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Blowflies Reared in Laboratory Conditions from Maggots Collected on Rat (*Rattus norvegicus* Berkenhout, 1769, Var Wistar) Carrions in Yaoundé (Cameroon, Central Africa)

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Authors' contributions

This work was carried out in collaboration among all authors. Author FDFY designed the study, perform the field work, the laboratory survey, the writing of manuscript and the identification of specimens, author PBN participated to the field work and the laboratory survey, authors YB and MHV conceptualize, revise the identification and perfomed the litterature revue, author CD-I managed the data analysis and author CFBB supervise the field work, data analysis and writing and revising of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Forensic entomology offers insects as physical evidence during legal procedures. Forensic entomologists have determined succession of arthropods on dead animals, but few published studies are available on necrophagous larvae collected on carcasses around the world. This study evaluated the diversity of arthropods associated with rat carcasses to identify species of forensic relevance. Larvae hatched from arthropod eggs were reared until the emergence of adult flies under ambient laboratory conditions. Adult flies were identified to species level. Overall, 6319 individuals belonging to 6 families, 13 genera and 21 species of Diptera emerged.

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1. INTRODUCTION

In the course of legal procedures, investigators are expected to produce evidence during inquiries at the court. Nowadays, it is a difficult task to produce palpable evidence without the intervention of scientists such as forensic entomologists, toxicologists, ballistic experts, etc. [1]. One milestones is to determine time elapsed since death, known as Post Mortem Interval (PMI), and the circumstances of the death. Judicial investigators need accurate data that help them to make decisions. Within three days (72 hours) after death, legal medicine can use autopsy to obtain data for the court [2]. After this time, accurate data can be obtained from studies targeting on necrophagous arthropods sampled on and around the corpse [3,4,5].

Forensic, judicial or medico-legal entomology is the science that deals with the study of necrophagous arthropods collected at crime scenes and assessed to inform cases in court [1]. The principle of predictable species succession on corpses usable in judicial inquiries was recognized by Megnin [6] who suggested eight successive waves of arthropods colonizing a carrion over the time. This principle has been debated because the delimitation of the different waves is a difficult task [7-11]. The time of arrival of a specific insect on the cadaver can be monitored according to biogeographic variation [12]. To gather data for each environment, it is crucial to conduct local studies and determine the insect species involved in decaying corpses [10].

Africa is the stronghold of over a dozen necrophagous blowfly species, several of which are found on other continents [13]. These flies have been intensively studied over the world, but papers referring to African countries are scarce [14] or absent, except in South Africa [15,4,10,14,16], Nigeria [17,18], Namibia [19], Algeria [20-25] and Cameroon [26,27]. Consequently, crime cases solved with entomological evidence and exploitation of the ecological database are too poor or remain scarce in Africa because (1) a lot of spiritual value is attached to corpses, (2) some investigators or researchers do not know enough about forensic entomology and, (3) judicial employees rarely ask for scientific or palpable proof during their procedures [1,28].

The aim of this study is to identify and document all forensic relevant blowflies by rearing larvae hatched from eggs until the emergence of adults under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Study Site

This study was conducted in an experimental station located on the premises of the University of Yaounde I (11°33′01″E 3°51′35″N), Cameroon. The climate is equatorial and characterized by four distinct seasons: a long rainy season from mid-November to February, a short rainy season from March to June, a short dry season from July to August and a long rainy season from September to mid-November. The annual mean temperature is about 23-24°C and the rainfall fluctuates between 1500 and 2000 mm [29,30]. The landscape of this part of the campus suburban and characterized by the presence some trees like *Elaeis guineensis* (Arecaceae) and *Musa* sp. (Musaceae).

