universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Morphology and diversity of sexual dimorphic characters of the ultimate legs in selected lithobiomorph centipedes (Myriapoda, Chilopoda, Lithobiomorpha)"

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2021 / Vienna, 2021

Acknowledgements

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Abstract

Many lithobiomorph centipedes present a pronounced sexual dimorphism reflected in remarkable modifications and structures on the ultimate legs of male specimens, including thickenings, grooves, tubercles, greater glandular systems and sensory structures. There is not much known about their diversity, detailed morphology and their possible role during reproduction. In this study, nine species of the two lithobiid genera *Lithobius* Leach, 1814 and *Eupolybothrus* Verhoeff, 1907 were investigated using light microscopy and scanning electron microscopy (SEM) to examine and document the detailed morphology of secondary sexual characters on male ultimate legs. Secondary sexual cuticular modifications were often associated with sensilla, interpreted here as chemo- and mechanoreceptors, and with pores that were arranged in clusters, a hitherto undescribed distribution. The cuticular modifications of the species *Lithobius nodulipes* Latzel, 1880 were additionally examined with micro-computed tomography (μ-CT) and histological semi-thin sections, which revealed that the clustered pores are probably related to the glandular system found inside the cuticular modifications. The presence of a high number of sensory and glandular structures associated with secondary sexual characters indicate a possible role during courtship and mating. Closely related species show similarities in their secondary sexual characters, but still these structures are highly species-specific. This study illustrates different degrees of sexual dimorphism in lithobiid centipedes, ranging from subtle to highly complex. The detailed comparative data on a microstructural level presented here, along with the histological information and physiological interpretations, is perhaps a first step towards understanding some aspects of the reproductive biology of the group.

Keywords: sexual dimorphism, cuticular modifications, microstructures, sensilla, glandular system, glandular pores, SEM, μ-CT, histology, *Lithobius*, *Eupolybothrus*

Introduction

Males and females of sexually reproducing species naturally differ in certain morphological aspects, a concept which is known as sexual dimorphism. These differences can be directly related to reproduction, such as different reproductive organs, which are then called primary sexual characters (Darwin 1871, Andersson 1994). If those differences are not directly or not at all related to reproduction, they are considered as secondary sexual characters (Darwin 1871, Andersson 1994). Secondary sexual characters can be further distinguished between those linked to different ecological needs of the sexes and those that are, however not directly related, facilitating reproduction (Darwin 1971, Andersson 1994). Such can be for example enhanced sensory and locomotory skills (usually) of the male to find a partner, female organs ensuring nourishment or protection of the offspring, size dimorphism and (again most frequently in males) weapons and ornaments (Darwin 1871). In arthropods, sexual dimorphism is very common in the form of modifications of their appendages, for example the pedipalps in pseudoscorpions (Zeh 1987), pedipalps and antenniform legs in whip spiders (McArthur et al. 2018), cheliceres in harvestmen (Willemart et al. 2006), different legs in crustaceans (Hume et al. 2005, Ohtsuka and Huys 2001), legs in collembolans (Palacios-Vargas and Castaño-Meneses 2009), mandibles in orthopterans (Bateman and Toms 1998, Gwynne and Jamieson 1998) or the sex combs on the legs of *Drosophila melanogaster* males (Massey et al. 2019). The appendages of arthropods generally show a high morphological plasticity and may serve, besides reproduction, functions related to locomotion, prey capture, feeding, respiration and signal perception (Boxshall 2004, Williams and Nagy 2001).

The arthropod subphylum Myriapoda is characterized by several leg-bearing trunk segments, whose legs are overall very similar and usually consist of coxae and five-segmented telopodites (for morphology and terminology of centipede legs see Fig. 1A), although the terminology of the articles differs between taxa (Minelli and Koch 2011, Koch 2015). There are cases of specialisation in some leg-pairs in different taxa, for example the gonopods (genitalia) in millipedes used for the transfer of the aflagellate sperm or spermatophores, or the forcipules (venomous claws) of centipedes used for injecting venom into preys (Minelli and Koch 2011, Koch 2015). The transformation of the first pair of walking legs into venomous claws is considered an apomorphy of the class Chilopoda (Minelli 2011, Lewis 1981). Another type of leg modification observed in this group is displayed by their ultimate legs (Fig. 1A). These are often elongated, orientated backwards and equipped with considerably more sensory and glandular structures than the anterior walking legs (Fig. 1A) (Minelli and Koch 2011,

Kenning et al. 2017, Kenning et al. 2019). Different types of sensilla trichoidea on the ultimate legs of centipedes were already described from different taxa and their function has been discussed (Keil 1976, Ernst et al. 2009, Sombke et al. 2011, Sombke and Ernst 2014). Moreover, the ultimate legs show different cuticular modifications (Kenning et al. 2017, Zapparoli and Edgecombe 2011). These modifications vary between different taxa from moderate to extraordinary differentiations (Kenning et al. 2017, Zapparoli and Edgecombe 2011). Although some modifications of ultimate legs in centipedes are known, many remain to be described (see Kenning et al. 2017 for review).

In the centipede order Lithobiomorpha, modifications of ultimate legs are very diverse across different taxa and often sexually dimorphic (Zapparoli and Edgecombe 2011, Lewis 1981). Lithobiomorph centipedes possess 15 leg-bearing trunk segments, showing heterotergy with an alternation of longer (macro-) and shorter (micro-)tergites (Fig. 1A) (Lewis 1981). The legs typically increase in length from anterior to posterior, the last three to four pair of legs (also called the ultimate legs) being particularly elongated (Lewis 1981). Frequently, the 14th and 15th pair of legs bear several characters that distinguish them from the anterior legs (Zapparoli and Edgecombe 2011, Eason 1964, Kenning et al. 2017). Modifications include thickenings of the articles and the presence of dorsal sulci (Lewis 1981, Eason 1964, Zapparoli and Edgecombe 2011). An increase in density and abundance of setae and pores in the posterior legs and segments is also common (Farzalieva et al 2017, Kenning et al. 2019). Six different types of sensilla on walking und ultimate legs were recently recorded for *Lithobius forficatus* (Linnaeus, 1758) (Kenning et al. 2019). Furthermore, three types of pores were identified so far on the ultimate legs of Lithobiomorpha: coxal pores, which are present on the 12th-15th leg-pair, small epidermal glands ("kleine Epidermisdrüse") and elliptical pores of the telopodal glands ("telopodal pores") (Keil 1975, Rosenberg et al. 2011). The latter are highly abundant and known to secrete a lipid-protein-containing substance as a defense mechanism (Blower 1952, Keil 1975, Kenning et al. 2019, Rosenberg et al. 2011).

The order Lithobiomorpha comprises two families: Henicopidae Pocock, 1901 and Lithobiidae Newport, 1844, with sexual dimorphism of the ultimate legs restricted to the latter one, comprising six subfamilies (Lewis 1981, Zapparoli and Edgecombe 2011). Here, sexual dimorphism is reflected in various cuticular modifications, including protuberances, warts, grooves and several other structures occurring only in males (Lewis 1981, Zapparoli and Edgecombe 2011). Generally, males show thickened and furrowed ultimate legs, like in the genera *Lithobius* Leach, 1814, *Eupolybothrus* Verhoeff, 1907, *Gosibius* Chamberlin, 1912, *Pseudolithobius* Struxberg, 1875 and *Dispaheorobius* Attems 1927 (Chamberlin 1917, Eason

1964, Farzalieva et al. 2017, Zapparoli and Edgecombe 2011). In some species, females show thickenings and sulci as well, but less distinct than males (see Eason 1964, Zapparoli and Edgecombe 2011). Another form of secondary sexual modifications in males are swellings or enlargements of the entire or part of the articles, as well as pits and grooves (Zapparoli and Edgecombe 2011, Stoev et al. 2010, Akkari et al. 2018). Another type of modifications in male's ultimate legs are processes and projections, which often occur in combination with "setae", present on different articles. They display different forms, such as a directed wart-like process in male *Lithobius calcaratus* Koch, 1844, on the 15th femur or a rounded lobe on the 14th tibia of male *Gosibius intermedius* Chamberlin, 1917 (Eason 1964, Chamberlin 1917). In the subgenus *Schizopolybothrus* Verhoeff, 1934 the males of all species (except for *Eupolybothrus* (*Schizopolybothrus*) *tabularum* Verhoeff, 1937) possess a proximal prefemoral knob bearing setae on their 15th prefemora, which varies in shape, location and type of setae between species (Akkari et al. 2017). Although modifications of the 14th and 15th leg-pairs are most common, other leg-pairs may present sexual dimorphism as well, such as in the genus *Pleurolithobius* Verhoeff, 1899 that additionally shows modifications on the 13th leg-pair (Zapparoli and Edgecombe 2011, Stoev 2002).

Additionally, several lithobiid species show secondary sexual structural differences in posterior tergites, for example the intermediate tergite (= tergite posterior to the 15th tergite) which is usually more slender in males than in females (Lewis 1981, Farzalieva et al. 2017, Zapparoli and Edgecombe 2011, Eason 1964, Bonato et al. 2010). The species *Disphaerobius svenhedini* (Verhoeff, 1934) and *Disphaerobius loricatus* (Sseliwanoff, 1881) represent a remarkable example, where posterior tergites in males are serrated and broadened (Farzalieva et al. 2017). In *Disphaerobius loricatus*, the 14th tergite of males is very enlarged and in *Disphaerobius svenhedini* the 12th tergite of the males is extremely enlarged and forms two lateral wing-like lobes (Farzalieva et al. 2017). There are but a few cases of sexual dimorphism in anterior structures, for example *Lithobius muticus* C. L. Koch, 1847, with a broadened head of the males (Koren 1992). Another special case of sexual dimorphism is found in the American species *Paitobius zinus* (Chamberlin, 1911) in which the forcipules of the males show an elongation and distortion (Crabill 1960). While in some species modifications are restricted to one body part, there are species such as *Disphaerobius svenhedini*, where forcipules, posterior tergites and ultimate legs of the males are modified (Farzalieva et al. 2017). On the other hand, sexual dimorphism of ultimate legs, tergites or forcipules can also be completely absent, as for example in *Lithobius forficatus* (Linnaeus, 1758), *Ottobius hopanus*

Chamberlin, 1952, the genera *Cruzobius* Chamberlin, 1942, *Tropobius* Chamberlin 1943 and *Texobius* Chamberlin and Mulaik, 1940 (Koren 1992, Zapparoli and Edgecombe 2011).

Most of the secondary sexual characters are recorded in the literature, being also useful taxonomic traits, however, knowledge on their possible function(s) is particularly poor (Stoev 2010, Farzalieva et al. 2017, Akkari et al. 2017). The ultimate legs have been considered to play a role in courtship, for example for tactile signals, though they do not play a primary role in sperm transfer (Klingel 1959, Kenning et al. 2017, Minelli 2011, Akkari et al. 2018). The aim of this study is to identify sexual dimorphic structures of the ultimate legs and to describe them in detail with special regard to their associated microstructures. Investigations of the external and internal morphology of sensory and glandular structures may aid in assessing the putative physiological functions of the microstructures as well as the putative role of the sexual dimorphic characters in lithobiomorph centipedes' reproduction.

Material & Methods

Material

For this study material from the scientific collection of the Natural History Museum Vienna (Naturhistorisches Museum Wien, NHMW) and freshly collected material was investigated. The material is stored in 70% ethanol (Table 1). Fresh material was collected from soil and leaf litter in Mauerbach, Niederösterreich, near the Kartause Mauerbach (48.24922 N, 16.17119 E) using a sifter. The freshly collected material was fixed in FAA (ethanol 70%, formaldehyde 35%, acetic acid in the ratios 10:3:1), then transferred to 70% ethanol and incorporated in the NHMW collection (Inventory Number NHMW 10213).

Methods

Light microscopy

External characters were studied and photographed using a Nikon SMZ25 stereomicroscope (Nikon Corporation, Tokyo, Japan). Images were taken with a Nikon DS-Ri2 camera (Nikon Corporation, Tokyo, Japan) mounted on the stereomicroscope, using NIS-Elements Microscope Imaging Software (version 5.02; Nikon Corporation, Tokyo, Japan) with an Extended Depth of Focus (EDF) patch.

Scanning electron microscopy (SEM)

Material of the scientific collection of the NHMW, stored in 70% ethanol, was used. The ultimate legs were detached from the posterior segment using forceps. Specimens were cleaned using different methods depending on the amount of dirt attached to the specimen and on the specimen's fragility. Highly contaminated and robust specimens were cleaned with ultrasound for 20 seconds up to 10 minutes. Very fragile specimens were cleaned manually with a fine brush and eyelashes and by several exchanges of the ethanol (with a pipette) or with a KOH treatment. For the KOH treatment, a 5% KOH solution was used and applied on the specimens for 1.5 to up to 8.5 hours, depending on their size and the amount of dirt. Subsequently, specimens were put into either glacial acidic acid for 15-20 minutes and afterwards distilled water for 15 minutes or only into distilled water for 15 minutes. Then, they were returned to 70% ethanol. After cleaning, the ultimate legs were dehydrated in an ascending alcohol series

comprising the steps 70%-80%-80%-90%-90%-96%-96% for 10 minutes each. Next, the specimens were left in 96% ethanol for about 20 minutes, then ethanol was removed and a few drops of hexamethyldisilazane (HMDS) were added under an air exhauster and left to dry. One specimen was transferred from 96% ethanol into acetone and left there for 15 minutes. Then acetone was removed and the specimen was air-dried (approximately 3 minutes) and mounted directly. The dried ultimate legs were mounted on aluminium tape attached to aluminium stubs. The mounted specimens were sputter-coated with platinum at 30 mA for 250 seconds using a Leica EM SCD500 sputter coater (Leica Microsystems, Wetzlar, Germany). Specimens were investigated with a JEOL JSM 6610 (JEOL ltd., Akishima, Tokio, Japan).

Micro-computed tomography (µ-CT)

Two male individuals of *Lithobius nodulipes* of the scientific collection of the NHMW, stored in 70% ethanol, were used for micro-computed tomography. They were transferred from 70% ethanol into alcoholic Bouin's solution (1:1 Bouin's solution:ethanol), left there for approximately 1 hour and then retransferred into 70% ethanol. After one washing step with 70% ethanol they were transferred into 90% ethanol and left there for approximately 10 minutes. They were stained in a 1% iodine in 90% ethanol solution in which they remained for approximately 3.5 hours. The specimens were mounted in agarose in plastic tubes. Scans were made with an Xradia MicroXCT-200 system (Carl Zeiss X-Ray Microscopy, Inc., 4385 Hopyard Rd., Suite 100, Pleasanton, California 94588, USA) equipped with a micro-focus tungsten source (Hamamatsu L9421-02). Scans were made of the whole animal (objective lens: 1x, exposure time: 25 sec., X-ray source voltage: 60 kV, power: 5 W, reconstructed voxel size: 6.5 µm), for the posterior end (objective lens: 4x, exposure time: 35 sec., X-ray source voltage: 40 kV, power: 5 W, reconstructed voxel size: 3.6 µm) and for the ultimate legs only (objective lens: 4x, exposure time: 15 sec., X-ray source voltage: 40 kV, power: 5 W, reconstructed voxel size: 2.0 µm). Reconstruction was performed using Xradia XMReconstructor (version 8.2; Xradia, Inc. Concord, California, USA) software.

Semi-thin sections

Histological semi-thin sections of the 15th legs of *Lithobius nodulipes* were made. Specimens from the scientific collection of the NHMW, stored in 70% ethanol and freshly collected spec-

imens were investigated. Identification of living specimens of *L. nodulipes* used later for histology was performed by anesthetizing the animals with ca 10% ethanol and checking key taxonomic characters under the light microscope. Identified specimens were fixed in FAA (ethanol 70%, formaldehyde 35%, acetic acid in the ratios 10:3:1). Ultimate legs were dissected by detaching them at the coxa from the posterior segment using forceps. An alcohol series was performed, comprising the steps 70%-70%-70%-80%-90%-96%-100%-100%-100% with approximately 10 minutes between each step. Afterwards, the specimens were transferred into 100% acetone with two changes after 10 minutes each. They were subsequently transferred from acetone into a 1:1 mix of acetone and resin. After 21 hours, the amount of resin was increased to 3:7 (acetone:resin) and after another 5 hours, the specimens were transferred into 100% resin and left there for 20 hours. The legs were embedded into the resin in small forms and incubated at 40°C at 150 mbar for 45 minutes. Finally, they were dried at 60°C for about 24 hours to fully polymerize.

Trimming was done manually and the sectioning using a Leica UC6 ultra cut ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany) and a Histo- Jumbo diamond knife (Diatome AG, Biel, Switzerland) at speed: 1.00 mm/s, making sections of 1 µm thickness. After sectioning, bands were placed on slides and put on a heating plate (ca 60°C). The sections were stained with toluidine blue in bromide at ca 60°C. Specimens were checked every 5 seconds under a compound microscope to decide if staining was already strong enough. Therefore, staining time depended on the specimen and ranged between 5-20 seconds. Sections were photographed using a Nikon DS-Ri2 camera mounted on a Nikon Eclipse Ni compound microscope (Nikon Corporation, Tokyo, Japan), using NIS-Elements Microscope Imaging Software with an Extended Depth of Focus (EDF) patch.

Image Processing

Light microscopy and SEM images were edited using Photoshop CS6 (version 13.0 x64; Adobe Systems Software Ireland Limited, Dublin, Republic of Ireland), µ-CT scans were edited using Amira (Amira, Konrad-Zuse-Zentrum Berlin (ZIB), Germany) and FIJI (ImageJ 1.53c, Schindelin et al. 2012). Images were assembled to figure plates using InDesign CS6 (version 8.0; Adobe Systems Software Ireland Limited, Dublin, Republic of Ireland).

