in GenBank	Accession no. of closest match	Nucleotide identify, %	Closest matches in GenBank	Accession no. of closest match	Protein identify, %
***E. reticulatus* sp.n.** X-BIOTU St-56, OP960175					
Stigmaeidae sp. BIOUG06909-F08	KM831849	93.99–90.80	Stigmaeidae sp. BIOUG01099-17	JX835485	98.62–98.42
BIOUG01099-17	JX835485		DPMIT-34-55	KR069793	
DPMIT-34-55	KR069793		DPMIT-34-34	KR070345	
DPMIT-34-34	KR070345				
BIOUG01937-90	JX838177				
BIOUG30841-H03	MN352486				
BIOUG01937-89	JX834484				
Eustigmaeus collarti X-BIOTU St-33	OL863255	90.76–90.48	*Eustigmaeus collarti* X-BIOTU St-33	OL863255	96.73–95.86
X-BIOTU St-44	OP960166		X-BIOTU St-53	OP960162	
X-BIOTU St-53	OP960162		X-BIOTU St-44	OP960166	
***E. pseudolacunus* sp.n.** X-BIOTU St-60, OP960176					
Stigmaeidae sp. 08DPMIT-0077	JX835074	96.13–94.98	Stigmaeidae sp. CHARS00089-E02	MN677435	97.69–97.17
08DPMIT-0076	JX834470		CHARS00089-D11	MN666904	
08DPMIT-0073	JX834813				
08DPMIT-0075	JX835763				
08DPMIT-0074	JX837936		*Eustigmaeus rhodomelus* X-BIOTU St-15	OL863247	94.38–93.17
CHARS00089-B10	MN680399			OP960172	
CHARS00089-D11	MN666904		X-BIOTU St-51		
CHARS00089-B01	MN674356				
CHARS00137-E03	MN680977				
CHARS00089-E02	MN677435				

DISCUSSION

Wainstein and Kuznetsov (1978) reported and illustrated *Eustigmaeus clavatus* (Canestrini and Fanzago, 1976) from European Russia. However, the illustration of this species is identical to the descriptions of *E. jiangxiensis*. In particular, both species lack setae *4a* and have the same shape of dorsal idiosomal setae and the same dorsal ornamentation (Faraji *et al*. 2007; Stathakis *et al.* 2016). Undoubtedly, *E. clavatus* sensu Wainstein and Kuznetsov (1978) is conspecific with *E. jiangxiensis*. *Eustigmaeus clavatus* was originally described and illustrated by Canestrini and Fanzago (1876) from Italy. Later, Canestrini (1889) provided a supplementary description and illustrated the dorsal idiosomal seta and dorsal ornamentation. Summers (1957) redescribed this species based on material from North America. Wood (1973) revised the collection of Antonio Berlese and the type material of *E. clavatus*. He confirmed that the specimens from the Berlese collection were the same as the ones described by Summers (1957) from North America. Since then,

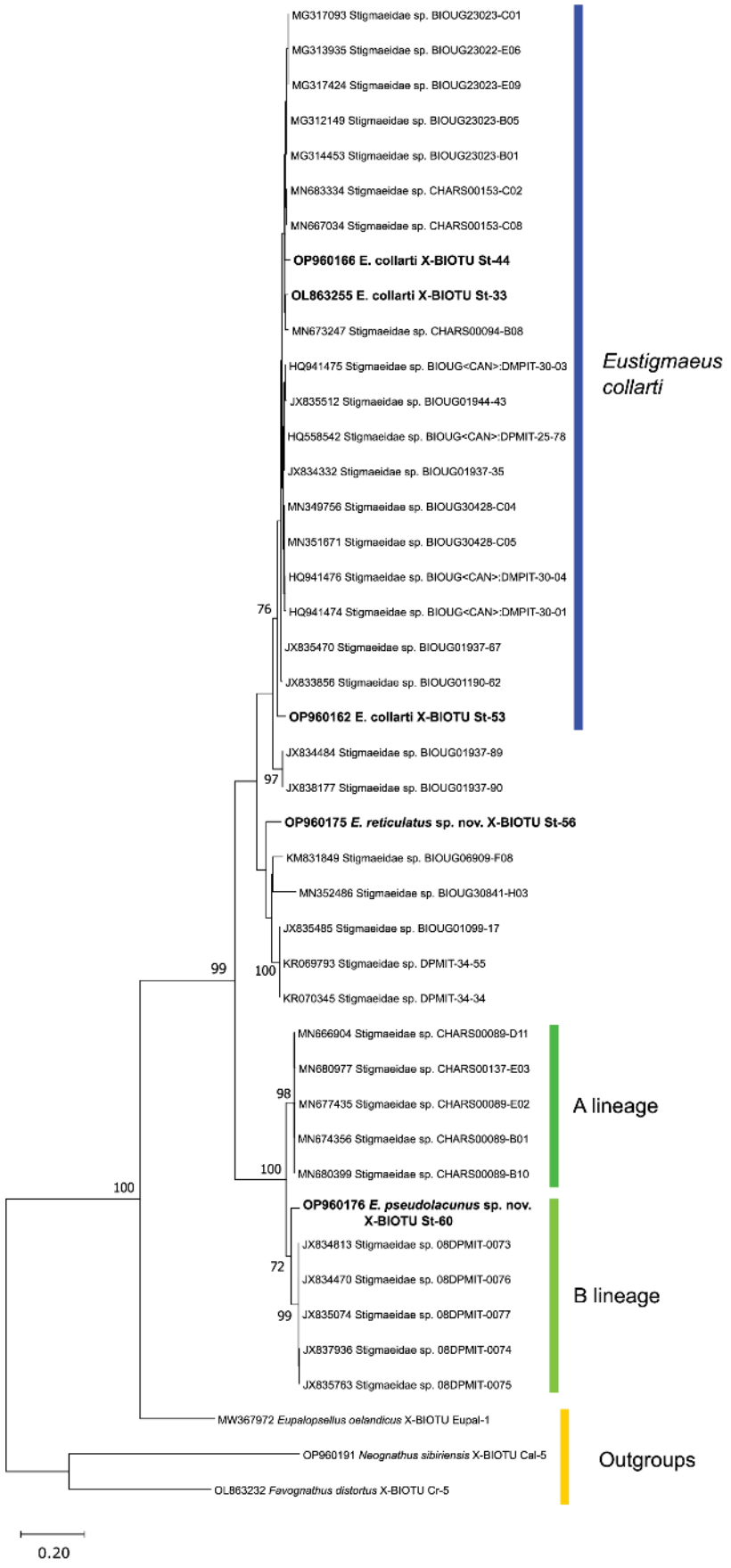

Fig. 18. Maximum Likelihood tree based on 43 COI sequences of stigmaeid mites (*Eustigmaeus* spp. from eastern Russia and "Stigmaeidae sp." from Canada) and three outgroups (Eupalopsellidae, Caligonellidae, Cryptognathidae).

no one has checked the type material of *E. clavatus* and at present, the identifications of this species are based on the redescriptions by Summers (1957) or Wainstein and Kuznetsov (1978). Most likely, *Eustigmaeus clavatus* from Italy is conspecific with *E. jiangxiensis*, but not with specimens redescribed from North America as *E. clavatus* by Summers (1957). This is indicated by the fact that this species has never been redescribed based on European materials from such well-studied Mediterranean countries as Greece and Turkey; however, *E. jiangxiensis* has been recorded (Stathakis *et al.* 2016). For the final clarification of the possible synonymies of *Eustigmaeus clavatus* and *E. jiangxiensis*, an examination of the Berlese collection material is necessary.

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