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First evidence of forensic entomology revealed the presence of arthropods on rabbit carrion in Cotonou, Benin (West Africa)

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Abstract
The study aimed to document the entomofauna of medico-legal importance in Benin, by studying arthropod's succession pattern on the rabbit carrion and the variation of their density associated with temperature and humidity was also investigated. The study was carried out on the rabbit carrion in Cotonou by the collection of necrophagous arthropods trapped during the stages of decomposition. The identification of arthropods on the basis of their morphological characteristics and their statistical analysis revealed that the orders of Diptera, Coleoptera, Hymenoptera and Aranea were those which colonized the rabbit carrion. With a Simpson Index (Is) of 0.69, *Chrysomia sp*. was the most abundant arthropod with a maximum of 990 individuals at the bloated stage ($p < 0.001$). Air temperature and relative humidity had significant effects on the numbers of arthropods collected. The present study showed that Diptera can be used for the determination of indices during criminal investigations in Benin.

Keywords: rabbit carrion, arthropods succession, forensic entomology

1. Introduction
Arthropods are important in medico-criminal identification of a human death. Forensic entomology is the study of the insects associated with a dead body to determine the time elapsed since death [1]. Many types of information can be extracted from the study of arthropods at crime scenes. After death, a different group of sarcosaprophagous arthropods, especially insects invade the carrion in a predictable way. Some are attracted to the remains which are used as a medium for oviposition or feeding, while others are attracted by the aggregation of other arthropods that are used as a food source [2]. Insects attraction and their succession on a dead body are dependent on environmental factors[3,4]. Knowledge of insect succession is very interesting because of helpful for estimating postmortem interval, cause of death and toxicological investigations. The use of insects to clarify cases of crimes has increased considerably in recent years. Except in United States, Europe, Britain and Australia, the literature in the field of forensic entomology are not well known in Africa. There is a lack of information regarding forensic entomology in this continent. Just a few countries such as South Africa, Egypt, Nigeria, Cameroon, Ghana and Zimbabwe [2] have studied insects associated with decomposing dead bodies. In Benin, forensic entomology research is new and no medical or criminal investigation using forensic entomology has been used. Forensic entomology has not been included into criminal investigations, despite many homicide and holdups cases being reported almost every month in Cotonou. This first study can provide basic data and evidence for a possible use of forensic entomology in Benin. The objectives of this study are therefore to document the entomofauna of forensic importance in Bénin and its succession pattern in relation to the decomposition stages of rabbit carrion and its dependence in numbers of factors temperature and humidity. The results of this study could be very useful for further forensic work in Cotonou.

2. Materials and methods
2.1 Site description
The Center of Entomological Research of Cotonou (CREC) is the framework where the present study was conducted. This center is located in the industrial zone of Cotonou (Figure 1). The climate is sub-equatorial and marked by two dry seasons (August- September and December-March), and two rainy seasons (April- July and October-November). The monthly average temperatures extend from 26 to 32°C. The annual mean rainfall is 1300 mm and the average annual temperature fluctuates between 19°C to 33°C (Suchel, 1987). The landscape of this part of Cotonou is characterized by the presence of *Elaeis guineensis* (Arecaceae) and *Anacardium occidentale* (Anacardiaceae).