Table 1: Studied material with inventory numbers (of the Natural History Museum Vienna) and information on the used method.
Abbreviations: LM – light micsroscopy SEM – scanning electron microscopy, µ-CT – micro computed tomography

Species	Sex	Method	Specimen	Inventory Nr.
Eupolybothrus cavernicolus	male	LM	posterior end	NHMW 9212
Eupolybothrus liburnicus	male	LM	posterior end	CHP545
Eupolybothrus liburnicus	male	LM	posterior end	CHP538
Eupolybothrus liburnicus	female	LM	posterior end	CHP8409
Eupolybothrus liburnicus	subadult male	LM	posterior end	CHP543
Eupolybothrus liburnicus	subadult male	LM	posterior end	CHP544
Eupolybothrus fasciatus	males + females	LM	posterior end	NHMW 1366
Eupolybothrus fasciatus	female	SEM	15th leg	NHMW 10234
Eupolybothrus fasciatus	female	SEM	left 15th leg	NHMW 10236
Eupolybothrus fasciatus	male	SEM	left 15th leg	NHMW 10233
Eupolybothrus fasciatus	male	SEM	left 15th leg	NHMW 10235
Eupolybothrus fasciatus	male	SEM	right 15th leg	NHMW 10237
Eupolybothrus fasciatus	male	SEM	right 15th leg	NHMW 10238
Eupolybothrus fasciatus	male	SEM	left 15th leg	NHMW 10239
Eupolybothrus grossipes	female	LM	posterior end	NHMW 9175
Eupolybothrus grossipes	male	LM	posterior end	NHMW 9176
		SEM	15th leg-pair	
Lithobius forficatus	male	LM	posterior end	NHMW 9138
		SEM	right 15th leg	
Lithobius forficatus	female	LM	posterior end	NHMW 8959
Lithobius forficatus	male	LM	posterior end	NHMW 10225
		SEM	right 15th leg	
Lithobius forficatus	female	LM	posterior end	NHMW 10226
		SEM	15th leg-pair	
Lithobius forficatus	female	SEM	left 15th leg	NHMW 10227
Lithobius forficatus	male	SEM	15th leg	NHMW 10228
Lithobius mutabilis	males + females	LM	posterior end	
Lithobius mutabilis	Female	LM	posterior end	NHMW 9126
		SEM	right 14th leg, right 15th leg	
Lithobius mutabilis	male	LM	posterior end	NHMW 1227
		SEM	14th leg-pair, 15th leg-pair	
Lithobius muticus	male	LM	posterior end	NHMW 10215
Lithobius muticus	male	LM	posterior end	NHMW 10216
Lithobius muticus	male	LM	posterior end	NHMW 10217
Lithobius muticus	male	LM	posterior end	NHMW 10218
Lithobius muticus	female	LM	posterior end	NHMW 10218
Lithobius muticus	Female	SEM	left 14th leg, left 15th leg	NHMW 10202
Lithobius muticus	male	SEM	posterior end with left 14th leg and left 15th leg	NHMW 10203
Lithobius nodulipes	male	LM	posterior end	NHMW 10219
Lithobius nodulipes	male	LM	posterior end	NHMW 10220

Lithobius nodulipes	female	LM	posterior end	NHMW 10221
Lithobius nodulipes	female	LM	posterior end	NHMW 10222
Lithobius nodulipes	male	LM	posterior end	NHMW 10223
Lithobius nodulipes	subadult male	LM	posterior end	NHMW 10223
Lithobius nodulipes	female	LM	posterior end	NHMW 10204
		SEM	15th leg-pair	
Lithobius nodulipes	female	SEM	right 15th leg	NHMW 10205
Lithobius nodulipes	male	SEM	posterior end with 15th leg-pair	NHMW 10206
Lithobius nodulipes	male	SEM	left 15th leg	NHMW 10209
Lithobius nodulipes	male	LM	posterior end	NHMW 10211
		SEM	right 15th leg	
Lithobius nodulipes	female	histology	15th leg-pair	NHMW 10207
Lithobius nodulipes	male	histology	15th leg-pair	
Lithobius nodulipes	female	histology	15th leg-pair	NHMW 10208
Lithobius nodulipes	male	histology	15th leg	NHMW 10208
Lithobius nodulipes	male	LM	posterior end,	NHMW 10212
		histology	15th leg-pair	
Lithobius nodulipes	male	histology	15th leg-pair	NHMW 10213
Lithobius nodulipes	male	histology	15th leg-pair	NHMW 10213
Lithobius nodulipes	male	histology	15th leg-pair	NHMW 10213
Lithobius nodulipes	male	µ-CT	whole specimen	NHMW 10210
Lithobius nodulipes	male	µ-CT	whole specimen	NHMW 10214
Lithobius pelidnus	male	LM	posterior end	NHMW 6441
Lithobius pelidnus	female	LM	posterior end	NHMW 6444
Lithobius pelidnus	female	LM	posterior end	NHMW 10229
		SEM	left 14th leg, left 15th leg	
Lithobius pelidnus	male	LM	posterior end	NHMW 10230
		SEM	right 14th leg, right 15th leg	
			left 14th leg, left 15th leg	NHMW 10231
Lithobius pelidnus	male	LM	posterior end	NHMW 10232
		SEM	right 15th leg	

Results

1. Ultimate legs

Leg length increases from anterior to posterior and particularly in the last four leg-pairs, referred here as ultimate legs (Fig. 1A). The last (= 15th) leg-pair is extremely elongated and oriented backwards in both sexes (Fig. 1A). The length of the 15th leg-pair is about ⅓-½ of body length in the *Lithobius* species, and over ½ the body length in the *Eupolybothrus* species. In most species several articles of ultimate legs are thickened. Most often this affects the prefemora, femora and tibiae or only prefemora and femora. Differences between the sexes include the presence and extent of the thickenings (Table 2). For example, in *E. liburnicus*, only males show slight thickenings of their 14th and 15th prefemora and femora. In *L. muticus*, *L. mutabilis* and *L. pelidnus*, the difference between males and females occurs in the extent of the thickening, which is more pronounced in males. *L. pelidnus* and *L. mutabilis* show a thickening of 13th, 14th and 15th prefemora, femora and tibiae, also more prominently in males. A notable case of sexual dimorphism related to leg expansions can be found in *L. muticus*, where females show only slightly enlarged 14th and 15th prefemora, femora and tibiae, while males have stout and tremendously thickened 14th and 15th legs (Fig. 1H-I).

Table 2: Comparison of the thickened articles in ultimate leg-pairs of the studied species. The thickenings occur with different degrees from - absent, (+) slightly, + noticeable to ++ prominent. **Abbreviations:** pr = prefemur, f = femur, ti = tibia. No data (x) for female *E. cavernicolus*.

Species	Leg-pair	Male			Female		
		pr	f	ti	pr	f	ti
Eupolybothrus cavernicolus	14	(+)	(+)	-	x	x	x
	15	+	(+)	-	x	x	x
Eupolybothrus liburnicus	14	(+)	(+)	-	-	-	-
	15	+	+	-	-	-	-
Eupolybothrus fasciatus	14	++	++	+	+	+	+
	15	++	++	+	+	+	+
Eupolybothrus grossipes	14	+	+	(+)	+	+	(+)
	15	+	+	(+)	+	+	(+)
Lithobius forficatus	14	+	+	(+)	+	+	(+)
	15	+	+	(+)	+	+	(+)
Lithobius nodulipes	14	+	+	+	+	+	+
	15	+	+	+	+	+	+
Lithobius muticus	14	++	++	++	+	+	+
	15	++	++	++	+	+	+
Lithobius mutabilis	13	++	++	++	+	+	+
	14	++	++	++	+	+	+
	15	++	++	++	+	+	+
Lithobius pelidnus	13	+	+	+	+	+	+
	14	++	++	++	+	+	+
	15	++	++	++	+	+	+

Another character of the ultimate legs is the presence of dorsal sulci, mainly on prefemora and femora, but in some species also on the tibiae (Fig. 1, Table 3). These sulci are fine indentations in the cuticle already visible under the light microscope as thin lines (Fig. 1). Most often they occur in pairs, extending parallel along the whole article. The expression of these sulci differs across species from quite prominent (for example in *E. grossipes* and *E. fasciatus*) to barely noticeable (for example in *L. forficatus* and *L. nodulipes*) (Fig. 1F-G). The sulci may be present in both sexes, but they are also commonly related to sexual dimorphism in their expression. Frequently females show less conspicuous sulci on fewer articles (for example in *E. fasciatus* and *L. pelidnus*). In some species, sulci are even only present in males (for example *L. mutabilis*). However, since both sexes as well as immature individuals may possess dorsal sulci, they are not considered to be sexual dimorphic characters, only different in their expression. Furthermore, the expression varies also between mature individuals of the same sex (for example *L. nodulipes*). Here it is important to mention, that there are also indentations beside these "regular" sulci, which are usually broader and larger, in this study referred to as grooves, which actually are only found in males (see: 3. Male cuticular structures on the ultimate legs).

Table 3: Comparison of the occurrence and expression of dorsal sulci on articles of the ultimate leg-pairs of the studied species. The sulci occur in varying degrees from - absent, (+) slightly, + noticeable to ++ prominent. **Abbreviations:** pr = prefemur, f = femur, ti = tibia. No data (x) for female *E. cavernicolus*.

Species	Leg-pair	Male			Female		
		pr	f	ti	pr	f	ti
Eupolybothrus cavernicolus	14	+	+	x	x	x	x
	15	-	+	x	x	x	x
Eupolybothrus liburnicus	14	(+)	+	(+)	-	(+)	-
	15	-	+	(+)	-	(+)	-
Eupolybothrus fasciatus	14	++	++	++	+	+	(+)
	15	++	++	++	+	+	-
Eupolybothrus grossipes	14	++	++	+	++	++	(+)
	15	++	++	+	++	++	(+)
Lithobius forficatus	14	(+)	(+)	(+)	(+)	(+)	(+)
	15	(+)	(+)	(+)	(+)	(+)	(+)
Lithobius nodulipes	14	-	(+)	-	-	(+)	-
	15	-	(+)	-	-	(+)	-
Lithobius muticus	14	-	+	-	-	(+)	(+)
	15	+	+	-	-	(+)	(+)
Lithobius mutabilis	14	-	+	-	-	-	-
	15	-	+	-	-	-	-
Lithobius pelidnus	14	+	+	+	(+)	(+)	(+)
	15	-	(+)	+	(+)	(+)	(+)

Figure 1: Habitus and ultimate legs of Lithobiomorpha, light photographs. **A** Habitus and ultimate leg of *L. nodulipes*, female with ultimate legs elongated and orientated backwards. **B** Posterior end of female *L. forficatus*, ventral view (black arrow pointing to coxal pores of the left 14th leg). **C-E** Coxal pores on ultimate legs: **C** *E. fasciatus*. **D** *L. forficatus*. **E** *L. mutabilis*. **F-H** 15th leg-pair of *E. fasciatus*: **F** Male, black arrows pointing to prominent dorsal sulci of the right femur. **G** Female, black arrows pointing to less pronounced dorsal sulci of the right femur. **H-I** Ultimate segments and leg-pairs of *L. muticus*, dorsal view: **H** Male, with stout and thickened 14th and 15th legs. **I** female, with only slightly thickened 14th and 15th leg. Scale bars: A, B, F, G: 1 mm; C, H, I: 0.5 mm. D, E: 0.1 mm. **Abbreviations:** coxa (c), femur (f), prefemur (pr), tibia (t), first tarsal article (ta-1), second tarsal article (ta-2), trochanter (tr)

2. Microstructures

The ultimate legs are equipped with different types of sensilla and pores (Fig. 2). Herein, three types of sensilla and four types of pores were identified.
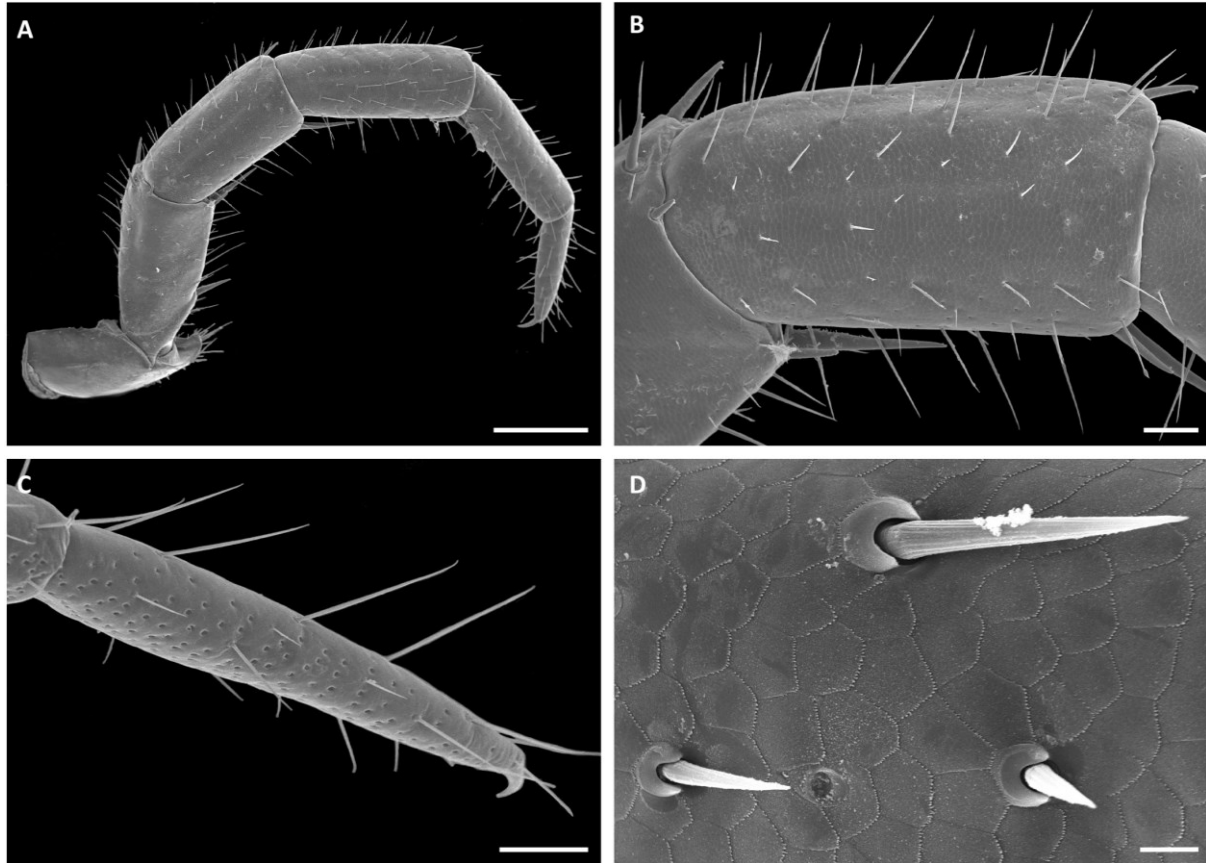

Figure 2: Microstructures of the ultimate legs, SEM. **A-B** *L. muticus*, female: **A** Entire 15th leg. **B** Various sensilla trichoidea and pores on the 15th femur. **C-D** *L. nodulipes*, male: **C** Telopodal pores, sensilla mesotrichoidea and macrotrichoidea on the 15th tarsus. **D** Sensilla mesotrichoidea on the 15th prefemur. Scale bars: A: 500 µm, B, C: 100 µm, D: 10 µm.

2.1. Sensory structures (sensilla)

The most abundant sensory structures found on the ultimate legs are sensilla trichoidea (Figs. 2, 3). The sensilla are either regularly arranged in rows or distributed irregularly. In both cases, they have the same direction, with their tips pointing distad (Fig. 2D). A sensillum trichoideum consists of a pedestal, a shaft and an apex (Figs. 2, 3). The sensilla trichoidea of the ultimate legs differ in length and certain morphological characters. Three main length classes of sensilla were found and distinguished according to the terminology of sensilla proposed for *Lithobius forficatus* (Keil 1975, Kenning et al. 2019):

a. sensilla microtrichoidea (approximately 10 µm; Fig. 3A-C)

b. sensilla mesotrichoidea (approximately 20-90 µm, mainly either 20-40 µm or 60-90 µm; Fig. 3D-I)

14

c. sensilla macrotrichoidea (over 100 µm; Fig. 3J-L)

The different sensilla showed a great variation in morphological features among the studied species and individuals and were therefore not further categorized.

2.1.1. Morphological characters of sensilla

The pedestal is slightly elevated, crescent-shaped and often associated with one or many small, round pores located at its side(s) (Figs. 2D, 3A-C, E-I, K-L). The number of these pores varies from one to two (*L. forficatus*, *E. grossipes*, *E. fasciatus*, *L. mutabilis*) up to three and more (*L. pelidnus*, *L. muticus*, *L. nodulipes*) (Fig. 3). Pedestals of sensilla microtrichoidea were either with (Fig. 3B) or without associated pores (Fig. 3A, C). In sensilla mesotrichoidea and sensilla macrotrichoidea the pedestals were associated with at least one pore (Fig. 3F, H, L) but most often with many (Fig. 3G, K).

The shaft is either ribbed or sometimes with trabecular structures between the ribs (Fig. 3). In sensilla microtrichoidea and sensilla macrotrichoidea the ribs were mainly parallel or only slightly twisted (Fig. 3A, C, J), seldom strongly twisted (ex. Fig. 3B). Sensilla mesotrichoidea showed shafts with parallel (Fig. 3E, G, H) or twisted (Fig. 3D, 3F) ribs and in some cases with trabecular structures between the ribs (Fig. 3F).

The apex can be either straight or slightly bent and, in many cases, it features a terminal pore (Fig. 3A, C, E, J). In sensilla microtrichoidea the apex was mostly straight (Fig. 3B-C) and in a few cases slightly bent (Fig. 3A). The apex of sensilla mesotrichoidea and sensilla macrotrichoidea was either straight (Fig. 3D, J) or bent (Fig. 3E). Sensilla microtrichoidea and sensilla mesotrichoidea featured a terminal pore (Fig. A, C, l), for sensilla macrotrichoidea a terminal pore could not be verified.

