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

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Effects of miniaturization in the anatomy of the minute springtail *Mesaphorura sylvatica* (Hexapoda: Collembola: Tullbergiidae)

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Smaller animals display pecular characteristics related to their small body size, and miniaturization has recently been intensely studied in insects, but not in other arthropods. Collembola, or springtails, are abundant soil microarthropods and form one of the four basal groups of hexapods. Many of them are notably smaller than 1 mm long, which makes them a good model for studying miniaturization effects in arthropods. In this study we analyze for the first time the anatomy of the minute springtail *Mesaphorura sylvatica* (body length 400 µm). 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 of *M. sylvatica*, some organ systems, e.g., muscular and digestive, remain complex. On the other hand, the nervous system displays considerable changes. The brain has two pairs of apertures with three pairs of muscles running through them, and all ganglia are shifted posteriad by one segment. The relative volumes of the skeleton, brain, and musculature are smaller than those of most microinsects, while the relative volumes of other systems are greater than or the same as in most microinsects.

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

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ABSTRACT

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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 (e.g. Hanken & Wake, 1993; Polilov, 2016a). Many arthropods are comparable in size with unicellular organisms and are of great interest for studying miniaturization in animals. Miniaturization implies major morphological changes of structures and is often accompanied by allometric changes in many organs (Polilov, 2015a). The tremendous size changes that occurred in the evolutionary history of arthropods may have allowed them to occupy a vast range of niches. Studies on miniaturization in different arthropods can help us understand what limits ~~the~~ body size in organisms and how they evolved.

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 (Eberhard & Wcislo, 2011; Dunlop, 2019; Gross et al., 2019).

Studies on the miniaturization of insects and the anatomy of the smallest insects (adult body length smaller than 2 mm) show significant changes in the anatomy of microinsects correlated with their size. Some changes are commonly shared by remotely related taxa, and seem~~s~~ to be straightforward adaptions to physical constrains, e.g., the reduction of circulatory and tracheal systems, absence of midgut musculature; compactization, oligomerization, and asymmetry of central nervous system (Polilov, 2016a). However, some microinsect~~s~~ taxa possess their own original modifications, such as the complete shift of the brain into the thorax in the adult (Polilov

& Beutel, 2010) in *Mikado* sp., or the lysis of cell bodies and nuclei of neurons in *Megaphragma* sp. (Polilov, 2012, 2017).

~~A size~~ range of Collembola is from 0.12 to 17 mm. ~~Nevertheless, such factors~~ as soil interstices space and humidity levels affect size reduction of collemblans. Thus, many collembolan genera include especially minute species (< 500 µm), and therefore represent interesting models for research on miniaturization in arthropods.~~.~~ However, 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 (Table S1). 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 he studied only the muscles associated with the digestive system, and the excretory system was not mentioned in his study. 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 *A. 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 *O. cincta* (Verhoef et al., 1979), *T. flavescens*, *A. maritima*, *N. muscorum*, *Friesea mirabilis*, *Brachystomella parvula*, *Odontella armata* (Wolter, 1963), and *Sminthurus fuscus* (Willem & Sabbe, 1897), excretory system of *Onychiurus quadriocellatus* (Altner, 1968), *Tomocerus minor*, *Lepidocyrtus curvicollis* (Humbert, 1975), and *Orchesella rufescens* (Philiptschenko, 1907), respiratory system of *S. 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), *O. villosa* (Dallai et al., 2008), *A. maritima* (Lécaillon, 1902a), *Anurophorus laricis* (Lécaillon, 1902b). Schaller (1970) and Hopkin (1997) gave partial reviews of the above in their ~~respective~~ synthetic books on Collembola. The genus *Mesaphorura* ~~of Collembola~~ includes some of the smalle~~r~~ species, the adults some of them are only 0.4 mm long (Zimdars and Dunger, 1994). The external morphology of *Mesaphorura* has been completely and thoroughly investigated for systematic and phylogenetic purposes (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 sylvatica* for the first time and describe the effects of miniaturization in this species.

MATERIALS AND METHODS

~~C~~omplex anatomical study was conducted based on methods ~~of~~ study~~ing~~ microinsects~~,~~ described in previous papers (Polilov, 2016a, 2017; Polilov & Makarova, 2017).

Materials

Specimens of *Mesaphorura sylvatica* Rusek, 1971 were collected in September 2015 on a sand beach, on the bank of the 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 in a series of increasing ethanol solutions (in 70% and 95% for an hour, and twice in 100% for 30 min) and in acetone (twice in 100% for 30 min), and afterwards embedded in Araldite M (kept in a mixture of araldite and acetone 1:1 for a night, then twice in araldite for four hours, following polymerization at 60˚C for two days). 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 LED illumination source and ToupTek camera (5 MP). The resulting stack was then aligned and calibrated. 3D reconstructions were created in the program Bitplane Imaris 7.2 using the function of creating vector surface manually by outlining contours of structures on a series of slides. In addition, we processed the reconstructions with the functions of surface smoothing and rendering in the Autodesk Maya 2015 program.

Measurements

The body length was measured using SEM images. Linear measurements were based on images of histological slides in the program Bitplane Imaris. Volumes of organs (Table S2) and the body were calculated using the statistical module of Bitplane Imaris, as described in an earlier study (Polilov & Makarova, 2017). For all measurements we calculated means, n-number, and minimum and maximum values, where it was possible with a given sample size.

