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‘Cejkaian tubules’ in the posterior midgut of terrestrial Enchytraeidae (Oligochaeta)

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Abstract

More than one hundred years ago, Bohumil Čejka described peculiar elongate tubules in the posterior region of the intestine of *Hepatogaster birulae*, a new terrestrial enchytraeid species collected in North-East Siberia. The tubules have no cilia but a proper epithelium and they run parallel to the longitudinal axis of the intestine over several segments, inside the intestinal epithelium but in close contact with the blood sinus. The tubules end blindly anteriorly and with a porus to the intestinal lumen posteriorly. The number of tubules increases from posterior to anterior due to bifurcations, and their diameter decreases. Čejka hypothesized that these tubules are glands that provide secretions for the final process of digestion or that aid in the egestion of faeces. He found them only in one species, *Hepatogaster birulae*, which was later synonymized with *Henlea ochracea*. In recent years we screened a large number of terrestrial enchytraeids in vivo and found these peculiar tubules in two further species of *Henlea*, in one species of *Oconnorella* and in thirteen species of *Fridericia*. The pores of these tubules are always located near the transition from midgut to hindgut. The tubules vary among species in extent and branching pattern, and several types can be distinguished. We suggest naming the structures ‘Cejkaian tubules’ in honour and memory of the finder, whose last publication dates from 1914. ‘Cejkaian tubules’ were not found in every species of *Henlea* and *Fridericia*, and they seem to be absent in other genera, but techniques other than in vivo light microscopy are required to confirm their absence with certainty. As to their function, we hypothesize the opposite of Čejka, not secretion but resorption, possibly of water, similar to the colon in tetrapod vertebrates.

Keywords Intestine | invertebrates | Clitellata | Annelida | live investigation

1. Introduction

Knowledge and understanding of the digestive tract in enchytraeids is important for at least four reasons: 1) In contrast to other microdrile oligochaete taxa, differentiations of the intestine in Enchytraeidae are highly diverse and are used to recognize genera and species (Nielsen & Christensen 1959, Kasprzak 1984, Schmelz & Collado 2010). 2) The pattern of characters associated with the gut may be useful for phylogenetic reconstruction, for understanding evolutionary pathways or for interpreting differences in the autecology and lifestyle of the species. 3) In more general terms, a better knowledge of the different gut regions should increase our understanding of the process of digestion, which includes several steps such as physical and chemical diminution of particles, ingestion of nutrients and absorption of water. Different gut regions may be specialized for specific functions, however the simple question ‘what happens where’ in the gut of enchytraeids has rarely been addressed (but see Gelder 1984, Mothes-Wagner et al. 1996, Reichert et al. 1996). 4) Finally, enchytraeids feed on the medium in which they live, like many other oligochaetes, and alter it during passage through the gut (Didden 1993, Haimi & Siira-Pietikäinen 2003). Therefore knowledge of gut structure and function

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should help to understand the contribution of this group of animals to the formation, maintenance or alteration of the ecosystems of which they are part.

The digestive tract of enchytraeids follows the general oligochaete model of a 'tube within a tube', running straight or with slight sinuosities through the body from the anterior mouth to the posterior anus (Fig. 1). In cross-section it consists of the gut epithelium proper, surrounding blood sinus and an envelope of mesodermal tissue, differentiated into musculature and chloragocytes, the latter in contact with the coelomic fluid. A dorsal blood vessel rises from the blood sinus in the anterior body region and projects anteriad to the head region (Fig. 1). In developmental terms, there are three gut regions, the ectodermal foregut, the endodermal midgut and the ectodermal hindgut, accordant with the ground plan of annelids (Westheide & Rieger 2006). In functional terms, five gut regions are distinguished in enchytraeids: buccal cavity, pharynx, oesophagus, intestine and rectum (Gelder 1984, Mothes-Wagner et al. 1996).

The greatest variety of light-microscopically distinguishable differentiations is found in the posterior foregut and the anterior midgut, a region equivalent to pharynx and oesophagus, and the variations comprise three types of outgrowth structures and several cellular modifications. 1) Pharyngeal glands are strongly developed in all enchytraeids, but are of varying shape and arrangement among taxa. These solid glands form a functional unit together with the dorsal pharyngeal pad, the organ of food uptake in most oligochaetes (Stephenson 1930, Jamieson 1981, Purschke 2003). According to Ude (1977), all digestive enzymes are produced here. 2) Oesophageal appendages are tube- or sponge-like outfoldings of the gut epithelium in segments III to VII, and they differ among taxa in presence/absence, shape, size, histology and mode of attachment to the gut (Schmelz & Westheide 2000). 3) Intestinal diverticula are sac- or pouch-like outgrowths of the intestine located more posteriorly, usually in segments VI to VIII. Again presence/absence, shape, size and position are taxon-specific (Schmelz & Collado 2010). Cell modifications include the so-called 'chylus cells' (Michaelsen 1886, Schmelz 2003) with intracellular canals, present only in *Fridericia*.

Only few morphological or histological differentiations have been described in the posterior gut region. A field of ventrally inflated and vesicular gut epithelium, named 'pars tumida' in Schmelz et al. (2008) was found in terrestrial taxa (Rota 1995, Rota & Healy 1999, Schmelz et al. 2008, 2011, Schmelz & Collado 2013) and appears to be part of the standard equipment of the enchytraeid gut. Rota et al. (2007) distinguished a caudal gut dilatation in some species of the exclusively marine genus *Grania* and termed it 'rectal ampulla'. Gelder (1984) documented a gut region-specific presence of various digestive enzymes in *Lumbricillus lineatus* (Müller) and Mothes-Wagner et al. (1996) described region-specific ultrastructural differentiations of the intestinal epithelium; both studies include the posterior gut region.