Sensilla mesotrichoidea appeared to be the most variable sensilla form, as the number of associated pores and morphology of the shaft varied both within and among species as well as the combinations of these characters.
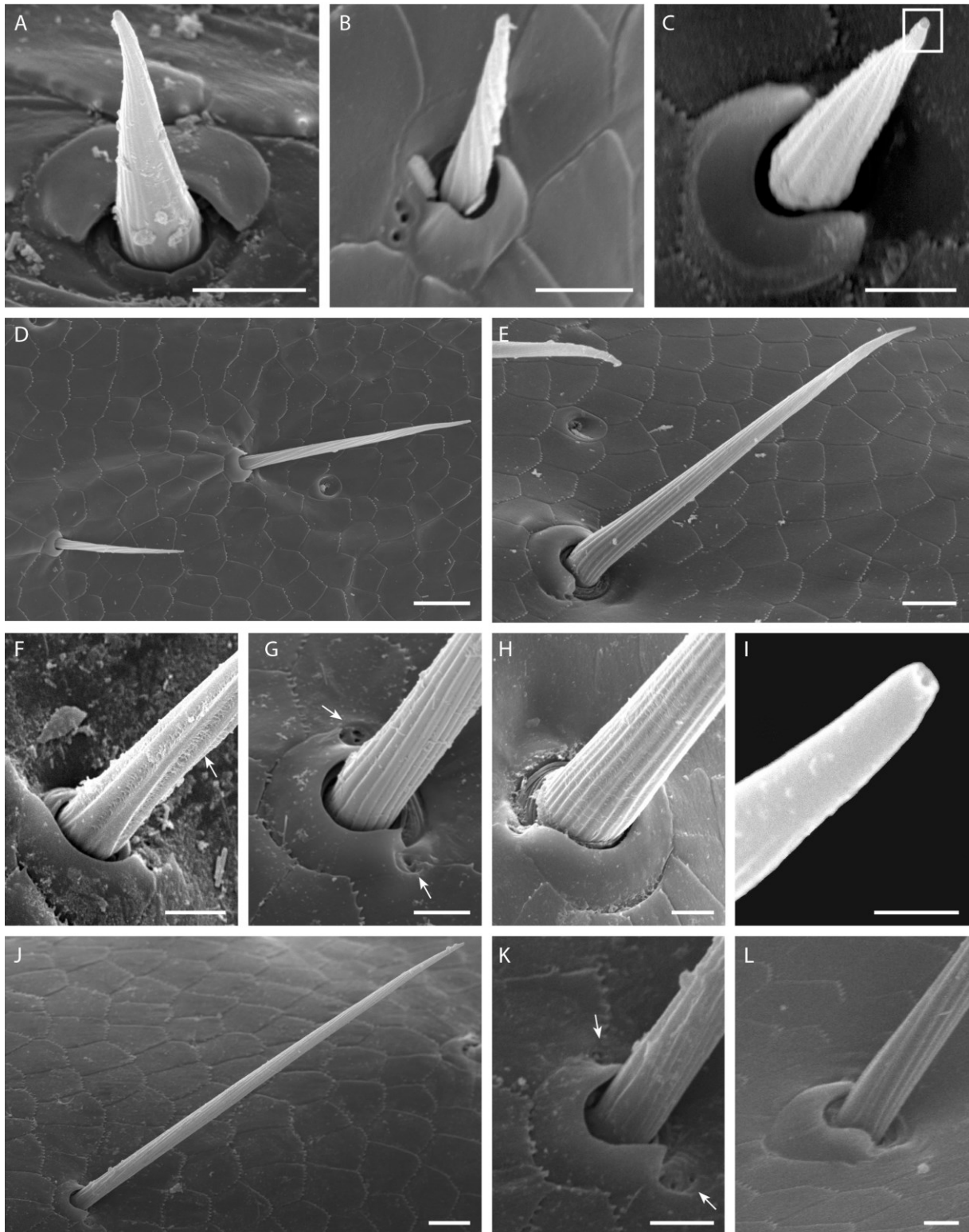
Figure 3: Different types of sensilla trichoidea, SEM. **A-C** Sensilla microtrichoidea: **A** 15th Coxa of male *L. giganteus*. **B** 15th prefemur of male *L. pelidnus*, pedestal of sensillum showing three associated pores. **C** 15th prefemur of male *L. nodulipes*, terminal opening visible in white box. **D-I** Sensilla mesotrichoidea. **D** 15th femur of male *L. pelidnus*, two sensilla of different length-classes. **E** The 15th prefemur of male *L. forficatus*. **F-H** Pedestal and shaft of sensilla mesotrichoidea: **F** 15th femur of male *L. forficatus*, white arrow pointing to trabecular structure of the shaft. **G** 14th femur of female *L. muticus*, white arrows pointing to pedestal with many associated pores. **H** 15th prefemur of female *L. forficatus*, pedestal with a single associated pore. **I** 15th femur of male *L. forficatus*, detail of terminal opening. **J-L** Sensilla macrotrichoidea: **J** 14th tarsus of male *L. muticus*. **K** 14th tarsus of male *L. muticus*, white arrows pointing to pedestal with many associated pores. **L** 15th tibia of female *L. forficatus*, pedestral without associated pores. Scale bars: A-C, F, G, H, K-L: 5 µm; D: 20 µm, E, J: 10 µm, I: 1 µm.

2.1.2. Distribution of sensilla types on the ultimate legs

Sensilla trichoidea cover all articles of the ultimate legs in both sexes of all investigated species. All three types of sensilla (sensilla microtrichoidea, sensilla mesotrichoidea and sensilla macrotrichoidea) were observed in both sexes of all examined species. The composition of sensilla types changed between the articles. Generally, there was an increase in the number of longer sensilla on the distal articles. Also, sensilla density increased from proximal to distal articles. No major differences in sensilla density and the ratio of different sensilla types were found between the sexes, however these often varied across species. The ratio of sensilla mesotrichoidea and sensilla macrotrichoidea varied especially on tibia and tarsus across species, while between sexes it was quite similar. In the species *L. muticus*, *L. mutabilis*, and *L. pelidnus*, in which both 14th and 15th legs were examined, sensilla density of the 15th leg was higher than on the 14th leg.

    a. Sensilla microtrichoidea were found on coxa and prefemur and two times on femur and tibia (*E. fasciatus* and *L. mutabilis*).

    b. Sensilla mesotrichoidea were present on all articles. On coxa and trochanter, they were mainly located on the distal margin organized in dense rows, as well as scattered over the lateral surface of the coxa. On prefemur and femur they were usually the most abundant sensilla type. Their density increased from prefemur to tarsus.

    c. Sensilla macrotrichoidea were present on prefemur, femur, tibia and tarsus, with their density increasing from prefemur to tarsus. In some species few sensilla macrotrichoidea were also found on the coxa. On tibia and tarsus, they were the most abundant sensilla type in all species, except for the *L. mutabilis*, where sensilla mesotrichoidea dominated.

2.2. Glandular structures (pores)

Three different types of glandular openings, referred to as "pores" herein, were present on the ultimate legs in both sexes of all examined species and a fourth type was found in six species, but restricted to males (Figs. 1, 2, 4).

2.2.1. Different types of glandular pores

    a. Coxal pores: round or oval pores on the last four leg-pairs ($12^{th} - 15^{th}$). Either arranged irregularly as in the genus *Eupolybothrus* or in regular rows in the genus *Lithobius* (Fig. 1B-E).

b. Telopodal pores: large and round pores that cover most parts of the ultimate legs (Fig. 4A-B). A telopodal pore consist of a round hollow pit located on oval cuticular scutes. The size of the pit differs among species (Table 5). Inside this pit, a smaller circular opening exists. The margin of the opening may appear frayed (Fig. 4B, asterisk). The openings sometimes appear to be closed by lid-like structures (Fig. 4A, arrows), but whether these structures are functional or just the remains of secretions is not clear.

Table 5: diameter (in μm) of hollow pits of telopodal pores of the studied species.

Species	E. fasciatus	E. grossipes	L. forficatus	L. mutabilis	L. muticus	L. nodulipes	L. pelidnus
Diameter pit (μm)	12.5 - 15	10 - 13	8 - 10	5 – 8.5	7 - 10	6-10	7-11

c. Small epidermal glands: small round pore openings in the cuticle irregularly distributed over all articles of the legs (Fig. 4B).

d. Small clustered pores: 2-13 simple small round openings in the cuticle with a diameter of approximately 0.7 μm, arranged in clusters of approximately 8 μm width (Fig. 4D-F). These pores were observed in males of *E. fasciatus*, *E. cavernicolus*, *L. nodulipes*, *L. mutabilis*, *L. muticus* and *L. pelidnus* on their secondary sexual structures.
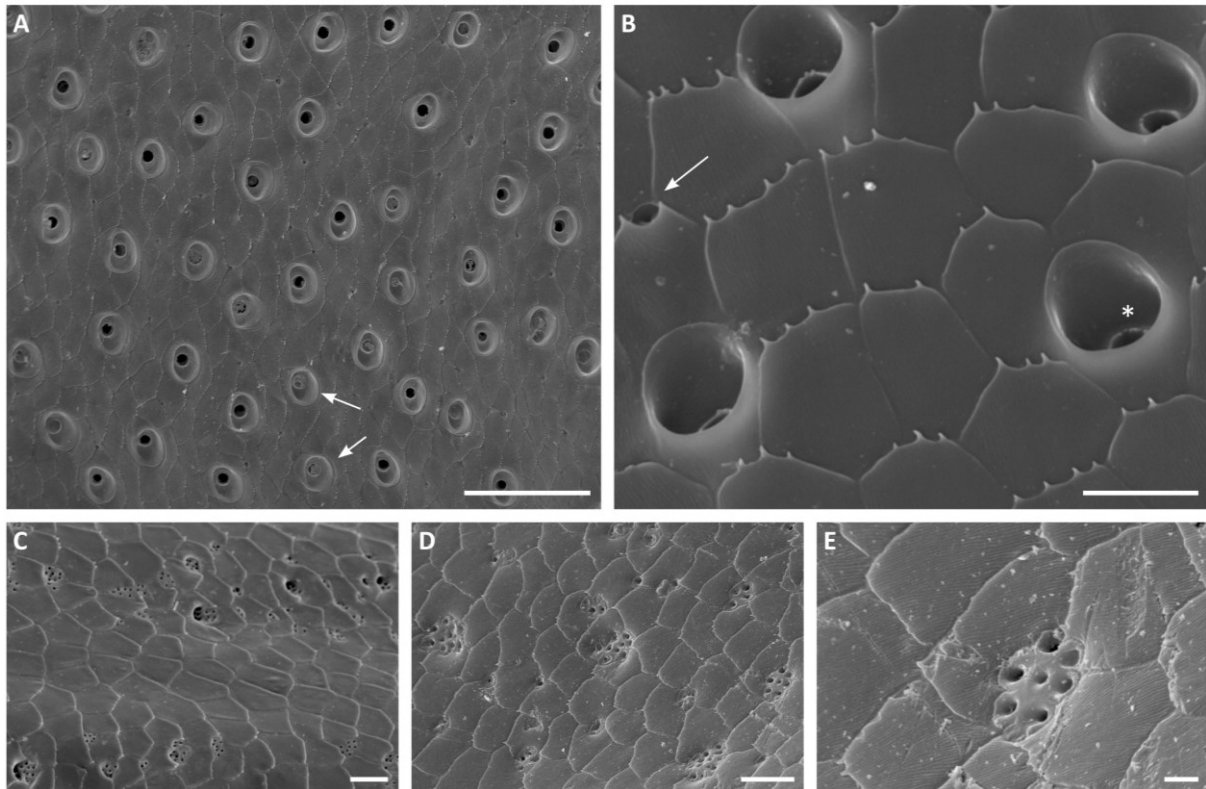
Figure 4: Different types of pores, SEM. **A** Telopodal pores on the 15th prefemur of female *E. fasciatus*, arrows pointing to pores that seem to be closed by a lid-like structure. **B-C** *L. pelidnus*, male. **B** Telopodal pores on 15th tarsus, asterisk marks pore with a fringed margin of the circular opening and white arrow points to a small epidermal glandular pore. **C** clustered pores on 15th femur, arranged in two parallel lines. **D-E** Clustered pores on 15th femur of male *E. fasciatus*. **D** Many clusters. **E** Close-up of one cluster. Scale bars: A: 50 μm, B-D: 10 μm, E: 2 μm

2.2.2. Distribution of pore types on the ultimate legs

a. The coxal pores are found on the coxa of the 12th – 15th leg-pair of each species (fig. 1B). The arrangement of coxal pores varies between the two genera *Eupolybothrus* and *Lithobius* and number and shape varies among species (Fig. 1C-E). Within species, the number of pores sometimes varies slightly between individuals, however, no sexual dimorphism was observed in any species regarding the coxal pores.

b. The telopodal pores are regularly distributed all over the legs in *E. fasciatus* and *E. grossipes*, while in all examined *Lithobius* species they show typical distributions on the different articles: the ventral sides of the ultimate legs are equipped with numerous pores, while dorsal, medial and lateral sides show fewer glandular openings (Fig. 2A-C). Pore density increases in the distal articles, in which they frequently cover the medial and ventral area (Fig. 2C). Coxa and trochanter present a few scattered telopodal pores, but no big differences were detected between species or sexes. In both sexes the prefemur is irregularly covered with pores, with highest density occurring either dorsally or ventrally depending on the species. On femur, tibia and tarsus pore density increases especially on the medial and ventral areas in both sexes of all species. On the femur it varies among species, whether dorsal and lateral pores are distributed regularly or irregularly. In all investigated species, tibia and tarsus have the highest density of pores. In general, the telopodal pores increase in density from proximal to distal articles in all species. However, their distribution and density vary among species, but not between the sexes.

c. The small epidermal glands are very irregularly and loosely distributed over all articles of the legs and no sexual dimorphism was observed.

d. The clustered pores are only present on male secondary sexual structures of *E. fasciatus*, *E. cavernicolus*, *L. nodulipes*, *L. mutabilis*, *L. muticus* and *L. pelidnus* (Fig. 7-12).

Table 4: Different sensilla types and distance between sensilla (in µm) on different articles of the studied species. **Abbreviations:** micro = sensilla microtrichoidea; meso = sensilla mesotrichoidea; macro = sensilla macrotrichoidea; •• = mainly, • = some, o = absent

Sensilla type		Article	Prefemur				Femur				Tibia				Tarsus			
			micro	meso	macro	distance (µm)	micro	meso	macro	distance (µm)	micro	meso	macro	distance (µm)	micro	meso	macro	distance (µm)
Eupolybothrus fasciatus	m	15th leg	•	••	○	100-200	•	••	○	100-200	○	••	•	<150	poor data			
Eupolybothrus fasciatus	f	15th leg	•	••	○	>200	•	••	○	100-300	○	••	•	<150	○	•	••	100
Lithobius forficatus	m	15th leg	•	••	○	>100	○	•	•	50-100	○	•	•	50-100	○	•	•	50-100
Lithobius forficatus	f	15th leg	•	••	○	>100	○	•	•	50-200	○	•	•	50-100	○	•	•	50-100
Lithobius nodulipes	m	15th leg	••	••	○	>50	○	•	•	25-150	○	•	••	<50	○	•	•	50-150
Lithobius nodulipes	f	15th leg	○	•	••	>100	○	•	•	50-100	○	•	••	<100	○	○	•	50-150
Lithobius muticus	m	14th leg	○	••	○	50-115	○	••	○	30-140	○	••	••	40-160	○	•	••	75-125
Lithobius muticus	m	15th leg	○	•	○	50-100	○	••	○	50-110	○	••	•	45-60	○	••	••	65-108
Lithobius muticus	f	14th leg	•	•	••	50-120	•	••	•	45-120	○	••	••	70-100	○	•	••	60
Lithobius muticus	f	15th leg	•	••	•	50-100	○	••	••	50-100	○	••	••	60-100	○	•	••	35-70
Lithobius mutabilis	m	14th leg	•	••	○	50-100	○	••	••	50-100	○	••	••	40-100	○	••	•	50-100
Lithobius mutabilis	m	15th leg	○	•	○	50-80	○	•	○	60-100	•	••	••	20-75	○	••	••	50-100
Lithobius mutabilis	f	14th leg	•	•	•	100	○	•	•	100	○	•	••	50-100	○	•	••	65-85
Lithobius mutabilis	f	15th leg	○	••	••	50	○	••	•	50-100	○	○	••	50-100	○	○	•	50-100
Lithobius pelidnus	m	14th leg	••	••	○	30-125	○	••	o	40-130	○	○	•	75-200	○	•	○	75
Lithobius pelidnus	m	15th leg	○	••	○	20-75	○	••	o	50-100	○	•	○	60-100	poor data			
Lithobius pelidnus	f	14th leg	•	••	••	90-120	○	••	•	25-100	○	••	•	60-100	○	••	••	80-130
Lithobius pelidnus	f	15th leg	○	••	••	35-75	○	••	••	65-135	○	••	••	50-100	○	••	••	100

3. Male cuticular structures on the ultimate legs

The following structures, which are cuticular modifications of the ultimate legs, were found only in males of the examined lithobiid species:

a. Projections: distinct extension of the cuticle, mostly roundish to oval-shaped, of different sizes; include for example knobs, protuberances, nodules

b. Swellings and extreme thickenings: prominent enlargements of certain parts of articles or whole articles, different from the "regular" thickenings of the entire ultimate legs, which occur in both sexes

c. Grooves: prominent wide indentations in the cuticle, different from the "regular" sulci, which appear much thinner and occur in both sexes

d. Pits: small deep indentations in the cuticle, mostly roundish

These cuticular modifications showed a considerable diversity across species regarding position, shape and size (Fig. 5). They appear on prefemora, femora and tibiae on the 14th, 15th or both 14th and 15th leg-pair. In the following part, the microstructural morphology of these male secondary sexual characters is described (see also Table 6).

