

Spatial Distribution of *Myxobolus Pethericii* and *Henneguya Pethericii* on the Gills of an African Anabantid *Ctenopoma Petherici* from the Sange River, Cameroon

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Abstract

The spatial distribution of *Myxobolus pethericii* Fomena, Lekeufack folefack and Tang II, 2007 and *Henneguya pethericii* Fomena, Lekeufack folefack and Bouix, 2008 gills parasites of *Ctenopoma petherici* Gunther, 1864 was investigated. The gill apparatus of each host individual was divided into arbitrary regions and the number of cysts of each parasites species in each gill region was determined. Results were analysed at parasite species and xenocommunity levels. Site specificity was determined by application of Chi-square test to the data. At the same time, mean cyst loads were compared between different gill regions. The mean cyst load of the xenocommunity was higher on arches II and III than on arch IV. *M. pethericii* encysted more on outer hemibranch of arch IV. The medial segment of the gill was more colonized by *M. pethericii* and the combination made of *M. pethericii*+*H. pethericii*. The greater mean cyst load of *M. pethericii* was observed on medial segment. The specific preferences of a gill region might be affected by the interaction of factors such as the possible heterogeneity of the gill system, possible differences in the hydrostatic pressure of the branchial pump and the water current over different parts of the gill surface.

Keywords Myxosporidia; *Myxobolus Pethericii*; *Henneguya Pethericii*; *Ctenopoma Petherici*; gill spatial distribution; Gill arches; Cameroon

Introduction

Members of the phylum Myxozoa Grasse, 1970 mainly infect fish. About 2300 species of Myxosporeans have been described so far [1]. It is known that the two largest genera of Myxosporeans (*Myxobolus* and *Henneguya*, including approximately 904 and 189 species respectively) are histologic [2,3]. They are commonly described on the gills of teleost fishes [2,3]. Molnar [4] reported that Myxosporeans have host, organs or tissues specificities. The tissue specificity is the most important. Knowledge related to the preferred gill area for the establishment of the Myxosporeans may facilitate the identification of the parasite species. Such knowledge is therefore relevant for the species description [5]. Only few authors have reported data on the spatial distribution of Myxosporeans species on the gills [6-8].

The original observation that some parasites have higher affinities for specific organs within the host was first reported by Cerfontaine et al. [9,10]. This observation has been greatly extended and refined. According to Dogiel [11], the host and its environment are the overall environment of the parasite. This finding is particularly approved as concerning the gill parasites which are in direct contact with the external environment of the host. The gills, commonly known as the most infected body part of the host by parasites are deemed a rather complex organ. Numerous authors have so far investigated the microhabitat of gill-living parasites [6-8,12-21]. Therefore, in the overall situation, the parasite species coexistence is studied in the context of site segregation [19,22]. Several authors have studied the spatial distribution of various Monogenea. They have reported some

specificity for particular areas of attachment of these parasites by arbitrarily dividing each gill arch into several regions.

This study aim at investigating the spatial distribution of *Myxobolus pethericii* and *Henneguya pethericii* on the gills of *Ctenopoma petherici*.

Material and Methods

Fish host *Ctenopoma petherici* Günther, 1864 (Anabantidae) were sampled in Wouri bassin on monthly basis during 15 months (January 2008-march 2009), from the Sange River at Ntonde (between latitude 4°12' N to 4°17' N and Longitude 10°0' E to 10°8' E), located in the Littoral Region of Cameroon. Fishes were captured using a 1 cm² mesh gill net. The collected sample were then preserved in a 10% formalin solution and transported to the laboratory (The University of Yaounde I) in a plastic container. In the laboratory, each specimen was dissected for the extraction of the gills. Both sides of the gills were examined with a stereoscopic microscope (Olympus Bo 61) to search for cysts. Myxosporidia cysts found on gill filaments or bony arch were counted and crushed between a slide and cover-slide and their content identified using an objective 100X of a Wild M-20 microscope. Parasitic species were identified according to Lom et al. [23]. Prior to the observation of the parasites location on the gills, arbitrary division of the gill arches was made according to Turgut et al. [24] modified. Therefore, the gill arches from each side of the fish were numbered I, II, III, IV from the anterior gill arch below the operculum to its posterior part. The surface of each hemibranch was designated as outer (i.e. that surface being the nearest to the operculum) and inner, and each hemibranch was divided into three approximately equal segments: anterior, medial and posterior. The bony part of the gill arch was also divided into three equal sections (Figure 1). The infection rate,

the percentage (or occurrence) of infection and the cyst load were also evaluated as the parasites prevalence and intensity. Prevalence and intensity were defined according to Bush et al. [25].

