Miniaturization effects in the anatomy of the minute springtail *Mesaphorura sylvatica* (Hexapoda: Collembola: Tullbergiidae) (#37277)

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Miniaturization effects in the anatomy of the minute springtail *Mesaphorura sylvatica* (Hexapoda: Collembola: Tullbergiidae)

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Animals display unique characteristics related to small body size, and in recent years miniaturization has been intensely studied in insects. However, the effects of miniaturization have been poorly studied in other arthropods. Collembola, or springtails, are abundant soil microarthropods and part of a basal hexapod group. Many of them are notably smaller than one millimeter long, which makes them a good model for studying miniaturization effects in arthropods. In this study we analyze the anatomy of the minute springtail *Mesaphorura sylvatica* (body length 400 µm) for the first time. It is described using light and scanning electron microscopy and 3D computer reconstruction. Possible effects of miniaturization are revealed based on a comparative analysis of data from this study and from studies on the anatomy of larger collembolans. Despite the extremely small size, some systems of organs, e.g., muscular and digestive, remain complex. On the other hand, considerable changes in the nervous system have been observed. The brain has two pairs of apertures with three pairs of muscles running through them, and all ganglia shifted in their position posteriad by one segment. The relative volumes of the skeleton, brain, and musculature are smaller than the ones of most microinsects, while the relative volumes of other systems are greater than or same as in most microinsects. Comparison of the effects of miniaturization in collembolans with those found in insects has shown that most of the miniaturization-related features of *M. sylvatica* have also been found in other microinsects (shift of the brain into the prothorax, reduced heart, absence of midgut musculature, etc.), but also has revealed unique features (brain with two apertures and three pair of muscles going through them), which have not been described before.

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ABSTRACT

Animals display unique characteristics related to small body size, and in recent years miniaturization has been intensely studied in insects. However, the effects of miniaturization have been poorly studied in other arthropods. Collembola, or springtails, are abundant soil microarthropods and part of a basal hexapod group. Many of them are notably smaller than one millimeter long, which makes them a good model for studying miniaturization effects in arthropods. In this study we analyze the anatomy of the minute springtail *Mesaphorura sylvatica* (body length 400 µm) for the first time. It is described using light and scanning electron microscopy and 3D computer reconstruction. Possible effects of miniaturization are revealed based on a comparative analysis of data from this study and from studies on the anatomy of larger collembolans.

Despite the extremely small size, some systems of organs, e.g., muscular and digestive, remain complex. On the other hand, considerable changes in the nervous system have been observed. The brain has two pairs of apertures with three pairs of muscles running through them, and all ganglia shifted in their position posteriad by one segment. The relative volumes of the skeleton, brain, and musculature are smaller than the ones of most microinsects, while the relative volumes of other systems are greater than or same as in most microinsects.

Comparison of the effects of miniaturization in collembolans with those found in insects has shown that most of the miniaturization-related features of *M. sylvatica* have also been found in other microinsects (shift of the brain into the prothorax, reduced heart, absence of midgut musculature, etc.), but also has revealed unique features (brain with two apertures and three pair of muscles going through them), which have not been described before.

Keywords: miniaturization, morphology, anatomy, Collembola, body size

INTRODUCTION

Miniaturization plays an important role in morphological changes in animals and has become a popular area of research (Hanken & Wake, 1993; Polilov, 2016a; etc.). Many arthropods are comparable in size with unicellar organisms and are of great interest for studying miniaturization in animals.

Morphological traits (rev.: Polilov, 2015a; Polilov, 2016a; Minelli & Fusco, 2019), scaling of organs (Polilov & Makarova, 2017), and even cognitive abilities (van der Woude et al., 2018; Polilov et al., 2019) associated with miniaturization have been studied in insects. Studies on other minute Panarthropoda are scarce (Dunlop, 2019; Gross et al., 2019).

Studies on the miniaturization of insects and the anatomy of the smallest insects show significant changes in the anatomy of microinsects ~~due to~~ their size. Some of these changes have been found among several taxa, e.g., the reduction of circulatory and tracheal systems, absence of midgut musculature; compactization, oligomerization, and asymmetry of central nervous system; and many more (Polilov, 2016a). However, some of the changes are unique to particular microinsects, such as complete shift of the brain into the thorax at the adult stage (Polilov & Beutel, 2010) in *Mikado* sp., or the lysis of cell bodies and nuclei of neurons in *Megaphragma* sp. (Polilov, 2012, 2017).

Many collembolan genera tend to evolve towards smaller body size, and they might become an interesting model for research of miniaturization in arthropods. However, such studies on the effects of miniaturization in collembolans have not been performed yet, and data on the anatomy of minute collembolans are extremely scarce. Previous studies on collembolan anatomy were based mostly on larger species. Moreover, the majority of them were concentrated on specific systems only. Lubbock (1873) described the anatomy of several species, but studied only the largest muscles of the body, and the head musculature was not mentioned. Fernald (1890) described the anatomy of *Anurida maritima*, but of muscular system he studied only the muscles associated with the digestive system, and the excretory system was not mentioned. Willem (1900) briefly described the anatomy of 12 species, but the muscular and excretory systems were not mentioned. Prowazek (1900) described the embryology and anatomy of both larvae and adults of *Isotoma grisea* and *Achorutes viaticus*, but the head musculature was not mentioned. Denis (1928) described the anatomy of *Anurida maritima*, *Onychiurus fimetarius*, and *Tomocerus catalanus*, but the reproductive system and musculature of the body (except the head musculature) were not mentioned. Mukerji (1932) described the digestive, nervous, and excretory systems, and partly the head musculature of *Protanura carpenteri*. In addition, there were several studies on the muscular system of *Orchesella cincta* (Folsom, 1899; Bretfeld, 1963), *Neanura muscorum* (Bretfeld, 1963), *Tomocerus longicornis* (Lubbock, 1873), *Tomocerus* spp. (Eisenbeis & Wichard, 1975), *Orchesella villosa*, *Isotomurus palustris*, *Podura aquatica*, and *Sminthurus viridis* (Imms, 1939), digestive system of *Tomocerus flavescens* (Humbert, 1979), digestive and excretory systems of *Orchesella cincta* (Verhoef et al., 1979), *Tomocerus flavescens*, *Anurida maritima*, *Neanura muscorum*, *Friesea mirabilis*, *Brachystomella parvula*, *Odontella armata* (Wolter, 1963), and *Sminthurus fuscus* (Willem & Sabbe, 1897), excretory system of *Onychiurus quadriocellatus* (AlPster, 1968), *Tomocerus minor*, *Lepidocyrtus curvicollis* (Humbert, 1975), and *Orchesella rufescens* (Philiptschenko, 1907), respiratory system of *Sminthurus viridis* (Davies, 1927), nervous system of *Folsomia candida*, *Protaphorura armata*, and *Tetrodontophora bielanensis* (Kollmann et al., 2011), reproductive system of *Allacma fusca* (Dallai et al., 2000), *Orchesella villosa* (Dallai et al., 2008), *Anurida maritima* (Lécaillon, 1902a), *Anurophorus laricis* (Lécaillon, 1902b). There were also several reviews (Schaller, 1970; Hopkin, 1997).

The genus *Mesaphorura* of Collembola includes some of the smaller species, some of them only 0.4 long (Zimdars and Dunger, 1994). The external morphology of the genus *Mesaphorura* has been completely and thoroughly investigated as a key to the identification and comparison of this genus to other taxa (Zimdars & Dunger, 1994; D'Haese, 2003), but its internal morphology has never been described. The aim of this work is to study the anatomy of *Mesaphorura sylavtica* for the first time and analyze the effects of miniaturization.

MATERIALS AND METHODS

Materials

Specimens of *Mesaphorura sylvatica* Rusek, 1971 were collected in September 2015 ~~at~~ a sand beach, Pirogovskoye Reservoir, Moscow Oblast, Russia, using the flotation method. The material was fixed in alcoholic Bouin's solution and stored in 70% ethanol.

Scanning electron microscopy (SEM)

External morphology was studied using a Jeol JSM-6380 scanning electron microscope following critical point drying (Hitachi HCP-2) and sputter coating of samples with gold (Giko IB-3).

Histology

The fixed material was dehydrated and embedded in Araldite M. The blocks were cut into series of cross sections 1 μm thick and longitudinal sections 0.5 μm thick using a Leica RM2255 microtome. These sections were stained with toluidine blue and pyronine.

Three dimensional computer reconstruction (3D)

The sections were photographed using a Motic BA410 microscope with a ToupTek camera. The resulting stack was then aligned and calibrated. 3D reconstructions were created in the program Bitplane Imaris using the function of manual segmentation. In addition, we processed the reconstructions with the functions of surface smoothing and rendering in the Autodesk Maya program. Volumes of organs and the body were calculated using the statistical module of Bitplane Imaris (Polilov & Makarova, 2017).

Nomenclature

The names of morphological elements are based on Folsom (1899), Snodgrass (1935), Bretfeld (1963), Bitsch (2012). The description of the musculature and abbreviations of muscles are based on Folsom (1899) for the head, Bretfeld (1963) for the thorax and abdomen, and Eisenbeis and Wichard (1975) for the ventral tube with some additions. Muscles are named according to the nomenclatures used for insects. The following abbreviations are used in descriptions of muscles: O, origin; I, insertion.

