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Authors: Seniczak, Stanisław, Kaczmarek, Sławomir, and Seniczak, Anna

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## Morphological ontogeny of *Proteremaeus oralensis* sp. nov. (Acari: Oribatida: Eremaeidae) from Kazakhstan, and comments on *Proteremaeus* Piffel

STANISŁAW SENICZAK<sup>1\*</sup>, SŁAWOMIR KACZMAREK<sup>1</sup> & ANNA SENICZAK<sup>2</sup>

<sup>1</sup>Department of Evolutionary Biology, Faculty of Biological Sciences, Kazimierz Wielki University, Bydgoszcz, Poland

<sup>2</sup>Department of Natural History, University Museum of Bergen, University of Bergen, Bergen, Norway

\*Corresponding author: [stseni@ukw.edu.pl](mailto:stseni@ukw.edu.pl)

### Abstract

The morphological ontogeny of *Proteremaeus oralensis* sp. nov. from the elderberry litter (*Sambucus nigra* L.) from Kazakhstan is described and illustrated. The adult of this species is the most similar to that of *P. macleani* Behan-Pelletier, 1982, but differs from it mainly in the shape of lamellar complex, posterior notogastral tip, distribution of genital setae, and some leg characters. The juveniles of *P. oralensis* are oval, light-brown, with short prodorsal and gastrontal setae and clavate bothridial seta. The nymphs are quadrid deficient and eupheredermous, i.e. they lack setae of *d*-series and carry the exuvial scalps of previous instars on the gastrontum. In the nymphs, setae  $p_1$  and  $h_1$  are inserted close to each other, and seta  $h_2$  is placed approximately at similar distances from seta  $p_1$  and  $p_2$ .

**Keywords:** oribatid mites, juveniles, exuvial scalps, leg setation, stage structure

### Introduction

While working on the oribatid mites from elderberry litter (*Sambucus nigra* L.) from Kazakhstan, we found a new species from *Proteremaeus* Piffel, 1965 (Eremaeidae), which was rather abundant and included all developmental instars. This species was the only member of Eremaeidae, so the juveniles and adults undoubtedly belong to it.

*Proteremaeus*, with the type species *P. jonasi* Piffel, 1965 comprises nine nominative species that occupy a Palearctic region (Subías 2004, updated 2020). Diagnosis of the adult of this genus gave Behan-Pelletier and Rjabinin (1991), with the main morphological characters: lamellar seta at tip of lamellar costula, interlamellar seta lateral of lamellar costula and anterior of bothridium; notogaster with 10 or 11 pairs of setae, each with porose ring; discidium present, two pairs of anal and three pairs of adanal setae, ventral plate heavily sclerotized posteriorly, femora with unequal paraxial and anti-axial carinae.

The juvenile stages of *Proteremaeus* are insufficiently known. Based on the catalogue of oribatid juveniles by Norton and Ermilov (2014), the full morphological ontogeny of *P. punctulatus* Bayartogtokh, 2000 has been described, which constitutes 11% of all species of this genus.

The aim of this paper is to describe and illustrate the morphological ontogeny of *P. oralensis* sp. nov., and compare the morphology of this species with congeners.

## Material and methods

The juveniles and adults of *P. oralensis* for this study were collected on 11 July 2019 by Kaczmarek S. from moist litter under elderberry (*Sambucus nigra* L.) growing dense on a slope (150–200 m<sup>2</sup>) in a meadow plain, about 10 m from Derkul river, one km east from Dachi-Novostroyka village (51°16'49"N, 51°17'70"E, 52 m a. s. l., West Kazakhstan). In this habitat, we investigated the density and stage structure of mites, and based on 30 randomly selected specimens, the sex ratio, number of gravid females and carried eggs, and length and width of the body. We measured total body length (from tip of rostrum to posterior edge of notogaster) in lateral aspect and body width (widest part of notogaster) in dorsal aspect, and size of anal and genital openings and setae perpendicularly to their length in  $\mu\text{m}$ .

The illustrations of instars are limited to the body regions of mites that show substantial differences between instars, including the dorsal and lateral aspect and some leg segments of the larva, tritonymph and adult, and ventral regions of all instars. Palp and chelicera of the adult are also illustrated. Illustrations were prepared from individuals mounted temporarily in lactic acid. In the text and figures we used the following abbreviations: rostral (*ro*), lamellar (*le*), interlamellar (*in*) and exobothridial (*ex*) setae, lamellar costula (*Cos*), bothridium (*bo*), bothridial seta (*bs*), notogastral or gastronotal setae (*c*-, *d*-, *l*-, *h*-, *p*-series), exuviae of larva (L), protonymph (Pn) and deutonymph (Dn), lyrifissures or cupules (*ia*, *im*, *ip*, *ih*, *ips*, *iad*, *ian*), integumental pit (*em*), opisthonotal gland opening (*gla*), pedotectum (*Pd*), discidium (*Dis*), subcapitular setae (*a*, *m*, *h*), cheliceral setae (*cha*, *chb*), Trägårdh organ (*Tg*), palp setae (*sup*, *inf*, *l*, *d*, *cm*, *acm*, *it*, *vt*, *ul*, *su*) and solenidion  $\omega$ , epimeral setae (*1a*–*c*, *2a*, *3a*–*c*, *4a*–*c*), adanal and anal setae (*ad*-, *an*-series), aggenital (*ag*) and genital setae (*g*), leg solenidia ( $\sigma$ ,  $\phi$ ,  $\omega$ ), famulus ( $\epsilon$ ) and setae (*bv*, *ev*, *d*, *l*, *ft*, *tc*, *it*, *p*, *u*, *a*, *s*, *pv*, *pl*, *v*). Terminology used follows that of Grandjean (1939, 1949, 1953) and Norton and Behan-Pelletier (2009). The species names follows Subías (2004, updated 2020).

### *Proteremaeus oralensis* sp. nov.

(Figs. 1–15)