Using the statistical package SPSS version 16.0, the data analysis was based on: (1) the χ^2 test for the comparison of the infection rates of *M. pethericii*, *H. pethericii* and *M. pethericii* + *H. pethericii* according to the side of the gill, the gill's arches, the inner and outer hemibranches and the gill segments of *C. pethericii*; (2) the Kruskal-Wallis H test for the comparison of the mean cyst load of *M. pethericii*, *H. pethericii* and *M. pethericii* + *H. pethericii* between the gill's arches and the gill segments; (3) The Mann-Whitney U test for the comparison of the paired mean cyst load of *M. pethericii*, *H. pethericii* and *M. pethericii* + *H. pethericii* depending on the side of the gill, the gill arches, the inner and outer hemibranches and the gill segments of *C. pethericii*.

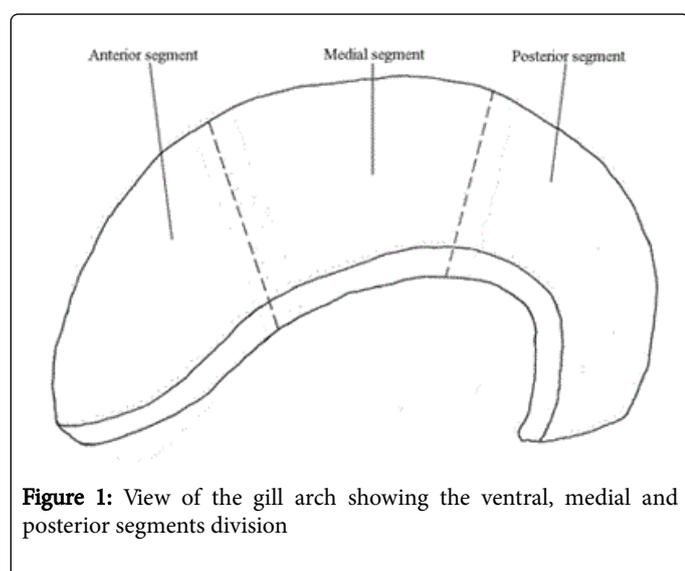


Figure 1: View of the gill arch showing the ventral, medial and posterior segments division

According to Bush et al. [25], the infection rate was estimated as the number of individuals of *C. pethericii* infected with one or more individuals of a particular Myxosporean species divided by the total number of *C. pethericii* examined. Referring to the mean intensity defined by Bush et al. [25], the mean cyst load was calculated as the average number of cysts of a particular species of Myxosporean among the infected members of *C. pethericii* found in the sample divided by the number of *C. pethericii* infected with that Myxosporean species. All statistical tests were considered significant at $P < 0.05$.

Results

A total of 364 specimens of *Ctenopoma pethericii* were examined, among which 245 (67.3%) were infected by *Myxobolus pethericii* and 201 (55.2%) by *Henneguya pethericii*. From this host sample, 166 (45.6%) individuals harbored both *M. pethericii* and *H. pethericii*. As for the cases of mono infection, it appears that 79 (21.7%) specimens of

C. pethericii harbored *M. pethericii* cysts alone while 35 (9.62%) individuals were only infected by *H. pethericii*. Among the examined fish specimens, 84 (23.08%) were free of parasites. A total of 31952 Myxosporean cysts were found on the gills of examined fishes; among which 3446 (10.8%) cysts were of *M. pethericii* and 28506 (89.2%) were of *H. pethericii*. The mean cyst load was 144.7 ± 214.9 (3-1470) for the xenocommunity (*M. pethericii* + *H. pethericii*), 14.1 ± 23.6 (1-196) cysts for *M. pethericii* and 141.8 ± 216.4 (1-1464) cysts for *H. pethericii*. Parasites species studied were found among the above considered gill regions, their infection rate and the mean cyst load are respectively presented in Figure 2 and Table 1. No gill area was free of parasites.