2.2 Experimental Procedure

At the experimental station, two rats *Rattus norvegicus* Berkenhout, 1769, var Wistar were euthanized with carbon dioxide and then strangulated. These two carcases were immediately deposited on a 10 cm layer of sterilized soil inside a rectangular (40 cm x 20 cm) plastic container. These two carcasses were protected against animal scavengers with a wooden cage (120 cm x 120 cm x 120 cm) covered with a net of 5 cm mesh size to allow colonization of the carcasses by insects only. The cage had an entrance allowing visiting and checking for larvae migrating to the sterilized soil. At the end of each search, the remains were again placed on top of fresh sterilized soil. The collected larvae and the cadaveric liquid were covered with a net to avoid eventual contamination by subsequent insect visitors and taken to the laboratory for rearing to maturity under ambient air conditions.

The plastic box was inspected twice daily, at 08:00 GMT and 16:00 GMT, to record the emergence of adult flies. The litter was immediately transferred to another plastic box and the adult flies were reared and fed for 48 hours with honey put on cotton and placed inside the box [31,32]. After this period of time, these adult flies were captured using flexible plastic forceps and preserved in 70% ethanol in microreaction tubes for further morphological identification. This exercise was repeated for five days and stopped when no new emergence was noticed.

Atmospheric temperature and relative humidity were recorded using a data logger Testo.

2.3 Fly Identification

The identification of adult flies was performed in two phases. The first phase, done using a stereomicroscope (M3Z,Herbrugg Switzerland), wa based on identification keys [33-36] [19,37,38,26,39,13]. The second step was performed successively at the Microtraces Laboratory of the National Institute of Criminalistic and Criminology of Brussel and the entomology section of the laboratory of Royal Museum of Central Africa at Tervuren, all in Belgium. It used the identification keys of Kurahashi & Chowanadisal [40], Rognes & Paterson [41], Kurahashi & Kirk-Spriggs [19], Couri [42], Carvalho & Mello-Patiu [43], Nihei & Carvalho [38] and LinLong et al. [44].

2.4 Data Analysis

The rate of occurrence (C) of each species was evaluated according to the formula [45] $C = (p*100)/P$, where p is the number of occurrences of a given species during the survey and P = 5 i. e. the total number of replicates. The results are interpreted as $C > 50\%$ = consistant species; $25\% \le C \le 50\%$ = accessory species; $C < 25\%$ = rare species.

3. RESULTS

3.1 Abiotic Parameters

During the experiment, the mean daily temperature was 28.5℃ (21.4-33.5℃) and the mean daily relative humidity was 64.65% (56.9-85.1%).

3.2 Overview of Emerged Entomofauna

The entomofauna that emerged was made up of 6 families, 13 genera and 21 species. Amongst the families, the Braconidae, Diapriidae and Pteromalidae are parasitoid wasps. Braconidae predominated among them and were present at each trial. When any of these families occurred together, the decreasing order of abundance was always Braconidae > Diapriidae > Pteromalidae (Table 1). With the exception of the first rearing experiment where only 7 species emerged, during the other trials 16 to 19 species emerged. The decreasing order of abundance was Calliphoridae, Muscidae, Braconidae, Sarcophagidae, Diapriidae and Pteromalidae (Table 1).

During the first trial, Calliphoridae, Muscidae, Sarcophagidae and Braconidae represented 42.85%, 37.71%, 11.9%, and 9.52% of the entomofauna. The two main species were *Hydrotaea* sp. (35.71%) and *Lucilia* sp. (28.57%) (Fig. 1).

During the second trial, the frequencies of insects were 84.72%, 12.9%, 2.02%, and 0.4% for Calliphoridae, Muscidae, Sarcophagidae and Braconidae respectively. The main species were Calliphoridae, namely *Hemipyrellia fernandica* (40.03%), *Chrysomya putoria* (19.50%) and *C. albiceps* (14.49%) (Fig. 1).

During the third trial, the entomofauna was made up of Calliphoridae (84.40%), Muscidae (7.50%), Braconidae (4.50%), Diapriidae (2.3%), Sarcophagidae (1.2%) and Pteromalidae (0.1%). The main species were *Hemipyrellia fernandica* (43.60%), and *C. putoria* (20.07%) (Calliphoridae) (Fig. 1).