Table 6: Comparison of male secondary sexual characters on the ultimate legs of the studied species. For more detailed information see descriptions below.

| Species	Leg-pair	Article	Cuticular structure(s)
E. cavernicolus	15th	prefemur	proximal knob with cluster of sensilla; dorsomedial ridge; distal protuberance with dense aggregation of sensilla
E. fasciatus	15th	femur	proximal basal pit with clustered pores; proximal cluster of sensilla; distal swelling with clustered pores
E. grossipes	15th	femur	proximal basal pit
E. liburnicus	15th	prefemur	proximal knob with cluster of sensilla; dorsomedial ridge; distal protuberance with dense aggregation of sensilla
L. forficatus	-	-	-
L. mutabilis	14th	tibia	dorsal groove with clustered pores; protuberance with sensilla
	15th	tibia	dorsal groove with clustered pores; slight rising with sensilla
L. muticus	14th	tibia	distal protuberance covered with sensilla and clustered pores
L. nodulipes	15th	tibia	distal nodule covered with sensilla and clustered pores
L. pelidnus	14th	prefemur	distal swelling pointing mediad; sulci with clustered pores
	14th	femur	dorsal sulci with clustered pores
	14th	tibia	dorsal sulci with clustered pores
	15th	prefemur	distal swelling pointing mediad; clustered pores
	15th	femur	dorsal cluster of sensilla encircled by clustered pores
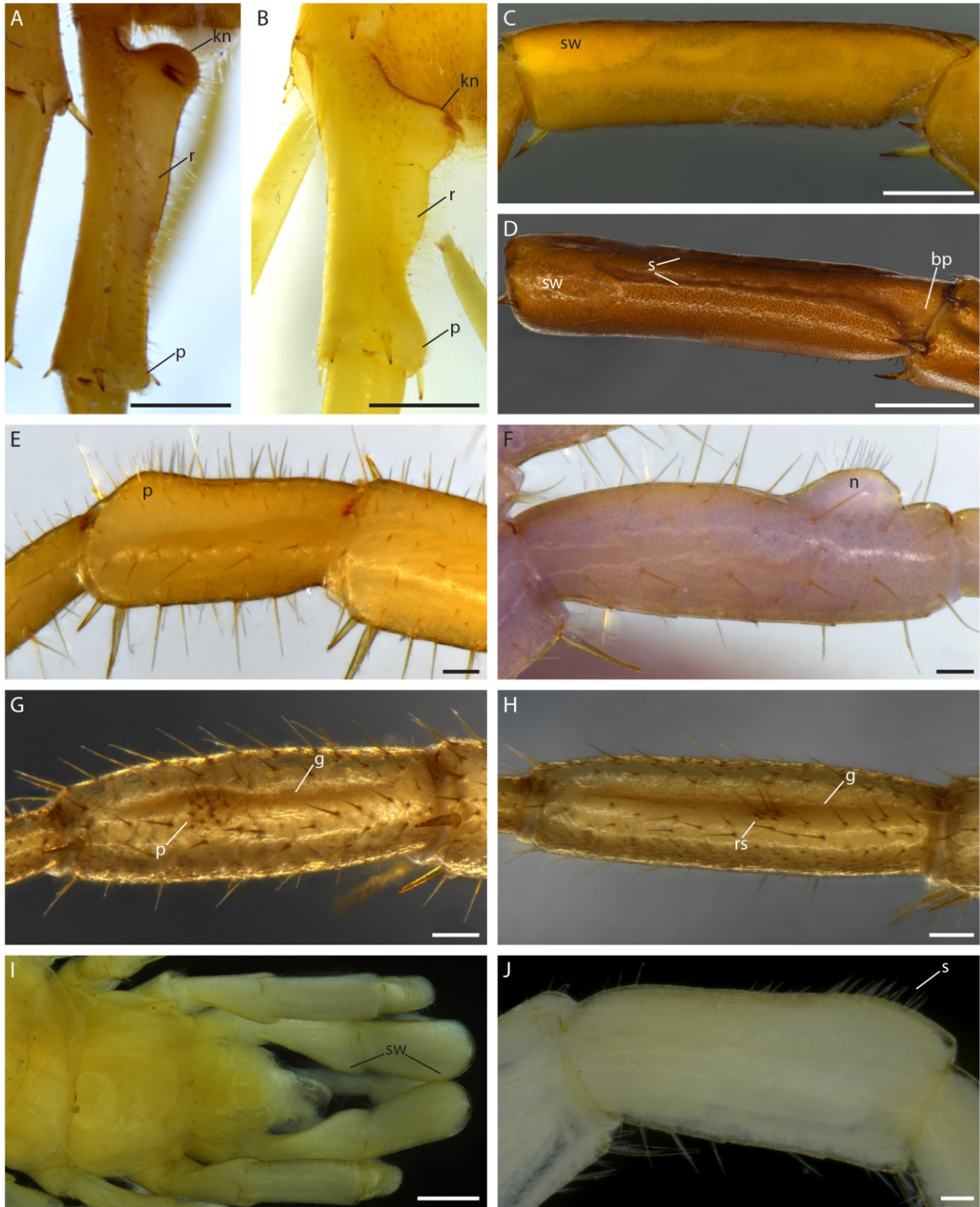
Figure 5: Male cuticular structures on the ultimate legs, light photographs. **A** 15th prefemur of *E. cavernicolus* with proximal round knob (kn) protruding mediad and bearing long sensilla, a dorsal ridge (r) and a distal circular protuberance (p). **B** 15th prefemur of *E. liburnicus* with proximal roundish-triangular knob (kn) protruding mediad and bearing long sensilla, a dorsal ridge (r) and a distal circular protuberance (p). **C-D** 15th femur of *E. fasciatus*: **C** Mesal view; distal globular swelling (sw) showing a slight brighter colour. **D** Dorsomedial view; proximal basal pit (bp), dorsal two sulci (s) and distal globular swelling (sw). **E** 14th tibia of *L. muticus*, dorsal with a distal protuberance (p), covered with sensilla. **F** 15th tibia of *L. nodulipes*, with a distal nodule (n) covered with sensilla. **G-H** *L. mutabilis*: **G** 14th tibia with dorsal groove (g) and small protuberance (p) that bears sensilla. **H** 15th tibia with dorsal groove (g) and a slight rising (rs) with a cluster of sensilla. **I-J** *L. pelidnus*: **I** Posterior end; 14th and 15th prefemora and femora strongly swollen (sw). **J** 15th femur, with a dense cluster of sensilla (s) distally. Scale bars: A-D: 1 mm, I: 0.5 mm, E- H, J: 0.1 mm. **Abbreviations:** basal pit (bp), groove (g), knob (kn), nodule (n), protuberance (p), ridge (r), rising (rs), sensilla (s), swellings (sw)
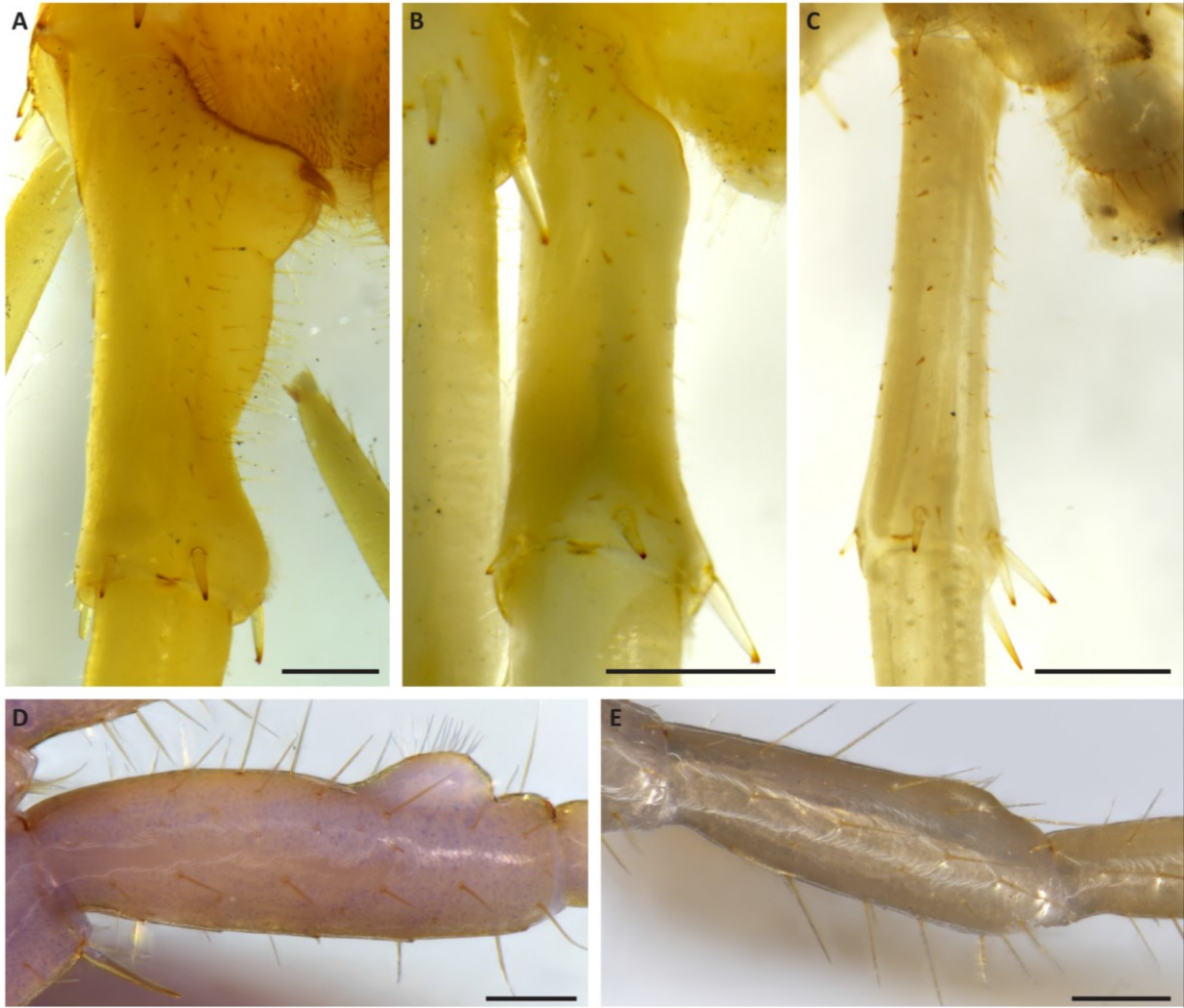
Figure 6: Development of secondary sexual modifications on ultimate legs of *E. liburnicus* and *L. nodulipes*, light photographs. **A-C** *E. liburnicus*: **A** Adult male, prefemoral knob fully developed. **B** Subadult male, prefemoral knob not fully developed. **C** Early subadult male, prefemoral knob not developed. **D-E** *L. nodulipes*: **D** Adult male, nodule fully developed. **E** Subadult male, nodule not fully developed. Scale bars: A-C: 0.5 mm, D-E: 0.2 mm.

3.1. *Eupolybothrus cavernicolus* and *Eupolybothrus liburnicus*

The most prominent cuticular structures in these two species are the prefemoral knobs on the 15th leg-pair (Figs. 5A, B, 7). Both species show thickened 15th prefemora, *Eupolybothrus liburnicus* even more than *E. cavernicolus* (compare Fig. 5A to 5B). Proximally, these knobs protrude mediad as round to triangular projections of the prefemora. In *E. cavernicolus*, the knob is round with a diameter of about 0.7 mm (Figs. 5A, 7A). In *E. liburnicus*, the knob is located proximal at the broadest part of the thickened and broadened prefemur (Figs. 5B, 7B). It is rather triangular, with a length of about 0.6 mm. The prefemoral knobs bear sensory structures in both species. The dorsal side of the knob bears a cluster of sensilla macrotrichoidea of ca. 170-250 µm length, with a slightly elevated crescent-shaped pedestal, a striated shaft and pointed tips (Fig. 7A-B). A terminal pore was not found. The inner and ventral side of the knob is densely covered with sensilla mesotrichoidea and macrotrichoidea (Fig. A-B).

The prefemoral knob of *E. cavernicolus* is covered with clustered pores (Fig. 7A). In *E. liburnicus* it was unclear, due to poor image data, whether clustered pores were present or not. In *E. liburnicus*, specimens of different developmental stages were studied (see also Akkari et al. 2017), showing a gradual growth of the knob with increasing age and body size. In early stages, the knobs are small round bulges, becoming larger and more triangular in older stages (Fig. 6A-C). Only the knobs of fully-grown individuals bear sensilla. Except for these prominent proximal knobs, both species show a dorsomedial ridge (Fig. 5A-B) and a small protuberance between the distal spines (Figs. 5A-B, 7C-D). In *E. cavernicolus* this protuberance is more distinct (compare Fig. 7C to Fig. 7D). The protuberance bears a dense aggregation of sensilla mesotrichoidea of approximately 75 µm in length that have an elevated, crescent-shaped pedestal and a striated shaft. No terminal pore was detected.
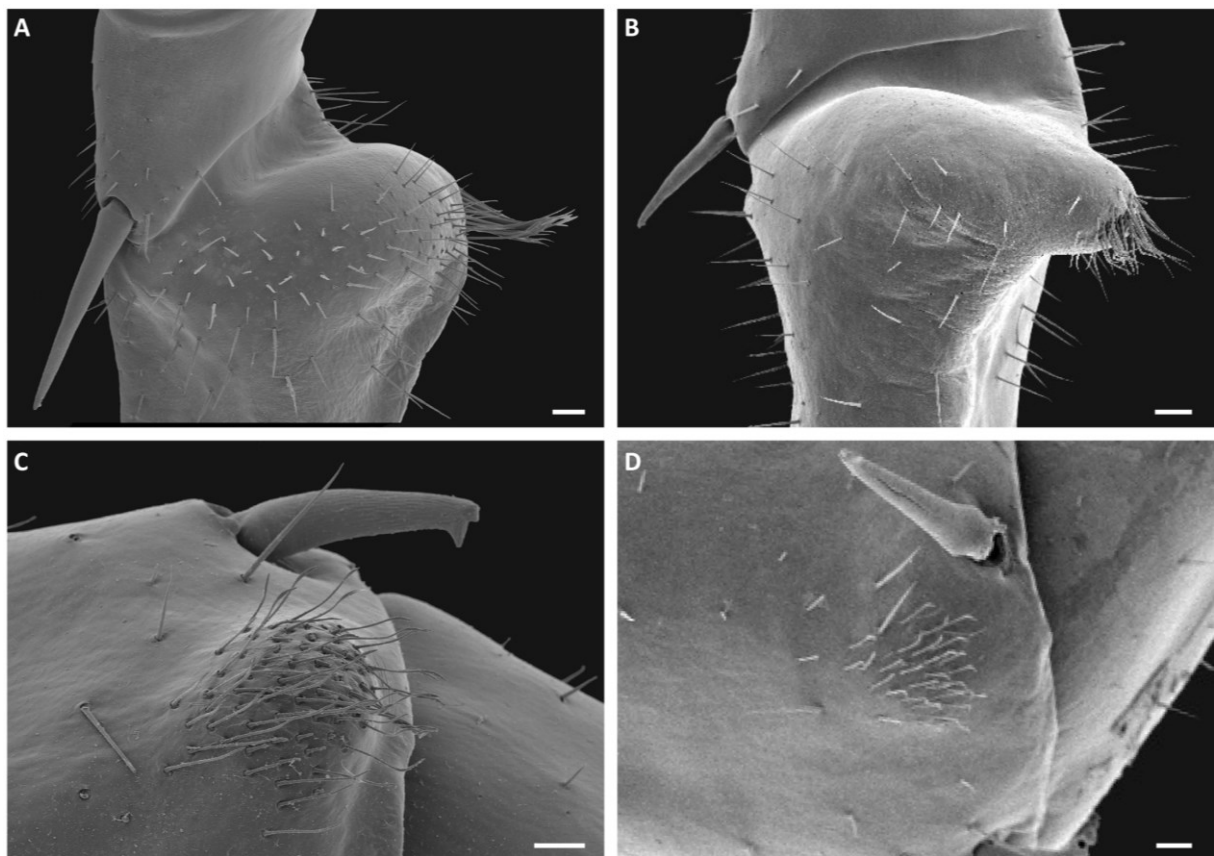

Figure 7: Modifications of the 15th prefemur of male *E. cavernicolus* and *E. liburnicus*, SEM. **A-B** Proximal prefemoral knob: **A** *E. cavernicolus*. **B** *E. liburnicus*. **C-D** Distal prefemoral circular protuberance **C** *E. cavernicolus*. **D** *E. liburnicus*. Scale bars: A-B: 100 µm, C-D: 50 µm. **N. Akkari courtesy**

