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Diversity of Necrophagous Arthropod Fauna of Dog (Canis lupus familiaris Linnaeus, 1758) Carcasses Exposed to the Natural Environment at Nsimalen Area, Yaounde Neighborhood, Cameroon

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Abstract

A field experiment was conducted on a dead dog (Canis lupus familiaris) carcass exposed to the natural environment from 4th December 2020 to 15th April 2021 in Nsimalen area, Yaounde neighborhood to understand the necroarthropod fauna assemblage under natural environmental conditions. This period covers the dry and wet season. A total of 1846 arthropod specimens belonging to 3 classes, 11 orders, 12 families, 17 genera and 20 species were identified. Hexapoda with 1708 (92,52%) of all invertebrate specimens census was the most abundant class. The main orders were successively Diptera with 728 (39,44%) of all the collected specimens and Hymenoptera [441 (49,16%)]. The dominant families were Calliphoridae [384 (20,80%)] and Muscidae [243 (13,16%)]. The most abundant species were Pheidole megacephala, *Hemipyrellia fernandica, Chrysomya laxifrons, Dichaetomya sp., Hemipyrellia sp*. and C. putoria with respectively [648 (35,10%)], [79 (4,28%)], [69 (3,74%)], [69 (3,74%)], [65 (3,52%)] and [45 (2,44%)]. Viewing the evolution of the decay process, the fresh state was colonized only by *Hemipyrellia fernandica*, the bloated state by C. putoria, *Hemipyrellia sp*., C. *laxifrons, Dichaetomya sp*., *Lucilia sericata, Musca sp*. and *Pheidole megacephala*, the putrefied stage by *C. albiceps, Hydrotaea sp*., *C. putoria, Dichaetomya sp*. and *C. vanemdeni*, the dried stage by *Iulus sp., Periplaneta sp*. and *Hydrotaea sp*.

Keywords: Cameroon, Necrophagous, Forensic entomology, *Hemipyrellia*

Introduction

After death, a carcass becomes a microhabitat with its own microclimate which is colonized by an abundant and diverse necrophagous arthropod fauna. Decomposition and insect colonization are closely related processes. The progression of these two phenomena are subject to abiotic and biotic factors [1-3]. The specific assemblage is composed mainly of necrophagous insects which are those which feed, lay eggs and develop on remains [3-5], saprophagous insects which are the decayers of organic matter (household waste, human and animal faeces eaters) [6], predators [7], hematophagous insects [8,9], and parasitoïds [10-12]. This cadaveric fauna comprises insects which are the most diverse group of animals estimated at over 5.5 million species worldwide [13-15]. There is a large number of unexplored or underexplored insect species that may improve knowledge about forensic entomology in Africa. Forensic entomology uses the information gathered from the study of insect or other related organisms collected on and around the body to help solve the crime [5]. In forensic science,

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the estimation of the time since death is extremely important in a murder case [16,12]. The estimation of the time elapsed since death, also called the Post Mortem Interval (PMI) is critical in any forensic entomology investigation. This time helps to determine the date of the disappearance of the animal or human. This parameter provides the basis of the queries of judicial workers. The precision of PMI assessment decreases with increasing time since death [17]. Knowledge of the distribution, biology, ecology and behavior of insects colonizing the body can provide information on when, where, how a crime was committed and the neglect of elders or elder abuse [18-23, 8-10, 24-28], and the identification of unknown deceased persons [29-32].

Medico-legal or criminal entomology deals with the information gathered from the study of necrophagous insects and their relatives on a body. Blow fly development rates can be used to provide an estimate of the time between death and discovery of the carcass [5,26], but these data are most useful when a full account of species ecology, seasonality, and distribution is known [5,4]. Studies on insect carrion ecology have been carried out worldwide except in Africa where only South Africa has good databases on forensic entomology. Forensic entomology in Cameroon is in the early stages and so, the knowledge of the discipline is limited; therefore, literature on this field is very scant, meaning that there is very little information on the diversity, biology and distribution of forensic insects in our area. Hence, the behavior, the life cycle as well as the aggregation of these flies remain unknown. The scientific research focused on the necrophagous entomofauna in Cameroon are only those of *Feugang Youmessi, et al.,* [8,9,24], Feugang Youmessi [3,33,34], Feugang Youmessi and Djonga [12], *Feugang Youmessi, et al,.* [28] done on the carcasses of rats (Rattus norvegicus Berkenhout, 1769).

The above papers result from research carried out only in the University of Yaoundé 1 campus. So, there is a need for research in other areas of Cameroon since, as argued by Catts and Goff [35], *Introna, et al.,* [36], Anderson [37] and *Carvalho, et al.,* [38], necrophagous insects can indicate that a corpse has been moved from one locality to another. In such an investigation, information on endemic insect fauna is crucial for all habitats in a given region in order to determine whether or not the carcasses have been moved. Evidence of relocation of a corpse can be revealed by identifying insect species from the corpse that are foreign to the area where the body was discovered [39]. As said by *Shaalan, et al.,* [40], the type and composition of attracted taxa to a dead corpse usually changes alongside the decomposition process and the pattern of succession is specific to the local conditions in which a carcass is found. Then, since taxa can vary greatly with location, it is necessary to identify the forensically important insects that are specific to an area before post mortem interval estimations can be made [40-45] outlining the importance of regional surveys of necrophagous fauna. Therefore, we conducted a census experiment at Nsimalen area/Yaounde to document forensically useful information. The goals of the present study were to present faunistic records for arthropods, all collected from decomposing tissues of animal origin in order to increase the database of that specific fauna within the residential area of Yaounde city in Cameroon.