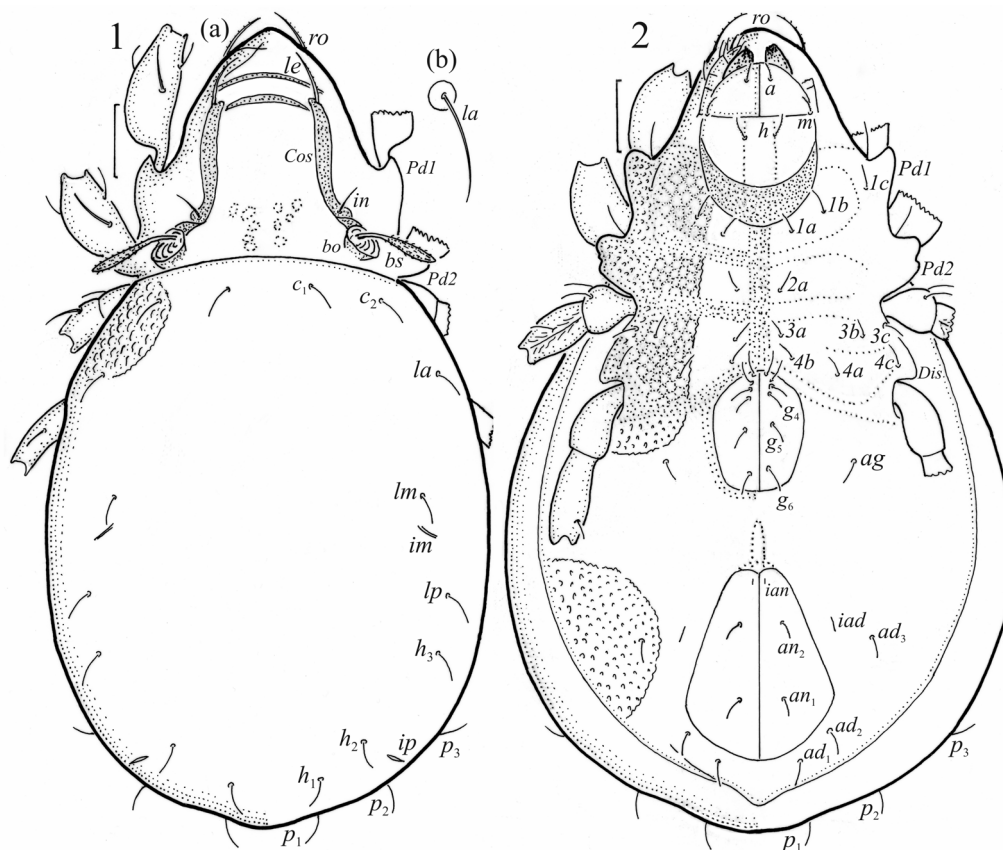
#### Diagnosis

Adult of medium size (507–572), with characters of *Proteremaeus*. Rostral and lamellar setae of medium size and finely barbed, interlamellar seta short and smooth. Lamellar costula long, s-shaped, with seta *le* and *in* in anterior and posterior part, respectively; transverse ridge present between anterior part of lamellar costula, and small triangular ridge between lamellar costula and bothridium. Bothridial seta clavate, with narrow, barbed head. Notogaster with reticulate pattern and 11 pairs of short setae, posterior tip of notogaster rounded. Adanal and anal setae with basal porose ring.

Juveniles oval, light-brown. Prodorsum without ridges in central part, and with short setae, but *le* longer than *in*. Bothridium rounded, bothridial seta clavate, with barbed head. Gastronotum with 12 pairs of short setae, nymphs quadridentate and eupheredermous, carrying exuvial scalps of previous instars, most setae inserted in peripheral part of gastronotum. Posterior setae *p*<sub>1</sub> and *h*<sub>1</sub> inserted close to each other, seta *h*<sub>2</sub> inserted approximately at similar distances from seta *p*<sub>1</sub> and *p*<sub>2</sub>.

#### Morphology of adult

**Measurements.** Mean length (and range) of females 565.0±8.2 (553–572, n= 13) and males 525.8±10.9 (507–540, n= 17), mean width (and range) of females 352.0±8.7 (338–358) and males 307.2±22.8 (267–332).



**FIGURES 1–2.** *Proteremaeus oralensis* sp. nov., female, legs partially drawn, scale bars 50  $\mu$ m. 1. (a) Dorsal aspect, (b) seta *la* (enlarged). 2. Ventral aspect.

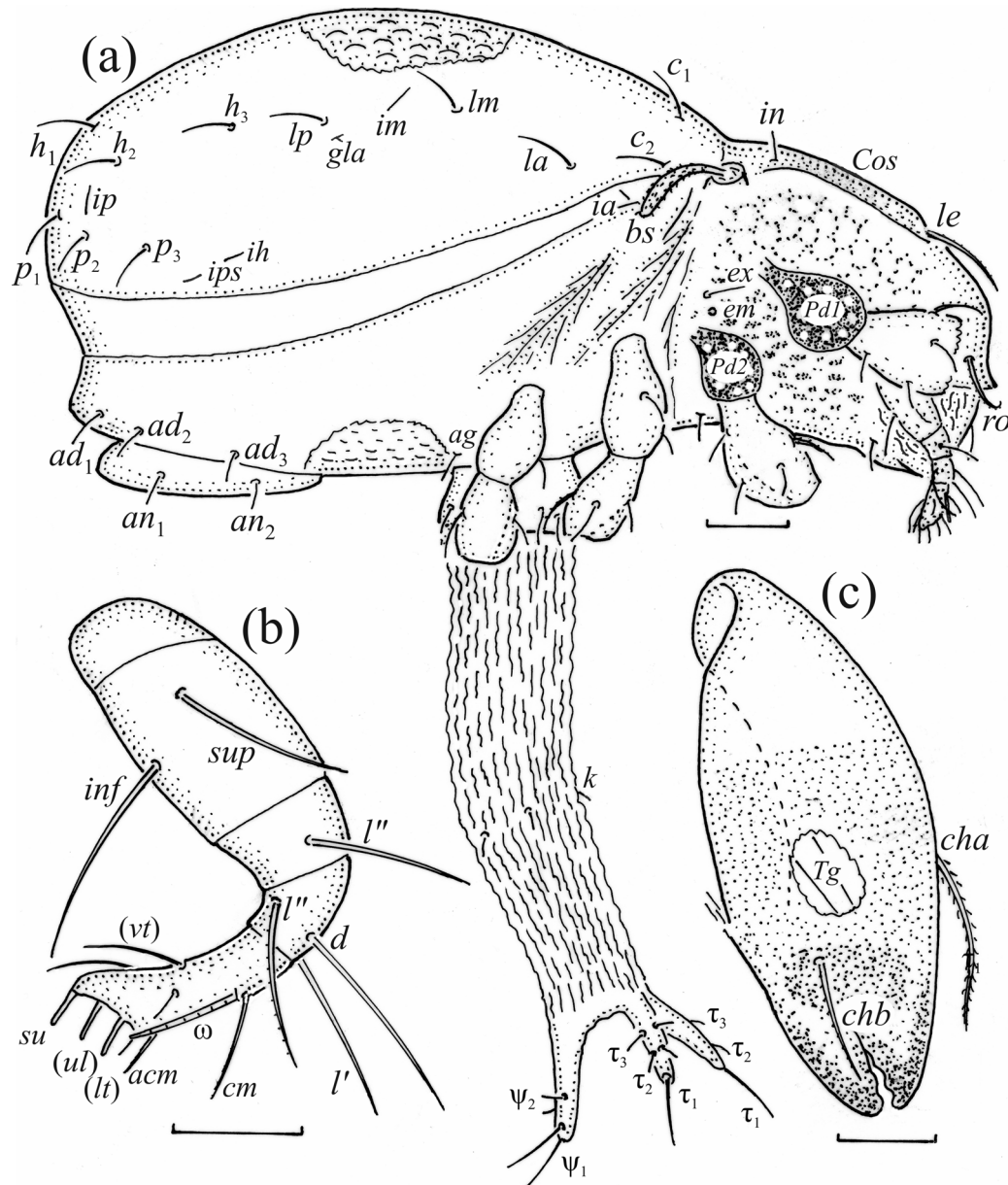
**Integument.** Most parts of body with reticulate pattern, well observed on notogaster, lateral parts of prodorsum, tectopodia I and II and epimeres (Figs. 1, 2, 3a, 5, 6), and covered with granular cerotegument.

**Prodorsum.** Rostrum rounded, rostral seta of medium size (length 28–30), finely barbed, inserted on lateral part of rostrum (Figs. 1, 2, 3a, 5, 6a). Lamellar costula long, narrow (90–101  $\times$  5), s-shaped, located on lateral part of prodorsum, with seta *le* and *in* in anterior and posterior part, respectively; small triangular ridge present between lamellar costula and bothridium. Lamellar seta slightly longer (33–35) than rostral seta (27–29), both with very short barbs, interlamellar seta short (12) and smooth. Two transverse ridges present, longer anterior to setal pair *le* and shorter between pair *le*. Bothridium rounded, bothridial seta (*bs*, 42–45) clavate, with narrow, flattened and barbed head (Figs. 1, 3a, 5, 6). Exobothridial seta (*ex*, 15) thin and smooth, inserted closer to pedotectum II than to bothridium. Integumental pit *em* present between seta *ex* and pedotectum II.