Nomenclature

The names of morphological elements are based on Folsom (1899), Snodgrass (1935), Bretfeld (1963), and Bitsch (2012). The description of the musculature and abbreviations of muscles (Table S3) 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 nomenclature~~s~~ used for insects (Friedrich & Beutel, 2008; Wipfler et al., 2011). The following abbreviations are used in descriptions of muscles: O, origin; I, insertion.

RESULTS

General morphology

The body is from 313 to 492 µm (M = 425, n = 4) in length (Fig. 1), uniformly white in color. ~~The j~~umping organ (furca), tenaculum and eyes are absent. It is important to note that the name "furca" is also ~~used regarding~~ endoskeletal structures in thorax of hexapods ("furca-like structures" mentioned below). 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 0.79~~nL~~.

Skeleton

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

The inner skeletal structures are highly developed. A complex pseudotentorium (pst) (Fig. 3A–B, D–F) is situated in the head. Its body (bp) consists of a mandibular tendon in the middle, which continues posteriorly into a thinner longitudinal endoskeletal connective. There is a pair of dorsal suspensory arms (dsa), connecting the structure with the head capsule anteriorly on the frons.

The glossa is prolonged posteriorly into a pair of chitinous stalks (ful), called the posterior tentorial apodemes by Koch (2000), or fulcra (Denis, 1928). They lie externally to the mid~~dle~~ line. The enlarged base of the stalk is called the foot (fo), and the foot underlies the cardo (car) of the maxilla (Mx). They seem to connect with the head capsule posteriorly possibly with some endoskeletal connectives. A pair of connecting arms (ca) (bras d'union, Denis, 1928) extend from the pseudotentorial plate downwards and are fused with the posterior tentorial apodemes. A pair of lateral arms (la) (bras latéraux, Denis, 1928) extend from the anterior part of the pseudotentorial plate, go upward and outward and are inserted into the head.

In *Orchesella cincta*, 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 (ce), which is the 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 (Fig. 2C). Additionally, there is a simple rectangular endosternite in the first abdominal segment.

The volume of the skeleton is about 0.044nL (5.8% of the body volume).

Digestive and excretory systems

The alimentary canal (Fig. 4A–B) 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 (oes). The slender pharynx is about 4.2 µm (M = 4.2, n = 8). The oesophagus passes through the suboesophageal ganglion and leads into the thicker midgut (mg) at the level of the metathorax (around the fourth abdominal segment). The midgut consists of one layer of cells (6–8 cells in cross section). The oesophagus has one pair of muscles 1(Oe) (Fig. 3E, F). The first half of the midgut is ~~round~~ in cross section, about 21.8 µm (M = 24.3, n = 8) 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, the hindgut extends into the wider rectum (rt) with four pairs of muscles. The latter continues posteriad and terminates ventrally at the anus with three anal lobes in the last abdominal segment.

Labial nephridia (laneph), or tubular glands, the main excretory organs of collembolans, are found in the posterior half of the head (Fig. 4). Each nephridium is composed of a sac, a labyrinth, and a duct. The sac is situated posteriorly and continues anteriad 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 0.68 n~~L~~ (8.6% of the body volume).

Nervous system
The nervous system (Fig. 4C–D) consists of a supraoesophageal ganglion, or brain (cer), suboesophageal ganglion (soeg), and three thoracic ganglia. The brain extends from the bases of the antennae to the anterior part of the first thoracic segment. The brain 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 the head and the first thoracic segment. Itterminates 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 (Fig. 3E–F). The suboesophageal ganglion (Fig. 3E; Fig 4C) 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 gangliashift their position by one segment: the first ganglion (gg1) lies in the mesothorax, the second one (gg2) is in the metathorax, and the third one (gg3) is in the first abdominal segment. They are interconnected by longitudinal cords in intersegments (one in each). As in all collembolans, the abdominal ganglia (ag) are fused with the third thoracic ganglion.
The volume of the central nervous system is about 0.051 n~~L~~ (6.3% of body volume). The volume of the brain is about 0.016 n~~L~~ (2.2% of the body volume).

Muscular system
Musculature of head (Fig. 3, Table 1). *M. sylvatica* has 24 pairs of muscles in the head. Two of them are connected to pseudotentorium, one – to antennae, nine – to mandibles, six – to maxillae, one – to oesophagus, and two of them are the dorsal lateral pairs of muscles. In addition, there are three very small obscure muscles that are connected to the hypopharynx. Two mandibular muscles 8(Mn) cross each other and attach to the opposite sides of the head.
Musculature of thorax (Fig. 5, Table 2).
Prothorax. *M. sylvatica* has 14 pairs of muscles in the prothorax. Two of them are dorsal longitudinal, one —ventral longitudinal, two —intersegmental dorsoventral, five —dorsoventral, and four —sterno-coxal.
Mesothorax. *M. sylvatica* has 19 pairs of muscles in the mesothorax. Compared to the prothorax, it has more dorsoventral (eight) and sterno-coxal (six) pairs of muscles.
Metathorax. *M. sylvatica* has 18 pairs of muscles in the mesothorax. Compared to the mesothorax, only one pair of muscles (III scm4) is absent.
Musculature of abdomen (Fig. 6, Table 3). *M. sylvatica* has 61 pairs of muscles and one unpaired muscle in the abdomen. Ten pairs of muscles are dorsal longitudinal, three —ventral longitudinal, eight —intersegmental dorsoventral, 29 —dorsoventral. In addition, there are 11 pairs of muscles that are connected to the ventral tube. One unpaired transversal muscle connects abdominal endosternites of both sides.

