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A preliminary study of arthropod associated with carrion in Yaounde, Cameroon: A first step in forensic entomology in Central Africa

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The first investigation of arthropods associated with carrion in Cameroon was carried out within the campus of the University of Yaounde I (Cameroon) from 17th January, to 3rd April, 2006. Carcasses of rats (Rattus norvegicus Berkenhout, 1769 var WISTAR) were exposed for colonization by the local fauna of arthropods. The visiting and colonizing arthropods were collected daily during the study periods. 1980 arthropods of forensic importance belonging to 4 classes, 16 orders, 39 families and 3 subfamilies were identified. Among the insects assessed were Coleoptera: Cleridae, Curculionidae, Dermestidae, Histeridae, Mordellidae, Ptiliidae, Scarabaeidae, Silphidae, Staphylinidae (subfamily Tachyporinae), Trogidae; Dermaptera; Diptera: Anthomyiidae, Calliphoridae, Cecidomyiidae, Culicidae, Drosophilidae, Ephydridae, Fanniidae, Heleomyzidae, Lauxaniidae, Muscidae (subfamily Phaoniinae), Phoridae, Piophilidae, Sarcophagidae, Sciaridae, Sepsidae and Sphaeroceridae; Hemiptera: Aphididae, Anthocoridae, Cicadidae; Hymenoptera: Formicidae (subfamilies Myrmicinae (genera Pheidole, Tetramorium and Monomorium), Formicinae (Paratrechina longicornis and Oecophylla longinoda) and Dolichoderinae), Braconidae, Proctotrupidae, Scelionidae, Chalcididae; Lepidoptera: Tineidae; Psocoptera, and Thysanura. Other arthropods such as Arachneida and Myriapoda (Chilopoda and Diplopoda) were also collected. This study illustrates the diversity of the cadaver fauna in Cameroon and provides an approximation of the succession pattern of arthropods associated with carrion through a field experiment (77days).

Key words: Cadaver fauna, arthropods, chronological succession, Rattus norvegicus, Calliphoridae.

INTRODUCTION

Within the framework of a judicial inquiry following the discovery of a cadaver, the determination of time of death is a very important issue for the legal authorities (police officers, magistrates, coroners, etc.). Such estimation is more difficult to establish when the cadaver has reached an advanced stage of decomposition. In this case, the entomological evidence can be one of the few sources to make such estimation. However, the process of decay of organic material is highly complex and numerous interrelated factors influence it: macroclimate. microclimate, availability and accessibility of insects to the carcasses (Braack, 1981). Therefore, it is necessary to study each region of necrophagous entomofauna. However, with the exception of South Africa where Braack (1981 and 1987) studied the succession of the insects on carcasses in the Kruger National Park; Williams and Villet (2006), the history of the Southern African research relevant to forensic entomology; Kelly et al. (2009), the influence of clothing and wrapping on

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carcass decomposition and arthropod succession; and Okiwelu et al. (2008), the arthropods associated with mammalian carcasses in Rivers State (Nigeria), carrionfeeding insects are not well studied in the Afrotropical Region. In Cameroon, there is no information available regarding insects associated with animal carrion and human cadaver. Since 2005, there has been strong enthusiasm from the Cameroonian authorities to introduce forensic science to improve the quality and efficiency of legal investigations. But there has been no development in forensic entomology because biology, ethology and ecodynamics systematics. of the nercophagous entomofauna are yet to be elucidated.

The aim of this paper is to present initial results of research on the arthropods associated with carrion in Cameroon, in order to create a database.

MATERIALS AND METHODS

The present study has been carried out in two sites, 200 m apart, with similar conditions at the campus of the University of Yaounde I (11°33'01"E 3°51'35"N) Yaounde, Cameroon.

The climate, named "Yaoundean" is equatorial and characterised by four distinct seasons (Suchel, 1987): a long rainy season (March to June), a short rainy season (September to mid November), a long dry season (mid November to February) and a short dry season (from July to August). The annual mean rainfall is 1600 mm and the average annual temperatures fluctuate between 19 to $27 \,^{\circ}$ C. The landscape of this part of the city is characterized by the presence of *Elaeis guineensis* (Arecaceae) and *Musa* sp. (Musaceae).

