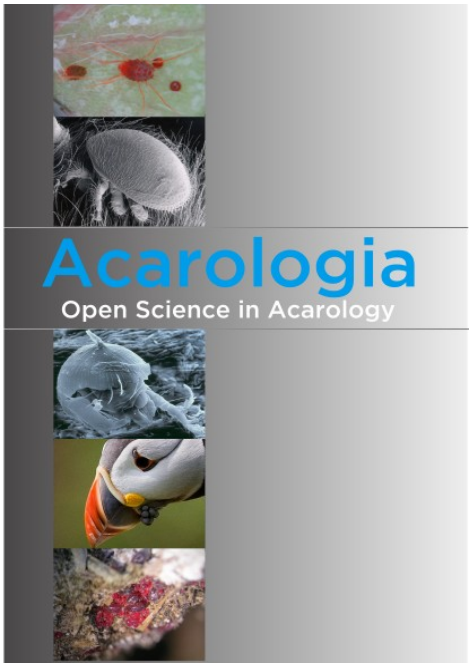

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Stylostome of the trombiculid mite larvae *Neotrombicula talmiensis* (Schluger, 1955) (Acariformes, Trombiculidae) feeding on two host species in the Russian Far East

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Original research

ABSTRACT

Stylostome and skin inflammatory reaction during feeding of *Neotrombicula talmiensis* (Schluger, 1955) (Acariformes, Trombiculidae) larvae on the naturally infected host animals – voles *Myodes rufocanus* Sundevall, 1846 and Asian chipmunk *Tamias sibiricus* (Laxmann, 1769) were studied by histological methods. In addition, larvae were studied in scanning electron microscope (SEM). The apical hypostomal portions form a temporal sucker, which applies to the host skin during feeding. Larval feeding on both naturally infected voles and chipmunks causes an epidermal hyperkeratosis and a permanent delayed inflammation with predominance of neutrophil leukocytes, dilation of dermal capillaries and local hemorrhages. Larvae tend to feed in tight groups and may attach themselves to both 'living' epidermis and hypertrophic stratum corneum. The stylostome is organized nearly identically in the two host species, which points to the species-specific character of the feeding tube in trombiculid larvae. The stylostome does not penetrate the epidermis through, so it may be classified as belonging to the epidermal type. The stylostome is produced by a solidifying larval secretion and composed of the proximal eosinophil cone and the main stylostome tube, both pale-pink in azure-II-eosin with a greyish peripheral portion more pronounced in voles. No longitudinal and transverse stratification is found in the stylostome composition. In contrast with other trombiculid larvae studied so far, larvae of *N. talmiensis* also ingest, besides liquefied nutrients, a pure blood that reveals a possibility for trombiculid larvae to be natural bloodsuckers.

Keywords larvae; mouth apparatus; feeding tube; histology; trombiculid mites; Acariformes

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Introduction

Larvae of trombiculid mites (chiggers) are known as ectoparasites of the wide range of vertebrate animals, several species serve as vectors of tsutsugamushi disease agents – *Orientia tsutsugamushi* (Hayashi). Scrub typhus, or tsutsugamushi fever, is an acute infection disease, which is widely distributed in countries of the Eastern hemisphere from Tajikistan to Papua New Guinea and, in particular, of South-Eastern Asia (Kulagin and Tarasevitch 1972; Kawamura *et al.* 1995; Takahashi *et al.* 2004). Therefore, the medical and epidemiological importance of this mite group cannot be overrated. During a parasitizing period, larvae of all trombiculid species produce a special feeding tube – stylostome – in the skin of the affected animals for

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better obtaining of the appropriate nutrients – lymph and tissue liquids (Shatrov 2009). A pure blood was not recorded as typical food of trombiculid larvae.

The stylostome is found to have different organization in different parasite and host species (Hoeppli and Schumacher 1962; Schumacher and Hoeppli 1963; Voigt 1970; Hase *et al.* 1978; Arnold 1986; Goldberg and Holshuh 1992; Shatrov 2009, 2018; Shatrov and Stekolnikov 2011; Shatrov *et al.* 2014; Shatrov and Mirolubov 2015). Nevertheless, there is still no evidence that its structure is species-specific, irrespective of the host species and feeding site. However, Hase *et al.* (1978) have shown that different *Leptotrombidium* larvae feeding on laboratory mice produce three different stylostome types – epidermal, mesenchymal and mixed – in accordance with the depth of penetration and position of the feeding tube in the host skin. While the organization of the stylostome may vary, the host skin reaction was found to be rather similar in its type but quite variable in degree in different host species and could depend on the number of feeding larvae and the duration of infection (Hoeppli and Schumacher 1962). The intensity of skin reaction, as supposed by Audy (1951), may serve as a measure of the novelty of the host for the given parasite species. The hyaline mass of the stylostome tube as such reveals a very low capability to react with different histological stains and may contain only some amount of acid mucopolysaccharides (Schumacher and Hoeppli 1963; Shatrov 2009).

The trombiculid mite *Neotrombicula talmiensis* (Schluger, 1955) is one of the most widespread species of the large and complex genus *Neotrombicula* Hirst, 1925. The species was first described from the Russian Far East and later it was found in the natural focus of tsutsugamushi disease in this region (Kudryashova and Tarasevitch 1964). It was later recorded in a broad range from Korea to Central Europe (Stekolnikov 2001, Stekolnikov *et al.* 2014) and revealed to be highly variable morphologically. It was shown that the name *N. talmiensis* had actually been used for a group of closely related species (Stekolnikov 2001, 2002).

Elucidation of the stylostome organization in this trombiculid species, in particular, its position within the host tissue as well as the intensity of the accompanied tissue reaction in two different host species – vole and chipmunk – is important in terms of the capability of these larvae to serve as a vector of the disease agents. In addition, disclosure of the stylostome structure may throw light upon the problem of its species-specific organization.

Material and methods

Trombiculid larvae of *Neotrombicula talmiensis* (Schluger, 1955) were collected by A.B. Shatrov in August 2019 from the voles *Myodes rufocanus* Sundevall, 1846 and Asian chipmunk *Tamias sibiricus* (Laxmann, 1769) in the Silinskiy forest, Komsomolsk-on-Amur (50°34′29′′N, 137°02′09′′E). Animals were captured using standard Gero traps. In total, five voles and four chipmunks were captured, trombiculid larvae parasitized all of them with the minimum number of around ten feeding larvae on a chipmunk. Larvae fed within ears in tight groups at different feeding stages. Some skin samples with feeding larvae were fixed in 70% ethyl alcohol for their further identification and systematical consideration, which were performed by A.A. Antonovskaia, some were fixed for histological examination in parallel.

Specimens selected for morphological identification were processed after alcohol fixation. Five larvae were mounted on microscopic slides in Faure-Berlese medium (Neuhaus *et al.* 2017) and then examined under a Micromed-3 Professional microscope (Ningbo Sheng Heng Optics & Electronics, Gao Qiao, China) equipped with phase contrast and a ToupCam camera (ToupTek Photonics, Hangzhou, China). Measurements were made from photos by the calibrated software ToupView (ToupTek Photonics, Hangzhou, China). The morphological terminology follows Kudryashova (1998) and Stekolnikov (2013). To distinguish between species of the *talmiensis* group, we also used functions calculated by Stekolnikov (2001). The examined specimens are deposited at the Department of Entomology, Faculty of Biology, Lomonosov Moscow State University.

For histological examinations, small skin samples with feeding larvae from three voles and three chipmunks were cut out from ears and immediately fixed in a compound histological

fixative fluid composed of 40% formalin (30 ml), 96% ethyl alcohol (10 ml) and glacial acetic acid (3 ml) for 3-4 hours. After fixation in the field, the samples were preserved in 96% ethyl alcohol and kept at 4-6 °C for some months. In the laboratory, the skin samples were treated with celloidin and then they were embedded in paraffin. Paraffin blocks were serially sectioned at 5-7 µm, and then the sections were stained with azure II-eosin that allows differentiating both basophilic and oxyphilic tissue properties (Lillie 1965). The sections were then studied and photographed with a Leica DM LS-2 light-optical microscope combined with a Leica EC-3 digital camera at the objective magnifications from x10 to x100 (oil immersion). In total, around several tens of stylostomes were examined in the two host species.

For investigation of the optical anisotropy of tissues, a plane-polarized emission was applied to sections with the help of a special device coupled with a light microscope and provided with the polarizer and the analyzer.

For Scanning Electron Microscope (SEM) examination, alcohol-preserved larvae that had dropped off their hosts as well as small skin samples with still attached larvae were washed in graded alcohol series and treated with hexamethyldisilazane (HMDS) for 5–10 min. Some larvae were additionally washed in an ultrasonic cleaner for 10-30 sec and then were treated with HMDS. The larvae, after placement on the microscope stubs, were then covered with a platinum layer in an Eiko IB-5 ion coater and examined with a SEM Quanta-250 (FEI Company) at 15 kV.

The histological and SEM preparations are deposited at the Zoological Institute of the Russian Academy of Sciences, St-Petersburg.

Results

Larvae

The morphological characters of the collected larvae match the diagnosis of *N. talmiensis* (Kudryashova 1998; Stekolnikov 2001). The examined larvae are characterized by branched galeal setae, palpal claw with 3 prongs (trifurcate), branched palpal setae, sensilla with 7–9 long branches, 30–34 dorsal setae, 29–34 ventral setae (SIF = 7BS-B-3-3111-1000; fPp = B/B/NBB or B/B/BBB; fCx = 1-1-1; fSt = 2.2; fD = 2H-8-6-6-4-4-2, 2H-8-6-6-6-4-2, etc.) (Table 1, Figure 1A-G).

SEM of *N. talmiensis* larvae has also revealed two eye lenses closely positioned on one plate on both sides of the scutum (Figure 1A-B) and long trifurcate palpal claws looking down and back nearby the sucker (Figure 1D). Like in other trombiculid larvae (Shatrov 2000), the apical hypostomal lips form a temporal sucker during larval feeding (Figure 1D). This sucker closely attaches to the host skin from the outside sealing the wound thus facilitating the action of the pharyngeal pump. The cheliceral movable digits (cheliceral blades), curved dorsally, protrude forward in active position (Figure 1C) and reveal a certain groove on the inner surface (Figure 2A), as well as barely noticeable barbs at their tips (Figure 2B) (tricuspid cap) (Kudryashova 1998). SEM of the host skin samples shows the sites of the larval attachment with the stylostome substance protruding from the place of puncture above the epidermal surface (Figure 2C-D). This substance reveals imprints of the hypostomal sucker outside and the cheliceral movable digits inside it (Figure 2C-D) thus disclosing the ‘starting point’ of the stylostome canal. The stylostome canal remains free from any material. As seen from the equal orientation of ridges of the stylostome substance in the larval puncture remaining after loosely shifted cheliceral blades, larvae attach to the host skin in the same general position (Figure 2C). This, obviously, may help larvae to feed in the tightest groups. It is also seen that the trifurcate palpal claws do not pierce the host skin and do not penetrate into it because there are no traces of such perforation found on the epidermal surface.