The volume of the muscular system is about 0.038 n~~L~~ (5.2% of the body volume).

Reproductive system

The female reproductive system has been studied in detail (Fig. 4E–F). The ovary is unpaired with three lobes. The largest lobe (eg) probably contains eggs, while two other, smaller lobes (ova) 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 (va), the margins of which are indistinct. The vagina opens ventrally on abdominal segment 5 with a transverse reproductive orifice (gonopore).

The volume of the reproductive system is about 0.15 n~~L~~ (18.9% of the body volume).

Circulatory system and fat body

Organs of the circulatory system are absent, the system is represented by hemolymph in the body cavity. 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 n~~L~~ (55.2% of the body volume).

Respiratory system

Organs of the respiratory system (tracheae) 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, in contrast with 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 (Lubbock, 1873; Kollmann 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. In larger collembolan species, an extension of the metathoracic ganglion to the first abdominal segment was reported (Lubbock, 1873; Hopkin, 1997. 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 Ptiliidae (Polilov & Beutel, 2009), adults and larvae of Corylophidae (Polilov & Beutel, 2010), larvae of Scydmaenidae (Jałoszyński et al., 2012), larvae of Hydroscaphidae (Beutel & Haas, 1998), adults of Sphaeriusidae (Yavorskaya et al., 2018), and larvae of Strepsiptera (Beutel et. al., 2005).

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 those of minute adult Hymenoptera and Paraneoptera larvae, and greater than those of adult Paraneoptera of the same size (Polilov & Makarova, 2017). Such small relative volume can beexplained by 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. The smaller relative volume of *M. sylvatica* compared to the ones 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 absence of flight and eyes (Jordana et al., 2000).

Circulatory system and fat body

The circulatory system of *M. sylvatica* is simplified, heart or vessels are absent. Most of the body cavities of *M. sylvatica* are filled with the fat body. In larger collembolan 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, also the case of *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 the fat body in excretion in Collembola, where excretory products are stored (Schaller, 1970).

Female reproductive system

The female reproductive system of *M. sylvatica* consists of unpaired ovaries and oviducts. In larger species, it consists of paired ovaries, oviducts and accessory glands, and an unpaired spermatheca. We did not observe accessory glands and spermatheca in *M. 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, an absence of which is also a common trait among minute insects (Polilov, 2016a).

M. sylvatica has a pair of labial nephridia, but lacks other head glands (acinous glands and antennal nephridia) that are present in larger collembolan species (Wolter, 1963). In microinsects, Malpighian tubes do not disappear, but their number decreases (Polilov, 2015a).

The relative volume of the digestive and excretory systems 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).

Musculature

The muscular system of *M. sylvatica* is reduced in number, compared to those of larger collembolan species. *M. sylvatica* 24 pairs of muscles in the head, 51 in the thorax, and 61 in the abdomen (and 1 unpaired muscle); 136 pairs in total. The total number of muscles of all tagmata have not been studied in any single species of springtails, which limits the possible comparison of our results with previous studies.

It is difficult to compare head muscles 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 addition, he noted 20 pairs of muscles associated with both pharynx and oesophagus (seven of them are the ventral dilators of the 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 specify number. 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 the maxilla and mandible in *A. 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 the number of muscles of the labium. Moreover, he described the groups of muscles of the heads of two larger species *Onychiurus fimetarius* and *Tomocerus catalanus*, but he compared them to *A. maritima*, without providing details on exact numbers. With *T. catalanus* he refer~~s~~ to the study of Hoffmann (1908), whodescribed 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~~ with pharynx, and 3 ~~muscles~~ with glossa. Hoffmann (1908) also mentioned the presence of tentorial muscles, but did not specify the number ~~of them. There are 15 pairs of muscles i~~n *M. sylvatica* associated with maxilla and mandible, one pair of antennal muscles, one pair of oesophageal 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 such as *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 the~~m~~ 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 fewer 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). Muscles associated with legs have not been described in that study. We found 36 pairs of muscles not associated with the 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. We describe two of them, Lb dlm1 and Lb dlm2, 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 larva~~e~~), 52 (*Mikado* sp., ~~the~~ last instar larva~~e~~) 63 (*S. lateralis*, ~~the~~ first instar larva~~e~~) or 64 (*S. lateralis*, ~~the~~ last instar larva~~e~~) 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., 2017) 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 smaller.

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*.

To sum up, the musculature system of *M. sylvatica* shows minor reductions in the number of muscles compared to larger collembolan species. In the head, absent muscles such as some mandibular retractors or maxillary adductors are not unique, and other muscles, present in *M. sylvatica*, have the same function (Folsom, 1899). In thorax and abdomen, ventral and dorsal longitudinal~~,~~ intersegmental muscles are present in full amount in *M. sylvatica*, but many dorsoventral muscles are absent. Nevertheless, they most likely do not differ in function from the dorsoventral muscles~~,~~ present in *M. sylvatica*. The reduction in total number of muscles in *M. sylvatica* does not seem to ~~refl~~ect any abilities of *M. sylvatica* to move. 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 the absence of flight musculature.

CONCLUSIONS

We have studied the anatomy of the minute collembolan *M. sylvatica* for the first time. We show that, despite the small body size, some systems (the highly developed elements of endoskeleton, or complex~~ity of the~~ musculature system) are not greatly changed compared to larger relatives. We revealed possible miniaturization effects; 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).