RESULTS

General morphology

The body is around 400 μm in length, uniformly pigmented, white in color (Fig. 1). Most of the head is occupied by the brain, the suboesophageal ganglion, the mouthparts and the complex pseudotentorium; the prothorax is occupied by part of the suboesophageal ganglion, while the meso- and metathorax are occupied by the wide midgut and fat body; the abdomen is mainly occupied by the reproductive system, with the digestive system above it (Fig. 2, Fig. S1). All tagmata have well-developed musculature.

The body volume of *M. sylvatica* is about 0.8 nl.

Skeleton

The cuticle thickness is 0.31–1.24 μm (M = 0.57, n = 80). Tergites are well-developed, sclerites and pleurites are hardly distinguishable.

The inner skeletal structures are highly developed. A complex pseudotentorium (Fig. 3) is situated in the head. Its body consists of a mandibular tendon in the middle, which continues into a thinner longitudinal endoskeletal connective posteriorly. There is a pair of dorsal suspensory arms, connecting the structure with the head capsule anteriorly on the frons. The glossa is prolonged behind into a pair of chitinous stalks, called posterior tentorial apodemes by Koch (2000), or fulcra (Denis, 1928). They lie externally to the middle line. The enlarged base of the stalk is called the foot, and the foot underlies the cardo of the maxilla. They seem to connect with the head capsule posteriorly possibly with some endoskeletal connectives. A pair of connecting arms (bras d'union, Denis, 1928) extend from the pseudotentorial plate downwards and are fused with the posterior tentorial apodemes. A pair of lateral arms (bras latéraux, Denis, 1928) extend from the anterior part of the pseudotentorial plate, go upward and outward and are inserted into the head.

According to Folsom (1899) there is also a chitinous rod, which is attached to the base of the lobe of the lacinia. The chitinous rod has a chitinous expansion, which is an attachment site for several maxillary muscles. It is shown in our model as a part of maxilla.

Antecostae are submarginal ridges near the anterior edges of the inner surface of the tergum with several body muscles attached to them.

Three ventral furca-like structures are branched and found in the thorax between the first and the second thoracic segments, between the second and the third thoracic segments, and between the third thoracic segment and the first abdominal segment. Additionally, there is a simple rectangular endosternite in the first abdominal segment.

The volume of the skeleton is about 0.05 nl (5.8% of the body volume).

Digestive and excretory systems

The alimentary canal (Fig. 4) is shaped as a straight tube without loops or diverticula, extending from the anterior and ventral area of the head into the terminal abdominal segment. It is divided into the fore-, mid-, and hindgut.

The slender foregut is round in cross section and extends posteriorly from the oral cavity. It is divided into the pharynx and oesophagus. The slender pharynx is about 4.2 μm. The oesophagus passes through the suboesophageal ganglion and leads into the thicker midgut at the level of the metathorax (around the fourth abdominal segment), which consists of one layer of cells (6–8 cells in cross section). The oesophagus has one pair of muscles 1(Oe). The first half of the midgut is round in cross section, about 27.8 μm in diameter. The second half is oval in cross section. The border between the midgut and the hindgut is indistinguishable. At around the sixth abdominal segment, it extends into the wider rectum with four pairs of muscles. The latter continues backwards and terminates ventrally at the anus with three anal lobes in the last abdominal segment.

Labial nephridia, or tubular glands, the main excretory organs of collembolans, are found in the posterior half of the head (Fig. 4). ~~It~~ is composed of a sac, a labyrinth, and a duct. The sac is situated posteriorly and continues forward into the labyrinth. The labyrinth follows a hardly distinguishable winding course and forms a loop. The labyrinth continues as the duct, which opens in the buccal cavity. Other head glands (anterior and posterior salivary glands, globular, or acinous glands, and antennal nephridia) were not found in *M. sylvatica*.

The volume of the digestive and excretory systems is about 0.07 nL (8.6% of the body volume).

Nervous system

The nervous system (Fig. 4) consists of a supraoesophageal ganglion (brain), suboesophageal ganglion, and three thoracic ganglia. The brain extends from the bases of the antennae to the anterior part of the first thoracic segment. It fills the dorsal portion of the head, but narrows in the posterior portion of the head (beyond the beginning of the suboesophageal ganglion) and extends at the boundary between ~~of~~ the head and the first thoracic segment, it terminates in the anterior half of the latter. The brain has a unique structure, with two pairs of apertures with one pair of oesophageal 1(Oe) and two pairs of pseudotentorial suspensory muscles 1(Pst), 2(Pst) running through them. The suboesophageal ganglion lies in the ventral portion of the head, starting at its middle, and continues to the distal margin of the first thoracic segment. Three large ventral thoracic ganglia, one in each segment, shift their position by one segment: the first ganglion lies in the mesothorax, the second one is in the metathorax, and the third one is in the first abdominal segment. They are interconnected by longitudinal cords in intersegments (one in each). As in all collembolans, the abdominal ganglia are fused with the third thoracic ganglion.

The volume of the central nervous system is about 0.05 nL (6.3% of body volume). The volume of the brain is about 0.02 nL (2.2% of the body volume).

Muscular system

Musculature of head (Fig. 3, Table S1). 1(Pst) (M. craniotentorialis lateralis): O, medial surface of frons, laterad of 2(Pst); I, dorsal surface of pseudotentorial plate, laterad of 2(Pst). 2(Pst) (M. craniotentorialis medialis): O, medial surface of frons, mediad of 1(Pst); I, dorsal surface of pseudotentorial plate, mediad of 1(Pst). 1(An) (M. antennotentoralis): O, lateral face of first antennal segment; I, pseudotentorium. 3(Mn) (M. craniomandibularis posterior): O, posterior surface of gena I, dorsolateral surface of basal ridge of mandible, posterad of 4(Mn). 4(Mn) (M. craniomandibularis anterior): O, frons, mediad of 3(Mn); I, dorsolateral surface of basal ridge of mandible, anterad of 3(Mn), posterad of 5(Mn). 5(Mn) (M. tentoriomandibularis): O, anterior arm of pseudotentorium; I, dorsolateral surface of mandible, anterad of 4(Mn). 7(Mn) (M. craniomandibularis): O, frons, along with 1(Mx), mediad of 10(Mn); I, ventroposterior area, outer angle of large triangular opening of mandible along with 8(Mn), 10(Mn). 8(Mn) (M. craniomandibularis): O, posterior surface of frons, crossing median plane, posterad of 7(Mn); I, ventroposterior area, outer angle of large triangular opening of mandible along with 7(Mn), 10(Mn). 9(Mn) (M. tentoriomandibularis): O, base of pseudotentorium; I, large triangular opening (median surface) of mandible. 10(Mn) (M. craniomandibularis): O, frons, laterad of 7(Mn); I, ventroposterior area, outer angle of large triangular opening of mandible along with 7(Mn), 8(Mn). 11(Mn) (M. craniomandibularis): O, anterior surface of area antennalis, near antennal base, ventrad of 12(Mn); I, lateral surface of mandible, laterad of 12(Mn). 12(Mn) (M. craniomandibularis): O, anterior surface of area antennalis, dorsad of 11(Mn); I, lateral surface of mandible, mediad of 11(Mn). 1(Mx) (M. craniocardinalis): O, dorsomedial area of occiput; I, lateral edge of cardo, dorsad of 5(Mx). 2(Mx) (M. craniostipitalis medialis): O, posterior surface of gena, ventrad of 3(Mx); I, lateral edge of chitinous expansion, mediad of 3(Mx). 3(Mx) (M. craniostipitalis lateralis): O, posterior surface of gena, dorsad of 2(Mx); I, median edge of chitinous expansion, laterad of 2(Mx). 4(Mx) (M. maxillaris internus 1): O, anterior surface of cardo; I, dorsoventral surface of chitinous expansion. 5(Mx) (M. tentoriocardinalis): O, base of pseudotentorium; I, concavity of cardo, ventrad of 1(Mx). 7(Mx) (M. maxillaris internus 2): O, median surface of stipes; I, dorsolateral surface of chitinous expansion. 1–3(Hy) (M. craniohypopharyngealis): O, anterioventral area of the head capsule; I, hypopharynx. Three very small obscure muscles. 1(Oe) (M. cranioesophagialis): O, anteriomedial surface of area antennalis; I, dorsal surface of oesophagus. dlm1 (M. occiputo-cranialis medialis): O, occiput, mediad of dlm2; I, medial surface of frons, mediad of dlm2. dlm2 (M. occiputo-cranialis lareralis): O, occiput, laterad of dlm1; I, medial surface of frons, laterad of dlm1.

Musculature of thorax (Fig. 5, Table S1). Prothorax. I dlm1 (M. antecosta-occipitalis medialis): O, antecosta I, mediad of I dlm2; I, occiput, mediad of I dlm2. I dlm2 (M. antecosta-occipitalis lateralis): O, antecosta I, laterad of I dlm1; I, occiput, laterad of I dlm1. I ism1 (M. antecosta-pseudotentoralis): O, antecosta I; I, posterior area of fulcrum. I ism2 (M. profurca-occipitalis): O, dorsal area of profurca-like structure; I, dorsolateral area of occiput. I dvm1 (M. cervico-coxalis): O, dorsolateral cervical membrane; I, anterior procoxal rim. I dvm3 (M. pronoto-coxalis lateralis; two bands): O, anterior region of pronotum; I, lateral procoxal rim and anterior procoxal rim along with instertion of I dvm2. I ldvm1 (M. pronoto-coxalis medialis): O, anterolateral part of pronotum; I, anterior procoxal rim. Lb dvm1 (M. occiputo-pseudotentoralis; two bands): O, dorsal area of occipitale; I, posterior area of fulcrum. Lb dvm2 (M. occiputo-cervicalis): O, dorsal area of occipitale; I, ventral cervical membrane. I vlm (M. profurca-pseudotentoralis): O, anterior part of profurca-like structure; I, union arm of pseudotentorium. I scm1 (M. profurca-coxalis 1): O, anterior face of profurca-like structure; I, posterior procoxal rim, laterad of I scm2. I scm2 (M. profurca-coxalis 2): O, ventral face of profurca-like structure; I, posterior procoxal rim, mediad of I scm1. I scm3 (M. profurca-coxalis 3): O, ventral face of profurca-like structure along with I scm4; I, lateral procoxal rim. I scm4 (M. profurca-coxalis 4): O, ventral face of profurca-like structure along with I scm3; I, posteriolateral procoxal rim.

