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First records on five species of Calliphoridae (Diptera) reared from maggot collected on rat carrions corpse during a forensic entomology experiment in the campus of the University of Yaounde I-Cameroon

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Abstract

Carcasses of laboratory-bred red rats (Rattus *rattus*, Berkenhout, 1769 var Wistar) were exposed in wooden cages on the campus of the University of Yaounde I (Cameroon) to take a census and identify insects of forensic importance. Amongst the 1613 insects obtained from the emergence of maggots reared in the laboratory under ambient air temperature, the family Calliphoridae (Diptera) represents 72% (1161). The species were distributed between *Chrysomya putoria* (Wiedemann, 1830), *Chrysomya laxifrons* (Villeneuve, 1814), *Chrysomya albiceps* (Wiedemann, 1819), *Hemipyrellia fernandica* (Macquart, 1855) and *Hemipyrellia* sp.

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Introduction

The use of insects to search for crime evidence has become useful procedure all over the world: Europe (Smith, 1986) North America; (Catts and Goff, 1992; Anderson and Van Laerhoven, 1996); Asia (Sukontason et al., 2003); South America (Carvalho et al., 2004) and recently in Africa (Williams and Villet, 2006; Richards, 2007; Richards et al., 2009). This discipline, called forensic entomology, can be defined as the application of the information obtained from the study of insect behaviour in solving crimes.

Kurahashi and Kirk-Spriggs (2006) wrote a general key for the identification of the Calliphoridae of Namibia. Recently some researchers have undertaken research work on forensic insects in Nigeria (Okiwelu et al., 2008; Ekanem and Dike, 2010) and in South Africa (Williams and Villet, op. cit.), but none of them produced a practical identification key for forensic purposes. Although the Afrotropical fauna includes some of the most important species of Diptera in terms of medical, veterinary, and forensic importance (Pont, 1980), no specific key for the identification of carrion-feeding insects is available for the afrotropical region. This paper is therefore the first key for the identification of Calliphoridae flies emerged from maggot in the laboratory during a forensic experiment in Cameroon.

Materials and methods

In order to initiate a medico-legal entomology database in Cameroon, the experiment was carried out on rat carrions within the Campus of The University of Yaounde I. Sixteen carcasses of laboratory-bred rats (*Rattus norvegicus*, Berkenhout, 1769 var Wistar) were exposed inside four wooden cages (100x100x100cm) (figure 1) on the campus, inside a small bush made up of mainly palm tree. Each cage had 5x5 cm mesh (Figure 1) to allow entrance to the insects. The cages were separated from each other by 50 meters. Rats were labelled, weighed, brought into the bush, killed and immediately placed inside the cages. Four carcasses were exposed inside each cage. As done by Carvalho et al. (2004), two of these were placed on top of a lattice-work deposited on a 10cm layer of sterilized soil and the two others on the ground. Adding to the protocol of this author, we used sterilized soil that was put inside transparent plastic boxes (18x18x28cm); the sampling of the insects was performed three times a day during the first post mortem week, and once a day until the remaining carcasses consisted of only bones.

(a) (b)

Fig. 1. Empty wooden cages (100x100x100cm) (a) and with rats corpse (b) used during our experiment.

After the maggot migration into the underlying soil, the lattice-work was removed with rat carrion on top and placed into a different plastic box containing 10 cm of sterilized soil. The boxes containing the migrated maggots were taken to the laboratory for rearing purpose. In the laboratory, the rearing box was covered with 1x1mm mesh cloths (figure 2), tied with elastic rope and placed for rearing on shelves in the laboratory under ambient temperature conditions. Every day, each box was checked for any adult insect emergence.

(a) (b)

Fig. 2. Boxes containing maggot uncovered (a) and covered (b) with a 1mm mesh cloth for laboratory rearing.

After the emergence, the insects were fed with honey put on cotton and placed on top of the covering clothes. After a 48-hour feeding period, the strong insects were sprayed with 70% alcohol. After 10 minutes, they were caught and kept in 70% ethanol for future identification.

The identification up to the family level was partially performed in the zoology laboratory of the University of Yaoundé I, using the identification keys of Smith (1986) and Delvare and Alberlenc (1989). For further identification, the samples were taken to the Royal Museum of Central Africa based in Tervuren Belgium, which offered us a grant for an identification training course. The identifications were carried out according to the identification keys of Mc Alpine et al. (1981) and Kurahashi and Kirk-Spriggs (2006). The terminology used in this paper is based on that of McAlpine et al. (1981).

Results

During this study, the arthropod fauna constituted of about 1600 individuals amongst which 1161 specimens were Calliphoridae. For this paper, only the specimens of Calliphoridae were examined since their life cycle is completely undertaken on or around the carcasses. They are also the most study forensic insect all over the world . The detailed results are given in Table 1. Adults of this family were amongst the initial colonizers of the corpse, arriving shortly after exposure of the carrion, and laying their eggs into the natural orifices. Table 1 summarizes the number of Calliphoridae collected within the study site during the experiment. The specimens were classified by subfamily and genera and were then identified to the species level.