Finally, we found some unique features in 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, giving us perspective on physical constrains of size limit. Studying miniaturization can also bring us further understanding on successful diversification of animals. Collembola is a highly diversified group of terrestrial arthropods with many extremely reduced in size species. Therefore, it is crucial to study anatomical changes in other minute collembolans to broaden our knowledge of miniaturization in animals.

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Table 1(on next page)

Head muscle origins and insertions.

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

1

Table 2(on next page)

Thoracic muscle origins and insertions.

1

Abbrev.	Name	Origin	Insertion
I dlm1	M. antecosta-occipitalis medialis	antecosta I, mediad of I dlm2	occiput, mediad of I dlm2
I dlm2	M. antecosta-occipitalis lateralis	antecosta I, laterad of I dlm1	occiput, laterad of I dlm1
I vlm	M. profurca-pseudotentoralis	anterior part of profurca-like structure	union arm of pseudotentorium
I ism1	M. antecosta-pseudotentoralis	antecosta I	posterior area of fulcrum
I ism2	M. profurca-occipitalis	dorsal part of profurca-like structure	dorsolateral area of occiput
I dvm1	M. cervico-coxalis	dorsolateral cervical membrane	anterior procoxal rim
I dvm3	M. pronoto-coxalis lateralis; two bands	anterior region of pronotum	lateral procoxal rim and anterior procoxal rim along with instertion of I dvm2
I ldvm1	M. pronoto-coxalis medialis	anterolateral part of pronotum	anterior procoxal rim
Lb dvm1	M. occiputo-pseudotentoralis; two bands	dorsal area of occipitale	posterior area of fulcrum
Lb dvm2	M. occiputo-cervicalis	dorsal area of occipitale	ventral cervical membrane
I scm1	M. profurca-coxalis 1	anterior face of profurca-like structure	posterior procoxal rim, laterad of I scm2
I scm2	M. profurca-coxalis 2	ventral face of profurca-like structure	posterior procoxal rim, mediad of I scm1
I scm3	M. profurca-coxalis 3	ventral face of profurca-like structure along with I scm4	lateral procoxal rim
I scm4	M. profurca-coxalis 4	ventral face of profurca-like structure along with I scm3	posteriolateral procoxal rim
II dlm1	M. antecosta-antecostalis medialis	antecosta II; mediad of II dlm2	antecosta III; mediad of II dlm2
II dlm2	M. antecosta-antecostalis lateralis	antecosta II; laterad of II dlm1	antecosta III; laterad of II dlm1
II vlm	M. profurca-mesofurcalis	lateral part of profurca-like structure	lateral part of mesofurca-like structure
II ism1	M. profurca-antecostalis medialis	lateral part of profurca-like structure; mediad of II ism2	antecosta III
II ism2	M. profurca-antecostalis lateralis	lateral part of profurca-like structure; laterad of II ism1	antecosta III
II dvm1	M. mesonoto-profurcalis anterior	dorsolateral part of profurca-like structure; anterior to II dvm2	mesonotum (middle of segment); anterior to II dvm2
II dvm2	M. mesonoto-profurcalis posterior	dorsolateral part of profurca-like structure; posterior to II dvm1	mesonotum (middle of segment); posterior to II dvm1
II dvm3	M. mesonoto-coxalis; two bands	lateral part of mesonotum (middle of segment)	anterior face of mesocoxa
II dvm4	M. mesonoto-subcoxalis anterior; two bands	anterolateral part of mesonotum	anterior border of mesosubcoxa
II dvm5	M. metanoto-subcoxalis posterior	posterolateral part of mesonotum	ventral border of mesosubcoxa
II ldvm1	M. mesonoto-coxalis anterior	posterolateral part of mesonotum	anterior mesocoxal rim