Here we describe one more differentiation of the intestinal epithelium. Its discovery goes back to Bohumil Čejka (1910). More than one hundred years ago, he described two new enchytraeid species collected in North-East Siberia and ascribed them to a new genus, *Hepatogaster*. According to Čejka (1910), one of these species, named *H. birulae*, has peculiar elongate tubules in the posterior region of the intestine (Fig. 2). The tubules have no cilia but a proper epithelium and they run parallel to the longitudinal axis of the intestine over several segments, inside the intestinal epithelium but in close contact with the blood sinus. The tubules end blindly anteriorly, and a posterior porus connects with intestinal lumen. The number of tubules increases from posterior to anterior due to bifurcations, and their diameter decreases. Čejka hypothesized that these tubules are glands that provide secretions for the final process of digestion or that aid in the egestion of faeces. He states explicitly that these tubules are absent in the second species, *H. sibiricus*, but according to a figure (see Fig. 2D), they seem to be present also in this species.

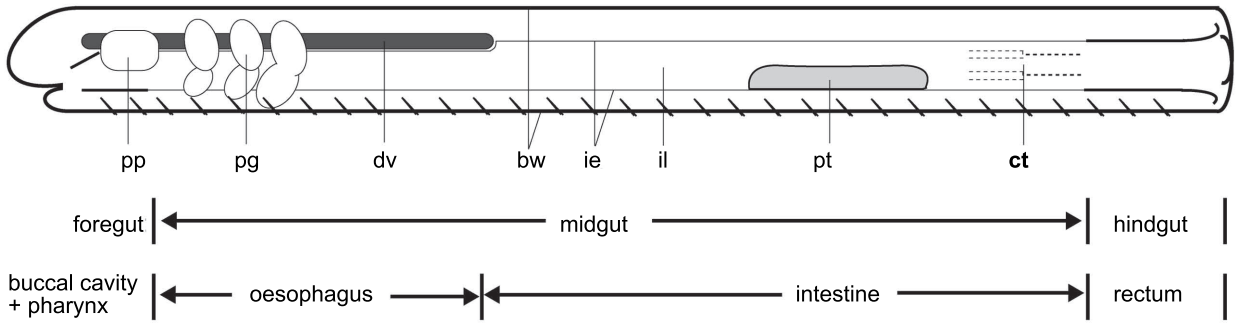

Figure 1. Gut regions in enchytraeids, a generalized scheme, lateral view, mouth to the left.

Thick lines – cuticle, **bw** – body wall with chaetae (lateral chaetae omitted), **ct** – Cejkaian tubules, **dv** – dorsal blood vessel, **ie** – intestinal epithelium, **il** – intestinal lumen, **pg** – pharyngeal gland, **pp** – pharyngeal pad, **pt** – midgut pars tumida.

In the subsequent literature these tubules found little interest and were mentioned only as possible distinguishing characters between the genera *Hepatogaster* and *Henlea* (Welch 1920, Stephenson 1922, 1930), an option rejected by Černosvitov (1931). Later the genus was united with *Henlea* (Nielsen & Christensen 1959) and the species was united with *Henlea ochracea* (Eisen, 1878) (Nurminen 1973) - hence the nomenclaturally valid name of Čejka's species with gut tubules is currently *Henlea ochracea*, pending a future revision of the nominal species. Nurminen did not investigate these tubules in his specimens from the Canadian Arctic Archipelago; he considered them a 'useless character' even at the species level (Nurminen 1973: 408).

In recent years we screened a large number of terrestrial enchytraeids in vivo, and we found these peculiar tubules in two further species of *Henlea*, in one species of *Oconnorella* and in thirteen species of *Fridericia*. We also found them to be species-constant and morphologically diverse among species. A short note on these tubules in *Fridericia* was published previously (Schmelz 2003: 43). We suggest naming the structures 'Cejkaian tubules' in honour and memory of the finder, whose last publication is from the ominous year 1914. In the following we present Čejka's original text, translated from German to English by the first author, and we describe these gut structures based on observations in living specimens.