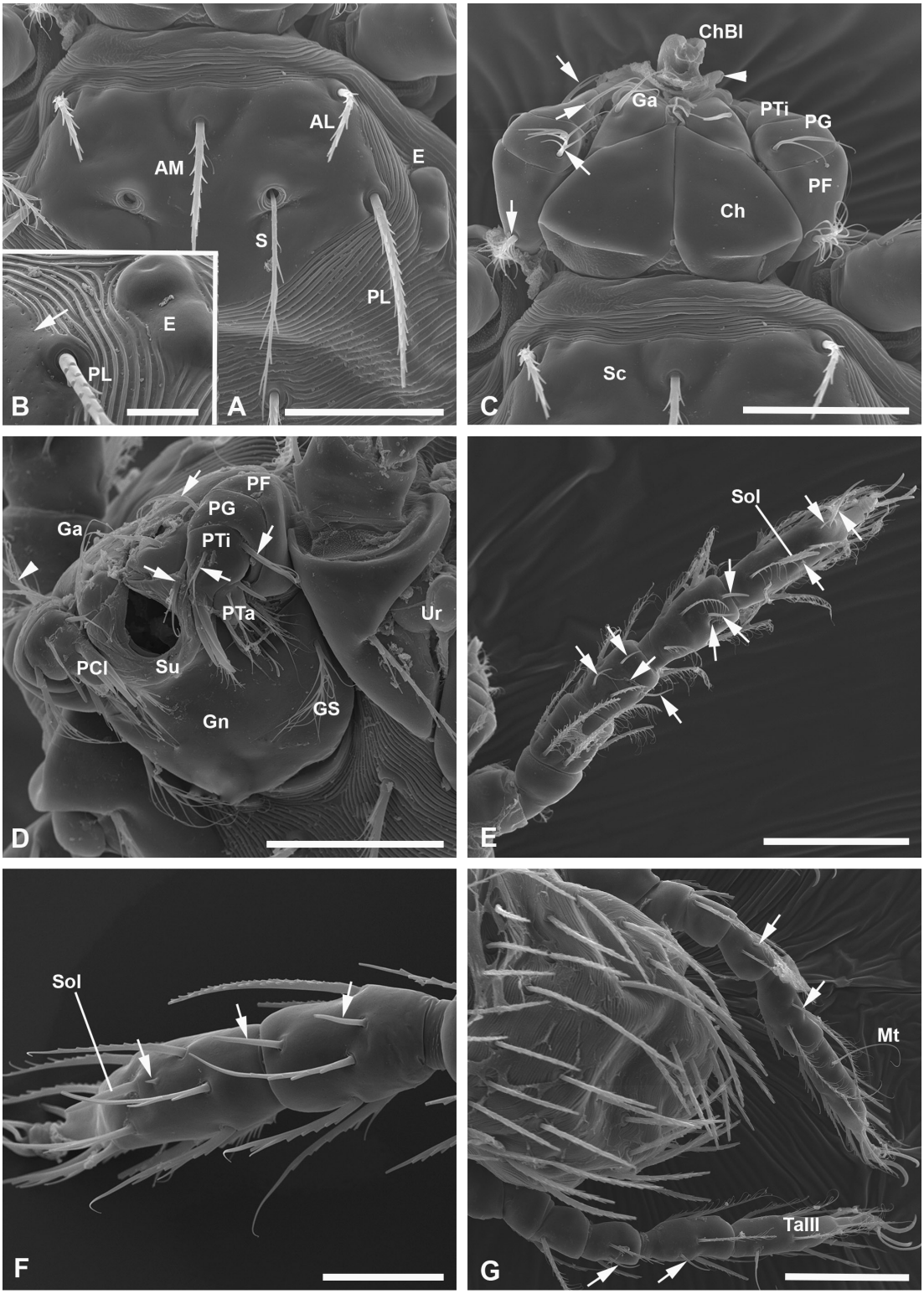

Figure 1 Organization of *Neotrombicula talmiensis* (Schluger, 1955) larvae. SEM. A – Scutum (one of sensilla broken). Scale bar – 40 μm; B – Paired eye and posterolateral seta of scutum. *Arrow* points to a pore on the scutal surface. Scale bar – 10 μm; C – Gnathosoma, dorsal view (palpal claws not visible). *Arrows* show branched setae on palpal femur, genu, and tibia (ventral seta of tibia and palpal tarsus not shown). *Arrowhead* points to a sucker formed of the turned back apical hypostomal lips. Scale bar – 40 μm; D – Gnathosoma, ventral view. Note the long trifurcate palpal claws adjacent to the sucker. *Arrows* show branched setae (dorsal, ventral, and lateral) on palpal tarsus and branched setae on tibia. *Arrowhead* shows branched seta on palpal femur (not in focus). Scale bar – 40 μm; E – Leg I. *Arrows* show specialized nude setae. Scale bar – 50 μm; F – Leg II, tarsus and tibia. *Arrows* show specialized nude setae. Scale bar – 20 μm; G – Posterior portion of the larval body showing disposition of the dorsal setae, and legs III. *Arrows* show specialized nude setae. Scale bar – 50 μm. — AL – anterolateral seta, AM – anteromedian seta, Ch – chelicera, ChBl – cheliceral blades (cheliceral movable digits), E – paired eyes, Ga – branched galeal seta, Gn – gnathocoxal plate, GS – gnathocoxal seta, Mt – mastitarsala, PCl – palpal claw, PF – palpal femur, PG – palpal genu, PL – posterolateral seta, PTa – palpal tarsus, PTi – palpal tibia, S – sensillum (trichobothrium), Sc – scutum, Sol – solenidion, TaIII – tarsus of leg III, Ur – urstigma (Claparède organ)

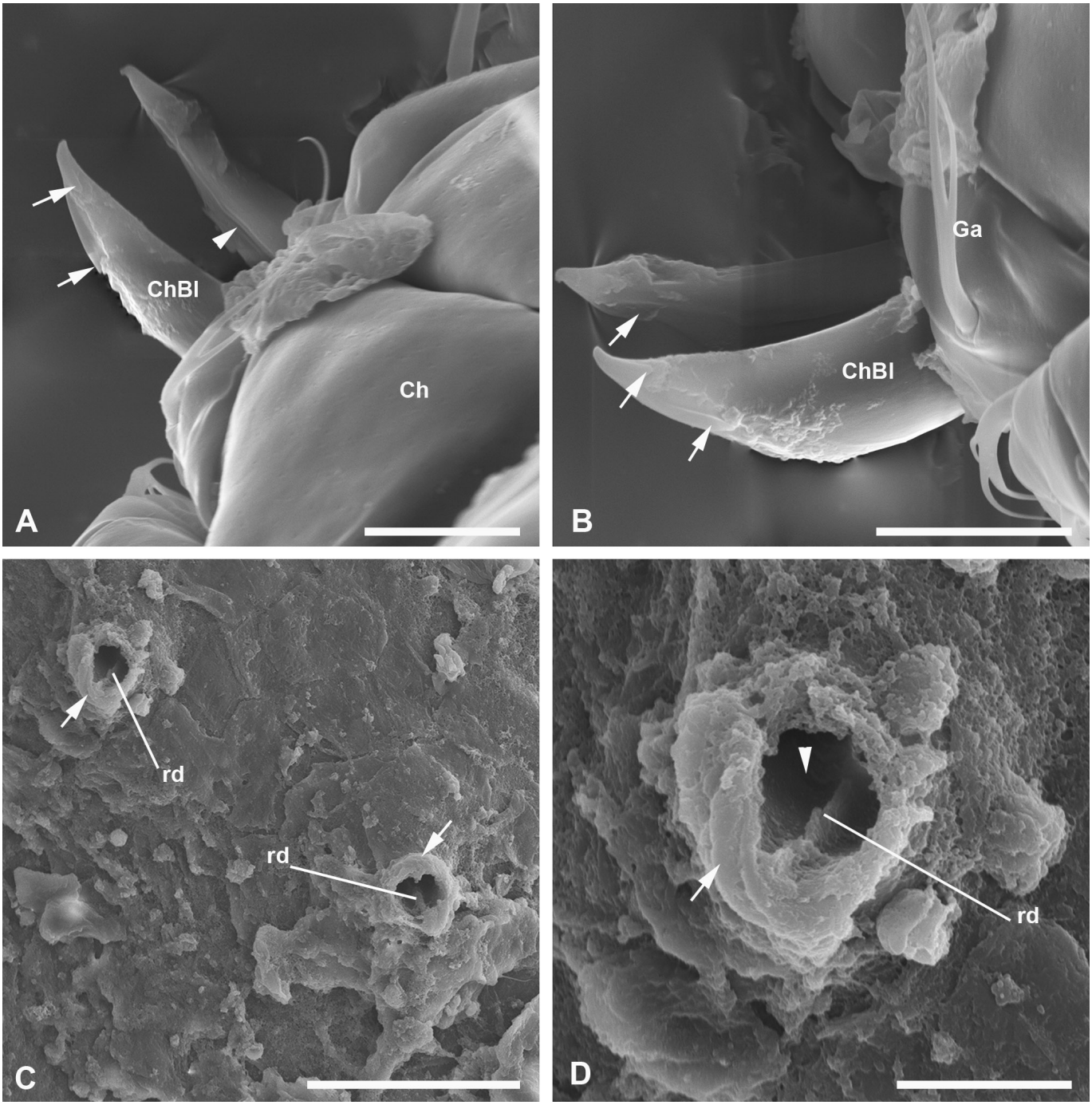

Figure 2 Cheliceral blades and sites of attachment of *Neotrombicula talmiensis* (Schluger, 1955) larvae. SEM. A – Cheliceral blades, dorsal view. *Arrows* show two small barbs on the tip of the cheliceral blades, the third barb is not visible. *Arrowhead* points to a groove on the medial surface of the cheliceral blade. Scale bar – 10 μm; B – Cheliceral blades, lateral view. *Arrows* indicate hardly noticeable barbs on the tip of the cheliceral blades (tricuspid cap). Scale bar – 10 μm; C – Sites of the larval attachment on the vole skin (*arrows*). Scale bar – 40 μm; D – Larval attachment site/puncture with an extruded material of the eosinophil cone (*arrow*) and a groove remaining from the cheliceral blade (*arrowhead*). Note the ridge of the stylostome substance along the axis of the stylostome canal. Scale bar – 10 μm. — Ch – basal cheliceral segment, ChBl – cheliceral blade, Ga – branched galeal seta, rd – ridge of the stylostome substance remaining from cheliceral blades