**Notogaster.** Longer (368–415) than wide (267–358), with 11 pairs of short setae (26–35). Lyrifissure *ia* posterolateral to seta *c*<sub>2</sub>, *im* posterior to seta *la*, *ip* posterolateral to seta *h*<sub>2</sub>, *ips* and *ih* anterolateral and anterior to seta *p*<sub>3</sub>, respectively, opisthonotal gland opening anterolateral to seta *lp* (Figs. 1, 2, 3a). Posterior tip of notogaster rounded.

**Gnathosoma.** Infracapitulum diarthric, subcapitular setae short (21–25) and barbed (Figs. 2, 7a). Apical part of palp (71) relatively wide, most setae relatively long and smooth, except for barbed tibial *l'*, solenidion  $\omega$  separated from seta *acm* (Figs. 3b, 7a), formula of setae (trochanter to tarsus

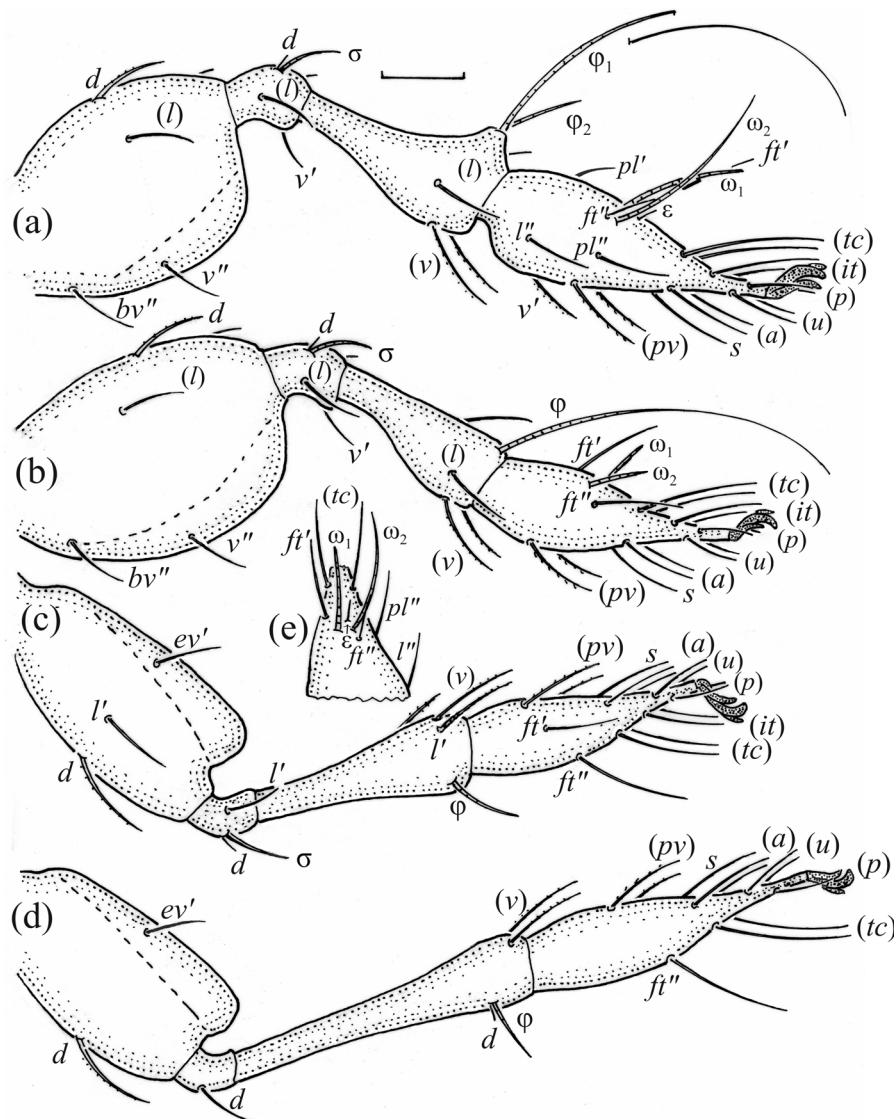
+ solenidion  $\omega$ ): 0-2-1-3-9(1). Chelicera (122 x 55) chelate, seta *cha* longer (35) than *chb* (21), both barbed, barbs on *cha* clearly longer than on *chb* (Figs. 3c, 7a).



**FIGURE 3.** *Proteremaeus oralensis* sp. nov., adult. (a) Lateral aspect, legs partially drawn, scale bar 50  $\mu$ m; mouthparts, right side, scale bars 20  $\mu$ m; (b) palp, (c) chelicera (Trägårdh organ in transparent area).

**Ventral and lateral regions.** Apodemes I, II and IV strongly developed and fused with sternal ridge, apodeme III short. Epimeral setae short (12–15) and smooth, formula of epimeral setae 3-1-3-3. Genital setae (6 pairs), aggenital setae (1 pair), adanal setae (3 pairs) and anal setae (2 pairs) as short as epimeral setae, adanal and anal setae with basal porose ring. Lyrifissure *iad* anteromedial to seta *ad*<sub>3</sub>, *ian* anterior to seta *an*<sub>2</sub>. Ovipositor long, with relatively short apical setae (Fig. 3a).

*Legs.* Trochanters III and IV and all femora flattened, with ventral carina and porose areas on paraxial side. Most leg setae finely barbed or smooth. Seta *d* present on all genua and tibia IV, close to proper solenidion (Fig. 4). Formulae of leg setae (and solenidia, trochanter to tarsus): I—1-5-4(1)-4(2)-20(2); II—1-5-4(1)-4(1)-15(2); III—2-3-2(1)-3(1)-15; IV—1-2-1-3(1)-12. Leg tarsi tridactylous.

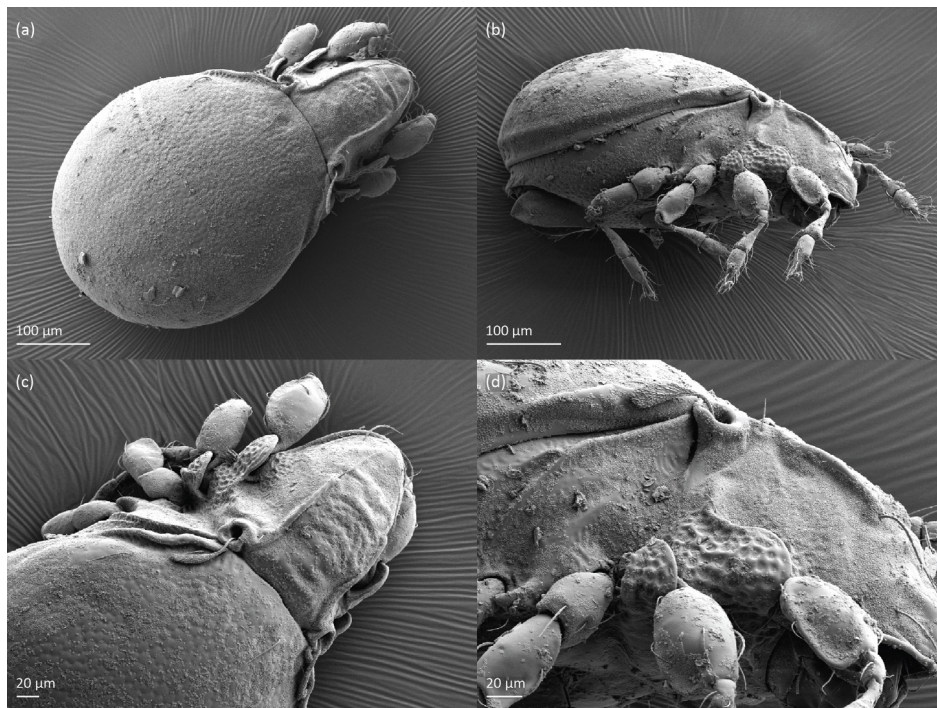


**FIGURE 4.** *Proteremaeus oralensis* sp. nov., leg segments of adult (part of femur to tarsus), right side, antiaxial aspect, scale bar 20  $\mu$ m. (a) Leg I; (b) leg II; (c) leg III; (d) leg IV, (e) location of solenidia on tarsus I.

