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Influence of soil physicochemical parameters on the abundance of *Paracoelophrya polymorphus* (Ciliophora: Radiophryidae) commensal of earthworms (Annelida: Glossoscolecidae) collected in Bambui (North-West Cameroon)

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Key words: *Alma nilotica*, astomatous ciliates (*Paracoelophrya polymorphus*), physico-chemical parameters of the soil, Bambui.

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Abstract

Earthworms of the genus *Alma nilotica* belonging to the family Glossoscolecidae, lodges an abundant fauna of astomatous ciliates in its digestive tract. Each species is located in a well-defined portion of the gut and the reasons for this stratification still remain unclear. In order to assess the influence of physico-chemicals parameters of the soil on the distribution of one of these astomatous ciliates (*Paracoelophrya polymorphus* Fokam, 2012) along the digestive tract of *Alma nilotica*, a study was carried out from April to September 2013 in Bambui on the banks of Fansaah river. The volumic density (VD) of worms (number /dm^3 soil) was examined. A total of 60 worms were used for the counting of ciliates. The observation of *Paracoelophrya polymorphus* was done after staining with methylene blue. Twelve physico-chemicals parameters of the soil from the site of collection of worms were analyzed. The degree of binding between the VD of worms, the abundance of ciliates and physico-chemicals parameters was performed for different portions of the digestive tract. VD of the earthworms shows a positive and significant correlation with the Cation Exchange Capacity CEC (r = 0.771; P < 0, 05) and with Mg (r = 0.886; P < 0, 01). *Paracoelophrya polymorphus* is located in the foregut and midgut of its host but, its preferential zone is the foregut. In the foregut, a negative and significant correlation was found between available phosphorus (P) and *P. polymorphus* abundance (r = -0,943; P < 0, 01). In the midgut, a positive and significant correlation was obtained between the Cation Exchange capacity (CEC) (r = 0.754; P < 0, 05), magnesium (r = 0.812; P < 0, 05), calcium (r = 0.754; P < 0, 05) and *P. polymorphus* abundance. These chemical parameters influence directly the density of the earthworms and indirectly the stratification of *P. polymorphus*.

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Introduction

Soil is a living, dynamic ecosystem. Healthy soil is full of microscopic and larger organisms that perform many vital functions including converting dead and decaying matter and provide nutrients to plants (Ruiz *et al.*, 2008). Among these larger organisms, earthworms have gained widespread attention due to their influence on a diverse array of soil processes including aggregation, residue decomposition, nutrient mineralization, aeration, and water infiltration (Lee, 1985). Their ability to affect soil functioning by such varied mechanisms has earned them recognition as ecosystem engineers (Jones *et al.,* 1994; Lavelle *et al.,* 1997).

The digestive tract of the earthworms lodges a complex microbial community (Lavelle *et al.*, 2006) among which ciliates have been targeted for studies by many investigators (Albaret, 1975; de Puytorac, 1969, 1994; de Puytorac & Dragesco, 1968, 1969; Ngassam, 1983; Njiné & Ngassam, 1993; Ngassam & Grain, 1997, 2000; Lynn, 2008; Fokam *et al.,* 2011; Fokam *et al.*, 2012; Fokam *et al.*, 2013; Fokam *et al.*, 2014; Nana *et al.* 2014).

The studies on the ecological conditions of Astomatous ciliates demonstrated that several species may be found simultaneously in the same worm, each of them living in a given portion that is favorable to its development (de Puytorac & Mauret, 1956). Up to now, the reason of this stratification still remains unclear and very few data are available on the living conditions of these endocommensal ciliates. Some authors investigated on the reasons of this stratification and showed that the physico-chemical factors of intestinal liquid of the host such as pH, water content (Nana *et al.,* 2012, 2014), composition of nutrients (Barois & Lavelle, 1986), osmotic pressure (Ngassam, 1983) could influence the distribution of each species or group of species of ciliates along the digestive tract of their hosts. Barois & Lavelle (1986) working on *Pontoscolex corethurus* (Glossoscolecidae, Oligochaeta) showed that the soil ingested by the earthworms, is gradually degraded by specific enzymes during gut transit. The degradation of soil releases nutrients whose composition varies according to each portion of the digestive tract. They also reported that some physico- chemicals properties of the soil seems to be more or less affected by the enzymes during the gut transit. The soil as a natural habitat of the earthworms could have some physico-chemicals properties that could influence the stratification of ciliates especially the species *Paracoelophrya polymorphus* Fokam, 2012. The present paper deal with soil physico-chemical parameters that may influence the density of earthworms (*Alma nilotica*) on the one hand, and on the other hand, on the abundance of an astomatous ciliate (*Paracoelophya polymorphus*) living in the gut of this earthworm.

