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Original Article

Differences in lobed gland structure and distribution in terrestrial isopods (Oniscidea)

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ABSTRACT

Lobed tegumental glands are found only in terrestrial isopods and are thought to have evolved in response to challenges associated with life on land. By examining 30 terrestrial isopod species, we aimed to determine the factors that influence the structure and distribution of the lobed glands. We discovered that lobed glands are found not only in the lateral plates and the uropods but also in the cephalothorax, the antennae, the tergites, and the pereopods of some species. The structure of the lobed glands is influenced by the phylogenetic position of an isopod. Namely, species of Diplocheta, Microcheta, and Synocheta have only one nucleus in the secretory cell, while two nuclei occur in the secretory cells of Crinocheta. By contrast, the distribution of the lobed glands in the body is influenced by the eco-morphotype of a species. Clingers and runners have numerous large lobed glands throughout the body. Creepers possess well-developed uropod glands, while lobed glands are absent or small and few in number in their pereon. Rollers have fewer lobed glands and some species apparently lack them. We also showed that several species possess several types of lobed glands. The correlation of lobed gland distribution with the eco-morphotype speaks in favor of the proposed defensive function of lobed glands.

KEYWORDS

Crustacean histology, defense, eco-morphotype, predation, secretion

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INTRODUCTION

Tegumental glands of crustaceans are exocrine glands associated with the integument and open through a pore on its surface. They are present in all crustaceans examined so far. They possess a vast structural diversity and have many different functions, including a role in copulation (Beninger and Larocque, 1998), antimicrobial defense by secreting specific enzymes (Tuchina et al., 2014), and the production of pheromones (Kamiguchi, 1972a; 1972b; Sarojini et al., 1982).

Terrestrial isopods, the Oniscidea, are the only crustacean group known to possess lobed glands, which do not resemble any tegumental glands found in other Crustacea. They are tricellular, consisting of a secretory cell, a ramifying intermediate cell, and an elongated canal cell (Weirich and Ziegler, 1997) (Fig. 1). According to previous research, lobed glands are located in uropods and lateral plates of terrestrial isopods (Collinge, 1921; Gorvett, 1951; Weirich and Ziegler, 1997). Collinge (1921), who described the distribution of lobed glands in *Porcellio dilatatus* Brandt, 1833, and Weirich and Ziegler (1997), who described the ultrastructure of lobed glands in *Porcellio scaber* Latreille, 1804, reported that lobed glands located in the thoracic lateral plates open on the edges of the lateral plates, while lobed glands in the uropods and pleonites 3–5 open on the lateral surface of the uropod protopodite and exopodite. By contrast, Gorvett (1951) observed that lobed glands in the pleon also have openings on the lateral plates of the pleon, not only on the uropods in *Po. scaber.* In this way, he distinguishes two types of lobed glands: uropod glands, that have openings along the outer edges of the uropods, and lateral plate glands, which open near the distal edge of lateral plates. It is thought that lobed glands have evolved as an adaptation to terrestrial life, probably having a defensive function (Gorvett, 1951; 1956). Although behavioral studies have been undertaken to support this hypothesis (Gorvett, 1956; Deslippe et al., 1996), their exact function remains subject to debate.

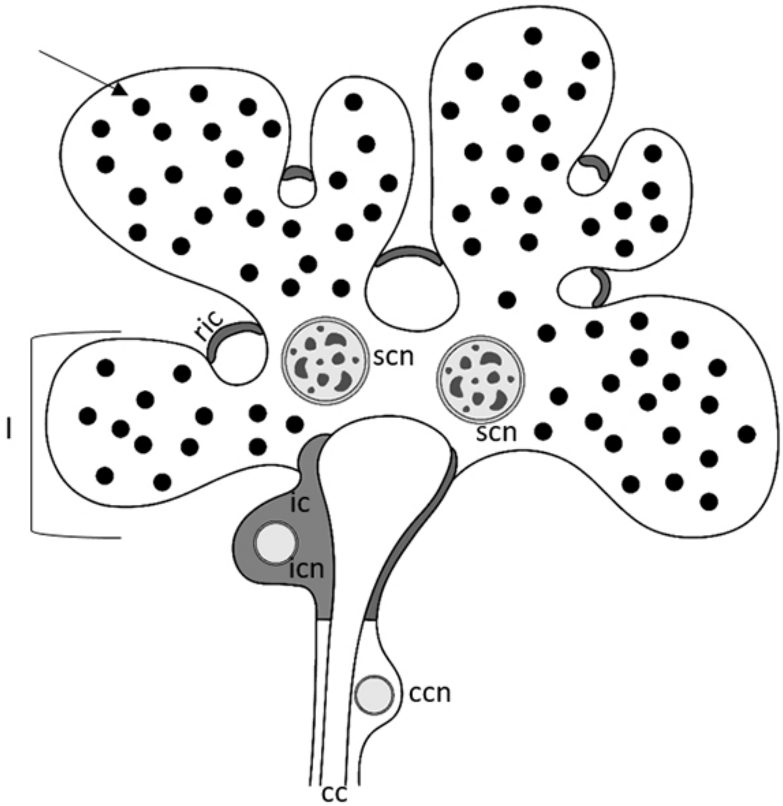

Figure 1. Schematic representation of the binucleated lobed gland. The lobed glands consist of three cells. The secretory cell is filled with secretory granules (arrow), may have two nuclei (scn), and forms several lobes (l). The intermediate cell (ic), possesses one nucleus (icn) and branches into ramifications (ric) that connect to the secretory cell. It connects with the canal cell (cc) that possesses only one nucleus (ccn).

With the transition from water to terrestrial habitats, Oniscidea evolved specific behavioral and morphological features, correlating with the environment they inhabit and their anti-predatory strategies. Based on these features, Schmalfuss (1984) distinguishes six main eco-morphotypes of terrestrial isopods. Runners have long pereopods and narrow bodies with smooth tergites. They are very mobile and run away to hide when attacked. Clingers have flat and broad bodies. They have short, strong pereopods, are generally slow and cling to the substrate for defense. Creepers are small, endogean forms with convex, cylindrical bodies and longitudinal ridges. Rollers are conglobating forms with highly convex bodies. Spiny forms possess spiny tergal protuberances. Species that do not fit in any of these groups are categorized as non-conformists. Transitional forms between all the above eco-morphotypes have also been described (Schmalfuss, 1984).

This research aimed to broaden our knowledge about the structure and distribution of lobed glands in different terrestrial isopod taxa, including previously unstudied groups like Mesoniscidae and Tylidae. Furthermore, the selected species inhabit diverse ecological environments, ranging from extremely dry habitats to supralittoral areas. We determined whether there is more similarity among phylogenetically more closely related species with different eco-morphological strategies or among more distantly related species that defend themselves similarly. Using the data of the lobed gland structure, histochemical composition, and spatial distribution across species in relation to eco-morphological strategies, we attempted to provide additional insight into the function of the lobed glands.

MATERIALS AND METHODS

In the section "Materials and methods" and "Results", systematics is consistent with WoRMS (World Register of Marine Species; https://www.marinespecies.org/). Accordingly, the analyzed species belong to the suborder Oniscidea, consisting of the three parvorders: Diplocheta, Tylida, and Orthogonopoda. Parvorder Orthogonopoda is further divided into three sections: Microcheta, Synocheta, and Crinocheta. The number of examined species and the selection criteria are given for each method separately.

Serial histological sections

We examined specimens of 30 Oniscidea species belonging to different eco-morphological types and two aquatic representatives of non-oniscidean isopods (Tab. 1). Live crustaceans were fixed in either 3.7% formaldehyde in 0.1 M HEPES buffer, except for *Ligia italica* Fabricius, 1798 and *Tylos ponticus* Grebnicki, 1874 (3.7% formaldehyde in seawater), *Mesoniscus graniger* (Frivaldsky, 1865) (70% ethanol), and *Po. dilatatus* and *Levantoniscus bicostulatus* Cardoso, Taiti, Sfenthourakis, 2015 (96% ethanol). In cases of species living in caves and those not native to Central Europe, fixed material was kindly provided by researchers working on the group. Information on the origin of material is provided in Appendix 1. Isopods were decalcified in a 10% aqueous EDTA solution (pH = 7.2) for two days, then washed, dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Sections (7 µm) were cut using a microtome and collected on glass slides. Deparaffinization with xylene and rehydration in a descending ethanol series to distilled water followed. Serial histological sections were stained with hematoxylin and eosin (Kiernan, 1990), dehydrated in an ascending series of ethanol followed by xylene, and covered using Pertex (Medite).

Histochemical analysis

We performed a histochemical analysis on paraffin sections of six different species: *Po. scaber*, *Armadillidium versicolor* Stein, 1859, *Cylisticus convexus* (De Geer, 1778), *Oniscus asellus* Linnaeus, 1758, *Trichorhina tomentosa* (Budde-Lund, 1893), and *Ligidium germanicum* Verhoeff, 1901. The analysis was limited to these species, which are easily maintained in culture and were selected to cover different families while also representing diverse eco-morphological types.

Acid polysaccharides were demonstrated using the Alcian blue technique at pH 2.5 (Kiernan, 1990). The presence of acid polysaccharides is indicated by blue staining.

For the staining of proteins, the bromophenol blue technique was used, staining proteins blue to red (Kiernan, 1990).