Materials and Methods

Study Site

The present research was carried out from 4th December 2020 to 15th April 2021 at the southern entrance of Yaounde, specifically at Nsimalen (11° 33 '01''E 3° 51' 35''N) which has an estimated population of 3000. Yaounde has been urbanized quickly with the level of violent crimes increasing as a consequence. According to Kengne Fodouop and Atangana [46], the climate is equatorial, characterized by a specific climate called "Yaoundean climate" characterized by four distinct seasons: two dry seasons and two wet 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 dry season from September to mid –November. Also, based on the data from Yaounde meteorological station and that of our data logger Testo 174 T, the average climatological values of annual mean temperature fluctuated between 23 and 34°C and the precipitation varied between 1500 and 2000 mm per annum [47,46]. The landscape of the study site is suburban and characterized by the presence of some common trees such as Elaeis guineensis Jacq. (Arecaceae) and Musa sp. (Musaceae). The experimental set-up was situated in a corner of an abandoned house yet to be finished with twelve (12H) hours of sun daily and away from anthropic activities and scavengers.

Methods

Biological Substratum

We used one domestic dog (Canis lupus familiaris Linnaeus, 1758) for one cage and replicates within three cages at the same study period. They were weighing approximately 15 kg, similar to *Barbosa, et al.,* [48]. The animal was examined then euthanized by a veterinarian using carbon dioxide (CO2) following animal care protocol. The carcass was then carefully wrapped and carried by vehicle to the study site situated approximately thirty-five minutes from the slaughterhouse. At the study site, the carcass was placed immediately inside a wooden cage covered on all sides with a metallic wire mesh to exclude scavenging by vertebrates and to protect it from people's curiosity while allowing the necrophagous arthropod fauna access to the corpse [35,49]. This square cage was pinned down vertically at ground level with 1 m iron stake (50 cm inside the ground, 50 cm out and tied to the wood with a strong rope) so that the first vertical stick was touching the ground.

During each visit to the field, carrion pictures were taken, the remains were meticulously observed based on visual morphological changes and physical modifications due to the decay progress were noted in our field notebook for the assessment of the decompositional stages of the corpse.

Sampling Protocol

The necrophagous arthropod fauna were collected during 20 minutes periods using three complementary procedures; three times daily at 0800, 1200 and 1600 h for the first two weeks after death then at 1200 until the end of the experiment [3]. Flying insects were caught with a hand net of 1 mm mesh, then brought back to the laboratory and sprayed with 70% alcohol to asphyxiate them. After 10 minutes, the asphyxiated insects were preserved in 70% alcohol for further taxonomic identification. Larvae were collected from carcasses using flexible forceps, then divided into two sub samples: the first one preserved inside tubes containing 70% alcohol and the other reared in the laboratory to adulthood as done by *Smith, et al.,* [50] during their research in the Metro Vancouver region of British Columbia, Canada. After the emergence, insects were fed with honey for two days, then preserved for taxonomic identification. Larvae were reared in the laboratory until emergence of adults flies. After emergence, insects were fed with honey for two days, preserved for taxonomic identification. Non-flying/teneral flies were collected directly with flexible forceps and other small insects were caught using 4 pitfall traps placed around the corpse at the head, the left and right side of the body and near the legs at a distance of 10 cm from the body [51]. These traps were made of plastic round cups (6 cm diameter and 15 cm high 2/3 filled with 70% alcohol) and placed in holes so that their entrance was at the same level as the surface of the soil to collect active fauna. The pitfall traps were emptied by us every two days in the morning at 0800 h during the decay process and the specimens stored in labeled plastic tubes containing 70% alcohol and kept in the laboratory for further identification [52]. As suggested by *Cruickshank and Wall,* [53] and *Yapo, et al.,* [54] temperature and relative humidity were collected at the cage site, using a data logger, Testo 174 T placed approximately 50 cm from the carcass.

Identification

Collected arthropods were identified to various taxonomic levels using a dissecting microscope and various dichotomous keys [55-61] and a reference collection of necrophagous entomofauna of the Laboratory of Zoology of the Faculty of Science of University of Yaounde 1.

Statistical Analysis

Statistical processing of the data was done using specific richness and relative abundance of each taxa according to the decay stage. The specific wealth or specific richness is the total number of the species collected and identified during the experiment [62]. The relative abundance of each taxa was computed using the following relation: $Ar=n_i/N \times 1000$ where Ar is relative abundance, n_i number of individuals for i species and N total number of individuals collected. Based on their relative abundance, the identified species were classified following this scale: $Ar \geq 10\%$, the species are very abundant; $5\% \leq Ar < 10\%$, the species are abundant; $1\% \leq Ar < 5\%$, the species are quite abundant; $Ar < 1\%$, the species are scarce. We used the Kruskal-Wallis ($\chi 2$) to test the effect of the decaying stage on the variation of abundance of the main necrophagous arthropod fauna during the decomposition process. The data were analysed using R software (4.4.2) and the results were calculated at a 5% confidence level.