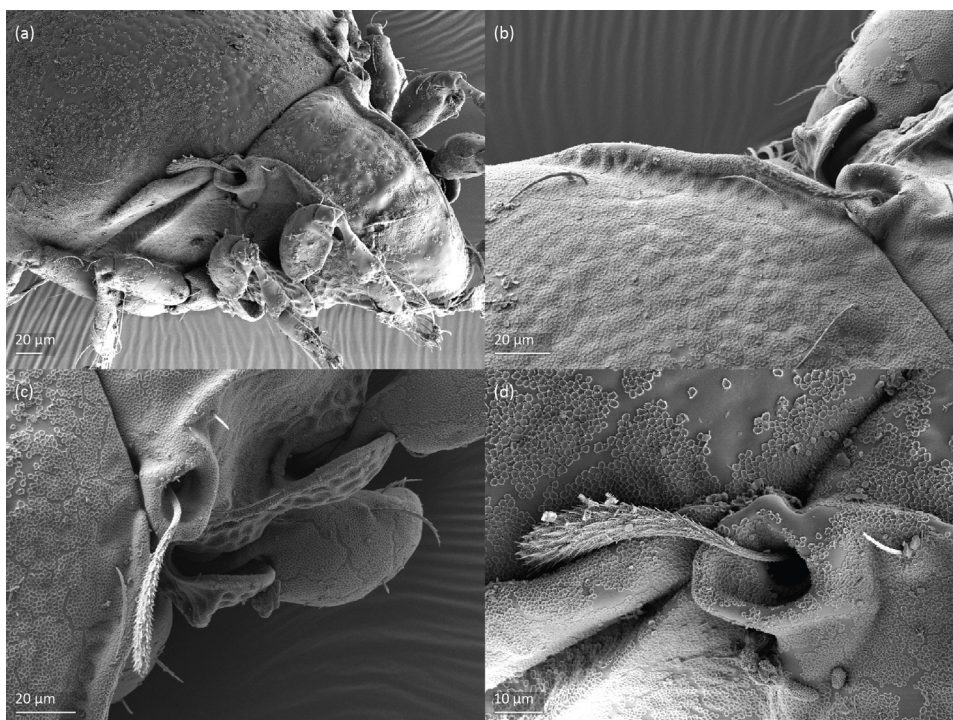
#### Description of juveniles

Larva oval (Fig. 8), body light-brown. Prodorsum subtriangular, without distinct ridges in central part, and with short (Table 1) and smooth setae, *ro* and *le* longer than other setae. Mutual distance between setal pair *le* about two times longer than between pair *ro*, between setal pair *in* about four times longer than between pair *ro*, pair *le* inserted closer to pair *ro* than *in*. Opening of bothridium rounded, bothridial seta clavate, with barbed head. Transverse and inclined folds in medial and posterior part of prodorsum. Integumental pit *em* present posterolateral to seta *ex* (Fig. 10a).





**FIGURE 5.** *Proteremaeus oralensis* **sp. nov.**, adult, SEM micrographs. (a) Dorsal view, (b), lateral view, (c) anterior part of body, dorsolateral view, (d) anterior part of body, lateral view.



**FIGURE 6.** *Proteremaeus oralensis* **sp. nov.**, SEM micrographs. Adult, (a) anterior part of body, dorsolateral view, (b), (c) (d) bothridium and bothridial seta, different views and magnifications.

Gastronotum of larva with 12 pairs of setae, including  $h_3$  inserted lateral to medial part of anal opening (Fig. 9a), all short (Table 1) and smooth. Gastronotum with transverse and inclined folds in anterior and medial parts, and longitudinal folds in lateral parts. Paraproctal valves (segment PS) with two pairs of small setae. Cupule  $ia$  posterolateral to seta  $c_3$ ,  $im$  posterior to seta  $lm$ ,  $ip$  posterior to seta  $h_2$ ,  $ih$  lateral to anterior part of anal valves (Fig. 9a). Gland opening located lateral to seta  $h_2$ . Anal region with transverse and inclined folds, lateral parts of gastronotum with longitudinal folds. Most leg setae smooth or finely barbed (Fig. 11), seta  $d$  present at solenidia on all genua, but thin, short and difficult to observe.

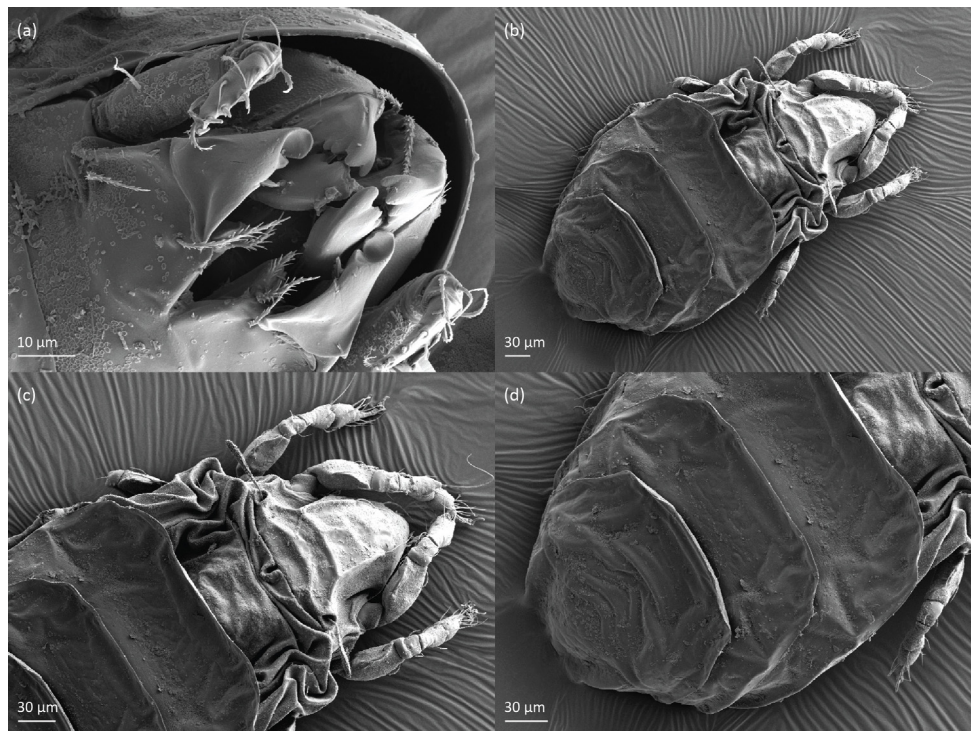
**TABLE 1.** Measurements of some morphological characters of juvenile stages and adult of *Proteremaeus oralensis* sp. nov., (mean measurements of 2–10 specimens in  $\mu\text{m}$ ); Nd: not developed.