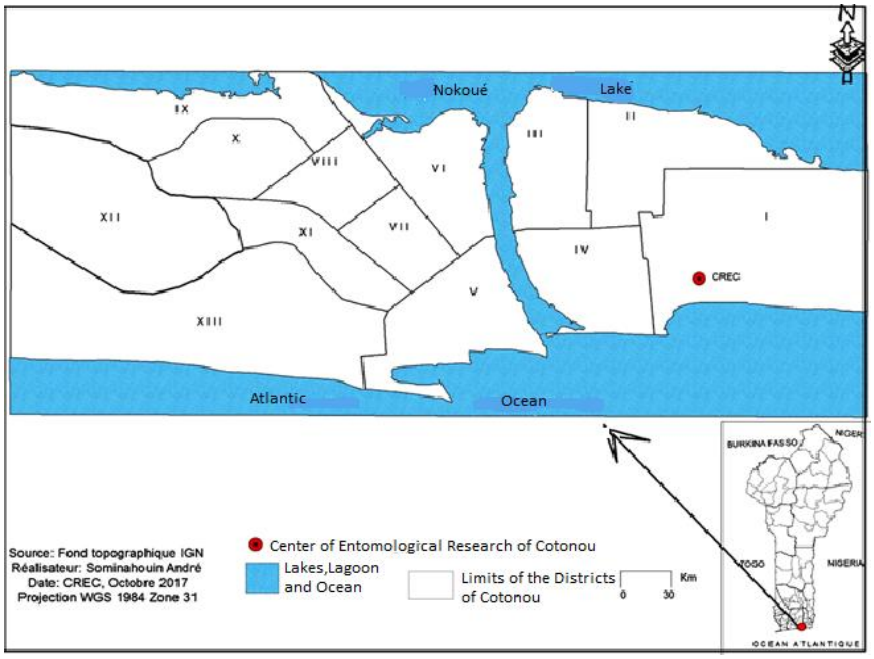

Fig 1: Map of study area.

2.2. Study animal, insect collection and identification

The present study received the approval of the National Ethics Committee in accordance with principles of laboratory animal care. Two rabbits were used to maximize the number of individuals caught. One of the rabbits weighed 2.75kg and the second 2.60Kg. Each of them was killed by brusque beatings carried on their heads with a stick. Then each one was placed in the fly trap type (Figure 2) and placed open 25m apart in the courtyard of the Entomological Research Center of Cotonou, which has maximum sunshine and an open environment facilitating access to flying insects. The trap and rabbit dead bodies were protected from the birds by a metal grid (Figure 2). A thermometer suspended inside the cage allows the daily ambient temperature to be measured. Sampling is carried out using a sampling bottle where the insects are mounted. The sampling was carried out twice a day at 9 am and 1 pm. Sampling began on 22 September 2015 and ends on December 19, 2015. Manual sampling was used to collect insects crawling on the carcasses and the surrounding soil. The collected insects were anesthetized with diethyl ether and stored in 70% ethanol. The captured arthropods were observed using a camera microscope and identified using dichotomous keys [5, 6, 7, 8].

Fig 2: Rabbit carrion trap for the collection of necrophagous arthropods.

2.3. Statistical analysis

The software R Core Team (Version 3.3.1 -2016) and PAST and GraphPad Prism were used for data processing. The chi-square test of multiple comparison of the proportions allowed to have the p-values; the generalized linear regression, followed by the Wald tests of significance of the coefficients and that of the ratio of likelihood made it possible to calculate the odds ratio and the p-value of the tests. The Simpson and Equitability index were calculated using the PAST software. The equitability index varies between 0 and 1, tends to 0 when almost all the numbers are concentrated on one species; it is 1 when all species have even abundance. Its formula is:

$$Eq = \frac{1}{Log2(S)} \sum_{i=1}^{S} \frac{n_i}{N} Log2\left(\frac{n_i}{N}\right)$$

S: number of species; ni : number of the population of the species i; N: sum of species numbers.

The Simpson index being the probability that two randomly selected individuals in the population are of the same species, then the diversity would be higher as the probability is closer to 0.

3. Results

3.1 Numerical importance of orders of necrophagous Arthropods collected on the rabbit carrion.

Figure 3 indicates that the Arthropod orders collected were Diptera, Coleoptera, Hymenoptera and Aranea. The Diptera order was significantly (P <0.001) the largest in number with 2267 collected. The other orders have numerical numbers 480; 17 and 5 (P <0.001) respectively for Hymenoptera, Coleoptera and Aranea.

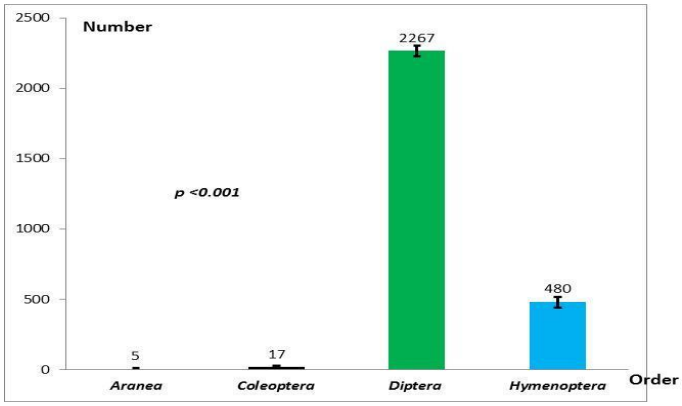

Fig 3: Numerical importance of orders of necrophagous arthropods collected on the rabbit carrion.