Results

Carrion Decomposition

Five decomposition stages were recognized, fresh, bloated, putrefied, dried and skeletonized. Each with characteristics summarized in (Table 1).

Table 1: Summary of physical characteristics observed during the domestic dog carrion decay process.

Decay stages	Characteristics of the Carcass
Fresh (Day 1 to day 2)	Carcass looks fresh with pliant/flexible skin, no perceptible odor by human nostrils Clusters of eggs located at the natural openings
Bloated (Day 3 to day 4)	Slight distention of the skin Odor perceptible at about 5m by human nostrils
	Inflation of the abdomen with some hairs on the ground
Putrefied (Day 5 to Day 11)	Deflation of the cadaver alongside increasing odor which was very strong Many more sloughed hairs on the ground near the cadaver Increasing number of larvae present
	Skin beginning to breakdown.
Dried (Day 12 to Day 45)	Odor reduced and not detectable by day 14 Sloughed hair on ground increasing
	Larvae have left carcass, skin breaking down further and few insects present
Skeletonized (Day 46 to 15 April)	Almost all soft tissue removed, except some remaining at joints, some tendons remain. Skeletonized

Carrion Colonization

Insects first explored the carcass for about five minutes after first arrival to assess the quantity and quality of the resource before ovipositing eggs in clusters in natural orifices. Adults also fed on the remains.

A total of 1846 arthropod specimens were collected over the experimental period, belonging to three classes, 11 orders, 13 families, 18 genera and 21 species.

Variation of Arthropods Abundance at Class Level: Between the three arthropods classes identified, insects were the most abundant with 92.52% of 1846 arthropods collected. In regard to the whole decay process, insects were the most dominant taxa and represented more than 80 % of the arthropods collected. During the fresh and bloated stage, insects were the only taxon found on the carcass. Other groups appeared during the putrefied, dried and skeletonized stages. The variation of Insect Abundance during different corpse alteration was significant ($\chi2$ = 18.87; ddl = 4; p = 0.003) (Table 2).

Table 2: Summary of the statistical analysis of the different classes of arthropod fauna (number of specimens) captures during the decomposition of a dog carcass.

Class	Decomposition stages					Total	χ^2 (Kruskal-Wallis)
	Fresh	Bloated	Putrefied	Dried	Skeletonized		
Arachnida	0(0.00)	0(0.00)	1(0.32)	20(2.23)	17(4.66)	38(2.06)	χ^2 =1.44, p= 0.83 ns
Diplopoda	0(0.00)	0(0.00)	0(0.00)	64(7.13)	36(9.86)	100(5.42)	χ^2 =9.15, p= 0.06 ns
Insecta	48(100.0)	221(100.0)	314(99.68)	813(90.64)	312(85.48)	1708(92.52)	χ^2 =18.87, p= 0.003**
Total	48	221	315	897	365	1846	

Note*: Degree of freedom = 4; ** highly significant and ns : non-significant difference at 5 % level.

Variation of Arthropods Abundance at Order Levels: Eleven orders of arthropods were identified during decomposition, with Diptera and Hymenoptera being the most abundant with 39.44 % and 36.02 % of the total individuals sampled, respectively. Orthoptera were the least abundant with less than 1 % of the individuals sampled. Diptera were present throughout decomposition. They were most abundant at the fresh, bloated and putrefied stages with 48 (100 %), 218 (98.64 %) and 253 (80.32 %) of the total individuals sampled respectively. Diptera were followed by the order Hymenoptera with 445 (49.61%) and 162 (44.38%) of the fauna collected at the dried and skeletonized steps successively. This finding highlights the voracious role of these Hymenoptera on the necrophagous arthropod fauna proving that they predate on the usual carrion insects. Coleoptera were the next most numerous order, appearing at the dried (13.49%) and skeletonized stages (23.56%). There was a significant difference in abundance between the different decomposition stages for Diptera ($\chi2$ = 14.37, df=4; p= 0.007), Collembola ($\chi2$ = 14.24, df=4; p= 0.007) and Hymenoptera ($\chi2$ = 12,09, df=4; p= 0,02). The remaining orders did not show a significant difference (Table 3).

Table 3: Variation of arthropod abundance at the order level in relation the decomposition stages of the carcass.