Material and methods

The study area

The present study was carried out in the locality of Bambui, Tubah subdivision, Mezam division of North West region in Cameroon during April 2013 to September 2013. The samples of the earthworms were collected along the banks of the stream Fa-sa'ah (06°00'54" N; 10°16'09.7" W; elevation of 1457 m) where the soil is made up mainly of sand, rocks and mud (Fig. 1).

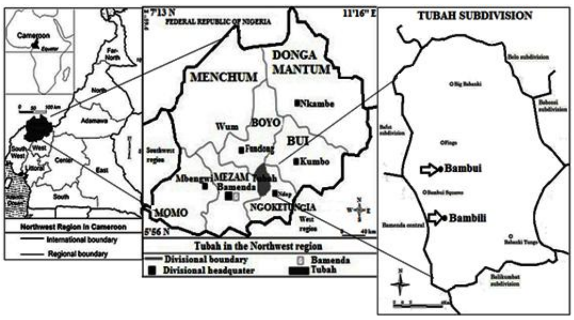

Fig. 1. The study area (modified from Olivry, 1986).

Sample collection

Collection of the earthworms and the soil in the field

In many randomly spots of the banks, the sampling was done in digging quickly the soil with the help of a spade till a depth of 30 cm of soil, near of the earthworms' casts. The soil containing earthworms were gathered in plastic containers of 120 ml of capacity. Further, worms Glossoscolecidae of the genus *Alma* were identified according to the keys of Omodeo (1958) and counted. Soil samples from different spots were mixed to form a composite sample and a proportion was packed in the container of 120 ml of capacity. The composite soil sample is air dried during one week, crushed, sieved, packaged and labeled. This exercise was repeated on a monthly basis during 6 months.

Soil sample analysis

Soil analysis was carried out according to the standard procedures (Pauwels *et al.,* 1992). Thirteen parameters have been analysed. Two physical parameters: soil moisture and granulometry and eleven chemical parameters: pH-water, pH- KCL, organic matter (OM), organic carbon (OC), total nitrogen (TN), cation exchange capacity (CEC), exchangeable bases (Calcium, Magnesium, Potassium and Sodium) and available phosphorus.

Moisture content of the soil

Moisture content of fresh soil samples was determined after oven drying them at 105°C and expressed as a percentage of weight of the soil samples. The weight of the soil samples were measured using an electronic balance of mark Mtech BL-310 s. The following formula was used to obtain percentage of soil water content:

$$\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Granulometry

Survey of the different granulometric constituents consisted of separating clayey, sandy and silt particles after destruction of the organic matter with oxygenated water. The sand was separated by sifting under water on a sifter of 2 mm in diameter. The scattering of the silt and the clay was done with sodium carbonate. The clay was isolated by successive decantation after the sedimentation of silt. The basic suspension of clay thus obtained was then neutralized with hydrochloric acid, and then aggregated by addition of the sodium chloride. The clay was finally ridded of salts by successive washing with water and ethanol until complete elimination of the chloride. The silt fraction was washed with water in order to eliminate the rest of the clay. The obtained results permitted with the help of the soil texture triangle of the USDA (United States Department of Agriculture) soil taxonomy system to determine the texture of every sample.

pH

The pH of soil was measured with the help of a pH meter of the type CG822 provided with a combined pH electrode. The real acidity (pH- water) was measured in a soil - water suspension of ratio of 1/2.5 (10 g of soil in 25 ml of water), at least 16 hours after the preparation. As for the potential acidity (pH-KCL), it is measured in a soil - KCL suspension of the same ratio, 10 minutes after the preparation.

Organic matter

The measurement of organic matter (OM) was effectuated by a wet way from the measurement of the organic carbon (OC). The method of determination of the OC is based on the oxidization of the sample by potassium dichromate ($K_2Cr_2O_7$) in a concentrated acidic medium (H_2SO_4). Excess $K_2Cr_2O_7$ is titrated with the help of ferrous sulphate ($FeSO_4.7H_2O$) in order to deduct the quantity of dichromate neutralized by the OC. The equivalence point is indicated by purple diphenylamine turning to green. The organic matter containing 58 % of OC on average, its content is gotten by the following relation: MO % = CO% x 1, 724.

