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Distribution of Astomatia Schewiakoff, 1896 and Hysterocinetidae Diesing, 1866 (Ciliophora, Oligohymenophora) along the digestive tract of *Alma emini* (Oligochaete, Glossoscolecidae) is correlated with physico-chemical parameters

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Summary

The paper demonstrates the influence of physico-chemical parameters on the distribution of endocommensal ciliates through the gut of the earthworm *Alma emini*. We measured physico-chemical parameters of the intestinal liquid extracted with the vacuum aspiration technique and concomitantly recorded biological parameter (species abundance). Furthermore, correlation analysis between physico-chemical parameters and biological parameter was performed in different compartments. In the foregut, among the eleven species of Astomatia recorded, correlation was significant between *Metaracoelophrya intermedia, Coelophrya roquei* and Water Content (WC = 46.94 ± 7.77%). In the midgut, among the nine species of Hysterocinetidae recorded, a significant correlation was observed between *Metaptychostomum ebebdae, Ptychostomum macrostomum* and Electric Conductivity (EC = 84.55 ± 12.94 µS/cm). In the same compartment, a significant correlation was also observed between *Ptychostomum macrostomum* and Total Dissolved Substance (TDS = 16.20 ± 3.46%). In the hindgut, eight species of Astomatia were found, among which significant correlation was obtained between *Coelophrya roquei* and Hydrogen potential (pH = 7.35 ± 0.16). In the same compartment, taking into account the eleven species of Hysterocinetidae recorded, a significant correlation was also obtained between *Ptychostomum macrostomum* and pH; *Ptychostomum commune* and WC (28.84 ± 3.97%). These results suggest that each part of the digestive tract of *A. emini* can be considered as a set of natural microhabitats in which certain physico-chemical factors generate ecological niches suitable for one or another group of species.

Key words: *Alma emini*, Astomatia, endocommensal ciliates, electric conductivity, intestinal liquid, hydrogen potential, Hysterocinetidae, total dissolved substance, water content

Introduction

Oligochaeta represent a major component of the soil macrofauna. They are grouped into three ecological categories: epigeic, anecic and endogeic (Bouché, 1972, 1977). *Alma emini* which measures 51 cm on average and weighs 3.8 g is an anecic species belonging to the family of Glossoscolecidae. This fairly pigmented worm is found in the wet soil, near the less polluted rivers. Like all Oligochaeta, it is a hermaphrodite and creates more or less deep galleries, probably, in response to various constraints such as the content of food and water, the temperature or the degree of oxygenation (Jégou et al., 2000). These galleries increase soil macroporosity and, consequently, contribute to its aeration (Lavelle, 1997) and to water infiltration. They also facilitate root soaking in the soil as well as the movements of invertebrates (Jégou et al., 2002). The role of *A. emini* in the formation, dynamics and fertility of soil has been long known (Darwin, 1881). Besides its role of "the engineer" of the soil, *A. emini* is regarded as a microhabitat as its digestive tract lodges an important microbial fauna (protozoa, bacteria, and viruses). The protozoa are mainly represented by ciliates belonging to Heterotrichida Stein, 1859 (Albaret, 1975; Albaret and Njiné, 1975), Hysterocinetidae Diesing, 1866 (Njiné and Ngassam, 1993; Ngassam et al., 1993; Ngassam and Grain, 1997, 2000) and Astomatia Schewiakoff, 1896 (de Puytorac, 1968, 1969; de Puytorac and Dragesco, 1969a, 1969b; Ngassam, 1983; Fokam et al., 2008, 2012).

These studies demonstrated that several species of ciliates may be found simultaneously in the same worm, each of them living in a given compartment favorable to its development. Up to now, the reason of this stratification still remains unclear. Very few data were known on the living conditions of these endocommensal ciliates from the digestive tract of their host.

The aim of this study is to assess whether physico-chemical parameters (Hydrogen potential; Electric Conductivity; Total Dissolved Substance and Water Content) may influence the distribution and abundance of ciliate species along the digestive tract of *Alma emini*.