Ten carcasses of laboratory-bred rats (*Rattus norvegicus* Berkenhout, 1769 var WISTAR), each weighing about 180 g, were used as models. The rats were sacrificed by strangulation on January 17th, 2006 and immediately placed in one cage (120 cm x 120 cm x 120 cm) in each locality (each cage containing five carcasses) covered with 5 cm mesh to allow colonization of the carcasses by insects while preventing scavengers' attacks. Each cage was visited three times on each day, during the first and second stages of decomposition (from day 1 to 4). After this, sampling was undertaken daily at 12 h for 20 min. Sampling stopped when the carcasses became only bones on 3^{rd} April.

During each sampling session, carcasses were filmed, observations of the physical modification due to decay were made and the ambient air temperature was recorded using a mercury column thermometer (Miras et al., 1998; Catts and Haskell, 1990; Bharti and Singh, 2003; Amendt et al., 2006; Gaudry et al., 2007). One thermometer was held inside each cage 5 cm above ground. The maximum, minimum and mean temperatures were 27.85, 21.33 and 27.6 ℃.

Larvae, pupae and puparia were collected from the carcasses using flexible forceps. Flying insects were caught with a hand net of 1 mm mesh. Insects caught with the net were brought back to the laboratory and sprayed with 70% ethyl alcohol. After 10 min, the insects were preserved in 70% ethyl alcohol for taxonomic identification. The larvae and pupae were divided into two parts: one part preserved and the other one reared in the laboratory until the emergence of the adults flies. After the emergence, the insects were fed with honey within two days and then captured and preserved for taxonomic identification.

The inventory and identification of the samples were partially completed in the Zoology Laboratory of the University of Yaounde I, using keys of Delvare and Alberlenc (1989) for families; and keys of Smith (1986), Claudio and Cátia (2008), Prins (1982), Couri (2007) and Regina (2002) for genera and species. A selection of 103 plastic tubes containing about 200 samples was sent to the Forensic Science Institute of the French Gendarmerie (IRCGN-Department of Forensic Entomology) for further identifications.

RESULTS AND DISCUSSION

All arthropods were assigned to the ecological categories proposed by Smith (1986) and Magaña (2001) in Martinez et al. (2007) (Table 1). As other researchers have found, (Tullis and Goff, 1987; Anderson and VanLaerhoven, 1996; Wolff et al., 2001; Wyss and Cherix, 2006; Martinez et al., 2007; Okiwelu et al., 2008 and Velásquez, 2008), Diptera were the predominant insects collected during the first, second and third stages of decomposition. Their number decreased gradually and successively while the beetles' numbers progressively increased following the decay of the corpse (Table 2). This result is different from that recorded by Braack (1987). This author obtains Coleoptera (Histeridae) at the first stage of decomposition during his research.

According to the morphological modifications of the cadavers, just like Benecke (2004) five stages of decomposition were observed in this study: fresh, bloated, decayed, dried and skeletonised. The sequence of different stages recorded during our study (five: fresh, bloated, putrefied, dried and skeletonized) are similar to that observed by Martinez et al. (2007) and Moretti et al. (2008) although durations of their stages were shorter. The results are, however, different from those recorded by Braack (1981) and Kelly et al. (2009). During their study on the insect succession on the carcasses in South Africa they obtain four stages of decomposition (fresh, bloat, active decay and advanced decay). This difference can be explained by the fact that South Africa was situated in a region where there is a very slow change of the temperatures although the duration of decomposition was similar. Added to this, the study was conducted on small carcasses in contrast to those used by Kelly et al. (2009) where each study site had its own particular climatic and environmental conditions and the carcasses were bigger.

During the fresh stage (Day 1 to 2), there were no noticeable morphological changes on the carcasses. On the second day maggots were observed to be emerging from the eggs. At the end of the second day, the odours were faintly noticeable in the immediate surroundings of the carcasses; and the carrion had started to bloat. The only family of insects found on the carcasses were Calliphoridae. The first individuals to lay eggs were *Lucilia* sp., *Calliphora vicina* Robineau-Desvoidy and *Chrysomya* sp. Females laid eggs in the natural orifices (nose, mouth and eyes) during Day 1 and 2.