Total nitrogen

The determination of the content in total nitrogen was done according to the Kjeldahl method (Bremner, 1960). It consists of a complete mineralization of the organic nitrogen by a mixture of hot concentrated sulphuric acid and salicylic acid (350°C). The mixture is distilled by steam practice of water. The distillate is collected in boric acid and is then titrated with a solution diluted sulphuric acid.

Exchangeable bases and cation exchange capacity (CEC) at pH 7

The determination of the contents of exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+) was done after extraction with ammonium acetate at 1N at pH 7. The measurement of Na and K was done by atomic spectrophotometry and that of Ca and Mg by complexiometry with EDTA (ethylene diamine tetra acetic acid).

The determination of the CEC at pH 7 is realized after washing with alcohol in order to eliminate the saturating solution of NH_4. The measurement of NH_4^+ is done by Kjeldahl distillation after quantitative desorption by the KCl.

Available phosphorus

Available phosphorus was determined by the method of Bray II (Bray and Kurst, 1945). This method combines the extraction of the phosphorus in an acidic medium (HCl 0, 1 M) from the complex of ammonium fluoride (NH_4F 0, 03 M) bound to the phosphorus. The measurement of the phosphorus is made by colorimetry to molybdenum blue with the help of a molecular absorption spectrophotometer.

Dissection of the earthworms and counting of the ciliates

The worms were carefully washed with tap water and the length in extension was measured with the help of a graduated ruler. They were cut alive from the prostomium to the pygidium. The anterior part just after the clitellum comprising pharynx, the esophagus, crop and gizzard is removed. The second part is further divided into three equivalent parts: foregut, midgut and hindgut. Each part was still divided into three fragments (anterior, middle and posterior). Using the pair of forceps and a blade, each fragment was dilacerated in a Petri dish of 10cm in diameter containing 10-15 ml of mineral water Supermont (Ca^{2+} 30mg/L ; Mg^{2+} 5.9 mg/L ; Na^+ 0.0 mg/L ; K^+ 3.8 mg/L ; Cl^- 1.3 mg/L ; NO_3^- 0.0 mg/L ; SO_4^- 0.0 mg/L ; HCO_3^- 134 mg/L ; pH 7.1).

Ciliates found in these different portions of the earthworms were identified according to the keys previously described (de Puytorac 1968, 1969; de Puytorac & Dragesco 1969; Ngassam, 1983; Fokam *et al.,* 2008, 2012). They were individually extracted using a micropipette, sorted and counted under a binocular dissecting scope Wild M5 (Heerbrugg, Germany) at 250 x magnification.

Observation of the ciliates

To the content of the Petri dish, and using a pipette, two drops of methylene blue (1%) were added and allowed for 3-6 minutes. Methylene blue then diffused by capillarity to stain the ciliates. These ciliates were later observed under a light microscope Optic Ivymen System® at 200 x magnification. Drawings were performed with the aid of a camera lucida attached to a Wild M20 microscope.

Data analysis

Ten earthworms were used for the counting of ciliates each month during 6 months with a total of 60 studied worms. The software Microsoft excel 2007 was used for the calculations of the means and to draw graphs and charts. Volumic density is the number of the earthworms per dm^3 of the soil and relative abundance is the number of ciliate per portion of the digestive tract. The Software SPSS 16.0 package was used for the correlations. The correlation tests were used to assess the degree of binding between the physico-chemical parameters of the soil and the ciliate abundance in different portions of the digestive tract of the earthworms. Since our variables do not follow a normal distribution, we applied correlation test 'rho" of Spearman to analyze our data. P-values were used to assess the degree of significance of correlation between physic-chemical parameters and ciliate abundance. $P < 0.05$ were set as significant.

Results and discussion

Results

Description and temporal variation of density of the ciliate's hosts

The Oligochaete earthworm *Alma nilotica* belongs to the family of Glossoscolecidae and the genus *Alma*. It is a small pigmented worm with an average weight of 1.25 g which measures on average 17cm in extension (Fig. 2). The specimens are more located at the topsoil horizon and mime the color of the mud. The density of these worms varies over the six months of analysis. The highest volumic density has been obtained in April (25 individuals/dm^3) and the lowest density is in August (5 individuals/dm^3) (Fig. 3).