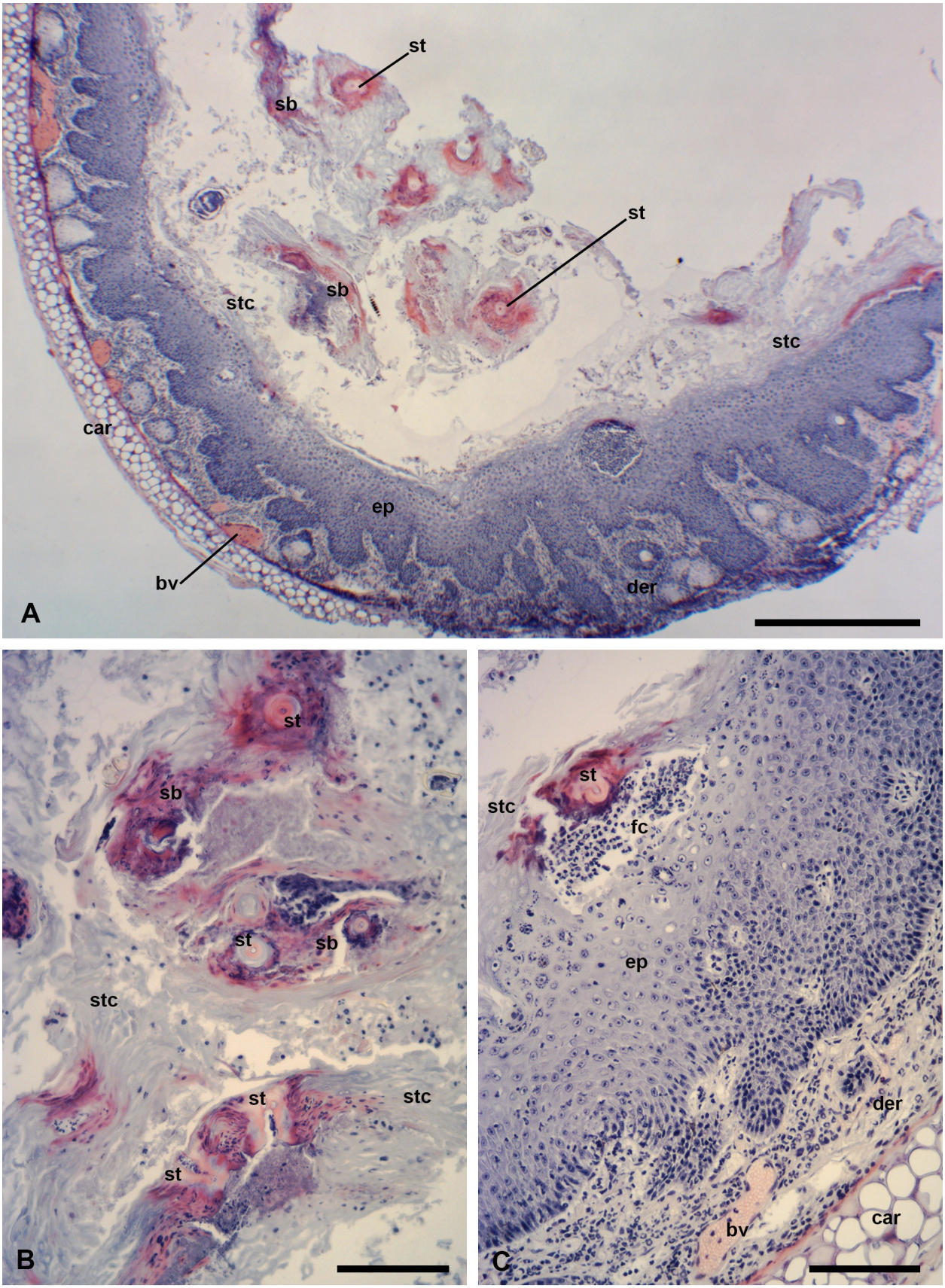

Figure 3 Skin injury of voles *Myodes rufocanus* Sundevall, 1846 during feeding of *Neotrombicula talmiensis* (Schluger, 1955) larvae. Histological sections of aural cavity. Azure II-Eosin. A – General view of aural cavity damaged by feeding larvae. Scale bar – 200 µm; B – Stylostomes evolved within the hypertrophic stratum corneum. Scale bar – 100 µm; C – Single young stylostome obliquely sectioned evolved within the epidermis. Note the feeding cavity filled with inflammatory cells. Scale bar – 100 µm. — bv – blood vessel, car – aural cartilage, der – dermis, ep – epidermis, fc – feeding cavity, sb – scab, st – stylostome, stc – stratum corneum

Stylostome

In both vole and chipmunk, the epidermis-dermis tissue system in the auricles is organized similarly (Figures 3A, 6A, B). It reveals the well-developed papillary layer of the dermis and the thick epidermal layer with the expressed stratum spinosum – spiked layer of the epidermis, whereas the stratum granulosum – granular layer, and the stratum lucidum – shiny layer of the epidermis are significantly thinner. At the same time, in the highly immunized naturally infected animals, like voles and chipmunks, the epidermis in the very deep portions of the auricles, mostly affected by feeding larvae, undergoes hyperplasia and hyperkeratosis. This leads to the significant increase of the overall thickness of the epidermis and, especially, the stratum corneum (horn layer) as well. However, larvae may attach themselves irrespectively in both greatly keratinized sites (Figure 3B) and 'normal' epidermis (Figures 3C, 6B, C) and usually tend to feed close to each other by tight groups (Figures 3A, B, 6A, B).

The initial stages of the stylostome formation in larvae of *N. talmiensis* are identical in both host species. In the case of the 'normal' epidermis, where the stratum corneum is not thick, larvae cut this layer by the cheliceral blades and reach the stratum lucidum – shiny layer of the epidermis by their tips (Figures 4A, 7A, B). The stylostome begins to evolve from this level. On the longitudinal sections to the stylostome axis, the first stylostome portion is a cone-shaped structure, of which the lateral angles generally radiate between the stratum lucidum and the stratum corneum (Figures 4A, 7A, B). In turn, its upper margins may protrude beyond squamae of the stratum corneum as seen in SEM images (see above). The color of this cone-shaped structure in azure-eosin staining, as in other trombiculids, is variably pale-pink, so it is termed 'eosinophil cone' (Shatrov 2009). Remarkably, this structure apparently reveals viscous properties because it shows high meniscuses with the cheliceral blades inserted into and adhered to it (Figures 4A, 7B). The narrow portion of the growing stylostome just beneath the eosinophil cone may be light grayish on this initial stage of the stylostome formation (Figure 7A, B). The cheliceral blades are moved apart by around 10 µm thus forming the most proximal portion of the stylostome canal (Figure 4A). Immediately after formation of the eosinophil cone, a particular clear cavity (the so-called feeding cavity) evolves in the upper portions of the stratum granulosum just beneath the eosinophil cone (Figures 3C, 4A, 7A, B) as a result of dissolution of the epidermal cells by action of the larval salivary secretion. Importantly, this

Table 1 Standard measurements of *Neotrombicula talmiensis* (Schluger, 1955) larvae from this study (n=5).

	Range, µm	Median, µm		Range, µm	Median, µm
AW	76–79	78	Vmin	29–30	30
PW	91–100	92	Vmax	44–54	47
SB	33–37	36	pa	281–311	287
ASB	31–35	32	pm	242–279	262
PSB	25–29	26	pp	305–319	306
SD	56–63	61	Ip	830–896	862
P-PL	24–30	27	DS	30–34	33
AP	26–30	28	VS	29–34	32
AM	44–47	47	NDV	62–66	64
AL	41–45	44	TaIII (L)	73–79	77
PL	63–70	67	TaIII (W)	16–18	16
S	70–73	72	dmt	12–14	13
H	62–68	66	m-t	0,16–0,19	0,17
Dmin	40–46	46			
Dmax	57–65	61			

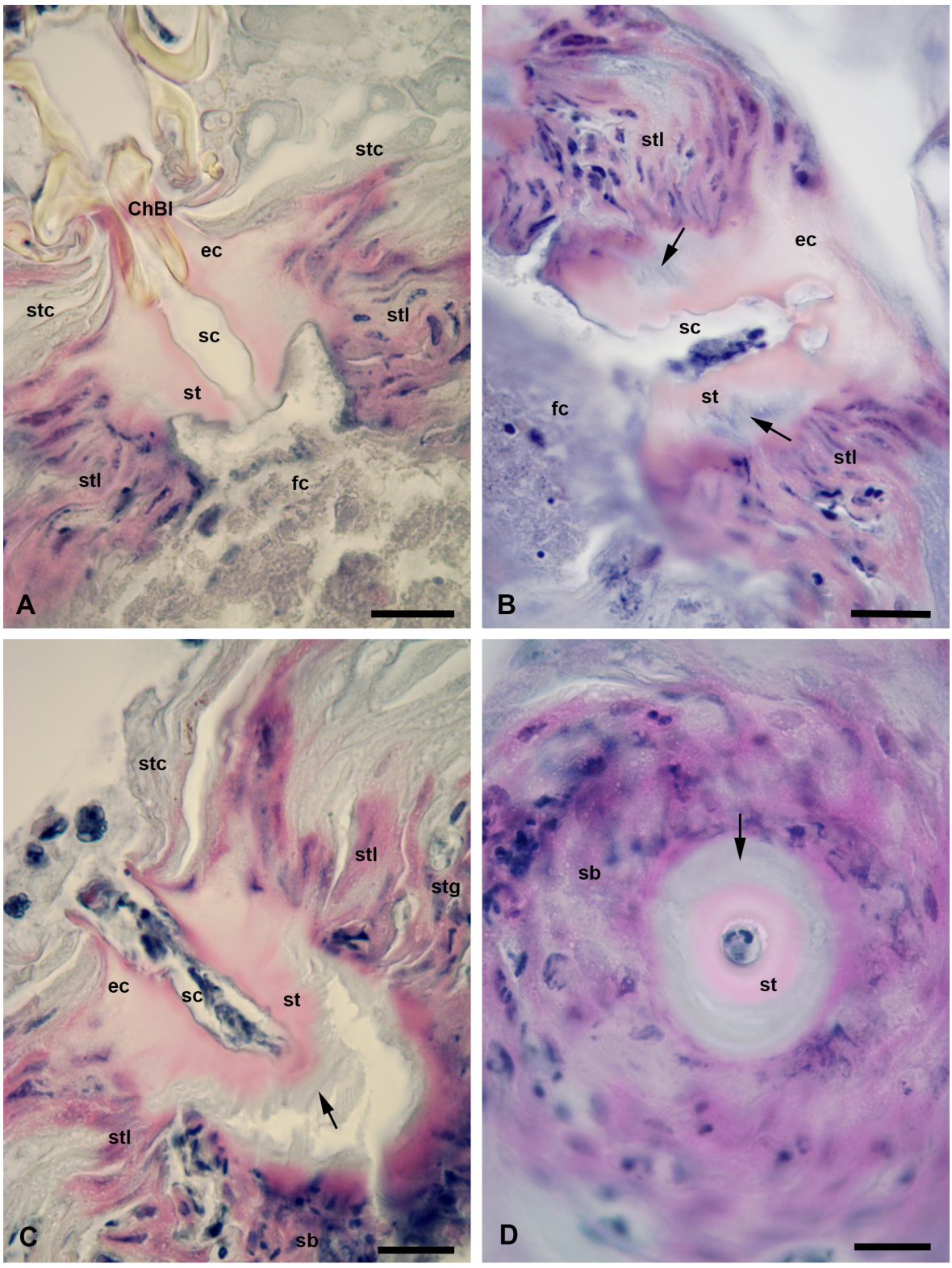

Figure 4 Stylostomes of *Neotrombicula talmiensis* (Schluger, 1955) larvae in the skin of voles *Myodes rufocanus* Sundevall, 1846. Histological sections. Azure II-Eosin. A – Young stylostome evolved in the hypertrophic stratum corneum. Note the active stratum lucidum surrounding growing stylostome and feeding cavity filled with a flocculent material without inflammatory cells. Stylostome canal is also empty. Scale bar – 20 μm; B – Stylostome at the advanced stage also evolved in the hypertrophic stratum corneum and tightly surrounded by cells of the stratum lucidum. Note the greyish substance on the periphery of the stylostome (*arrows*). The stylostome canal contains cell debris, although feeding cavity is devoid of inflammatory cells. Scale bar – 20 μm; C – Stylostome at the advanced stage evolved against the scab underneath it, so the feeding cavity is not obviously manifested. The stylostome canal is filled with debris. Note the greyish substance on the periphery of the stylostome (*arrow*). Scale bar – 20 μm; D – Transverse section of a stylostome evolved within the moderately expressed scab. Note the greyish substance on the periphery of the stylostome (*arrow*). Scale bar – 20 μm. — ChBl – cheliceral blade, ec – eosinophil cone, fc – feeding cavity, sb – scab, sc – stylostome canal, st – stylostome, stc – stratum corneum, stg – stratum granulosum, stl – stratum lucidum

