INFLUENCE OF IONIC CONTENTS ON THE STRATIFICATION OF ASTOMATIA AND HYSTEROCINETIDAE (CILIOPHORA, OLIGOHYMENOPHORA) ALONG THE DIGESTIVE TRACT OF ALMA EMINI (OLIGOCHAETE, GLOSSOSCOLECIDAE) FROM THE CENTER REGION OF CAMEROON

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To cite this version:


HAL Id: hal-01114626
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ARTICLE INFO

**Article History:**
Received 16th September, 2013  
Received in revised form  
30th October, 2013  
Accepted 23rd December, 2013  
Published online 26th January, 2014

**Key words:**
Alma emini, Astomatia, Hysterocinetidae, Intestinal liquid, Ionic contents, Microhabitat.

ABSTRACT

The intestinal liquid of *A. emini* was extracted using the vacuum aspiration technique, followed by measurement of ion concentrations and concomitantly recorded species abundance. Nitrate (NO$_3^-$) concentration decreased from the foregut to the hindgut. Nitrates (NO$_3^-$), ammoniums (NH$_4^+$) and orthophosphates (PO$_4^{3-}$) reached their greatest levels in the midgut. Furthermore, correlation analyses between ion contents and ciliate abundance in different compartments were performed. In the foregut, a positive and significant correlation was found between the abundance of the Paracoleophrya ebebendensis and PO$_4^{3-}$. In the midgut, a positive and significant correlation was observed between the number of the ciliates Dicoelophrya mediovacuolata; Coelophrya roquei; Coelophrya ovales; Ptychostomum commune and NO$_3^-$; Almophrya mediovacuolata and NH$_4^+$. In this same compartment, a positive and significant correlation was also observed between the abundance of *Ptychostomum prolaxis* and NO$_3^-$; In the hindgut, a positive and significant correlation was found between the values of PO$_4^{3-}$ and *Ptychostomum variabilis* abundance. These results suggest that each portion of the digestive tract of *A. emini* can be considered as a set of natural micro-habitat in which a large number of ions generate ecological niches suitable for the survival of a specific group of ciliate species.

INTRODUCTION

The oligochaeta annelids 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 Glossoscoleididae. 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 (Jégu 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 (Jégu 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 due to the fact that its digestive tract lodges an important microbial fauna (protozoa, bacteria, and viruses). The protozoa are mainly represented by ciliates belonging to Heterotrichida (Allart 1975, Albaret and Njindjop 1975), Hysterocinetidae (Njiné and Ngassam 1993; Ngassam et al., 1993, Ngassam and Grain 1997, 2000) and Astomatia (de Puytorac 1968, 1969; de Puytorac and Dragescu 1969a, b; Ngassam 1983; Fokam et al., 2008, 2011, 2012).  

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These studies demonstrated that several species of ciliates may be found simultaneously in the same worm, each of them living in a given compartment that is favorable to its development. Up to now, the reason of this stratification still remains unclear. Until now, very few data is available on the living conditions of these endocommensal ciliates of the digestive tract of their host. The aim of this study is to assess whether ionic contents (Nitrate, Nitrites, ammoniums, and orthophosphates) may influence the distribution and abundance of ciliate species along of digestive tract of Alma emini.

MATERIALS 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 (Figure 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 ion content and abundance of ciliate species present in their digestive tract.

Measurements of ions 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) (Figure 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 ions, 25 earthworms were used for each series of experiment. Thirty series of identical experiments were performed during the whole study. The concentrations of the various ions were obtained by colorimetry with spectrophotometer HACH DR/2800 (Hach Lange, Germany). The reagents used are: the reagent of

Figure 1. Map of the centre region of Cameroon showing the sampling site for A. emini
Nessler for NH$_4^+$, Nitriver III (Permachem Reagents, Germany) for NO$_2^-$, Nitraver VI (Permachem Reagents, Germany) for NO$_3^-$ and Phosver III (Permachem Reagents, Germany) for PO$_4^{3-}$.

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) (Figure 2). Each portion was dilacerated in a Petri dish (10 cm 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, b; 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 250X magnification. This experiment was performed on 30 earthworms.

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

Statistical analyses

Correlation tests were used to assess the degree of binding between the ion concentrations 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 less than 0.05 were set as significant. The means of various ion concentrations 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.