Morphological characters	Larva	Protonymph	Deutonymph	Tritonymph	Adult
Body length	281	340	377	527	553
Body width	174	185	241	325	357
Length of prodorsum	54	80	89	99	152
Length of seta $ro$	18	18	21	30	28
seta $le$	17	17	23	33	34
seta $in$	4	4	4	5	12
seta $bs$	33	39	43	51	43
seta $c_1$	3	4	5	8	27
seta $c_2$	4	4	5	7	26
seta $c_3$	4	5	6	8	lost
seta $da$	3	lost	lost	lost	lost
seta $dp$	4	lost	lost	lost	lost
seta $la$	3	4	5	6	35
seta $lp$	4	5	5	7	28
seta $h_1$	4	5	5	6	28
seta $h_3$	3	4	5	6	28
seta $p_1$	Nd	4	5	6	27
seta $p_3$	Nd	4	4	5	27
genital opening	Nd	29	45	63	78
anal opening	61	79	98	112	126

Protonymph more stocky (Fig. 9b) than larva, body light-brown. Prodorsum, prodorsal setae and bothridium as in larva, but bothridial seta with slimmer head than in larva. Gastronotum of protonymph with 12 pairs of setae because  $p$ -series appearing and remaining in deutonymph and tritonymph (Figs. 12a, 12b), and setae of  $d$ -series lost and remaining absent in all nymphs (Figs. 10b, 13), all short and most inserted in peripheral part of gastronotum. In protonymph, one pair of genital setae appearing on genital valves, and two pairs added in deutonymph and tritonymph each (Figs. 12a, 12b), all short and smooth. In deutonymph, one pair of aggenital setae and three pairs of adanal setae appearing, and remaining in tritonymph, all short and smooth. In protonymph and deutonymph, anal valves glabrous, in tritonymph two pairs of short and smooth anal setae present (Figs. 9b, 12a, 12b). All nymphs carrying exuvial scalps of previous instars on gastronotum (Figs. 7a–c, 10b, 13). After removal of these exuviae, dorsocentral part glabrous. In light microscope, prodorsum of tritonymph with transverse and inclined folds, in SEM micrographs with two longitudinal ridges and



transverse ridges (Figs. 7b, 7c, 13). Setae  $p_1$  and  $h_1$  inserted close to each other, seta  $h_2$  inserted approximately at similar distances from seta  $p_1$  and  $p_2$  (Figs. 9b, 12a, 12b). Cupule  $ia$  and  $im$  located as in larva, cupule  $ip$  posterolateral (protonymph, deutonymph) or posterior to seta  $h_2$  (trityonymph), cupule  $iad$  lateral to anterior part of anal opening, cupule  $ips$  and  $ih$  displaced anterolateral and lateral from cupule  $iad$ , respectively, cupule  $ian$  anterior to seta  $an_2$ , gland opening anterolateral to seta  $ad_3$  (Figs. 9b, 10b, 12, 14b–d). Anogenital region with transverse and inclined folds, lateral parts with longitudinal folds. Most leg setae smooth or finely barbed (Fig. 15), seta  $d$  present at solenidia on all genua and tibia IV, but thin, short and difficult to observe, at other solenidia on tibiae this seta not observed.



**FIGURE 7.** *Proteremaeus oralensis* sp. nov., SEM micrographs. (a) Mouthparts of adult, ventral view; tritonymph, dorsal view, (b) whole body, (c) anterior and medial part of body, (d) posterior part of body.

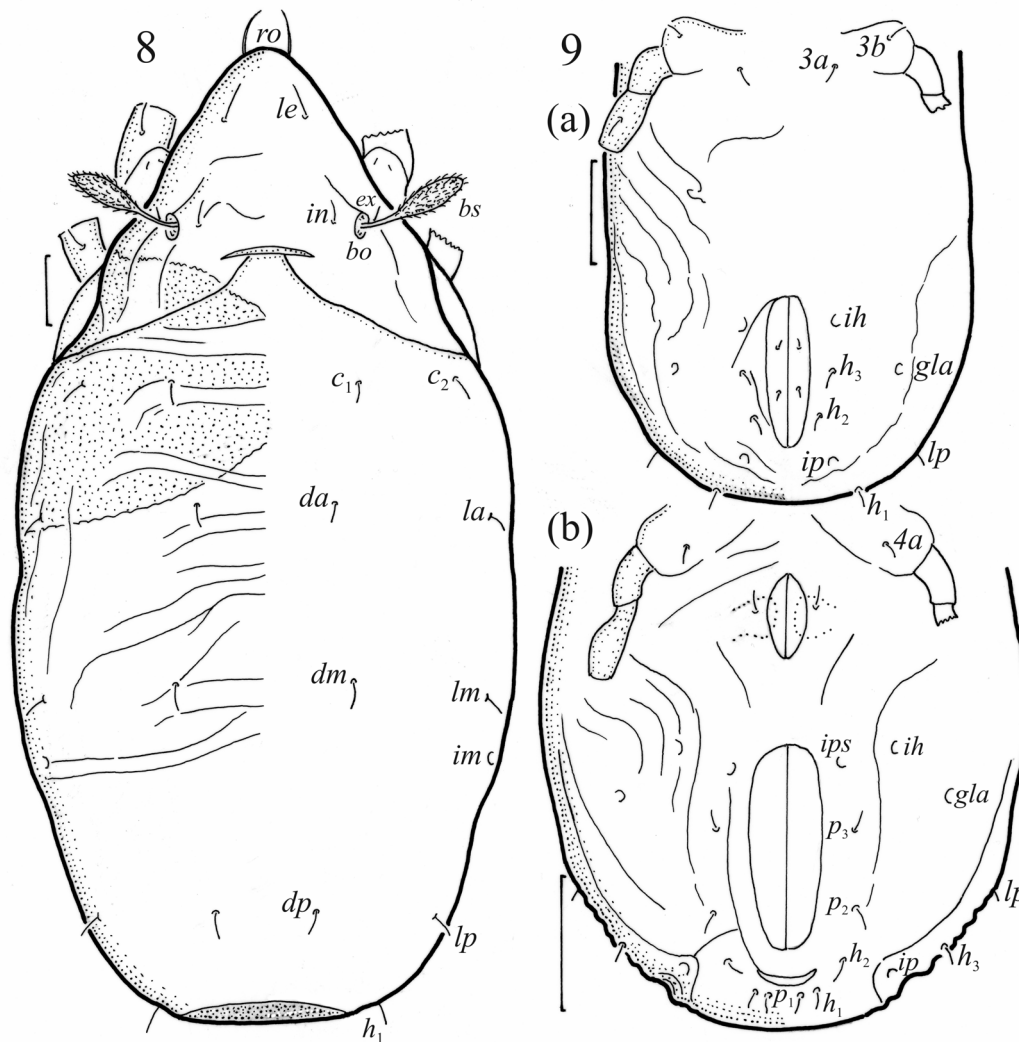
#### Summary of ontogenetic transformations

In all juveniles, the prodorsal setae are short, whereas in the adult  $ro$  and  $le$  are of medium size, and other setae are short. The bothridium is rounded in all instars, and the bothridial seta is clavate, but in the larva the head of the bothridial seta is relatively thicker than in the nymphs and adult. The larva has 12 pairs of gastronotal setae and so have the nymphs ( $p$ -series appears,  $d$ -series lost). The notogaster of the adult loses seta  $c_3$ , such that and 11 pairs of setae remain. The formula of gastronotal setae in *P. oralensis* is 12-12-12-12-11 (larva to adult), the formulae of epimeral setae are: 3-1-2 (larva, including scaliform  $lc$ ), 3-1-2-1 (protonymph), 3-1-2-2 (deutonymph) and 3-1-3-3 (tritonymph and adult). The formula of genital setae is 1-3-5-6 (protonymph to adult), that of aggenital setae is 1-1-1 (deutonymph to adult), and the formula of segments PS-AN is 23333-0333-022. The ontogeny of leg setae and solenidia of *P. oralensis* is given in Table 2.

#### Ecology and biology

*Proteremaeus oralensis* was relatively abundant in moist elderberry litter (134 indiv./500cm<sup>3</sup>) near Dachi-Novostroyka village (West Kazakhstan). In this habitat, the juveniles dominated,

constituting 53% of all individuals. The stage structure of this species was the following: 2 larvae, 7 protonymphs, 70 deutonymphs, 62 tritonymphs and 126 adults. In this population, the sex ratio (females to males) was 1:1.3, and only two females were gravid, carrying 1 large egg each (290 × 132), which comprised 51% of the length of females.