II ldvm2	M. metanoto-subcoxalis; two bands	posterolateral part of mesonotum	posteroventral border of mesosubcoxa and posterior border of mesocoxal rim
II ldvm3	M. metanoto-coxalis posterior	posterolateral part of mesonotum	anterior face of mesocoxa
II scm1	M. mesofurca-coxalis 1	anterior face of mesofurca-like structure	posterior mesocoxal rim, laterad of II scm2
II scm2	M. mesofurca-coxalis 2	ventrolateral face of mesofurca-like structure along with II scm4	posterior mesocoxal rim, mediad of II scm1
II scm3	M. mesofurca-coxalis 3	anterior face of mesofurca-like structure	lateral mesocoxal rim, laterad of II scm4
II scm4	M. mesofurca-coxalis 4	ventrolateral face of mesofurca-like structure along with II scm2	lateral mesocoxal rim, mediad of II scm3
II scm5	M. profurca-coxalis lateralis	posterior face of profurca-like structure	anteriolateral mesocoxal rim, laterad of II scm6
II scm6	M. profurca-coxalis medialis	posterior face of profurca-like structure	anteriolateral mesocoxal rim, mediad of II scm5
III dlm1	M. antecosta-antecostalis medialis	antecosta ll, mediad of lll dlm2	antecosta lll, mediad of lll dlm2
III dlm2	M. antecosta-antecostalis lateralis	antecosta ll, laterad of lll dlm1	antecosta lll, laterad of lll dlm1
III vlm	M. mesofurca-metafurcalis	posterolateral face of mesofurca-like structure	anterolateral face of metafurca-like structure
III ism1	M. antecosta-mesofurcalis anterior	lateral face of mesofurca-like structure, anteriad of lll ism2	antecosta lll, anteriad of lll ism2
III ism2	M. antecosta-mesofurcalis posterior	lateral face of mesofurca-like structure, posteriad of III ism1	antecosta lll, posteriad of lll ism1
III dvm1	M. metanoto-mesofurcalis anterior	lateral face of mesofurca-like structure, anteriad of lll dvm2	lateral part metanotum (middle of segment), anteriad of lll dvm2
III dvm2	M. metanoto-mesofurcalis posterior	lateral face of mesofurca-like structure, posteriad of lll dvm1	lateral part of metanotum (middle of segment), posteriad of lll dvm1
III dvm3	M. metanoto-coxalis; two bands	anterolateral part of metanotum	anterior metacoxal rim
III dvm4	M. metanoto-subcoxalis anterior; two bands	anterolateral part of metanotum	anterior border of metasubcoxa
III dvm5	M. metanoto-subcoxalis posterior	posterolateral part of metanotum	ventral border of metasubcoxa
III ldvm1	M. metanoto-coxalis anterior	lateral part of metanotum (middle of segment)	anterior metacoxal rim
III ldvm3	M. metanoto-coxalis posterior	lateral part of metanotum (middle of segment)	anterior face of metacoxa
III ldvm2	M. metanoto-subcoxalis; two bands	lateral part of metanotum (middle of segment)	posteroventral border of metasubcoxa
III scm1	M. metafurca-coxalis 1	ventromedial face of metafurca-like structure	posterior metacoxal rim
III scm2	M. metafurca-coxalis 2	ventrolateral face of metafurca-like structure	lateral metacoxal rim
III scm3	M. metafurca-coxalis 3	lateral face of metafurca-like structure	lateral metacoxal rim
III scm5	M. mesofurca-coxalis lateralis	posterior face of mesofurca-like structure	anteriolateral metacoxal rim, laterad of III scm6
III scm6	M. mesofurca-coxalis medialis	posterior face of mesofurca-like structure	anteriolateral metacoxal rim, mediad of III scm5

2

Table 3(on next page)

Abdominal muscle origins and insertions.

1

Abbrev.	Name	Origin	Insertion
AI dlm1	M. antecosta-antecostalis medialis	antecosta III, mediad of AI dlm2	antecosta IV, mediad of AI dlm2
AI dlm2	M. antecosta-antecostalis lateralis	antecosta III, laterad of AI dlm1	antecosta IV, laterad of AI dlm1
AI vlm	M. metafurca-endosternalis	lateral face of metafurca-like structure	endosternite
AI ism1	M. antecosta-metafurcalis anterior	antecosta IV, anteriad of AI ism2	lateral face of metafurca-like structure, anteriad of AI ism2
AI ism2	M. antecosta-metafurcalis posterior	antecosta IV, posteriad of AI ism1	lateral face of metafurca-like structure, posteriad of AI ism1
AI dvm1	M. tergo-metafurcalis anterior	middle region region of tergum, anteriad of AI dvm2	lateral face of metafurca-like structure, anteriad of AI dvm2
AI dvm2	M. tergo-metafurcalis posterior	middle region region of tergum, posteriad of AI dvm1	lateral face of metafurca-like structure, posteriad of AI dvm1
AI dvm3	M. pleuro-pleuralis	lateral wall of segment	tendon system
AI dvm4	M. tergo-pleuralis	posterior region of tergum	tendon system
AI ldvm1	M. pleuro-metafurcalis	lateral wall of segment	lateral face of metafurca-like structure
AI dvm VT	M. tergo-pleuralis	anterior region of tergum	tendon system
AI lm	M. ventratubularis lateralis 1	base of ventral tube	valva of ventral tube
AI pm1	M. sterno-coxalis proximalis anterior 1	base of ventral tube	metafurca-like structure
AI pm2	M. sterno-coxalis proximalis anterior 2	base of ventral tube, anteriad of AI pm3, ventrad of AI pm4	tendon system
AI pm3	M. sterno-coxalis proximalis anterior 3	base of ventral tube, posteriad of AI pm2, AI pm4	tendon system
AI pm4	M. coxalis proximalis posterior 1	base of ventral tube, anteriad of AI pm3, dorsad of AI pm2	tendon system
AI dm1	M. sterno-vesicularis	anterior face of ventral tube, laterad of AI dm3	metafurca-like structure
AI dm2	M. tergo-vesicularis anterior	vesicles of ventral tube, ventrad of AI dm4	endosternite
AI dm3	M. coxo-vesicularis	anterior face of ventral tube, mediad of AI dm1	metafurca-like structure
AI dm4	M. tergo-vesicularis posterior	vesicles of ventral tube, laterad of AI dm2	endosternite
AI dm5	M. coxo-vesicularis anterior	posterior face of ventral tube	tendon system
AII dlm1	M. antecosta-antecostalis medialis	antecosta IV, mediad of AII dlm2	antecosta V, mediad of AII dlm2
AII dlm2	M. antecosta-antecostalis lateralis	antecosta lV, laterad of AII dlm1	antecosta V, laterad of AII dlm1
AII vlm	M. endosterno-antecostalis	endosternite	antecosta V
AII ism1	M. antecosta-endosternalis anterior	antecosta V, anteriad of AII ism2	endosternite, anteriad of AII ism2
AII ism2	M. antecosta-endosternalis posterior	antecosta V, posteriad of AII ism1	endosternite, posteriad of AII ism1
AII dvm1	M. tergo-endosternalis anterior	middle region of tergum, anteriad of AII dvm2	endosternite, anteriad of AII dvm2