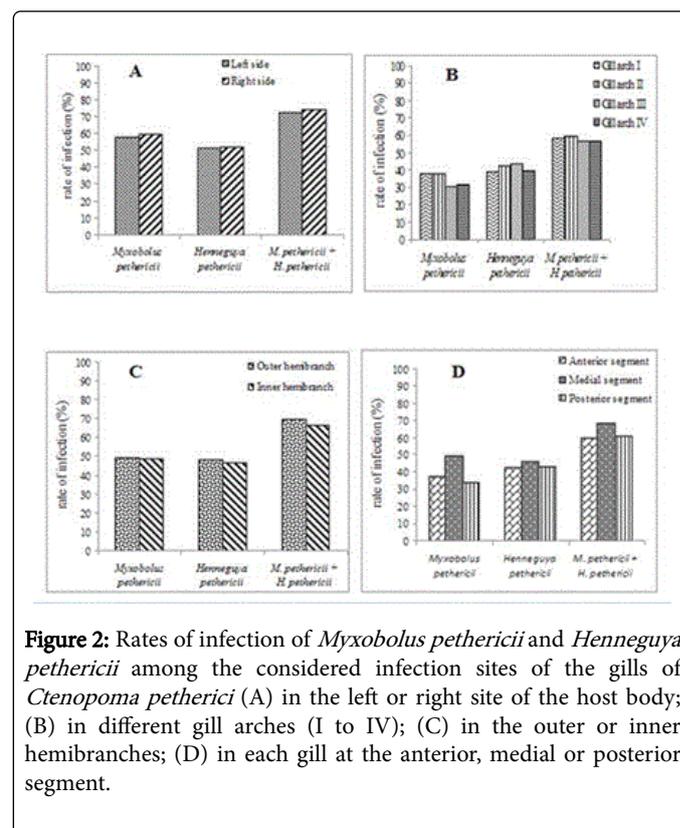


Figure 2: Rates of infection of *Myxobolus pethericii* and *Henneguya pethericii* among the considered infection sites of the gills of *Ctenopoma pethericii* (A) in the left or right site of the host body; (B) in different gill arches (I to IV); (C) in the outer or inner hemibranches; (D) in each gill at the anterior, medial or posterior segment.

Data analysis showed no statistically significant difference in the variation of the occurrence and the mean cyst load of *M. pethericii* ($\chi^2=0.204$; $P > 0.05$) and *H. pethericii* ($\chi^2=0.022$; $P > 0.05$) between the right and left gill arches of *C. pethericii* (Figure 2A). The branchial system of *C. pethericii* is formed of four pairs of gill arches; but due to the absence of difference in the variation of the occurrence and the mean cyst load of parasites species between the right and left gill arches of *C. pethericii*, only one set of gill arches has been considered in the following analysis.

Parameter	<i>Myxobolus pethericii</i>			<i>Henneguya pethericii</i>			<i>M. pethericii</i> + <i>H. pethericii</i>		
	n	$\bar{x} \pm S$ (min-max)	Test value	n	$\bar{x} \pm S$ (min-max)	Test value	n	$\bar{x} \pm S$ (min-max)	Test value
Left side	220	8.17 \pm 12.79(1-100)	U=22509	186	76.4 \pm 119.54(1-868)	U=17150.5	268	59.79 \pm 95(1-874)	U=35158
Right side	216	8 \pm 12.83(1-96)		188	76.03 \pm 106.07(1-596)		263	60.33 \pm 105.39(1-596)	