Table 1. List of examined species and description of the distribution, number and structure of lobed glands. Approximate numbers of lobed glands are given in parentheses. * not examined; # not discernible; <10 less than 10 glands; >10 more than 10 glands; >>10 significantly more than 10 glands

species	ecomorphotype	lateral plates of the pereon	lateral plates of the pleon	uropods	cephalothorax	antennae	pereopods	tergite	number of nuclei in the gland cell
Asellota									
Asellus aquaticus									
Sphaeromatidea									
Sphaeroma serratum									
Oniscidea, Diplocheta									
Ligiidae									
Ligia italica	runner	+ (1)	+ (1)	+ (1)	+	+	–	–	1
Ligidium germanicum	runner	+ (<10)	+ (<10)	+ (<10)	+	+	+	–	1
Oniscidea, Tylida									
Tylidae									
Helleria brevicornis	roller	–	–	–	–	–	–	–	–
Tylos ponticus	roller	–	–	–	–	–	–	–	–
Oniscidea, Microcheta									
Mesoniscidae									
Mesoniscus graniger	creeper	+ (<10)	+ (>10)	+ (>10)	–	–	–	–	1
Oniscidea, Synocheta									
Trichoniscidae									
Haplophtalmus danicus	creeper	–	–	+ (#)	–	–	–	–	1
Haplophtalmus mengii	creeper	–	–	+ (#)	–	–	–	–	1
Alpioniscus heroldii	runner	+ (~10)	+ (~10)	+ (~10)	+	*	+	-	1
Androniscus roseus	creeper	+ (1)	+ (>10)	+ (>10)	–	–	–	–	1
Hyloniscus adonis	runner	+ (~10)	+ (>10)	+ (>10)	–	–	+	–	1
Hyloniscus riparius	runner	+ (~10)	+ (>10)	+ (>10)	–	–	+	–	1
Tithanetes albus	runner	+ (<10)	+ (>10)	+ (>10)	–	–	–	–	1
Oniscidea, Crinocheta									
Platyarthridae									
Platyarthrus hoffmannseggii	non-conformist	–	+ (#)	+ (>10)	–	–	–	–	2
Trichorchina tomentosa	creeper	–	+ (<10)	+ (>10)	–	–	–	–	2

Table 1. Cont.

species	ecomorphotype	lateral plates of the pereon	lateral plates of the pleon	uropods	cephalothorax	antennae	pereopods	tergite	number of nuclei in the gland cell
Oniscidae									
Oniscus asellus	clinger	+ (<10)	+ (>10)	+ (>10)	–	–	–	–	2
Halophiloscidae									
Halophiloscia couchii	runner	+ (<10)	+ (>10)	+ (>10)	+	–	–	–	2
Philosciidae									
Philoscia muscorum	runner	+ (>10)	+ (>10)	+ (>10)	*	*	*	–	2
Cylisticidae									
Cylisticus convexus	conglobating clinger	+ (~10)	+ (~10)	+ (>10)	+	+	–	+	2
Armadillidiidae									
Armadillidium nasatum	roller	+ (~10)	+ (~10)	+ (>10)	*	*	*	–	2
Armadillidium palasii	roller	+ (~10)	+ (~10)	+ (>10)	-	–	*		2
Armadillidium versicolor	roller	+ (~10)	+ (~10)	+ (>10)	*	–	*	–	2
Armadillidae									
Armadillo officinalis	roller	–	–	–	–	–	–	–	–
Cubaris murina	roller	–	–	–	–	–	–	–	–
Porcellionidae									
Porcellio dilatatus	clinger	+ (>>10)	+ (>>10)	+ (>>10)	–	–	–	–	2
Porcellio scaber	clinger	+ (>>10)	+ (>>10)	+ (>>10)	–	–	–	–	2
Porcellionides pruinosus	runner	+ (>>10)	+ (>>10)	+ (>>10)	–	–	–	–	2
Trachelipodidae									
Levantoniscus bicostulatus	creeper	-	(~10)	(~10)	–	–	–	–	2
Trachelipus rathkii	clinger	+ (>>10)	+ (>>10)	+ (>>10)	+	*	*	+	2
Trachelipus ratzeburgii	clinger	+ (>>10)	+ (>>10)	+ (>>10)	+	*	*	+	2
Agnaridae									
Protracheoniscus politus	runner	+ (>>10)	+ (>>10)	+ (>>10)	+	*	*	+	2

Periodic acid/Schiff was used for the demonstration of carbohydrates, and the Ninhydrin/Schiff technique for the demonstration of primary amino groups, indicative of amino acids and peptides (Kiernan, 1990). Positive reactions are indicated by magenta coloration.

For the detection of lipids, we prepared cryosections of the same six species. Prior to cryosectioning, isopods were fixed in 3.7% formaldehyde in 0.1 M HEPES buffer and decalcified as described for paraffin sections, after which they were incubated overnight in 30% sucrose and frozen in Tissue freezing medium® (Leica). Sections were stained with Sudan black (Kiernan, 1990). A positive reaction is indicated by dark blue to black coloration.

Resin sections

Resin sections were prepared only when it was necessary to obtain more detailed structural information, supplementing the results of serial paraffin sections. For the preparation of resin sections, specimens were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M HEPES buffer (pH = 7.2). Following fixation, specimens were washed using 0.1 M HEPES buffer, decalcified in a 10% aqueous EDTA solution (pH = 7.2) for 2 days, postfixed in 1% aqueous osmium tetroxide, washed again and dehydrated in an ascending ethanol series. Samples were then embedded in Spurr's resin (SPI Supplies). Sections (0.5 µm) were cut using an Ultracut S ultramicrotome (Reichert), transferred to glass slides and stained with methylene blue/Azure II according to Richardson et al. (1960).

Microscopic imaging of sections

Light micrographs of paraffin, frozen, and resin sections were recorded using an AxioImager Z.1 microscope (Zeiss) equipped with an HRc Axiocam camera.

Whole-body imaging

Two species were analyzed: *Po. scaber* and *Am. versicolor*. The species were selected as they are easily maintained in culture and due to the presence of two types of lobed glands, as determined by the examination of paraffin sections. All specimens were fixed in two different fixatives, either 3.7% formaldehyde in 0.1 M HEPES (pH = 7.2) or a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M HEPES buffer (pH = 7.2). They were decalcified for two days in 10% aqueous EDTA solution and dehydrated in an ascending series of ethanol. For tissue clearing, we used a mixture of benzyl alcohol and benzyl benzoate (1:2). Cleared tissues were imaged with fluorescence and transmitted light microscopy. Micrographs were recorded using an MZ FLIII Leica fluorescence stereomicroscope. For fluorescence excitation, we used three filter sets: UV (330–380 nm excitation), GFP1 (457–487 nm excitation) and ET mCHER (542–582 nm excitation).

Optical sections

Porcellio scaber, *Cy. convexus*, *Am. versicolor*, *Armadillidium vulgare* (Gorvett, 1951), *Hyloniscus riparius* (C. Koch, 1838), and *Lm. germanicum* were dissected and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M HEPES buffer (pH = 7.2), and then incubated in 0.004% methyl green in 0.1 M HEPES buffer (pH = 7.2) for two days at 4 °C. Samples were then washed in HEPES buffer and mounted using ProLong Glass (Thermo Fisher). Tissue autofluorescence was imaged using UV excitation (405 nm) and methyl green fluorescence using red excitation (633 nm). Images were acquired using an SP8 TCS confocal microscope (Leica).

Measurement of the lobed gland size

On images of paraffin sections, we measured the largest and smallest diameter of lobed glands using Fiji (Schindelin et al., 2012). Then we calculated an average value from both measurements of an individual lobed gland. We report the size of the largest measured lobed gland from the largest examined specimen of each investigated species.

Counting of lobed glands

In all species, we determined the number of lobed glands by counting them on paraffin sections. Since many glands overlapped on sections and were often difficult to count precisely, the numbers of lobed glands are provided as estimates.

RESULTS

The histological analysis of serial sections provided information on the structure and distribution of lobed glands in the body. In addition, we used semi-thin and optical sections for a detailed analysis of their structure in cases when it was unclear on paraffin sections. As we will show, differences between taxa may be found in the distribution, the size and number of lobed glands, the depth of the lobes, and the number of nuclei in the secretory cell (Tab. 1). Examined representatives of Diplocheta (Fig. 2A), Microcheta, and Synocheta (Fig. 2B) have only one nucleus per secretory cell, while binucleated secretory cells appear in Crinocheta (Fig. 2C). Finally, differences are found in the staining intensities of the secretory granules, not just between different species, but also among different lobed glands in a single body region of an isopod.

The distribution and size of lobed glands in different isopods

The distribution and size of the lobed glands are more species-specific than the structure itself. Therefore, we have analyzed these features in more detail, considering the different taxa and eco-morphotypes the isopods belong to. Details are provided in Tab. 1. In all species that possess lobed glands, they are present in the lateral plates of the pleon and in the uropods. Lobed glands may be present also in the lateral plates of the pereon, and in some species, under the tergites (*Cy. convexus*, *Trachelipus rathkii* (Brandt, 1833), *Trachelipus ratzeburgii* (Brandt, 1833), *Protracheoniscus politus* (C. Koch, 1840), in the cephalothorax (*Li. italica*, *Lm. germanicum*, *Alpioniscus heroldii* (Vehoeff, 1931), *Halophiloscia couchii* (Kinahan, 1858), *Cy. convexus*, *T. rathkii*, *Ts. ratzeburgii*, *Pr. politus*), the antennae (*Li. italica*, *Lm. germanicum*, *Cy. convexus*), and the pereopods (*Lm. germanicum*, *Al. heroldii*, *Hyloniscus adonis* (Verhoeff, 1927), *Hy. riparius*) (Fig. 3).

We only observed lobed glands in Oniscidea but not in the other examined aquatic isopod species (*Asellus aquaticus* (Linnaeus, 1758) and *Sphaeroma serratum* (J.C. Fabricius, 1787)). In Oniscidea, lobed glands are present in the examined representatives of Diplocheta (*Li. italica* and *Lm. germanicum*) but we did not observe them in Tylida (*Ty. ponticus* and *Helleria brevicornis* Ebner, 1868). In Orthogonopoda, we detected them in all species, except in the examined representatives of Armadillidae (*Armadillo officinalis* Duméril, 1816 and *Cubaris murina* Brandt, 1833) (Tab. 1).

We examined two representatives of Diplocheta: *Li. italica* and *Lm. germanicum*, both belonging to the runner eco-morphotype. They possess small lobed glands with shallow lobes and only one nucleus in the secretory cell. The diameter of their lobed glands does not exceed 60 µm. Lobed glands are present in all lateral plates, the uropods, the cephalothorax, and the antennae (Fig. 3B, C). In *Lm. germanicum*, we also found lobed glands in the pereopods. They are present in each pair of pereopods, with the anterior five pairs having somewhat smaller lobed glands, while they are bigger and more numerous in the posterior two pairs. In *Li. italica*, we found only one lobed gland in each lateral plate, while *Lm. germanicum* has several lobed glands per lateral plate.

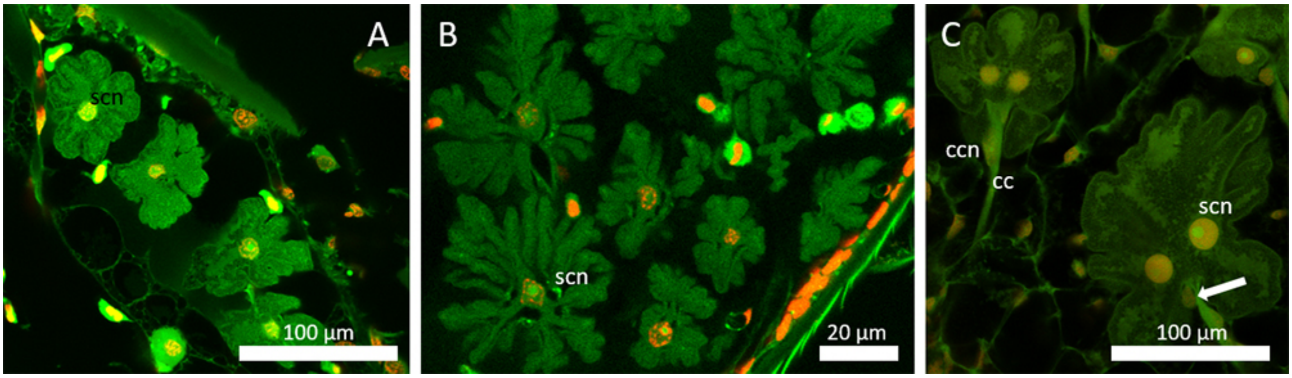

Figure 2. Structure of lobed glands as seen in optical sections obtained using confocal microscopy. Each secretory cell has only one nucleus in lateral plate glands of *Ligidium germanicum* (**A**) and *Hyloniscus riparius* (**B**). By contrast, two nuclei are present in each secretory cell in lateral plate glands of *Cylisticus convexus* (**C**) Abbreviations: scn: secretory cell nucleus, cc: canal cell, ccn: canal cell nucleus, arrow: intermediate cell nucleus. Scale bar: **A**, **C** = 100 µm; **B** = 20 µm.