AII dvm2	M. tergo-endosternalis posterior	middle region of tergum, posteriad of AII dvm1	endosternite, posteriad of AII dvm1
AII ldvm1	M. tergo-sternalis	lateral wall of segment	sternum
AII dvm3	M. tergo-sternalis anterior	anterior border of tergum, anteriad of AII dvm4	lateral border of sternum, anteriad of AII dvm4
AII dvm4	M. tergo-sternalis posterior	lateral area of tergum, posteriad of AII dvm3	lateral border of sternum, posteriad of AII dvm3
AII trm1	M. endosterno-endosternalis	inner surface of endosternite	inner surface of endosternite (opposite side)
AIII dlm1	M. antecosta-antecostalis medialis	antecosta V, mediad of AIII dlm2	antecosta Vl, mediad of AIII dlm2
AIII dlm2	M. antecosta-antecostalis lateralis	antecosta V, laterad of AIII dlm1	antecosta Vl, laterad of AIII dlm1
AIII ism1	M. antecosta-antecostalis medialis	antecosta VI, mediad of AIII ism2	ventral area of antecosta V, mediad of AIII ism2
AIII ism2	M. antecosta-antecostalis lateralis	antecosta VI, laterad of AIII ism1	ventral area of antecosta V, laterad of AIII ism1
AIII dvm1	M. tergo-antecostalis anterior	middle region of tergum, anteriad of AIII dvm2	ventral area of antecosta V, anteriad of AIII dvm2
AIII dvm2	M. tergo-antecostalis posterior	middle region of tergum, posteriad of AIII dvm1	ventral area of antecosta V, posteriad of AIII dvm1
AIII dvm3	M. tergo-sternalis anterior	anterior border of tergum, anteriad of AIII dvm4	lateral border of sternum, anteriad of AIII dvm4
AIII dvm4	M. tergo-sternalis posterior	lateral area of tergum, posteriad of AIII dvm3	lateral border of sternum, posteriad of AIII dvm3
AIII ldvm1	M. tergo-sternalis	lateral wall of segment	sternum
AIV dlm1	M. antecosta-antecostalis medialis	antecosta Vl, mediad of AlV dlm2	antecosta VII, mediad of AlV dlm2
AIV dlm2	M. antecosta-antecostalis lateralis	antecosta Vl, laterad of AlV dlm1	antecosta VII, laterad of AlV dlm1
AlV vlm	M. antecosta-antecostalis	antecosta V	antecosta VII
AIV ism1	M. antecosta-antecostalis	dorsal part of antecosta VI	ventral part of antecosta VII
AIV dvm1	M. tergo-sternalis posterior	anterior border of tergum, posteriad of AIV dvm3	posterior border of sternum, posteriad of AIV dvm3
AIV dvm2	M. tergo-sternalis 1	lateral wall of segment	sternum,along with AIV ldvm5
AIV dvm3	M. tergo-sternalis anterior	anterior border of tergum, anteriad of AIV dvm1	lateral board of sternum, anteriad of AIV dvm1
AIV ldvm3	M. tergo-antecostalis	posterior region of tergum	ventral part of antecosta VII
AIV ldvm4	M. tergo-sternalis 2	posterior region of tergum	lateral board of sternum
AIV ldvm5	M. tergo-sternalis 3	posterior region of tergum	sternum, along with AIV dvm2
AIV ldvm7	M. tergo-sternalis 4	lateral wall of segment	lateral board of sternum
AV dlm1	M. antecosta-antecostalis medialis	antecosta VII, mediad of AV dlm2	antecosta VIII, mediad of AV dlm2
AV dlm2	M. antecosta-antecostalis lateralis	antecosta VII, laterad of AV dlm1	antecosta VIII, laterad of AV dlm1
AV ism1	M. tergo-intersegmentalis	anterior region of tergum	intersegmental area between 5th and 6th segments
AV ldvm1	M. tergo-sternalis	lateral wall of segment	sternum
AVl sm1	M. sterno-rectalis 1	posteriolateral board of sternum	rectum, posteriad of AVI sm1
AVI sm2	M. sterno-rectalis 2	lateral board of sternum	rectum, anteriad of AVI sm2
AVl dvm1	M. tergo-rectalis	anterior region of tergum	rectum, anteriad of AVI dvm2
AVI dvm2	M. tergo-sternalis 1	central region of tergum	rectum, posteriad of AVI dvm1
AVI dvm3	M. tergo-sternalis 2	posterior region of tergum	dorsal anal lobe
AVl dvm4	M. tergo-sternalis 3	lateral wall of segment	lateral anal lobe

2

Table S1. Sizes of collembolan species with studied anatomy.