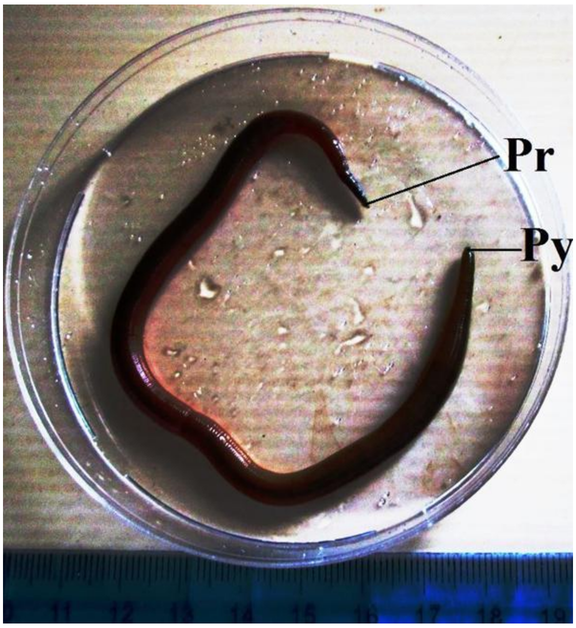

Fig. 2. General view of *Alma nilotica* Pr: Protosmium; Py: Pygidium.

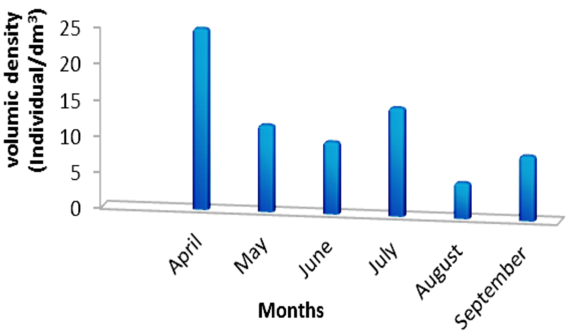

Fig. 3. Variation of Earthworm (*Alma nilotica*) density over Time (April to September 2014).

Physico-chemical characteristics of the soil

- Physical parameters of the soil

The moisture content of the soil varies from 31.11 % to 48.4 % (Fig. 4). The maximum value was observed in July while the minimum value was in May.

The granulometry analysis of the soil shows that it is composed of 23-27% of sand, 57-63% of silt and 14-17% of clay. According to the soil textural triangle (USDA), the texture type of the studied soil is silt loam (Fig. 5).

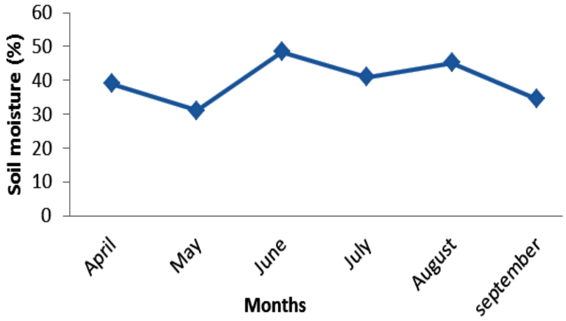

Fig. 4. Temporal variation of soil moisture.

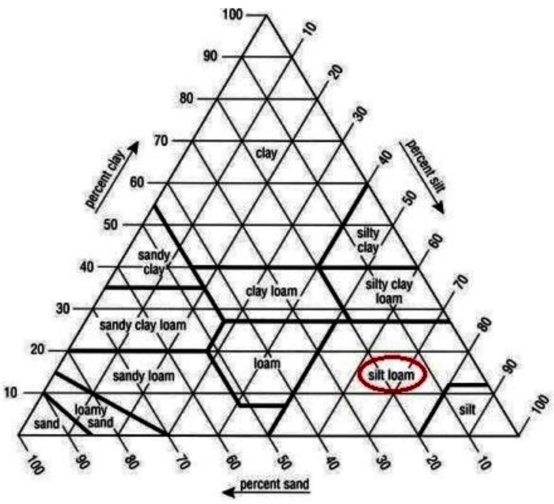

Fig. 5. Soil typology.