3.2 Evolution of numbers and species of Arthropods according to the stages of decomposition of the rabbit

Table 1 shows that among the captured Arthropods the Diptera order was represented by the Sarcophagidae, Chrysominae, Muscidae, Luciliinae, Lauxaniidae, Ephydridae, Phoridae and Piophilidae families; that of the Coleoptera by the families Dermestidae, Sylphidae, Trogidae, Histeridae and Scarrabeidae; that of the Hymenoptera by the family of the Formicidae and that of the Aranae by the families Pisauridae and Agelenidae. It was at the bloated stage that the largest number of arthropods was collected (1972; 71. 21%). The number of arthropod species increased from the fresh stage (5 species) to the decay stage (11 species). This number did not increase at the dry stage. At this dry stage, the Phoridae and *Piophila casei* present at the decay stage were not observed. However, the Lauxaniidae *Nicrophorus sp*. and Scarrabeidae absent at the stage of decomposition appeared at the dry stage. According to the value of the Simpson Index (Is) equal to 0.69 (Table 2), *Chrysomia sp.* was the most abundant species with a maximum of 990 individuals at the bloated stage (p <0.001). At the fresh stage, Formicidae were the most numerous (p <0.001). At the decay stage they were a high number of *Lucilia sp.* and *Musca domestica* (p <0.001). But at the dry stage, *Lucilia sp*. was more present with 13 individuals (p <0.001).

Table 1: Variation of the numbers and species of Arthropods according to the stages of decomposition of the rabbit.

Period Arthropods	Fresh 22-23 Sep (S=5)	Bloated 24-26 Sep (S=7)	Stage Decay 27 sep-01oct (S=11)	Dry 02oct-19dec (S=11)	Total	P-value
Pisauridae	0	0	2^{a}	2^{a}	4	-
Agelenidae	0	0	0	1^{a}	1	-
Chrysomia sp.	105^{a}	990^{a}	4^{a}	1^{a}	1100	< 0.001
Trogidae	0	5^{b}	0	0	5	-
Histeridae	0	0	2^{a}	0	2	-
Dermestes sp.	0	4^{b}	3^{a}	1^{a}	8	-
Discomyza incurva	0	0	2^{a}	1^{a}	3	-
Formicidae Ud1	475^{b}	0	0	0	475	-
Formicidae Ud 2	0	0	5^{a}	0	5	-
Lauxaniidae	0	0	0	2^{a}	2	-
Lucilia sp	70^{c}	850^{c}	29^{b}	13^{b}	962	< 0.001
Musca domestica	25^{d}	100^{d}	6^{a}	8^{ab}	139	< 0.001
Nicrophorus sp	0	0	0	1^{a}	1	-
Phoridae	0	3^{c}	5^{a}	0	8	-
Piophila casei	0	0	4^{a}	0	4	-
Sarcophaga haemorrhoidalis	25^{d}	20^{e}	2^{a}	2^{a}	49	< 0.001
Scarrabeidae	0	0	0	1^{a}	1	-
Total	700 (25.27%)	1972 (71.21%)	64 (2.31%)	33 (1.21%)	2769 (100%)	< 0.001

sep: september; oct: october; dec: december; S: number of species per stage; Ud : Undeterminated; p-value : p-value comparison of species abundance between stages; For a same parameter of the table, values which carry different letters in exposant were significantly differents ($p < 0.001$).

Table 2 : Values of diversity index.

Diversity index	value	IC-95%
Simpson's Index (I_S)	0.69	[0.68 – 0.70]
Equitability (E)	0.48	[0.46 – 0.49]

IC-95%: 95% confidence interval

3.2 Influence of temperature and relative humidity on the numbers of Arthropods

Table 3 shows that the odds ratio related to humidity and temperature are lower than 1. Wald test and Likelihood ratio test suggest a significant effect of relative humidity and temperature on the numbers of arthropods caught on the rabbit's carrion.

Table 3: Effects of temperature and relative humidity on the number of arthropods collected.

Average number of arthropods collected	Parameters	OR	IC-95% OR	p-waldtest	p-lrtest
54.29	Relative humidity	0.944	[0.94 - 0.95]	0	< 0.001
	Temperature	0.7	[0.75 – 0.79]	0	< 0.001

p-lrtest : p-value of likelihood ratio test ; OR : odds ratio ; IC-95%OR : 95% confidence interval of OR; p-waldtest : p-value of significance testing of the logistic regression coefficients.

