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# Polyparasitism by monogenea in wild *Hemichromis elongatus* (Guichenot, 1861) (Pisces: Cichlidae): A study model encouraging the prevention of heavy parasitic infections in fish farms

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**Abstract**
The present paper aims to study the occurrence of monogenean species infecting the gills of wild *Hemichromis elongatus* (Pisces). Fish specimens were caught with nets, fixed in 10% formalin and then dissected. Monogenean specimens found were removed under a stereomicroscope with a needle, and then mounted between slide and cover slip in a drop of hematoxylin eosin. Among the five parasite species found, only *O. voltaensis* and *C. euzeti* were found infesting their host alone. There were significant differences between mono-parasitism and polyparasitism both in parasite load and infection rate, which were higher in simultaneous infestations. These monogenean ectoparasites best exploit their common host when they co-occur. This phenomenon observed in natural conditions is a threat in fish farming where high host densities favor parasite transmission. We therefore recommend quarantining native fish specimens captured in the wild, periodic pond sanitization and deworming.

**Keywords:** Co-occurrence, gill parasites, fish, river mefou, Cameroon

## 1. Introduction

Interspecific interactions are generally considered one of the important factors contributing to community structure of parasites [1]. It is worth noting that host-parasite interactions generally involve communities of parasites [2, 3]. However, in a given system and due to the aggregated nature of parasites, not all combinations of potential infecting species are encountered in each host individual [1]. According to Vaumourin *et al*. [4], coinfections may result when hosts are independently infected by different parasite taxa at the same time or during a sequential infection as well as when interactions among parasitic species facilitate co-occurrence. This implies that some parasite species which settle first can modulate the host immunity and promote its secondary colonization by other parasitic species [5, 2]. In other words, interactions may be synergistic (the presence of one parasite may facilitate subsequent infection by other parasites) or antagonistic: the presence of one parasite may inhibit subsequent infection by other parasites [4]. In this context, Petney *et al.* [6] then Wood *et al*. [7] argued that interactions rather than associations among parasites play a major role in structuring parasite populations (both within and among hosts). It is known that adult stages of monogenean parasites are more dangerous to fish health depending on factors such as modes of attachment, size and weight of host [8]. These ectohelminths are responsible for localized hyperplasia, disturbance of osmoregulation and mortality of the host [9, 10]. They can also result in secondary infections in the host from viruses, bacteria and fungi [11, 12]. Indeed, the parasitic diseases of fish reduce the amount of food available to people around the globe [13] and affect the marketability of commercially produced fish, thus raising many public health concerns [14]. Thus, the investigation of these interactions can contribute to fight against heavy losses in fish farming caused by parasites. The aim of this work was to study the occurrence of monogenean species parasitizing the gills of wild *Hemichromis elongatus* (Guichenot, 1861) from the Mefou River in Cameroon and to exploit the results acquired in the domestication any fish species. It was recently shown that at least in our area, this species was mistaken for *H. fasciatus* Peters, 1857 [15].

## 2. Materials and Methods

The present study was conducted in the upstream course (11°27'N; 3°40'E), the middle course (3°51'N; 11°29'E) and downstream course (11°32'N; 3°38'E) of the Mefou River. Fish were sampled from December 2017 to September 2019 by nets. After capture, they were immediately introduced in a jar containing 10% formalin then transferred to the laboratory for subsequent procedures. Monogeneans were dislodged from the gill filaments using a needle and were mounted between slide and cover slip in a drop of hematoxylin eosin [16]. The species were identified under a Leica DM2500 microscope by the morphological characteristics of the sclerotized parts of the haptor and the copulatory organs according to Paperna [17, 18] and Dossou and Birgi [19]; they were then counted. In this work, the terms prevalence (Pr), intensity (I), mean intensity (MI) and abundance are defined according to [20], while infrapopulation, infracommunity, xenocommunity are defined according to Combes [21]. The mean intensity (even the intensity) is categorized in our environment as follows: very low (MI ≤ 10), low (10 < MI ≤ 50), average (50 < MI ≤ 100) and high (MI > 100) after Bilong Bilong and Njiné [22]. Mean values are expressed as follows: ± SE. The Chi-square ($\chi^2$) test permitted to compare prevalences. The Kruskal-Wallis test (K) allowed the comparison of several (>2) means while Mann-Whitney test (U) was used for pairwise comparisons. Sperman's rank correlation coefficient ($r_s$) was calculated to seek the existence of any meaningful association between mean intensity and species richness (sr). All the analyses were performed using STATISTICA 6.0 software and Quantitative Parasitology 3.0. *P*-values less than 5% were considered significant.