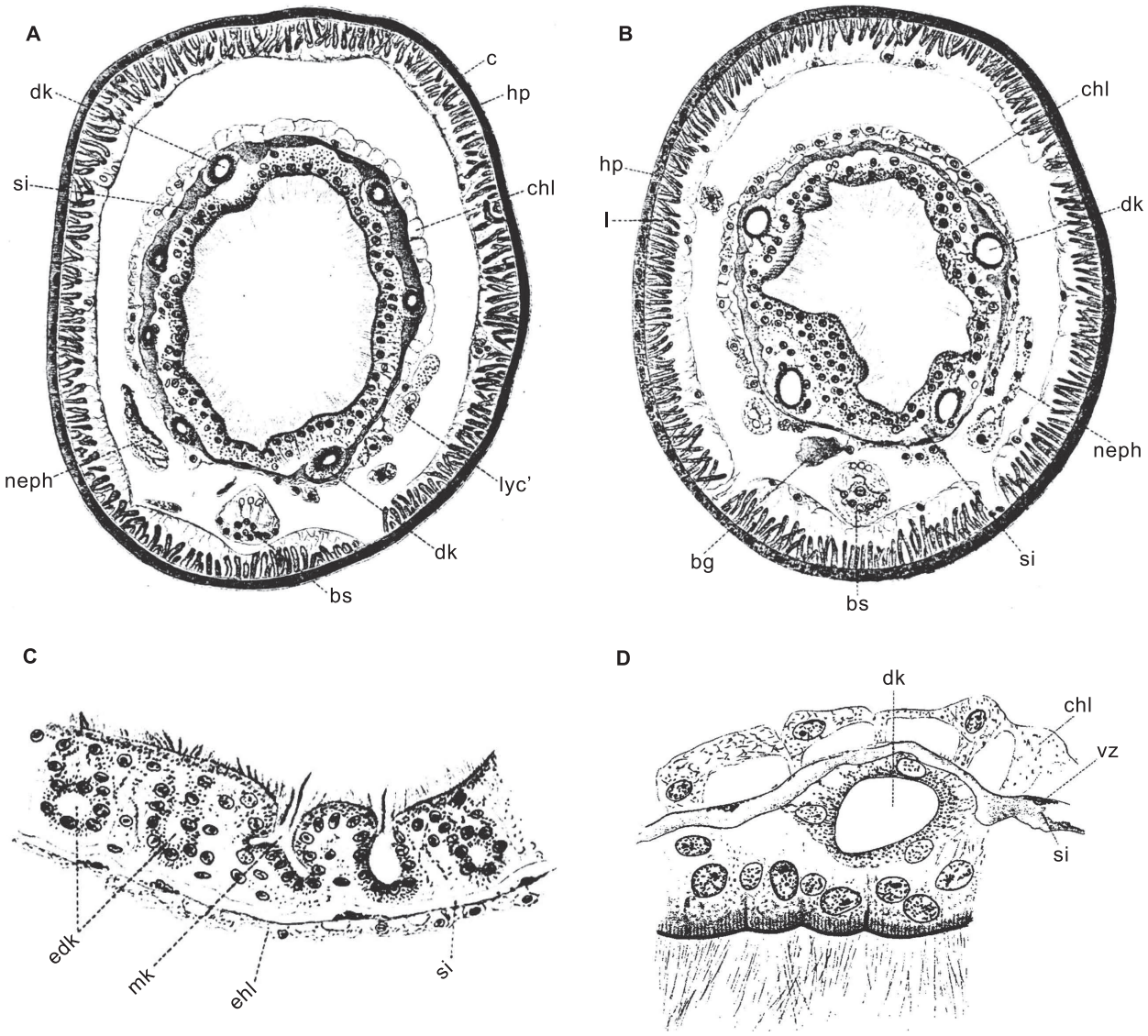

Figure 2. Illustrations of intraintestinal ('Cejkaian') tubles in Čejka (1910). (**A–C**) *Hepatogaster birulae*. (**D**) *Hepatogaster sibiricus*. (**A**) Posterior body part, cross-section (Čejka 1910, Pl. II Fig. 17). (**B**) Posterior body part, cross section, posterior to section in A (Čejka 1910, Pl. II Fig. 21). (**C**) Opening of intestinal tubules (Čejka 1910, Pl. II Fig. 20). (**D**) Intestinal tubule, cross-section, detail (Čejka 1910, Pl. III Fig. 26). **bg** – ventral blood vessel, **bs** – ventral nerve cord, **c** – cuticle, **chl** – chloragocytes, **dk** – intestinal (Cejkaian) tubule, surrounded by blood sinus, **hp** – epidermis, **l** – longitudinal muscle layer of body wall, **lyc** – coelomocytes, **neph** – nephridium, **si** – blood sinus, **vz** – 'vasothelial' cells inside intestinal blood sinus. Acronyms original, explanations translated (R. M. Schmelz).

2. Material and Methods

Observations were made during the process of identifying large numbers of individuals to species level for various projects in the field of soil ecology and soil ecotoxicology in Europe. Most of the results are not yet published (projects LOEWE and EcoFINDERS, but see Holmstrup et al. 2012, Coulson et al. 2013, Schmelz & Collado 2013). Some species from freshwater and marine sediments were included as well. Altogether more than 30.000 individuals and more than 100 species were screened, covering the majority of genera known from Europe, except *Hemienchytraeus*, *Guaranidrilus*, *Bryodrilus* and *Grania*. Observations were made on specimens in vivo, using a light microscope with interference (Nomarski) optics, and gently pressing specimens between slide and coverslip in a drop of water (see Schmelz & Collado 2010 for details). After recording the presence or absence of the structures in question, their pattern and anatomical details were scrutinized in suitable specimens and documented via descriptions, freehand drawings and photographs.

3. Results

3.1. Historical account

The following is a translation of Čejka's account, written in German (Čejka 1910: 12–13).

'Gut tubules [Darmkanälchen]. Amongst the most curious traits of the new genus are peculiar long tubules in the gut epithelium, which run, right below the blood sinus, from the middle of the body down to the XII. segment – counted from the end. To my knowledge, these canaliculi have hitherto not been described and illustrated in the genera of enchytraeids. They do not occur in any genus of the Annulata and not in any other animal either, and hence require a detailed description.

Initially I dedicated little attention to the hind parts of the intestinal tract, because these parts are not used at all in the determination of genera. Besides, the majority of specimens in the collection is bent in various ways, which complicates the arrangement of the series of sections considerably. The few stretched specimens were sectioned sagitally and transversally and the traits of the organs in question as ascertained here are as follows: I refer here only to results of the transverse section, because in longitudinal sections these traits cannot be ascertained in detail. I pursued the tubules from the rear to the front and found that they are narrowed towards the front with the effect that they can be overlooked easily here. The opening occurs in the XII. segment – counted from the hind end of the body. The orifice of the tubules is not conspicuous, so if one does not examine the serial sections very carefully, it cannot be determined where the tubules open. The best traits to ascertain the orifices of the tubules are the denser arrangement of nuclei in recesses of the epithelium and the long cilia at the orifice, which probably serve to empty the secretions of the tubule (Fig. 20, cdk) [see Fig. 2C].