Orders	Decomposition stage					Total	χ^2 (Kruskal-Wallis)
	Fresh	Bloated	Putrefied	Dried	Skeletonized		
Acaria	0(0.00)	0(0.00)	0(0.00)	31(3.46)	31(8.49)	62(3.36)	χ^2 =1.36, p= 0.85 ns
Araneida	0(0.00)	0(0.00)	1(0.32)	8(0.89)	8(2.19)	17(0.92)	χ^2 = 1.60, p= 0.81 ns
Chilopoda	0(0.00)	0(0.00)	0(0.00)	30(3.34)	14(3.84)	44(2.38)	χ^2 = 2.75, p=0.60 ns
Coleoptera	0(0.00)	1(0.45)	5(1.59)	121(13.49)	86(23.56)	213(11.54)	χ^2 =4.92, p= 0.37 ns
Collembola	0(0.00)	0(0.00)	0(0.00)	30(3.34)	3(0.82)	33(1.79)	χ^2 = 14.24, p= 0.007**
Dictyoptera	0(0.00)	0(0.00)	0(0.00)	5(0.56)	15(4.11)	20(1.08)	χ^2 = 3.93, p= 0.42 ns
Diptera	48(100.0)	218(98.64)	253(80.32)	187(20.85)	22(6.03)	728(39.44)	χ^2 = 14.37, p= 0.007**
Glomerida	0(0.00)	0(0.00)	0(0.00)	34(3.79)	22(6.03)	56(3.03)	χ^2 = 4.99, p= 0.29 ns
Hymenoptera	0(0.00)	2(0.90)	56(17.78)	445(49.61)	162(44.38)	665(36.02)	χ^2 = 12.09, p= 0.02*
Lepidoptera	0(0.00)	0(0.00)	0(0.00)	5(0.56)	2(0.55)	7(0.38)	χ^2 = 1.47, p= 0.90 ns
Orthoptera	0(0.00)	0(0.00)	0(0.00)	1(0.11)	0(0.00)	1(0.05)	χ^2 = 1.03, p= 0.90 ns
Total	48	221	315	897	365	1846	

Note*: Degree of freedom = 4; ** highly significant and ns: non-significant difference at 5 % level.

Variation of Arthropod Abundance at the Family Level: The highest number of specimens collected were those of Formicidae with 441 (49.16%) and 162 (44.38%) specimens collected at the dried and skeletonized stages respectively. These were followed by the Calliphoridae with 151 specimens (68.33%) at the bloated stage and 139 (44.13%) at the putrefied stage. The family Ptiliidae were also present alongside the other families with 90 (10.03%) and 59 (16.36%) specimens at the dried and skeletonized decomposition stages. The comparison of the abundance of the different families of invertebrates between the decompositional stages using the non-parametric Kruskal-Wallis test is highly significant ($\chi2 = 30.03$, p= 0.0001, ddl = 4) at 5% for the family Calliphoridae and ($\chi2 = 19.36$, p= 0.0001, ddl = 4) at 5% for the family Muscidae. Entomobryidae was highly significant with $\chi2 = 14.23$ and p= 0.007, ddl = 4. Formicidae was significant with $\chi2 = 12.09$ and p= 0.02, ddl = 4. The remaining families were non-significant (Table 4).

Table 4: Variation of arthropod abundance at the family level during decomposition of the carcass.

Families	Decomposition Stage					Total	χ^2 (Kruskal-Wallis)
	Fresh	Bloated	Putrefied	Dried	Skeletonized		
Blattidae	0(0.00)	0(0.00)	0(0.00)	5(0.56)	15(4.11)	20(1.08)	$\chi^2 = 3.92$, p= 0.42 ns
Calliphoridae	42(87.50)	151(68.33)	139(44.13)	52(5.80)	0(0.00)	384(20.80)	$\chi^2 = 30.03$, p<0.0001***
Entomobryidae	0(0.00)	0(0.00)	0(0.00)	30(3.34)	3(0.82)	33(1.79)	$\chi^2 =14.23$, p= 0.007**
Formicidae	0(0.00)	2(0.90)	56(17.78)	441(49.16)	162(44.38)	661(35.81)	$\chi^2 = 12.09$, p= 0.02*
Glomeridellidae	0(0.00)	0(0.00)	0(0.00)	0(0.00)	22(6.03)	22(1.19)	$\chi^2= 12.13$, p= 0.02*
Julidae	0(0.00)	0(0.00)	0(0.00)	34(3.79)	0(0.00)	34(1.84)	$\chi^2 = 14.85$, p= 0.005 ns
Muscidae	5(10.42)	57(25.79)	106(33.65)	72(8.03)	3(0.82)	243(13.16)	$\chi^2 = 19.36$, p<0.0001***
Phthiracaridae	0(0.00)	0(0.00)	0(0.00)	31(3.46)	31(8.49)	62(3.36)	$\chi^2 = 1.37$, p= 0.85 ns
Ptiliidae	0(0.00)	0(0.00)	0(0.00)	90(10.03)	59(16.16)	149(8.07)	$\chi^2 = 6.53$, p= 0.16 ns
Scolopendrellidae	0(0.00)	0(0.00)	0(0.00)	30(3.34)	14(3.84)	44(2.38)	$\chi^2 = 2.75$, p= 0.60 ns
Sepsidae	0(0.00)	0(0.00)	2(0.63)	26(2.90)	4(1.10)	32(1.73)	$\chi^2 = 2.35$, p= 0.67 ns
Staphylinidae	0(0.00)	0(0.00)	5(1.59)	21(2.34)	2(0.55)	28(1.52)	$\chi^2 = 3.84$, p= 0.43 ns
Others	1(2.08)	11(4.97)	7(2.22)	65(7.25)	50(13.70)	134(7.26)	
Total	48	221	315	897	365	1846	

Note*: Degree of freedom = 4; ** highly significant and ns: non-significant difference at 5 % level.