3.2. *Eupolybothrus fasciatus* and *Eupolybothrus grossipes*

Both species show a proximal dorsal pit on their femora of the last leg-pair (referred to as basal pit; Figs. 5C-D, 8A-C). The femora also bear two dorsal sulci, the medially positioned sulci emerge from the basal pit (Figs. 5D, 8A). In *Eupolybothrus grossipes* no further modifications are present. However, *E. fasciatus* shows additional modifications of the 15th femora adjacent to the basal pit and the dorsal sulci. Clustered pores appear distally to the pit and extend in a broad band along the dorsal sulci, tapering towards the end of the proximal quarter or third of the femur (Fig. 8C, E). The clustered pores could not be observed in *E. grossipes* due to poor image data. Furthermore, *E. fasciatus* presents a cluster of long sensilla trichoidea that is located proxomedially on the 15th femora, next to the basal pit (Fig. 8C-D). These sensilla are slender and may be up to 320 µm long. They are filiform with a slightly striated shaft, a deeply embedded crescent-shaped pedestal and a terminal pore (Fig. 8C-D). Broken sensilla showed that they are hollow inside. The number of sensilla varies between 7 and 15 among individuals. Furthermore, the 15th femora of *E. fasciatus* shows a large, round distomedial swelling. The size of the swelling differed across the investigated specimens. It bears a few sensilla and it is densely covered with clustered pores (Fig. 8B, F). The margin of the swelling is free from pores and sensilla. Single scattered telopodal pores are located around the swelling, but on the swelling telopodal pores are absent, resulting in a much brighter colour of the swelling compared to the rest of the femur (Figs. 5C, 8B).
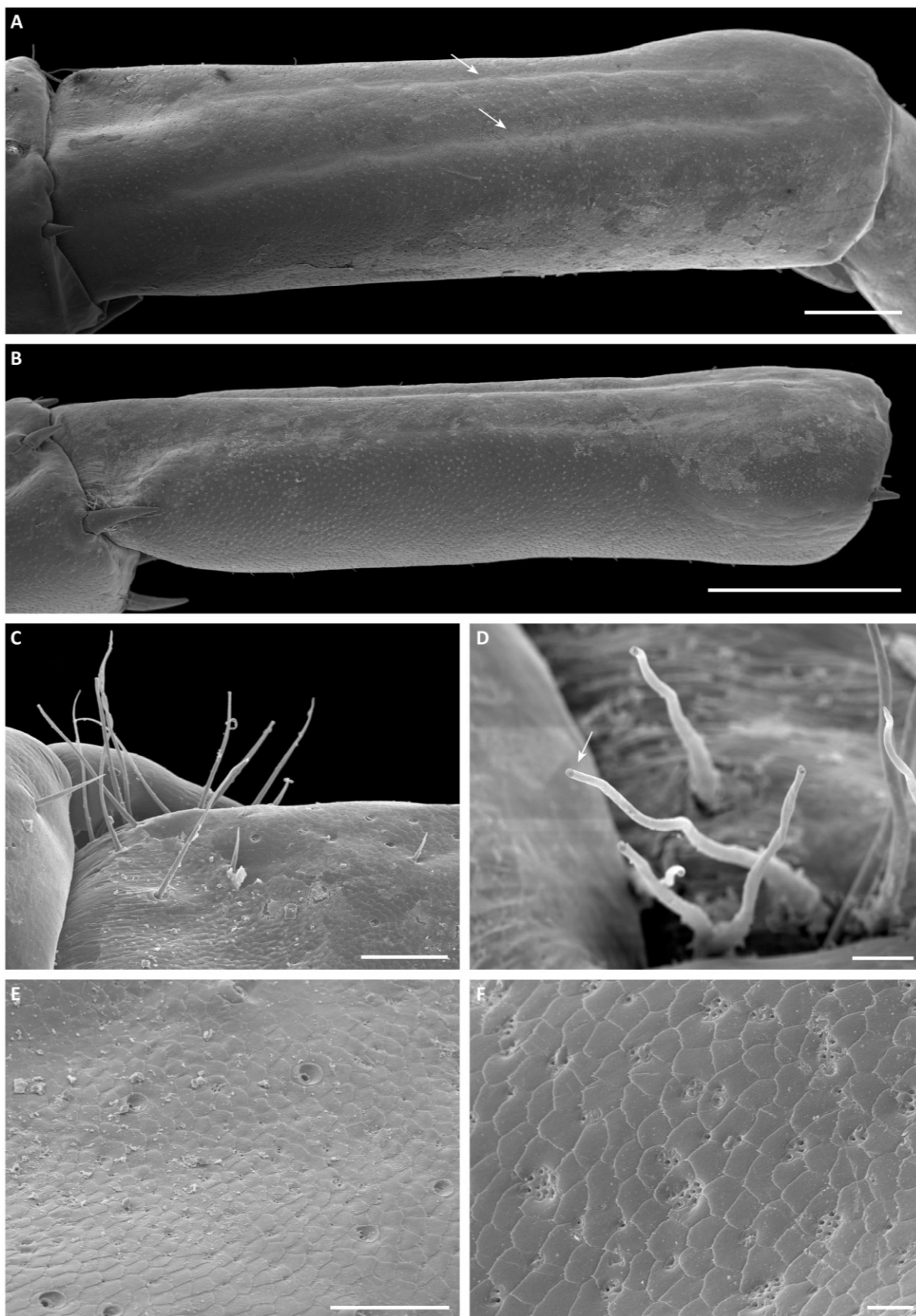
Figure 8: Modifications of the 15th femur of male *E. fasciatus*, SEM. **A** Dorsolateral view, arrows pointing to the dorsal sulci. **B** Mesal view. **C** Basal pit with cluster of sensilla. **D** Cluster of sensilla, arrow pointing to terminal pore of a sensilla. **E** Transition surface between the basal pit and the rest of the femur. **F** clustered pores on the distomedial swelling. Scale bars: A-B: 1 mm, C: 100 μm, D, F: 20 μm, E: 50 μm.

3.3. *Lithobius nodulipes*

As emphasized by its name, the cuticular modification of this species is a lobe-like projection, referred to as nodule, which is located dorsally on the distal end of the 15th tibiae (Figs. 5F, 9A). The nodule is oval-shaped and has a length of 0.15-0.2 mm, a width of 0.12-0.14 mm and a height of about 0.1 mm (Fig. 9A-B). The dorsal side of the nodule is covered with sensilla mesotrichoidea (with a length of about 50 µm). In some specimens, sensilla microtrichoidea were also found (Fig. 9B-C). The sensilla bear a terminal pore. The dorsal side of the nodule is furthermore covered with clustered pores (Fig. 9B-C). The margin and sides of the nodule are free of pores or sensilla. Different developmental stages were studied and showed that the nodule grows with age and increasing body size, since subadult stages showed only a slightly developed nodule compared to adults (Fig. 6D-E).
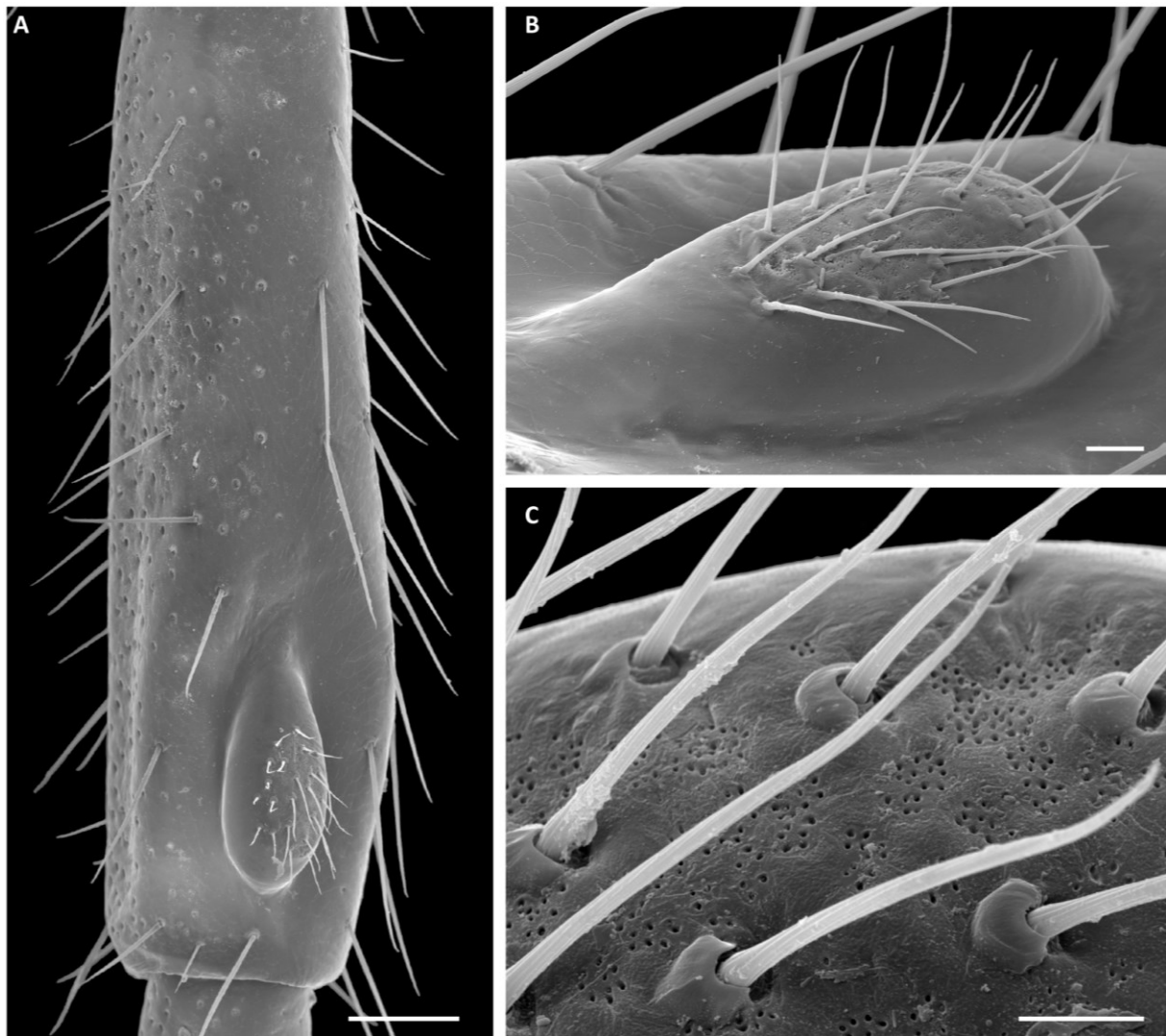

Figure 9: Modifications of the 15th tibia of male *L. nodulipes*, SEM. **A** Tibia with distal nodule. **B** Nodule. **C** Sensilla and clustered pores located on the nodule. Scale bars: A: 100 µm, B: 20 µm, C: 10 µm.

3.4. *Lithobius muticus*

This species has a cuticular modification on the 14th tibiae, expressed by a dorsal protuberance of approximately 0.2 mm length that points slightly mediad (Figs. 5F, 10A). The protuberance is densely covered (distances of about 25 µm) with 50-80 µm long sensilla mesotrichoidea with strongly elevated, crescent-shaped sockets (Fig. 10B-D). Terminal pores of the sensilla could not be observed. Clustered pores are located around the protuberance as well as on it, between the sensilla (Fig. 10B-D).

3.5. *Lithobius mutabilis*

Modifications occur on both the 14th and the 15th pair of legs (Figs. 5G-H, 11A, E). On the 14th tibiae, a dorsal groove extends almost over its entire length (Figs. 5G, 11A). In the groove clustered pores are arranged in a row (Fig. 11B). A small cuticular protuberance lies laterally to the groove, located at the distalmost third of the tibia (Fig. 11A, C). The protuberance bears a dense cluster of sensilla mesotrichoidea of approximately 50-80 µm length with thick, elevated, crescent-shaped pedestals (Fig. 11C-D). No terminal pores were observed. On the protuberance clustered pores appear as well. On the 15th tibiae a dorsal groove is present too (Figs. 5H, 11E). It is much wider and a bit shorter than that of the 14th leg (Fig. 11E) and bears clustered pores (Fig. 11F). At half length of the tibia the cuticle is slightly elevated medially, next to the groove, recognizable as a slight rising with 4-5 densely arranged 46-90 µm long sensilla mesotrichoidea (Fig. 11E, G). They show crescent-shaped pedestals without associated pores (Fig. 11G-H). No terminal pores were detected.
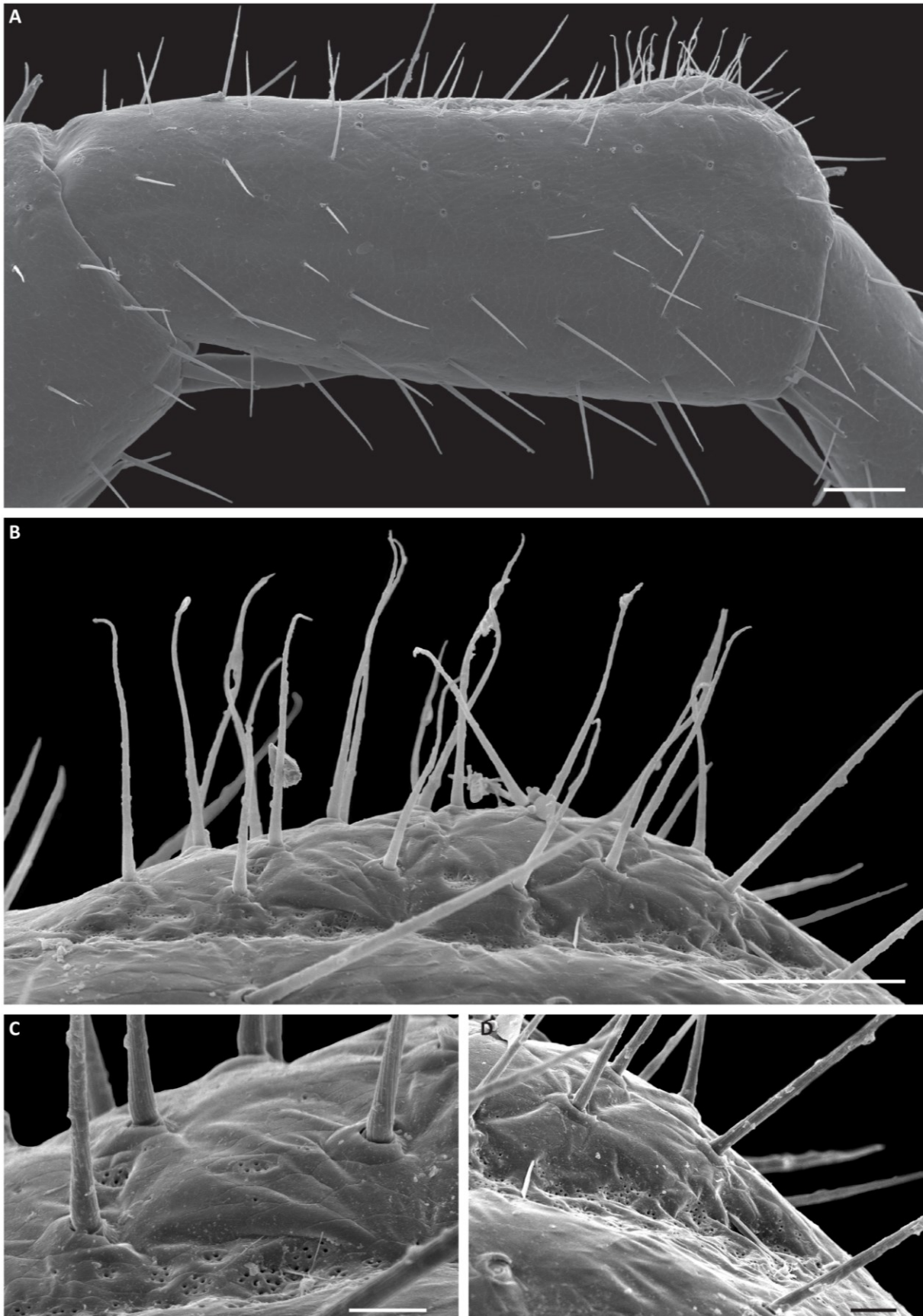
Figure 10: Modifications of the 14th tibia of male *L. muticus*, SEM. **A** Tibia, lateral view. **B** Distal protuberance with sensilla and pores. **C-D** Sensilla and pores on the protuberance and clustered pores that surround the protuberance. Scale bars: A: 100 µm, B: 50 µm, C-D: 10 µm.
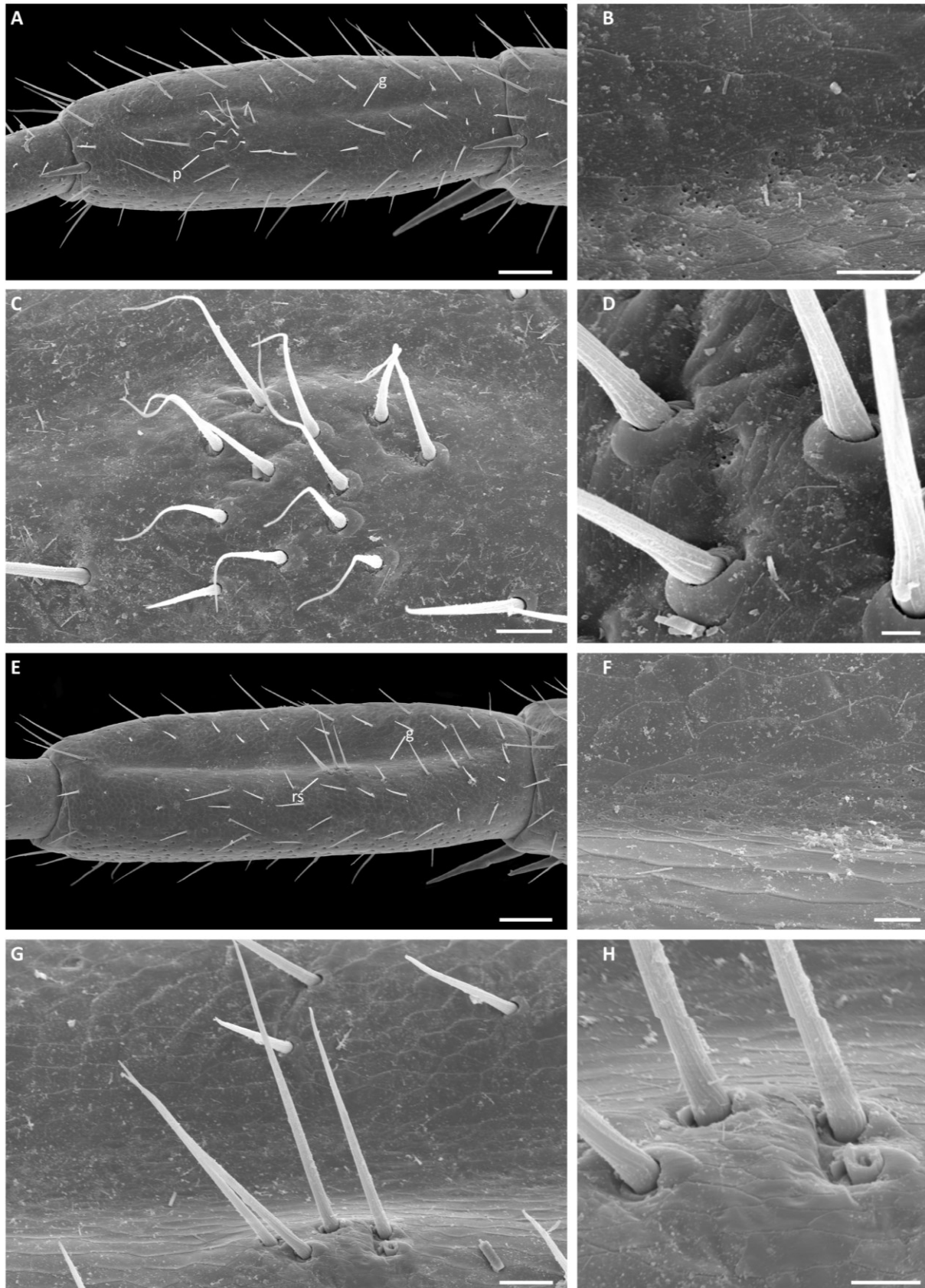
Figure 11: Modifications of the 14th and 15th tibiae of male *L. mutabilis*, SEM. **A-D** 14th tibia. **A** Dorsal view, dorsal groove (g), cuticular protuberance (p) with sensilla. **B** Clustered pores running along the dorsal groove. **C** Close-up of cuticular protuberance with sensilla. **D** Pedestals of sensilla of the cuticular protuberance. **E-H** 15th tibia. **E** Dorsal groove (g) and the slight rising of the cuticle (rs) with sensilla. **F** Band of clustered pores, running along the dorsal groove. **G** Aggregation of sensilla on slight cuticular rising. **H** Close-up showing pedestals of sensilla without associated pores. Scale bars: A, E: 100 µm, B, F, H: 10 µm, C, G: 20 µm, D: 5 µm. Abbreviations: groove (g), protuberance (p), rising of the cuticle (rs)