These tubules run from the rear to the front, few in number at the beginning, but in the XIV. or XV. segment (counted from the end) they branch into several ducts and these then run forward in parallel beneath the blood sinus; they do not form any further anastomoses, nor do they communicate with the gut here. Their number is, as far as I can see, quite variable among different individuals; I counted 8 – 12 – 20. The more numerous the tubules, the narrower they are, of course. Their course is interesting. They always proceed forward in parallel, and in some parts they are surrounded by sinus to such a degree that they appear to penetrate the blood sinus (Fig. 17) [see Fig. 2B]. Throughout the course I did not find anastomoses or communications with the gut – the posterior part near the orifice excepted – and the lumen diminishes towards the middle of the body, to a degree that about in the middle of the body, where the high cylindrical epithelium begins, the tubules can be recognized only by the arrangement of the nuclei, until this last trace vanishes as well – the tubules are blind-ending. It cannot be specified in which segment they end, because the segment number varies considerably among individuals.

The histology of these tubules is quite simple. They are formed by cubic or somewhat flat cells with finely striated plasma. The inner surface of the tubules is smooth, aciliate, as shown in Fig. 26dk [see Fig. 2D].

The whole arrangement and structure of the intestinal tubules suggests that we are dealing here with peculiar glands, which empty their secretions into the gut lumen. The formation of the secretory substance is apparently under control of the blood (in the blood sinus) and it must contain the secretion for the final digestive processes, or some suitable enzyme for the defecation of food residues' (Čejka 1910: 12–13).

3.2. Observations

Cejkaian tubules were found in 16 species: 13 species of *Fridericia*, 2 species of *Henlea* and 1 species of *Oconnorella* (Table 1). They were absent or indistinguishable in other species of these genera and also in species of *Achaeta*, *Cernosvitoviella*,

Table 1. List of enchytraeid species with Cejkaian tubules. Types ('A, B, C') are explained in the text.

Genus	Species	Author, year	Type
Henlea	*ochracea*	(Eisen, 1878)	A
= *Hepatogaster*	*birulae*	Čejka, 1910	
Henlea	*nasuta*	(Eisen, 1878)	A
Henlea	*heleotropha*	Stephenson, 1922	A
Oconnorella	*cambrensis*	(O'Connor, 1963)	B
Fridericia	*benti*	Schmelz, 2002	B
Fridericia	*bisetosa*	(Levinsen, 1884)	A
Fridericia	*bulboides*	Nielsen & Christensen, 1959	B
Fridericia	*brunensis*	Schlaghamerský, 2007	A
Fridericia	*ciliotheca*	Schmelz & Collado, 2013	B
Fridericia	*connata*	Bretscher, 1902	A
Fridericia	*cylindrica*	Springett, 1971	A
Fridericia	*maculata*	Issel, 1905	B
Fridericia	*minor*	Friend, 1913	B
Fridericia	*paroniana*	Issel, 1904	B
Fridericia	*roembkei*	Schmelz & Collado, 2013	B
Fridericia	*sylvatica*	Healy, 1979	A
Fridericia	*ulrikae*	Rota & Healy, 1999	C

Cognettia, *Enchytraeus*, *Enchytronia*, *Hemifridericia*, *Lumbricillus*, *Marionina* s. l., *Mesenchytraeus*, *Stercutus* and in the recently erected genus *Globulidrilus* (Christensen & Dózsa-Farkas 2012). The tubules extend over one to twelve segments, with a diameter ranging from 0.5 to 6 µm. They are branched or unbranched, and anastomoses may occur. Posteriorly each tube opens independently with a porus into the intestinal lumen (Fig. 3A); this porus was, however, rarely seen in species with narrow tubules. In species with wide tubules (Fig. 3B), four posterior pores are present, all at the same level, equidistant from one another in cross-section, and in dorso-lateral and ventro-lateral position, respectively. This pattern agrees with the one described by Čejka (Fig. 2B). The tubules extend anteriad in various modes, branched or unbranched, either strictly parallel to the gut's longitudinal axis, or meandering, or branching out in several directions, covering the entire gut circumference (Fig. 4). Loops with a 180° turn may occur as well (Fig. 5B). Extension, shape and branching pattern of the tubules are species-specific, details are described below. The tubules end at some distance from the pars tumida midgut region (Fig. 1), only in *Henlea nasuta* do both regions overlap. The exact anterior end is often indistinguishable, since the tubules thin out in diameter anteriad. They appear to end blindly, anterior pores were never observed.

The posterior pores are located in a gut region which is characterized by an abrupt antero-posterior change from a strongly ciliated epithelium with an apical double lining, probably representing microvilli, to an epithelium without distinguishable cilia and a more conspicuous inner surface without a double lining (Fig. 3A). The latter continues over 1–8 segments down to the anus and may be considered to be the rectum; peristaltic gut contractions in antero-posterior direction can be observed here in living specimens, actively extruding faeces. We identify this border between intestine and rectum as the transition from the endodermal midgut to the ectodermal hindgut, pending further evidence from ultrastructural or embryological investigations. The pores are located close to this transition, but still within the ciliated part (Figs 3A, 4C). Midgut and hindgut were sometimes seen closed against each other by muscular contraction at the point of transition, but a permanent sphincter is not present.