Variation of Arthropod Abundance at the Genera Level: Pheidole with 648 (35.10%) specimens was the most abundant genus followed by Chrysomya with 188 (10.18%) specimens. These genera were followed in decreasing order by : Ptilii1 [149 (8.07%], *Hemipyrellia* [144 (7.80%)], Dichaetomya [82 (4.44%)], Hydrotaea [67 (3.63%)], Lucilia [46 (2.49%)], Musca [37 (2.00%)], Iulus [34 (1.84%)], Atherigona [37 (2.00)] and Periplaneta [20 (1. 08%)]. The genera analysis indicated that predators, namely Pheidole were common at the dried and skeletonized stages with 441 (49.16%) and 162 (44.38%) specimens respectively. The genera Chrysomya with 104 (33.02%) dominated the putrefied stage while the Ptiliidae genus were also most active at the dried [90 (10.03%)] and skeletonized [59 (16.16%)] stages. The comparison of the abundance of the different genera of invertebrates between the various decomposition stages using the non-parametric Kruskal-Wallis test showed that the variation of the genera Chrysomya ($\chi2 = 27.08$, p= 0.0001) and *Hemipyrellia* ($\chi2 = 39.77$, p= 0.0001) is highly significant. The variation of the genera Dichaetomya ($\chi2 = 20.32$, p = 0.001), Hydrotaea ($\chi2 = 19.45$, p = 0.001) and Pheidole ($\chi2 = 14.42$, p = 0.006) is highly significant while that of Atherigona ($\chi2 = 11.91$, p = 0.02%) and Lucilia ($\chi2 = 11.23$, p = 0,02) is significant at the level of 5% (ddl = 4) (Table 5).

Table 5: Variation of arthropod abundances at genera level during the decay stages of the carrion.

Genera	Decomposition stage					Total	χ^2 (Kruskal-Wallis)
	Fresh	Bloated	Putrefied	Dried	Skeleton-ized		
Genera of Acaria	0(0.00)	0(0.00)	0(0.00)	31(3.46)	31(8.49)	62(3.36)	χ^2 = 1.37, p= 0.85 ns
Atherigona	2(4.17)	3(1.36)	17(5.40)	14(1.56)	1(0.27)	37(2.00)	χ^2 = 11.91, p= 0.02*
Genra of Chilopoda	0(0.00)	0(0.00)	0(0.00)	30(3.34)	14(3.84)	44(2.38)	χ^2 =2.75 , p= 0.60 ns
Chrysomya	0(0.00)	42(19.00)	104(33.02)	42(4.68)	0(0.00)	188(10.18)	χ^2 = 27.08, p<0.0001***
Genera of Colembola	0(0.00)	0(0.00)	0(0.00)	30(3.34)	3(0.82)	33(1.79)	χ^2 = 14.24, p= 0.007**
Dichaetomya	0(0.00)	16(7.24)	38(12.06)	26(2.90)	2(0.55)	82(4.44)	χ^2 = 20.32, p<0.001**
Genera of Glomeridel-lida	0(0.00)	0(0.00)	0(0.00)	0(0.00)	22(6.03)	22(1.19)	χ^2 = 12.13, p= 0.02*
Hemipyrellia	42(87.50)	92(41.63)	9(2.86)	1(0.11)	0(0.00)	144(7.80)	χ^2 =39.77 , p<0.0001***
Hydrotaea	0(0.00)	16(7.24)	40(12.70)	11(1.23)	0(0.00)	67(3.63)	χ^2 = 19.45, p<0.001**
Iulus	0(0.00)	0(0.00)	0(0.00)	34(3.79)	0(0.00)	34(1.84)	χ^2 =14.85 , p= 0.005**
Lucilia	0(0.00)	17(7.69)	20(6.35)	9(1.00)	0(0.00)	46(2.49)	χ^2 = 11.28, p= 0.02*
Musca	0(0.00)	19(8.60)	0(0.00)	18(2.01)	0(0.00)	37(2.00)	χ^2 = 9.02, p= 0.06 ns
Periplaneta	0(0.00)	0(0.00)	0(0.00)	5(0.56)	15(4.11)	20(1.08)	χ^2 = 3.93, p= 0.42 ns
Pheidole	0(0.00)	2(0.90)	43(13.65)	441(49.16)	162(44.38)	648(35.10)	χ^2 = 14.42, p= 0.006**
Genera of Ptiliida	0(0.00)	0(0.00)	0(0.00)	90(10.03)	59(16.16)	149(8.07)	χ^2 = 6.53, p= 0.16 ns
Genera of Sepsidae	0(0.00)	0(0.00)	2(0.63)	26(2.90)	4(1.10)	32(1.73)	χ^2 = 2.35, p= 0.67 ns
Genera of Staphylinida	0(0.00)	0(0.00)	5(1.59)	21(2.34)	2(0.55)	28(1.52)	χ^2 = 3.84, p= 0.43 ns
Others	4(8.33)	14(6.33)	37(11.75)	68(7.58)	50(13.70)	173(9.37)	
Total	48	221	315	897	365	1846	

Note*: Degree of freedom = 4; ** highly significant and ns : non-significant difference at 5 % level.