The fourth tiral made it possible to obtain Calliphoridae (82.50%), Muscidae (16.27%), Braconidae (0.5%), Sarcophagidae (0.41%) and Pteromalidae (0.20%). The main species were *Hemipyrellia fernandica* (46.07%), *Chrysomya putoria* (15.86%) and *Chrysomya* sp. (11.00%) (Fig. 1).

At last during the fifth trial, insects that emerged were Calliphoridae (90.50%), Muscidae (6.60%), Braconidae (2.15%), Sarcophagidae (0.9%), Diapriidae (0.50%) and Pteromalidae (0.20%). The main species were *Hemipyrellia fernandica* (58.65%) and *Chrysomya putoria* (15.93%) (Fig. 1).

During the successive trials, only three families (Calliphoridae, Muscidae and Sarcophagidae) constituted the dominant necrophagous entomofauna. Calliphoridae predominated and represented 47.40% (18 individuals), 86.5% (639 individuals), 90.70% (1188 individuals), 83.20% (1596 individuals) and 93. 20% (1971 individuals) amongst that necrophagous entomofauna (Table 1). As far as necrophagous arthropods are concerned, almost all species were constant (C > 50%), except *Sarcophaga zumpti*, which was accessories (C =40%).

4. DISCUSSION

Several works have reported faunistic inventories of necrophagous insects over the world, but data on emerged adult flies from larvae collected on cadavers are scarces [10]. The current work is the first initiative in Central Africa to rear larvae during a forensic entomology experiment within ambient laboratory conditions.

4.1 Carrion Models

A variety of baits are used for forensic purposes. In the present laboratory study, rats were used as trapping bait like in other experiments already documented [46,20,21,9,2,47,48,49]. [50-53] used carcasses of pigs as trapping substrates. [54,55,56,12,57] utilized multiple trapping baits while [58,59] employed fish when [60,61] used chicken liver as bait. This multitude of biological models of trapping substrates gives new opportunities to authors to enrich their database on forensic entomology.

4.2 Emerged Necrophagous and Parasitoid Entomofauna

The present survey revealed 6 families, 13 genera and 21 species. This entomofauna diversity is greater than that published by [62-67]. These scholars censured twelve to fourteen species excluding beetles within their different study areas. This contrast can be due to differences in climatic conditions and local arthropod assemblages since their studies were carried out in Europe, Asia, North America and Australia respectively. This supports the fact that the biodiversity of these insect is governed by the environmental parameters specific to each biogeographic area [28,68] and also the body size of the bait.