The course of the tubules is intracellular, inside the intestinal epithelial cells, and here closer to the periphery

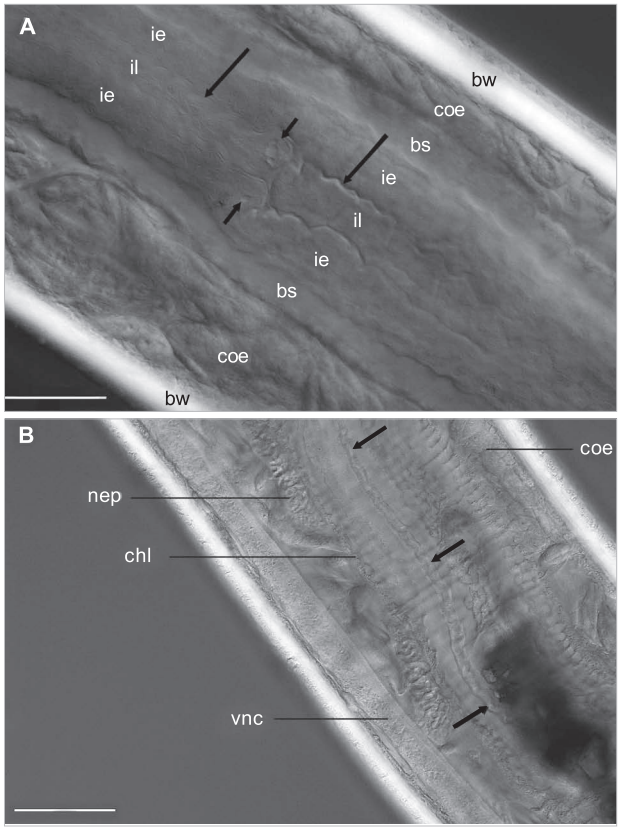

Figure 3. Cejkaian tubules, live photographs. (**A**) *Fridericia ulrikae*, transition zone from posterior midgut to hindgut, with posterior pores of tubules (short arrows). Posteriorly (bottom right half), apical border of gut epithelium distinct and single-lined (long arrow). Anteriorly (top left half), apical border of gut epithelium double-lined (microvillous, long arrow); gut lumen (**il**) filled with long and strongly moving cilia, visible here as undulations. The tubules themselves are in a different focusing plane and therefore not visible. (**B**) *Fridericia bisetosa*, unbranched canals (arrows), gradually and slightly tapering from posterior (bottom right) to anterior (top left).

bs – peri-intestinal blood sinus, **bw** – body wall, **chl** – chloragocytes, **coe** – body cavity, filled with coelomocytes, **ie** – intestinal epithelium, **il** – intestinal lumen, **nep** – nephridium, **vnc** – ventral nerve cord. The dark shadow is gut contents. Scale bars = 100 µm.

than to the gut lumen. The diameter of the tubules is species-specific and age-dependent. The larger tubules are surrounded by a wall or lining which may be a proper epithelium (Figs 3B, 5B). The finer tubules (Figs 4C,D, 5B) appear as canals without a lining. Ciliar movements or a double lining suggesting microvilli were not observed inside the tubules.

So far we have distinguished three different types of tubules, named A, B and C type in the following:

Type A tubules (Figs 3B, 4A,B) run strictly in parallel to the longitudinal axis of the intestine. There are four of them and they extend over several segments (8–12), bifurcating in some species once or twice during their course anteriad. Few stump-like branches may be present, and transient or non-transient meanders may occur, but without abandoning the overall longitudinal orientation. The tubules have a distinct lining, probably a proper epithelium. At their posterior end they are conspicuous due to their large diameter (5–10 µm). Further anteriad they become less conspicuous, as the tubule diameter decreases from posteriad to anteriad, often abruptly after the bifurcation. No anastomoses were observed. Their most anterior end is rarely distinguishable. The A type of tubules is the one that most closely resembles Čejka's description (see below). We found type A tubules in *Fridericia bisetosa*, *F. brunensis*, *F. connata*, *F. cylindrica*, *F. monochaeta*,