3.6. *Lithobius pelidnus*

Several cuticular modifications occur on the different podomeres of the 14th and 15th leg-pairs (Figs. 5I-J, 12). On the 14th legs, the prefemora, femora and tibiae each show two dorsal sulci covered with rows of clustered pores running along the whole length of these articles (Fig. A-D). On the prefemora the clusters are more condensed proximally, while distally they show increasing distances to the other clusters (Fig. 12B). Prefemora and femora are thickened, especially distally, and the prefemora show a small median swelling (Figs. 5I, 12A-C). On the 15th leg, modifications become more pronounced. Distally, the 15th prefemora show a median swelling that is larger than on the 14th leg, so that the distal end of the prefemur is double the width than the proximal part (Figs. 5I, 12E). Additionally, a longitudinal assemblage of clustered pores and single small epidermal pore openings are found dorsally. The 15th femora show a distal cluster of approximately 20 sensilla mesotrichoidea that are approximately 50-70 µm long (Figs. 5J, 12F-J). The sensilla have a slightly elevated, crescent-shaped pedestal and a terminal pore (Fig. 12G-H). Some sensilla show associated pores near the pedestals (Fig. 12H). Dorsally, the pores are arranged in two lines starting at the proximal end of the femora, where they appear as single pore openings, before aggregating distally to clusters. The two lines of pores unite distally and enclose the cluster of sensilla (Fig. 12G, I, J). Clustered pores appear between the sensilla of the distal sensilla-cluster as well (Fig. 12G-I). The 15th tibia shows dorsal sulci with clustered pores (Fig. 12K).

L. forficatus does not show any secondary sexual modifications of cuticular structures on the ultimate legs.
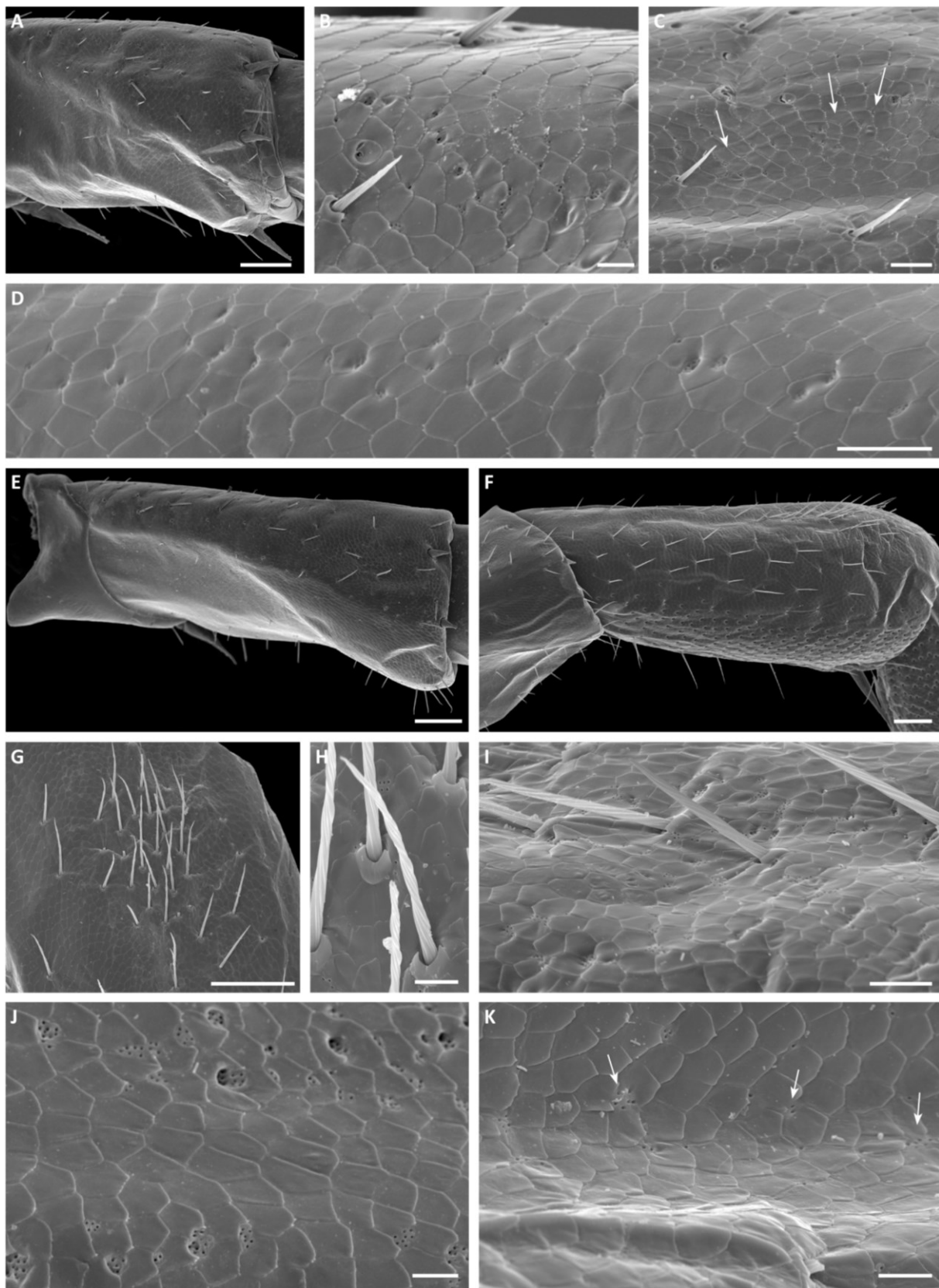
Figure 12: Modifications of the 14th and 15th leg of male *L. pelidnus*, SEM. **A-B** 14th prefemur: **A** Distal swelling of 14th prefemur (note that the cuticle shows an artefact due to collapsing). **B** Band of clustered pores on 14th prefemur. **C** Band of clustered pores (arrows) on the 14th femur. **D** Band of clustered pores on 14th tibia. E 15th prefemur with a distomedial swelling. **F-J** Modifications of the 15th femur. **F** 15th femur, mesal view. **G** Sensilla and clustered pores of the distal cluster of sensilla. **H** Close-up of the sensilla of the cluster. **I** Arrangement of clustered pores encircling the cluster of sensilla. **J** Two bands of clustered pores. **K** Groove on 15th tibia, arrows indicating clustered pores. Scale bar: A, E-G: 100 μm, C-D, I: 20 μm, B, H, J-K: 10 μm.

4. internal anatomy of the cuticular nodule of *Lithobius nodulipes*

The tibial nodule of *L. nodulipes* is neither solid nor hollow but filled with tissue (Fig. 13). The multi-layered cuticle surrounding the nodule and its internal structures shows a constant thickness of about 9-10 μm (Fig. 13-16). The endocuticle (with visible striation), the exocuticle (dense) and the epicuticle (very dense thin layer) were also identified (Fig. 14B). Channel-like structures perforate the cuticle in various parts (Fig. 14A, C, D). Inside the leg, a thin membrane (basement membrane) encircles a cavity, where muscles, vessels and haemolymph are present (Fig. 14A, C, D). Between basement membrane and cuticle, epidermal cells and glandular tissue are found (Fig. 14A, C, D). In some sections, nervous tissue is identifiable. On the ventral side of the tibia, the area between basement membrane and cuticle is filled with large cells of about 30-40 μm size, which are possibly telopodal gland cells (Fig. 14A-C). On the dorsal side, males and females show a great difference: while females have a thin lining of epidermal and glandular cells (Fig. 14A, D), males have a significantly larger glandular system occupying the dorsal side of the two distal thirds of the tibia (Figs. 14C, 15, 16). The glandular system comprises two large lobes on both sides of the tibia, which are interconnected dorsally (Fig. 15C-H). This glandular system is composed of several secretory cells filled with vesicles and epidermal cells located between the secretory cells (Fig. 15A-B). Additionally, fibre-like structures that may belong to cellular structures or to muscles are located in this region (Fig. 15B).
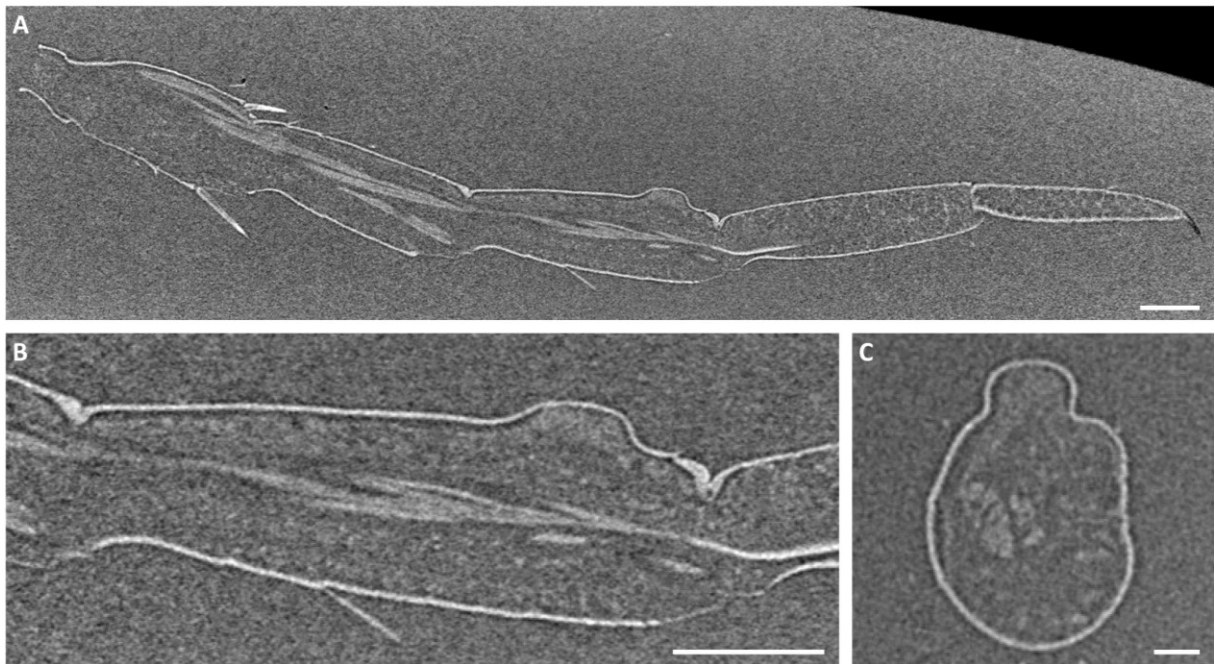

Figure 13: μ-CT scans of the 15th leg of male *L. nodulipes*. **A** Sagittal section of 15th leg. **B** Sagittal section of 15th tibia. **C** Cross section of 15th tibia. Scale bars: A, B: 200 μm, C: 50 μm.
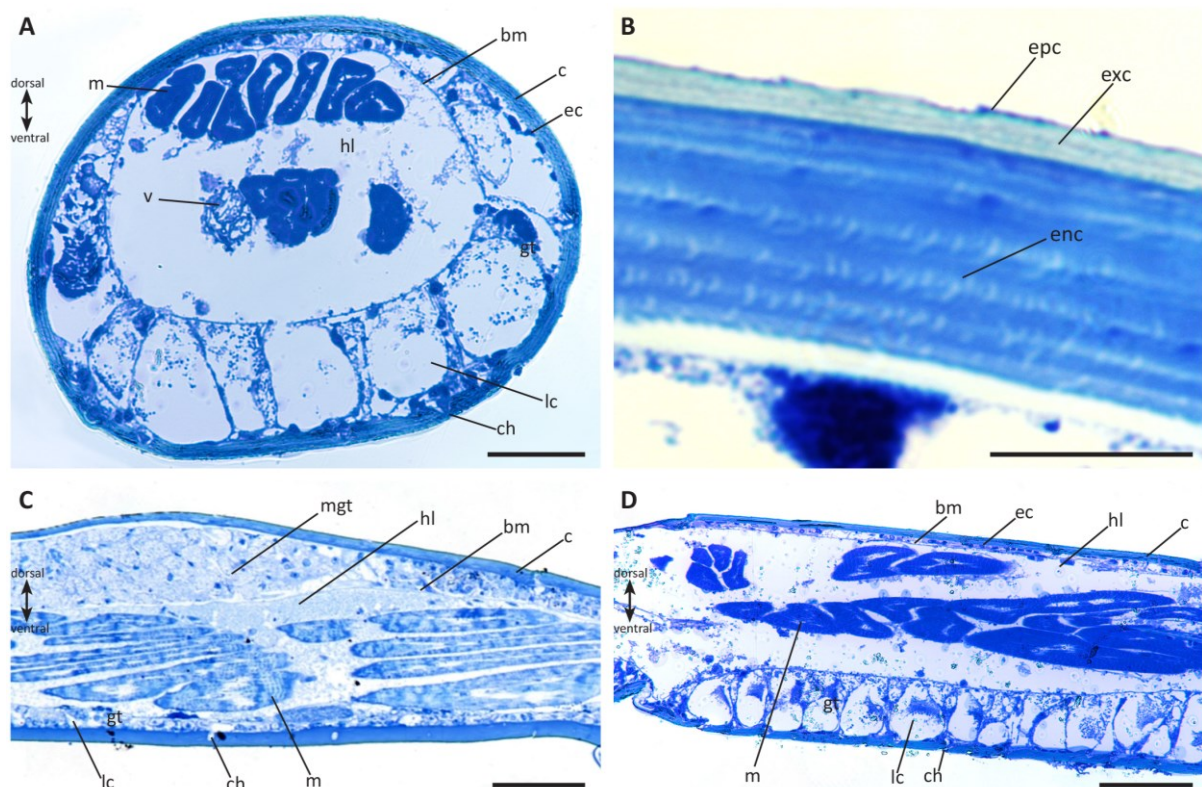
Figure 14: Histological cross and sagittal sections of 15th tibia of *L. nodulipes*, light photographs. **A** Female, cross section. **B** Male, cross section showing detail of cuticle with striated endocuticle, dense exocuticle and thin epicuticle. **C** Male, sagittal section. **D** Female, sagittal section. Scale bars: A 50 µm, B: 10 µm, C-D: 100 µm. **Abbreviations:** basement membrane (bm), cuticle (c), channel-like structures that perforate the cuticle (ch), epidermal cells (ec), endocuticle (enc), epicuticle (epc), exocuticle (exc), glandular tissue (gt), haemolymph (hl), large empty cells, probably of glandular tissue (lc), male glandular tissue (mgt), muscles (m), vessels (v)