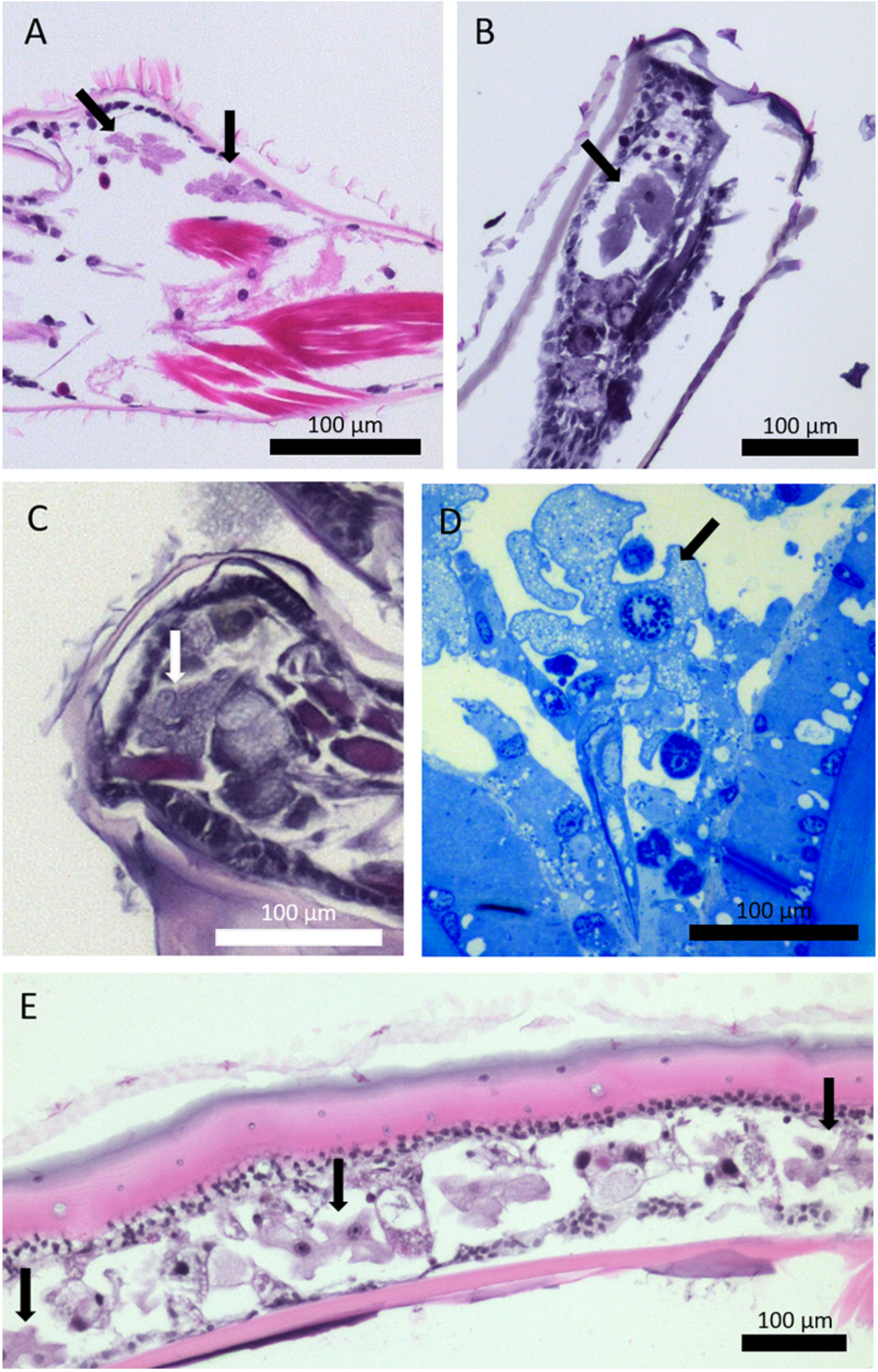

Figure 3. The presence of lobed glands in different body segments. **A**, *Alpioniscus heroldii*: paraffin section of a pereopod. Many small lobed glands (arrow) with one nucleus in the secretory cell are present; **B**, *Ligidium germanicum*: paraffin section of a lobed gland (arrow) in the antennae. The secretory cell has one nucleus; **C**, *Ligia italica*: paraffin section of a lobed gland (arrow) in the cephalothorax. The secretory cell possesses one nucleus; **D**, *Cylisticus convexus*: resin section of a lobed gland (arrow) in the cephalothorax; **E**, *Cy. convexus*: paraffin section of lobed glands (arrow) distributed dorsally, under the tergites. Scale bar: **A** – **E** = 100 µm.

We did not observe lobed glands in *He. brevicornis* and *Ty. ponticus*, endoantennal rollers belonging to Tylida.

In *M. graniger*, a Microcheta species that can be considered a creeper, few lobed glands with shallow lobes are present in the lateral plates of the pereon. Their diameter is around 30 µm. By contrast, lobed glands are numerous and have pronounced lobes in the lateral plates of the pleon and in the uropods, where they reach sizes of approximately 60 µm and 100 µm, respectively.

Among Synocheta, we examined seven species of Trichoniscidae. *Haplophthalmus danicus* Budde-Lund in Meinert, 1880, *Haplophthalmus mengii* (Zaddach, 1844), and *Androniscus roseus* (C. Koch, 1838) are creepers, while *Hy. riparius* and *Hy. adonis* are runners. *Titanethes albus* (C. Koch, 1841) and *Al. heroldii* are amphibious cave species that can morphologically also be classified as runners. In all creepers, lobed glands are less well developed in the pereon than in the pleon and uropods. *Androniscus roseus* has more than ten lobed glands with deep lobes in the uropods and each lateral plate of the pleon, with a diameter of around 50 µm. By contrast, each lateral plate of the pereon has only one small lobed gland (around 20 µm in diameter) with shallow lobes. In *Hs. danicus* and *Hs. mengii*, lobed glands in the lateral plates are completely lacking, and only lobed glands with a few shallow lobes are present in the uropods (Fig. 4). *Titanethes albus* has more than ten lobed glands in all lateral plates and the uropods. They have deep lobes and are larger than the lobed glands in other Synocheta species, around 100 µm in diameter. *Hyloniscus riparius* and *Hy. adonis* have more than 10 lobed glands in all lateral plates and uropods, and lobed glands are also present in the pereopods. In both species, all lobed glands reach sizes of 50 to 60 µm. In *A. heroldi,* lobed glands are present in all lateral plates, the uropods, the cephalothorax, and the pereopods (Fig. 2A). They reach approximately 60 µm in diameter and have deep lobes.

We included 18 species of Crinocheta, belonging to five eco-morphotypes. *Trichorhina tomentosa* and *Le. bicostulatus* are creepers, *Platyarthrus hoffmannseggii* Brandt, 1833 is an endogean non-conformist living in association with ants, *O. asellus*, *Po. scaber*, *Po. dilatatus*, *Ts. rathkii*, and *Ts. ratzeburgii* are clingers, *Philoscia muscorum* (Scopoli, 1763), *Ha. couchii*, *Pr. politus*, and *Porcelionides pruinosus* (Brandt, 1833) are runners, while *Am. versicolor*, *Armadillidium nasatum* Budde-Lund, 1885, *Armadillidium pallasii* Brandt, 1833, *Ao. officinalis*, and *C. murina* are rollers. *Cylisticus convexus* is a transitional form, classified as a conglobating clinger. In *Ta. tomentosa*, and *P. hoffmanseggii* (Fig. 5A), we found prominent lobed glands with deep lobes in the lateral plates of the pleon and uropods. They reach a size of around 100 µm and 70 µm in each species, respectively. Small glands that are visible in the pereon of *Ta. tomentosa* and *P. hoffmanseggii* (Fig. 5B) cannot be considered lobed glands, as their morphology is different, and we did not find lobed glands in the pereon of these two species. In *O. asellus*, the largest uropod glands reach a size of around 130 µm (Fig. 5C), but smaller lobed glands are visible as well. In the lateral plates of the pereon of *O. asellus*, there are only a few lobed glands with shallow lobes (Fig. 5D). They are significantly smaller than the uropod lobed glands and the lobed glands of the lateral plates of the pleon, as their size does not exceed 70 µm. *Halophiloscia couchii* possesses prominent lobed glands with deep lobes in the lateral plates and the uropods, and there are also large lobed glands in the cephalothorax. Its lobed glands reach a diameter of 110 µm in the uropods and cephalothorax and around 60 µm in the lateral plates of the pleon. *Philoscia muscorum* has very numerous and large lobed glands with deep lobes in the lateral plates and uropods. The largest uropod lobed glands in *Ph. muscorum* reach 160 µm, while the lateral plate lobed glands reach around 100 µm in size.

In *Ts. rathkii*, and *T. ratzeburgii*, we found many large lobed glands with deep and pronounced lobes, both in the lateral plates and the uropods. In both species, lateral plate lobed glands exceed 150 µm in diameter, and uropod lobed glands are larger than 200 µm. Lobed glands are also present in the cephalothorax and under tergites in these species. These glands are somewhat smaller, reaching a size of 80 to 100 µm. In *Le. bicostulatus*, we found lobed glands in the lateral plates of the pleon and in the uropods, reaching a diameter of approximately 90 µm and pronounced lobes. We did not notice lobed glands in the lateral plates of the pereon.