Material and methods

Collection and identification of earthworms

Earthworms were collected on Sanaga River bank in Ebebda village, located between 11°30' and 11°50' of Eastern longitude and 4°00' and 4°30' of Northern latitude, 60 km north of Yaoundé-Cameroon (Central Africa) (Fig. 1). Worms were then identified according to the keys described by Sims and Gerard (1999). These worms were randomly divided into two batches for the assessment of physico-chemical parameters and abundance of ciliate species present in their digestive tract.

Measurements of physico-chemical parameters of the earthworm's intestinal liquid

Once in the laboratory, the first batch of earthworms was carefully washed with tap water, and then fixed using formalin (10%). The digestive tract of each of these worms was then separated from the rest of its body and stretched on a filter paper. Once the blood and the coelomic liquid dried up, the intestine of worms was divided into three equivalent portions (fore-, mid- and hindgut) (Fig. 2). The content of each portion of the digestive tract was emptied in an earthenware dish by applying a slight pressure to the walls of the intestine, moving from the middle towards the extremities. The intestinal content was placed on a glass with very fine meshes (1-2 µm). The yellowish liquid was aspirated using a vacuum pump and collected in a flask. This technique, developed by de Puytorac and Mauret (1956), is fast and allows the collection of three to four drops of the intestinal liquid deprived of any particles. In order to collect sufficient amount of the earthworm's digestive liquid for direct measurements of physico-chemical parameters, 15 earthworms were used for each series of experiment. Thirty tree series of identical experiments were performed during the whole study.

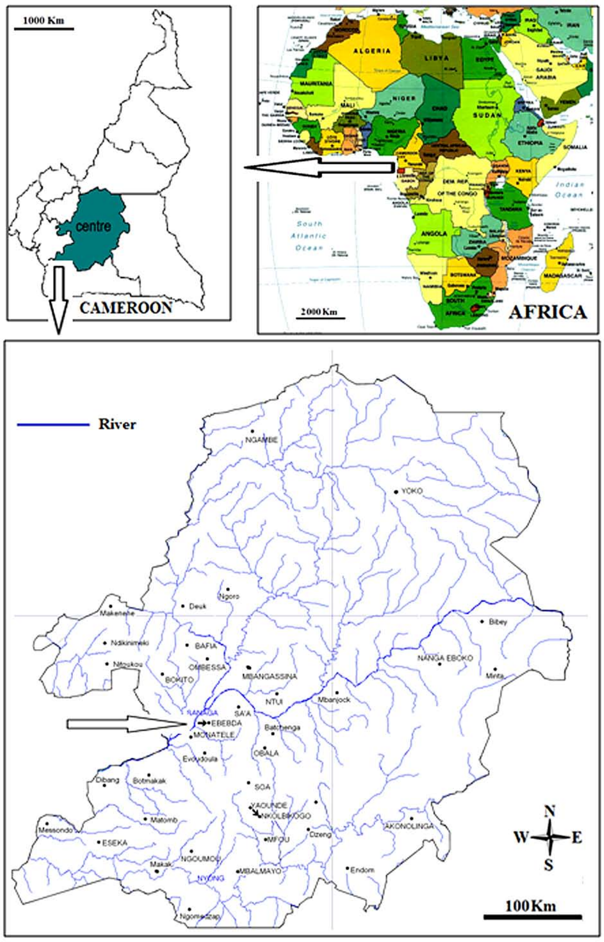

Fig. 1. A map showing collection site for earthworms.