The bloated stage (Day 2 to 4) started with the swelling and deflation of the carrion. "Cadaver" odours were at this point noticeable for a distance of 5 m from the carcasses. In addition to Calliphoridae, during the third **Table 1.** Inventory of arthropods associated with carrion in Yaounde (Cameroon) during the dry season (January 17^{th} to April 3^{rd} , 2006). X = present (unidentified until species taxonomic level); E = eggs; I = immature; A = adults; F = fresh; B = bloated; D1 = decay; D2 = dried; S = skelotonized. The classification of ecological categories is based on Smith (1986) and Magaña (2001) in Martinez et al. (2007).

						State of decomposition										
Taxonomy						F(0-2 days)			B (3-4 D ⁻ days) d		5-9 /s)	D2 (10-73 days)		S(d	S(74-77 days)	
					Ecological category	E	I	Α	Е	ΙΑ	ΕI	Α	ΕI	Α	ΕI	IA
Class	Order	Family	Subfamily	Species												
		Calliphoridae		Lucilia sp. Chrysomya sp. Calliphora vicina (R-D)		х		34 31	x x x	122		9		23		
		Muscidae		Ophvra sp.					x							
		Sarcophagidae										9		23		
		Fanniidae								4		•		17		
		Sphaeroceridae			Necrophagous									8		
	Diptera	Sepsidae										6		26		
	1	Piophilidae												8		
		Phoridae										7		16		
	Coloortoro	Silphidae										3				
	Coleoptera	Dermestidae												56		30
	Lepidoptera	Tineidae												2		5
		Staphylinidae	Aleocharinae							10		28		230		
		Histeridae			Predators					3		7		97		
	Coleoptera	Cleridae										3		96		6
	Dermaptera											1				
Hexa	Hemiptera	Anthocoridae												1		
		Chalcididae						4		4		7				
			Myrmicinae	<i>Pheidole</i> sp. <i>Tetramorium</i> sp. <i>Monomorium</i> sp.	Predators/			11		5		2		9		1
		Formicidae	Formicinae	Paratrechina longicornis Oecophylla longinoda	parasitoids			2		3		13		7		8

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Table 1. Contd.

	Hymonoptora		Delichederinge				6	
	riymenoptera	Braconidae	Donchodennae				0	5
		Proctotrupidae			3			0
		Scelionidae			1		2	
					•		-	
		Cecidomyiidae					1	
		Drosophilidae		Phytophagous	6	7		
	Distant	Muscidae	Phaoniinae		101	20	x 10	
	Diptera	Anthomyiidae			7	9	31	
		Heleomyzidae			47	3	3	
		Ephydridae		Saprophagous		6	6	
		Sciaridae					3	
		Lauxanniidae				6	4	
	Coleoptera	Ptiliidae					554	52
	Dictyoptera	Blattidae		Omnivorous		6	13	7
	Collembola						3	
Arach	Araneida					14	17	22
/	Acaria	Haplozetidae		Opportunist			60	36
	5							
Myria	Diplopoda					1	4	1
	Chilopoda					17	28	2
	Diptera	Culicidae		Hematophyl		1	2	
	0	Irogidae			1		1	
	Coleoptera	Scarabaeidae				1	3	
		Mordellidae	-				22	
		Staphylinidae	lachyporinae				5	
	Hemiptera	Cicadidae		Incidental	1		-	
Hexa		Aphididae					3	
	Homoptera	Undetermine			4	23	35	20
	Orthoptera	Gryllidae					18	12
	Psocoptera						_	1
	Thysanura						5	

Legend: hexa, Hexapoda; Arach, Arachnida ; Myria, Myriapoda.

Decay state							
Orders	0-2 fresh	3-4 bloated	5-9 putrefied	10-73 dried	74-77 skeletonized	rotar	
Coleoptera		16	42	1121	88	1267	
Diptera	65	287	83	181		616	
Acaria				60	36	96	
Heteroptera		4	23	35	20	82	
Araneida			14	17	22	53	
Chilopoda			17	28	2	47	
Orthoptera				18	12	30	
Dictyoptera			6	13	7	26	
Lepidoptera				2	5	7	
Diplopoda			1	4	1	6	
Thysanura				5		5	
Homoptera				3		3	
Collembola				3		3	
Hemiptera			1	1		2	
Dermaptera			1			1	
Psocoptera					1	1	
Hymenoptera	17	18	22	24	14	95	
Total	82	325	210	1515	208	2340	

Table 2. Total number of individual carrion-feeding arthropods of rat carrion in Yaoundé (Cameroon) during the dry season (January 17th to April 3rd, 2006).

day, Muscidae, Heleomyzidae, and Fanniidae frequented the body. Drosophilidae appeared on the fourth day. At the same time, coleopteran families (Staphylinidae, Histeridae and Trogidae) were also recorded. Maggots continued to feed, grow and maggot masses extend to the abdominal region of the rat carrion.