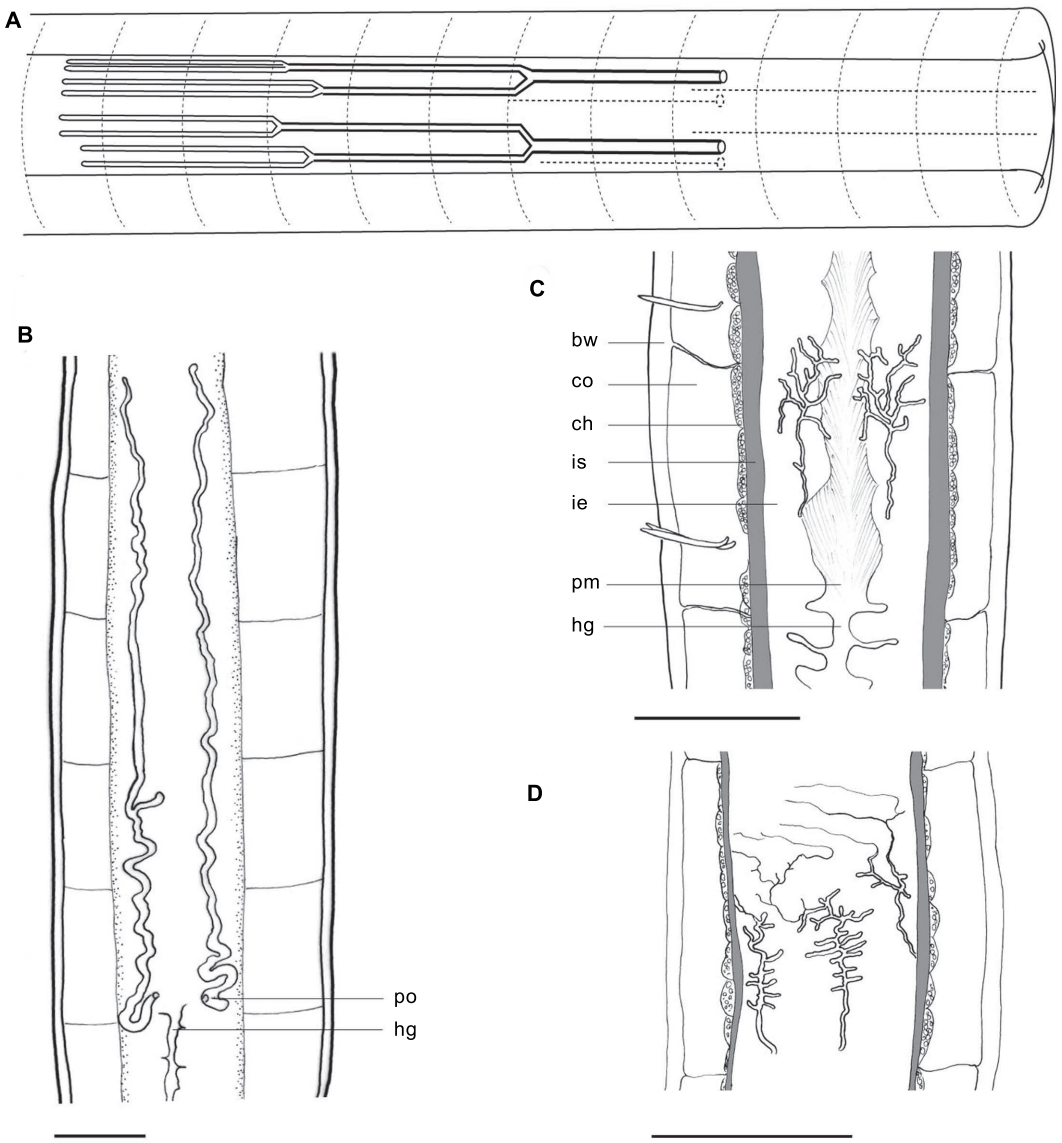

Figure 4. Cejkaian tubules, different types. (**A**) Type A, bifurcating twice, as seen in *Henlea nasuta*, schematic. (**B**) Type A, unbranched, *Fridericia brunensis*. (**C**) Type B, *Fridericia bulboides*. (**D**) Same as C, different specimen, here tubules with very thin projections, not seen in C.
bw – body wall, **ch** – chloragocytes, **co** – coelom, body cavity, **hg** – hindgut lumen, **ie** – intestinal epithelium, **is** – peri-intestinal blood sinus, **pm** – end of posterior midgut, lumen, **po** – pore of tubule. Scale bars = 100 µm.

F. sylvatica, *Henlea heleotropha* and in *H. nasuta*. Together with *Henlea ochracea* (Eisen) (the senior synonym of *Hepatogaster birulae* and *H. sibiricus* according to Nurminen 1973, see above), type A tubules are recorded here in nine species of two genera of Enchytraeidae.

Canals are not bifurcate in *F. connata*, *F. brunensis* and *G. monochaeta*, but small stump-like branches may occur. The widest and most conspicuous tubules were seen in *F. monochaeta*. In *F. brunensis*, the tubules meander immediately before the posterior pore (Fig. 4B). In *F. monochaeta* and *F. brunensis*, backward loops (U-turns) were observed (comp. Fig. 5B), but the species-constancy of this character was not ascertained. In the single juvenile specimen of *H. nasuta* that we investigated up to now, the tubules extended over a length of 12 segments and invaded even the pars tumida region.

Type B tubules are thin, often less than 1 µm wide, and difficult to observe. It seems that four independent tubule systems are present, but the exact number was not ascertainable. The tubules extend over not more than one to four segments and they cover the gut perimeter by multiple branches in different directions (Fig. 4C,D). Anastomoses were seen sometimes but not identified with certainty nor documented. The most posterior stretch of each tubule branching system runs parallel to the gut longitudinal axis.

Type B tubules were seen in *Fridericia benti*, *F. bulboides*, *F. maculata*, *F. minor*, *F. paroniana*, in the two recently described species *Fridericia roembkei* and *F. ciliotheca* (Schmelz & Collado 2013) and in *Oconnorella cambrensis*, altogether in eight species of two genera. However, they were not seen in all specimens of the aforementioned species. When the canals are collapsed, e.g. due to prolonged pressure of the worms between slide and coverslip, they disappear completely. Furthermore, chloragocyte vesicles easily obscure the structures beneath. The tubules are best observed in juvenile specimens with a poorly developed chloragocyte layer, but here the species identity is often uncertain. In *Oconnorella cambrensis* they are always conspicuous; in this species all life stages can be identified to species level.

Type C tubules are more complex and so far a peculiarity of *Fridericia ulrikae* (Fig. 5A,B). Four unbranched tubules with a lining extend anteriad over several segments, running strictly in parallel to the longitudinal axis of the intestine. After a conspicuous loop (Fig. 5A, short arrows), each canal runs backwards, in parallel and closely attached to its own stretch, gradually thinning out. At a point which is difficult to localize, this thin canal turns again anteriad and branches out into a network of narrow and anastomosing canals that fill the space between its own double-tubule and the one of the neighboring tubule (Fig. 5B, long arrow). Type C tubules are conspicuous in juvenile and in adult specimens.