Four physico-chemical parameters were assessed for each compartment of the digestive tract. The pH and Electric Conductivity (EC) were measured respectively by introducing the electrodes of a portable pH-meter (Shott Gerate CG 812, England) and electrodes of a portable conductimeter (Hanna series HT 8733, Germany) in a flask containing the intestinal liquid. The values of pH were expressed in conventional units and EC in micro Siemens per centimeter (µS/cm). The Total Dissolved Substace (TDS) and Water Content (WC) were evaluated before and after a complete evaporation of samples at 80°C in an oven (Gallenkamp, Germany). Weighs were recorded using a balance (Sartorius, France). Note that we measured WC of the total intestinal content and not of the intestinal liquid only, 33 earthworms were used during the study.

Identification and enumeration of ciliates

Worms of the second batch were cut alive in three compartments from the prostomium to the pygidium as above (fore-, mid-, and hindgut) (Fig. 2). Each portion was dilacerated in a petri dish (10 cm in diameter) containing 10-15 ml of mineral water (Volvic™, France). Ciliates found in these different portions of the earthworms were identified according to the keys previously described (de Puytorac 1968, 1969; de Puytorac and Dragesco 1969a, 1969b; Ngassam, 1983; Njiné and Ngassam, 1993; Ngassam et al., 1993; Ngassam and Grain, 1997, 2000; Fokam et al., 2008, 2012). They were sorted and counted under a binocular dissecting scope Wild M5 (Heerbrugg, Germany) at 250× magnification. This experiment was performed on 33 earthworms.

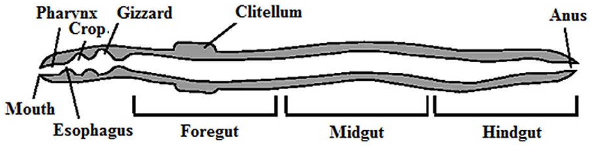

Fig. 2. Diagram of the digestive tract of earthworms (from Horn et al., 2003, modified).

Statistical analyses

Correlation tests were used to assess the degree of binding between the physico-chemical parameters and ciliate abundance in different portions of digestive tract. Since our variables do not follow a normal distribution, we applied correlation test 'r' of Spearman to analyze our data. P-values were used to assess the degree of significance of correlation between physico-chemical parameters and ciliate abundance. P less than 0.05 were set as significant.

The means of various physico-chemical parameters in different portions of the digestive tract were compared using the Kruskal Wallis 'H' test. The 'U' Mann-Whitney test was used to compare the means of each parameter two by two. The criterion for significance was set at $P<0.05$. Values presented in the tables and figures are the mean ± standard deviation of the mean (sdm, n = 33).

Results

During this study, a total of 561 earthworms were dissected: 528 worms were used for measurements of physico-chemical parameters and 33 for studies of biodiversity of ciliates along the digestive tract.

Physico-chemical variables

The pH varied from 6.22 ± 0.43 in the foregut, 7.13 ± 0.17 in the midgut, and 7.35 ± 0.16 in the