Mesothorax. II dlm1 (M. antecosta-antecostalis medialis): O, antecosta II, mediad of II dlm2; I, antecosta III, mediad of II dlm2. II dlm2 (M. antecosta-antecostalis lateralis): O, antecosta II, laterad of II dlm1; I, antecosta III, laterad of II dlm1. II ism1 (M. profurca-antecostalis medialis): O, lateral part of profurca-like structure, mediad of II ism2; I, antecosta III, mediad of II ism2. II ism2 (M. profurca-antecostalis lateralis): O, lateral part of profurca-like structure, laterad of II ism1; I, antecosta III, laterad of II ism1. II dvm1 (M. mesonoto-profurcalis anterior): O, dorsolateral part of profurca-like structure, anterad of II dvm2; I, mesonotum (middle of segment), anterad of II dvm2. II dvm2 (M. mesonoto-profurcalis posterior): O, dorsolateral part of profurca-like structure, posterad of II dvm1; I, mesonotum (middle of segment), posterad of II dvm1. II dvm3 (M. mesonoto-coxalis; two bands): O, lateral part of mesonotum (middle of segment); I, anterior face of mesocoxa. II dvm4 (M. mesonoto-subcoxalis anterior; two bands): O, anterolateral part of mesonotum; I, anterior border of mesosubcoxa. II dvm5 (M. metanoto-subcoxalis posterior): O, posterolateral part of mesonotum; I, ventral border of mesosubcoxa. II ldvm1 (M. mesonoto-coxalis anterior): O, posterolateral part of mesonotum; I, anterior mesocoxal rim. II ldvm2 (M. metanoto-subcoxalis; two bands): O, posterolateral part of mesonotum; I, posteroventral border of mesosubcoxa and posterior border of mesocoxal rim. II ldvm3 (M. metanoto-coxalis posterior): O, posterolateral part of mesonotum; I, anterior face of mesocoxa. II vlm (M. profurca-mesofurcalis): O, lateral part of profurca-like structure; I, lateral part of mesofurca-like structure. II scm1 (M. mesofurca-coxalis 1): O, anterior face of mesofurca-like structure; I, posterior mesocoxal rim, laterad of II scm2. II scm2 (M. mesofurca-coxalis 2): O, ventrolateral face of mesofurca-like structure along with II scm4; I, posterior mesocoxal rim, mediad of II scm1. II scm3 (M. mesofurca-coxalis 3): O, anterior face of mesofurca-like structure; I, lateral mesocoxal rim, laterad of II scm4. II scm4 (M. mesofurca-coxalis 4): O, ventrolateral face of mesofurca-like structure along with II scm2; I, lateral mesocoxal rim, mediad of II scm3. II scm5 (M. profurca-coxalis lateralis): O, posterior face of profurca-like structure; I, anteriolateral metacoxal rim, laterad of II scm6. II scm6 (M. profurca-coxalis medialis): O, posterior face of profurca-like structure; I, anteriolateral metacoxal rim, mediad of II scm5.

Metathorax. All muscles that are present in mesothorax, are found in metathorax, except: III scm4.

Musculature of abdomen (Fig. 6, Table S1). AI dlm1 (M. antecosta-antecostalis medialis): O, antecosta III, mediad of AI dlm2; I, antecosta IV, mediad of AI dlm2. AI dlm2 (M. antecosta-antecostalis lateralis): O, antecosta III, laterad of AI dlm1; I, antecosta IV, laterad of AI dlm1. AI vlm (M. metafurca-endosternalis): O, lateral face of metafurca-like structure; I, endosternite I. AI ism1 (M. antecosta-metafurcalis anterior): O, antecosta IV, anterad of AI ism2; I, lateral face of metafurca-like structure, anterad of AI ism2. AI ism2 (M. antecosta-metafurcalis posterior): O, antecosta IV, posterad of AI ism1; I, lateral face of metafurca-like structure, posterad of AI ism1. AI dvm1 (M. tergo-metafurcalis anterior): O, middle region region of tergum, anterad of AI dvm2; I, lateral face of metafurca-like structure, anterad of AI dvm2. AI dvm2 (M. tergo-metafurcalis posterior): O, middle region region of tergum, posterad of AI dvm1; I, lateral face of metafurca-like structure, posterad of AI dvm1. AI ldvm1 (M. pleuro-metafurcalis): O, lateral wall of segment; I, lateral face of metafurca-like structure. AI dvm VT (M. tergo-pleuralis): O, anterior region of tergum; I, tendon system. AI lm (M. sterno-pleuralis): O, base of ventral tube; I, valva of ventral tube. AI pm1 (M. sterno-pleuralis): O, base of ventral tube; I, metafurca-like structure. AI pm2 (M. sterno-pleuralis): O, base of ventral tube; I, tendon system. AI pm3 (M. sterno-pleuralis): O, base of ventral tube; I, tendon system. AI pm4 (M. sterno-pleuralis): O, base of ventral tube; I, tendon system. AI dvm3 (M. pleuro-pleuralis): O, lateral wall of segment; I, tendon system. AI dvm4 (M. tergo-pleuralis): O, posterior region of tergum; I, tendon system. AI dm1 (M. sterno-pleuralis): O, anterior face of ventral tube, laterad of dm3; I, metafurca-like structure. AI dm2 (M. sterno-pleuralis): O, vesicles of ventral tube; I, endosternite I. AI dm3 (M. sterno-pleuralis): O, anterior face of ventral tube, mediad of dm1; I, metafurca-like structure. AI dm4 (M. sterno-pleuralis): O, vesicles of ventral tube; I, endosternite I. AI dm5 dm4 (M. sterno-pleuralis): O, posterior face of ventral tube; I, tendon system. All dlm1 (M. antecosta-antecostalis medialis): O, antecosta IV, mediad of AII dlm2; I, antecosta V, mediad of AII dlm2. All dlm2 (M. antecosta-antecostalis lateralis): O, antecosta IV, laterad of AII dlm1; I, antecosta V, laterad of AII dlm1. All vlm (M. endosterno-antecostalis): O, endosternum I; I, antecosta V. All ism1 (M. antecosta-endosternalis anterior): O, antecosta V, anterad of AII ism2; I, endosternum I, anterad of AII ism2. All ism2 (M. antecosta-endosternalis posterior): O, antecosta V, posterad of AII ism1; I, endosternum I, posterad of AII ism1. All dvm1 (M. tergo-endosternalis anterior): O, middle region of tergum, anterad of AII dvm2; I, endosternum I, anterad of AII dvm2. All dvm2 (M. tergo-endosternalis posterior): O, middle region of tergum, posterad of AII dvm1; I, endosternum I, posterad of AII dvm1. All ldvm1 (M. pleuro-sternalis): O, lateral wall of segment; I, sternum. All dvm3 (M. tergo-sternalis anterior): O, anterior border of tergum; I, lateral border of sternum. All dvm4 (M. tergo-sternalis posterior): O, lateral area of tergum; I, lateral border of sternum. All trm1 (M. endosterno-endosternalis): O, inner surface of endosternum I; I, inner surface of endosternum I (opposite side). Alll dlm1 (M. antecosta-antecostalis medialis): O, antecosta V, mediad of AIII dlm2; I, antecosta Vl, mediad of AIII dlm2. Alll dlm2 (M. antecosta-antecostalis lateralis): O, antecosta V, laterad of AIII dlm1; I, antecosta Vl, laterad of AIII dlm1. Alll ism1 (M. antecosta-antecostalis medialis): O, antecosta VI, mediad of AIII ism2; I, ventral area of antecosta V, mediad of AIII ism2. Alll ism2 (M. antecosta-antecostalis lateralis): O, antecosta VI, laterad of AIII ism1; I, ventral area of antecosta V, laterad of AIII ism1. Alll dvm1 (M. tergo-antecostalis anterior); O, middle region of tergum, anterad of AIII dvm2; I, ventral area of antecosta V, anterad of AIII dvm2. Alll dvm2 (M. tergo-antecostalis posterior): O, middle region of tergum, laterad of AIII dvm1; I, ventral area of antecosta V, laterad of AIII dvm1. Alll dvm3 (M. tergo-sternalis anterior): O, anterior border of tergum; I, lateral border of sternum. Alll dvm4 (M. tergo-sternalis posterior): O, lateral area of tergum; I, lateral border of sternum. Alll ldvm1 (M. pleuro-sternalis): O, lateral wall of segment; I, sternum. AIV dlm1 (M. antecosta-antecostalis medialis): O, antecosta Vl, mediad of AIV dlm2; I, antecosta Vll, mediad of AIV dlm2. AIV dlm2 (M. antecosta-antecostalis lateralis): O, antecosta Vl, laterad of AIV dlm1; I, antecosta Vll, laterad of AIV dlm1. AlV vlm (M. antecosta-antecostalis): O, antecosta V; I, antecosta VII. AIV ism1 (M. antecosta-antecostalis): O, dorsal part of antecosta VI; I, ventral part of antecosta VII. AIV dvm1 (M. tergo-sternalis posterior): O, anterior border of tergum; I, posterior border of sternum. AIV dvm2 (M. pleuro-sternalis): O, lateral wall of segment; I, sternum, along with AIV ldvm5. AIV dvm3 (M. tergo-sternalis anterior): O, anterior border of tergum; I, posterior border of sternum. AIV ldvm3 (M. tergo-antecostalis): O, posterior region of tergum; I, ventral part of antecosta VII. AIV ldvm4 (M. tergo-sternalis): O, posterior region of tergum; I, lateral board of sternum. AIV ldvm5 (M. tergo-sternalis): O, posterior region of tergum; I, sternum, along with AIV dvm2. AIV ldvm7 (M. pleuro-sternalis): O, lateral wall of segment; I, lateral board of sternum. AV dlm1 (M. antecosta-antecostalis medialis): O, antecosta Vll, mediad of AV dlm2; I, antecosta Vlll, mediad of AV dlm2. AV dlm2 (M. antecosta-antecostalis lateralis): O, antecosta Vll, laterad of AV dlm1; I, antecosta Vlll, AV dlm1. AV ism1 (M. tergo-intersegmentalis): O, anterior region of tergum; intersegmental area between 5th and 6th segments. AV ldvm1 (M. pleuro-sternalis): O, lateral wall of segment; I, sternum. AVl sm1 (M. sterno-rectalis): O, sternum; I, rectum. AVI sm2 (M. sterno-rectalis): O, lateral board of sternum; I, rectum. AVl dvm1 (M. tergo-rectalis): O, anterior region of tergum; I, rectum. AVI dvm2 (M. tergo-sternalis): O, central region of tergum; I, rectum. AVI dvm3 (M. tergo-sternalis): O, posterior region of tergum; I, dorsal anal lobe. AVl dvm4 (M. pleuro-sternalis): O, lateral wall of segment; I, lateral anal lobe.