In the decay stage (Day 5 to 9), the abdomen organs were completely consumed by maggots and there was an outpouring of the decomposition fluid Together with the maggot migration from the cadaver to the ground. During this stage, odour perceptibility decreased from day to day. Calliphoridae decrease significantly from days 5 to 8. The new families first collected at this stage were Sarcophagidae (Diptera) and Silphidae (Coleoptera). On day 6, Sepsidae (Diptera), Phoridae (Diptera) and Cleridae (Coleoptera) started to appear; on day 7, Lauxaniidae (Diptera) and Scarabaeidae (Coleoptera) arrived and Ephydridae (Diptera) on day 8. Only one individual of Culicidae was caught, and this was on the ninth day of decay. This putrefaction stage ended with the complete degradation of the viscera and the migration of the maggots.

At the fresh stage, the first insects to lay eggs on the carrion were *Calliphora vicina* (Robineau-Desvoidy), *Lucilia* sp. and *Chrysomya* sp. This result is similar to that of Martinez et al. (2007); except for the fact that they also capture Muscidae (*Dasymorellia seguyi*, *Fannia* sp. and *Limnophora* sp.) at this stage. These results resemble those of Bharti and Singh (2003) who in addition to Calliphoridae recorded the presence of Sarcophagidae.

This difference can be explained by the fact that, as shown by the study conducted by Martinez et al. (2007), the bloated and putrefied stages were mainly characterized by Calliphoridae (122 individuals), Muscidae (101 individuals) and Heleomyzidae (47 individuals) although the duration (started from Day 4 to 16 after death) of these stages was different.

The presence of a large number of individuals of these families is the main reason for the disappearance of the abdominal organs due to maggots, the outpouring of the cadaver liquid and maggot migration from the cadaver to the ground. In addition to these fauna, Braack (1981, 1987) captured *Chrysomya albiceps*, ants, Histeridae, Staphylinidae and Silphidae; this difference in insects could have been the consequence of the fact that their study site is a semi-arid region with an average rainfall of 438.1 mm; it could also be because of the biogeographical variations of the species and the stability of the environmental parameters.

In the most long dried stage (Day 10 to 73), decay stopped. Only skin, cartilage, bones, nails and teeth remained. Odours were almost imperceptible. Skin started to split on day 10, hair and bones were already delocalised. We found Dermestidae (Coleoptera) at day 10 and Sphaeroceridae (Diptera) at day 11; Mordellidae (Coleoptera) at day 13 and Ptiliidae (Coleoptera) at day 15; Piophilidae (Diptera) at day 21 and Sciaridae (Diptera) at day 36; Haplozetidae (Acari) at day 41, Staphylinidae (subfamily Tachyporinae (Coleoptera)) at day 42 and Cecidomyiidae (Diptera) at day 73. In the skeleton stage (74 to 77 days), only dried piece of skins and bones were left. During this stage, no new families were recorded but there was a decrease in Ptiliidae, consuming the tough and dried material.

The abundance of the Coleoptera mainly Ptilidae which feed on dry remains looks like that of Kelly et al. (2009). In the skeletal stage (74-77 days), the stability of the environmental parameters may be the reason for the increase in the number of Ptilidae.

Conclusion

This study presents, for the first time a list of the assemblages of arthropods on rat carrion in Central Africa. The carrion-feeding fauna is defined by three major groups: the Diptera, Coleoptera and Acaria. This first study did not show clear evidence of insect succession on the carrion but enables us to get the overview of the necrophagous entomofauna of the area. We can also have an idea of the different stages of the carcasses decomposition according to our specific environmental parameters.

The further studies of the necrophagous entomofauna, the determination of the different state of the carrion decomposition process, the rearing of the maggot in the laboratory, the measurement of the various environmental parameter (temperature (ambient air temperature, carcasses internal temperature, soil temperature), humidity, hygrometry.... will be done on rat (*R. norvegicus* Berkenhout, 1769 var. WISTAR) and pic *Sus scrofa* in the campus of the University of Yaounde I (Cameroon) in order to create a database of the forensic insects of the region.

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