Species	Studied by	Modern name	Body size	Source of body size	Comments
Anurida maritima	Fernald (1890), Willem (1900), Lécaillon (1902), Denis (1928), Wolter (1963)	*Anurida maritima*	3 mm	Fjellberg, 1998	
Isotoma grisea	Prowazek (1900)	uncertain	~1.8 mm	Fjellberg, 1998	~~So far *Desoria grisea* was recovered as the member of the genus *Desoria* and has a strict understanding. In older publications the identification of this species is uncertain~~. The average size for the genus is given.
Achorutes viaticus	Prowazek (1900), Willem (1900)	*Hypogastrura viatica*	1.9 mm	Fjellberg, 1998	
Onychiurus fimetarius	Denis (1928)	*Onychiurus (sensu lato)* sp.	>1.3 mm	Gisin, 1960	In older publications the identification of this species is uncertain. Any species with complex PAO and without anal spines could be ~~named as 'fimetarious'~~. A very approximate average size for such forms is given.
Tomocerus catalanus	Denis (1928)	*Tomocerus catalanus*	4.5 mm	Denis, 1924	
Protanura carpenteri	Mukerji (1932)	uncertain	>1mm		

Orchesella cincta	Folsom (1899), Bretfeld, (1963), Verhoef et al. (1979)	*Orchesella cincta*	3 mm	Fjellberg, 1998	
Neanura muscorum	Bretfeld, (1963), Wolter (1963)	*Neanura muscorum*	3.5 mm	Fjellberg, 1998	
Tomocerus longicornis	Lubbock, (1873)	*Pogonognathellus longicornis*	4-5 mm	Fjellberg, 1998	
Orchesella villosa	Imms (1939), Dallai et al. (2008)	*Orchesella villosa*	4 mm	Fjellberg, 1998	
Isotomurus palustris	Imms (1939)	*Isotomurus* sp.	2.5 mm	Fjellberg, 1998	In older publications the identification of this species is uncertain. The average size for the genus is given.
Podura aquatica	Willem (1900), Imms (1939)	*Podura aquatica*	2 mm	Fjellberg, 1998	
Sminthurus viridis	Davies (1927), Imms (1939)	*Sminthurus viridis*	3 mm	Fjellberg, 1998	
Tomocerus flavescens	Wolter (1963), Humbert (1979)	*Pogonognathellus flavescens*	4-5 mm	Fjellberg, 1998	
Friesea mirabilis	Wolter (1963)	*Friesea mirabilis*	1.9 mm	Fjellberg, 1998	
Brachystomella parvula	Wolter (1963)	*Brachystomella parvula*	1.0 mm	Fjellberg, 1998	
Odontella armata	Wolter (1963)	*Xenyllodes armatus*	1.0 mm	Fjellberg, 1998	
Sminthurus fuscus	Willem & Sabbe (1897), Willem (1900)	*Allacma fusca*	3-4 mm	Fjellberg, 1998	
Onychiurus quadriocellatus	Altner (1968)	*Protaphorura quadriocellata*	2.2 mm	Fjellberg, 1998	

Tomocerus minor	Humbert (1975)	*Tomocerus minor*	4 mm	Fjellberg, 1998	
Lepidocyrtus curvicollis	Humbert (1975)	*Lepidocyrtus curvicollis*	3 mm	Fjellberg, 1998	
Orchesella rufescens	Philiptschenko (1907)	*Orchesella spectabilis*	4 mm	Fjellberg, 1998	*O.rufescens* is a junior synonym of *O.spectabilis* (collembola.org.).
Folsomia candida	Kollmann et al. (2011)	*Folsomia candida*	2.5 mm	Fjellberg, 1998	
Protaphorura armata	Kollmann et al. (2011)	*Protaphorura armata*	1.8 mm	Fjellberg, 1998	
Tetrodontophora bielanensis	Kollmann et al. (2011)	*Tetrodontophora bielanensis*	5-9 mm	Gisin, 1960	
Allacma fusca	Dallai et al. (2000)	*Allacma fusca*	3-4 mm	Fjellberg, 1998	
Anurophorus laricis	Willem (1900), Lécaillon (1902)	*Anurophorus* sp.	1.4 mm	Fjellberg, 1998	In older publications the identification of this species is uncertain. The average size for the genus is given.
Lipura armata	Willem (1900)	*Protaphorura* sp.	1.8 mm		In older publications the identification of this species is uncertain. The average size for the genus is given.
Anura muscorum	Willem (1900)	*Neanura muscorum*	3.5 mm	Fjellberg, 1998	
Isotoma viridis	Willem (1900)	*Isotoma viridis*	3-4 mm	Fjellberg, 1998	
Tomocerus plumbeus	Willem (1900)	*Pogonognathellus flavescens*	4-5 mm	Fjellberg, 1998	So far *Tomocerus plumbeus* is considered to be a synonym of *P.flavescens* .
Papirius sp.	Willem (1900)	*Dicyrtomidae* g.sp.	2 mm	Fjellberg, 1998	The average size for the family is given.
Megalothorax minimus	Willem (1900)	*Megalothorax* sp.	0.5 mm	Schneider et d'Haese, 2013	In older publications the identification of this species is uncertain. The average size for the genus is given.

Additional reference

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Table S3. Nomenclatures of muscles used in the present stidy and in others.

Head

This study, *Mesaphorura*	**Folsom (1899), *Orchesella***
1(Oe)	dil. oe. x7
-	dil.phy. X4
-	dep.
-	1. rot. l.
-	2. abd.
3(Mn)	3. ret. rot.
4(Mn)	4. ret.
5(Mn)	5. pr't. l.
-	6. pr't. ms.
7(Mn)	7. rot.
8(Mn)	8. rot.
9(Mn)	9. add.
10(Mn)	10. rot.
11(Mn)	-
12(Mn)	-
1(Mx)	1. ret. abd.
2(Mx)	2. add.
3(Mx)	3. add.
4(Mx)	4. add.
5(Mx)	5. pr't. add.
-	6. prt. add.
7(Mx)	7. add.
-	8. ret. add.
-	9. pr't. add.
-	10. add.
-	1. dep. p.
2(Lb)	2. lvt. ms.
-	3. dep. a.
4(Lb)	4. lvt. m.
5(Lb)	5. lvt. l.
dlm1	-
dlm2	-
1(An)	-