The volume of the muscular system is about 0.04 nL (5.2% of the body volume).

Reproductive system

The female reproductive system has been studied in detail (Fig. 4). The ovary is unpaired with three lobes. The largest lobe probably contains eggs, while two other, smaller lobes contain no eggs and lie dorsad of the largest one. The anterior portion of the ovary lies between the abdominal segments 2 and 3, while its posterior portion ends between abdominal segments 4 and 5. The oviduct is small, short and unpaired, leading to the vagina, the margins of which are indistinct. The vagina opens ventrally on the fifth abdominal segment with a transverse reproductive orifice (gonopore).

The volume of the reproductive system is about 0.15 nL (18.9% of the body volume).

Circulatory system and fat body

Organs of the circulatory system are absent. The fat body occupies all cavities between organs in the head, thorax, and abdomen. It consists of cells of various shape.

The volume of the ~~circulatory system and~~ fat body is about 0.44 nL (55.2% of the body volume).

Respiratory system

Organs of the respiratory system (trachea) are absent.

DISCUSSION

We studied the anatomy of *M. sylvatica* to extend the knowledge on the anatomy of Collembola as well as to reveal possible miniaturization traits and compare them to the miniaturization effects discovered in microinsects and other minute arthropods. Moreover, we analyzed the relative volume of organs in *Mesaphorura sylvatica* in comparison with microinsects.

Skeleton

The endoskeletal structures of *M. sylvatica* are well-developed as in larger species (Manton, 1964), the complex pseudotentorium has multiple arms, and the furca-like structures are branched. However, reductions seem to have affected the abdomen, in which we observed only one endosternite, compared to *Neanura muscorum*, in which five endosternites were found (Bretfeld, 1963). In microinsects, elements of the endoskeleton tend to fuse (Polilov, 2015a; Polilov, 2015b). Of all adult microinsects, only booklice have a complex tentorium (Polilov, 2016b), and all furcae are developed in thrips, beetles of the family Corylophidae, and wasps of the family Mymaridae (Polilov and Beutel, 2010, Polilov and Shmakov, 2016, Polilov, 2016c).

The relative volume of the skeleton of *M. sylvatica* is similar to the one of adult Paraneoptera of the same size, but notably smaller than the ones of other microinsects (both larvae and adults) of the same size (Polilov & Makarova, 2017). The smaller relative volume of the skeleton of *M. sylvatica*, compared to most microinsects, could be related to the differences in cuticle thickness (due to the fragmented epicuticle of all collembolans) and to the flightlessness of collembolans.

Nervous system

Unlike those of larger species of Collembola (Hopkin, 1997; Kolmann et al., 2011), the brain and suboesophageal ganglion of *M. sylvatica* extend into the prothorax. The three thoracic ganglia of *M. sylvatica* shift their position by one segment posteriorly, reported by Lubbock (1873) in larger species. The brain and suboesophagal ganglion are situated close to each other; they are connected in the neck region. The central nervous system is symmetrical and displays moderate concentration and oligomerization of ganglia. Similar degrees of concentration and oligomerization of the central nervous system are found in adult booklice of the family Liposcelididae (Polilov, 2016b) and in adult thrips (Polilov & Shmakov, 2016).

The shift of different parts of the brain into the prothorax has been described in thrips larvae (Polilov & Shmakov, 2016), adults and larvae of beetles of the family Ptiliidae (Polilov & Beutel, 2009), adults and larvae of beetles of the family Corylophidae (Polilov & Beutel, 2010), larvae of ~~beetles of the family~~ Strepsiptera (Beutel et. al., 2005), larvae of beetles of the family Scydmaenidae (Jałoszyński et al., 2012), larvae of beetles of the family Hydroscaphidae (Beutel & Haas, 1998), adults of beetles of the family Sphaeriusidae (Yavorskaya et al., 2018).

The nervous system of *M. sylvatica* shows unique changes in the brain with two pairs of apertures and three pairs of muscles running through them. This feature has not been described in studies of the nervous system of larger collembolans (Lubbock, 1873; Hopkin, 1997; Kollmann et al., 2011) or microinsects (Polilov, 2015a; Polilov, 2015b).

The relative volume of the central nervous system of *M. sylvatica* is similar to the one of tiny adult Coleoptera, smaller than the ones of minute adult Hymenoptera and Paraneoptera larvae, and greater than the one of adult Paraneoptera of the same size (Polilov & Makarova, 2017). The same relative volume as in tiny adult Coleoptera possibly follows the tendency of the nervous system of microinsects to increase as the body size decreases (Polilov & Makarova, 2017). It is also supported by the fact that representatives of adult minute Paraneoptera have greater body size, but smaller relative volume of the central nervous system compared to the body size and the relative volume of *M. sylvatica*. The smaller relative volume compared to the one of minute adult Hymenoptera and Paraneoptera larvae could be related to better pronounced effects of miniaturization in adult Hymenoptera and Paraneoptera larvae of the same size.

The relative volume of the brain of *M. sylvatica* is similar to the one of adult Coleoptera of the same size, slightly greater than the one of minute adult Paraneoptera, and notably smaller than the ones of other microinsects of the same size (Polilov & Makarova, 2017). The smaller relative volume than in most microinsects of the same size could possibly be related to the simpler behavior and absence of eyes (Jordana et al., 2000).

Circulatory system and fat body

The circulatory system of *M. sylvatica* is simplified, ~~the~~ heart and ~~the~~ vessels are absent. Most of the body cavities of *M. sylvatica* are filled with fat body. In larger species, there is a heart with 2–6 pairs of ostia and an aorta (Fernald, 1890; Denis, 1928; Imms, 1957; Schaller, 1970). The same reduction as in *M. sylvatica* was observed in adults and larvae of beetles of the family Ptiliidae (Polilov, 2005; Polilov & Beutel, 2009), larvae of booklice of the family Liposcelididae (Polilov, 2016b), adult hymenopterans of the family Trichogrammatidae (Polilov, 2016d; Polilov, 2017), tardigrades (Gross et al., 2019), and some chelicerates (Dunlop, 2019). In microinsects, it is assumed that the diffusion of metabolites is sufficient enough for the transport between the organs (Polilov, 2008; Polilov & Beutel, 2009), which is, apparently, the same case in *M. sylvatica*.

The relative volume of the circulatory system and fat body of *M. sylvatica* is particularly great, greater than the ones of other microinsects of the same size except Paraneoptera and Coleoptera larvae (Polilov & Makarova, 2017). The high relative volume could be related to the importance of fat body in excretion in Collembola (Schaller, 1970).

Female reproductive system

The female reproductive system of *M. sylvatica* has unpaired structures (~~the~~ ovaries and oviducts). In larger species, it consists of paired ovaries, oviducts and accessory glands, and unpaired spermatheca. We did not observe ~~the~~ accessory glands and spermatheca in *Mesaphorura sylvatica*, what may be explained by the fact that they are hardly recognizable even in larger species (Schaller, 1970; Dallai, 2008). The same changes were observed in beetles of the family Ptiliidae, in which both sexes have unpaired structures (Polilov & Beutel, 2009).

The relative volume of the reproductive system of *M. sylvatica* is also particularly great; it is smaller only than the ones of some minute adult Coleoptera and minute Hymenoptera (Polilov & Makarova, 2017). The greater relative volume compared to those of most microinsects of the same size could be related to the relative egg size increase with decreasing body size (Polilov, 2016a).