- Chemical parameters of the soil

The pH-water ranges between 5.70 and 6.30 (Fig. 6A). According to 'Memento de l'agronome' (1993), the pH is slightly acidic. OC varies between 3.26 and 4.87 %. According to the classification of Dabin (1985), this soil is rich in organic carbon. OM ranges between 5.62 % in September and 8.4 % in May (Fig.

6B). As OM depends of OC, this soil is also rich in OM. The value of total nitrogen (TN) varies from 3.63 to 0.1 %. Available phosphorus has the higher value in September (10.54 ppm) and the lower value in June (1.49 ppm) (Fig. 6E).

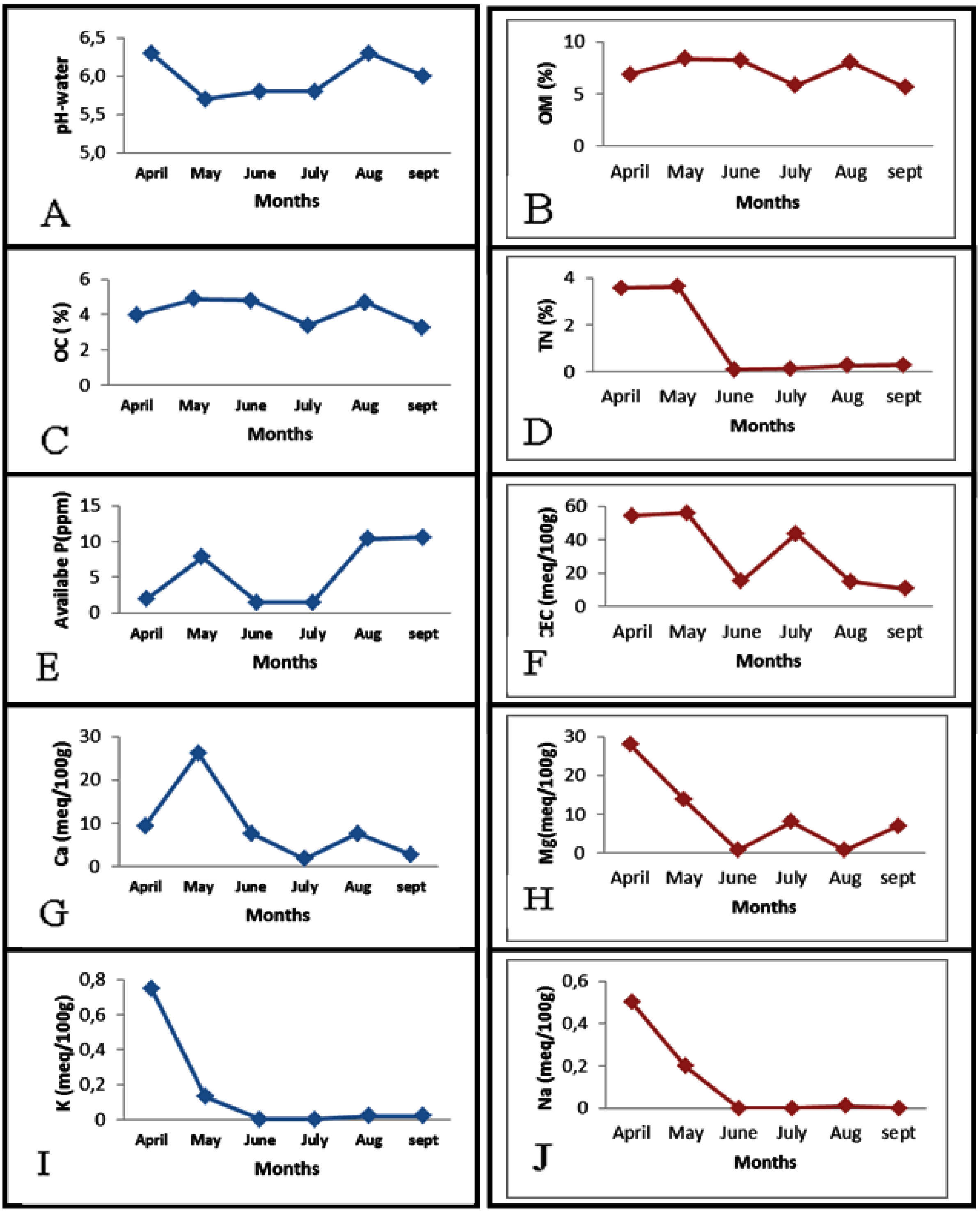

Fig. 6. Temporal variation of chemical parameters. A : pH; B: OM (Organic Matter) ; C: OC Organic Carbon); D: TN (Total Nitrogen) ; E: available P; F: Cation Exchange capacity CEC; G: Ca^{++}; H: Mg; I: K, and J : Na^{+}.