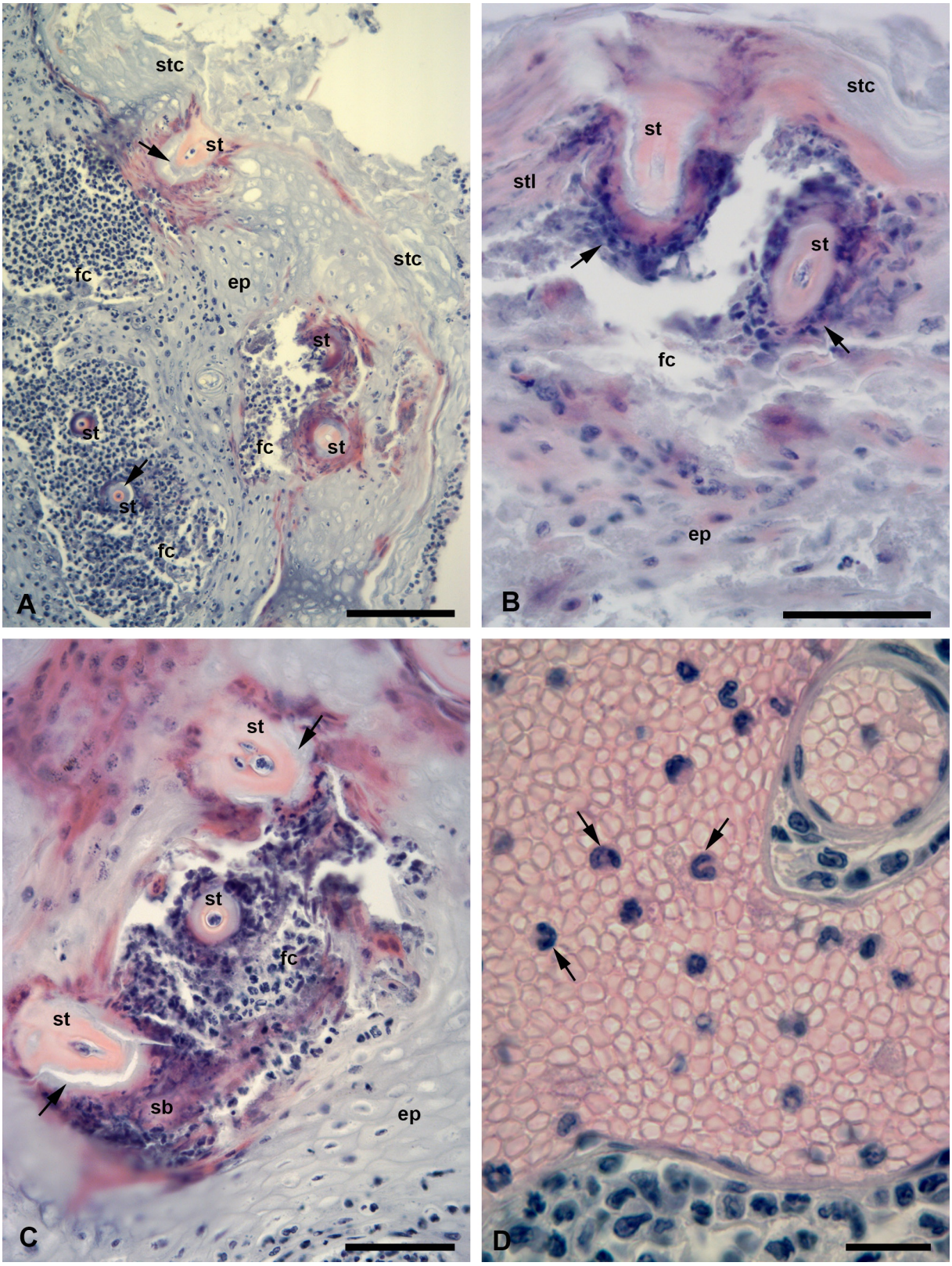

Figure 5 Skin injury of voles *Myodes rufocanus* Sundevall, 1846 and stylostomes of *Neotrombicula talmiensis* (Schluger, 1955) larvae. Histological sections. Azure II-Eosin. A – Several stylostomes evolving in both hypertrophic epidermis and penetrating into the strongly expressed feeding cavities filled with numerous infiltrating cells and cell debris. Note the grayish substance at the periphery of stylostomes (*arrows*). Scale bar – 100 µm; B – Two closely disposed stylostomes evolving within the cell association of the broken inflammatory and epidermal cells (*arrows*). Scale bar – 50 µm; C – Three differently arranged stylostomes evolving within the pronounced feeding cavity tightly packed with the broken inflammatory and epidermal cells fused to form a scab. Note the grayish substance at the periphery of stylostomes (*arrows*). Scale bar – 50 µm; D – Strongly dilated capillary in the papillary dermal layer tightly packed with erythrocytes and containing polymorphnonuclear leucocytes (neutrophils) (*arrows*). Scale bar – 20 µm. — ep – epidermis, fc – feeding cavity, sc – scab, st – stylostome, stc – stratum corneum, stl – stratum lucidum

cavity appears when there are still no leukocytes present in the epidermis. In the case of two or several closely attached larvae, the initial feeding cavities fuse to form a large common cavity in the upper epidermal layers (Figures 5A, C, 6C). In more or less developed condition, these cavities begin to fill up with numerous neutrophil leucocytes (Figures 3C, 5A, C, 6C, 7C, D) migrating here across the epidermis from the dilated blood vessels of the terminal vessel bed (see below). Leucocytes and their nuclear fragments may be also seen within the initial short stylostome canal and in the area between the cheliceral blades (Figure 4B, C). Nevertheless, the midgut of the feeding larvae never contains any intact host cells or their debris as on the initial so on the advanced feeding stages. In the developed inflammatory focus with the perforated epidermis, leucocytes may be also seen coming outside the epidermis and disposing among squamae of the stratum corneum as well as among feeding larvae.

The stylostome tube grows straight down from the base of the eosinophil cone (Figures 4B, C, 7C) but may extend into the epidermis at various angles to its surface (Figures 5A-C, 8B). In the more or less developed condition, the internal stylostome portion around the canal is always variably pale-pink and do not differ significantly from the eosinophil cone by its staining ability (Figures 4B, C, 5B, C, 7D, E). Sometimes, especially in the case of chipmunk, this staining may spread over the entire stylostome (Figures 7C-E, 8B). By contrast, the external and the peripheral (deepest) portions of the stylostome tube may be lighter and greyish and may be to some extent distinct from the internal stylostome portion (Figures 4B-D, 5A, C). This character seems to be more pronounced in the case of voles, whereas in the case of chipmunk the peripheral stylostome portions are only slightly lighter than the middle ones and are not distinctly divided from the latter. However, the general stylostome composition in both cases is quite similar and certain layers in the stylostome walls cannot be surely identified. The stylostome canal may show some dilations throughout its length and is always opened at its end (Figures 4A, C, 7B). The diameter of the canal varies within 10-15 µm. The stylostome measurements are indicated in Table 2.

Differences in the stylostome size in two host species generally depend on the place of the larvae attachment and may reflect only particular and local variations. The stylostome is typically shorter and wider when it evolves within the already existed feeding cavity or when it is surrounded only by epidermal cells (Figures 4B, 7C, E). By contrast, the stylostome may be longer and narrower when it develops in the scab formed of numerous broken necrotic lymphoid cells on the epidermal surface (Figures 5B, 7D). Nevertheless, due to a great variety of the particular tissue condition in the site of the larval attachment (Figures 3B, 5A, 8A, B), no exact regularity in the stylostome size may be apparently ascertained.

The study of stylostome with a polarizer-analyzer system reveals a very weak light in the internal pale-pink layer of the distal stylostome portions of the developed stylostomes. This optical anisotropy indicates that the distal stylostome portions may be processed by particular structural changes different from other stylostome substance without any anisotropy.

In the course of the stylostome formation, the cells of the stratum granulosum and the stratum lucidum become active and pronounced. They come to the growing stylostome tube in a perpendicular direction to its axial line (Figures 4A, C, 7C-E), obviously acting in displacing

Table 2 Measurements of stylostome of *Neotrombicula talmiensis* (Schluger, 1955) larvae feeding on two natural host species (n=7 from each host).