Digestive and excretory systems

The digestive system of *M. sylvatica* is least modified, compared to larger species (Lubbock, 1873; Folsom, 1899; Wolter, 1963; Schaller, 1970). It is straight, without loops or diverticula. Among microinsects, only larvae of booklice of the family Liposcelididae have no loops or pronounced bends (Polilov, 2016b). No salivary glands are found in *M. sylvatica*. Salivary glands in some microinsects are absent as a result of miniaturization (Polilov, 2015a; Polilov, 2015b). We did not observe any muscles of the midgut, ~~the~~ absence which is also a common trait among minute insects (Polilov, 2016a).

The relative volume of the digestive system of *M. sylvatica* is similar to those of most minute Coleoptera, notably smaller than those of minute Paraneoptera, but greater than the ones of Hymenoptera and some Coleoptera species of the same size (Polilov & Makarova, 2017).

Excretory system

~~The~~ specialized organs ~~of the excretory system~~ are absent in *Mesaphorura sylvatica*. In larger species, there might be small papillae instead of Malpighian tubes or labial nephridia in the head (Schaller, 1970). Moreover, in collembolans, there are also acinous glands and antennal nephridia (Wolter, 1963). In microinsects, Malpighian tubes do not disappear, but their number decreases (Polilov, 2015a).

Musculature

The muscular system of *M. sylvatica* is reduced, compared to those of larger species of Collembola, and has 24 pairs of muscles in the head, 51 in the thorax, and 60 in the abdomen (and 1 unpaired muscle); 136 pairs in total. The total number of muscles of all tagmata have not been studied for a single species of springtails, which makes it challenging to compare our results with previous studies.

It is challenging to compare muscles ~~of a head~~ in Collembola. Folsom (1899) described at least 47 pairs of muscles in the head of a large collembolan *Orchesella cincta* associated with the digestive system and mouthparts. There are 26 pairs of muscles associated with mouthparts (labrum, labium, maxilla, mandible) ~~in details~~. In addition, he noted 20 pairs of muscles associated with both pharynx and oesophagus (7 of them are the ventral dilators of pharynx, which he later classified as tentorial muscles), but he did not designate them. He mentioned the presence of tentorial muscles (dilators of pharynx, antennal, and muscles connected to the head), but did not even specify the number of these muscles. Folsom (1899) also mentioned two muscles of the palpi, but it is not clear whether he meant two pairs of muscles or two muscles in total. Denis (1928) described at least 73 pairs of muscles in the head of a large collembolan *Anurida maritima* associated with mouthparts, pseudotentorium and the digestive system. He divided the muscles of the head into several groups, but he specified the number of muscles only for some of them. For mouthparts (maxilla and mandible), he remarked that for some muscles he drew a single bundle that included several muscles, but he did not specify the number of them. There are at least 17 pairs of muscles associated with maxilla and mandible in *Anurida maritima*. Moreover, he described all tentorial muscles, and there are at least 45 of them (14 of those are the ventral dilators of pharynx). In addition, he mentioned superlingual muscles (the number was not given), suspensors of the atrium (3 pairs), 8 pairs of antennal muscles (excluding the muscles inside the antenna), and 5 muscles associated with epipharynx and pharynx. He did not specify a number of muscles of labium. Moreover, he described the groups of muscles of heads of two large species *Onychiurus fimetarius* and *Tomocerus catalanus*, but he compared them to *Anurida maritima*, without any details on exact numbers. With *Tomocerus catalanus* he refers to the study of Hoffmann (1908). Hoffmann (1908) described at least 53 pairs of muscles in the head of a large collembolan *Tomocerus plumbeus.* There are 35 muscles associated with mouthparts (maxilla, mandible, and labium), 15 muscles ~~of~~ pharynx, and 3 muscles of glossa. Hoffmann (1908) also mentioned presence of tentorial muscles, but did not specify the number of them. There are 15 pairs of muscles in *M. sylvatica* associated with maxilla and mandible, one pair of antennal muscles, one pair of oesophagal muscles, two pairs of suspensory pseudotentorial muscles, and three pairs of muscles, possibly associated with hypopharynx. Moreover, there are two pairs of dorsal longitudinal muscles, while this group of muscles was mentioned, but not described in the literature. In this study, we have not described any other tentorial muscles, except those mentioned above, and internal antennal muscles due to their small size. *M. sylvatica* has 15 pairs of muscles of maxilla and mandible, which is less than in larger collembolans *O. cincta* (20), *A. maritima* (17), and *T. plumbeus* (29). *M. sylvatica* does not have muscles of labium or labrum, while there are 6 pairs of them in *O. cincta* and at least 6 pairs in *T. plumbeus*. *M. sylvatica* has only one pair of dorsal dilators of the oesophagus and no dorsal dilators of the pharynx, which is less than in larger collembolans *O. cincta* (13), *T. plumbeus* (15).

A total of 51 pairs of muscles were described in the thorax of *Neanura muscorum* (Bretfeld, 1963) and a total of 37 pairs of muscles were described in the thorax of *O. cincta* (Bretfeld, 1963). Any muscles associated with legs have not been described in this study. We found 36 pairs of muscles not associated with legs in the thorax of *M. sylvatica* and 17 pairs of muscles associated with legs. There is a greater similarity between the muscles of *M. sylvatica* and *O. cincta* (both species have greater numbers of dorsoventral muscles) than between the muscles of *M. sylvatica* and *N. muscorum*. *M. sylvatica* lacks several dorsoventral and intersegmental muscles, while the amount of longitudinal muscles remain the same. It is important to note that Bretfeld (1963) described several muscles in the thorax as muscles possibly associated with the head. Two of them, Lb dlm1 and Lb dlm2, we describe as dlm1 and dlm2 in the section on the muscles of the head. A total of 52 pairs and 9 unpaired muscles were described in the abdomen of *N. muscorum* (Bretfeld, 1963) and a total of 45 pairs of muscles were described in the abdomen of *O. cincta* (Bretfeld, 1963). No muscles associated with the ventral tube, rectum, or anal lobes were described in these collembolans, except one in one species (VTm in *O. cincta*). We found 43 pairs and one unpaired muscle in the abdomen of *M. sylvatica*, not connected to the ventral tube, as well as 11 pairs of muscles associated with the ventral tube, four with the rectum, and two with the anal lobes. As for the thorax, *M. sylvatica* lacks many dorsoventral muscles, some intersegmental muscles and almost all transverse unpaired muscles.

Minute adult Coleoptera have 19 or 20 pairs of muscles in the head (*Sericoderus lateralis* and *Mikado* sp., respectively) and 48 or 49 pairs of muscles in the thorax (*Mikado* sp. and *S. lateralis*, respectively) (Polilov & Beutel, 2009; Polilov & Beutel, 2010). Compared to them, the number of the head pairs of muscles (24) and thoracic pairs of muscles (51) in *M. sylvatica* is slightly greater. Larvae of minute Coleoptera have 16 pairs of muscles in the head (*Mikado* sp. and *S. lateralis*) (Polilov & Beutel, 2009; Polilov & Beutel, 2010) and 46 (*Mikado* sp., the first instar larvae), 52 (*Mikado* sp., the last instar larvae) 63 (*S. lateralis*, the first instar larvae) or 64 (*S. lateralis*, the last instar larvae) pairs of muscles in the thorax. The number of thoracic pairs of muscles in minute *M. sylvatica* (51) is close to the number of thoracic muscles in the last instar larvae of *Mikado* sp., but smaller than in larvae of *S. lateralis*. Minute Hymenoptera have 18 (*Megapragma mymaripenne*, *Trichogramma evanescens*) (Polilov, 2016d; Polilov, 2017), or 20 (*Anaphes flavipes*) (Polilov, 2016c) muscles in the head and 45 (*M. mymaripenne*), 50 (*A. flavipes*), 52 (*T. evanescens*), or 53 (*Gonatocerus morrilli*) (Vilhelmsen et al., 2010) muscles in the thorax. Compared to them, the number of the head muscles of *M. sylvatica* (24) is slightly greater, but the number of the thoracic muscles of this species (51) is greater only compared to those of *M. mymaripenne* and *A. flavipes*. Minute adult booklice *Liposcelis bostrychophila* have 33 pairs of muscles in the head and 57 pairs of muscles in the thorax (Polilov, 2016b). The larvae of *L. bostrychophila* have 29 pairs of muscles in the head and 55 pairs of muscles in the thorax (Polilov, 2016b). Compared to both larvae and adults, the number of the head pairs of muscles (24) and thoracic pairs of muscles (51) of *M. sylvatica* is notably smaller. Minute adult thrips *Heliothrips haemorrhoidalis* have 19 pairs of muscles in the head and 60 pairs of muscles in the thorax (Polilov & Shmakov, 2016). Compared to them, the number of the head pairs of muscles (24) of *M. sylvatica* is greater, but the number of the thoracic pairs of muscles (51) is notably smaller. Larvae of *H. haemorrhoidalis* have 18 pairs of muscles in the head and 41 pairs of muscles in the thorax (Polilov & Shmakov, 2016). Compared to them, the number of the head pairs of muscles (24) and thoracic pairs of muscles (51) of *M. sylvatica* is notably greater. Minute Neuroptera *Coniopteryx pygmaea* (Randolf et al., 2016) have 46 pairs of muscles in the head. Compared to them, the number of the head pairs of muscles (24) of *M. sylvatica* is notably lesser.

In all studied microinsects, there are three groups of abdominal muscles: dorsoventral, dorsal longitudinal, and ventral longitudinal (Polilov, 2016a). All three groups are present in the abdomen of *M. sylvatica*.