## 3. Results

From the gills of the 73 *H. elongatus* hosts a total of 3318 monogenean specimens were collected among which five species were identified namely *Cichlidogyrus euzeti* Dossou & Birgi, 1984; *C. falcifer* Dossou & Birgi, 1982; *C. longicirrus* Paperna, 1965; *Onchobdella aframae* Paperna, 1968 & *O. voltaensis* Paperna, 1968. All autopsied fish harbored at least one monogenean species i.e. the overall infection rate was 100%. The overall gill monogeneans load varied from 1 to 249, the average values being low (45.45 ± 6.21 monogeneans per host individual). The five parasitic taxa aggregated in the host sample since always R > 1 and the prevalence of each of them was higher than 50 %, with that of *O. voltaensis* being the highest (Pr = 90.4 % ± 0.03) (see Table 1).

Moreover, during the study period, the MI appeared very low for *C. longicirrus* (MI = 3.8 ± 0.6) and low for the other species (10 < MI ≤ 50) (table 1).

A total of 17 different parasitic associations were recorded (table 2). The subsequent analysis mainly focused on associations with a prevalence or infection rate ≥ 5 %. The infrapopulations concerned were made up of: only *O. voltaensis* (A); only *C. euzeti* (B); while infracommunities comprised *O. voltaensis* and *C. longicirrus* (D); *O. voltaensis* and *C. euzeti* (E); *O. voltaensis, C. longicirrus C. euzeti* and *C. falcifer* (N); *C. euzeti*, *O. aframae*, *C. falcifer* and *O. voltaensis* (P); *C. euzeti*, *C. falcifer*, *O. aframae*, *C. longicirrus* and *O. voltaensis* (Q).

Among the five ecto-helminthic taxa, three namely, *C. falcifer*, *C. longicirrus* and *O. aframae* were never found parasitizing alone a *H. elongatus* host. In monogenean associations retained, in cases of polyparasitism, infection rate increased with the species richness (SR) (see Table 2).

Among the 73 fish examined, only 15 (20.55 %) were infested by a single monogenean species: 10 (13.7 %) by *O. voltaensis* and 5 (6.84 %) by *C. euzeti*. In these instances of mono-parasitism, *O. voltaensis* was more often hosted than *C. euzeti* although no significant difference was found between their prevalence ($\chi^2$ = 0.51; P= 0.17) and intensities (K = 0.07; P= 0.78). Polyparasitism was noticed in 58 *H. elongatus* hosts (infection rate of 79.45%). Therefore, there were differences in the average parasite load between cases of mono-parasitism (mean intensity = 1.9 ± 0.4) and polyparasitism (global mean load = 56 ± 7.1) (U = 17; P = 0.0001) as well as in their infection rate ($\chi^2$ = 37.73; P < 0.0001).

**Table 1:** Distribution of Monogenean gill parasites of *H. elongatus*

| Parasite species | N | $n_i$ | NP | Pr | MI | $S^2$ | R=$S^2$/MI |
|---|---|---|---|---|---|---|---|
| *C. euzeti* | 73 | 46 | 697 | 63±0.05 | 15.5±3.9 | 701.5 | 45.2 |
| *C. falcifer* | | 45 | 686 | 61.6±0.05 | 14.9±1.8 | 163.4 | 10.9 |
| *C. longicirrus* | | 46 | 171 | 63±0.05 | 3.8±0.6 | 16.9 | 4.4 |
| *O. aframae* | | 40 | 304 | 54.7±0.05 | 7.6±1.3 | 74.3 | 9.7 |
| *O. voltaensis* | | 66 | 1460 | 90.4±0.03 | 22.12±2.9 | 564.9 | 25.5 |

*N = Number of hosts examinated; $n_i$ = number of infected hosts; NP= number of parasite individuals collected from all host specimens studied; Pr = prevalence; MI = Mean Intensity; $s^2$= variance.

**Table 2:** The different parasite associations found and their frequencies.

| Parasite in free Population Infracommunity | *C. euzeti* | *C. falcifer* | *C. longicirrus* | *O. aframae* | *O. voltaensis* | Infection rate (%) | Mean global parasitic load | SR |
|---|---|---|---|---|---|---|---|---|
| A* | | | | | X | 13.7 | 2.1 ± 0.62 | 1 |
| B* | X | | | | | 6.84 | 1.6 ± 0.24 | 1 |
| C | | X | | | X | 1. 36 | 3 ± 0 | 2 |
| D* | | | X | | X | 5.47 | 6.5 ± 1.84 | 2 |
| E* | X | | | | X | 5.47 | 8.25 ± 3.9 | 2 |
| F | | X | X | | | 1.36 | 2 ± 0 | 2 |
| G | | | | X | X | 1.36 | 8 ± 0 | 2 |
| H | X | | X | | X | 1.36 | 19 ± 0 | 3 |
| I | X | X | | X | | 1.36 | 22 ± 0 | 3 |
| J | | X | X | | X | 1.36 | 7 ± 0 | 3 |
| K | | X | | X | X | 4.10 | 25 ± 16.52 | 3 |
| L | | | X | X | X | 1.36 | 8 ± 0 | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| M | X | X | | | X | 1.36 | 33 ± 0 | 3 |
| N* | X | X | X | | X | 6.84 | 23 ± 4.99 | 4 |
| O | X | | X | X | X | 1.36 | 14 ± 0 | 4 |
| P* | X | X | | X | X | 9.58 | 59 ± 8.77 | 4 |
| Q* | X | X | X | X | X | 35.62 | 94.84 ± 11.09 | 5 |

