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THE IMMATURE STAGES OF THREE CARRION BREEDING BLOWFLIES (DIPTERA: CALLIPHORIDAE) IN SOUTH EASTERN NIGERIA

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Abstract

Three calliphorid flies, *Chrysomya chloropyga* (Wiedemann), *C. albiceps* (Wiedemann), and *Hemipyrellia fernandica* (Macquart) which breed in animal carcasses in Uyo, Akwa Ibom State, Nigeria, were reared in the laboratory on beef. Each fly was reared through three generations. Their developmental rates were recorded as well as the morphological descriptions of each immature stage. *C. chloropyga* and *H. fernandica* had been studied before, while *C. albiceps* was studied for the first time in this area. Mean developmental periods of *C. chloropyga, H. fernandica,* and *C. albiceps* from eggs to adults were 8.1 ± 0.8 day, 15.1 ± 0.9 day, and 9.0 ± 0.3 day, respectively at 26° C – 29°C. Keys to eggs and larval instars are provided.

Key words: Calliphorids, eggs, larvae, pupae

Introduction

In Uyo and other towns in Akwa Ibom, South eastern Nigeria, the commonest non-biting flies are the calliphorid flies. Fly surveys of Akwa Ibom State by Ekanem (1994), and Idiong (2007) reported *C. chloropyga*, *C. albiceps* and *C. chloropyga*, respectively as the most abundant non-biting flies of the area. They are common sights in market stalls, abattoirs, refuse heaps, and even out of doors. These saprophagous flies feed and breed on organic wastes, and they are the principal invertebrate consumers of terrestrial carrion (Braack, 1986; and Putman, 1987). Calliphorid flies are termed 'Primary flies' (Payne, 1965) of carrion because they are among the first flies to be attracted to animal carcasses.

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According to Hall (1948) some calliphorids are extremely vagile and are able to locate a carcass and oviposit on it within a few hours of death. Their immediate response to the presence of carrion and subsequent breeding within it are of forensic importance, because, immature stages of insect picked up at a homicide scene may be used to estimate the post mortem interval (PMI) of the body.

Meanwhile, crucial to making accurate PMI estimates is the knowledge of the breeding biology and identities of the immature insects taken from the crime scene. Information such as these are lacking in this area, and this study was carried out to ascertain the identities of the immature calliphorid flies of carrion in this area, give information on their developmental rates, as well as their morphological descriptions.

Materials and methods

Three different groups of third instar larvae suspected to be calliphorids were collected from beef carcasses in Uyo, (5° 3′N, 7° 57′E), South eastern, Nigeria. Each group of larvae was reared on beef in a 250 ml rearing cup. The cup was covered with a cotton cloth, secured with a rubber band, and kept in a 4 litre bucket with floor covered with sterilized sand (4cm deep). The bucket was also covered with cotton cloth and secured with rubber band. At pupal stage, each group was transferred into a netted cage (45 litre capacity). Floors of cages were also covered with sterilized fine sand, to a depth of 4cm. Water, solution of glucose (Allenbury®) and powdered milk were placed in the cages for the adults (Greenberg and George, 1985; and Laurence 1988). Fresh fruits (bananas and pineapples) were also placed in the cages as additional sources of energy for the flies. Liquids were presented in 50 ml bottles with wicks. Soiled wicks and the milk were replaced daily. Pieces of beef in petridishes were placed in the cages as additional sources of protein, as well as oviposition medium. The beef (bait) was examined at hourly intervals for presence of egg batches. The adults of these three (wild) populations served as the parents of the first generation. Whenever an egg batch was observed on the oviposition medium, the time was noted. Each egg batch was placed on another/piece of beef in a 250 ml rearing cup and reared as described above. The cup contents (eggs, larvae, prepupae) were observed at two-hourly intervals under a dissecting microscope (Philip Harris) to note the time of egg hatching, the conditions of the anterior and posterior spiracles of the larvae, and presence of any moult casts. Records were taken of time intervals between stages of larval instar. At third instar, the covering over the breeding cups were removed to permit the subsequent prepupae free movement out of the wet rearing medium to other drier areas outside the cup. Prepupae often were found burrowed in the sand underneath the cup. Pupae were transferred to netted cages as previously described, where they latter emerged as adults. Adults were maintained as previously described. Their eggs were removed and reared as another generation. Three generations of each initial group of larvae (wild population) were reared. There were 30 recorded replicates of each developmental stage of each fly. Adult specimens from each group of flies were sent to the Insect Museum, Department of Crop Protection, Amadu Bello University, Zaria for identification. No artificial lighting was used throughout the rearing. Temperatures within the cages were taken with a thermometer and ranged from 27° – 31°C.