Type A and type C tubules are species-constant, juveniles included. Species-constancy of type B tubules was ascertained in *Oconnorella cambrensis*, but in the *Fridericia* species they were often not distinguished, either because of inconspicuousness or true absence.

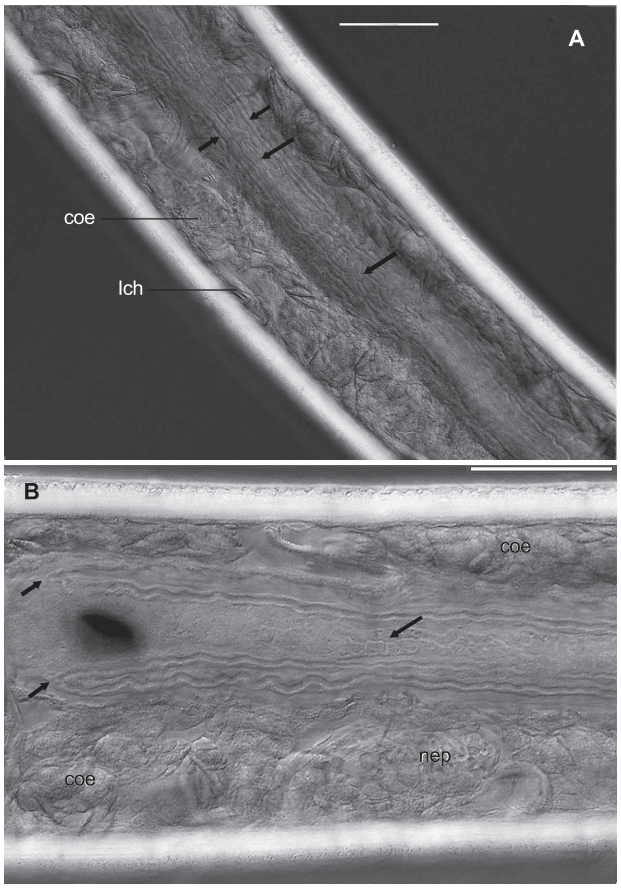

Figure 5. *Fridericia ulrikae*, Cejkaian tubules, type C. (**A**) Region with a complex pattern of thick, straight and unbranched tubules (short arrows) and thin, meandering and branched tubules (long arrows) in the same intestinal region. (**B**) Anterior end of gut region with Cejkaian tubules. Short arrows: anterior loops. Each loop belongs to a different tubule. As the tubules return towards the posterior end they become thinner and finally branch into several thin meandering tubules (long arrow).
coe – coelomocytes, **lch** – lateral chaetae, **nep** – nephridium.
Scale bars = 100 µm.

4. Discussion

In enchytraeids two types of gut structures or differentiations can be distinguished, those present in many if not all taxa and those present in only some taxa. The first are apparently part of the basic equipment, indispensable for a proper functioning of the digestive process, while

the second are specializations that may optimize or add digestive functions. To the first type belong the pharyngeal pad, pharyngeal glands and most probably also the midgut pars tumida (Schmelz pers. obs.), whereas oesophageal appendages, intestinal diverticula and chylus cells belong to the second type. With the Cejkaian tubules one more gut differentiation is added to the second group, which raises the question of its possible function.

The position of Cejkaian tubules at the posterior end of the midgut suggests water uptake if the function is resorptive, comparable to the function of the colon in vertebrates; however Čejka (1910) hypothesized liquefaction with some sort of enzymes to facilitate cast extrusion. An indication of their function may give the fact that Cejkaian tubules only occur in taxa that have oesophageal appendages. If the hypothesis in Schmelz & Westheide (2000) is correct that oesophageal appendages help to moisten food, then it would be suggestive to regard Cejkaian tubules as an additional device for retaining or recycling water more effectively before the casts are extruded. It will be a challenging task to design experiments that elucidate the true function of these organs.

Čejka's description is in good agreement with type A tubules as described in this paper. Note the number of four tubules with large diameter in the posterior region (Fig. 2B), and almost twice the number of tubules with narrower diameter in a more anterior region (Fig. 2A). The protrusion of the canals into the blood sinus explains the aspect of canals with walls as seen in living specimens (Fig. 3B). However there are two puzzling inconsistencies between text and figures: 1) Figure 2C (Čejka 1910, Pl. II Fig. 20) shows numerous narrow canals opening into the intestine, which would require a branching posteriad of the four large posterior tubules (Fig. 3B) into several small tubules before they open into the intestine. This is not described in the text and does not agree with our findings either. The number of openings, when they could be counted, was always four. 2) Figure 2D (Čejka 1910, Pl III Fig. 26) shows the cross section of a hindgut tubule in *Hepatogaster sibiricus*, although it is stated explicitly (Čejka 1910: 27) that tubules are absent in this species. These inconsistencies are not easily explained and require a reinvestigation of the original sections (possibly still present in the St. Peterburg Natural History Museum) or collection of new material from the type localities.