Gill arch I	137	4.09 ± 5.33(1-39)	H=5.85	142	23.33 ± 27.63(1-153)	H=7.8*	211	18.36 ± 24.95(1-153)	H=9.18*
Gill arch II	138	3.5 ± 5.46(1-37)		154	27.63 ± 37.42(1-278)		216	21.94 ± 33.63(1-278)	
Gill arch III	111	3.5 ± 5.13(1-32)		158	26.76 ± 34.13(1-167)		206	22.41 ± 31.68(1-167)	
Gill arch IV	116	2.55 ± 2.36(1-14)		144	17.33 ± 23.59(1-181)		205	13.61 ± 21.03(1-183)	
Outer hemibranch	179	5.21 ± 7.75(1-60)	U=14498.5	173	41.88 ± 54.88(1-311)	U=14667.5	253	32.32 ± 49.06(1-311)	U=30589.5
Inner hemibranch	177	4.49 ± 6.39(1-44)		171	41.23 ± 54.30(1-285)		242	32.42 ± 49.13(1-285)	
Anterior segment	136	3.45 ± 4.43(1-28)	H=6.8*	155	29.69 ± 35.43(1-175)	H=1.67	217	23.37 ± 32.60(1-175)	H=2.556
Medial segment	179	4.73 ± 6.69(1-45)		166	32.56 ± 41.40(1-214)		248	25.20 ± 36.93(1-214)	
Posterior segment	125	3.3 ± 5.03(1-42)		157	27.29 ± 35.34(1-207)		221	21.26 ± 31.34(1-207)	

*: significant difference at 5% level of confidence.

Table 1: Distribution of mean cyst load of *Myxobolus pethericii* and *Henneguya pethericii* on different parts of the gill apparatus in *Ctenopoma petherici*.

With no significant difference, *M. pethericii* ($\chi^2=7.163$; $P>0.05$) presented preference for arches I and II meanwhile *H. pethericii* ($\chi^2=2.032$; $P>0.05$) preferred arches II and III (Figure 2B). The mean cyst load of *M. pethericii* decreased in the antero-posterior direction without any significant difference ($H=5.845$; $P>0.05$). This pattern was not the same with *H. pethericii*. The median arches II and III harbored more cyst than arches I and IV but there was no significant difference ($H=7.799$; $P>0.05$) (Table 1). Our observations also showed that, for all parasite species with no significant difference, arch IV always harboured few cysts for all parasite species with no significant difference. At the xenocommunity level (*M. pethericii* + *H. pethericii*), the mean cyst load obtained on arches II ($U=19171$; $P<0.05$) and III ($U=17778.5$; $P<0.05$) are significantly higher compared to arch IV (Table 2).

	Gill arch I	Gill arch II	Gill arch III
Gill arch II	22134.5		
Gill arch III	20558.5	21710	
Gill arch IV	19278.5	19171*	17778.5**

*: significant difference at 5% level of confidence;
 **: significant difference at 1% level of confidence.

Table 2: Comparison of the xenocommunity mean cyst load between different gills arches (U value).

Data analysis showed no statistical significant differences in the infection rate of *M. pethericii* ($\chi^2=0.022$; $P>0.05$) and *H. pethericii* ($\chi^2=0.22$; $P>0.05$) on outer and inner hemibranches irrespective of the gill arch (Figure 2C). On Arch IV, *M. pethericii* significantly ($U=2064$; $P<0.01$) encysted more on outer hemibranch (Table 3).

Our observations showed that the medial segment of the gill was significantly more colonized by *M. pethericii* ($\chi^2=18.598$; $P<0.01$) and the combination made of *M. pethericii* + *H. pethericii* ($\chi^2=6.689$; $P<0.05$) (Figure 2D). The same result was obtained when comparing segments at the gill arch level (Table 4). There was a significant

difference between the mean cyst loads of *M. pethericii* on different gills arches segments ($H=6.8$; $P<0.05$). A greater mean cyst load of *M. pethericii* was observed on medial segment. At the gill arch level, only arch IV show significant different ($H=7.36$; $P<0.05$) mean cyst load between segments, the medial segment harbouring more cyst than the others. In single infection with *H. pethericii* ($H=1.67$; $P>0.05$) and mixed infection (*M. pethericii* + *H. pethericii*) ($H=2.556$; $P>0.05$), the medial segment also harbour more cysts without significant difference.