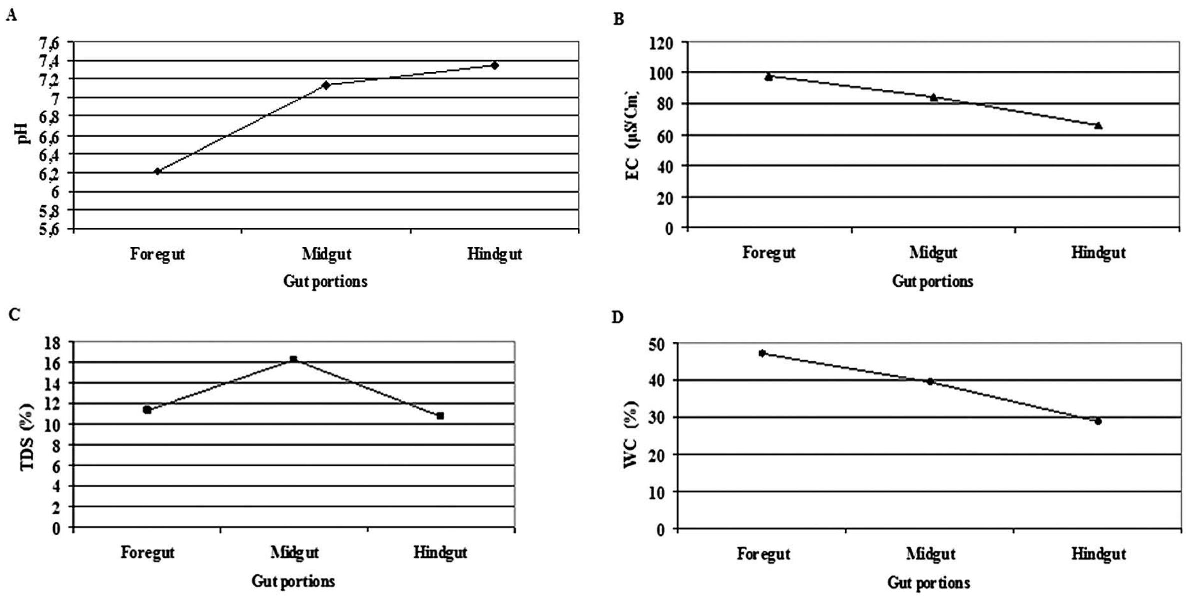

Fig. 3. Variation of physico-chemical parameters along the digestive tract of *Alma emini*. A – Hydrogen potential (pH), B – electric conductivity (EC), C – total contents in dissolved substances (TDS), D – water content (WC).

hindgut (Fig. 3A). Thus, the acid pH in foregut of the digestive tract of earthworms became alkaline in its mid and the hind portions. The average pH of the whole intestinal liquid of the worm was close to neutrality (6.90 ± 0.25).

The mean value of EC in the fore-, mid- and hindgut were 97.51 ± 11.18 µS/cm, 84.55 ± 12.94 µS/cm, and 66.22 ± 8.60 µS/cm respectively (Fig. 3B). Then, the greatest ionic concentration was obtained in the foregut.

The TDS was on average 11.38 ± 2.41% in the foregut, 16.20 ± 3.46% in the midgut, and 10.75 ± 3.76% in the hindgut (Fig. 3C). Globally, the mean value of this parameters 12.77 ± 3.21%.

The WC decreased gradually from the foregut to the hindgut, with average values of 46.94 ± 7.77% in the foregut, 39.27 ± 5.05% in the midgut, and 28.84 ± 3.97% in the hindgut (Fig. 3D).

The Kruskal Wallis 'H' test appeared significant for the whole of the measured parameters ($P<0.05$) (Table 1). Thus, for each variable, the Mann Whitney 'U' test showed a significant difference among the three portions of the digestive tract of *A. emini* taken two by two: foregut and midgut ($P<0.05$), foregut and hindgut ($P<0.05$), midgut and hindgut ($P<0.05$) (Table 2).

Table 1. Overall comparison of the different physico-chemical parameters in the different portions of the digestive tract*.

Parameters	pH	EC	TDS	WC
P-value	0.010	0.047	0.022	0.034

Notes: * – correlation is significant at the 0.05 level; EC – electric conductivity; pH – Hydrogen potential; TDS – total dissolved substances; WC – water content.

Ciliate biodiversity

Twenty three species belonging to nine genera of ciliates were found during this study. Twelve species belonged to the Subclass of Astomatia: *Almophryra bivacuolata* de Puytorac and Dragesco, 1969b; *Almophryra mediovacuolata* Ngassam, 1983; *Almophrya laterovacuolata* de Puytorac and Dragesco, 1969b; *Dicoelophrya almae* de Puytorac and Dragesco, 1969b; *Dicoelophrya mediovacuolata* Fokam et al., 2012; *Paracoelophrya intermedia* de Puytorac, 1969; *Paracoelophrya polymorphus* Fokam et al., 2012; *Paracoelophrya ebebdensis* Fokam et al., 2012; *Metaracoelophrya intermedia* de Puytorac and Dragesco, 1969a; *Coelophrya roquei* de Puytorac and Dragesco, 1969b; *Coelophrya ovales* Fokam et al., 2008; *Coelophrya ebebdensis* Fokam et al.,

Table 2. Values of the 'U' Mann-Whitney test*.