**FIGURES 8–9.** *Proteremaeus oralensis* sp. nov., larva, legs partially drawn. 8. (a) Dorsal aspect, scale bar 20  $\mu$ m. 9. Ventral part of hysterosoma, scale bars 50  $\mu$ m, (a) larva, (b) protonymph.

#### Type material deposition

Holotype female and five paratypes (two females and three males) are deposited in the University Museum of Bergen, University of Bergen, Bergen, Norway.

#### Etymology

The species name follows the Kazakhstanian name Oral (Uralsk), in which surroundings this species was found.

**TABLE 2.** Ontogeny of leg setae (Roman letters) and solenidia (Greek letters) in *Proteremaeus oralensis* **sp. nov.**

Leg	Trochanter	Femur	Genu	Tibia	Tarsus
Leg I					
Larva	–	<i>d, bv''</i>	<i>(l), dσ</i>	<i>(l), v', φ<sub>1</sub></i>	<i>(fi), (tc), (p), (u), (a), s, (pv), (pl), ε, ω<sub>1</sub></i>
Protonymph	–	–	–	–	<i>ω<sub>2</sub></i>
Deutonymph	–	<i>(l)</i>	–	<i>φ<sub>2</sub></i>	–
Tritonymph	<i>v'</i>	–	–	<i>v''</i>	<i>(it)</i>
Adult	–	<i>v''</i>	<i>v'</i>	–	<i>v', l''</i>
Leg II					
Larva	–	<i>d, bv''</i>	<i>(l), dσ</i>	<i>l', v', φ</i>	<i>(fi), (tc), (p), (u), (a), s, (pv), ω<sub>1</sub></i>
Protonymph	–	–	–	–	–
Deutonymph	–	<i>(l)</i>	–	<i>l''</i>	<i>ω<sub>2</sub></i>
Tritonymph	<i>v'</i>	–	–	<i>v''</i>	<i>(it)</i>
Adult	–	<i>v''</i>	<i>v'</i>	–	–
Leg III					
Larva	–	<i>d, ev'</i>	<i>l', dσ</i>	<i>v', φ</i>	<i>(fi), (tc), (p), (u), (a), s, (pv)</i>
Protonymph	–	–	–	–	–
Deutonymph	<i>v'</i>	<i>l'</i>	–	–	–
Tritonymph	<i>l'</i>	–	–	<i>l'</i>	<i>(it)</i>
Adult	–	–	–	<i>v''</i>	–
Leg IV					
Protonymph	–	–	–	–	<i>ft'', (p), (u), (pv)</i>
Deutonymph	–	<i>d, ev'</i>	<i>d</i>	<i>v', dφ</i>	<i>(tc), (a), s</i>
Tritonymph	<i>v'</i>	–	–	<i>v''</i>	–
Adult	–	–	–	–	–

Note: structures are indicated where they are first added and are present through the rest of ontogeny; pairs of setae in parentheses, dash indicates no additions.

### Comparison of morphology of *Proteremaeus oralensis* **sp. nov.** with congeners and remarks

Based on the mean length of adults of *Proteremaeus* species, the largest is *P. oralensis* **sp. nov.**, and smallest is *P. punctulatus*, while the body length of *P. jonasi* Piffel, 1965 is unknown (Table 3). In most species, the bothridial seta is clavate, but in *P. chadaevae* Golosova, 1983, *P. elongatus* (Rjabinin & Krivolutsky, 1975) and *P. nebaikini* Behan-Pelletier & Rjabinin, 1991 it is fusiform. Most species have the transverse ridge between lamellar setae, whereas in *P. chadaevae*, *P. jonasi*, *P. macleani* Behan-Pelletier, 1982 and *P. punctulatus* this ridge is absent. In most species, the posterior tip of notogaster is present, whereas in *P. chadaevae*, *P. jonasi* and *P. nebaikini* it is absent. In most species, the notogastral seta *c*<sub>1</sub> is absent, but in *P. macleani* and *P. oralensis* this seta is present. These species also differ from one another by the shape of posterior part of lamellar costula and shape of some setae (Table 3).

The morphological ontogeny of *P. oralensis* is similar to that of *P. punctulatus* investigated by Seniczak *et al.* (2013). The larva of both species differs slightly from each other in the length of seta *h*<sub>1</sub>, but the tritonymph of *P. oralensis* differs clearly from that of *P. punctulatus* by the location of setae *h*<sub>1</sub>, *h*<sub>2</sub> and *p*<sub>1</sub> on the posterior part of gastrnotum (Table 4). The adults of *P. oralensis* have 11

pairs of notogastral setae, including  $c_1$ , which is lacking in *P. punctulatus*. These species differ from each other also by the body size and shape of prodorsal seta *in* (Table 4). By contrast, the morphology of *P. oralensis* and *P. punctulatus* differs clearly from that of *Eueremaeus laticostulatus* Bayartogtokh, 2003, which has six pairs of adanal setae in the deutonymph, tritonymph and adult and 5–6 pairs of anal setae in the tritonymph and adult. Similar number of adanal and anal setae as *E. laticostulatus* have other species of *Eueremaeus* Mihelčič, 1963 and *Eremaeus* C.L Koch, 1835 (Behan-Pelletier 1993, Seniczak *et al.* 2013, 2014), and in species with known ontogeny, the location of setae  $h_1$ ,  $h_2$ ,  $p_1$  and  $p_2$  on the gastronomum has diagnostic value, as in *Proteremaeus* species.

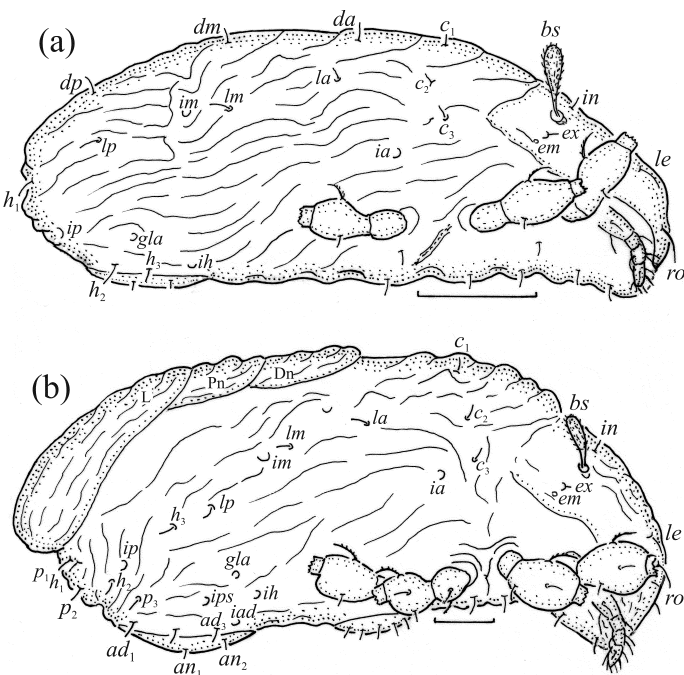
**TABLE 3.** Selective morphological characters of *Proteremaeus oralensis* **sp. nov.**, *P. punctulatus* and *Eueremaeus laticostulatus*.