Samples of eggs, larval instars of all generations were cleared in boiling 10% KOH, and their cephalopharyngeal skeletons anterior and posterior spiracles studied and described under a light microscope (Olympus). Following of methods of Greenberg and Szyska (1984) samples of eggs, the first and second instars, and pupae were preserved in 70% ethanol/glycerine solution (9:1), and the third instars were injected posterodorsally with 80% ethanol/glycerine/glacial acetic solution (8:1:1) to prevent decomposition (Greenberg and Szyska, 1984). A dichotomous key for the eggs and larval stages was

constructed from these studies. Details of the cuticular features of the pupae could not be made with the light microscope. Samples of specimens studied are kept in the collections of the Department of Zoology, University of Uyo, Uyo.

Results

The three flies, *Chrysomya chloropyga, C. albiceps Hemipyrellia fernandica* laid eggs in batches. The eggs are creamy white in colour. During hatching the whole egg batch appeared to melt and the creamy white first instar larvae emerged. They immediately chew into the meat and feed within or underneath the meat. Just before moulting, each larva remained motionless for a few seconds and crawled out of its moult cast. The second instar also fed actively within the carcass as the first instar, and also in a similar manner molted into the third instar. The third instar continued to feed within the carcass. After one day, the third instar became sluggish and eventually stopped feeding. The breeding medium became fluid at this time because of regurgitations of the larvae. This marked the onset of the prepupal stage. The pre-pupae began to migrate from the wet breeding medium inside the cup to outside of the cup. They burrowed in the sand beneath any objects placed on the sand. As the prepupa settled in its borrow its creamy colour changed to golden, and brown, and it became a pupa. There is no moult cast as the prepupa becomes a pupa, and the larval skin forms the pupal cuticle.

Descriptions of pre-adult stages

Chrysomya chloropyga (Wiedemann)

Egg: Creamy white in colour; mean length 1.5 ± 0.02mm (n=15) (Fig. 1); median area bifurcates at the micropylar collar forming a 'Y' shape.

First instar: Spine pattern as in third instar (Fig. 2); lateral fusiform areas present on segments 5–9, Cephalopharyngeal skeleton as in Fig. 3; dorsal and ventral cornu weakly sclerotized.

Second instar: Spine pattern similar to third instar (Fig. 2); lateral fusiform areas present on segments 5 or 6-9, Cephalo-pharyngeal skeleton as in Fig. 4; ventral cornu with window.

Third instar: Spine pattern as in Fig. 2; all segments completely encircled with spine bands; spine bands on segments 2-6 both anterior and posterior; spine bands on segments 7-11 only anterior, and single rows; spines pigmented; lateral fusiform areas on segments 5 or 6-9. Cephalopharyngeal skeleton as in Fig. 5. Anterior spiracles (Fig. 6) with 10±0.3 branches (n=16). Posterior spiracles (Fig. 7) heavily pigmented with incomplete peritremes; mean spiracular width 0.35 ±0.03mm (n=20); mean spiracular separation 0.21±0.02mm (n=10). Distances between inner tubercles on the upper stigmal field (Fig. 8) approximately equal to distance between the inner and median tubercles, and between the median and outer tubercles; all tubercles with spinose bases and sclerotized tips.

Puparium: Cuticular features (Fig. 9) same as third instar

***Chrysomya albiceps* (Wiedemann)**

Egg: Creamy white in colour; mean length 1.5±0.02m, (n=10) (Fig. 19), median area bifurcates as in C. chloropyga, but the 'Y' arms much longer.

First instar: Spine pattern as in third instar (Fig.20); four pairs of dorsal and lateral tubercles on segments 5-11; tips of tubercles not pigmented. Cephalo-pharyngeal skeleton as in Fig. 21.

Second instar: Spine pattern same as third instar (Fig. 20); segmental tubercles same as first instar; tips of tubercles pigmented. Cephalopharyngeal skeleton as in Fig. 22; dorsal cornu longer than ventral.