Parameters	Foregut	Midgut	Hindgut
pH	0.033	0.037	0.030
EC	0.024	0.020	0.019
TDS	0.027	0.035	0.031
WC	0.021	0.032	0.021

Notes: * – correlation is significant at the 0.05 level; EC – electric conductivity; pH – Hydrogen potential; TDS – total dissolved substances; WC – water content.

Table 3. Species richness and variation of ciliates abundance along the digestive tract of *A. emini*.

	Species	Digestive tract		
		Foregut (m ± sd)	Midgut (m ± sd)	Hindgut (m ± sd)
Astomatia	*Almophryra bivacuolata*	58 ± 11	25 ± 4	0
	Almophryra mediovacuolata	73 ± 10	28 ± 5	0
	Almophrya laterovacuolata	14 ± 3	7 ± 1	0
	Dicoelophrya almae	0	23 ± 7	5 ± 2
	Dicoelophrya mediovacuolata	0	20 ± 5	7 ± 2
	Paracoelophrya intermedia	48 ± 8	27 ± 5	3 ± 1
	Paracoelophrya polymorphus	33 ± 6	19 ± 4	1 ± 0
	Paracoelophrya ebebdensis	56 ± 7	34 ± 7	18 ± 7
	Metaracoelophrya. intermedia	32 ± 6	18 ± 8	1 ± 0
	Coelophrya roquei	62 ± 4	24 ± 3	1 ± 0
	Coelophrya ovales	27 ± 4	16 ± 3	0
	Coelophrya ebebdensis	59 ± 9	23 ± 6	0
Hysterocinetidae	*Metaptychostomum ebebdae*	0	17 ± 3	6 ± 2
	Metaptychostomum pirimorphus	0	14 ± 4	7 ± 2
	Ptychostomum sanagae	0	13 ± 4	2 ± 1
	Ptychostomum prolixus	0	2 ± 1	12 ± 4
	Ptychostomum commune	0	3 ± 1	17 ± 5
	Ptychostomum macrostomum	0	10 ± 2	13 ± 3
	Ptychostomum elongatum	0	0	12 ± 3
	Ptychostomum variabilis	0	4 ± 2	20 ± 6
	Proptychostomum commune	0	0	11 ± 3
	Proptychostomum simplex	0	4 ± 2	22 ± 3
	Preptychostomum microstomum	0	19 ± 6	6 ± 3

Notes: m –mean; sd –standard deviation.

2008, while the eleven others were Hysterocinetidae: *Metaptychostomum ebebdae* Ngassam and Grain, 1997; *Metaptychostomum pirimorphus* Ngassam and Grain, 2000; *Ptychostomum sanagae* Ngassam and Grain, 2000; *Ptychostomum prolixus* Njiné and Ngassam, 1993; *Ptychostomum commune* de Puytorac, 1968; *Ptychostomum macrostomum* Njiné and Ngassam, 1993; *Ptychostomum elongatum* Njiné and Ngassam, 1993; *Ptychostomum variabilis* Ngassam and Grain, 2000; *Proptychostomum commune* Ngassam and Grain, 1997; *Proptychostomum simplex* Ngassam and Grain, 1997; *Preptychostomum microstomum* Ngassam et al., 1993.

Among the 762 specimens of Astomatia recorded in the digestive tract of *Alma emini*, 462 were found in their foregut, 264 in their midgut and 36 in their hindgut. The abundance of Astomatia then significantly decreased gradually along the digestive tract of earthworms (Table 3).

The Hysterocinetidae ciliates were mostly found in the hindgut (128 specimen), while they were absent in the foregut and only 86 were found in the midgut.