Variation of Arthropod Abundance at the Species Level: The decreasing abundance of the various species are as follows : Pheidole megacephala (Fabricius, 1793) (648), *Hemipyrellia fernandica* (Macquart, 1855) (79), Chrysomya laxifrons (Villeneuve, 1914) and *Dichaetomya* sp. (69), *Hemipyrellia* sp. (65), C. putoria (Wiedemann, 1830) (45), *Hydrotaea* sp. (41), Lucila sericata (Meigen, 1826) (38), *Musca* sp. (37), C. albiceps (Wiedemann, 1819) (36), Iulus sp. (34), C. vanemdeni (Zumpt, 1953) and *Periplaneta* sp. (20). Based on the decomposition stages, the fresh and the bloated stages of decay were colonized by *Hemipyrellia* sp. with 65 (29.41%) and H. fernandica with 42 (87.50%) specimens. At the dried and putrefied stages, P. megacephala also dominated the fauna with 441 (49.16%) and 43 (13.65%) specimens respectively while the most abundant species were Pheidole megacephala with 162 (44.38%) at the skeletonized stage. The comparison of the abundance of the different species of invertebrates between the various decomposition stages using the non-parametric Kruskal-Wallis test yielded three groups of species organized as follows: Highly significant species: C. laxifrons ($\chi2$ = 19.85, p= 0.0005), C. putoria ($\chi2$ = 32.55, p= 0.0001), H. fernandica ($\chi2$ = 25.24, p= 0.0001), Hemipyrellia sp. ($\chi2$ = 30.5, p= 0.0001), C. albiceps ($\chi2$ = 16.26, p= 0.002), Colembola spp. ($\chi2$ = 14.24, P = 0.006), Dichaetomya sp. ($\chi2$ = 16.97, p= 0.002), Hydrotaea sp. ($\chi2$ = 14.63, p= 0.006), Iulus sp. (14.85, p= 0.005), L. sericata ($\chi2$ = 14.43, p= 0.006) and P. megacephala ($\chi2$ = 14.42, p= 0.006). The only significant species were Glomeridellida spp. ($\chi2$ = 12.13, p= 0.02) at the level of 5% (Table 6).

Relationship Between Various Decay Stages and Relative Abundance (Ar)

The relationship between the successive decay states and relative abundance is summarized in Table 7. It shows that some insects are linked to the specific decomposition stages.

Discussion

Sampling Procedures

The collection methods used for our experimentation were similar to the protocol performed by Kpama-*Yapo, et al.,* [63] in the Guinean zone of Côte d'Ivoire on pig corpses (Sus scrofa domesticus L.) even though our biological sampling bait was the dog (Canis

lupus familiaris) as used by *Barbosa, et al.,* [48]. Also, *Camine, et al.,* [64], *Biro, et al.,* [65], *Soler, et al.,* [31], *Lemos, et al.,* [32], *Schieweck, et al.,* [66], *Feugang Youmessi, et al.,* [24], *Koffi, et al.,* [51] and *Blau, et al.,* [30] collected three times daily at 0800, 1200 and 1600 h from an unidentified human body except the first authors who use domestic pig carcasses as trapping bait. Following authors such as *Dao, et al.,* [67] our sampling was done every day throughout the whole decomposition process until the skeletonized stage, but our pitfall distance varied from that of *Koffi, et al.,* [51] who installed theirs at a distance of 15 cm from the carcass.

Table 6: Variation of arthropod abundances at species level during the decay stages of the carrion.