Parasite species	Gill of <i>C. petherici</i>			
	arch I	arch II	arch III	arch IV
<i>Myxobolus pethericii</i>	4237	3804	2474	2064**
<i>Henneguya pethericii</i>	7053	8808	9164,5	6951
<i>M. pethericii</i> + <i>H. pethericii</i>	15717.5	1478	14768	12907

** : significant at 1% level of confidence.

Table 3: Comparison of parasites mean cyst load between hemibranches for the same gill arch (U value).

Parasites species	Gill of <i>C. petherici</i>			
	arch I	arch II	arch III	arch IV
<i>Myxobolus pethericii</i>	14.88**	13.43**	9.96**	17.96**
<i>Henneguya pethericii</i>	0.57	0.98	2.28	3.51
<i>M. pethericii</i> + <i>H. pethericii</i>	8.59*	3.39	6.82*	13.91**

*: significant at 5% level of confidence;
 **: significant at 1% level of confidence.

Table 4: Comparison of rate of infection between different gills segments (χ^2 value).

Discussion

Parasite specific richness of gill of *C. pethericii* consisting of two different species of Myxosporean (*Myxobolus pethericii* and *Henneguya pethericii*) may be responsible of significant losses in river Sange. According to Combes [26], the pathogenic effect is rarely due to a single parasite species. The sum effect of all species of *C. pethericii* gill Myxosporean could be the cause of host morbidity and even mortality. The large number of cyst observed can be the consequence of accumulation of vegetative forms of Myxosporean studied on the gills of *C. pethericii*. These cysts, which are firmly attached to the fish gill epithelium, can release their infective spore only after the death of the host.

Myxobolus pethericii and *Henneguya pethericii* are distributed on the entire bronchial apparatus. The present study indicated that only weak competitive relations exist between the studied parasite species. Most probably there is a reciprocal tolerance between *M. pethericii* and *H. pethericii*. On the basis of the performed study, one cannot talk about separate ecological niches occupied by different parasite species [27]. Moreover Lom et al. [28] reported in Myxosporean the paucity of inter-and intra-specific competition. According to these authors, the lack of competition would promote polyparasitism in hosts.

Myxobolus pethericii and *H. pethericii* affecting the gill of *C. pethericii* could be responsible for major pathological changes (haemorrhagic foci, inflammations in the gill epithelium). Fomena [29,30] noted that in an advanced stage of infection, the plasmodia of such parasites can fully occupy the gill lamellae and cause epithelial dilation and hyperplasia. A pronounced dilation of infected gill lamellae can create pressure on the neighbouring lamellae causing their deformation and ultimately a merger. In massive infection by Myxosporean cysts, reduction of the epithelial surface and compression of blood capillaries by these parasites can partially impair gill functions [31].

Studies on microhabitat distribution of Myxosporean on the gills of their host. Data available are less abundant and are those of [6-8]. The majority of works on the distribution of gill parasites are related to Monogenea [6,7,12-21].

The degree of colonization of different gill zones by the Myxosporean studied varied from one parasite species to another. Thus, preference of some regions was observed. Combes [26] noted that biotope heterogeneity creates a series of distinct microenvironments that are all habitats options for parasites species.

Differences between rate of infection and mean cystic load on left and right site of *C. pethericii* were not statistically significant at xenocommunity and parasite species level. Similar results were obtained by Tombi et al. [7] on *Myxobolus barbi* and *M. njinei* gill parasites of *Barbus martorelli* in Cameroon, and Saha et al. [8] on *Thelohanellus rohita*, a gill parasite of *Labeo rohita* in India. We believe that, the equal distribution of Myxosporean on both sides of the body of *C. pethericii* would be the consequence of bilateral symmetry of the host. Furthermore, we agree with Saha et al. [8] that this could be due to the fact that similar volumes of water flowing through the left and right sides of the gill might have brought equal amount of actinospore stages to the gill. However, preference for fish side has been recorded with some monogenes. Preference for the right side was observed on *Dactylogyrus amphibothrium* [32] and *Microcotyle mugilis* while preference for the left side was observed concerning *Metamicrocotyle cephalus* [14] and *Dactylogyrus valeti* [21].