We noted however, the existence of a buffer medium in the midgut where Hysterocinetidae and Astomatia (*Dicoelophrya almae, Dicoelophrya mediovacuolata*) ciliates dwelled together. In addition, we noted an effective cohabitation among species of the same genus (*Almophryra bivacuolata, Almophryra mediovacuolata* and *Almophryra laterovacuolata; Coelophrya ebebdensis* and *Coelophrya roquei; Ptychostomum prolixus* and *Ptychostomum commune*) (Table 3).

Correlation between the relative abundance of ciliates and the physico-chemical parameters of their host

Table 4 displays the relationship between the ciliate abundance and physico-chemical parameters of the three portions of the digestive tract of their host.

In the foregut, a positive and significant correlation was found between the abundance of the ciliates *Metaracoelophrya intermedia* (r = 0.694;

Table 4. Correlation between ciliate abundance and physico-chemical parameters in the different portions of the digestive tract.

	Species	Foregut				Midgut				Hindgut			
		pH	EC	TDS	WC	pH	EC	TDS	WC	pH	EC	TDS	WC
Astomatia	*Almophryra bivacuolata*	0.790	0.790	0.958	0.612	0.232	0.064	0.829	0.756	–	–	–	–
	Almophryra mediovacuolata	0.474	0.739	0.863	0.189	0.249	0.738	0.309	0.926	–	–	–	–
	Almophrya laterovacuolata	0.947	0.780	0.863	0.852	0.294	0.724	0.295	0.829	–	–	–	–
	Dicoelophrya almae	–	–	–	–	0.821	0.979	0.612	0.750	0.899	0.590	0.152	0.957
	Dicoelophrya mediovacuolata	–	–	–	–	0.473	0.729	0.258	0.252	0.630	0.198	0.290	0.428
	Paracoelophrya intermedia	0.926	0.474	0.527	0.831	0.164	0.415	0.728	0.544	0.822	0.977	0.417	0.061
	Paracoelophrya polymorphus	0.728	0.649	0.535	0.127	0.313	0.446	0.627	0.147	0.959	0.799	0.503	0.438
	Paracoelophrya ebebdensis	0.915	0.223	0.417	0.979	0.098	0.709	0.555	0.631	0.811	0.298	0.873	0.689
	Metaracoelophrya intermedia	0.728	0.738	0.177	0.018*	0.372	0.242	0.727	0.480	0.605	0.875	0.290	0.498
	Coelophrya roquei	0.589	0.170	0.830	0.036*	0.883	0.904	0.100	0.677	0.024*	0.367	0.460	0.792
	Coelophrya ovales	0.428	0.893	0.36	0.388	0.920	0.841	0.188	0.155	–	–	–	–
	Coelophrya ebebdensis	0.270	0.474	0.601	0.304	0.313	0.811	0.277	0.346	0.769	0.223	0.223	0.770
Hysterocinetidae	*Metaptychostomum ebebdae*	–	–	–	–	0.968	0.013*	0.059	0.431	0.850	0.305	0.291	0.333
	Metaptychostomum pirimorphus	–	–	–	–	0.896	0.140	0.590	0.323	0.788	0.480	0.099	0.524
	Ptychostomum sanagae	–	–	–	–	0.914	0.607	0.531	0.746	0.717	0.100	0.935	0.051
	Ptychostomum prolixus	–	–	–	–	0.733	0.849	0.775	0.448	0.141	0.261	0.979	0.082
	Ptychostomum commune	–	–	–	–	0.925	0.727	0.185	0.872	0.457	0.853	0.355	0.021*
	Ptychostomum macrostomum	–	–	–	–	0.562	0.012*	0.042*	0.530	0.007**	0.135	0.727	0.758
	Ptychostomum elongatum	–	–	–	–	–	–	–	–	0.251	0.322	0.931	0.214
	Ptychostomum variabilis	–	–	–	–	0.422	0.199	0.904	0.178	0.883	0.448	0.800	0.572
	Proptychostomum commune	–	–	–	–	–	–	–	–	0.899	0.748	0.936	0.873
	Proptychostomum simplex	–	–	–	–	0.063	0.060	0.674	0.787	0.423	0.821	0.408	0.630
	Preptychostomum microstomum	–	–	–	–	0.722	0.957	0.841	0.947	0.547	0.430	0.609	0.779

Notes: * – correlation is significant at the 0.05 level; ** – correlation is significant at the 0.01 level; "–" – no value; EC – electric conductivity; pH – Hydrogen potential; TDS – total dissolved substances; WC – water content.