Cejkaian tubules show some resemblance to the enteronephridia known from marine polychaetes of the family Nerillidae (Jouin 1967, Tzetlin et al. 1992, Tzetlin & Purschke 2005). These are also blind-ending intraepithelial canals of the intestine, running in parallel to the longitudinal gut axis. However, they are directed backwards and their opening is more anterior, in the posterior part of the stomach. The canals are unicellular, ciliated and microvillous (Tzetlin et al. 1992), whereas, following the light-microscopical account of Čejka (1910), the canals in enchytraeids are multicellular and not ciliated. Of course, a histological reinvestigation of Cejkaian tubules is necessary to confirm these differences. Enteronephridia are also known in earthworms, mainly in Megascolecidae and related families, but these are extra-intestinal structures, modified metanephridia that open into the intestinal lumen instead of to the exterior (Bahl 1947). Further comparable structures are the excretory systems of nematodes and the Malpighian tubules in tardigrades, arachnids and insects (Tzetlin et al. 1992).

Considering phylogeny it seems unlikely that Cejkaian tubules have evolved only once in the Enchytraeidae. Figure 6 shows a simplified version of the molecular tree in Erséus et al. (2010), the most comprehensive approximation currently available regarding the phylogenetic relationships of enchytraeid species and genera. *Fridericia* on the one hand and *Henlea* plus

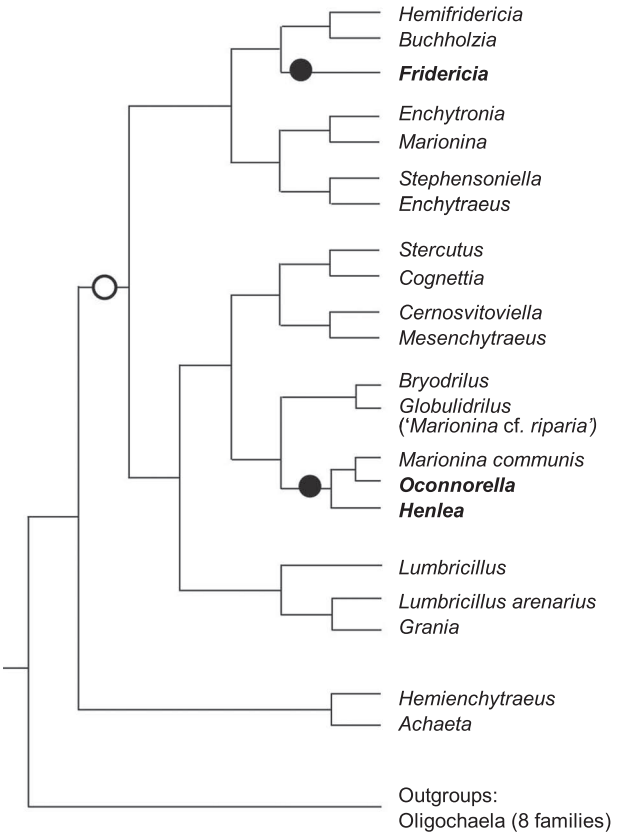

Figure 6. Molecular phylogenetic tree of Enchytraeidae after Erséus et al. (2010), simplified, and possible phylogenetic origin of Cejkaian tubules. Genera where Cejkaian tubules occur in bold type. *Fridericia* and the *Henlea* clade are positioned on distant branches of the tree, therefore to assume a two-fold independent origin (filled circles) is more parsimonious than to assume a common origin (open circle) with multiple subsequent loss concomitantly. Not shown is the further possibility of multiple independent origins within each genus, reflecting the fact that in *Henlea* and *Fridericia* only few species have these tubules.

Oconnorella on the other are positioned on distant branches of the tree, therefore the hypothesis of an at least two-fold independent origin (filled black circles) is more parsimonious than to assume a common origin and multiple subsequent losses in the neighbouring taxa (open circle). Note also that tubule types A and B are present on both branches (comp. Table 1). However, an only two-fold independent origin would imply multiple subsequent losses because of the absence of tubules in many species of *Henlea* and *Fridericia*. Therefore a multiple origin within genera is favoured here as the most likely scenario. This does not mean a de novo evolution in each species. For example, *Fridericia connata*, *F. monochaeta* and *F. brunensis*, all with conspicuous type A tubules, are also similar due to joint spermathecae and the presence of only one chaeta in lateral postclitellar bundles, the latter a rare character state in *Fridericia*. So there is support for the hypothesis that these three species form a monophyletic group, and its stem species may have possessed Cejkaian tubules. The same reasoning applies to *F. paroniana* and *F. benti*, two extremely similar species or species groups (comp. Schmelz & Collado 2010), and both with B type tubules.

Cejkaian tubules are intraepithelial structures of an organ that is only one cell layer thick, so careful focusing is necessary to recognize them or – even more difficult – to confirm their absence. In an unsectioned specimen, body wall, coelom with coelomocytes, and chloragocytes overlay the gut epithelium and may obscure the intestine, especially in well-fed or large specimens. Considering further that usually anterior segments are used to identify specimens, it seems not surprising that these tubules have been overlooked for such a long time.

Despite some uncertainty regarding type B tubules it seems that Cejkaian tubules in general are species-constant and present already in juveniles. Although not easily distinguishable in many specimens, they may eventually serve as useful additional characters that help in identifying specimens to species. This applies especially to *F. ulrikae* with its unique C type of tubules, but also to juvenile specimens of other species, where presence/absence and the type of tubules can help to decide between alternatives, when the species composition of a site is known.

5. Conclusion

Our description of the Cejkaian tubules is entirely based on observations in living specimens and is more a by-product of identification work rather than the outcome of a focused study. More detailed records are necessary regarding inter- and intraspecific variability, and ultrastructural, histochemical or embryological investigations are required. On the other hand it should be stressed that the transparency of living enchytraeids allowed anatomical studies in vivo without preparative effort, and therefore a large number of specimens of different taxa could be compared. It was the optimal method to study both the general pattern and diversity at the same time.

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