CEC has the highest value in April (54.4 meq/100g) and the lowest in September (10.54 meq/100g) (Fig. 6F). The mean value shows that this soil has higher CEC. Ca ranges between 26.16 meq/100g in May and

1.76 meq/100g in July while Mg varies from 13.68 to 0.64 meq/100g (Figs. 6G, 6H) .This soil is rich in Ca and Mg according to 'Memento de l'agronome' (1993). K varies between 0.75 and 0 meq/100g (Fig. 6I). The mean value shows that this soil is poor in K according to 'Memento de l'agronome' (1993). Na ranges between 0.5 and 0 meq/100g and shows that this soil is poor in Na. The highest value was observed in April and no value was observed from June to September (Fig. 6J).

Description of the ciliate

Paracoelophrya polymorphus Fokam, 2012 is a commensal ciliate of the anterior part of the digestive tract of *Alma nilotica*. This species lives in cohabitation with many astomatous ciliates of the genera *Almophrya*, *Coelophrya*, and *Anoplophrya*. It has an ovoid shape, more or less flattened with two longitudinal rows of contractile vacuoles on the both sides of the macronucleus. *P. polymorphus* is charactrised by a polymorphism of his representatives marked essentially by two main morphological shapes (longitudinal and ovoid). The micronucleus is spherical or lenticular. The cytoskeleton is flattened "V" shape skeletal branches made up of two unequal arms. The right branch has a hook at its left extremity and has 12-14 fibers which distort and thicken in form of arches at halfway (Fig. 7). This species colonizes the foregut and the midgut of *Alma nilotica* earthworms.

The study of the distribution of *P. polymorphus* along the digestive tract of its hosts shows that *P. polymorphus* has as preference portion the foregut and the midgut, the hindgut has no individuals; the maximum abundance was recorded in the foregut (Fig. 8). Concerning the temporal variation, the highest abundance of ciliates in the foregut is in June (54 individuals) while the lowest abundance recorded is in September (10 individuals). In the midgut, the highest abundance is in April (11 individuals) and the lowest abundance is in August (1 individual).

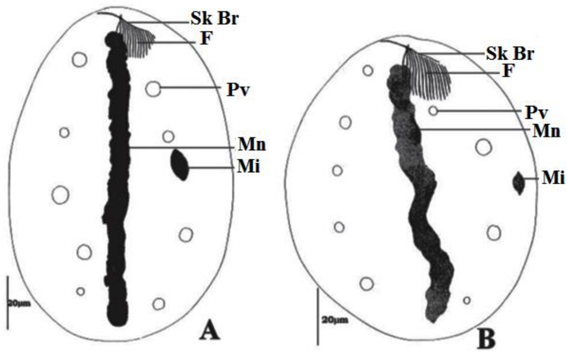

Fig. 7. *Paracoelophrya polymorphus* Fokam, 2012. A: General morphology of the elongated form, B: General morphology of the ovoid form. Pv: Pulsatile vacuole; F: Fibers; Mi: Micronucleus; Mn: Macronucleus; Sk. Br: Skeletal Branch.

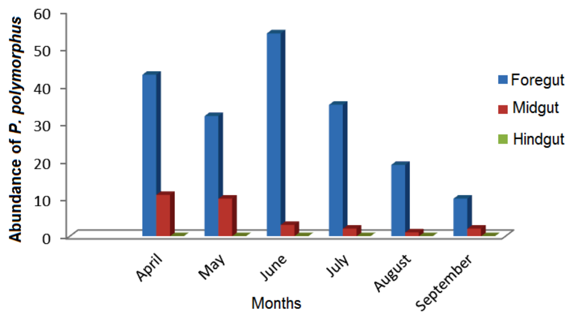

Fig. 8. Distribution of *P. polymorphus* along the digestive tract of *Alma nilotica* over time.