P<0.05), *Coelophrya roquei* (r = 0.628; P<0.05) and WC.

In the midgut, a negative and significant correlation was observed between the number of the ciliates *Metaptychostomum ebebdae* (r = -0.717; P<0.05), *Ptychostomum macrostomum* (r = -0.725; P<0.05) and EC. In the same compartment, a negative and significant correlation was also observed between the abundance of *Ptychostomum macrostomum* and TDS (r = -0.619; P<0.05).

In the hindgut, a positive and very significant correlation was found between the values of the pH and *Ptychostomum macrostomum* abundance (r = 0.755; P<0.01). A significant and positive correlation was also recorded between the number of *Ptychostomum commune* counted in the hindgut and the WC of the digestive tract of the latter (r = 0.682; P<0.05), while the correlation between the abundance of *Coelophrya roquei* and the pH was negative and significant (r = -0.669; P<0.05).

Discussion

The present paper aimed to assess whether the physico-chemical parameters (pH, EC, TDS and WC) of *Alma emini*'s digestive tract may influence the distribution and abundance of ciliate species.

The pH of the digestive tract of *A. emini*, collected in acidic soil of Sanaga River bank (Fokam, 2005), increased from the foregut to the hindgut. These results are in accordance with previous experiments carried out on the oligichaetes *Allolobophora savignyi* and *Lumbricus herculeus* collected in alkaline environment where similar variation of the pH of the digestive tract was observed (Simm, 1913; Krieg, 1923; Puh, 1940; de Puytorac and Mauret, 1956), suggesting that the pH of the habitat of the earthworm might not affect this parameter.

The gradual reduction in the total content of ions (TDS and EC) in the digestive tract of *A. emini* might be due to the action of deionizing bacteria present in the digestive tract. Indeed, some authors reported the presence of a strong and much diversified bacterial community in the digestive tract of oligochaetes (Hyun-Jung et al., 2004; Brito-Vega and Espinosa-Victoria, 2009). The progressive decrease of the values of EC observed is in good accordance with the maximum concentration of ions (chloride, sulfate, calcium, potassium, and sodium) observed in the foregut (Maluf, 1940) and detection of a great number of denitrifying organisms in the digestive tract of *Lumbricus rubellus*, *L. terrestris* and *Aporrectodea caliginosa* (Depkat-Jakob et al., 2010).

Regarding TDS in the three compartments of the digestive tract of *A. emini*, it seems to be concentrated in the first two parts (fore- and midgut), and to be assimilated in the hindgut, thus explaining its reduction in this latter portion of the intestine as suggested by de Puytorac and Mauret (1956).

We observed a progressive decrease, from the foregut to the hindgut, in WC. Such gradient of fluidity had already been reported by Maluf (1940), and then by de Puytorac and Mauret (1956) in *Lumbricus terrestris* and *Allolobophora savignyi* collected in various fields. This parameter seems to be influenced by the habitat or environment (porosity of the soil), and the physiological status of the worm, as previously reported by Edwards (1998) and Lavelle and Spain (2001).