Third instar: Anterior and posterior spine bands on segments 2-11 (Fig. 20); all segments and posterior spiracular field spinose (hairy) with single or grouped spines; spine distribution more dense dorsally than laterad; spines pigmented; segments 3 and 4 each with 3 pairs of tubercles or dorsum and laterad; segments 5-11 each with 4 pairs of dorsal and lateral tubercles as well as coupled ventrad tubercles; all tubercles with pigmented tips. Cephalopharyngeal skeleton as in Fig. 23. anterior spiracle (Fig. 24) with 10±0.02 branches (n=12). Posterior spiracles (Fig. 25) heavily pigmented with incomplete peritremes; mean spiracular separation 0.23 ± 0.01mm (n=8); mean spiracular width 0.36 ± 0.02mm (n=16). Tubercles on posterior spiracular field long with pigmented spinose tips (Fig. 26); distance between inner tubercles on the upper stigmal field approximately equal to distance between inner and median tubercles, but slightly greater then between the median and outer tubercles.

Puparium: Cuticular features of puparium (Fig. 27) same as third instars

***Hemipyrellia fernandica* (Macquart)**

Egg: Creamy white in colour; mean length 1.6 ± 0.02mm (n=15); median area bifurcates widely towards the micropyle so that the 'Y' arms are long (Fig. 10).

First instar: Spine pattern same as in third instar (Fig. 11). Cephalopharyngeal skeleton as in Fig. 12.

Second instar: Spine pattern same as third instar (Fig. 11). Cephalopharyngeal skeleton as in Fig 1.

Third instar: Anterior and posterior spine bands on segments 2-7,(Fig. 11); double to several rows of ventrad spines with few spines scattered on dorsum and laterad of segments 8-10; spinose bands posterior of segment 11; spines pigmented. Cephalo-pharyngeal skeleton as in Fig. 14. Anterior spiracles (Fig. 15) with 8.9±0.9 branches (n=20). Posterior spiracles (Fig. 16) surrounded with lightly pigmented, complete peritremes with buttons; mean spiracular width 0.27±0.03mm (n=20); mean spiracular separation 0.17±0.01mm (n=10). Distance between the inner dorsal tubercles on the upper stigmal field (Fig. 17) greater than distance between the inner dorsal tubercles and the median dorsal tubercles; distance between the median

dorsal tubercles and inner dorsal tubercles approximately equal to distance between median dorsal and outer dorsal tubercles.

Puparium: Cuticular features of puparium (Fig.18) same as in third instar.

Bionomics

Chrysomya chloropyga

The fly *C. chloropyga* has been described as a carcass breeder and a secondary myasis producer in man and animals by Greenberg and Syzska (1984). It has also been described as a primary carrion fly of Akwa Ibom area of the south easthern Nigeria, by Ekanem (2000) and Ekanem and Usua (2005). Its SI (Synanthropic Index) computed and categorized after Nuorteva (1963) classified the fly to be positively synanthropic in both dry and wet seasons with a preference for hemisynanthropic environments (Ekanem, 1994).

This fly had been reported as the most abundant non-biting cyclorrhaphous fly in Akwa Ibom, area of south eastern Nigeria by Ekanem (1994) for both dry(30.1%, n = 5604) and wet(39.2%, n = 9216) seasons. Idiong (2007) also recorded *C. chloropyga* as the second most abundant non-biting fly after *C. albiceps*

Chrysomya albiceps

Many authors have described C. albiceps as a carcass breeder, and a predator on other fly larvae (Hanski, 1977; Guimaraes *et al*., 1979; Baumgartner and Greenberg, 1984; and Wells and Greenberg, 1992). *C. albiceps* was implicated in the elimination of some native species in Madeira and the Canary Islands (Hanski, 1977); Brazil (Guimaraes *et al*., 1979) and Peru (Baumgartner and Greenberg, 1984).

A fly survey by Idiong (2007) reported of *C. albiceps* as the most abundant non-biting fly of the Akwa Ibom area, in dry (63.5%; n = 1745) and wet (39.8%; n = 2929) seasons. Its SI computed and categorized after Nuorteva (1963) classified it as hemisynanthropic with positive heliophily (Idiong 2007)

Hemipyrellia fernandica

Records of previous fly surveys in the Akwa Ibom area (Ekanem, 1994 and Idiong 2007) show that *H. fernandica* as compared with *C. chloropyga* and *C. albiceps* is not abundant in the Akwa Ibom area. The fly is a carcass breeder, and readily oviposits on any exposed fresh carcass. It is thus, a primary carcass fly of the Akwa Ibom area. In these surveys, this fly was noted to show aversion to faeces.