Correlation between volumic density of the earthworms and physico-chemical parameters of the soil

The table 1 displays the relationship between the volumic density of the earthworms and the physico-chemical parameters of the soil. Volumic density of the earthworms shows a positive and significant correlation with the CEC ($r = 0.771$; $P < 0.05$) and with Mg ($r = 0.886$; $P < 0.01$).

Correlation between abundance of P. polymorphus and physico-chemical parameters of the soil

The table 2 displays the relationship between the ciliate abundance and the physico-chemical parameters of the soil. In the foregut, a negative and significant correlation was observed between the abundance of *P. polymorphus* and the available phosphorus ($r = -0.943$; $P < 0.01$). In the Midgut, a positive and significant correlation was observed with the abundance of *M. polymorphus* and the CEC (r = -0.754; P < 0.05) Ca (r = 0.754; P < 0.05) and Mg (r = 0.812; P < 0.05).

Table 1. Correlation between volumic density of earthworms and physico-chemical parameters of the soil.

Physico-chemical elements	Earthworms Correlations
pH - water	0,117
pH - Kcl	0,029
OC	-0,029
OM	-0,029
TN	0,257
Available P	-0,543
CEC	**0,771***
Ca	0,257
Mg	**0,886****
K	0,294
Na	0,395
Soil moisture	-0,257

* : correlation is significant at the 0,05 level;
** :correlation is significant at the 0,01 level

Table 2. Correlation between relative abundance of *P. polymorphus* and physico-chemical parameters of the soil.

Physico-chemical elements	Foregut	Midgut	Hindgut
	Correlations		
pH water	-0,177	-0,224	-
pH Kcl	-0,145	0,015	-
OC	0,371	0,348	-
OM	0,371	0,348	-
TN	-0,371	0,493	-
Available P	**-0,943****	-0,377	-
CEC	0,486	**0,754***	-
Ca	0, 314	**0,754***	-
Mg	0,257	**0,812***	-
K	-0,177	0,567	-
Na	0,091	0,585	-
Soil moisture	0,486	-0,377	-

* : correlation is significant at the 0,05 level;
** : correlation is significant at the 0,01 level; -: no value

Discussion

Alma nilotica seems to be an anecic species as its sister species *Alma emini* (Nana *et al.*, 2012, 2014). These earthworms are more localized at the top soil and they are dorsally coloured. Anecic communities are more likely to modify the soil physical properties and enhance decomposition of plant material by burying and mixing it with soil (Lavelle, 1988). The variation of volumic density of the earthworms in the soil might be due to the abiotic factors except rainfall because the sampling was done during the rainy season. In general, the volumic density of *A. nilotica* is low and might be explained by the hydromorphic characteristics of the soil. Decaëns *et al.* (2003) reported that anecic species seem to be the most affected by soil hydromorphic conditions. Mather & Christensen (1988) assumed that, in flooded soils, earthworms' main physical stress could be due to the degree to which soil conditions are anaerobic. They concluded that both earthworm biomass and earthworm diversity are significantly lower in flooded soils than in non-flooded ones. While, others considered excess of water not to be a limiting factor for most earthworm species, as they are able to live in flooded conditions for long periods providing water is sufficiently oxygenated (Bouché, 1972; Edwards & Bohlen, 1996).

The soil occupied by these earthworms is rich in silt that has as medium properties the nutrient and water holding capacity, water infiltration and aeration of the soil. Our results corroborate those of Salomé *et al.* (2011) who reported that loamy and silt loamy texture with high organic carbon are found in mature forest stages and these conditions favoured the settlement of anecic species especially *Aporrectodea cali. nocturna*. The slightly acidic pH of the soil sample is due to the high Ca and Mg values observed. It is well known that the application of high quantities of calcium and magnesium help to neutralize soil acidity. Edwards & Bohlen (1996) cited soil pH as a limiting factor on earthworm distribution. In our study, no relationship was found between the pH and earthworms. The absence of significant correlation between soil moisture and earthworms density might be due to the excessive waterlogging of the soil. Duiker & Stehouwer (2008) noticed that the biomass of the earthworms is drastically reduced in flooding periods. The volumic density of the earthworms is positive and significant correlate with CEC and Mg. This may be explained by the higher organic matter of the soil that is mainly composed by the stable and complex organic matter called humus. Humus has a negative charge and allows the soil to hold these nutrients cations due to the attraction of charges. Soils with high clay or organic matter content will have a higher CEC (Jhonson, 2009). The high value of Ca and Mg observed is in accordance with the higher CEC observed in the soil. However, no significant correlation is found with organic matter. The relationship between earthworm abundance and organic matter is a complex one (Rossi *et al.*, 2006).