In the digestive tract of *A. emini*, astomes, in their great majority, proliferated in the acid foregut, rich in mineral elements and in fluid. Besides, we noted that the ciliates inhabiting the anterior part of the digestive tract were very mobile. In contrast, in the posterior portion, populated by Hysterocinetidae and characterized by alkaline pH and low content of mineral substances and water, ciliates were attached to the intestinal wall by their sucker. Along the same lines, de Puytorac and Mauret (1956) had already shown stratification of *Allolobophora savignyi* related to the physico-chemical variables. These results reveal high affinity between ciliates and physico-chemical variables prevailing in their respective biotope. In general, the ciliate abundance in each portion of the digestive tract of *A. emini* was variable, according to the conditions of the medium.

It is important to notice that the stratification of cells is observed not only in the digestive tract of earthworms. Gohre (1943) reported similar trend in three species of the genus *Gregarina*, parasites of the mealworm (*Tenebrio molitor*). Adam (1951) also showed that two successive ciliate fauna can respectively be found in the coecum and the rectum of the large intestine of horses.

Therefore, it appears that the stratification of ciliate species in the digestive tract of a given host might be largely associated with the physico-chemical parameters of this environment and to a lesser extent with the biotic conditions of the habitat of these hosts.

It is also important to notice that the parameters assessed in our study might not be the only factors affecting the abundance of ciliates. Some authors have not detected enzymes (cellulase and chitinase) in the pharynx, the esophagus, the jabot and the gizzard of many worms, but registered them in the anterior part of the intestine (Tracy, 1951; Laverack, 1963; Urbasek, 1990). If Hysterocinetidae (*Ptychostomum* *prolixus, Ptychostomum commune, Ptychostomum macrostomum, Ptychostomum elongatum, Ptychostomum variabilis, Proptychostomum commune, Proptychostomum simplex*) thus seem to be able to spare from such diastases, it is possible that Astomatia (*Almophryra bivacuolata, Almophryra mediovacuolata, Almophrya laterovacuolata, Coelophrya ovales*) have reversely great need for them. A similar case has been demonstrated in *Diplodinium* and *Entodinium* species of the paunch of ruminants. The first have cellulase and cellobiase which enables them to transform cellulose into glucose whereas the second are deprived of it (Tracy, 1951).

Variation of physico-chemical parameters and ciliate species along the digestive tract of earthworms suggests that passage of soil in digestive tract would influence not only physical and chemical properties of soil, but also its microbial biomass, as was previously suggested by Pedersen and Hendriksen (1993), Fischer et al. (1995, 1997), Houjian et al. (2002) and Depkat-Jakob et al. (2010). The density of soil nematodes, protozoa and coliformes also changes after its transit through the intestine of the epigeic earthworms, providing further evidence in favor of this hypothesis (Monroy et al., 2007). Falling into the line, the passage of soil through the digestive tract of earthworms could stimulate (Brito-Vega and Espinosa-Victoria, 2009) or inhibit (Byzov et al., 2007) the growth of microorganisms and mineralizing bacteria.

Conclusion

The present study reveals that the digestive tract of oligochaetes in general and that of *A. emini* in particular is a set of biotopes with specific physical and chemical parameters responsible for ecological conditions which can favor the development of a particular ciliated fauna. Contrary to Hysterocinetidae, Astomatia mostly proliferate in the acid foregut, rich in mineral elements and fluid. Despite the quite relevant information provided by our study, other factors influencing the distribution of ciliates in their host still remain to be assessed. The importance of various parameters of the medium differs and depends on the particular organism or a group of organisms (its sensitivity to the particular factor) and the amplitude of variations these factors undergo. In the case of endocommensal ciliates of *A. emini*, each parameter has certainly an essential and primordial action. The greatest concentration of cells of each group (Astomatia and Hysterocinetidae) seems to occur in the fore and hindgut, correspondingly. Nevertheless, specimens of these two groups can be observed in the midgut, qualified as a buffer medium, where tolerant species occur. Each of these species is probably more sensitive to the quality and quantity of substances present in the medium. It is also necessary to consider predation, rate of oxygen, osmotic pressure, nutrients and ions, specific enzymes, interaction with ciliates, bacteria and viruses in digestive tract of *A. emini* in further research.

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