Fig. 1

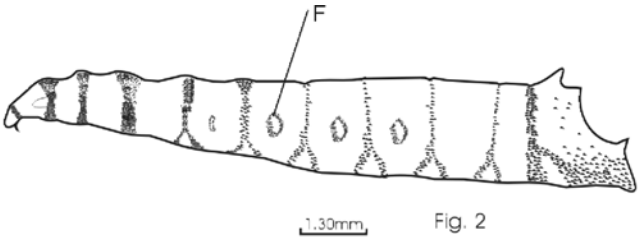


Fig. 2

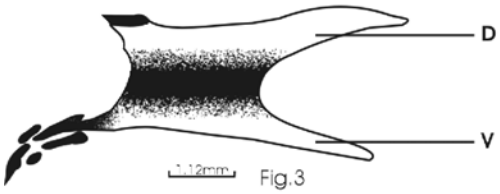


Fig.3

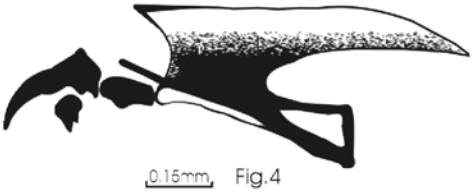


Fig.4

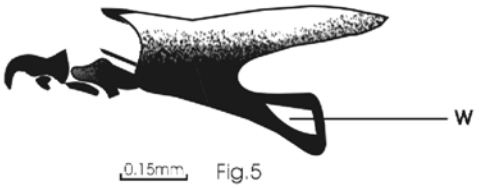


Fig.5

Fig.6

Fig.7

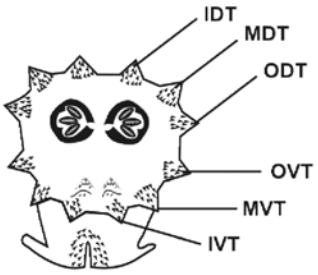


Fig.8

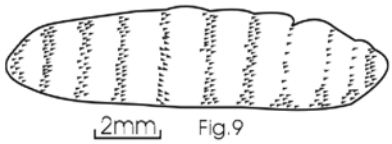
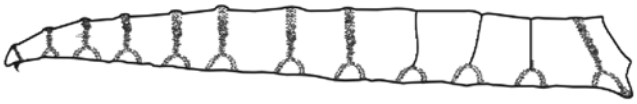


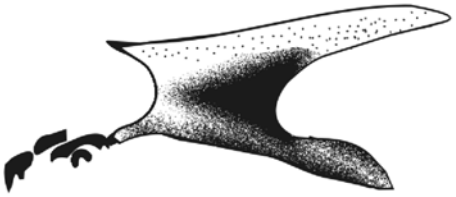
Fig.9

Figs.1-9 *Chrysomya chloropyga*., Egg (Fig.1);Third instar larva (Fig.2) Cephalopharyngeal skeletons of larval instars, first instar (Fig.3), second instar (Fig.4) third instar (Fig.5); anterior spiracle (Fig.6); right posterior spiracle (Fig.7); posterior surface (Fig.8); puparium (Fig.9); IDT (Inner dorsal tubercle), MDT (median dorsal tubercle), ODT (Outer dorsal tubercle), OVT (Outer ventral tubercle), MVT (median ventral tubercle), IVT (Inner ventral tubercle). F-fusiform area; P=peritreme D-Dorsal cornu; V- Ventral Cornu; W- Window (From Ekanem and Usua, 2000)

0.55mm Fig 10



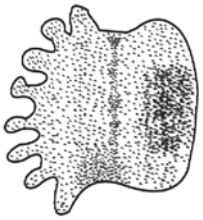
0.86mm Fig 11



0.07mm Fig 12

0.07mm Fig 13

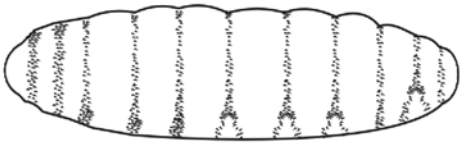
Fig 14 0.07mm



0.08mm Fig 15

0.09mm Fig 16

0.22mm Fig 17



1.00mm Fig 18

Fig.10-18 *Hemipyrellia fernandica;* Egg (Fig.10) Third instar larva (Fig.11) Cephalopharyngeal skeletons of larval instars, first instar (Fig.12), second instar (Fig.13), third instar (Fig.14); anterior spiracle (Fig.15); left posterior spiracle (Fig16) posterior surface (Fig.17); puparium (Fig.18); B=button; W=window (From Ekanem and Usua, 2000)

Discussion

Developmental rates of the preadult stages of *C. chloropyga, C. albiceps,* and *H. fernandica* show that *C. chloropyga* exhibited the shortest rate of development (8.1±0.8 d) from egg to adult, while *H. fernandica* exhibited the longest rate (15.1±0.9 d). There were however, no significant differences between the developmental rates.