Character	<i>P. oralensis</i>	<i>P. punctulatus</i> <sup>1</sup>	<i>E. laticostulatus</i> <sup>1</sup>
Formula of gastronotal setae	12-12-12-12-11	12-12-12-12-10	12-12-12-12-10
Pairs of adanal setae (Dn-Ad) <sup>2</sup>	3	3	6
Pairs of anal setae (Tn, Ad) <sup>2</sup>	2	2	5-6 <sup>3</sup>
<b>Adult</b>			
Body length in µm	507–572	471–540	584–640
Transverse ridge between setae <i>le</i>	Present	Absent	Absent
Shape of seta <i>in</i>	Smooth	Barbed	Barbed
Shape of gastronotal setae	Short, smooth	Short, smooth	Medium sized, barbed <sup>4</sup>
Location of setae <i>le</i> and <i>in</i>	On costula	On costula	Outside of costula
<b>Tritonymph</b>			
Body length in µm	527	462	553
Location of seta $h_2$	Close to $h_1$	Close to $p_1$	Close to $h_1$ and $p_1$
<b>Larva</b>			
Body length in µm	281	271	304
Length of seta <i>in</i>	Shorter than <i>le</i>	Shorter than <i>le</i>	Longer than <i>le</i>
Length of seta $c_3$	As short as $c_1$	As short as $c_1$	Longer than $c_1$
Length of <i>l</i> -series setae	As short as <i>c</i> -series	As short as <i>c</i> -series	Longer than <i>c</i> -series
Length of seta $h_1$	Shorter than $h_2$	As short $h_2$	Longer than $h_2$

<sup>1</sup>According to Seniczak *et al.* (2013), <sup>2</sup>Dn – deutonymph, Tn – tritonymph, Ad – adult, <sup>3</sup>unpaired seta also occurs, <sup>4</sup>seta  $h_2$  reaches insertion of seta  $h_1$ .

The adults of *Proteremaeus* have 10 or 11 pairs of notogastral setae, depending on seta  $c_1$ , which in *P. oralensis* and *P. macleani* is present, and in other species is absent. The number of *c*-series setae on the notogaster is an important character of Brachypylina (Circumdehiscence) that can explain the phylogeny of mites. According to Grandjean (1939, 1949, 1953) and Shaladybina (1972) during the phylogeny loss of notogastral setae starts with the *c*-series, but the former author thought that the first is lost seta  $c_1$ , and next  $c_3$  and only  $c_2$  remains, whereas the latter author observed this loss in an opposite order, e.g. from  $c_3$  via  $c_1$  to  $c_2$ . *Proteremaeus oralensis* and *P. macleani* lost one setae of *c*-series ( $c_3$ ), whereas other species lost two setae ( $c_3$  and  $c_1$ ), so the latter species are phylogenetically younger than *P. oralensis* and *P. macleani*. *Proteremaeus* loses setae of *c*-series according to Shaladybina (1972), similarly as Sphaerozetinae (Ceratozetidae) *sensu* Shaladybina (1975), where we can observe gradual loss of setae of *c*-series and *d*-series. For example, the most primitive *Ghilarovizetes* has 15 pairs of notogastral setae, and only  $f_1$  is lacking, comparing to holotrichous setal pattern of *Hermannia* Nicolet, 1855 (Seniczak *et al.* 2017a, b), whereas *Melanozetes* loses seta  $c_1$  and 14 pairs of notogastral setae remain (Shaladybina 1975; Seniczak *et al.* 1990, 2015). *Fuscozetes*

loses also seta  $c_3$ , and some species lose some or all setae of  $d$ -series, such as 10–13 pairs of notogastral setae remain, including  $c_2$  (Seniczak *et al.* 1990, 1991, 2016). However, the nymphs of Sphaeroretinae are apherodermous, and have 15 pairs of gastronal setae, and this subfamily loses setae of  $d$ -series between the tritonymph and adult, whereas the nymphs of *Proteremaeus* are eupherodermous, and lose setae of  $d$ -series between the larva and protonymph. Loss of setae of  $d$ -series in this genus makes the central part of gastronotum glabrous, which allows carry the dorsal exuvial scalps of previous instars.

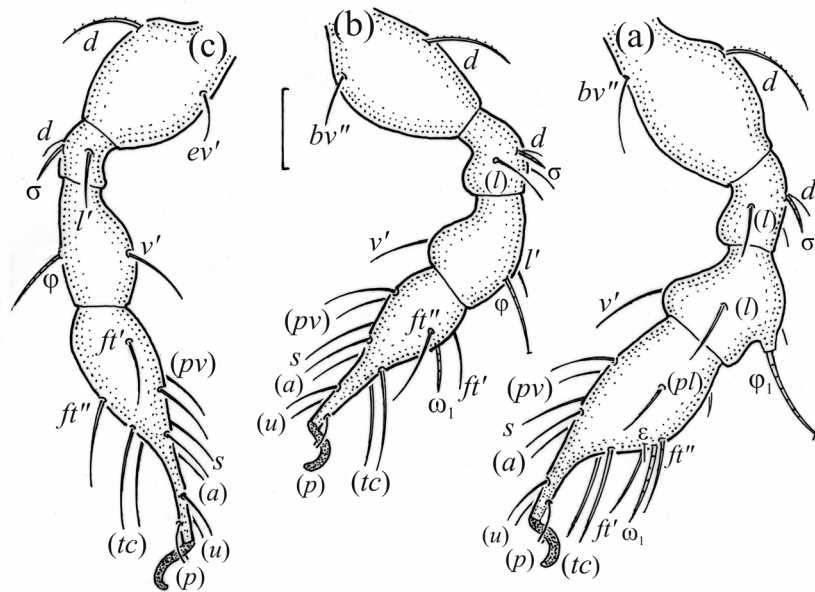


**FIGURE 10.** *Proteremaeus oralensis* sp. nov., lateral aspect, legs partially drawn, scale bars 50 μm. (a) Larva, (b) tritonymph.

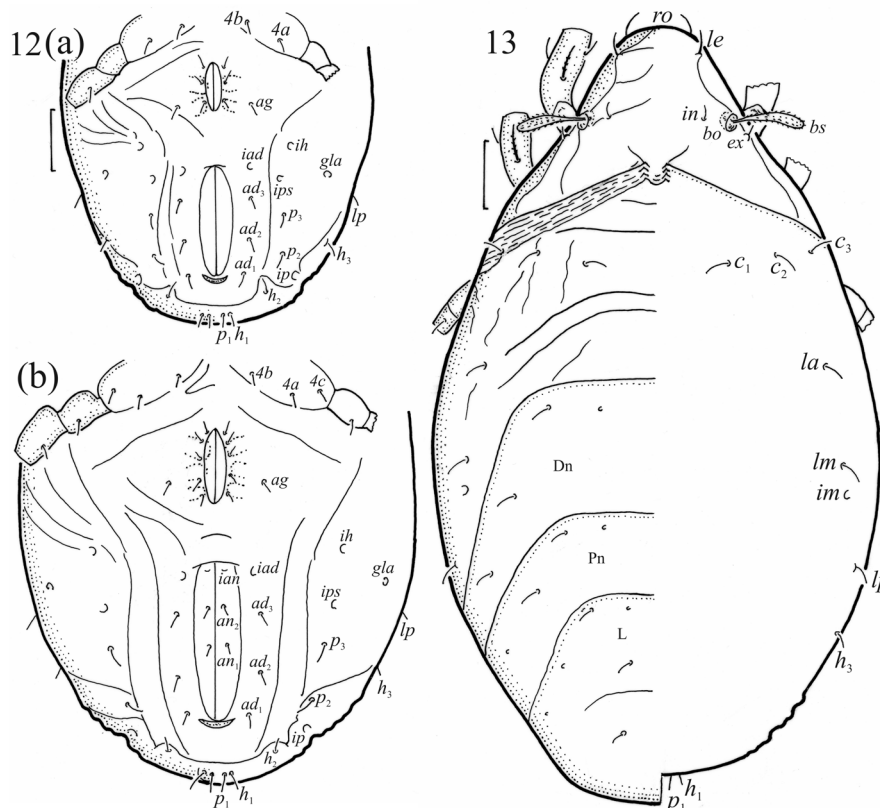
**TABLE 4.** Selected morphological characters of *Proteremaeus* species; Ng: notogaster (notogastral).