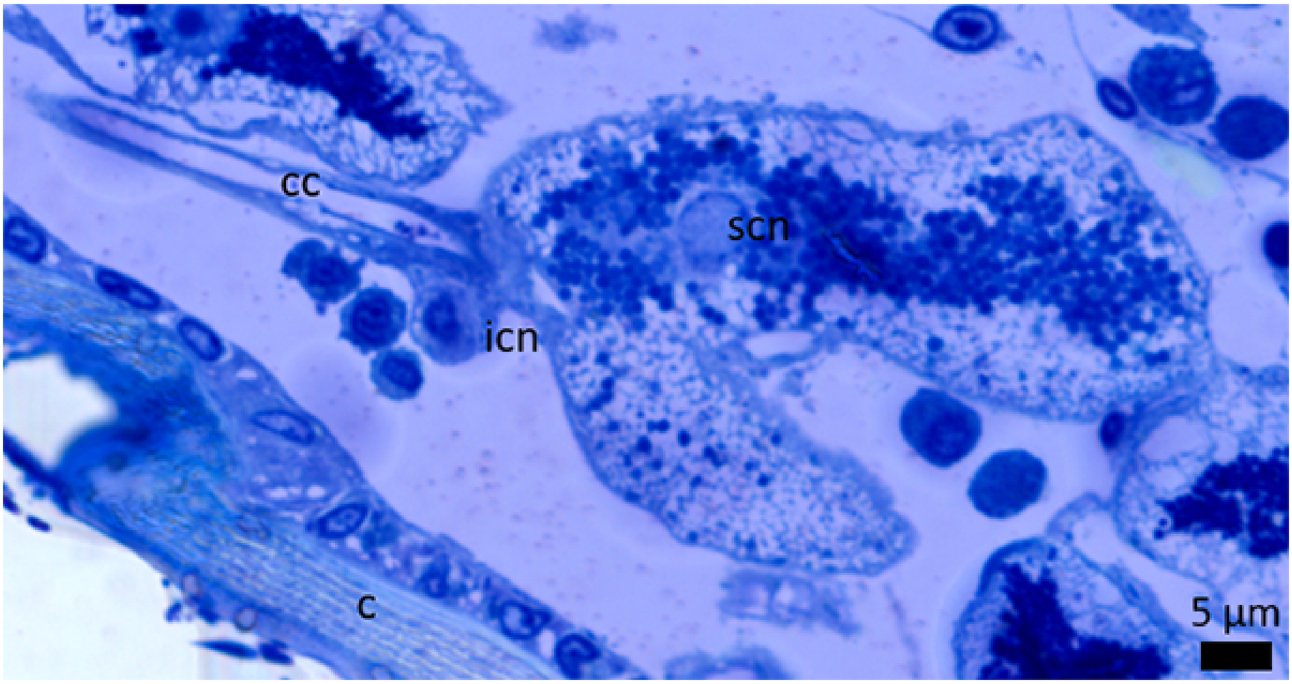

Figure 4. Uropod lobed glands in *Haplophthalmus danicus* as seen in semi-thin resin sections. While lobed glands are lacking in the lateral plates, they are present in the uropods and have the typical structure of lobed glands in Synocheta: a secretory cell with one nucleus (scn), an intermediate cell and a canal cell (cc). icn: intermediate cell nucleus, c: cuticle. Scale bar = 5 µm.

The distribution and size of lobed glands in *Pr. politus* are very similar to that observed in *Ts. rathkii* and *Ts. ratzeburgii*.

In *Cy. convexus*, lobed glands are present in all lateral plates, the uropods, the cephalothorax (Fig. 3D), the antennae, and dorsally under the tergites (Fig. 3E). They reach a size of around 80 µm and 120 µm in the lateral plates and uropods, respectively, and have intermediately pronounced lobes.

In *Po. scaber, Po. dilatatus*, and *Ps. pruinosus*, large lobed glands with deep and pronounced lobes are present in the lateral plates and uropods. The lateral plate glands reach more than 150 µm and uropod glands more than 200 µm in diameter.

Armadillidium versicolor, Am. nasatum, and *Am. pallasii* have fewer lobed glands than other Crinocheta species. They possess lobed glands in all lateral plates and the uropods. Lateral plate lobed glands in *Am. versicolor* and *Am. nasatum* measure approximately 80 µm in diameter, and the lobed glands in the uropods around 120 µm. In *Am. pallasii*, all lobed glands have a similar size, around 130 µm. We did not find lobed glands in *Ao. officinalis* and *Cu. murina* (different type of tegumental glands shown in Appendix 2).

Histochemical analysis of lobed glands

Histochemical properties of the lobed glands were investigated in six species: *Lm. germanicum, Ta. tomentosa, O. asellus, Cy. convexus, Po. scaber*, and *Am. versicolor.* Although lobed glands in the lateral plates of the pereon and those in the pleon and the uropods have a similar structure, the histochemical staining properties of their secretory product differs. Because the pattern of staining is similar in all examined species, results are only shown for *Po. scaber* (Fig. 6). Alcian blue does not stain the lateral plate lobed glands of the pereon but weakly stains the larger lobed glands of the pleon and the uropods, i.e., the uropod lobed glands, indicating the presence of macromolecules with a high density of negative charge in the uropod glands. The PAS reaction does not result in strong staining of lobed glands, which means that these glands do not contain elevated amounts of polysaccharides. Bromophenol blue stains all lobed glands, indicating

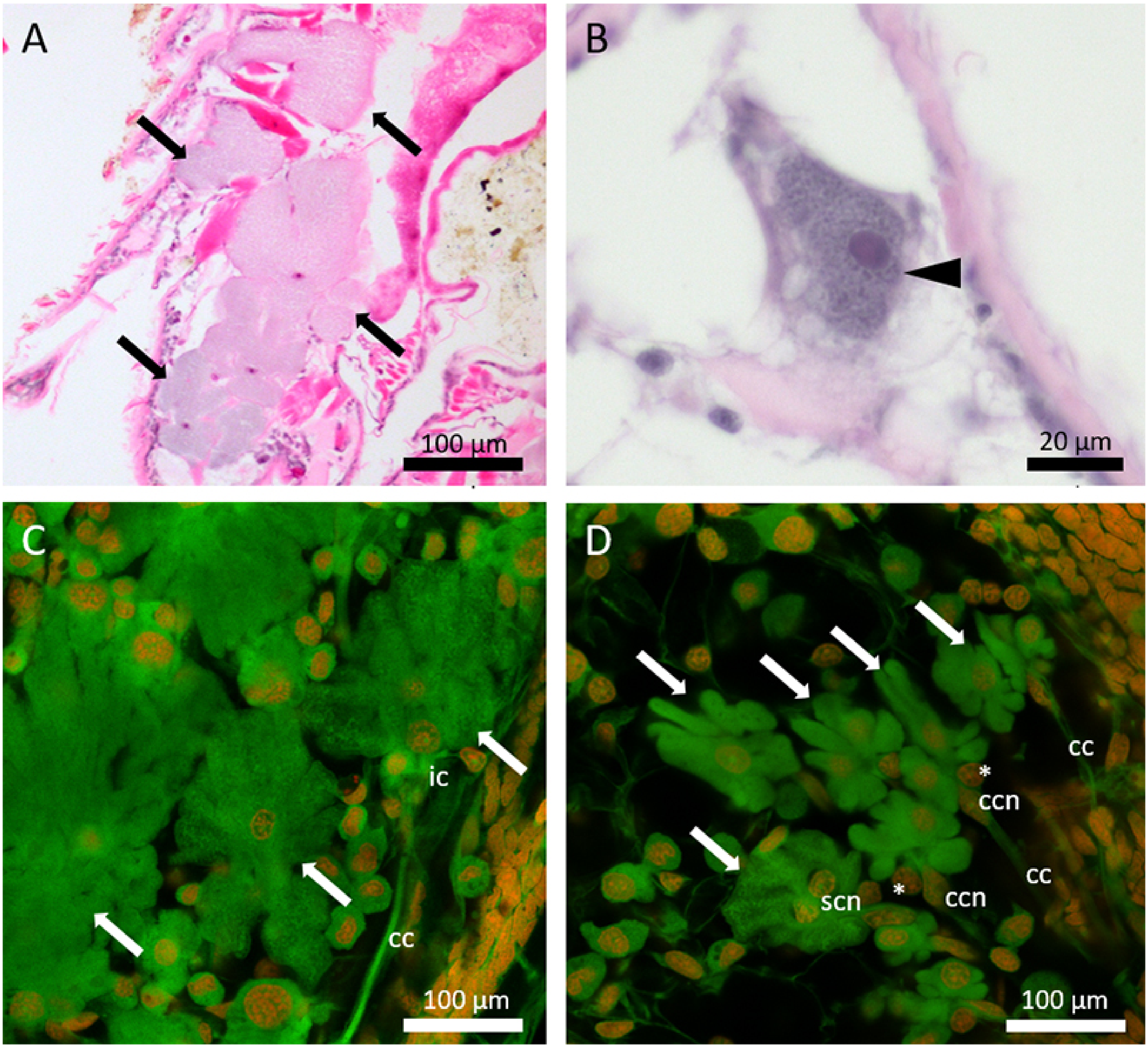

Figure 5. **A**, Lobed glands (arrows) in the uropods and lateral plates of the pleon in *Platyarthrus hoffmannseggii*. In these body segments, lobed glands are prominent; **B**, tegumental glands (arrowhead) in the lateral plates of the pereon in *Ph. hoffmannseggii* do not resemble lobed glands; **C**, uropod lobed glands (arrows) in *Oniscus asellus*. They are almost twice the size of lobed glands in the lateral plates of the pereon; **D**, lobed glands (arrows) in the lateral plates of the pereon in *O. asellus* are smaller and fewer in number. Abbreviations: scn: secretory cell nucleus; ccn : canal cell nucleus; cc: canal cell; asterisk: intermediate cell nucleus. Scale bar: **A**, **C**, **D** =100 µm; **B** = 20 µm.

the presence of proteins. Uropod lobed glands stain with greater intensity than lateral plate lobed glands, probably due to different amounts or types of proteins. Ninhydrin-Schiff staining confirms the presence of amino groups in all lobed glands, although the staining intensity is generally similar to other tissues. Sudan black does not stain the lobed glands, indicating an absence of lipids.