Species	Decomposition Stages					Total	χ^2 (Kruskal-Wallis)
	Fresh	Bloated	Putrefied	Dried	Skeletonized		
Acaria spp.	0(0.00)	0(0.00)	0(0.00)	31(3.46)	31(8.49)	62(3.36)	χ^2 = 1.37, p= 0.85 ns
Chilopoda spp.	0(0.00)	0(0.00)	0(0.00)	30(3.34)	14(3.84)	44(2.38)	χ^2 = 2.75, p= 0.6 ns
Chrysomya albiceps (Wiedemann, 1819)	0(0.00)	0(0.00)	36(11.43)	0(0.00)	0(0.00)	36(1.95)	χ^2 =16.26, p<0.002**
Chrysomya laxifrons (Villeneuve, 1814)	0(0.00)	18(8.14)	44(13.97)	7(0.78)	0(0.00)	69(3.74)	χ^2 = 19.85, p<0.0005***
Chrysomya putoria (Wiedemann, 1830)	0(0.00)	24(10.86)	19(6.03)	2(0.22)	0(0.00)	45(2.44)	χ^2 = 32.55, p<0.0001***
Chrysomya vanemdeni (Zumpt, 1953)	0(0.00)	0(0.00)	4(1.27)	16(1.78)	0(0.00)	20(1.08)	χ^2 = 2.80, p=0.60 ns
Colembola spp.	0(0.00)	0(0.00)	0(0.00)	30(3.34)	3(0.82)	33(1.79)	χ^2 = 14.24, p<0.006**
Dichaetomya sp. (Rondani, 1868)	0(0.00)	16(7.24)	25(7.94)	26(2.90)	2(0.55)	69(3.74)	χ^2 =16.97, p<0.002**
Glomeridellida spp.	0(0.00)	0(0.00)	0(0.00)	0(0.00)	22(6.03)	22(1.19)	χ^2 = 12.13, p<0.02*
Hemipyrellia fernandica (Macquart, 1855)	42(87.50)	27(12.22)	9(2.86)	1(0.11)	0(0.00)	79(4.28)	χ^2 =25.24, p<0.0001***
Hemipyrellia sp. (Towsend, 1918)	0(0.00)	65(29.41)	0(0.00)	0(0.00)	0(0.00)	65(3.52)	χ^2 = 30.5, p< 0.0001***
Hydrotaea sp. (Robineau-desvoidy, 1830)	0(0.00)	0(0.00)	38(12.06)	3(0.33)	0(0.00)	41(2.22)	χ^2 = 14.63, p< 0.006**
Iulus sp. (Leach, 1814)	0(0.00)	0(0.00)	0(0.00)	34(3.79)	0(0.00)	34(1.84)	χ^2 = 14.85, p<=0.005**
Lucilia sericata (Meigen, 1826)	0(0.00)	17(7.69)	20(6.35)	1(0.11)	0(0.00)	38(2.06)	χ^2 = 14.43, p< 0.006**
Musca sp. (Linnaeus, 1758)	0(0.00)	19(8.60)	0(0.00)	18(2.01)	0(0.00)	37(2.00)	χ^2 = 9.02, p= 0.06 ns
Periplaneta sp. (Burmeister, 1838)	0(0.00)	0(0.00)	0(0.00)	5(0.56)	15(4.11)	20(1.08)	χ^2 = 3.93, p= 0.42 ns
Pheidole megacephala (Fabricius, 1793)	0(0.00)	2(0.90)	43(13.65)	441(49.16)	162(44.38)	648(35.10)	χ^2 = 14.42, p< 0.006**
Ptiliida spp.	0(0.00)	0(0.00)	0(0.00)	90(10.03)	59(16.16)	149(8.07)	χ^2 = 6.53, p= 0.16 ns
Sepsida spp	0(0.00)	0(0.00)	0(0.00)	21(2.34)	4(1.10)	25(1.35)	χ^2 = 2.37, p= 0.67 ns
Staphylinida spp.	0(0.00)	0(0.00)	0(0.00)	17(1.90)	2(0.55)	19(1.03)	χ^2 = 3.19, p= 0.53 ns
Others	6(12.5)	33(14.93)	77(24.44)	124(13.82)	51(13.97)	291(15.76)	
Total	48	221	315	897	365	1846	

Note*: Degree of freedom = 4; ** highly significant and ns : non-significant difference at 5 % level.

Table 7: Relative abundance of the main necrophagous arthropod fauna species in relation to the decay stages.

Ar	Decomposition stages				
	Fresh	Bloated	Putrefied	Dried	Skeletonized
Very abundant species	*Hemipyrellia fernandica*	*C. putoria* *Hemipyrellia fernandica* *Hemipyrellia* sp.	*C. albiceps* *C. laxifrons* *Hydrotaea* sp. *Pheidole megacephala*	*C. vanemdeni* *Dichaetomya sp. Iulus* sp. *Musca* sp. *Pheidole megacephala*	*Periplaneta* sp *Pheidole megacephala.*
Abundant species		C. *laxifrons* *Dichaetomya sp.* *Lucilia sericata* *Musca* sp.	*C. putoria* *Dichaetomya* sp. *Lucilia sericata*	*C. laxifrons* *Periplaneta* sp.	
Quite abundant species			*C. vanemdeni* *H. fernandica*;	*C. putoria* *H. fernandica* *Hydrotaea* sp. *L. sericata*;	*Dichaetomya* sp.
Scarce species		*Pheidole megacephala*			

Diversity and Composition of Necrophagous Arthropod Fauna

The decomposition process went through five distinct decay phases which is consistent with that of *Silahuddin, et al.,* [68], *Dao, et al.,* [67], Anderson [69] and Maisonhaute and Forbes [70] who stated that abiotic factors such as the region, climate and others factor intrinsic to the carcasses guided the decomposition process.

Our field succession observations revealed a rich biodiversity of necrophagous fauna with 1846 individuals in 17 genera and 21 species, similar to that seen in Australia [5], in Ngaoundéré-Cameroon [27], in Zimbabwe [42], in the Eastern Region of the Kingdom of Saudia Arabia [40] indicating the high diversity of these organisms. This is in line with the results gathered by *Fantio, et al.,* [71] in Douala even though their work was done on ground beef meat in the laboratory but differed from those obtained by *Naman, et al.,* [72] at the botanical garden of Kaduna State University in Nigeria where their biodiversity very low. This is probably due to the methods used as these authors collected fauna only during the wet season in their area because biotic and abiotic conditions are very important in decomposition and insect succession (73, 40, 74).