In conclusion, the musculature system of *M. sylvatica* shows minor reductions in numbers of muscles compared to larger species. Studies on microinsects also show that the changes in musculature are minor, and this system is rather conserved (Polilov, 2015a). The number of muscles in *M. sylvatica* is slightly greater than those in most microinsects.

The relative volume of the musculature of *M. sylvatica* is smaller than those of other microinsects of the same size except Coleoptera larvae (Polilov & Makarova, 2017). The smaller relative volume compared to those of other microinsects could be explained by absence of flight musculature.

CONCLUSIONS

The anatomy of the minute collembolan *M. sylvatica* has been studied for the first time. It is shown that, despite the small body size, some systems (the highly developed elements of endoskeleton, or complexity of the musculature system) are not greatly changed compared to larger relatives.

Possible miniaturization effects are revealed; most of them are found in microinsects (the absence of organs of the circulatory system, unpaired ovaries and oviducts of the female reproductive system, absence of midgut musculature and salivary glands, reduction of some muscles).

We found unique features of the anatomy of *M. sylvatica*: two pairs of apertures in the brain with three pairs of muscles going through it.

Reduction in size leads to changes in different organs and organ systems. It is crucial to study miniaturization effects in other Panarthropoda including other minute collembolans to broaden our knowledge on miniaturization in living organisms.

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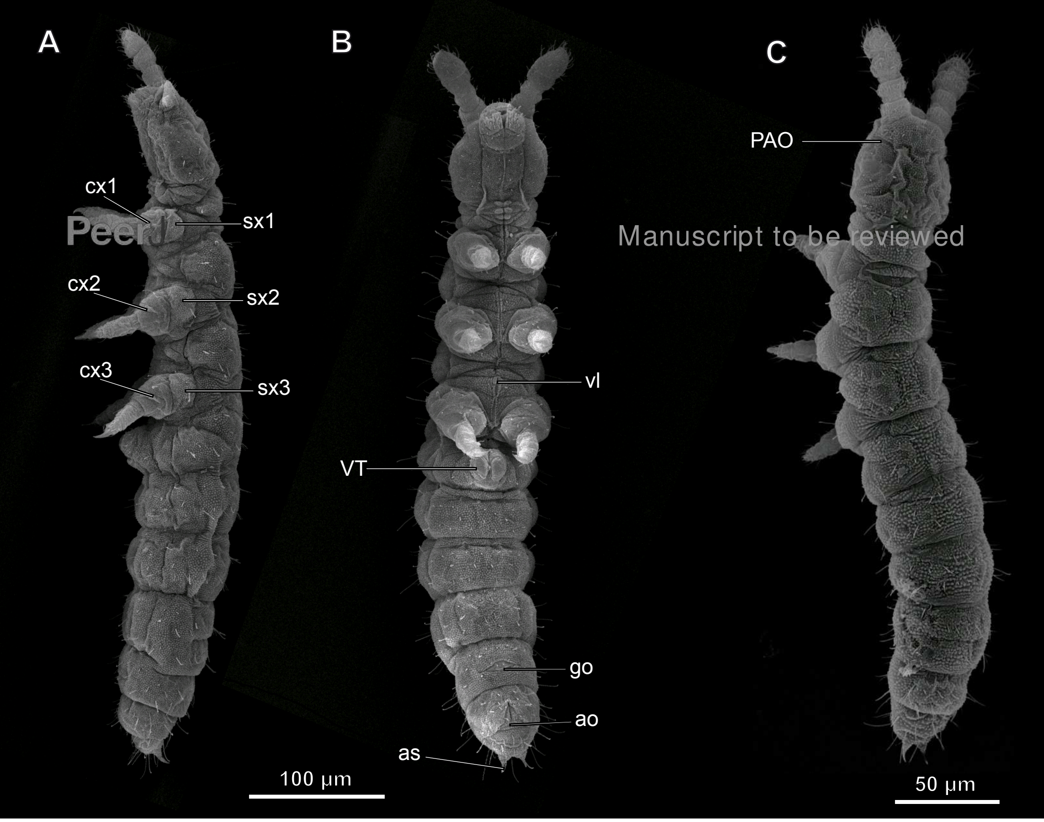
Figure 1(on next page)

Habitus of *Mesaphorura sylvatica*, SEM

(A) lateral view; (B) ventral view; (C) dorsal view; ao — anal opening, as — anal spine, cx1, 2, 3 — pro-, meso-, and metacoxae, go — genital opening, PAO — postantennal organ, sx1, 2, 3 — pro-, meso, and metasubcoxae, vt — ventral tube.

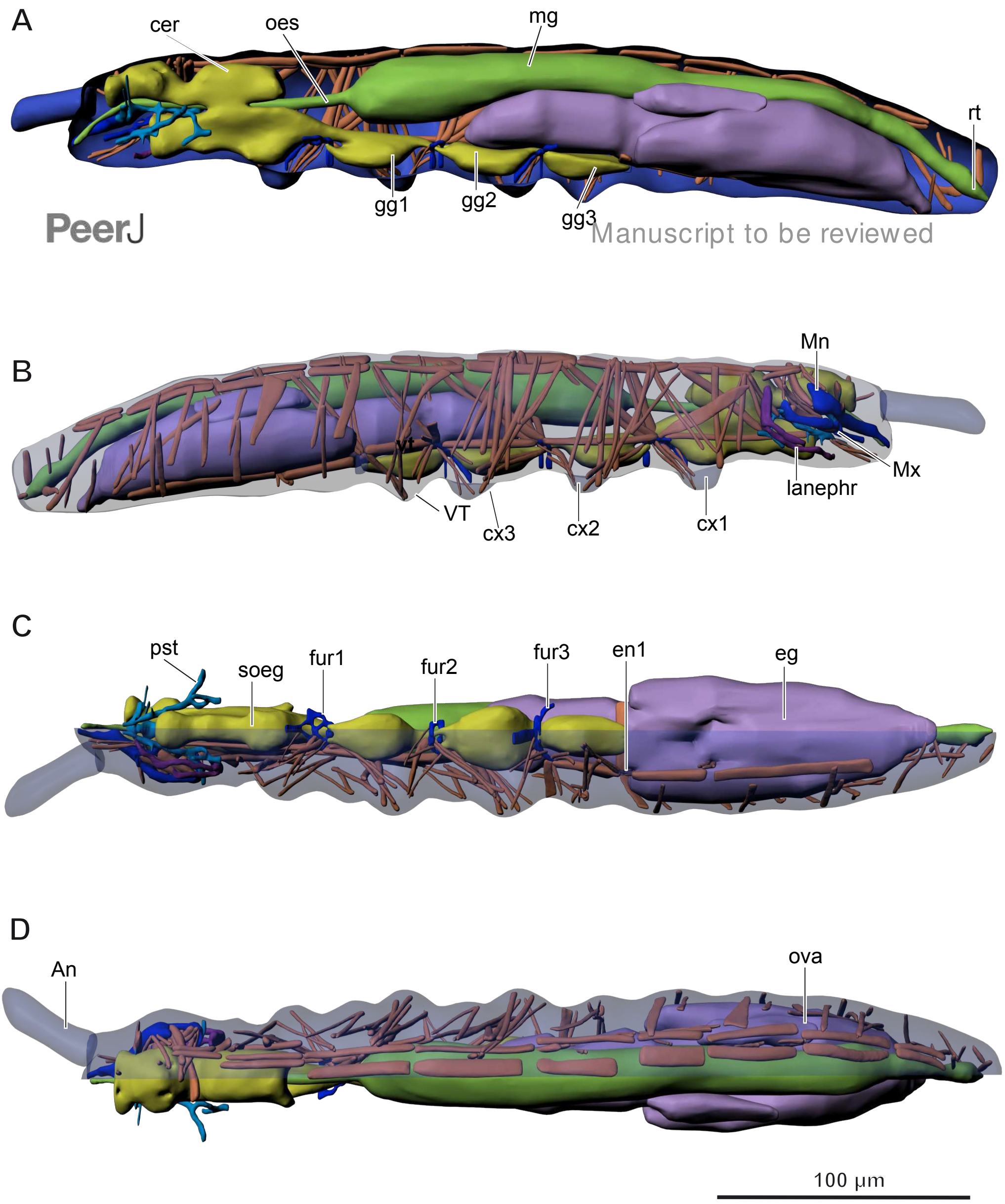


A
B
C
cx1
sx1
cx2
sx2
cx3
sx3
vl
VT
go
ao
as
100 µm
PAO
50 µm

Figure 2(on next page)