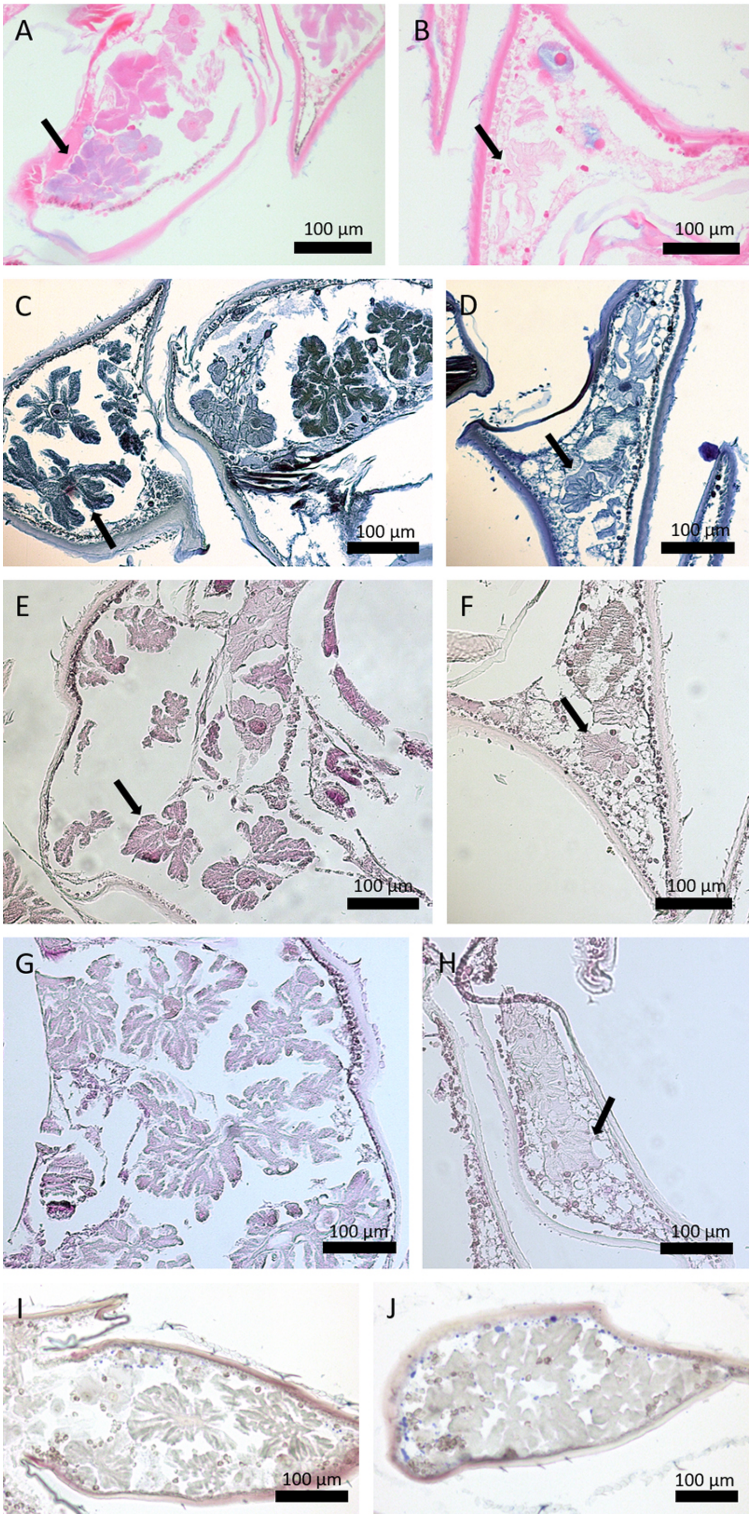

Figure 6. Different staining of lobed glands in *Porcellio scaber* as an indication of their histochemical composition. **A**, **B**, Uropod lobed glands (**A**) and lateral plate glands (**B**), stained with Alcian blue and eosin. Uropod glands stain weakly, while lateral plate glands do not. This indicates the presence of macromolecules with a high density of negative charge in the uropod glands; **C**, **D**, uropod lobed glands (**C**) and lateral plate glands (**D**), stained with bromophenol blue. Both types of glands stain strongly, confirming the presence of proteins or peptides; **E**, **F**, uropod lobed glands (**E**) and lateral plate glands (**F**), stained with the ninhydrin-Schiff stain. The staining of both types of glands demonstrates the presence of amino groups; **G**, **H;** uropod lobed glands (**G**) and lateral plate glands (**H**), stained with the periodic acid-Schiff stain. Both types of glands stain weakly, indicating that they do not contain elevated amounts of carbohydrates; **I**, **J**, uropod lobed glands (**I**) and lateral plate glands (**J**), stained with Sudan black. The absence of staining indicates that both types of glands lack demonstrable amounts of lipids. Scale bar = 100 µm.

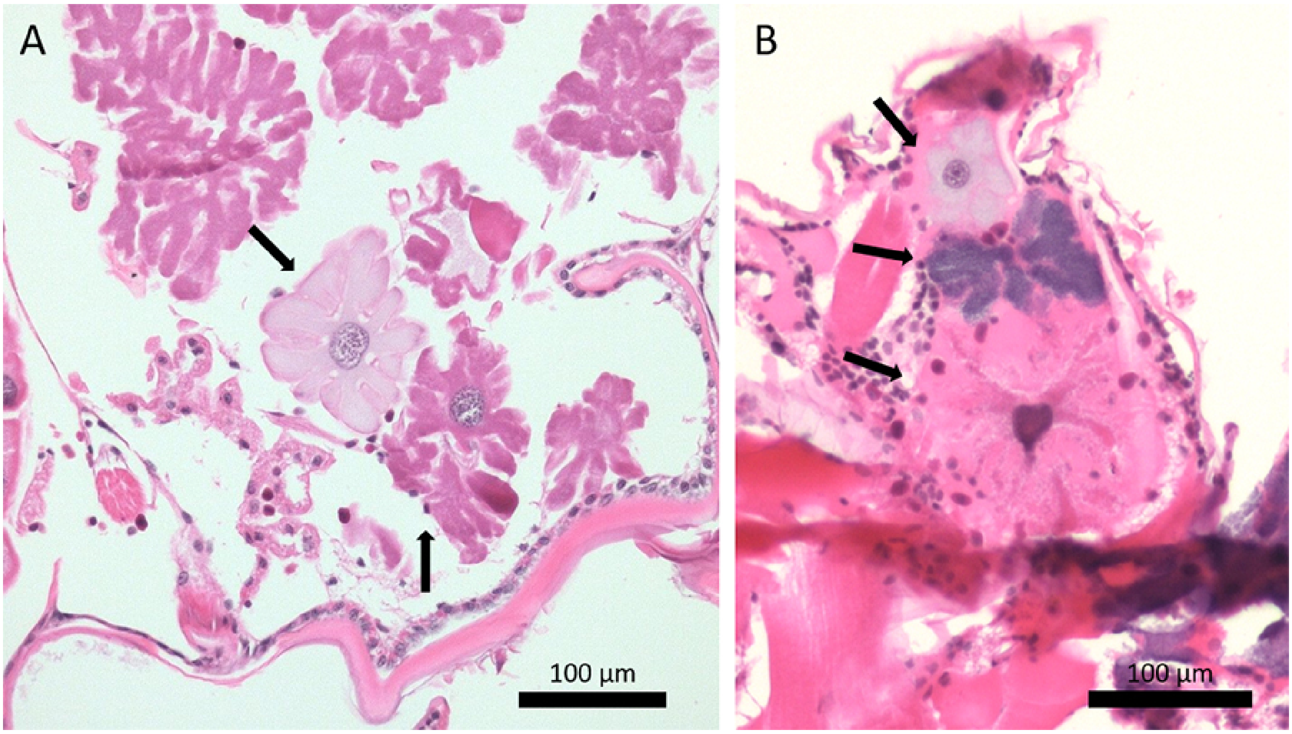

Figure 7. Paraffin sections of lobed glands stained with hematoxylin and eosin. In the same body region of an isopod, glands can stain with different intensities (arrows). **A**, Lateral plate glands of the pleon in *Porcellio scaber*. **B**, Uropod glands in *Trichorhina tomentosa*. Scale bar = 100 µm.

Certain isopods may possess several types of lobed glands

Serial histological sections stained with hematoxylin and eosin revealed that lobed glands in the same individual and body region may have different morphologies and stain with different intensities (Fig. 7). In addition to lobed glands with deep lobes, some species (in Porcellionidae and Armadillidiidae, among examined taxa) have lobed glands with two nuclei in the secretory cell but without pronounced lobes, as visible on paraffin sections (Fig. 8). Confocal microscopy shows that while lobes of the secretory cell in these glands are not visible in paraffin sections (Fig. 8A), it does possess shallow lobes (Fig. 8B, C). This implies that some species may possess several types of lobed glands.

As we noticed different types of lobed glands in the lateral plates, in serial histological sections in *Po. scaber* and *Am. versicolor*, we further performed whole-body imaging in these species. The results of whole-body imaging of cleared isopods revealed lobed glands of different colors and fluorescence properties. Some lobed glands are red in *Po. scaber* individuals when fixed in glutaraldehyde and observed with transmitted light. There are up to three such lobed glands in each lateral plate of the pereon and the third lateral plate of the pleon. Red lobed glands are absent in the remaining lateral plates of the pleon and in the uropods (Fig. 9A, B). The lobed glands are not red when *Po. scaber* is fixed in formaldehyde (results not shown). In *Am. versicolor* fixed in glutaraldehyde, no red lobed glands are visible (results not shown). Fluorescence imaging also shows lobed glands with different properties. In *Am. versicolor* fixed using glutaraldehyde, fluorescence excitation with UV light (Fig. 10A) reveals two rows of lobed glands in all lateral plates. Only the lobed glands in the proximal row fluoresce under green excitation (Fig. 10B).