Thorax

This study, *Mesaphorura*	**Bretfeld (1963), *Neanura***	**Bretfeld (1963), *Orchesella***
Prothorax	Prothorax	Prothorax
I dlm1	I dlm1	I dlm
I dlm2	I dlm2	I dlm
I vlm	I vlm	I vlm
I ism1	I ism1	I ism1
I ism2	I ism2	I ism2
-	-	I ism3
-	-	I ism4
I dvm1	I dvm1	-
I dvm2	I dvm2	-
-	I dvm3	-
-	I dvm4	-
-	I dvm5	-
-	I dvm6	-
I ldvm1	I ldvm1	I ldvm
dlm1 (in the head)	Lb dlm1	-
dlm2 (in the head)	Lb dlm2	-
-	Lb vlm1	Lb vlm1
-	Lb vlm2	Lb vlm2
-	-	Lb ism
Lb dvm1	Lb dvm1	-
Lb dvm2	Lb dvm2	-
-	Lb ldvm1	Lb ldvm?
-	Lb ldvm2	-
I scm1	-	-
I scm2	-	-
I scm3	-	-
I scm4	-	-
Mesothorax	Mesothorax	Mesothorax
II dlm1	II dlm1	II dlm1
II dlm2	II dlm2	II dlm2
II vlm	II vlm	II vlm
II ism1	II ism1	II ism1
II ism2	II ism2	II ism2
-	II ism3	II ism3
-	II ism4	II ism4
II dvm1	-	II dvm1
II dvm2	II dvm2	II dvm2
II dvm3	II dvm3	-
II dvm4	II dvm4	-
II dvm5	II dvm5	-
-	II dvm6	-
II ldvm1	II ldvm1	II ldvm1
II ldvm2	II ldvm2	II ldvm2
II ldvm3	II ldvm3	II ldvm3
-	II ldvm4	II ldvm4
II scm1	-	-
II scm2	-	-
II scm3	-	-
II scm4	-	-
II scm5	-	-
II scm6	-	-
Metathorax	Metathorax	Metathorax
III dlm1	III dlm1	III dlm1
III dlm2	III dlm2	III dlm2
III vlm	III vlm	III vlm
III ism1	III ism1	III ism1
III ism2	III ism2	III ism2
III dvm1	III dvm1	III dvm1
III dvm2	III dvm2	III dvm2
III dvm3	III dvm3	-
III dvm4	III dvm4	-
III dvm5	III dvm5	-
-	III dvm6	-
III ldvm1	III ldvm1	III ldvm1
III ldvm2	III ldvm2	III ldvm2
III ldvm3	III ldvm3	III ldvm3
-	III ldvm4	III ldvm4
III scm2	-	-
III scm3	-	-
III scm5	-	-
III scm6	-	-
III scm7	-	-

Abdomen

This study, *Mesaphorura*	**Bretfeld (1963), *Neanura***	**Eisenbeis (1976), *Tomocerus***
A I dlm1	I dlm1	-
AI dlm2	I dlm2	-
AI vlm	I vlm	-
AI ism1	I ism1	-
AI ism2	I ism2	-
AI dvm1	I dvm1	-
AI dvm2	I dvm2	-
AI dvm3	I dvm3?	-
AI dvm4	I dvm4?	-
AI dvm VT	I dvm VT	-
AI lm	-	lm
AI pm1	-	pm1
AI pm2	-	pm2
AI pm3	-	pm3
AI pm4	-	pm4? pm5? pm6?
AI dm1	-	dm1
AI dm2	-	dm2
AI dm3	-	dm3
AI dm4	-	dm4
AI dm5	-	dm5? dm6?
AI ldvm1	I ldvm1	-
AII dlm1	II dlm1	-
AII dlm2	II dlm2	-
AII vlm	II vlm	-
AII ism1	II ism1	-
AII ism2	II ism2	-
AII dvm1	II dvm1	-
AII dvm2	II dvm2	-
AII dvm3	II dvm3	-
AII dvm4	II dvm4	-
AII ldvm1	II ldvm1	-
AII trm1	II trm1	-
AIII dlm1	III dlm1	-
AIII dlm2	III dlm2	-
AIII ism1	III ism1	-
AIII ism2	III ism2	-
AIII dvm1	III dvm1	-
AIII dvm2	III dvm2	-
AIII dvm3	III dvm3?	-
AIII dvm4	III dvm4?	-
AIII ldvm1	III ldvm1	-
AIV dlm1	IV dlm1	-
AIV dlm2	IV dlm2	-
AIV vlm	IV vlm	-
AIV ism1	IV ism1? IV ism2?	-
AIV dvm1	IV dvm1	-
AIV dvm2	IV dvm2	-
AIV dvm3	-	-
AIV ldvm3	IV ldvm3	-
AIV ldvm4	IV ldvm4	-
AIV ldvm5	IV ldvm5	-
AIV ldvm7	IV ldvm7	-
AV dlm1	V dlm1	-
AV dlm2	V dlm2	-
AV ism1	V ism1? V ism2?	-
AV ldvm1	-	-
AVl sm1	-	-
AVI sm2	-	-
AVl dvm1	-	-
AVI dvm2	-	-
AVI dvm3	-	-
AVl dvm4	-	-