Internal morphology of *Mesaphorura sylvatica*, 3D

Colors: blue — cuticle, light blue – tentorium, green — digestive system, yellow — central nervous system, brown — musculature, purple — reproductive system, dark violet – excretory system: (A) lateral internal view; (B) lateral external view; (C) vetral view; (D) dorsal view; an — antennae, cer — brain, cx1, 2, 3 — pro-, meso-, and metacoxae, gg1, 2, 3+ag — pro-, meso-, and metathoracic+abdominal ganglia, mg — midgut, oes — oesophagus, ova — ovary lobe with eggs, ov — ovary lobe without eggs, rt — rectum, soeg — supraoesophagal ganglion, VT — ventral tube.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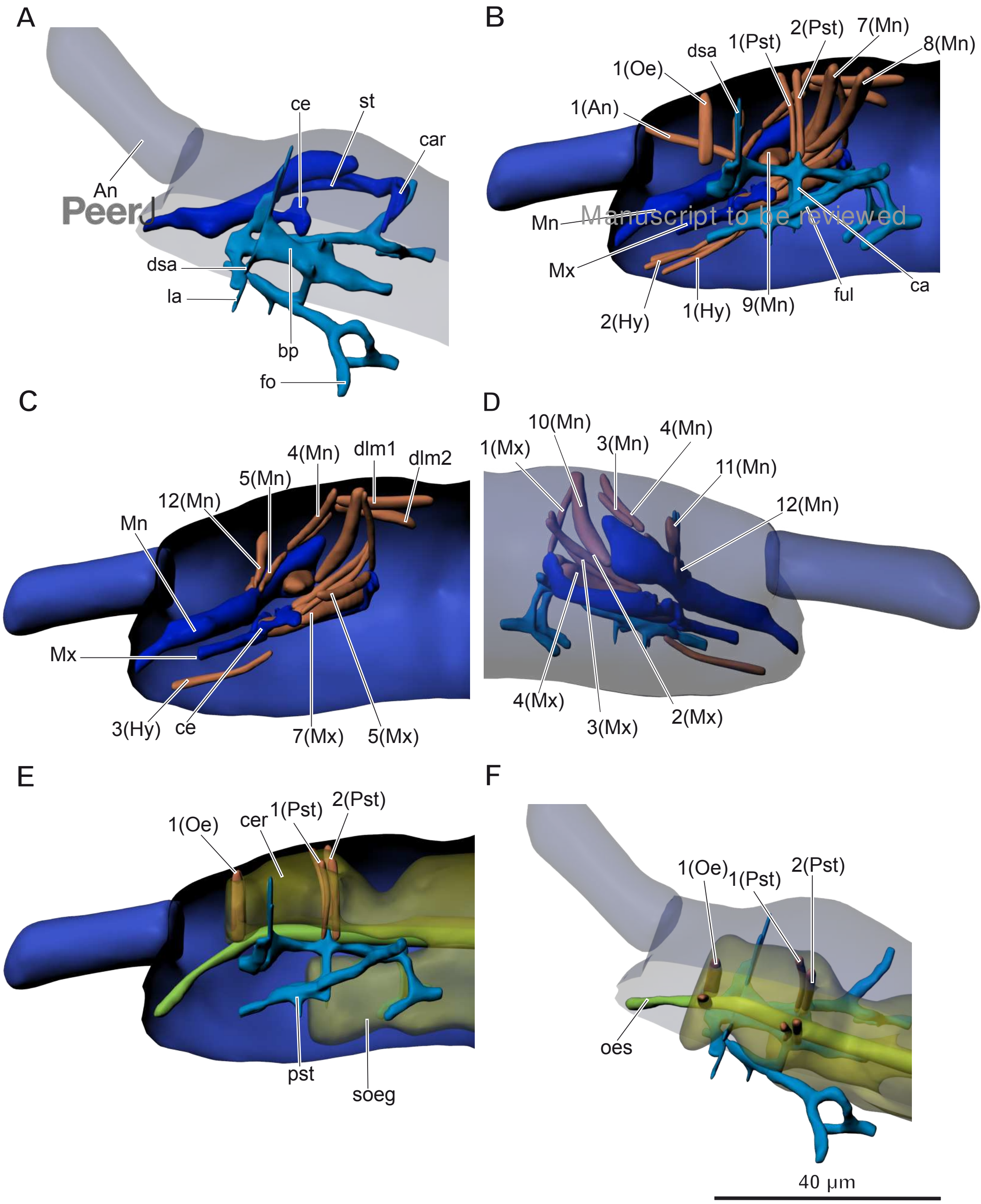
mg
rt
gg1
gg2
gg3
B
Mn
Mx
lanephr
VT
cx3
cx2
cx1
C
pst
soeg
fur1
fur2
fur3
en1
eg
D
An
ova
100 µm

Figure 3 (on next page)

Anatomy of head in *Mesaphorura sylvatica*, 3D

(A, F) dorsolateral view, (B, C, E) lateral internal view, (D) lateral external view. bp–body of pseudotentorium, ca–connecting arm, car–cardo, ce–chitinous expansion, cer — cerebrum, dsa–dorsal suspensory arm, fo–foot, ful–fulcrum, la — lateral arm, Mn — mandible, Mx — maxillae, oes–oesophagus, soeg — supraoesophagal ganglion, st–stipes. Musculature see text.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car
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la
bp
fo
B
1(Oe)
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7(Mn)
8(Mn)
1(An)
Mn
Mx
2(Hy)
1(Hy)
9(Mn)
ful
ca
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4(Mn)
dlm1
dlm2
5(Mn)
12(Mn)
Mn
Mx
3(Hy)
ce
7(Mx)
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10(Mn)
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3(Mn)
4(Mn)
11(Mn)
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cer
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2(Pst)
pst
soeg
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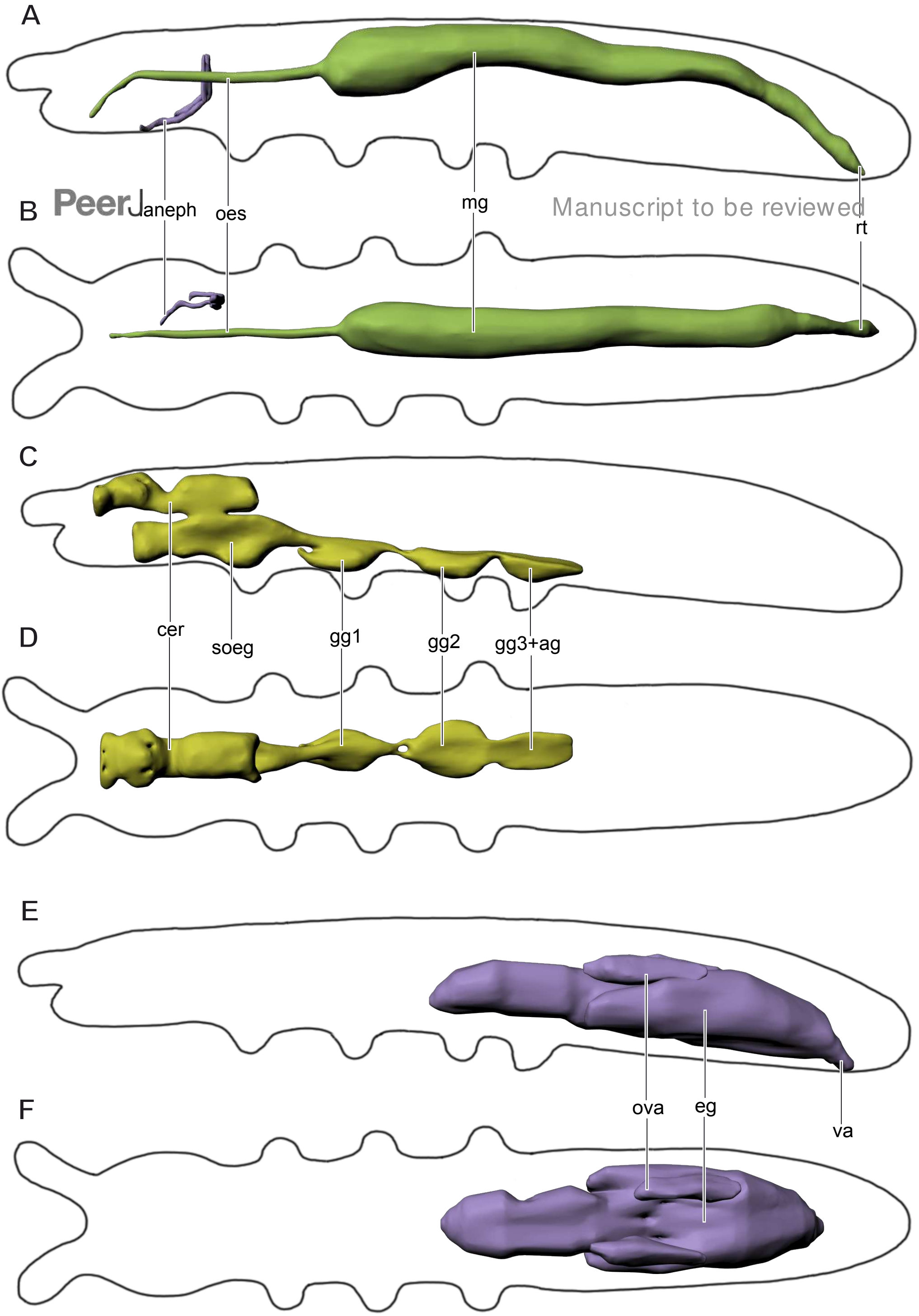
1(Oe)
1(Pst)
2(Pst)
oes
40 µm

Figure 4 (on next page)

Digestive and excretory (A, B), nervous (C, D), and reproductive (E, F) systems of *Mesaphorura sylvatica*, 3D

(A, C, E) lateral view; (B, D, F) dorsal view. cer — brain, eg — ovary lobe with eggs, gg1, 2, 3+ag — pro-, meso-, and metathoracic+abdominal ganglia, mg — midgut, oes — oesophagus, ova — ovary lobe without eggs, rt — rectum, soeg — supraoesophagal ganglion, va — vagina.


A
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gg3+ag
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ova
eg
va

Figure 5(on next page)

Musculature of thorax in *Mesaphorura sylvatica*, 3D

(A — C) lateral internal view. cx1, 2, 3 — pro-, meso-, and metacoxae, fur1, 2, 3 — pro-, meso-, and metafurca-like structures, pst — pseudotentorium. Musculature see text.