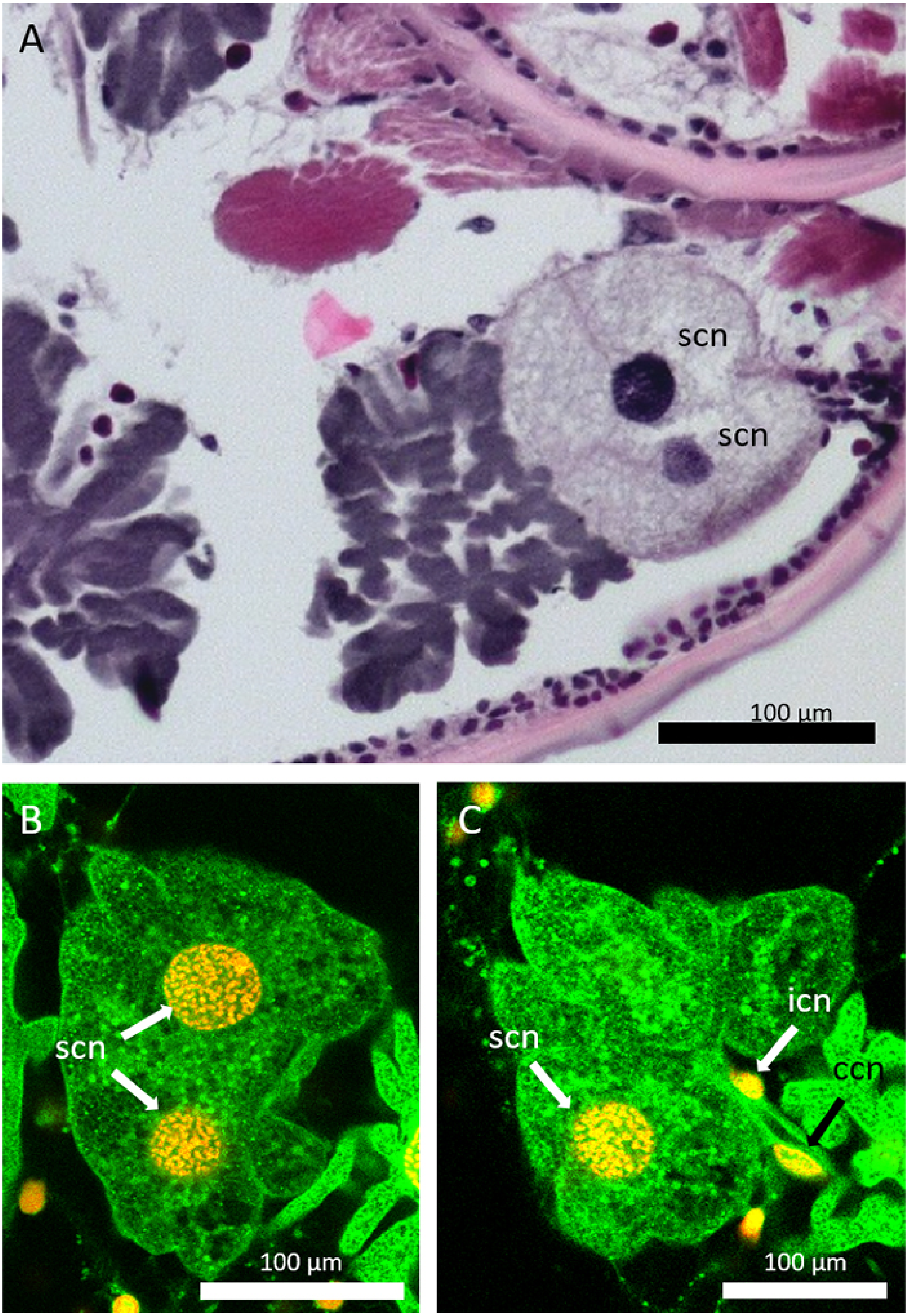

Figure 8. Various types of lobed glands in *Porcellio scaber*. **A**, Paraffin sections stained with hematoxylin and eosin; **B**, **C**, optical sections obtained with confocal microscopy. On image **A**, a binucleated gland without visible lobes is shown. On optical sections (**B**, **C**) we can see that these glands also possess a secretory cell with small lobes, a canal cell and an intermediate cell, and the location of their nuclei is similar as in other lobed glands with pronounced lobes. Abbreviations: scn: secretory cell nucleus, ccn: canal cell nucleus, icn: intermediate cell nucleus, l: lobes, cc: canal cell. Scale bar = 100 µm.

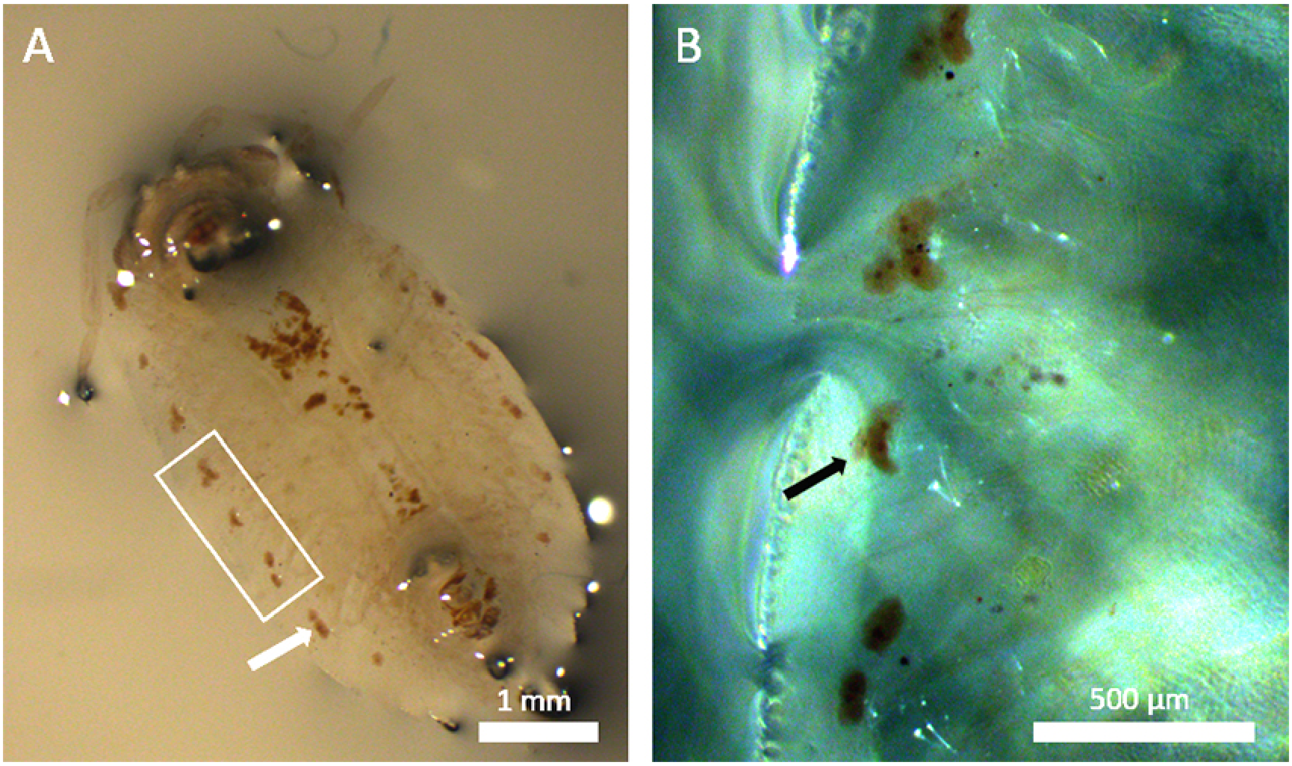

Figure 9. Red lobed glands in the lateral plates of the pereon and the third lateral plate of the pleon of *Porcellio scaber* fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde at low magnification (**A**) and higher magnification (**B**). Scale bar: **A** = 1 mm; **B** = 500 µm.

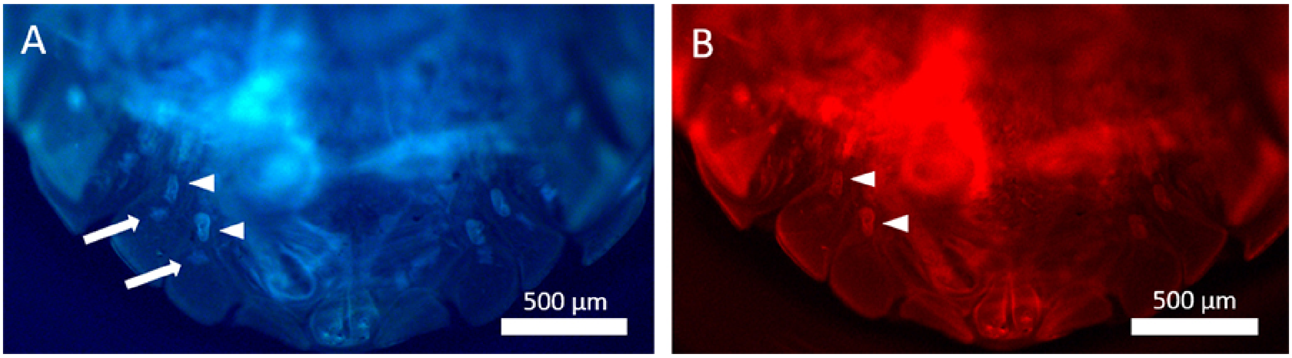

Figure 10. Lobed glands in the pleon of *Armadillidium versicolor* fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde. UV excitation reveals two sets of lobed glands (**A**), while under green excitation (**B**), only one set closer to the base of the lateral plates fluoresces. Scale = 500 µm.

DISCUSSION

Morphological characteristics of the different types of lobed glands

Lobed glands appear to be characteristic only of Oniscidea, but their presence, number, and distribution depend on the eco-morphological strategy of the species and, to some extent, on its phylogenetic position. Tricellular tegumental glands have also been reported in other Crustaceans, but their structure differs from that of the lobed glands (Talbot and Demers, 1993). Lobed glands comprise a secretory cell that is divided into several lobes, a ramified intermediate cell and an elongated duct cell, surrounded by a common basal lamina (Weirich and Ziegler, 1997). Paraffin sections of lobed glands revealed that not only do uropod lobed glands differ from the lateral plate lobed glands, but even lobed glands located in a single lateral plate or a uropod can stain with different intensities, as is the case in *Am. versicolor*, *Po. scaber*, and *Ta. tomentosa*. These differences also corresponded to differences in lobed gland morphology in the case of *Am. versicolor* and *Po. scaber*. Weirich and Ziegler (1997) reported differences in the appearance of secretory granules between uropod and lateral plate lobed glands, but our results show different staining intensities also among different lobed glands in the same body region of individual animals, indicating that some species have several types of lobed glands. Ide (1891) and Herold (1913) also noted lobed glands with different staining intensities on histological sections. They suggested that this difference is caused by differences in the penetration of the fixative into glands located at different distances from the surface. While it is possible that the variable staining intensities are due to different amount of secretory product in the glands caused by non-simultaneous discharge, we argue that differences in staining properties at least partly result from the presence of different lobed gland types. Nevertheless, the reproducible pattern of lobed glands with different staining intensities and colors following fixation, as well as differences in the shape and size of their secretory cells, show that there are several types of lobed glands at least in some isopod species. An example of this are the lobed glands in the lateral plates of *Po. scaber*. In addition to lobed glands with large and pronounced lobes, some lobed glands have barely visible lobes that stain less intensely with the selected dyes. Furthermore, *Po. scaber* possesses numerous lobed glands in each lateral plate, but only three turn red following glutaraldehyde fixation. One possible explanation is that some of the lobed glands contain substances that react with glutaraldehyde to give a red product. However, more detailed research is needed to determine the exact cause of this phenomenon.