4. Discussion

The present study provides basic information of the entomological fauna of forensic importance in Cotonou, southern Benin and their succession on the rabbit carrion according to the stages of decomposition and the link between their density, temperature and humidity factors. It revealed that Diptera, Coleoptera, Hymenoptera and Aranea are the Arthropods that invade the rabbit carrion. This study is necessary because no information on insects of forensic importance is not available in Benin, at a time when scavenger arthropods are helping to provide valuable information in the context of judicial investigations [9, 10, 11]. During our study, Diptera group insects were significantly ($P < 0.001$) high in number with a total of 2267 individuals collected. The numbers of other insect orders such as Hymenoptera, Coleoptera and Aranea were significantly different and were respectively 480; 17 and 5 for. These results confirmed those of other authors [2, 12], who collected only Diptera, Coleoptera and Hymenoptera on a rabbit carrion. However, they did not report the collection of Aranae. The presence of Diptera, Coleoptera, Hymenoptera and Aranae on the rabbit cadaver could be justified by the necrophagic nature of these arthropods. For the 4 orders recorded, Diptera insects were most represented and confirm the results of previous works [12, 13]. The invasion of the rabbit dead bodies by these necrophagous arthropods is justified by the emission of different odors related to the decomposition stages [14]. In the present study four stages of decomposition were observed: Fresh, Bloated, Decay and Dry [15, 16]. At the bloated stage we observed the largest number (ranged: 71-1972; 21%) of arthropods, but the number of arthropod species caught, increased from fresh stage (5 species) to bloated stage (7 species) then at the decay stage (11 species). These results were contrary to those of other authors [17] who found that the composition of insect species at the decay stage of a pig carcass was not different from those collected during the bloated stage. In addition, these authors revealed the presence of the families of Ulidiidae, Stratiomyiidae, Dermestidae, Cléridae, Muscidae, Sarcophagidae, Formicidae, Typhidae, Coreidae, Gryllidae, Pygomorphidae and Mantidae during the dry stage. In contrast, the present study reported during the dry stage the families of Pisauridae Agelenidae, Chrysominae, Dermestidea, Ephydridae, Lauxaniidae, Lucilidea, Muscidae, Sylphidae, Sarcophagidae and Scarrabeidae. Phoridae and *Piophila casei* present at the decay stage were not observed at the dry stage. But, Lauxaniidae, *Nicrophorus sp* and Scarrabeidae absent at the decay stage appeared at the dry stage. These various findings and the largest number of formicidae at fresh stage ($p < 0.001$), *Chrysomia sp*. at bloated stage ($p < 0.001$), *Lucilia sp*. and *Musca domestica* at the decay stage ($p < 0.001$) question Smith's idea of the eight squads proposed in his 1986 monograph [18] and confirm that the succession of insects is highly variable and that decomposition is strongly linked to the characteristics of the environment and the body [18]. Our results also revealed that Formicidae, *Chrysomia sp*., *Lucilia sp*., *Musca domestica*, and *Sarcophaga haemorrhoidalis* are the first insects to colonize rabbit carrion. These results are consistent with previous work [11, 20] and reveal Calliphoridae and Muscidae as potential arthropods for *estimations* of *Postmortem Intervals* (*PMI*) and determination of clues during criminal investigations in Cotonou. However, our results are contrary to those of Mabika *et al*. (2014) [2] who reported that Sarcophagids (*Sarcophaga sp*.) were observed as secondary colonizers arriving during the decomposition of the rabbit dead bodies. The significant effects of relative humidity and temperature on the numbers of arthropods collected on the rabbit dead bodies during the study may be justified by the dependence of the decomposition stages of the climate. This confirms previous studies [20] that report that higher air temperature and lower relative humidity leads to chemical reactions, leading to a faster decomposition of the corpse and greater attraction of insects and other Arthropods. The information provided by this study was limited to the morphological characters of arthropods and environmental factors in Cotonou city. The techniques of molecular biology and aspects of insect metamorphosis could be taken into account by future studies to enrich the present results.

5. Conclusion

According to the present study, the Diptera, Coleoptera, Hymenoptera and Aranea are the orders of arthropods that invade the rabbit carrion in Cotonou. Environmental factors such as relative humidity and temperature have had statistically significant effects on the numbers of arthropods collected. *Chrysomia sp*. was the most abundant species found. Further work on the molecular identification of this species, the biology and population dynamics of Diptera Calliphoridea during seasons in other regions in Benin can be performed for use in criminal investigations. Because the duration of metamorphosis in insects can vary from one region to another, and from one season to another. The present study should therefore be completed by further work to ensure that forensic entomology is at the service of justice in Benin.