Higher temperatures not only influence abundance of species of C*alliphorids* (Horenstein *et al.*, 2007) but also influence developmental rates of the immature stages (Vélez and Wolff, 2008). Development of *C. albiceps* from egg to adult was completed in 9.0±0.3 days at 27^{o} – 31^{o}C in the study area, while at 25^{o}C in Colombia, development was completed in 13-14 days (Vélez and Wolff, 2008).

Key to eggs

1. Median area bifurcates close to micropyle ……...…2
   Median area wide; arms of 'Y' end above level of micropyle ……*.H. fernandica.*
2. 'Y' arms long ……... *C. albiceps*
   'Y' arms short ……...…. *C. chloropyga*

Key to larval instars

1. Anterior spiracles absent … First instar
   Anterior spiracles present ……..….2
2. Posterior spiracles with two slits ……Second instar
   Posterior spiracles with three slits ……..Third instar

Key to first instar larvae

1. Segments 5-11 with tubercles …… *C. albiceps*
   Segments without tubercles …….. 2
2. Segments 5-9 with lateral fusiform areas ……...……*C. chloropyga*
   Segments without lateral fusiform areas ……..…..…….. *H. fernandica*
3. Cephalopharyngeal skeleton completely sclerotized ……..…… *C. albiceps*
   Cephalopharyngeal skeleton partially scleratized ……..…….. 4
4. Dorsal cornu unsclerotized; ventral cornu partially sclerotized ……..... *H. fernandica*
   Dorsal and ventral cornu unsclerotized …….. *C. chloropyga*

Key to second instar larvae

1. Segments 5-11 with tubercles ……...……. *C. albiceps*
   Segments without tubercles ….…… 2
2. Segments 5 or 6-7 with lateral fusiform areas ……...….*C. chloropyga*
   Segments without lateral fusiform areas …….*H. fernandica*
3. Ventral cornu bifurcated ……..…..... *C. albiceps*
   Ventral cornu with window …… 4
4. Dorsal cornu partially sclerotized ……..…… *H. fernandica*
   Dorsal cornu sclerotized; tip of dorsal arch sclerotized ……...*C. chloropyga*

Key to third instar larvae

1. Segments 5-11 with tubercles ……*C. albiceps*
   Segments without tubercles ……2
2. Segments 6-9 with lateral fusiform areas …….. ..*C. chloropyga*
   Segments without lateral fusiform areas ……..…… *H. fernandica*
3. Ventral cornu with window ……4
   Ventral cornu bifurcated…...*C. albiceps]*

4. Dorsal cornu unsclerotized …….. *C. chloropyga*
   Dorsal cornu partially sclerotized ……..*H. fernandica*
5. Peritreme lightly pigmented and with a button ……..*H. fernandica*
   Peritreme heavily pigmented and incomplete……..6
6. Posterior tubercles with sclerotized tips ……*C. albiceps*
   Posterior tubercles without sclerotized tips ……..…..*C. chloropyga*

Table 1. Developmental rates of preadult stages of *C. chloropyga, C. albiceps* and *H. fernandica*

Developmental Stage	*C. chloropyga*	*C. albiceps*	*H. fernandica*
Egg	12.4 ± 0.8h	14.9 ± 0.4h	10.3 ± 0.3h
1st Instar	13.2 ± 0.6h	16.2 ± 0.5h	11.6 ± 0.4h
2nd Instar	14.6 ± 0.4h	18.0 ± 0.5h	13.4 ± 0.4h
3rd Instar	1.3 + 0.1d	1.7 ± 0.3d	2.1 ± 0.2d
Prepupa	1.3 + 0.1d	1.1 ± 0.2d	2.1 ± 0.1d
Pupa	3.9 ± 0.4d	4.2 ± 0.3d	9.4 ± 0.6d
Egg-Adult	8.1 ± 0.8d	9.0 ± 0.3d	15.1 ± 0.9d

Values are means and standard deviations of 30 replicates; hour (h); days (d)

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