Host species	Indication\Sign	Range, µm	Median, µm
Myodes rufocanus Sundevall, 1846	Length	70-110	85
	Half-length width	20-50	34
Tamias sibiricus (Laxmann, 1769)	Length	80-130	105
	Half-length width	25-55	39

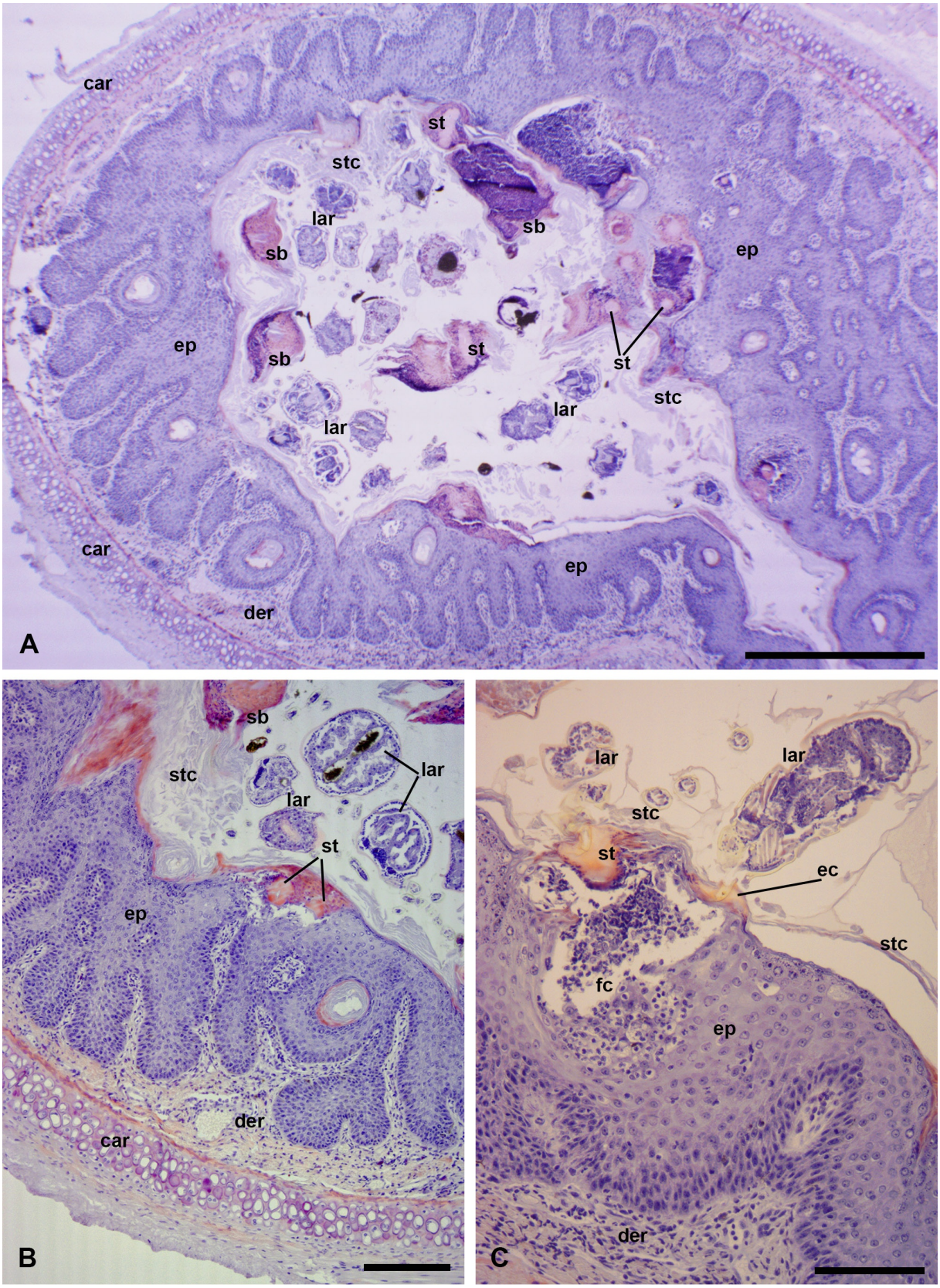

Figure 6 Skin injury of the Asian chipmunk *Tamias sibiricus* (Laxmann, 1769) during feeding of *Neotrombicula talmiensis* (Schluger, 1955) larvae. Histological sections of aural cavity. Azure II-Eosin. A - General view of aural cavity damaged by numerous feeding larvae. Note the differently developed stylostomes evolving within scabs. Scale bar – 500 μm; B – Two young closely disposed stylostomes evolving within the epidermis. Note the numerous larvae at different feeding stages occupying the aural cavity. The feeding cavity is barely expressed. Scale bar – 200 μm; C – Two young stylostomes of the recently attached larvae at different developmental stages evolving within the epidermis showing the large feeding cavity filled with numerous inflammatory cells. — car – aural cartilage, der – dermis, ec – eosinophil cone, ep – epidermis, fc – feeding cavity, lar – larvae, sb – scab, st – stylostome, stc – stratum corneum

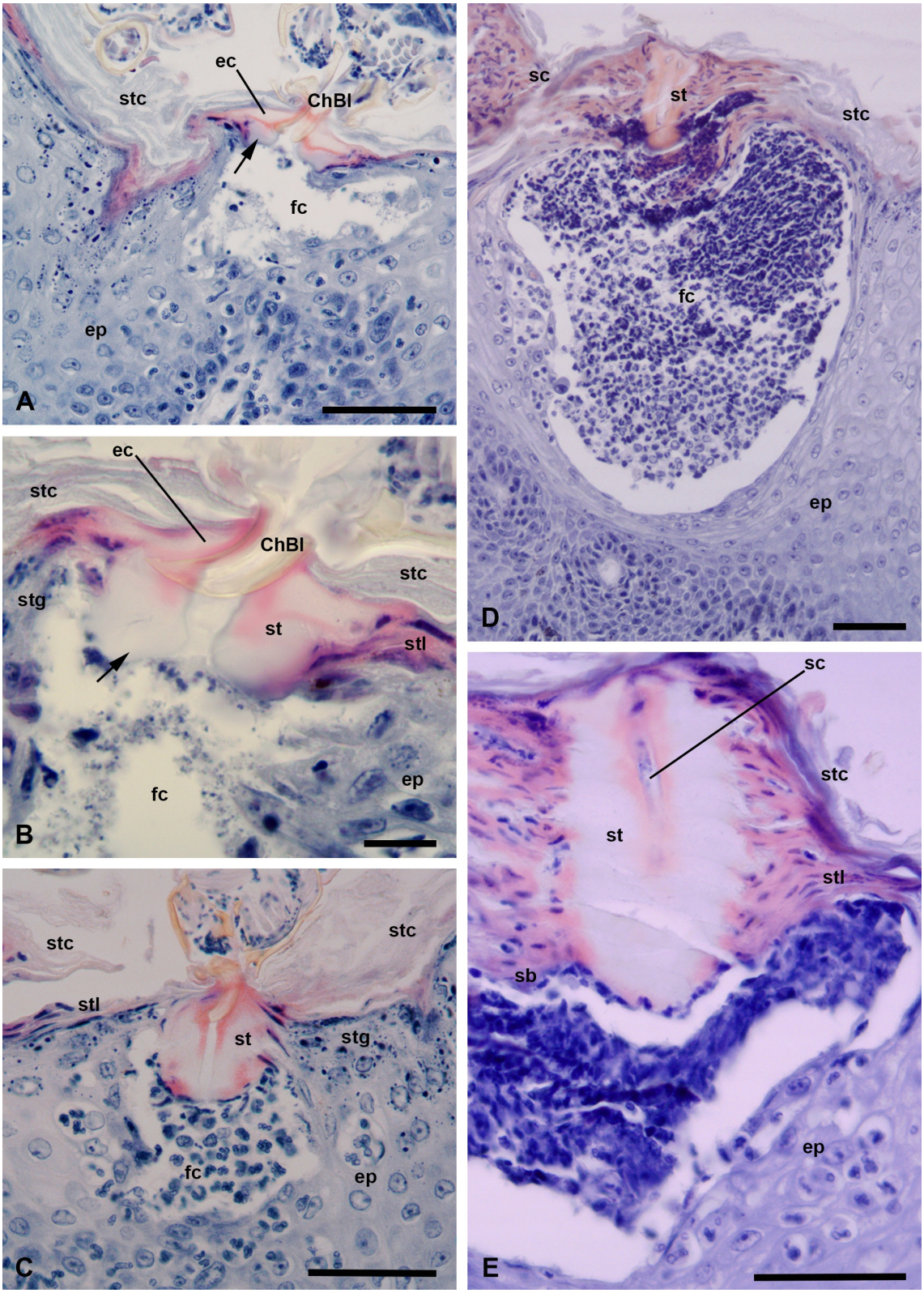

Figure 7 Stylostomes of *Neotrombicula talmiensis* (Schluger, 1955) larvae in the skin of the Asian chipmunk *Tamias sibiricus* (Laxmann, 1769). Histological sections of aural cavity. Azure II-Eosin. A – Young stylostome composed of one eosinophil cone with the already developed feeding cavity situated within the upper epidermal layers. Note the grayish substance underneath the eosinophil cone (*arrow*). Scale bar – 50 μm; B – Young stylostome at the initial stage of development with the nearly empty feeding cavity underneath it. Note that the pale-grey substance on the distal periphery of the stylostome (*arrow*) does not show any delimitations with the eosinophil cone. Scale bar – 20 μm; C – Stylostome at the advanced stage of development in the epidermis. Scale bar – 50 μm; D – Developed stylostome in the area of scabs and extensive feeding cavity filled with tightly infiltrating cells. Scale bar – 50 μm; E – Developed stylostome with walls of an equal homogeneous pale-pink staining. Note the stylostome encompassed by cells of the stratum lucidum showing a hyperactivity. Scale bar – 50 μm. — ChBl – cheliceral blades, ec – eosinophil cone, ep – epidermis, fc – feeding cavity, sb – scab, sc – stylostome canal, st – stylostome, stc – stratum corneum, stg – stratum granulosum, stl – stratum lucidum

and isolation of the alien object from the host organism. At the same time, inflammatory cells and their nuclear debris, located within the feeding cavity, typically tightly surround the most distal stylostome portions (Figures 5A, C, 7D). The cells of the stratum granulosum being in the area of the inflammatory focus are disorganized or even destroyed and take part in the formation of necrotic scab. Squamae of the stratum corneum remain outside the epidermal injury and being hyperkeratotic may nearly totally envelope the feeding larvae by sides (Figures 3A, B, 6B, 8A). Finally, the larval feeding leads to the ulceration of the epidermis. This ulceration is manifested in (i) the penetrability of the epidermis for cells of the internal environment, (ii) the extremely large cavities in the hyperplastic epidermis filled with inflammatory cells and (iii) formation of necrotic scabs on the epidermal surface composed of the tight and mixed associations of migrating inflammatory cells and destroyed epidermal cells. This situation on the epidermal surface within the auricles provokes larvae to attach themselves more and more distally.

Several variations of the attaching and feeding process may be obviously observed. Larvae attach themselves to (i) the 'pure' highly keratinized stratum corneum (Figure 3B), (ii) the previously hyperplastic stratum lucidum after already fed or simultaneously feeding larvae (Figures 4B, 7E), (iii) the scab consisting of tight associations of the epidermal and inflammatory cells (Figures 5B, C, 8A, B) and (iiii) the area of feeding cavity (Figures 3C, 6C). All these situations lead to slightly different results in the stylostome formation and, apparently, feeding process. In particular, a tightly packed tissue in the attaching site results in formation of the longer and narrower stylostome (Figures 5B, 7D) than in the case of the loosely packed tissue like feeding cavity. In all other cases of the 'distally' attaching larvae, a tight association of the destroyed cells surrounds the stylostome and prevents formation of the pronounced feeding cavity. Remarkably, however, leucocytes are still coming to the stylostomes through the damaged epidermis and may be even seen in the stylostome canal.

It is clear that in the observed feeding mode, the stylostomes do not pierce the epidermis through and reach the dermis. Nevertheless, the dermis in the damaged area undergoes a strong dilation of venules and capillaries of the terminal vessel bed. These vessels are filled with erythrocytes and contain polymorphonuclear neutrophil leucocytes (PMN) (Figure 5D), which penetrate through the vessel walls and infiltrate the area of the inflammatory focus in mass. Eosinophils and basophils were not identified with certainty as well as mast cells. Certain hemorrhages may be also found in the upper dermal layers and even penetrate through the damaged epidermis to discharge themselves among stylostomes and scabs. This type of tissue reaction during feeding period of trombiculid larvae *N. talmiensis* on both naturally infected voles and chipmunks may be characterized as a permanent delayed inflammation.