*A= only *O. voltaensis* present; B= only *C. euzeti* present; C= presence of *C. falcifer* and *O. voltaensis*; D= presence of *C. longicirrus* and *O. voltaensis*; E= presence of *C. euzeti* and *O. voltaensis*; F= presence of *C. falcifer* and *C. longicirrus*; G= presence of *O. aframae* and *O. voltaensis*; H= presence of *C. euzeti*, *C. longicirrus* and *O. voltaensis*; I= presence of *C. euzeti*, *C. falcifer* and *O. aframae*; J= presence of *C. falcifer*, *C. longicirrus* and *O. voltaensis*; K= presence of *C. falcifer*, *O. aframae* and *O. voltaensis*; L= presence of *C. longicirrus*, *O. aframae* and *O. voltaensis*; M= presence of *C. euzeti*, *C. falcifer*, *O. voltaensis*; N= presence of *C. euzeti*, *C. falcifer*, *C. longicirrus* and *O. voltaensis*; O= presence of *C. euzeti*, *C. longicirrus*, *O. aframaer* and *O. voltaensis*; P= presence of *C. euzeti*, *C. falcifer*, *O. aframae*, and *O. voltaensis*; Q= presence of P= *C. euzeti*, *C. falcifer*, *O. aframae*, *C. longicirrus* and *O. voltaensis*; SR= species richness; *Infection rate ≥ 5%.

### 3.1 Analysis of parasite associations retained

The prevalence of each parasite species (table 3) varied significantly between the different infracommunities in which it occurred ($P < 0.001$). A general trend was observed in this work: the mean intensity (MI) of each parasite species positively correlated with the species richness (sr) of the infracommunity, especially for *O. voltaensis* ($r_s = 0.86$, $P = 0.024$); in the other words the mean intensity increased significantly in the different parasite associations in which a monogenean occured, especially for *O. voltaensis* ($K = 25.4$, $P = 0.0001$), *C. euzeti* ($K = 19.15$, $P = 0.0002$) and *C. falcifer* ($K = 7.93$, $P = 0.01$) (table 3).

**Table 3:** Mean intensity of the five monogenean species in the different associations

| Parasite species | Parasite association | | | | | | | Statistics | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | D | E | N | P | Q | | |
| *O. voltaensis* | 2.1±0.6 | / | 4.75±1.3 | 7.25±3.9 | 10.2±3.2 | 28.7±3.5 | 38.7±5.3 | K=25.4 ; P= 0.0001 | $r_s$= 0.86 P=0.024 |
| *C. longicirrus* | / | / | 1.75±0.7 | / | 3.6±1.1 | / | 4.2±0.6 | K=5.8 P=0.12 | $r_s$=0.99 P=0.06 |
| *C. euzeti* | / | 1.6±0.2 | / | 1±0 | 4.2±1.5 | 8±2.1 | 22.8±6.5 | K=19.15 P=0.0002 | $r_s$=0.78 P=0.11 |
| *C. falcifer* | / | / | / | / | 5.2±2.6 | 13±3.7 | 20.5±2.5 | K=7.93 P= 0.01 | $r_s$=0.86 P=0.34 |
| *O. aframae* | / | / | / | / | / | 11±2.6 | 7.8 ±1.9 | U=49.5 P=0.06 | / |
| Species richness | 1 | 1 | 2 | 2 | 4 | 4 | 5 | | |

*A= only *O. voltaensis* present; B= only *C. euzeti* present; D= presence of *O. voltaensis* and *C. longicirrus*; E= presence of *O. voltaensis* and *C. euzeti*; N= presence of *O. voltaensis, C. longicirrus C. euzeti* and *C. falcifer*; P= presence of *C. euzeti*, *O. aframae, C. falcifer* and *O. voltaensis*; Q= presence of *C. euzeti*, *C. falcifer*, *O. aframae*, *C. longicirrus* and *O. voltaensis*.