RESULTS

During this study, a total of 780 earthworms of Alma emini were dissected: 750 worms were used for measurements of ions and 30 for studies of biodiversity of ciliates along the digestive tract.

Ionic contents

The various ionic concentrations varied from one to another portion of the digestive tract of A. emini. Nitrates varied from 7.02 ± 0.77 mg.L$^{-1}$ in the foregut, to 3.63 ± 0.23 mg.L$^{-1}$ in the midgut, and 3.40 ± 0.19 mg.L$^{-1}$ in the hindgut. It comes out from these results that the ionic content of NO$^3_3$ decreased gradually (Figure 3A). The mean concentration of NO$_2^-$ of whole intestinal liquid of the worm was approximately 4.68 ± 0.39 mg.L$^{-1}$. Nitrites concentrations were very weak compared to the other proportioned ionic, and varied very little. They were 0.19 ± 0.01 mg.L$^{-1}$ in the foregut, 0.22 ± 0.02 mg.L$^{-1}$ in the midgut, and 0.16 ± 0.01 mg.L$^{-1}$ in the hindgut (Figure 3B). The mean concentration of NO$_2^-$ of the whole digestive tract was 0.19 ± 0.01 mg.L$^{-1}$. The contents of PO$_4^{3-}$ in intestinal liquid of the worm slightly increased from the foregut (3.75 ± 0.03 mg.L$^{-1}$) to the midgut (4.80 ± 0.07 mg.L$^{-1}$), and finally diminished in the hindgut (3.60 ± 0.11 mg.L$^{-1}$) (Figure 3C). Finally, the mean content of PO$_4^{3-}$ of the whole digestive tract was 4.05 ± 0.07 mg.L$^{-1}$.

Figure 3. Variation of the ionic contents along the digestive tract of A. emini. A: nitrate; B: Nitrite; C: orthophosphate; D: ammonium
The ionic concentrations of $\text{NH}_4^+$ varied from 3.51 ± 0.38 mg.L$^{-1}$ in the foregut, to 5.50 ± 0.63 mg.L$^{-1}$ in the midgut, and 5.02 ± 0.78 mg.L$^{-1}$ in the hindgut (Figure 3D). The mean concentration of $\text{NH}_4^+$ of intestinal liquid of whole digestive tract was close to 4.68 ± 0.50 mg.L$^{-1}$. It appears from Figure 3 that concentrations of $\text{PO}_4^{3-}$, $\text{NO}_2^-$ and $\text{NO}_3^-$ were higher in the midgut than in the two other portions of the intestine.

**Biodiversity and abundance of ciliates**

Twenty three species belonging to nine genera of ciliates were found during this study. Twelve species belonged to the Subclass of Astomatia while the eleven others were Hysterocinetidae (Table 1).