Our observations showed that arch IV was always less colonized by almost all parasite species. Few studies had been done to determine whether all of the gill arches play an equal part in gaseous exchange or whether more of the respiratory current passes over some gill arches than others. Considering the size alone, one might suspect that at least in most freshwater fishes the most posterior gill arch, number IV, receives less water flow than the anterior ones. Paling [33] described a single method of estimating the relative volume of water flowing over the different gill arches. He found that in brown trout, most of the respiratory current flows over the second and third pair of gills, less flows over the first pair and least of all across the most posterior pair of gills. In the absence of more sophisticated methods producing more accurate results, Paling's [33] findings serve useful functions in providing estimates of the different volumes of water flowing over the four pairs of gill arches. His findings, therefore, was adopted, particularly in view of Hughes [34] work indicating that the degree of infection of the gills is directly related to the ventilation volume and the pattern of current flow over the gills. As far as differences in the water current over the different parts of gill surface can be considered important in determining the distribution of parasites on the gills [20,32], the strongest water current passes through the middle part of the gill arches, thus creating convenient conditions for parasite settlements. The volume of the passing water may influence the aerobic conditions in certain gill parts, thus facilitating parasite settlement but also reflected the greater surface area available for parasite attachment on these gills [32]. This result might explain the present findings that the greatest mean cyst load of the xenocommunity occurred on the second and third gill arches. *Myxobolus pethericii* average cyst load reduced gradually on gill arches without any significant difference in the anterior-posterior direction. The same observation was made by Tombi et al. [21] on the distribution of *Dactylogyrus amieti*, a gill parasite of *Barbus camptacanthus*, which follows the variation of host filaments number. This filament number decreased significantly from arch I towards arch IV, the posterior arch (arch IV) which harboured the smallest number of filaments was least infected. Although slightly more *H. pethericii* cysts occurred on the second and third gill arches of *C. pethericii*, the difference was not statistically significant. The results coincide with the findings of Tombi et al. [7] who found no statistical difference in mean cyst number of *M. barbi* and *M. njinei* between gill arches of *B. martorelli*.

In the present study, *M. pethericii* mean cyst load was statistically higher on the outer rather than the inner face of the arch IV hemibranch. Different observation was made by Saha et al. [8] on the distribution of *Thelohanellus rohita* on the gill of *Labeo rohita*. For this host species, posterior hemibranch of second gill arch was the most preferred site for parasite attachment. El Hafidi et al. [14] pointed that some monogenean species tend to attach to the inner hemibranch of the gill. On arch I, II and III, *M. pethericii* and were randomly distributed between the outer and inner hemibranches. This can be explained by the geometry of the gills that changes constantly during a single breathing cycle [35]; therefore, parts of the gill sieve are alternately exposed to and protected from the water flow.

A high occurrence of *M. pethericii* and the xenocommunity (*M. pethericii*+*H. pethericii*) on median segment of the gills arches was found in this work. Bychowsky [36] reported that the Monogenean Diplozoon paradoxum was predominant in the median sector of the gills. Similar preference was noted by Suydam et al. [37,38]. When studying spatial distribution of parasites species of the genus *Dactylogyrus* (monogenean) on the gills of the host fish, Turgut et al. [24] found a preference for specific regions of the gill arches. The

author concluded that these specific preferences might be affected by the interaction of several factors such as differences in the hydrostatic pressure of the branchial pump [39], coughing action [40], water current over the gill surface [32,33] during the respiratory cycle [41,42].

Compliance with Ethical Standards

Animal use followed a protocol approved and authorized by Institutional Animal Care and Use Committee at Animals Biology and Physiology Department, Faculty of Science, University of Yaounde 1, Cameroon.

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Conflict of Interest Statement

The authors declared: There is no conflict of interest.

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