Composition of the lobed gland secretion

Attempts to determine the composition of the lobed gland secretion have already been made, but included a limited set of species. In our research, we included six species, belonging to different phylogenetic groups and different eco-morphotypes. Histochemical analyses reveal that the lateral plate lobed glands of the pereon differ from the lateral plate lobed glands of the pleon and the uropods. The staining of the lobed glands suggests that they secrete proteins or peptides and not polysaccharides or lipids. The staining properties of the lobed glands in the pleon and the uropods differ slightly from those in the pereon in some species. The lobed glands in the pleon stain weakly with Alcian blue and hematoxylin, but do not stain with the PAS method, suggesting that they secrete strongly acidic, perhaps glycosylated, proteins. Weirich and Ziegler (1997), who observed ultrathin sections of lobed glands in *Po. scaber*, also report on differences in the appearance of secretory granules between lateral plate and uropod lobed glands. Data on the composition of the lobed gland secretions are scarce. According to Gorvett (1952), both secretions contain proteins incorporating phenol groups and arginine, but no tryptophan, sulfide groups, or carbohydrates. He determined the content of the secretion of both uropod and lateral plate lobed glands of *Po. scaber* using various chemical tests on droplets of secretion. Apart from amino acids (such as tyrosine and cysteine), Maccagno Paulucci (1952) suggested the presence of chlorides, urea, and uric acid. To determine the chemical composition of the secretion, she performed solubility and coagulation tests. Deslippe et al. (1996), by means of mass spectroscopy, confirmed that the secretion of uropod lobed glands in *O. asellus* is proteinaceous, with proteins containing high levels of glycine and proline and low levels of hydroxyproline, cysteine, and valine. According to several authors (Gorvett, 1952; Maccagno Paulucci, 1952; Deslippe et al., 1996; Weirich and Ziegler, 1997), proteins are the major component of the lobed gland secretion. Our histochemical staining results also indicate the secretion of proteins, as do the ultrastructural features of secretory cells, examined by Weirich and Ziegler (1997).

Lobed gland structure and distribution in correlation with the phylogenetic position and eco-morphotype of the terrestrial isopods

The main structural feature of the secretory cell in Diplocheta, Microcheta, and Synocheta is the presence of one nucleus, whereas in Crinocheta, the secretory cell is binucleated. The presence of one nucleus in the secretory cells of certain species and two nuclei in others was also observed by Gorvett (1951), but he attributed this difference to the size of the secretory cell, not the phylogenetic position of a species. Our results show that the number of nuclei per secretory cell does not depend on the size of the secretory cell, as lobed glands of various sizes can be found in different oniscidean groups, whereas the number of nuclei is consistent within each group.

Although a consensus regarding the phylogenetic relationships between groups within Oniscidea has not yet been reached, it is evident that regardless of which classification is followed, the structure of the lobed glands correlates with the phylogenetic position of the species, while their distribution in the body depends more on the eco-morphological strategy than the phylogenetic position of an isopod species.

We considered two published phylogenies of Oniscidea, one based on morphological traits (Schmidt, 2008) and a more recent one, proposed on the basis of a molecular analysis (Dimitriou et al., 2019). Our results show that lobed glands are present in Diplocheta, which are generally considered the sister group of all other Oniscidea (see Schmidt, 2008), while the molecular phylogeny by Dimitriou and co-workers (2019) suggested that *Ligia* Fabricius, 1798 forms a clade separate from the rest of the Oniscidea. Tabacaru and Giurginca (2020) disagree with this, stating that morphological characteristics of *Ligia* include synapomorphies of the Oniscidea that are unlikely to have arisen due to convergence and did not support the exclusion of *Ligia* from Oniscidea. In the examined representatives of Diplocheta, the distribution of the lobed glands in the body segments follows the pattern typical of most oniscidean runners.

Even though genetic markers show a close relationship between Sphaeromatidae and *Ligia* (see Dimitriou et al., 2019), we did not find lobed glands in *S. serratum*. The absence of lobed glands in *As. aquaticus* was expected since it has only a distant relationship with Oniscidea (Dimitriou et al., 2019; Tabacaru and Giurginca, 2020). Gorvett (1951) also did not observe lobed glands in *As. aquaticus*. Although there are many more isopod taxa that we could not examine, our results indicate that lobed glands are indeed characteristic of Oniscidea, if we consider *Ligia* a part of this group. One alternative is that *Ligia*, Oniscidea and certain other groups of isopods shared a common ancestor with lobed glands that were later either lost in some groups or the related taxa were not included in our analysis. Another alternative is that *Ligia* evolved lobed glands independently as it transitioned to land.

In the two examined representatives of Tylida, *Ty. ponticus* and *He. brevicornis*, we did not observe lobed glands. There are two possible explanations for their absence, one correlating with the phylogenetic position of this parvorder and the other with their eco-morphological strategy. The hypothesis that Tylida have an isolated position from other oniscids and separated early from the Ligiamorpha (see Tabacaru and Giurginca, 2020) may explain the lack of lobed glands. On the other hand, a reduction of the size and number of lobed glands is noticeable in other rollers as well.

The creepers *M. graniger*, *An. roseus*, *Ta. tomentosa*, *Ph. hoffmannseggii*, and *Le. bicostulatus* show similarities in lobed gland distribution, even though they are not closely related. Most of them have prominent lobed glands in the lateral plates of the pleon and in the uropods but have only a few small lobed glands in the pereon or even completely lack them in this body region. The most extreme case of lobed gland reduction in creepers was noticeable in *Haplophthalmus* Schöbl, 1860; while species from this genus have numerous tegumental glands, they only resemble lobed glands in structure in the uropods. Our findings are in agreement with Gorvett's results (1951). He noticed 5 lobed glands with few, very shallow lobes in the uropods of *Hs. danicus*, but none in the lateral plates. *Platyarthrus* Brandt, 1833 and *Trichorhina* Budde-Lund, 1908, presently included in the family Platyarthridae, are likely not closely related (Dimitriou et al., 2019), yet the lack of lobed glands in the lateral plates of the pereon is common to both species. At least in the case of *Ph. hoffmannseggii*, this is not in agreement with the reports of Gorvett (1951), who noticed one lobed gland per lateral plate of the pereon in *Ph. hoffmannseggii*, with single nuclei in the secretory cells. By contrast, Herold (1913), reports that lobed glands are not present in this species in the first four lateral plates of the pereon. He noticed lobed glands only in the lateral plates of pereonites 5 to 7, with only one lobed gland in each segment.

However, the structure of the glands that Herold (1913) describes is simpler than that of lobed glands. Ter-Poghosian (1909) described the glands found in these segments in *Ph. hoffmannseggii* as different from lobed glands. Similar to our findings, Ter-Poghosian (1909), Herold (1913) and Gorvett (1951) reported many lobed glands with binucleated secretory cells in the uropods. Lobed glands are also apparently only present in the pleon and the uropods of *Le. bicostulatus*, a creeper belonging to Trachelipodidae. In other species of this family that are clingers, lobed glands are generally abundant in the pereon as well as the pleon.

In clingers, lobed glands with pronounced lobes are abundant in all lateral plates and the uropods (Porcellionidae) and may additionally be present in the cephalothorax and under tergites (*Ts. rathkii*, and *Ts. ratzeburgii*). The clinger *O. asellus* possesses much smaller lobed glands in the lateral plates of its pereon than other clingers. This was also noticed by Gorvett (1951), who stated that while lobed glands in the lateral plates of the pereon are significantly smaller and less numerous, the number of the lobed glands in the uropods is the highest compared to all other species he examined. Verhoeff (1908), on the other hand, claimed that *Oniscus* Linnaeus, 1758 does not possess lobed glands or that there are only remnants present in pereonite 1, likely due to the difficulty of observing these small glands (Herold, 1913).

In runners, the distribution and abundance of lobed glands are similar as in clingers. In *Ps. pruinosus* (Porcellionidae), large lobed glands with well-defined lobes are distributed in the lateral plates of the pereon and pleon and in the uropods. In *Pr. politus* (Agnaridae), additional lobed glands are present in the cephalothorax and under tergites. Similarly, runners belonging to Synocheta (*Hy. riparius*, *Hy. adonis*, *Ti. albus*, and *Al. heroldii*) and Diplocheta (*Li. italica* and *Lm. germanicum*), have lobed glands in the lateral plates of the pereon, the pleon, and in the uropods. In addition, some runners from these two parvorders (*Hy. adonis*, *Hy. riparius*, *Al. heroldii*, and *Lm. germanicum*) have lobed glands in the pereopods, where they have not been reported before, or in the cephalothorax (*Al. heroldii*, *Lm. germanicum*, and *Li. italica*). To our knowledge, only Maccagano Pauluci (1951) reported on the presence of lobed glands in parts of the body other than lateral plates and uropods, finding typical lobed glands in the cephalothorax of *Leptotrichus panzerii* (Audouin, 1826). In the genus *Ligia*, Weber (1881) and Herold (1913) observed the presence of lobed glands in the uropods and lateral plates of *Ligia oceanica* (Linnaeus, 1767), although it is questionable if the glands they observed were indeed lobed glands (Gorvett, 1951). Huet (1883), on the other hand, did not observe these glands in the same species. Gorvett (1951) claimed that typical lobed glands, although in a rudimentary form, are only present in the uropods and lateral plates of young specimens of *Li. oceanica* but absent in mature individuals. To our knowledge, there have been no reports of the lobed gland presence in the cephalothorax and antennae of *Li. oceanica*. Regarding *Ligidium* Brandt, 1833, we only found information on *Ligidium hypnorum* (Cuvier, 1792) (Gorvett, 1951), reporting lobed glands in the uropods and lateral plates but not in the cephalothorax, antennae, or pereopods.

In rollers, we noticed a reduction in the number and size of the lobed glands. In the examined representatives of Armadillidiidae, the lobed glands are much less numerous than in most other Crinocheta. Roller species in Armadillidae lack typical lobed glands altogether, as do the tylid rollers *Ty. ponticus* and *He. brevicornis*. Regarding Armadillidae, Maccagno Paulucci (1951) reported the presence of secretory pores, but as she did not observe histological sections, we argue that the observed pores belong to tegumental glands of a different type. Another species capable of conglobation is *Cy. convexus* (Cylisticidae) but is considered a conglobating clinger (Schmalfuss, 1984). In this species, lobed glands are present in all lateral plates, the uropods, the cephalothorax, the antennae, and under the tergites, as in *Ts. ratzeburgii* and *Pr. politus*. As *Cy. convexus* is a transitional form, the distribution of glands in its body likely reflects that is not a true roller, as it resembles the distribution of trachelipodid clingers and the runner *Pr. politus*. Herold (1913) mentions that gland secretion is visible all over the dorsal body surface in specimens of *Cy. convexus* fixed in ethanol, an observation we can confirm.

We also studied amphibious species: *Al. heroldii* (see Culver and Sket, 2002; Bedek, 2019), *Ti. albus*, *Li. italica*, (runners), in which lobed glands are numerous and distributed across the body, and *Ty. ponticus* (roller) that lacks lobed glands completely. These results indicate that the habitat and humidity preferences of a species have little or no effect on the presence and distribution of lobed glands and that these are largely determined by the eco-morphotype of the species and its phylogenetic position.

Lobed gland distribution and defensive strategies in terrestrial isopods

The observed differences in lobed gland distribution between species may be explained in the context of the glands' defensive function. The smaller number of lobed glands observed in creepers and rollers may be due to the fact that the glands are less important to their anti-predatory defense. As much less is known about the mode of action of lateral plate lobed glands, we can only speculate why their reduction occurred in creepers, but it is possible that the small size of these animals and hiding in crevices exposes them to a limited set of predators, necessitating only the presence of uropod lobed glands. Rollers are difficult to prey upon due to their thick exoskeletons and globular shape, possibly reducing the need for defensive glands. On the other hand, the ventral side of the body in clingers that use tonic immobility as a defensive mechanism (Quadros et al., 2012; Tuf et al., 2015) is more susceptible to attacks, therefore the presence of glands on the tergites, cephalothorax and pereopods could be of significance as an additional protective method. Similarly, runners have long pereopods that are prone to attacks from predators, hence the need of lobed glands in the pereopods as well.