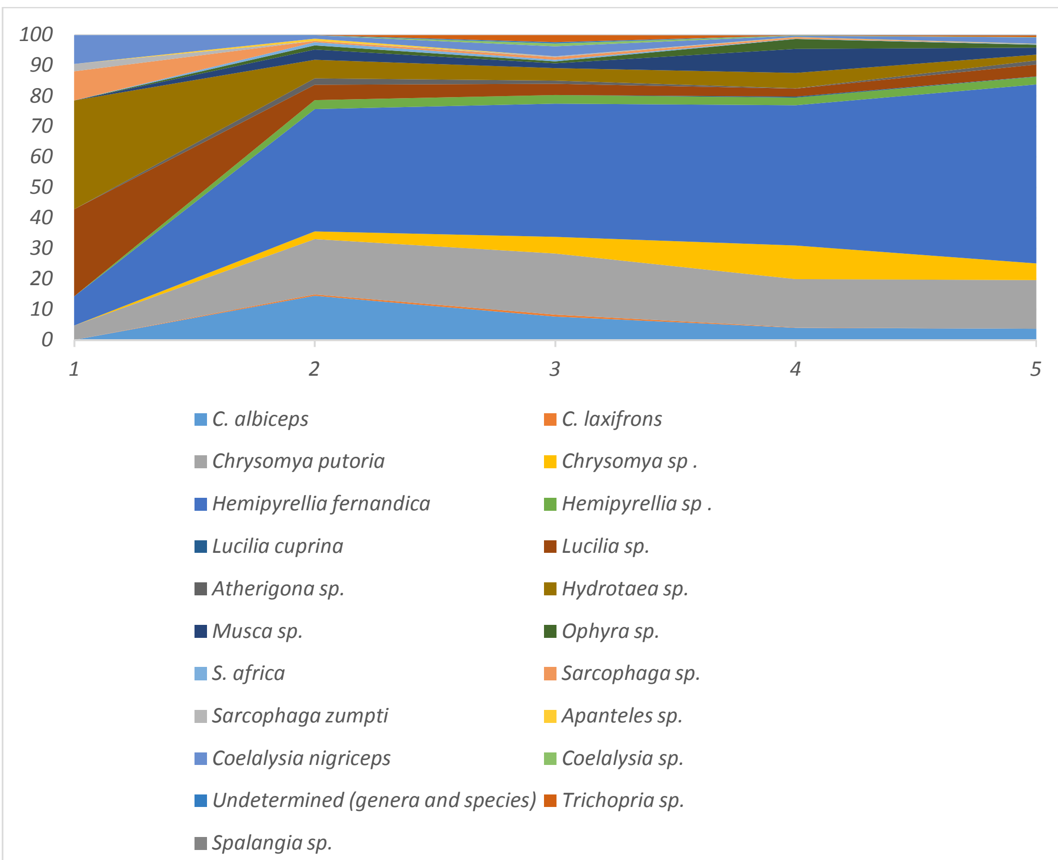

Fig. 1. Evolution of the entomofauna that emerged in the different trial

Table 1. Number (n) and proportion (%) of emerged flies in the different trial

Taxa	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5		Total per family			
	n	%	n	%	n	%	n	%	n	%	Species	%	Family	%
DIPTERA														
Calliphoridae														
C. *albiceps*			109	14.49	109	7.70	77	3.98	81	3.71	376	5.95		
C. *laxifrons*			3	0.40	8	0.57	2	0.10			13	0.21		
Chrysomya putoria	2	4.76	147	18.22	284	20.07	307	15.86	348	15.93	1078	17.06		
Chrysomya sp.			19	2.53	78	5.51	213	11.00	119	5.45	429	6.79	5402	85.49
Hemipyrellia fernandica	4	9.52	301	40.03	617	43.60	892	46.07	1281	58.65	3095	48.98		
Hemipyrellia sp.			22	2.93	40	2.83	48	2.48	57	2.61	167	2.64		
Lucilia cuprina							5	0.26	2	0.09	7	0.11		
Lucilia sp.	12	28.57	38	5.05	52	3.67	52	2.69	83	3.80	237	3.75		
Muscidae														
Atherigona sp.			16	2.13	15	1.06	1	0.05	31	1.42	63	1.00		
Hydrotaea sp.	15	35.71	46	6.12	58	4.10	98	5.06	42	1.92	249	3.94	665	10.52
Musca sp.			25	3.32	23	1.63	152	7.85	49	2.24	249	3.94		
Ophyra sp.			10	1.33	9	0.64	64	3.31	21	0.96	104	1.65		
Sarcophagidae														
S. africa			8	1.06	7	0.49	2	0.10	5	0.23	22	0.35		
Sarcophaga sp.	4	9.52	3	0.40	12	0.85	8	0.41	1	0.05	28	0.44	56	0.89
Sarcophaga zumpti	1	2.38			5	0.35					6	0.09		
HYMENOPTERA														
Braconidae														
Apanteles sp.			6	0.80			1	0.05	1	0.05	8	0.13		
Coelalysia nigriceps	4	9.52	8	1.06	45	3.18	10	0.52	47	2.15	114	1.80		
Coelalysia sp.					13	0.92	1	0.05	2	0.09	16	0.25	139	2.20
Undetermined (genera and species)			1	0.13	6	0.42					1	0.02		
Diapriidae														
Trichopria sp.					33	2.33			10	0.46	43	0.68	43	0.68
Pteromalidae														
Spalangia sp.					1	0.07	3	0.15	4	0.18	8	0.13	8	0.13
Total per trial	4	100	75	100	141	100	193	100	218	100	6319	100	6319	100
						2			2		5		6	4