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7. References

1. Gurafi LMA, Mohamed EAER. Decomposition and arthropod Succession on two monkeys (cercopithecus aethiopicus). S.J.S. 2012; 5:1-7.
2. Nyasha M, Ron M, Gilbert M. An initial study of insect succession on decomposing rabbit carrions in Harare, Zimbabwe. Asian Pac. J. Trop. Biomed. 2014; 4(7):561-565.
3. Voss SC, Cook DF, Dadour IR. Decomposition and insect succession of clothed and unclothed carcasses in Western Australia Sahsa. Forensic Sci. Int. 2011; 211(1-3):67-75.
4. Shi YW, Liu XS, Wang HY, Zhang RJ. Seasonality of insect succession on exposed rabbit carrion in Guangzhou, China. Insect Science. 2009; 16:425-439.
5. *Roth M. Initiation* à la *morphologie* la *systématique* et la biologie des insectes. ORSTOM, Initiations-Documentations Techniques Nº 23, Paris. 1980; 259.
6. Delvare G, Aberlenc HP. Les insectes d'Afrique et d'Amérique tropicale. Clés pour la reconnaissance des familles. CIRAD-GERDAT, Montpellier. 1989; 302.
7. *Rickenbach* A. *Sarcophagidae du Cameroun* (Dipt. Cyclorrhapha). Bull. Soc. Entomol. Fr. 1973; 78(34):150-159.
8. Marshall SA, Whitworth T, Roscoe L. *Blow flies* (*Diptera*; *Calliphoridae*) of *eastern Canada* with a *key* to *Calliphoridae subfamilies* and *genera* of *eastern North America*, and a *key* to the *eastern Canadian species* of *Calliphorinae*, *Lucilinae and Chrysominae*. Can. J. Arthropod Identif. 2011; 11:1-93.
9. Byrd JH, Castner JL. *Forensic Entomology*: The Utility of Arthropods in Legal Investigations. Edn 2, CRC Press, Boca Raton. 2010 ; 23:681.
10. Sanford MR. Insects and associated arthropods analyzed during medicolegal death investigations in Harris County, Texas, USA: January 2013- April 2016. PLoS One. 2017; 12(6).
11. Abajue MC, Ewuim SC, Akunne CE. Preliminary checklist of flies associated with pig carrions decomposition in Okija, Anambra State, Nigeria. Anim Res Int. 2014; 11(1):1899-1904.
12. Zeariya MGM, Hammad KM, Kabadaia MM. Frequency of forensic insects on dog and rabbit carcasses in different habitats: use of developmental data of *Chrysomya albicep*s in determining the postmortem interval. Int. J. Entomol. Res. 2017; 2(4):04-08.
13. Ewuim SC, Abajue MC. Forensic Entomology in Nigeria: The Journey So Far. Open Science Journal of Bioscience and Bioengineering. 2016; 3(1):1-4.
14. Megnin JP. La faune des cadavres: application de l'entomologie à la médecine légale. Gauthier-Villars et fils. 1894; 210.
15. Reed HB. A study of dog carcass communities in Tennessee, with special reference to the insects. Am. Midland Nat. 1958; 59:213-245.
16. Tantawi TI, El-Kady EM, Greenberg B, El-Ghaffar HA. Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. J. Med. Entomol. 1996; 33:566-580.
17. Abajue MC, Ewuim SC, Akunne CE. Preliminary checklist of flies associated with pig carrions decomposition in Okija Anambra State, Nigeria. Anim. Res. Int. 2014; 11(1):1899-1904.
18. Smith, KGV. A manual of forensic entomology. Trustees of the British Museum (Natural history), London. 1986; 205.
19. Wells JD, LaMotte LR. Estimating the postmortem interval. In: Byrd JH, Castner JL. Editors. Forensic Entomology. The Utility of Arthropods in Legal Investigations. CRC Press; Boca Raton, FL, USA, 2001; 263-285.
20. Carvallo LML, Linhares AX. Seasonality of insect succession and pig carcass decomposition in a natural forest area in south eastern Brazil. J. Forensic Sci. 2001; 46:604-608.
21. Ekanem MS, Dike MC. Arthropod succession on pig carcasses in South Eastern Nigeria. Pap Avulsos Zool (Sao Paulo) 2010; 50(35):561-570.