Species	Body length, μm	Bothridial seta	Transvers e ridge <sup>1</sup>	Posterior tip of Ng	Seta $c_1$	Shape of setae			Seta $d$ at leg solenidia	
						Ng	$le$	$in$	$\sigma$	$\phi$
<i>P. angarensis</i> (Rjabinin & Krivolutsky, 1975)	511	Clavate	Present	Present	Absent	Short, smooth	Smooth	Smooth	?	?
<i>P. chadaevae</i> Golosova, 1983	506	Fusiform	Absent	Absent	Absent	Medium sized <sup>2</sup> , smooth	Smooth	Barbed	?	?
<i>P. elongatus</i> (Rjabinin & Krivolutsky, 1975)	540	Fusiform	Present	Present	Absent	Short, smooth	Smooth	Smooth	?	?
<i>P. jonasi</i> Piffel, 1965	?	Clavate	Absent	Absent	Absent	Short, smooth	Smooth	Smooth	I	III, IV
<i>P. lawariensis</i> Hammer, 1977	500	Clavate	Present	Present	Absent	Short, barbed	Smooth	Smooth		
<i>P. macleani</i> Behan-Pelletier, 1982	504-552	Clavate	Absent	Present	Present	Short, smooth	Barbed	Smooth	I-III	I, III, IV
<i>P. mongolicus</i> (Golosova, 1983)	594	Clavate	Present	Present	Absent	Short, barbed	Barbed	Barbed	?	?
<i>P. nebaikini</i> Behan-Pelletier & Rjabinin, 1991	515-518	Fusiform	Present	Absent	Absent	Short, smooth	Barbed	Barbed	III	III, IV
<i>P. oralensis</i> sp. nov.	507-572	Clavate	Present	Present	Present	Short, smooth	Barbed	Smooth	I-III	VI
<i>P. punctulatus</i> Bayartogtokh, 2000	471-540	Clavate	Absent	Present	Absent	Short, smooth	Smooth	Barbed	I-III	I-IV

<sup>1</sup>Between lamellar setae, <sup>2</sup>seta  $h_2$  reaches insertion of seta  $h_1$ .

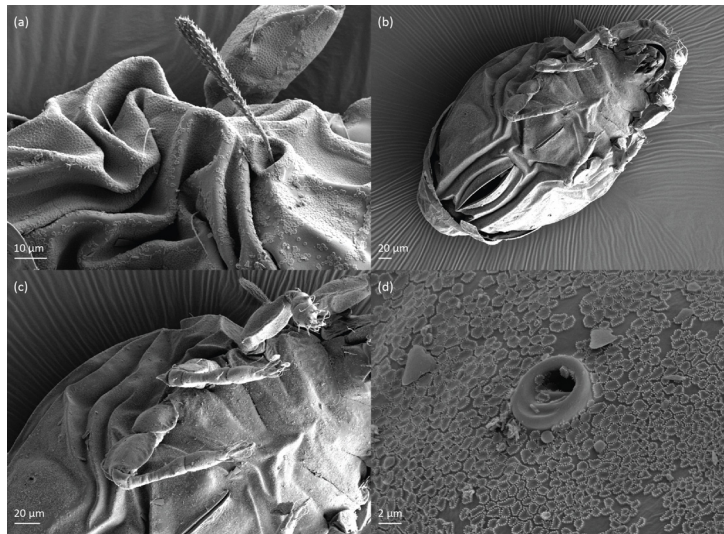


**FIGURE 11.** *Proteremaeus oralensis* sp. nov., leg segments of larva (part of femur to tarsus), right side, antiaxial aspect, scale bar 10  $\mu$ m. (a) Leg I; (b) leg II; (c) leg III.

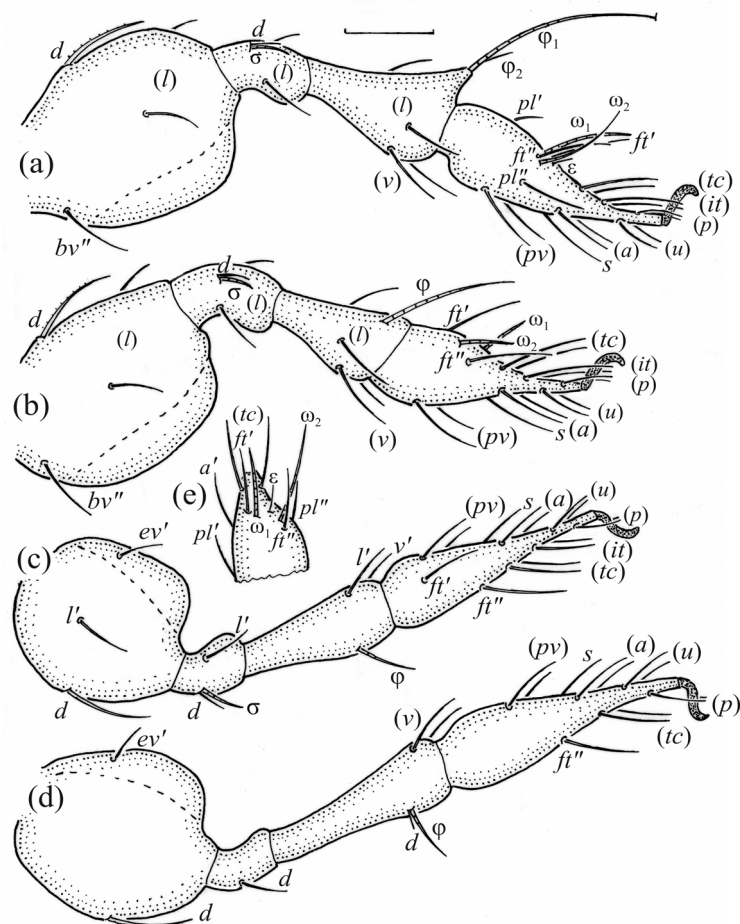


**FIGURES 12–13.** *Proteremaeus oralensis* sp. nov., legs partially drawn, scale bars 50  $\mu$ m. 12. Ventral part of hysterosoma, (a) deutonymph, (b) tritonymph. 13. Tritonymph, dorsal aspect.





**FIGURE 14.** *Proteremaeus oralensis* sp. nov., tritonymph, SEM micrographs. (a) Bothridium and bothridial seta, (b) whole body, ventral view, (c) medial part of body, ventral view, (d) gla opening.



**FIGURE 15.** *Proteremaeus oralensis* sp. nov., leg segments of tritonymph (part of femur to tarsus), right side, antiaxial aspect, scale bar 20 µm. (a) Leg I; (b) leg II; (c) leg III; (d) leg IV, (e) location of solenidia on tarsus I.

*Proteremaeus* has seta *d* at some leg solenidia, but this seta is usually short and thin or closely associated with corresponding solenidion (Bayartogtokh 2000), and therefore it is difficult to observe in the light microscope. For example, in *P. oralensis*, seta *d* is present on genua I–III and tibia IV, but this observation is based on several specimens, and different angles of observations. In other species of *Proteremaeus*, this seta was noted either at all leg solenidia on genua and tibiae, at some of them or leg setae of species are unknown (Table 4). Therefore, the diagnostic value of coupled seta *d* in *Proteremaeus* seems to be small, which is consistent with the observation of Behan-Pelletier (1993), who investigated the presence of seta *d* at solenidia of 42 North American species of close related genera *Eremaeus* and *Eueremaeus*; in 10 species she found this seta, in seven species this seta was present or absent, and in other species it was absent.

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