## 4. Discussion

Ecological studies on the monogenean gill parasites of *H. elongatus* from Cameroon have been conducted by Bilong Bilong and Euzet [16] and Bilong Bilong and Njiné [22]. These authors stated that *H. elongatus* from Cameroon hosted seven gill monogenean species, but two of them namely *Onchobdella bopeleti* Bilong Bilong & Euzet, 1995 and *Cichlidogyrus dageti* Dossou & Birgi, 1984 were not found in the current study. According to Bilong Bilong and Euzet [16], the absence of *C. dageti* in the Ozum Lake of Yaoundé, though found uncommonly in some watercourses around should be related to environmental conditions while that of *O. bopeleti* only found in the coastal plain should be related to the geomorphology. These arguments still seem valid to justify our findings. Our host-parasite system in the river Mefou (1 host species/5 parasitic species) is more complex than that studied by Madhi and Belghyti [23] made up of only two monogenean species. We can also use the argument of the permanent presence of vacant niches on the gill biotope [24] to explain the multi-specific parasitism of *H. elongatus*. However, while recognizing that interactions can still occur in a low density system, it is known that in case of two or more parasite species having similar optimal positions on the gill filaments, niche shifts are likely to occur [25]. We consider that this phenomenon could result in the built up of infracommunities with increased density rather than in their impoverishment. The data presented herein shows a significant difference between mono parasitism and polyparasitism of *H. elongatus* both in parasite load and infection rate. Specific average parasite load (intensity) and prevalence were significantly higher in simultaneous infections with higher specific richness. This result corroborates the finding of Madhi and Belghyti [23] who simply considered that positive interactions between fish ectoparasites in the same host individual are very frequent. In the current study, *O. voltaensis* and *C. euzeti* occurred in the majority of infracommunities found. Although the immunological effects of monogeneans on their hosts remain to be explored, Cloutman *et al.* [26] also argue that the presence of one parasitic species may be advantageous for another, thus allowing the establishment of polyparasitism. Thus the weakening of the host's immune response by the first parasitic species to settle in would favor the host colonization by other species which settle secondarily [5, 4]. In a literature review, Holmes [25] also reports other interference mechanisms which operate interspecifically such as modification of the (host) environment in ways that interfere with other parasites. In the natural environment, it was found that some taxa (*C. longicirrus, C. falcifer* and *O. aframae*) infest their common host only in presence of other parasitic species. At the level of our analysis, it is suggested that the latter argument by Holmes [25] could more likely explain the presence of *C. falcifer, C. longicirrus* and *O. aframae* only in infracommunities. In fact, anchors of these three species are relatively smaller than those of *C. voltaensis* and *C. euzeti* see [18, 19]. The two latter species could first plough the gill epithelium to make easy infestation by the other species. Relying on this idea, *O. voltaensis* was revealed as the main ectohelminthic monogenean species of *H. elongatus* in the Mefou watercourse. All the monogenean ectoparasites of *H. elongatus* best exploit this fish when they co-occur in the same host individual. This idea is supported by the fact that, the mean intensity and prevalence increased (due to parasite multiplication) with the species richness. In this context, Madhi and Belghyti [23] argued that the preference of parasites to attach themselves to already parasitized fish increases the parasitic load of affected hosts, and promotes aggregation of different parasite populations (i.e infrapopulation) within these hosts. This finding was very obvious in our complex host / parasites system. Consequently, under culture conditions where the density of fish is always high, infestation by several species could increase the pathogenic effects of the parasites and cause heavy losses through morbidity and / or mortality of the fish [27]. With reference to Vaumourin *et al.* [4], two sets of ecological factors may promote multiparasitism:

1. Those that influence host exposure, namely the spatial distribution of hosts [28],
2. Those that are intrinsic to host and influence their susceptibility namely host life-history traits [29, 30].

It has been established that waters especially in the middle and downstream of the Mefou River are very poor in quality [31]. This water status may influence negatively the host's susceptibility. *Hemichromis elongatus* which is widely distributed in Africa [32, 33], is very aggressive and territorial [34], pair-bonding [32]; parents guard the nest and larvae [35]. From our results, we suggest that the above behavioral characteristics may increase the exposure of this host species to parasites and may facilitate co-infections. It is also known that stressed or malnourished hosts are more likely to become infected [36]. This situation is more likely to occur in fish ponds where high host densities favor parasite transmission [37]). However, the threshold of the parasite load which could be harmful to the hosts in our environment remains to be determined.

## 5. Conclusion

In the Mefou River, *H. elongatus* hosted five monogenean gill parasite species during the study period, mostly in simultaneous infestations. Some species, never observed in monoparasitism would need the presence of other parasites to sinfest the hosts. Polyparasitism in this natural environment was more frequent; this favored the increase not only of infracommunity loads but first of specific mean intensities and prevalences. This phenomenon is an alert to fish farmers about the threats hanging over their productions, and we recommend quarantining native large fish specimens captured in the wild and used as sires in ponds, periodic pond sanitization, and possibly host deworming.

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## 7. Conflict of interest

The authors declare no conflicts of interest.

## 8. Acknowledgements

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