Stages of the larval feeding, i.e. filling of their midgut and the midgut cells of nutrients and nutrition globules correspond to the progressive grades of the stylostome development – the larger stylostome, the more advanced feeding stage. Importantly, however, that on the early stages of the stylostome formation no aliment is present in the larval midgut, even if the feeding cavity is already developed. Remarkably, while the debris of inflammatory and epidermal cells may be observed in the stylostome canal, no whole cell elements or even cell debris of the host tissues are usually present in the midgut lumen and the midgut cells of feeding larvae. During feeding, the midgut cells are greatly enlarged and become filled with round violet nutrition globules varying in size, whereas the midgut lumen become squeezed and contains only vacuolated blueish material with small dark grains. While feeding, the excretory organ of larvae is gradually filled with tightly packed brownish guanine granules showing an optical anisotropy, among which small violet globules are also present. These globules in the excretory organ may be seen even in the case when the midgut is still free from nutrition. During feeding, the excretory products are periodically evacuated through the excretory pore. As a result of feeding, larvae greatly increase in sizes and are filled with food.

In spite of these general characteristic of feeding, in one case larva on the middle feeding stage parasitizing chipmunk contained the whole blood in its midgut lumen – tightly packed

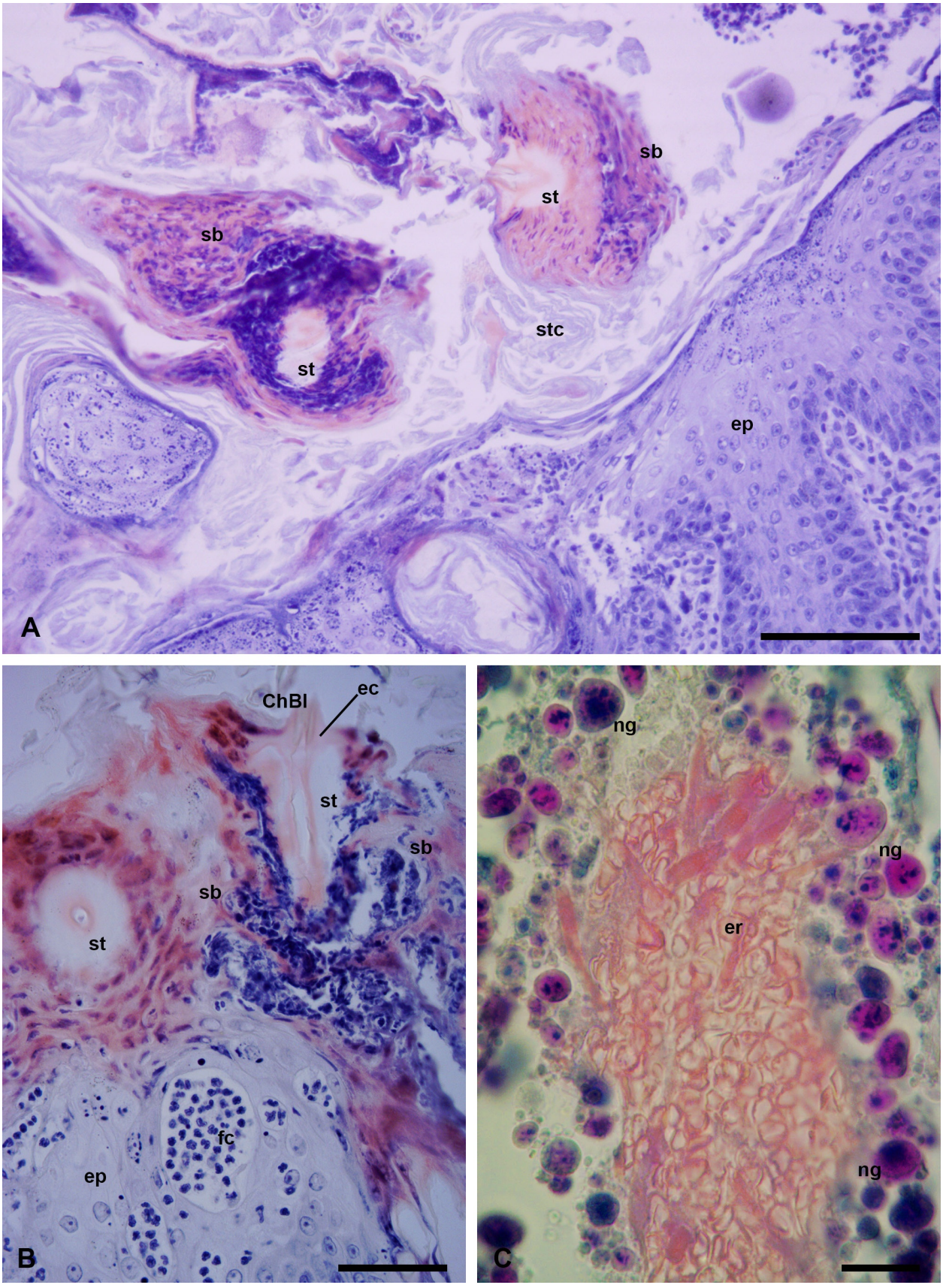

Figure 8 Stylostomes of *Neotrombicula talmiensis* (Schluger, 1955) larvae in the skin of the Asian chipmunk *Tamias sibiricus* (Laxmann, 1769). Histological sections of aural cavity (A-B). The midgut of larva (C). Azure II-Eosin. A – Stylostomes evolved within the extensive scabs outside the epidermis. Scale bar – 100 µm; B – Differently arranged stylostomes completely enclosed in scabs composed of mixed association of the broken inflammatory and epidermal cells. Scale bar – 50 µm; C – The midgut of larva filled with erythrocytes. Nutrition globules located in the midgut cells badly preserved. Scale bar – 20 µm. — ChBl – cheliceral blades, ec – eosinophil cone, ep – epidermis, er – erythrocytes, fc – feeding cavity, ng – nutrition globules, sb – scab, st – stylostome, stc – stratum corneum

erythrocytes (Figure 8C). Leucocytes were absent. This finding indicates that generally larvae of this species may engulf erythrocytes.

Discussion

The present investigation has evidently shown that larvae of the studied trombiculid species produce nearly identical stylostomes by their shape and localization in the two different host species. Because all studied stylostomes of *N. talmiensis* were restricted within the epidermis and never penetrated into the dermis, this type of the stylostome may be attributed as the epidermal ones (Hase *et al.* 1978). At the same time, the position of stylostome, depending on the site of the larval attachment, is highly variable – from within the 'living' epidermal layer up to the keratinized horn layer only. This situation may provoke the particular variations in the stylostome shape and size that may also depend on the activity of the surrounding host tissues. This activity seems to be more pronounced in the case of voles in which the external greyish portion of the stylostome walls appears to be a result of a complex interaction of tissue fluids and the external stylostome substances. However, the main stylostome configuration and the staining ability are restricted within the minimum variations.

Stylostomes of all previously studied trombiculid species also differ from each other (Jones 1950; Allred 1954; Aoki 1957; Clark and Stotts 1960; Hoeppli and Schumacher 1961, 1962; Schumacher and Hoeppli 1963; Voigt 1970; Hase *et al.* 1978; Goldberg and Holshuh 1992; Shatrov 2000, 2009, 2018; Shatrov and Stekolnikov 2011; Shatrov *et al.* 2014; Shatrov and Mirolubov 2015) that may point to a species-specific stylostome organization with regard to a given parasite species. The main function of any stylostome type, besides anchoring of the parasite to the host skin, is to obtain liquefied nutrients from the underlying tissues dissolved by the larval salivary secretion, ultimately from the loosely organized connective tissue layer of the host skin (Schumacher and Hoeppli 1963; Voigt 1970; Shatrov 2009). This phenomenon – feeding by the already dissolved liquid host nutrients – has been termed as the 'extra-oral' or 'extra-intestinal' digestion (Cohen 1995, 1998) highly characteristic for arthropods and, in particular, arachnids.

The initial stylostome portion – the eosinophil cone – is a constant characteristic of trombiculid larvae (Shatrov 2009) and shows an apparent viscosity because the cheliceral movable digits adhere to it and leave prints in its substance after the larval detachment. Immediately after discharging onto the host epidermal surface, this initial larval viscous fluid secretion supposedly transforms from the 'sol' into the 'gel' condition and firmly cements the larva to the host epidermis (see Schramlová 1978). Nearly the same situation was observed during attachment of the cattle-tick *Boophilus microplus* (Canestrini), when the tick just before piercing the host skin releases an immunological inactive fluid secretion – the proximal portion of the further cement cone (Moorhouse and Tatchell 1966). The further steps of attachment in both these cases are also quite similar. In the case of trombiculid larvae, immediately after piercing of the stratum corneum and adhering of the chelicerae to the solidifying substance of the eosinophil cone, the larva apparently injects a type of the liquid lytic secretion into the wound that liquefies the epidermal cells just beneath the perforation. This results in the formation of the particular feeding cavity (Shatrov 2009). Nearly the same fluid-filled cavity is formed underneath the cement cone during feeding of ixodid ticks (Moorhouse and Tatchell 1966; Banerjee *et al.* 1992). However, in the case of ixodids, the formation of feeding cavity is more attributable to the activity of neutrophil leukocytes (Tatchell and Moorhouse 1970; Brown and Knapp 1980; Banerjee *et al.* 1992; Amosova 1994). Conversely, trombiculid larvae form a feeding cavity within the upper epidermal layers immediately after attachment when there are no leukocytes still present in the wound. Nevertheless, this joint action – the mechanical damage and lytic secretion activity – causes the host response inflammatory reaction, which manifests itself in the epidermal hyperplasia, dilation of the terminal blood vessels and active migration of lymphoid cells to the inflammatory focus.

The subsequent stylostome formation is confined in the gradual building of the stylostome tube that is facilitated by simultaneous liquefying of the underneath host tissues by the lytic salivary secretion of the larva as well as by substances released from destroyed leukocytes. It is no doubt that the stylostome tube is a derivative of the feeding larva (Aoki 1957; Schumacher and Hoeppli 1963; Voigt 1970; Hase *et al.* 1978; Shatrov 2009; etc.). Nevertheless, it has been supposed that the eosinophil substance surrounding the proximal stylostome portions of larvae *Eutrombicula lipovskyana* (Wolfenbarger) during their feeding on spiny lizards *Sceloporus jarrovii* (Cope) is derived from coagulated proteins of the host tissues (Goldberg and Holshuh 1992). It is clear, however, that the external stylostome portions are more or less affected by the host tissue fluids that is more obvious in the case of voles in the present study. Throughout the entire feeding period of larvae, the stylostome continues to build, and, generally, the longer feeding period of the given trombiculid species – the longer stylostome (Shatrov 2000). However, the ultimate length of the stylostome tube that directly related to the stylostome age, is not random, totally depends on the parasite species and duration of its feeding. For example, the stylostome may reach 350 µm (Shatrov and Stekolnikov 2011) and even 926 µm (Allred 1954) significantly exceeding in the latter case the length of the larval body in a totally fed state. In the case of *Kepkatrombicula desaleri* (Methlagl) feeding on chamois in Europe, a comparatively thin stylostome penetrates deep into the host dermis (Shatrov and Stekolnikov 2011). The stylostome substance reveals an extremely low staining ability with some amount of acid mucopolysaccharides (Schumacher and Hoeppli 1963; Voigt 1970) and combination of glycoproteins (Schramlová 1978; Shatrov 2000). By this character, a stylostome is also quite similar to a combined cement substance of some ixodid ticks (Moorhouse and Tatchell 1966; Balashov 1967; Chinery 1973) showing the presence of carbohydrate-containing proteins and lipoproteins as well with a low antigenic activity. The latter prevents rapid elimination of the parasite from the host owing to its immunological response and provides growing of stylostome up to the appropriate condition allowing a successful feeding.