At the level of species, the species Pheidole megacephala, Hemipyrellia fernandica, Chrysomya laxifrons, *Dichaetomya* sp. and *Hemipyrellia* sp. were the most abundant overall. These diptereans and hymenopterans followed the same trends as those obtained by other authors such as *Biavati, et al.,* [4] in Central Brazil, *Feugang Youmessi, et al.,* [8,9] and Feugang Youmessi [33] at Yaounde-Cameroon, *Faria, et al.,* [75] at Minas Gerais-Brazil, *Dao, et al.,* [67] at Côte d'Ivoire and *Feugang Youmessi, et al.,* [76] at Yaounde-Cameroon. This result is in contrast with the results obtained by *Taleb, et al.,* [43] in Algeria who showed that the species Lucilia sericata and Calliphora vicina were the most abundant on carrion in their area. This is because fauna are specific for a given region, proving the correlation between local fauna and environmental variables [77,78]. Numerous published data on necrophagous insects highlight variability within the composition of their community due to factors like experimental design [79], geographical variation [24], sampling artifacts [80-83]. Overall, these results highlight the fact that blow flies are forensically very important in postmortem interval estimation since they are often the first invader of the carcass worldwide [84,85]. The presence of Lucilia sericata within our sample demonstrate its plasticity/ cosmopolitancy in growth, development and adaptations to adverse conditions either environmental or inter/heterospecific interactions [85,28].

Variation of Abundance of Necrophagous Arthropod Fauna

Diptera, Hymenoptera and Coleoptera families were recorded during our experiment. Among the Diptera and in line with our observations in Yaounde, *Kpama-Yapo, et al.,* [86], *Dao, et al.,* [67] also recorded that the first arrivals were Calliphoridae even though the colonization time was just some few hours in Yaounde contrary to a 24 hours delay (maybe due to environmental parameters of a given study site) observed by *Dao, et al.,* [67] using pig cadavers exposed to the open air in the Sub-Sudanese zone in Ivory Coast. These results are consistent with those of Picimbon, [87], *Lee, et al.,* [88], *Dekeirsschieter* [89], *Silahuddin, et al.,* [68], *Koffi, et al.,* [51], *Feugang Youmessi, et al.,* [24] and *Anderson,* [69]. Early colonization by these flies is the result of their highly developed olfactory organs which enable them to smell and detect volatile organic compounds over long distances at levels not perceptible by humans [90,54,51].

Our results showed that H. fernandica was the first and the only colonizer of the carcass during the fresh stage which is not the

common species usually cited in forensic research work worldwide. This finding is contrary to *Taleb, et al.,* [43] who use the same substratum like us and reported that L. sericata was the primary carrion feeders in their research conducted in the experimental station of the faculty of Nature and Life Sciences, Blida, Algeria. This suggests that H. fernandica is more common in the region of Yaounde and therefore need more added experiment on that specific species at Yaounde. *Hemipyrellia fernandica* is considered widespread in the Afrotropical Region and has been recorded from many other areas within the continent [60,93] which makes it surprising that it has not been found more commonly in other carrion studies. It has been found to colonize carcasses and feces and even a giant dung beetle [93]. It has mostly been recorded from small carcasses such as mice, birds and reptiles, which might be why it is less common in carrion studies which usually employ larger carcasses, such as this study, although it has been reported from larger vertebrates including humans [93-95].

Conclusion

This research highlights the forensic relevance of some necrophagous arthropod fauna especially insects that may be used to help in human death and neglect cases as well as wildlife crimes in this region. The most forensically relevant insects are Diptera families Calliphoridae: Chrysomya albiceps, Chrysomya laxifrons, Chrysomya putoria, C. vanemdeni, *Hemipyrellia fernandica*, *Lucilia cuprina*, *Lucilia sericata*, *Lucilia* sp., Hemipyrellia sp., *Chrysomya* sp.; *Muscidae*: *Dichaetomya* sp., *Hydrotaea* sp., *Musca* sp. and some Coleoptera such as Ptiliidae, Sepsidae and Staphylinidae. Some species seem to be related to a particular decomposition stage: fresh state (*Hemipyrellia fernandica*), bloated state (C. putoria, *Hemipyrellia* sp., C. *laxifrons*, *Dichaetomya* sp., *Lucilia sericata*, *Musca* sp. and *Pheidole megacephala*), putrefied stage (*C. albiceps*, *Hydrotaea* sp., *C. putoria*, *Dichaetomya* sp. and *C. vanemdeni*), dried stage (*C. vanemdeni*, *Iulus* sp., *Periplaneta* sp. and *Hydrotaea* sp.). The limitations of this research is the use of three carcass inside each cage replicated three time. It must be replicate several time for many years at the same area to gathered more information on forensic entomology of Yaounde.

Author Contributions

The first author conceived and designed the experiments, collected the data and wrote the paper. The second author defined the analysis tools, performed the analysis and wrote the paper.

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Conflict of Interest

None.

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