At the margin of the nodule numerous epidermal cells are located close to the cuticle (Figs. 15C-H, 16A-B). They have a thick, prominently stained membrane and sometimes a granulated appearance. The epidermal cells are also visible inside the nodule, positioned mostly near the cuticle but also in the centre of the nodule (Figs. 15E-H, 16A-C). The male glandular system protrudes into the nodule (Figs. 15E-H, 16A-B). Inside the nodule, fibrous strands lead from both sides of the tibia to the glandular structures (Figs. 15F-H, 16A-B). The strands become more prominent in the mid-sections of the nodule, where they often seem to be organised into two symmetrical strands (Fig. 15G-H). In the sagittal sections, many of these strands are visible and reveal that the glandular system contributes to the nodule along its entire anterior-posterior-axis (Fig. 16A-B). At the dorsal surface of the nodule, there is a dense condensation of fine fibril-like structures that diverge distally into numerous narrow openings that penetrate the cuticle (Figs. 15H, 16B-C). The openings are clustered in bundles of three to four (Fig. 16C).
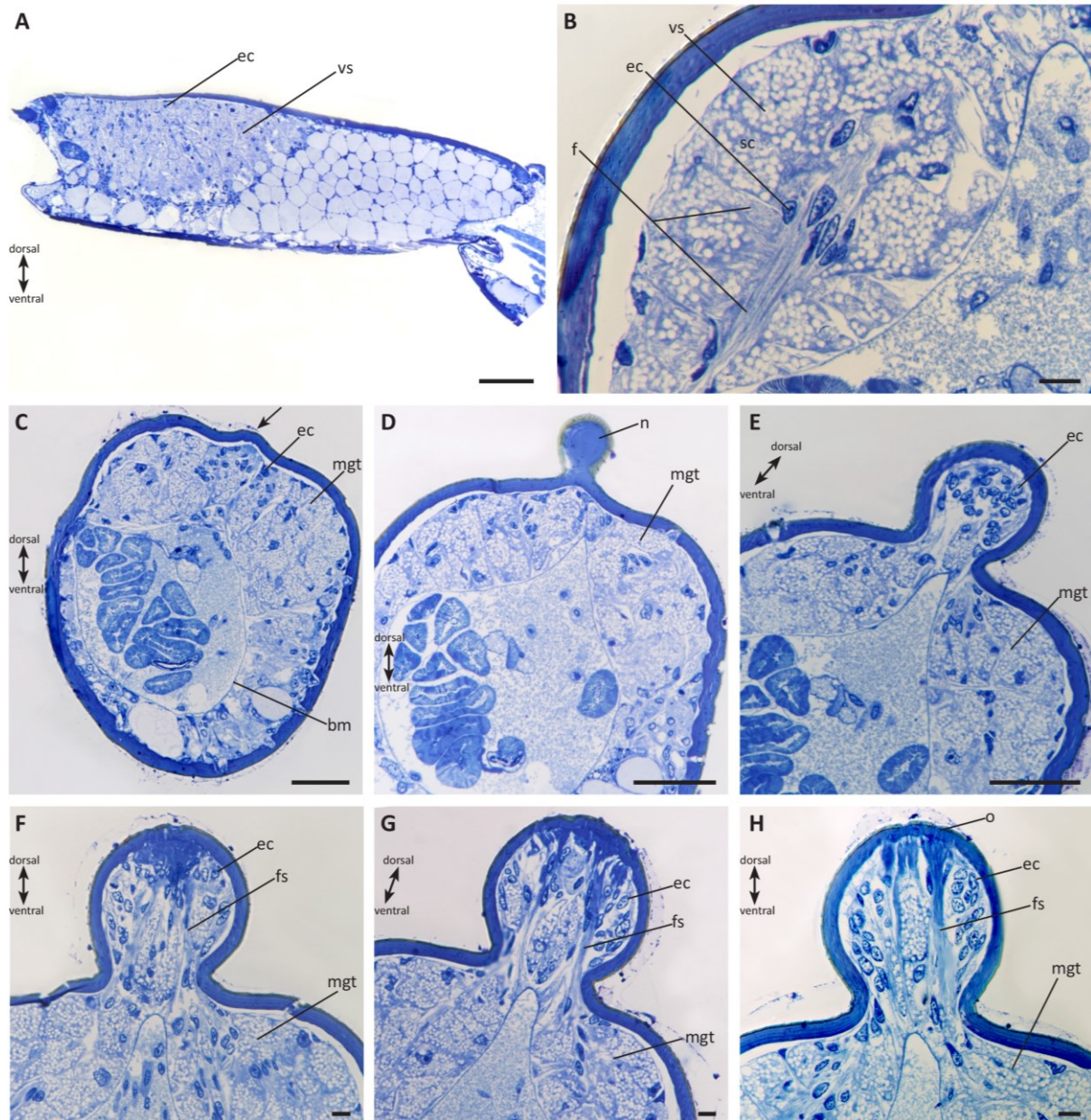
Figure 15: Histological cross and sagittal sections of 15th tibia of male *L. nodulipes*, light photographs. **A** Sagittal section, showing male glandular tissue (mgt) occupying most space of the distal part of the tibia. **B** Cross section, detail of male glandular tissue with epidermal cells (ec), secretory cells (sc) filled with vesicles (vs), and fibre-like structures (f). **C-H** Different cross sections of tibia along the anterior-posterior axis (nodule dorsal), showing male glandular system with two large lobes on the lateral and medial sides of the tibia, connected dorsally: **C** Out-folding of the cuticle (black arrow) contributing to the margin of the nodule. **D** Margin of nodule (n) is visible. **E** Nodule with several epidermal cells (ec) located mainly at the margin near the cuticle. **F-H** Different cross sections through the mid-part of the nodule, epidermal cells (ec) located at the margin of the nodule near the cuticle, fibrous strands (fs) of the glandular system changing their appearance: **F** Many fibrous strands of the male glandular tissue condensing dorsally. **G** Fibrous strands of the male glandular tissue forming two symmetrical bands inside the nodule that condense dorsally. **H** Higher condensation of symmetrical fibrous strands of the male glandular tissue, distally diverging into many narrow openings (o) that lead through the cuticle. Scale bars: A: 100 µm, C-E: 50 µm, B, F-H: 10 µm. **Abbreviations:** basement membrane (bm), epidermal cells (ec), fibre-like structure of male glandular tissue (f), fibrous strands in the nodule (fs), male glandular tissue (mgt), nodule (n), openings of male glandular tissue that lead through the cuticle (o), secretory cells (sc), vesicles (vs)
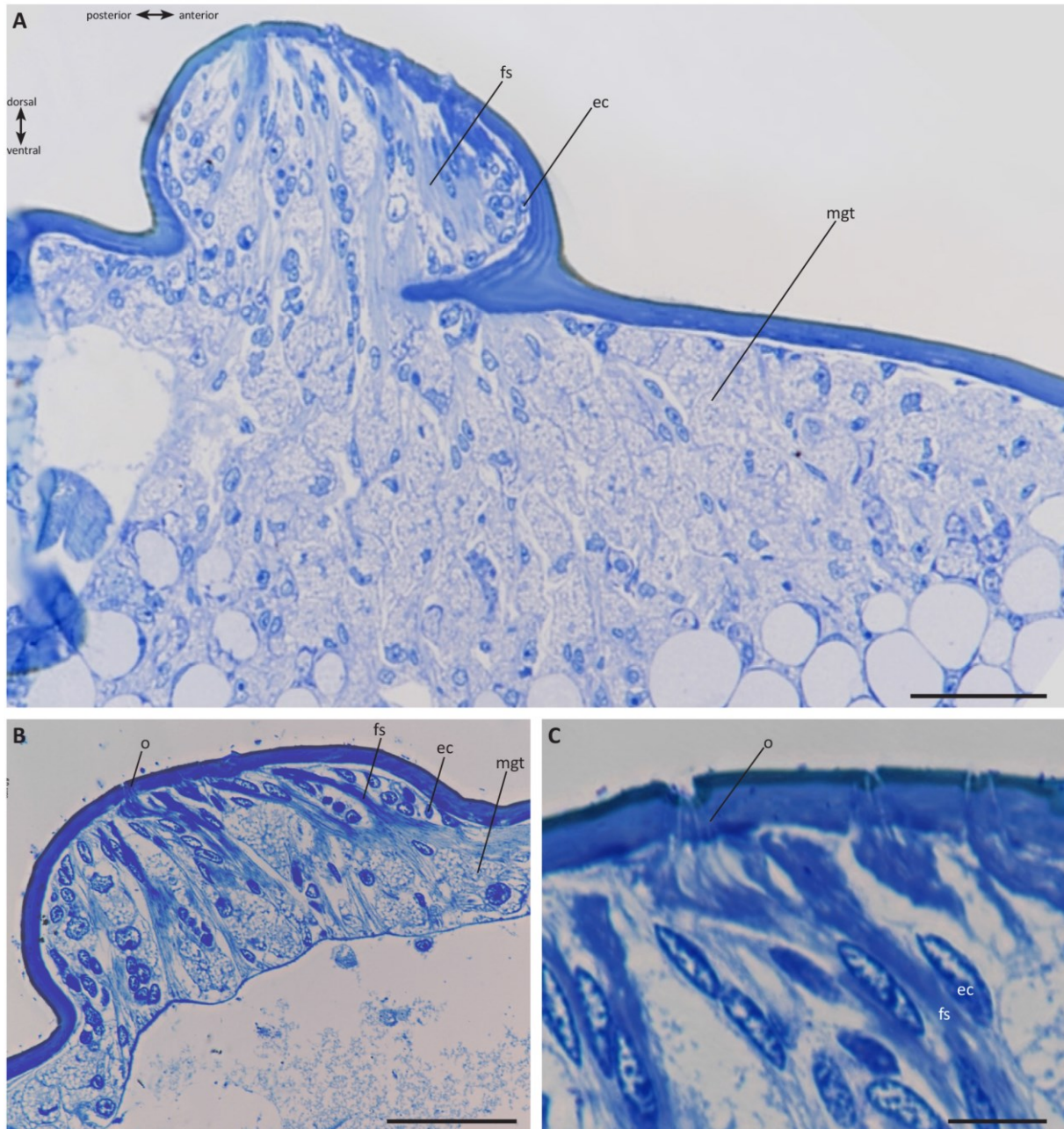
Figure 16: Histological sagittal sections through the distal end of the 15th tibia and the nodule of male *L. nodulipes*, light photo-graphs. **A-B** Sagittal sections of distal end of the tibia and nodule showing epidermal cells (ec) located at the margin of the nodule near the cuticle, many fibrous (fs) strands of the male glandular tissue (mgt) leading inside the nodule and condensate dorsally: **A** Male glandular tissue in the tibia consuming most space of the distal part of the tibia and protruding into the nodule. **B** Many fibrous strands of the male glandular tissue leading into the nodule, condensing dorsally, then diverging again distally into many narrow openings through the cuticle. **C** Detail of the openings (o) through the nodule's cuticle. Scale bars: A-B: 50 µm, C: 10 µm. **Abbreviations:** epidermal cells (ec), fibrous strands in the nodule (fs), male glandular tissue (mgt), openings of male glandular tissue that lead through the cuticle (o)

Discussion

1. Sexual dimorphism in lithobiid ultimate leg structures

1. 1. Sensory structures

The ultimate legs of centipedes are rarely used for locomotion but *inter alia* as sensory and defense structures or for warning and courtship behaviour (Klingel 1959, Kronmüller and Lewis 2015, Kenning et al. 2017). The observed increase in sensory structures on the ultimate legs compared to the anterior walking legs is therefore not surprising.

In all examined species, the sensory structures of the ultimate legs are dominated by sensilla trichoidea covering all articles of the ultimate legs in both sexes. Also, all three sensilla types (sensilla microtrichoidea, sensilla mesotrichoidea and sensilla macrotrichoidea) were present in both sexes of all examined species. There were no major differences in the sensilla type distribution and density between males and females of the studied species. The increase in sensory structures on the ultimate legs compared to the anterior walking legs seems therefore not to be associated with sexual dimorphism. However, males presenting cuticular modifications showed special arrangements of sensilla on those modifications. In these males, sensilla appear as tufts (for example on the prefemoral knob of *Eupolybothrus cavernicolus* and *E. liburnicus*, or next to the femoral basal pit of *E. fasciatus*, or along the tibial grooves of *Lithobius mutabilis*) dense clusters (for example on the 15th femur of L. pelidnus) or they even cover the whole surface of projections (for example the tibial nodule of *L. nodulipes* or the tibial distal protuberance of *L. muticus*).

1. 2. Glandular structures

Pores indicate the presence of glandular tissue, providing connections to the external environment (Rosenberg et al. 2011). Coxal pores vary in the number, shape and arrangement between genera and species, but no differences were observed between the sexes. Similarly, the telopodal pores and the epidermal glands ("kleine Epidermisdrüsen") do not differ between the sexes. The telopodal pores that cover large areas of the ultimate legs are openings of a typical gland type for Lithobiomorpha, the telopodal glands (Eason 1964, Lewis 1981, Blower 1952).

However, the clustered pores were only detected in males of the species *E. fasciatus*, *E. cavernicolus*, *L. nodulipes*, *L. mutabilis*, *L. muticus* and *L. pelidnus* and always associated with

male cuticular modifications. These pores are therefore considered sexually dimorphic characters. For *E. fasciatus* and *E. litoralis* a "pore-free-area" and a "pore-sieve" were described on the male cuticular modifications of the femur (Eason 1970). This "pore-sieve" might be the first observation of those clustered pores on the femoral swelling, which were then examined with light microscopy (Eason 1970).

Sexual dimorphism was also observed in the glandular tissue of the tibia of *L. nodulipes*, which differed noticeably in volume and morphology between males and females. Males show a vast glandular system beneath the nodule, which also extends dorsally, and to both sides of the tibia. In females, there was a thin lining of glandular tissue uniformly distributed throughout the entire tibia. The glandular tissue on the dorsal side of male tibiae differs from the tissue of the telopodal glands, regarding its size and histological features, such as the presence of many small vesicles, epidermal cells and fibre-like structures. Furthermore, the large gland cells, which are typical for the telopodal glands were absent (Rosenberg et al. 2011). The male glandular tissue found in the tibia of *L. nodulipes* therefore might be a different type of glandular tissue. There are no clear boundaries between the secretory cells and the fibre-like structures, which are highly condensed and don't seem to be separated. Therefore, this complex appears like a compound gland composed of many secretory cells (lobes on both sides) that discharge in many small pore openings. Since both the increased glandular tissue and the clustered pores are only found in males, the clustered pores are interpreted as the openings of this "male" gland. This is further supported by the morphology of the glandular tissue, which forms distally clustered channel-like structures that lead through the cuticle to the clustered pores. Moreover, there are fibrous strands associated with the glandular tissue which may belong to epithelial musculature. If this is the case, the musculature that leads to the canals and pores of glandular tissue may have the purpose to squeeze the ducts or close the openings.

According to the morphology of the clustered pores, this gland may belong to the "flexo-canal gland type", which is externally characterized by a simple glandular pore opening and a tendency to appear in clusters (Rosenberg et al. 2011). However, to confirm this gland type, further ultrastructural investigations should be carried on.

1. 3. Cuticular structures

Lithobiid centipedes show a wide spectrum of secondary sexual cuticular modifications of the ultimate legs in males. These include cuticular structures such as protuberances (for example

Lithobius pelidnus, *Eupolybothrus cavernicolus*), tubercles (for example *L. calcaratus*), projections (for example *L. curtipes*) and nodules (for example *L. nodulipes*), which are clearly sexual dimorphic traits since they are only present in adult male specimens (Eason 1964, Lewis 1981, Zapparoli and Edgecombe 2011).

The investigated cuticular modifications were mainly represented by enlargements of the cuticle, such as projections and swellings as well as indentations, such as grooves and pits. Prominent enlargements of the cuticle seem to be very common and widespread among male Lithobiidae (Eason 1964, Lewis 1981, Zapparoli and Edgecombe 2011). The histological sections through the tibial nodule of *L. nodulipes* show what we interpret as epidermal cells (compared to Rosenberg et al. 2011: 69-71, Lewis 1981: 53-55) inside the nodule, which means that this protrusion seems to be truly formed as an outgrowth or folding of the cuticle. Among the investigated species, projections were present in many different sizes, from very large (for example the proximal prefemoral knobs of *E. cavernicolus* and *E. liburnicus* or the distal protuberance of *L. muticus*) to rather small (for example the distal prefemoral protuberance of *E. cavernicolus* and *E. liburnicus* or the tibial protuberance of *L. mutabilis*). All projections were located on the dorsal side of the articles, except for the prefemoral knobs of *E. cavernicolus* and *E. liburnicus* which were pointing mediad. Furthermore, most projections were located at the distal part of the articles, such as the protuberance of *L. muticus* or the nodule of *L. nodulipes*. All projections were covered with sensilla and often associated with clustered pores. Another form of cuticular enlargement, the swellings, and extreme thickenings occurred also mainly distal (for example the prefemora in *L. pelidnus* and femora in *E. fasciatus*). The swellings were often directed mediad (for example in *E. fasciatus* and *L. pelidnus*) and also frequently associated with clustered pores. These swellings and extreme thickenings of specific articles or even only parts of articles in males are in contrast to the "regular" thickening of the entire ultimate legs, which frequently occur in both sexes (for example *E. grossipes*, *L. nodulipes*), however often more pronounced in males (for example *L. muticus*, *L. pelidnus*).

Indentations of the cuticle, like grooves and pits were also located dorsally on the articles. Grooves appeared dorsally on the 14th and 15th tibiae of male *L. mutabilis*. They were broader and deeper than the frequently observed "regular" sulci, which are narrower and not so deep. The presence of such "regular" sulci is very common in the ultimate legs of both sexes of numerous species (Lewis 1981, Eason 1964, Zapparoli and Edgecombe 2011). However, the "regular" sulci are sexually dimorphic on some species with regard to size and presence in different articles. In many species, the sulci are more prominent in males (e.g. *L. pelidnus*) as

well as they are sometimes associated with clustered pores only in males too. Thus, the presence of "regular" sulci on the ultimate legs (Fig. 1) may not constitute a sexual dimorphic characters *per se*, but the sulci might be expressed sexually dimorphic. Another form of indentations are the pits, which are again only present in males. The pits observed in *E. grossipes* and *E. fasciatus* were both located proximally on the femora and were also very similar in size.

The investigated species displayed different degrees of sexual dimorphic modifications of the cuticle. While some species possess just one modification (for example *L. nodulipes* or *E. grossipes*), some show several modifications also on different leg-pairs (for example *L. mutabilis* and *L. pelidnus*).

2. Putative role of cuticular, sensory and glandular structures for reproduction

Functions of sexual dimorphic characters are naturally related either to reproduction, which makes them mostly the result of sexual selection, or they can be related to different lifestyles of the sexes (Andersson 1994, Allen et al. 2011). In the latter case, several factors may influence these differences, like different ecological niches of the sexes, mating system or population density (Andersson 1994, Allen et al. 2011, Mori et al. 2017). Whether males and females of the order Lithobiomorpha show ecological differences has never been documented.

The investigation of the associated microstructures present on the secondary sexual cuticular structures is a potential approach to evaluate whether they possess any sensory or secretory function and moreover play a role in reproduction. The finding that many of the male secondary sexual modifications usually include sensory and/or glandular structures indicates at least a function in perception and/or secretion of certain substances and stimuli. Thus, these structures may help males perceive signals or secrete substances to facilitate courtship and mating. Sexual dimorphic glands are commonly found in all kind of animals and often exhibit a reproductive function (Juberthie-Jupeau and Lopez 1991, Murayama and Willemart 2015). Many of these modifications of male lithobiid centipedes include thickenings and swellings, which provide a larger cuticular surface for sensilla and pores. Grooves and sulci are frequently associated with sensory and/or glandular structures (clustered pores) as well. Increased surface area serving the purpose of an increased amount of sensory structures have often been reported to be sexual dimorphic modifications in other groups of invertebrates (Fea et al.

2019, Dumas et al. 2010, Mark et al. 2017). This phenomenon is supported by the findings in the external morphology and histology of this study. For example, the clustered pores often lie in grooves and sulci, which suggests that the function of these indentations may be the accumulation of specific substances.