The individuals of the Calliphoridae are Diptera characterized by:

- wings well-developed, longer than thorax with more than one longitudinal vein and cross vein;
- flagellomere with not more than six articles, the last one longer than the others and with a long setae called arista;
- second segment of the antenna with longitudinal cleft on dorsal face;
- frontal view of the head fitted with frontal scar;
- vein Cu2 long and not joining A1 before wing margin;
- hind and mid coxae close together medially, body not flattened;
- mouthparts well-developed, greater ampulla present and situated below wing base, lower and upper calypters well-developed;
- meron bare or fit with a row of erected bristles, sometimes with scattered fine hairs in addition to bristles;
- meron with row of strong bristles near its hind margin or sometimes with additional whitish hairs;
- subscutellum poorly developed or absent;
- abdo

en and thorax usually green or blue metallic lustrous, palpus yellowish to orange-like.

Table 1. Subfamily, species and number of specimens of Calliphoridae examined during the redaction of this identification key.

Subfamily and species	Number of specimens
Chrysomyinae	
Chrysomya putoria (Wiedemann,1830)	467
Chrysomya laxifrons (Villeneuve, 1814)	81
Chrysomya albiceps (Wiedemann, 1819)	22
Luciliinae	
Hemipyrellia fernandica (Macquart, 1855)	573
Hemipyrellia sp.	18

According to Carvalho and Mello-Patiu (2008), the identification of the carrion flies faces two majors problems: the lack of taxonomists and the lack of keys, that is the reason why we propose a key for the identification of four species of Calliphoridae (Diptera) reared from maggots collected on rat carrion during a forensic entomology experiment on the campus of the

University of Yaounde I,

Key for the identification of the subfamilies and species of Calliphoridae recorded within the Campus of the University of Yaounde I, (Central Africa region) and found on rats carrion (based on the key of Kurahashi and Kirk-Spriggs, 2006).

0 – Diptera found on a cadaver → 1

1 - Dorsal view of the basal section of the stem vein with distinct setulae → Chrysomyiinae

1' - Dorsal view of the basal section of the stem vein without distinct setulae; **→ 2**

2 - Small well-defined lateral black sclerite with the presence of posterior parasquamal tuft of setulose. → Luciliinae

2' - Small well-defined lateral black sclerite without posterior parasquamal tuft of erect hairs, lower calypter more or less covered with black hairs on its upper surface → Calliphorinae

Although we did not obtain Calliphorinae from the rearing of the maggot during our experiment, Kurahashi and Kirk-Spriggs (2006) point out the presence of Calliphorinae in Cameroon.

Subfamily Luciliinae

The genus *Hemipyrellia* Townsend, 1918 has only been recorded with one species formely identified as *H. fernandica* (Macquart, 1855). Among the Calliphoridae, this genus is characterized by the presence of several long, upstanding and erect hairs on katatergite. Among the African species of this genus, *H fernandica* could be identified by the antenna basally fuscous and separated by more than the width of ocellar triangle. A second species belonging to the genus *Hemipyrellia* was collected but did not fit to any species in our keys.

Subfamily Chrysomyiinae

All of our collected species shared the presence of white to yellow anterior spiracles. Species with dark-colored anterior spiracles can not be determined with this key. Our collected species can be separated as follows:

1 - Face, 1st article of antenna blackish except for the suture area with pedicel which is reddish; anterior part of fronto-orbital plate blackish; wing hyaline (transparent) and sometimes more or less infuscated on its base; colors of lower calypter variable **→ 2**

1' - Face, 1st article of antenna bright yellowish to orange-like; fronto-orbital plate broad, reddish brown anteriorly and darkened posterior half to two/third in both sexes; wing widely infuscate on its anterior margin; lower calypter largely pale brown and infuscated narrowly along its posterior margin → *Chrysomya laxifrons* (Villeneuve, 1814).

2 – Presence proepimeral bristles; gena entirely blackish outer vertical bristle on head absent in male; → *Chrysomya putoria* (Wiedemann, 1830)

2' - Proepimeral bristles absent; anterior half or more of gena reddish; outer vertical bristle on head well developed; → *Chrysomya albiceps* (Wiedemann, 1819)

The line 2 can also lead to *Chrysomya chloropyga*. Although this species has been signaled in Cameroon (Kurahashi and Kirk-Spriggs, 2006), we did never observed it during the emergence of adults flies from maggot rearing during our experiment. The differences with C. *putoria* are as follow:

-Presence of L-shaped pattern on each side of the presutural area of scutum, presutural dorso-central setae weak and inconspicuous, abdomen with last two segments shiny brassy green and contrasting strongly with bluish preceding segments → *Chrysomya chloropyga* (Wiedemann, 1818).

Conclusion

In agreement with the results of the work done by (Velasquez, 2008), amongst the insects collected, the family Calliphoridae was the most abundant. The emergence of the adult flies reared in the laboratory showed that the species *Hemipyrellia fernandica* and *Chrysomya putoria* are the most abundant. They may

be the most important for use in forensic science in the study area. Further study will be done in order to identify all the insects reared from the maggots and to produce the list of the Diptera of forensic importance in Cameroon.

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