### Table 1. Biodiversity and abundance of the ciliates along the digestive tract of *A. Eminii*

<table>
<thead>
<tr>
<th>Species</th>
<th>Digestive tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foregut (m ± sd)</td>
</tr>
<tr>
<td><em>Almophrya bivacuolata</em> de Puytorac and Dragesco, 1969b</td>
<td>58 ± 11</td>
</tr>
<tr>
<td><em>Almophrya mediovacuolata</em> Ngassam, 1983</td>
<td>73 ± 10</td>
</tr>
<tr>
<td><em>Almophrya lateroacuolata</em> de Puytorac and Dragesco, 1969b</td>
<td>14 ± 3</td>
</tr>
<tr>
<td><em>Dicoelophrya almae</em> de Puytorac and Dragesco, 1969b</td>
<td>0</td>
</tr>
<tr>
<td><em>Dicoelophrya mediovacuolata</em> Fokam et al., 2012</td>
<td>0</td>
</tr>
<tr>
<td><em>Paracelophrya intermedia</em> de Puytorac, 1969</td>
<td>48 ± 8</td>
</tr>
<tr>
<td><em>Paracelophrya polymorphus</em> Fokam et al., 2012</td>
<td>33 ± 6</td>
</tr>
<tr>
<td><em>Paracelophrya ebbebdensis</em> Fokam et al., 2012</td>
<td>56 ± 7</td>
</tr>
<tr>
<td><em>Metaracelophrya intermedia</em> de Puytorac and Dragesco, 1969a</td>
<td>32 ± 6</td>
</tr>
<tr>
<td><em>Coelelophrya roquei</em> de Puytorac and Dragesco, 1969b</td>
<td>62 ± 4</td>
</tr>
<tr>
<td><em>Coelelophrya ovales</em> Fokam et al., 2008</td>
<td>27 ± 4</td>
</tr>
<tr>
<td><em>Coelelophrya ebebdensis</em> Fokam et al., 2008</td>
<td>59 ± 9</td>
</tr>
<tr>
<td><em>Metaptychothomum ebbebdae</em> Ngassam and Grain, 1997</td>
<td>0</td>
</tr>
<tr>
<td><em>Metaptychothomum primorphus</em> Ngassam and Grain, 2000</td>
<td>0</td>
</tr>
<tr>
<td><em>Pycthotomum sanagae</em> Ngassam and Grain, 2000</td>
<td>0</td>
</tr>
<tr>
<td><em>Pycthotomum prolixus</em> Njiné and Ngassam, 1993</td>
<td>0</td>
</tr>
<tr>
<td><em>Pycthotomum commene</em> de Puytorac, 1968</td>
<td>0</td>
</tr>
<tr>
<td><em>Pycthotomum macromorphus</em> Njiné and Ngassam, 1993</td>
<td>0</td>
</tr>
<tr>
<td><em>Pycthotomum elongatum</em> Njiné and Ngassam, 1993</td>
<td>0</td>
</tr>
<tr>
<td><em>Pycthotomum variabilis</em> Ngassam and Grain, 2000</td>
<td>0</td>
</tr>
<tr>
<td><em>Propycthotomum commune</em> Ngassam and Grain, 1997</td>
<td>0</td>
</tr>
<tr>
<td><em>Propycthotomum simplex</em> Ngassam and Grain, 1997</td>
<td>0</td>
</tr>
<tr>
<td><em>Propycthotomum microstomum</em> Ngassam et al., 1993</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2. Correlation between ciliates abundance and ionic contents in the different portions of the digestive tract

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Foregut</th>
<th>Midgut</th>
<th>Hindgut</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NO}_3^-$</td>
<td>0.213</td>
<td>0.503</td>
<td>0.473</td>
</tr>
<tr>
<td>$\text{NH}_4^+$</td>
<td>0.239</td>
<td>0.274</td>
<td>0.435</td>
</tr>
<tr>
<td>$\text{PO}_4^{3-}$</td>
<td>0.436</td>
<td>0.792</td>
<td>0.819</td>
</tr>
</tbody>
</table>

* *: correlation is significant at the 0.05 level; **: correlation is significant at the 0.01 level; : no value; $\text{NO}_2^-$: nitrates; $\text{NO}_3^-$: nitrates; $\text{NH}_4^+$: ammonium; $\text{PO}_4^{3-}$: orthophosphates

Among the 762 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 1). The Hysterocinetidae ciliates were mostly found in the hindgut (128 specimens), 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 (*Almophrya bivacuolata*, *Almophrya mediovacuolata* and *Almophrya lateroacuolata*; *Coelophrya ebebdensis* and *Coelelophrya roquei*; *Pycthotomum prolixus* and *Pycthotomum commune*) (Table 1).
Correlation between abundance of ciliates and ion concentrations

Table 2 displays the relationship between the ciliate abundance and ion concentrations 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 Paracoelophrya ebebedensis and PO₄³⁻ (P<0.05). In the midgut, a positive and significant correlation was observed between the number of the ciliates Dicoelophrya mediovacuolata; Coelophrya roquet; Coelophrya ovales; Ptychostomum commune and NO₃⁻ (P<0.05); Almphrya mediovacuolata and NH₄⁺ (P<0.05). In this same compartment, a positive and highly significant correlation was also observed between the abundance of Ptychostomum prolulus and NO₃⁻ (P<0.01). In the hindgut, a positive and highly significant correlation was found between the values of PO₄³⁻ and Ptychostomum variabilis abundance (P<0.01).