The connection between the distribution of the lobed glands in the body and the eco-morphotype of the species points to the association of the glands with their specific strategy of defense against predators. Gorvett (1956) and Bristowe (1941) supported the hypothesis that the secretion of lobed glands is distasteful to spiders, resulting in them avoiding terrestrial isopods as prey, although only the reactions of spiders that are not specialist woodlouse predators, were observed. Gorvett and Bristowe graded the distastefulness of five species, *Po. scaber*, *Am. vulgare*, *O. asellus*, *Ph. muscorum*, and *Ph. hoffmannseggii* and claimed that the distastefulness to spiders correlates with the relative degree of development of the lobed glands. According to them, *Ph. muscorum* is the most tasteful species, whereas other species are less tasteful due to more abundant lobed glands. An exception to this principle is *Ph. hoffmannseggii* that is protected by living in association with ants. Our results do not agree with their grading since we showed that *Ph. muscorum* has more abundant lobed glands than *O. asellus* and most likely *Am. vulgare* as well, considering our results on the closely related roller, *Am. versicolor*, as well as other reports on the number of lobed glands in *Am. vulgare*. Tretzel (1961) examined prey preferences of the spider *Coelotes terrestris* (Wider) and observed that this species got entangled in the sticky secretion that isopods released from their uropods when attacked. According to Sutton (1970), the lobed gland secretion repelled lycosid spiders only if more adequate prey was available. Experiments that involved woodlouse specialists, such as *Dysdera* Latreille, indicated that the lobed gland secretion is not an effective repellant against this spider genus (Bristowe 1941; Pekar et al., 2015). Nevertheless, the results of various studies on the effect of lobed glands on spider predation showed that generalist spiders truly fail to overcome the defense mechanisms of isopods (Gorvett, 1956; Toft and Macías-Hernández, 2017), while specialist spiders might be well adapted to preying on isopods and are therefore able to overcome these defenses, including the secretion of lobed glands (Toft and Macías-Hernández, 2017). Deslippe et al. (1996) noticed the same repelling effect of the uropod secretion of *O. asellus* on ants, which withdrew after contacting the secretion. The secretion affected the mobility of the ants by gluing their legs together or even gluing together clusters of ants (Deslippe et al., 1996). They concluded that the uropod gland secretion acts as a mechanical deterrent and does not have a toxic effect, since the ants that managed to free themselves from the hardened secretion recovered without consequences. Deslippe et al. (1996) did not notice a discharge from the lateral plate lobed glands, even though they are present in *O. asellus*. Experiments performed with the myrmecophilous species *Ph. hoffmannseggii* (see Gorvett and Taylor, 1960) resulted in similar observations. When attacked by ants from a different nest, *Ph. hoffmannseggii* secreted from the uropods. The discharge was only stimulated after ants bit the uropods. As reported in *O. asellus*, Gorvett and Taylor (1960) found that an ant attack did not cause secretion from the lateral plates in *Ph. hoffmannseggii*. They attributed this to lateral plate lobed glands being poorly developed in this species, but we demonstrated that this species actually lacks lobed glands in the pereon. Based on their observations, they argued that lobed glands first evolved as a deterrent to spiders, but they can protect from any invertebrate with a similar mode of predation.

CONCLUSIONS

By analyzing the distribution and characteristics of the lobed glands, we reached the following conclusions:

- Lobed glands are present in Oniscidea but have not been observed in other examined isopods.
- Only Crinocheta representatives have a binucleated secretory cell, indicating that the number of nuclei per secretory cell does not depend on the size of the secretory cell itself, but on the phylogenetic position of the species.
- The eco-morphological strategy of isopods is the most important factor influencing the distribution of lobed glands in their bodies. Habitat preferences do not demonstrably affect the distribution of lobed glands.
- Lobed glands are not present only in the lateral plates and uropods, but in some species also in the cephalothorax, the antennae, under tergites, and in the pereopods.
- Clingers and runners have the most prominent lobed glands, present in almost all body segments. In creepers, lobed glands are reduced in number or lost in the pereon, while in rollers they are reduced in number or lacking altogether.
- Histological and optical sections, as well as tissue-clearing, confirmed that there are several types of lobed glands present in some terrestrial isopods.
- Histochemical analyses suggest that the secretory product of uropod glands slightly differs from the secretory product of lateral plate glands. However, the secretory product of lobed glands in all body segments contains proteins or peptides.
- Lobed glands probably serve defensive functions.

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Author Contributions

Conceptualization and Design: NC, MV. Performed research: NC, MV. Acquisition, analysis and interpretation of data: NC, MV, VŽ. Preparation of figures and tables: NC, MV. Writing – original draft: NC. Writing – critical review and editing: MV, VŽ.

Consent for publication

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

Competing interests

The authors declare no competing interest.

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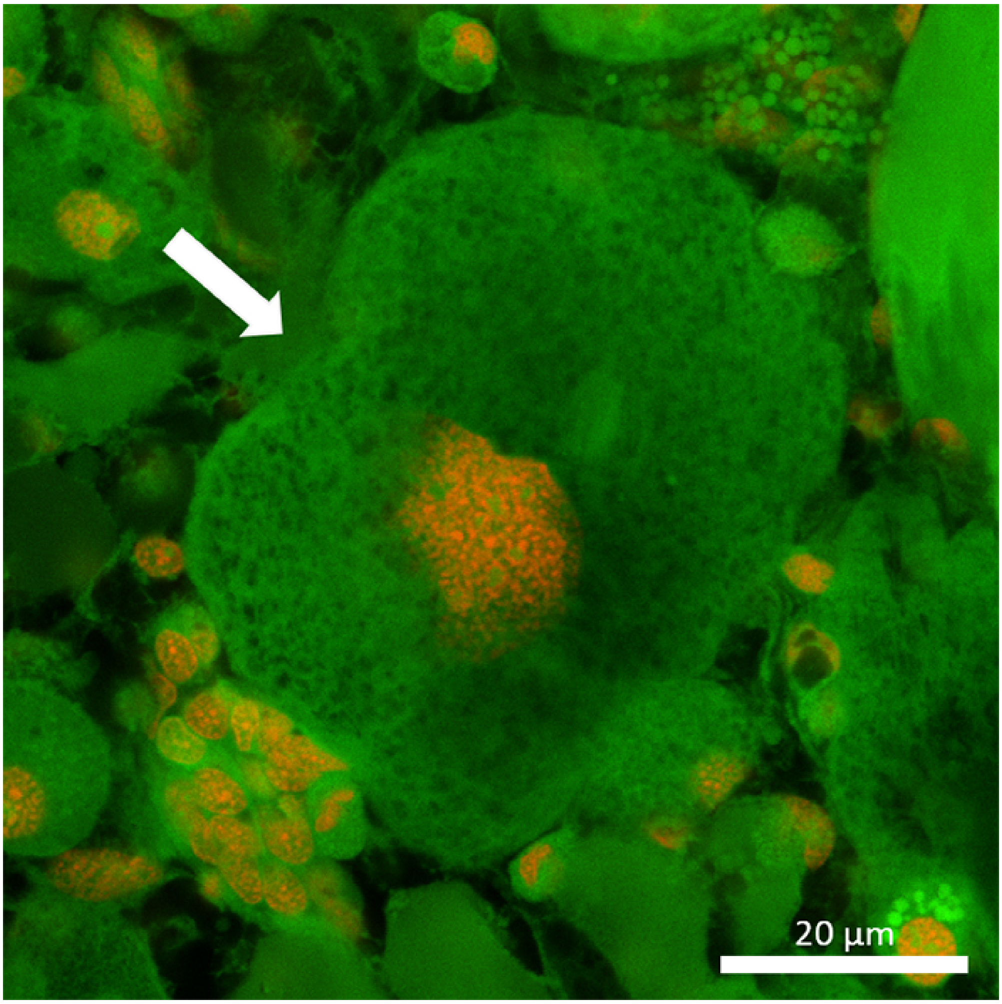
Appendix 1.

List of examined species with the localities from which they were collected

Species	**Locality**
Asellota	
Asellus aquaticus	Ljubljana, Slovenia
Sphaeromatidea	
Sphaeroma serratum	Ankaran, Slovenia
Oniscidea, Diplocheta	
Ligia italica	Strunjan, Slovenia
Ligidium germanicum	Lubljana, Slovenia
Oniscidea, Tylida	
Helleria brevicornis	Purchased from Insectenliebe.de
Tylos ponticus	Rizokarpaso, Cyprus
Oniscidea, Microcheta	
Mesoniscus graniger	Veternica Cave, Croatia
Oniscidea, Synocheta	
Haplophtalmus danicus	Črnomelj, Slovenia
Haplophtalmus mengii	Središče ob Dravi, Slovenia
Alpioniscus heroldii	Vjetrenica Cave, Bosnia and Herzegovina
Androniscus roseus	Črnomelj, Slovenia
Hyloniscus adonis	Cerknica, Slovenia
Hyloniscus riparius	Središče ob Dravi, Slovenia
Tithanetes albus	Planina cave, Slovenia
Oniscidea, Crinocheta	
Platyarthrus hoffmannseggii	From culture of Mátyás Farkas (Budapest, Hungary)
Trichorchina tomentosa	Črnomelj, Slovenia
Oniscus asellus	Stuttgart, Germany
Halophiloscia couchii	Strunjan, Slovenia
Philoscia muscorum	Hotederšica, Slovenia
Cylisticus convexus	Črnomelj, Slovenia
Armadillidium nasatum	Deskle, Slovenia
Armadillidium palasii	Komen, Slovenia
Armadillidium versicolor	Drava River, Slovenia
Armadillo officinalis	Purchased from Insectenliebe.de
Cubaris murina	Purchased from Insectenliebe.de
Porcellio dilatatus	São Miguel Island, Azores, Portugal
Porcellio scaber	Črnomelj, Slovenia
Porcellionides pruinosus	Spodnji Kamenščak, Slovenia
Levantoniscus bicostulatus	Filousa, Cyprus
Trachelipus rathkii	Črnomelj, Slovenia
Trachelipus ratzeburgii	Hotederšica, Slovenia
Protracheoniscus politus	Lubljana, Slovenia

Appendix 2.



A tegumental gland in a uropod of *Cubaris murina*. Even though *Cu. murina* possesses numerous large tegumental glands, they do not resemble lobed glands. Scale bar = 20 µm.