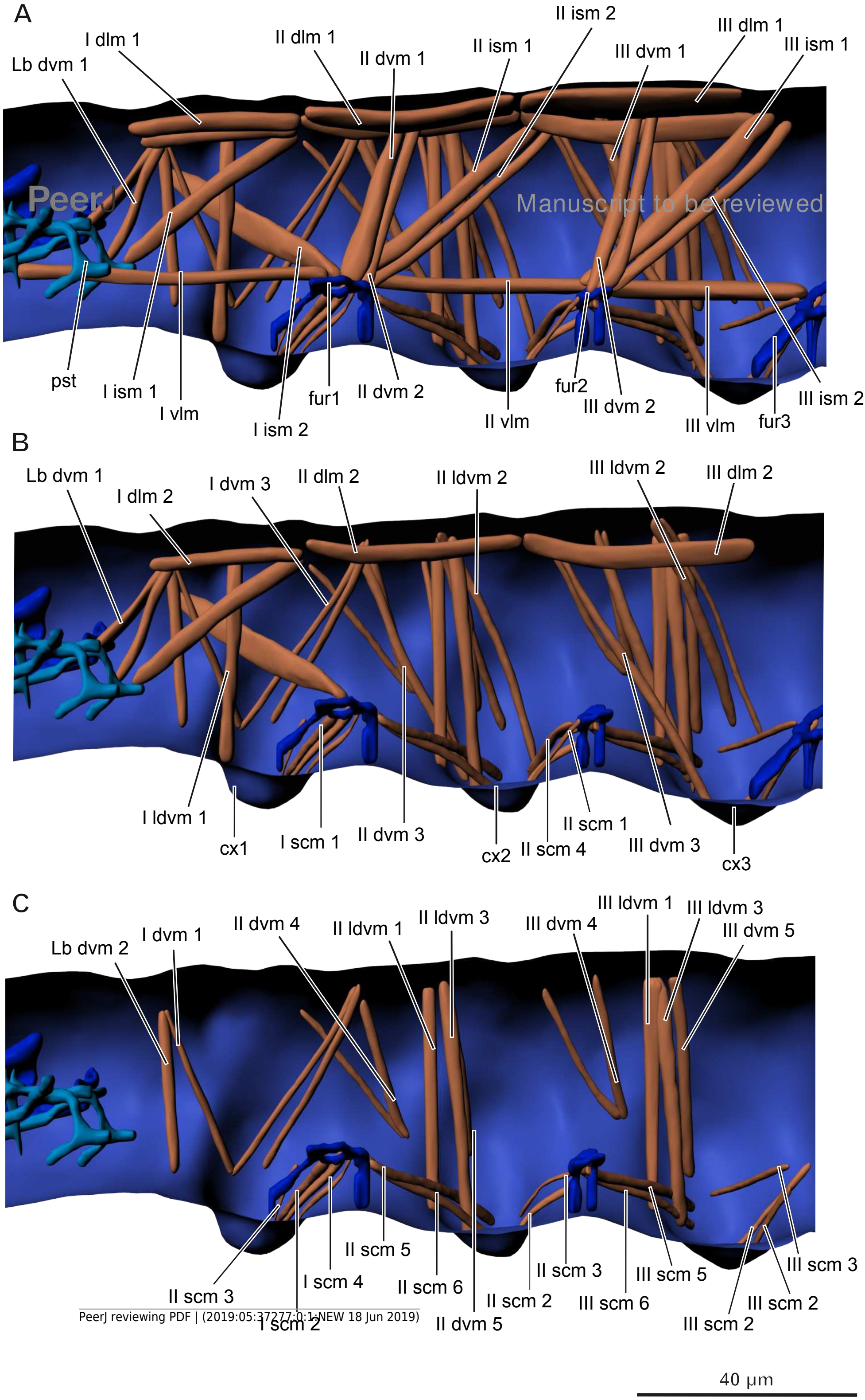
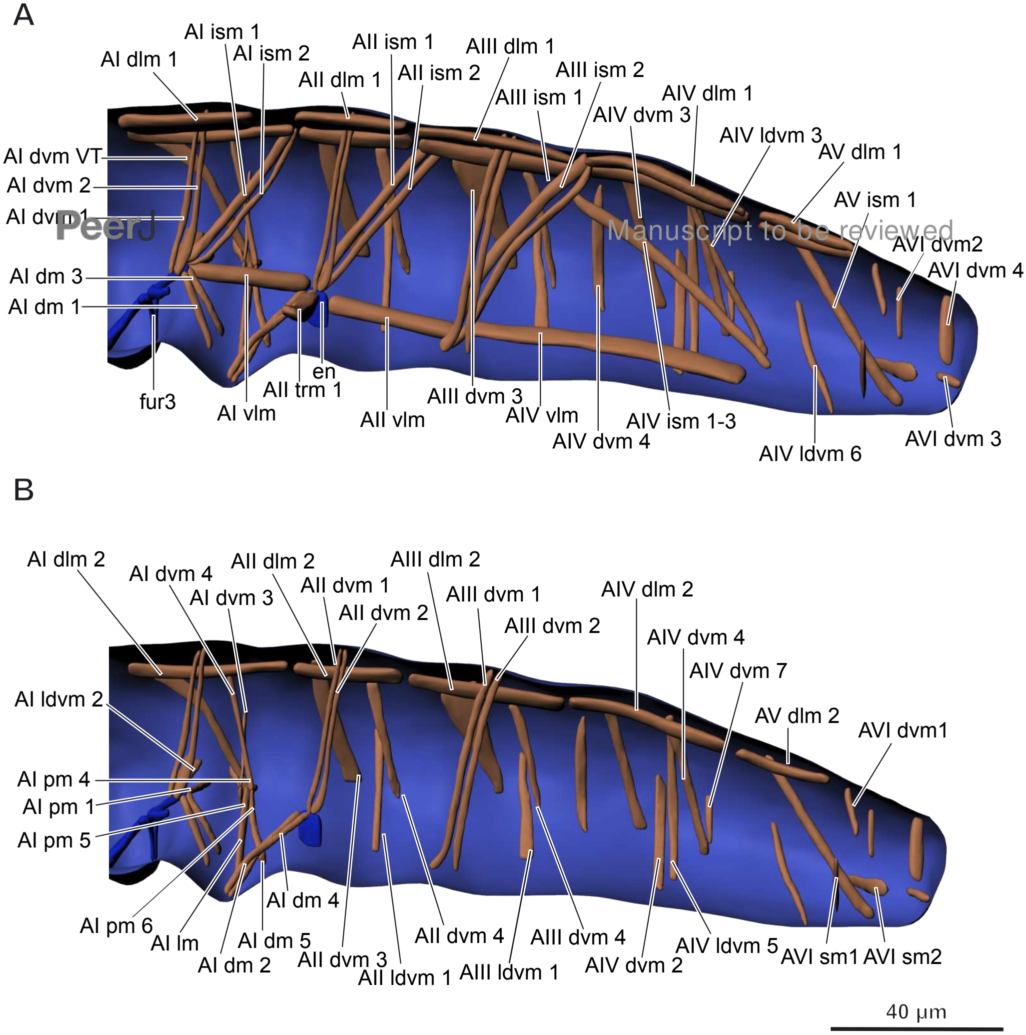

A
I dlm 1
Lb dvm 1
II dlm 1
II dvm 1
II ism 1
II ism 2
III dvm 1
III dlm 1
III ism 1
pst
I ism 1
I vlm
I ism 2
fur1
II dvm 2
II vlm
fur2
III dvm 2
III vlm
fur3
III ism 2
B
Lb dvm 1
I dlm 2
I dvm 3
II dlm 2
II ldvm 2
III ldvm 2
III dlm 2
I ldvm 1
cx1
I scm 1
II dvm 3
cx2
II scm 4
II scm 1
III dvm 3
cx3
C
Lb dvm 2
I dvm 1
II dvm 4
II ldvm 1
II ldvm 3
III dvm 4
III ldvm 1
III ldvm 3
III dvm 5
II scm 3
I scm 2
I scm 4
II scm 5
II scm 6
II dvm 5
II scm 2
II scm 3
III scm 6
III scm 5
III scm 2
III scm 3
III scm 2
40 µm

Figure 6(on next page)

Musculature of abdomen in *Mesaphorura sylvatica*, 3D

(A — B) lateral internal view. fur3 — metafurca-like structure, en — endosternite.

Musculature see text.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AI dlm 1
AI ism 1
AI ism 2
AII ism 1
AII dlm 1
AII ism 2
AIII dlm 1
AIII ism 1
AIII ism 2
AIV dvm 3
AIV dlm 1
AIV ldvm 3
AV dlm 1
AV ism 1
AVI dvm2
AVI dvm 4
AI dvm VT
AI dvm 2
AI dvm 1
AI dm 3
AI dm 1
fur3
AI vlm
AII trm 1
en
AII vlm
AIII dvm 3
AIV vlm
AIV dvm 4
AIV ism 1-3
AIV ldvm 6
AVI dvm 3
B
AI dlm 2
AI dvm 4
AI dvm 3
AII dlm 2
AII dvm 1
AII dvm 2
AIII dlm 2
AIII dvm 1
AIII dvm 2
AIV dlm 2
AIV dvm 4
AIV dvm 7
AV dlm 2
AVI dvm1
AI ldvm 2
AI pm 4
AI pm 1
AI pm 5
AI pm 6
AI lm
AI dm 2
AI dm 5
AI dm 4
AII dvm 3
AII ldvm 1
AII dvm 4
AIII ldvm 1
AIII dvm 4
AIV dvm 2
AIV ldvm 5
AVI sm1
AVI sm2
40 μm