Concerning the possible action of the mite salivary secretion in the wound and the mechanism of the stylostome formation as such, two opposite opinions exist. In accordance with the first one, an unreactive stylostome, produced by one type of secretion, provides only the mechanical penetration into the host skin, whereas another type of secretion, injected alternately with the cemented secretion, possesses hydrolytic properties, liquefies the host tissues and acts mostly upon dermis (Schumacher and Hoeppli 1963; Schramlová 1978). According to another idea, the one-time portion of the injected saliva serves for both building of the solidifying stylostome walls and dissolution of the host tissues, and formation of stylostome continues for the entire feeding period (Voigt 1970), as was mentioned above. Most likely, however, that, starting with formation of the eosinophil cone, several salivary fractions, differently targeted, function in a strict sequence in every given trombiculid species that leads to the species-specific stylostome characteristics. Differences in action of different saliva fractions are clearly seen from the comparison of feeding larvae of *Euschoengastia rotundata* (Schluger) and *Cheladonta costulata* (Willmann). Larvae of *E. rotundata* form a type of deep capsules on the ventral body regions of voles *Myodes rufocanus* Sundevall composed of solidifying secretion spreading upon the epidermal surface that is accompanied by an edema and a weak inflammatory response (Shatrov 2000). Conversely, larvae of *Ch. costulata* feeding on voles *Microtus arvalis* (Pallas) cause strong ulceration with degradation of the host epidermis and partial encapsulation owing not to a larval secretion but to a strong tissue edema (Schramlová 1978).

While the stylostome substance shows weak antigenic properties, the liquid hydrolytic fraction injected into the epidermal perforation provokes the cell inflammatory response and formation of the skin ulceration in the feeding site. It was found that in naturally immunized highly sensitized hosts like voles, a strong specific inflammatory response is evolved against feeding larvae (Wright *et al.* 1988; Wrenn 1996). The latter is especially evident in the case of deeply penetrating the so-called 'mesenchymal' stylostomes, which 'accumulate' many leukocytes, in particular, neutrophils, lymphocytes and macrophages around themselves (Hase *et al.* 1978; Shatrov and Stekolnikov 2011). It is considered that a

strong inflammation and numerous destroyed host inflammatory cells in the attachment site around deeply immersed stylostomes is a good background for transmission of disease agents from a parasite to a recipient (Boese 1972; Hase *et al.* 1978). It was shown in this regard that larvae of *Leptotrombidium*, which were recorded as vectors of tsutsugamushi disease agents (*O. tsutsugamushi*), reveal the comparatively long stylostomes penetrating deep into the dermis, and their feeding is accompanied by an intensive inflammatory reaction with dilation of capillaries and intensive hemorrhages (Hase *et al.* 1978). A particular role in this process can be attributed to macrophages because it was shown that rickettsia might survive and even reproduce themselves in these cells for some time (Boese 1972).

In the case of *N. talmiensis*, an intensive larval feeding cause, however, a moderate inflammatory response both in voles and chipmunk with the not so strong skin ulceration, formation of scabs on the epidermal surface composed of the broken inflammatory and epidermal cells with predominance of neutrophil leukocytes in the focus. The venules and capillaries are found dilated. At the same time, the initial thickness of the epidermis prevents a rapid and intensive incoming of leukocytes to the wound. Other leukocytes like eosinophils and basophils, as well as mast cells were not identified with certainty in the tissue damage. The latter may indicate that feeding of *N. talmiensis* on both naturally infected host animals provokes not so strong inflammatory response to attract mast cells to the damage, which would release histamine to attract, in turn, eosinophils, as it was shown in the case of feeding of some ixodid ticks (e.g. Tatchell and Moorhouse 1970; Brown and Knapp 1980; Amosova 1994). Although larvae perforate the epidermis by the action of the saliva secretion, the stylostomes do not penetrate beyond the epidermal layer into the dermis. Nevertheless, owing to this feeding characters, one can suppose that generally this trombiculid species under certain conditions can serve as a potential vector of pathogens and could transmit disease's agents if they would occur in the epidemic foci. In any case, this feature can be defined not by the type and length of stylostome as such but most likely by the type and intensity of the accompanied inflammation. Several pathogenic bacteria (i.e. *Borrelia burgdorferi* s.l.) were detected in species of the *N. talmiensis* group in Europe (Fernandez-Soto *et al.* 2001; Kampen *et al.* 2004; Literak *et al.* 2008). Although their importance in maintaining the natural foci is not proven.

The infection agent of scrub typhus, *O. tsutsugamushi*, was detected in several species of *Neotrombicula* (Elliot *et al.* 2019), but there have been no proven vectors of *O. tsutsugamushi* among the genus members. *Orientia tsutsugamushi* has been mostly recorded as transmitted by *Leptotrombidium* mites in Japan (Kawamura *et al.* 1995), and representatives of this genus were also found in the Far East of Russia (i.e. *Leptotrombidium pallidum, L. orientale, L. palpale*) (Kulagin and Tarasevitch 1972; Kudryashova 1998). In this region, the infections caused to humans were reported and high rates of the disease agents were found in trombiculid mites and small mammals in the 1960s (Kulagin and Tarasevitch 1972). Although since then, there were seldom data about these foci, and the infection rates have decreased (Urakami *et al.* 1999). In Primorye, *N. talmiensis* is abundant species and it could probably take part in maintaining epidemiological foci of scrub typhus in the Far East of Russia (Kulagin and Tarasevitch 1972). For example, during co-feeding with infected vector species or on the infected hosts (Frances *et al.* 2000). Nevertheless, to our best knowledge, there have been no experiments on acquisition of *O. tsutsugamushi* by *Neotrombicula* larvae. Small mammals maintain the natural focus of scrub typhus, and *O. tsutsugamushi* was detected both in *T. sibiricus* and in *M. rufocanus* (Elliot *et al.* 2019). However, this question needs further investigations.

Previously, erythrocytes have not been found in the midgut lumen of trombiculid larvae, and so a pure blood was not attributed to potential nutrients of these mites (Shatrov 2000). Typically, the midgut lumen of larvae contains a totally non-cellular weakly stained substance without any cell fragments. Conversely, the midgut cells gradually accumulate nutrients in the form of large round violet globules throughout feeding (Shatrov 2000). The same situation is also seen in the case of *N. talmiensis*. Nevertheless, an accidental discovery of the pure blood in the midgut lumen of a feeding larva evidently points to a possibility for larvae to be bloodsuckers in a particular but still unknown eco-physiological situation. This is indirectly

confirmed by the fact that erythrocytes were also found in the stylostome canal of the very long stylostomes of another trombiculid species – *K. desaleri* feeding on chamois in Europe (Shatrov and Stekolnikov 2011). It may be supposed that this feature is a new evolutionary acquisition of trombiculid larvae. While the erythrocytes can be only occasionally found in the stylostome canal, the fragments of the lymphoid cells have been frequently seen throughout the stylostome canal, but they have also never been found in the midgut lumen.

Conclusion

Trombiculid mites are the only group of the parasitic arthropods, whose parasitic larvae, feeding on vertebrates, produce a particular structure specially designed for two main purposes – (i) anchoring of the parasite to the host body and (ii) obtaining an appropriate food, typically not blood, from the skin layer both within and beneath the epidermis. The stylostome of trombiculids is one of the leading factors in the question of the origin of parasitism in trombiculid mites and other Parasitengona (Shatrov 2001). Other Parasitengona groups feeding on insects also produce stylostomes designed for similar purposes but of a quite different structure, which cannot be compared in detail with the stylostome of trombiculids (e.g. Davids 1973; Åbro 1984; Lanciani and Smith 1989; Smith 2003; Mohamed and Hogg 2004; Shatrov and Felska 2017; Felska *et al.* 2020). In particular, these stylostomes may be extremely long, branched and blindly ended. Nevertheless, the first above-mentioned function is characteristic for both stylostomes of all Parasitengona and special 'cement substance' of some ixodid ticks from the subfamily Amblyomminae (Moorhouse and Tatchell 1966; Chinery 1973; Banerjee *et al.* 1992; Jaworski *et al.* 1992; etc.). Both structures – stylostome and cement cone – are produced by the parasites' salivary glands (Jaworski *et al.* 1992) and appear to be characterized by low antigenic properties. The latter prevents rapid elimination of the parasite from the host and provides an effective feeding. All these reasons make these two systematically and phylogenetically distant groups effective and evolutionary progressive parasites with surprisingly similar feeding mechanisms evolving independently in the course of evolution.

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References

Åbro A. 1984. The initial stylostome formation by parasitic larvae of the water mite genus *Arrenurus* on zygopteran imagines. Acarologia. 25: 33-45.

Allred D.M. 1954. Observations on the stylostome (feeding tube) of some Utah chiggers. Proc. Utah Acad. Sci., Arts and Letters, 31: 61-63.

Amosova L.I. 1994. Ultrastructural features of histopathological changes at the attachment site of the ixodid tick *Ixodes ricinus*. Parasitologiya, 28: 356-363 [In Russian].

Aoki T. 1957. Histological studies on the so-called stylostome or hypopharynx in the tissues of the host parasitized by the trombiculid mites. Acta Med. Biol., 5: 103-120.

Arnold E.N. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. Biol. J. Linn. Soc., 29: 1-21. doi:10.1111/j.1095-8312.1986.tb01767.x

Audy J.R. 1951. Trombiculid mites and scrub itch. Aust. J. Sci., 14: 94-96.

Balashov Yu.S. 1967. Blood-sucking ticks (Ixodoidea) - vectors of human and animal diseases. Leningrad: "Nauka". pp. 320 [In Russian].