2. 1. Putative function of the sensilla

The sensory modalities of arthropod sensilla have been investigated and discussed by several authors (e.g. Keil 1975, Keil 1976, Zacharuk 1980, Ernst 1983, Ernst et al. 2009, Sombke et al. 2011, Sombke and Ernst 2014). Though the terminology is lacking clarity (see appendix, Table 7) and the external morphological features may vary, there are certain consistent aspects of sensilla morphology that help to assess the sensory modality of a sensillum (Keil 1975, Keil 1976, Ernst 1983, Ernst et al. 2009, Sombke et al. 2011, Sombke and Ernst 2014, Kenning et al. 2019). A chemoreceptive function is indicated by the presence of pores, either at the apical tip or in the shaft of the sensillum (Keil 1976, Zacharuk 1980). According to the number of pores they can be divided into uniporous and multiporous chemosensilla (Zacharuk 1980). A mechanoreceptive function is usually indicated by both the presence of a fibrillary membrane at the pedestal to enable deflection of the sensillum and the presence of a tubular body (Ernst 1983, Keil 1976). Ultrastructural investigations showed that sensilla trichoidea on the antenna of *Cryptops hortensis* (Donovan, 1810) (Scolopendromorpha) have one tubular body, which validates a mechanoreceptive function for sensilla trichoidea (Ernst et al. 2009). Since there are also many records that these sensilla have a terminal pore, sensilla trichoidea are also suggested to have an additional chemoreceptive function for perceiving mechanical stimuli and the chemical composition and humidity of the ground (Keil 1976, Ernst et al. 2009, Sombke et al. 2011, Sombke and Ernst 2014). Sensilla microtrichoidea are thought to function as proprio-mechanoreceptors, since they are often located in rows at the base of antennal segments and can therefore sense changes in the position of the antenna (Ernst 1983, Keil 1975, Sombke and Ernst 2014). They might also function as mechano- as well as contact-chemoreceptors, because of their positions and by the presence of a terminal pore (Keil 1975).

The presence of numerous sensilla trichoidea and sensilla microtrichoidea on the ultimate legs of Chilopoda, indicates that those legs have a sensory function in perceiving both mechanical stimuli to obtain information on the environment and on the orientation of their articles to each other (proprioreceptors) as well as chemosensory stimuli to get information on the chem-

ical composition of their environment. The ultimate legs may serve as a functional equivalent to the antennae on the opposite end of the body (Kenning et al. 2017). For all species with secondary sexual modifications, the associated sensilla trichoidea showed external features of the pedestal which fit in the descriptions of mechanoreceptive sensilla in the literature (Ernst et al. 2009, Keil 1976, Kenning et al. 2019, Müller et al. 2011). The sensilla associated with the secondary sexual modifications of *E. fasciatus*, *L. nodulipes* and *L. pelidnus* definitely showed a terminal pore, which indicates an additional chemoreceptive function (Keil 1976, Zacharuk 1980). The combination of these two sensory modalities are suggested to result in the function of contact-chemoreceptors (Sombke et al. 2011, Sombke and Ernst 2014). For all other investigated species, it remains unclear whether their sensilla possess a chemoreceptive function too, or just function as mechanoreceptors, since the presence of terminal pores could not be confirmed. Among other putative functions, the male cuticular modifications studied here can therefore be thought to enhance detection of mechanical stimuli and chemical signals. The length of the sensilla associated with male sexual cuticular structures seems to be very similar in all investigated species of the genus *Lithobius*. The species *Eupolybothrus cavernicolus* and *E. liburnicus* show sensilla that are over 170 µm long, and in *E. fasciatus* up to 320 µm long. This difference might be related to body size because the *Eupolybothrus* species are generally larger than *Lithobius* species.

2. 2. Putative function of the glandular structures

The telopodal glands are considered to have a defensive function via the release of a sticky substance (Keil 1975, Rosenberg et a. 2011, Kenning et al. 2017). Remains of this secretion are often visible on the SEM images (Fig. 4). For the coxal glands, a secretory function of pheromones seems to be likely but the possibility of a multifunctionality of the coxal organs, both pheromone release and osmoregulation, cannot be excluded (Littlewood 1991). Since the functions of both telopodal and coxal glands are vital, it is not surprising that no sexual dimorphism was detected in these structures. This is different for the clustered pores which are only present on the sexual dimorphic leg modifications of males and might therefore be responsible for the secretion of substances important or at least useful for a successful mating. The drastic increase in the size of the glandular system (Figs. 14-16), which at least partly discharges into these pores in male *L. nodulipes*, supports the assumption that the clustered pores secrete substances that serve an important role during courtship and/or mating. The tasks of such released substances can have multiple purposes: the attraction of a potential

partner, stimulation, providing information on one's condition, or marking of territory (Andersson 1994, Murayama and Willemart 2015, Johansson and Jones 2007, Steiger and Stöckl 2014). A key for a better understanding of the function of the sexual dimorphic gland and its clustered pores would be the chemical analysis of the secretion of these glands to find out whether they are composed of pheromones or other signalling molecules.

2. 3. Putative functions of male secondary sexual modifications of the ultimate legs

In centipedes, sexual reproduction includes the deposition of a spermatophore by the male and the uptake of this spermatophore by the female that later lays the fertilised eggs and, in case of Lithobiomorpha, does not show any brood care except for hiding the eggs (Klingel 1959, Minelli 2011). The courtship behaviour of *L. forficatus* (which possess no secondary sexual characters) involves several phases of repetitive touching of the ultimate legs of the male by the female with its antennae (Klingel 1959). The female is almost permanently in contact with the ultimate legs of the male, which are moved in certain patterns by the male (Klingel 1959). The female follows the male, which produces a net and deposits a spermatophore on it (Klingel 1959). Finally, the female grabs the spermatophore by crawling on the back of the male until its posterior end is above the male's genital segment (Klingel 1959).

The female may leave the male during courtship, so it seems that the female is in control whether spermatophore transmission takes place or not and therefore may choose its mating partner (Klingel 1959). Since this decision of the female is crucial for the quality of its offspring, the female is supposed to check the quality of the potential mating partner. These so called "indicator-mechanisms" were already proposed for various animal taxa by different authors (reviews: Møller 1990, Andersson 1994, Fitzpatrick et al. 1995, Andersson 2006), some of these examples including fish (Milinski and Bakker 1990, Haude 1999), birds (Hamilton and Zuk 1982, Hill et al. 1994, Nowicki et al. 1998) amphibians (Green 1991, Forsman and Hagman 2006) and arthropods (Hoefler et al. 2008). The secondary sexual characters of the males' ultimate legs in lithobiid centipedes may be such indicators used by the female to verify the quality and maturity of the male, like for example ornaments in some bird species (Andersson 1994). This assumption is supported by the fact that these secondary sexual characters grow with age, so that only mature and fully-grown individuals show most prominently expressed characters. There is evidence from this study to support these assumptions in *L. nodulipes* and *E. liburnicus* (Fig. 6) and also from previous studies on *E. liburnicus* (Akkari et

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al. 2017). Age- and condition-dependent secondary sexual characters are known for many animals and may act as important indicators for maturity and quality of the mating partner (Andersson 1994, Gotoh et al. 2014). It is also reported from some *Lithobius* species that after the loss of legs that bear the secondary sexual modifications, these would be absent on regenerated legs but sometimes expressed on different legs (Eason 1993). Perhaps, this could be already an indicator for the female on the male's condition and whether the male is "intact".

Another pathway of communication through the male ultimate legs is suggested here: females may perceive chemical signals emitted from the posterior end of the males that provide information on species identity and indications on the males' quality, like for example in the arctiid moth species *Utetheisa ornatrix* (Linnaeus, 1758) (Kelly et al. 2012). Furthermore, they may help the female to orientate and position itself properly during spermatophore transfer. Here, it would be interesting to investigate whether there is also a sexual dimorphism in the sensory structures on the antennae, since one could expect an increase in sensory sensilla on female antennae in order to obtain all the information provided by the male. This is for example the case in a recently studied cave cricket species (*Pachyrhamma waitomoensis* Richards, 1958), where females have more sensilla types on their antennae than males (Fea et al. 2019).

Another function of these cuticular structures is most likely the male's ability to better sense mechanical stimuli and in some cases chemical signals. The cuticular structures may provide an increased surface for sensory structures to be situated on and a more suitable position for them to enhance the detection of motion and contact with the female. The sensory structures on the prefemora of *E. liburnicus* were suggested to help the male to get information whether the female is correctly positioned and prepared for spermatophore uptake (Akkari et al. 2018).

However, many male lithobiomorph species do not possess such cuticular modifications and show functioning courtship behaviour (for example *L. forficatus*). Therefore, sexual dimorphic cuticular modifications may play an additionally role during courtship, independent from behaviour. To answer that question, whether these structures are in support of the male's courtship behaviour or independent signals, behavioural experiments are needed.

3. Systematic implications

Sexual dimorphism in the ultimate legs occurs in many species of family Lithobiidae. In this study, representatives of the two genera *Lithobius* and *Eupolybothrus* were investigated and a

large variety of modifications in nine species of these genera are presented. Cuticular modifications were observed on different legs, different articles, and in various combinations.

Similarities were found in two species pairs. *Eupolybothrus cavernicolus* and *E. liburnicus* both possess a proximal prefemoral knob, a dorsomedial ridge and a distal protuberance. *E. fasciatus* and *E. grossipes* both show a proximal basal pit on their femora. Both species pairs are closely related, which is supported by recent phylogenetic analyses (Stoev et al. 2013, Ganske et al. 2020). *E. liburnicus* and *E. cavernicolus* belong to the subgenus *Schizopolybothrus* and share the presence of a prefemoral knob in the males' 15th leg-pair with seven other *Schizopolybothrus*-species (Akkari et al. 2017). The taxon sampling of this study does not allow for a comparison within the genus *Lithobius*, which comprises over 500 species (Zapparoli and Edgecombe 2011).

Spermatophore deposition and transfer occurs at the posterior body end, which helps to explain why most sexual dimorphic characters are found on the ultimate legs in lithobiid centipedes. The fact that there are only few records of sexual dimorphism in anterior regions of the body (Crabill 1960, Farzalieva et al. 2017) supports this assumption. It appears that the position of modifications on the legs are conserved (at least in some lineages) but each species has its own unique characteristic of the modification for example the shape of the prefemoral knob of *E. cavernicolus* and *E. liburnicus*, which is roundish in *E. cavernicolus* and more triangular in *E. liburnicus* (for a comparison of the prefemoral knobs in the subgenus *Schizopolybothrus* see Akkari et al. 2017). Which legs are affected might be determined by sexual selection for each lineage. Although the location of those sexual dimorphic characters is supposed to be fixed for each species, there are also records of *Lithobius* species showing variation on which leg modifications occur, especially after leg loss and regeneration (Eason 1993).

Since there are many examples in Lithobiidae without sexual dimorphic ultimate legs (for example *L. forficatus*), such cuticular modifications do not seem to be crucial for courtship and mating in some species.

Conclusion

Sexual dimorphism in ultimate legs of lithobiid centipedes comprises several cuticular modifications in males, showing an important diversity in form and position across the studied taxa. Sensory structures as well as coxal, telopodal and small epidermal pores did not seem to have noticeable differences between sexes. The secondary sexual cuticular modifications of males are associated with sensilla, which could be identified as mechano- and/or chemoreceptors. In six species, a hitherto unrecorded distribution of pores was found and described – the clustered pores. These clustered pores are probably linked to the glandular system, found in the male tibial projection of *L. nodulipes*, indicating that male secondary sexual characters on the ultimate legs are functional, probably sensory and secretory, during courtship and mating. The closely related investigated species of *Eupolybothrus* possess comparable secondary sexual characters, however they are still highly species-specific. Sexual dimorphism in lithobiid centipedes remains to be a widely unexplored field. This study contributes new comparative morphological data on a microstructural level, showing that some lineages of lithobiid centipedes exhibit complex sexual dimorphism.

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Zusammenfassung

Die Männchen einiger Arten der Hundertfüßer-Ordnung Lithobiomorpha verfügen über auffällige sekundäre Geschlechtsmerkmale auf den letzten Beinpaaren, unter anderem Schwellungen, Höcker, Rillen und einem Zuwachs an sensorischen und glandulären Strukturen. Über die Diversität, Morphologie und mögliche Rolle dieser kutikulären Modifikationen während der Fortpflanzung ist bisher nicht viel bekannt. In dieser Studie wurde die Morphologie sekundärer Geschlechtsmerkmale von neun Arten der Gattungen *Lithobius* Leach, 1814 und *Eupolybothrus* Verhoeff, 1907 unter der Verwendung von Lichtmikroskopie und Rasterelektronenmikroskopie (REM) untersucht und detailliert beschrieben. Die sekundären Geschlechtsmerkmale der Männchen sind häufig mit Sensillen assoziiert, die sehr wahrscheinlich mechano- und chemorezeptiv sind. Zusätzlich finden sich auf ihnen gruppierte kutikuläre Poren, welche in dieser Formierung bisher unbeschrieben waren. Von der kutikulären Modifikation der Art *Lithobius nodulipes* Latzel, 1880, einem tibialen Höcker, wurden zusätzlich micro-computer tomographische (μ-CT) Scans und histologische Semi-dünnschnitte angefertigt, welche zeigen, dass die gruppierten Poren wahrscheinlich in Verbindung mit dem Drüsengewebe, das in dem tibialen Höcker gefunden wurde, stehen. Das Vorhandensein von derart vielen glandulären und sensorischen Elementen auf den geschlechtsdimorphen Strukturen der Männchen deutet auf eine mögliche Rolle dieser Strukturen während der Paarung hin. Nah verwandte Arten zeigten ähnliche geschlechtsdimorphe Merkmale, jedoch waren die Strukturen in ihren Details artspezifisch. Diese Studie bildet einen Teil der Vielfältigkeit der geschlechtsdimorphen Strukturen von lithobiiden Hundertfüßern ab. Die genauen Beschreibungen der Morphologie und Histologie dieser Strukturen, sowie deren physiologische Interpretationen sind ein erster Schritt, um Aspekte der Reproduktionsbiologie dieser Tiergruppe zu verstehen.

Appendix

Terminology of sensilla trichoidea and sensilla microtrichoidea in Myriapoda

Sensilla trichoidea and sensilla microtrichoidea were recorded in many taxa of Diplopoda and Chilopoda (Ernst et al. 1983, Ernst et al. 2009, Keil 1975, Keil 1976, Kenning et al. 2019, Sombke et al. 2011, Sombke and Ernst 2014). Although several attempts have been made to classify the sensilla trichoidea (mainly based on length and morphological characteristics of shaft, pedestal and apex), many of these categories are overlapping (Table 7) and not appliable for different species (Keil 1975, Keil 1976, Ernst 1983, Ernst et al. 2009, Sombke et al. 2011, Sombke and Ernst 2014, Kenning et al. 2019). A standard terminology for chilopod and/or diplopod sensilla is hitherto lacking. Probably, a classification mainly based on length would be the best way to have a comparable parameter of these sensilla. However, it is anyways questionable, whether different length classes or morphological variants of sensilla trichoidea display any functional and in a broader sense biological entities and if it is useful to classify them.

Table 7: Sensilla trichoidea and sensilla microtrichoidea of different myriapod species in literature.

Class, order	Species	Bodypart	Terminology	Length	Pedestal	Shaft	Apex	Reference
Diplopoda: Polydesmida	*Oranmorpha guerinii*	antenna	sensilla trichoidea	70-165 µm	symmetrical, circular and slightly elevated	slender, helically ribbed surface	presence of terminal pore unclear	Sombke and Ernst 2014
			sensilla microtrichoidea	13-17 µm		slender, conical and smooth	terminal pore was not documented	
Chilopoda: Scutigeromorpha	*Scutigera coleoptrata*	antenna	long sensilla trichoidea	48-73 µm				Sombke et al. 2011
			short sensilla mesotrichodea	27-40 µm	with bidentate scale	distinctly striated	with terminal pore	
Chilopoda: Lithobiomorpha	*Lithobius forficatus*	antenna	sensilla trichoidea	100-160 µm	sickle-shaped, with two associated pores	helically striated	with terminal pore	Keil 1975, 1976
		antenna	sensilla microtrichoidea	5-10 µm	miniaturized sensilla trichoidea	miniaturized sensilla trichoidea	miniaturized sensilla trichoidea	
		walking and ultimate legs	sensillum macrotrichodeum type I	114–173 µm	crescent-shaped socket, associated with one glandular pore	fine and slightly curled ribs	gently curved, without terminal pore	Kenning et al. 2019
		tarsus of walking legs	sensillum macrotrichodeum type II	112–128 µm	crescent-shaped, multidentate	finely grooved and slightly contorted shaft	without terminal pore	
Chilopoda: Lithobiomorpha	*Lithobius forficatus*	walking and ultimate legs	sensillum mesotrichodeum type I	37.4–54.7 µm	crescent-shaped, associated with one glandular pore	fewer, straight and rather stout ribs	without terminal pore	
		walking legs	sensillum mesotrichodeum type II	25–40.5 µm	crescent-shaped, associated with two pores	sponge-like surface between pronounced ribs	slightly contorted and occasionally bent, with terminal pore	
		walking and ultimate legs	sensilla microtrichoidea	13.0–14.5 µm	crescent-shaped,	few shallow and only slightly curled ribs	with terminal pore	
Chilopoda: Scolopendro-morpha	*Cryptops hortensis*		type 1 - sensilla trichoidea	17-87 µm	collar-like cavity, sometimes with one associated pore	longish, ribbed, tapering to the end	with terminal pore	Ernst et al. 2009
		antenna	type 2 - sensilla trichoidea	5.8-16.3 µm	-	short compact sensilla, densely ribbed	slightly curved, with larger terminal pore	
			sensilla microtrichoidea	6.45-11.7 µm	halfmoon-like cavity	slender, hair-like, with slight striation	slightly curved tips	
Chilopoda: Geophilomorpha	*Geophilus longicornis*	antenna	sensilla microtrichoidea	7.5 -17.5 µm	ring-like collar	tapering, smooth surface	slightly bent, presence of terminal pore unclear	Ernst et al. 1983