DISCUSSION

The ionic concentration in NO₃⁻ decreased gradually from foregut to hindgut. This gradual decrease might be related to the presence of denitrifying and fermentable bacteria in the digestive tract of the worm (Wüst et al., 2011). This observation was previously reported on the oligochaeta Lumbricus rubellus, Lumbricus terrestris, and Aporrectodea caliginosa by Depkat-Jakob et al., (2010), Karsten & Drake (1997), Horn et al., (2003, 2006) and Wüst et al., (2009).

These various authors highlighted role of denitrifying bacteria in the digestive tract of Oligochaeta in general. The strong ionic concentrations in NO₃⁻, PO₄³⁻ and NH₄⁺, obtained in midgut could be explained by fact that there would be first of all, an accumulation of minerals, then their assimilation. Our results corroborate those of de Puytorac & Mauret (1956) who worked on the oligochaeta Alloblophora savignyi and Lumbricus hercules. Moreover, Maluf in 1940 revealed that the maximum ionic concentration in chloride, sulfate, calcium, potassium, and sodium, was obtained in midgut of the digestive tract of worms. The variation of ionic concentrations along the digestive tract of A. emini could be also related to frequency of use of ions by ciliates that colonized each portion of the digestive tract of the earthworm. Astomes might use few amount of NO₃⁻ compared to Hysteroctinetidae that would have great need for it. These parameters could be influenced by conditions of the surrounding medium and physiological state of the worm as reported by Lavelle & Spain (2001).

In the digestive tract of A. emini, Astomatia (Almphrya bivacuolata; Almphrya mediovacuolata; Almphrya laterovacuolata; Metaracoelophrya intermedia; Coelophrya ebebedensis; Coelophrya roquet; Coelophrya ovales, and Paracoelophrya intermedia) proliferated in the foregut where NO₃⁻ concentration was elevated. On the other hand, hindgut, less rich in ions, was populated by Hysteroctinetidae (Ptychostomum prolulus; Ptychostomum commune; Ptychostomum macrostomum; Ptychostomum elongatum; Ptychostomum variabilis; Propycho stomum commune, and Propycho stomum simplex).

Let us retain that the variation of density is not only ensuing of the activity of ciliates of the digestive tract of earthworms. Brito-Vega & Espinosa-Victoria (2009) described the variation of abundance of the bacteria along the digestive tract of the worms. Gohre (1943) highlighted a similar ensuing of activity of three species of Gregarines of genus Gregarina, parasites of larva of insect Tenebrio molitor. The cohabitation between Astomes and Hysteroctinetidae in midgut confirms the theory of “the association of the species” (Pinel-Alloul et al., 1990). The stratification of the species might depend obviously on various physico-chemical and biotic conditions, successively offered by the biological environment to ciliates species, according to needs of each one of them. We could ask ourselves whether in such stratification does not intervene, in addition to external medium itself, a competition among the various genera. If there is such an antagonism in the case of ciliates, its importance does not appear considerable under normal conditions. Indeed, when one of the species has suddenly missed, the place it was occupying in the portion of the digestive tract remains generally free. These observations do not exclude the possibility of such a competition originally of the settlement of digestive tract of worms. The various ions of medium do not have an equal importance. The importance of each one of them depends on organisms or group of organisms considered. It is function of more or less large sensitivity that this organism expresses with amplitude of the variations that these factors undergo. In the case as of endocommensal ciliates of A. emini, each parameter would have certainly an essential and paramount action. The greatest concentration of cells of each species seems to occur at level of digestive tract extremities; except for some species known as tolerant, gathered in buffer zone. Each one of these species is probably more sensitive to quality of products present in the medium and to their respective content, than to their total quantity.

Variation of ion contents and abundance of ciliate 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 proposed previously suggested 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

Our data shows that some correlation was found between the abundance of ciliates and the ionic concentration in the different portions of digestive tract of A. emini. The stratification of Astomatia and Hysteroctinetidae along the digestive tract of A. emini is influenced by its ionic contents. 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 ion has certainly an essential and primordial action. The greatest concentration of cells of each group (Astomatia and Hysteroctinetidae) seems to
occurs in the fore and the hindgut, respectively. Nevertheless, relative abundance of species 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 the ion present in the medium. It would be necessary to consider in further work the predation, rate of oxygen, osmotic pressure, specific enzymes, bacteria and viruses in the digestive tract of A. emini.

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