Six families (Table 1) were obtained in this work, whereas [67] collected individuals belonging to Fanniidae, Piophilidae, Sepsidae, Sphaeroceridae, Phoridae, and Heleomyzidae during their experiment in Central Quebec, Canada, an environment dominated by a snowy winters and humid and temperate summers [69]. This suggests that type of vegetation and abiotic conditions are determinant factors during forensic entomology studies.

Our experiment showed the predominance of Calliphoridae (*Lucilia* sp*., Lucilia cuprina, Chrysomya albiceps, C. putoria, C. laxifrons, Hemipyrellia fernandica, Hemipyrellia* sp. and *Chrysomya* sp). This result confirms the work of Ekenem & Dike [17] in Nigeria, despite the fairly differences in the genera/species richness, and also corroborates the results of Matuszewski et al. [70,71] in Central Europe, Fremdt and Amendt [72], Bernhardt et al. [73] in Germany, and Thyssen et al. [54] in Brazil.

Muscidae were the second-most prevalent family in the current work, as observed in other studies performed in other biogeographic areas. In fact, [52,74] also reported the presence of Muscidae during a forensic set up. The Muscidae species that emerged in the present work are different from those obtained by Pitner et al. [75] in northern Germany. This could be the effect of environmental parameters which guide the geographical distribution of necrophagous flies, through their thermophysiological tolerances [76,77]. Consequently, forensic entomology experiments should be carried out in every region due to biogeographical specificity. This differences might also be due to vegetation, phylogenetic history or even season.

Sarcophagidae also emerged in our trials. That was the case in South Africa [78,10], Central Spain [79], Iran [80-82], central Quebec at Canada [67], and Nigeria [17]. The difference in species diversity may be the result of differences of geographic location and environmental factors (temperature, photoperiod, light, water or moisture and relative humidity), since the biodiversity and abundance of necrophagous flies are influenced by these abiotic parameters [28].

Braconidae, Pteromalidae and Diapriidae emerged during the present rearing experiments contrary to the results reviewed by Villet [10,81]. The genera *Nasonia* (= *Mormoniella*) on the one hand, and *Nasonia* and *Brachymeria* on the other, were obtained in South Africa [10] and Iran [81], respectively. Grassberger & Franck [83] in Punjabi India, Turchetto et al. [84] in Italy and Voss et al. [85] in Western Australia, collected other different parasitoid wasps during their studies. In a similar experimental design by Marchiori [86] and Horenstein & Salvo [86] in Brazil, Pteromalidae species also emerged from reared Calliphoridae larvae; this suggests that wasps of this taxon also infest necrophagous entomofauna larvae worldwide. In our environment, Braconidae may occur alone or be associated with Diapriidae and/or Pteromalidae; the two latter families being weakly represented.

5. CONCLUSION

The determination of necrophagous entomofauna is a fundamental milestone for the determination of post mortem interval. This pioneer experimental design under laboratory conditions in Cameroon has enabled us to have an idea of arthropods that emerged from rearing maggots. Many necrophagous arthropods species invade rat carcasses for oviposition, larviposition and/or feeding. Their adults emerged along with some parasitoid wasps. These flies can be primarily considered as forensic entomology indicators. Further experiments will be performed to confirm these preliminary results in Central Africa, since the accuracy of Post Mortem Interval estimation is based on (1) many replicates of necrophagous insect studies, (2) effects of abiotic environmental parameters on insects development and (3) the quantity of energy used by necrophagous insects to complete their life cycle, which varies with temperature.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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