Banerjee D.P., Momin R.R., Samantaray S. 1992. Histopathological changes at *Hyalomma anatolicum anatolicum* feeding sites on tick-resistant calves. Ind. J. Anim. Sci., 62: 24-27.
Boese J.L. 1972. Tissue reactions at the site of attachment of chiggers. J. Med. Entomol., 9: 591.
Brown S.J., Knapp F.W. 1980. *Amblyomma americanum*: sequential histological analysis of larval and nymphal feeding sites on guinea pigs. Exp. Parasitol., 49: 188-205. doi:10.1016/0014-4894(80)90116-2
Chinery W.A. 1973. The nature and origin of the "cement" substance at the site of attachment and feeding of adult *Haemaphysalis spinigera* (Ixodidae). J. Med. Entomol., 10: 355-362. doi:10.1093/jmedent/10.4.355
Clark G.M. Stotts V.D. 1960. Skin lesions on black ducks and mallards caused by chigger (*Womersia strandtmanni* Wharton 1947). J. Wildl. Man., 1: 106-108. doi:10.2307/3797371
Cohen A.C. 1995. Extra-oral digestion in predaceous terrestrial Arthropoda. Ann. Rev. Entomol., 40: 85-103. doi:10.1146/annurev.en.40.010195.000505
Cohen A.C. 1998. Solid-to-liquid feeding: The inside(s) story of extra-oral digestion in predaceous Arthropoda. Amer. Ent., 44: 103-116. doi:10.1093/ae/44.2.103
Davids C. 1973. The water mite *Hydrachna conjecta,* bionomics and relation to species of Corixidae. Neth. J. Zool., 23: 363-429. doi:10.1163/002829673X00012
Elliott I., Pearson I., Dahal P., Thomas N. V., Roberts T., Newton P. N. 2019. Scrub typhus ecology: a systematic review of *Orientia* in vectors and hosts. Parasites & Vectors, 12: 1-36. doi:10.1186/s13071-019-3751-x
Felska M., Mąkol J., Shatrov A.B. 2020. Stylostome formation by parasitic larvae of *Allothrombium fuliginosum* (Trombidiformes: Trombidiidae): morphology of feeding tubes and factors affecting their size. Exp. Appl. Acarol., 82: 359-378. doi:10.1007/s10493-020-00553-8
Fernandez-Soto P., Perez-Sanchez R., Encinas-Grandes A. 2001. Molecular detection of *Ehrlichia phagocytophila* genogroup organisms in larvae of *Neotrombicula autumnalis* (Acari. Trombiculidae) captured in Spain. J. Parasitol., 87: 1482-1483. doi:10.1645/0022-3395(2001)087[1482:MDOEPG]2.0.CO;2
Frances S.P., Watcharapichat P., Phulsuksombati D., Tanskul P. 2000. Transmission of *Orientia tsutsugamushi*, the etiological agent for scrub typhus, to co-feeding mites. Parasitology, 120: 601-607. doi:10.1017/S0031182099005909
Goldberg S.R., Holshuh H.J. 1992. Ectoparasite-induced lesions in mite pockets of the yarrow's spiny lizard, *Sceloporus jarrovii* (Phrynosomatidae). J. Wildl. Dis., 28: 537-541. doi:10.7589/0090-3558-28.4.537
Hase T., Roberts L.W., Hildebrandt P.K., Cavanaugh D.C. 1978. Stylostome formation by *Leptotrombidium* mites (Acarina: Trombiculidae). J. Parasitol., 64: 712-718. doi:10.2307/3279967
Hoeppli R., Schumacher H.H. 1961. Histological and histochemical reactions to trombiculid mites. Ann. Rept. Res. Act. Liber. Inst.: 54-58.
Hoeppli R., Schumacher H.H. 1962. Histological reactions to trombiculid mites, with special reference to "natural" and "unnatural" hosts. Z. Trop. Parasitol., 13: 419-428.
Jaworski D.C., Rosell R., Coons L.B., Needham G.R. 1992. Tick (Acari: Ixodidae) attachment cement and salivary gland cells contain similar immunoreactive polypeptides. J. Med. Entomol., 29: 305-309. doi:10.1093/jmedent/29.2.305
Jones B.M. 1950. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. Parasitology, 40: 247-260. doi:10.1017/S0031182000018096
Kampen H., Schöler A., Metzen M., Oehme R., Hartelt K., Kimmig P., Maier W.A. 2004. *Neotrombicula autumnalis* (Acari, Trombiculidae) as a vector for *Borrelia burgdorferi* sensu lato? Exp. Appl. Acarol., 33: 93-102. doi:10.1023/B:APPA.0000029975.92510.90
Kawamura A., Tanaka H., Tamura A. 1995. Tsutsugamushi Disease. Tokyo: University of Tokyo Press. pp. 362.
Kudryashova N.I. 1998. Chigger mites (Acariformes, Trombiculidae) of East Palaearctics. Moscow: KMK Scientific Press. pp. 342 [In Russian].
Kulagin S.M., Tarasevitch I.V. 1972. Tsutsugamushi fever. Moscow: "Meditsina". pp. 230 [In Russian].
Lanciani C.A., Smith B.P. 1989. Constancy of stylostome form in two water mite species. Canad. Entomol., 121: 439-443. doi:10.4039/Ent121439-6
Lillie R.D. 1969. Histopathological technic and practical histochemistry. Moscow: "Mir" Publishing House. pp. 639 [Translation to Russian].
Literak I., Stekolnikov A.A., Sychra O., Dubska L., Taragelova V. 2008. Larvae of chigger mites *Neotrombicula* spp. (Acari: Trombiculidae) exhibited *Borrelia* but no *Anaplasma* infections: a field study including birds from the Czech Carpathians as hosts of chiggers. Exp. Appl. Acarol., 44: 307-314. doi:10.1007/s10493-008-9150-1
Mohamed A.M.A., Hogg D.B. 2004. The attachment and stylostome of *Trombidium newelli* (Acari: Trombidiidae), an ectoparasitic mite on adults of alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae). Exp. Appl. Acarol., 34: 323-333. doi:10.1023/B:APPA.0000049217.01667.78
Moorhouse D.E., Tatchell R.J. 1966. The feeding processes of the cattle-tick *Boophilus microplus* (Canestrini): A study in host-parasite relations. Parasitology, 56: 623-632. doi:10.1017/S003118200007164X
Neuhaus B., Schmidt T., Riedel J. 2017. Collection management and study of microscope slides: storage, profiling, deterioration, restoration procedures, and general recommendations. Zootaxa, 4322: 173. doi:10.11646/zootaxa.4322.1.1
Schramlová J. 1978. Skin lesion produced by the larva of *Cheladonta costulata* (Willmann, 1952) (Acarina: Trombiculidae) and the feeding mechanism of the parasite. Folia Parasitol., 25: 261-270.
Schumacher H.H., Hoeppli R. 1963. Histochemical reactions to trombiculid mites, with special reference to the structure and function of the "stylostome". Z. Trop. Parasitol., 14: 192-208.
Shatrov A.B. 2000. Trombiculid mites and their parasitism on vertebrate hosts. St.-Petersburg: St.-Petersburg University Publishers. pp. 276 [In Russian with English summary].

Shatrov A.B. 2001. On the origin of parasitism in trombiculid mites (Acariformes: Trombiculidae). Acarologia, 41: 205-214.
Shatrov A.B. 2009. Stylostome formation in trombiculid mites (Acariformes: Trombiculidae). Exp. Appl. Acarol., 49: 261-280. doi:10.1007/s10493-009-9264-0
Shatrov A.B. 2018. On the life cycle and parasitism of the trombiculid mite *Hirsutiella hexasternalis* (Kudryashova, 1998) (Acariformes, Trombiculidae). Soil Organisms, 90: 157-170.
Shatrov A.B., Felska M. 2017. Comparative stylostome ultrastructure of *Hirsutiella zachvatkini* (Trombiculidae) and *Trombidium holosericeum* (Trombidiidae) larvae. Exp. Appl. Acarol., 72: 339-365. doi:10.1007/s10493-017-0172-4
Shatrov A.B., Mirolubov A.A. 2015. Stylostome and feeding of the trombiculid larva *Neotrombicula tianshana* Shao et Wen, 1984 (Acariformes: Parasitengona) from the Baikal region. Int. J. Acarol., 41: 537-550. doi:10.1080/01647954.2015.1079238
Shatrov A.B., Stekolnikov A.A. 2011. Redescription of a human-infesting European trombiculid mite *Kepkatrombicula desaleri* (Acari: Trombiculidae) with data on its mouthparts and stylostome. Int. J. Acarol., 37: 176-193. doi:10.1080/01647954.2010.548342
Shatrov A.B., Takahashi M., Noda S., Misumi H. 2014. Stylostome organization in feeding *Leptotrombidium* larvae (Acariformes: Trombiculidae). Exp. Appl. Acarol., 64: 33-47. doi:10.1007/s10493-014-9809-8
Smith B.P. 2003. Diversity of stylostome structure among parasitic larval water mites Prostigmata: Hydrachnidia). In: Smith I.M. (Ed). An acarological tribute to David R. Cook - From Yankee Springs to Wheeny Creek. West Bloomfield, Michigan: Indira Publishing House. p. 239-255.
Stekolnikov A.A. 2001. New species and sympatric relationships of the chigger mite species group *talmiensis* (Trombiculidae, *Neotrombicula*). Parazitologiya, 35: 296-518 [In Russian].
Stekolnikov A.A. 2002. Variability of chigger mites of the *talmiensis* group, genus *Neotrombicula* Hirst, 1925 (Acari, Trombiculidae) in the areas of sympatry in the Western Caucasus. Entomol. Obozr., 81: 761-777 [In Russian].
Stekolnikov A.A. 2013. *Leptotrombidium* (Acari: Trombiculidae) of the World. Zootaxa, 3728: 1-173. doi:10.11646/zootaxa.3728.1.1
Stekolnikov A.A., Ballardini B., Mignone W., Scapin P., Ulbano M., Marsan A., Balduzzi A. 2014. First finding of the chigger mite *Neotrombicula talmiensis* (Acari: Trombiculidae) in Italy. Int. J. Acarol., 40: 419-420. doi:10.1080/01647954.2014.942361
Takahashi M., Misumi H., Urakami H., Nakajima S., Furui S., Yamamoto S., Furuya Y., Misumi M., Matsumoto I. 2004. Mite vector (Acari: Trombiculidae) of scrub typhus in a new endemic area in Northern Kyoto, Japan. J. Med. Entomol., 41: 107-114. doi:10.1603/0022-2585-41.1.107
Tatchell R.J., Moorhouse D.E. 1970. Neutrophils: Their role in the formation of a tick feeding lesion. Science, 167: 1002-1003. doi:10.1126/science.167.3920.1002
Urakami H., Tamura A., Tarasevich I.V., Kadosaka T., Shubin F.N. 1999. Decreased prevalence of *Orientia tsutsugamushi* in trombiculid mites and wild rodents in the Primorye region, Far East Russia. Microbiol. Immunol., 43: 975-978. doi:10.1111/j.1348-0421.1999.tb03358.x
Voigt B. 1970. Histologische Untersuchungen am Stylostom der Trombiculidae (Acari). Z. Parasitenk., 34: 180-197. doi:10.1007/BF00259695
Wrenn W.J. 1996. Immune responses to mange mites and chiggers. In: Wikel S. (Ed). The Immunology of Host-Ectoparasitic Arthropod Relationships. Wallingford, UK: CAB International. p. 259-289.
Wright S.M., Wikel S.K., Wrenn W.J. 1988. Host immune responsiveness to the chigger, *Eutrombicula cinnabaris*. Ann. Trop. Med. Parasitol., 82: 283-293. doi:10.1080/00034983.1988.11812245