The ciliate *Paracoelophrya polymorphus* collected in Bambui is morphologically almost identical to the specimens collected in the center region for the original description by Fokam *et al.* (2012), but differs lightly just by the reduction of size: 81-110 x 63-94 µm instead of 95-125 x 70-102 µm. This observation shows that the geographical area of repartition of *Alma nilotica* seems to extend up to the region of the Nord west region of Cameroon and the trophic condition the worms may justify the decrease in size of the specimens of Bambui.

The stratification of *P. polymorphus* along the digestive tract of the *A. nilotica* earthworms shows that *P. polymorphus* are more abundant in the foregut than in the midgut and is absent in the hindgut this observations are in accordance with the findings of the previous authors (Fokam *et al.*, 2012). Some authors (de Puytorac and Mauret, 1956; de Puytorac, 1994) observed that one host may lodge several species of astomatous ciliates, each of them living in a given portion of the digestive tract favorable to its development. The evaluation of the relationship between the physico-chemical parameters of the habitat of the earthworms and the stratification of this ciliate shows a negative correlation with the available phosphorus in the foregut and positive correlation with CEC, Ca and Mg in the Midgut. This might be explained by the fact that the soil ingested by the earthworms is gradually degraded by specific enzymes (Barois & Lavelle, 1986) or by the microorganisms such as bacteria (Wüst *et al.*, 2011) releasing nutrients. The nutrients would be accumulated in the first portions of the digestive tract then, are assimilated. Nana *et al.* (2012), in determining the ionic contents in the intestinal liquid of the digestive tract of *Alma emini*, obtained an increase content of orthophosphates (PO_4^{3-}) from foregut to midgut and strong ionic concentration of nitrites (NO_2^-) and ammoniums (NH_4^+) in the Midgut. The higher abundance of *P. polymorphus* seems to live in the medium poor in (PO_4^{3-}) content. The studies of Maluf (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 variations of ionic concentrations and water content in the digestive tract could be influenced by the conditions of the surrounding medium (porosity of the soil) and the physiological status of the earthworms (Edwards, 1998; Lavelle & Spain, 2001). These results revealed that the internal physico-chemicals parameters of the earthworms are influenced by the parameters of the soil; in turn, the internal parameters (pH, electric conductivity, total dissolved substances, water content and ionic contents) influence the distribution of ciliates along the digestive tract of the earthworms (Nana *et al.*, 2012, 2014). Therefore, a high CEC in the soil indirectly could influence the abundance of ciliates in the midgut. The pH of the soil in our study does not influence the stratification of the ciliate. This results is in accordance with those of some authors (de Puytorac & Mauret, 1956; Nana *et al.*, 2012) who working with different species of earthworms coming from acidic and alkaline soils respectively, were showed that the pH of the digestive tract increase from foregut to midgut.

Besides these abiotic factors, we have also the biotic factors such as interspecific competition and predation. Fokam (2005), hypothesised that the heterotrichous ciliate *Pronyctotherus camerounensis* living in the foregut and midgut of Megascolecidae worms can ingest the Astoms of small size such as *Metaradiophrya simplex* living in these mediums. However, it cannot ingest the astomatous ciliate of high size *Eudrilophrya complanata* present in the three portions of the gut because it cannot pass through the mouth of the heterotrichous ciliate. This observation seems to confirm the fact that the predation could influence the stratification of ciliates along the digestive tract of the earthworms.

Conclusion

The present study shows that *P. polymorphus* colonizes the foregut and the midgut of the digestive tract with the foregut as preferential zone. The CEC and Mg are two important parameters of the soil that seem influence both the volumic density of the earthworms and the stratification of *P. polymorphus* along the digestive tract of its hosts. Also, available phosphorus and calcium are not the lesser importance in the distribution of this ciliate. These chemical parameters influence directly the physico-chemical parameters of the intestinal fluid and indirectly the stratification of ciliates. This study also reveals that the digestive tract of Oligochaete in general and that of *Alma nilotica* in particular can be considered as a set of biotopes with specific physical and chemicals parameters influenced by the conditions of habitat of the worms.

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