Distinction among the Puparia of Three Blowfly Species (Diptera: Calliphoridae) Frequently Found on Unburied Corpses

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Calliphorid larvae are important in the decomposition of carrion. Since these larvae are present in the primary stages of succession on carcasses, they may be important indicators of death time and the movement of corpses in homicide investigations. In this study we examined the morphological differences among puparia of Chrysomya megacephala, C. putoria *and* Cochliomyia macellaria. *Puparia of the three species (N=30, each) were obtained from the F2 generation bred in culture medium at 25ºC, and 60% relative humidity on a 12 h photoperiod. The interspecific differences found were related to the conspicuousness of six tubercles located in the region near the posterior spiracles and to the distance between the two peritrema involving the spiracles. The latter were (mean ± SD) 15.2 ± 3.1 µm for* C. megacephala, *18.8 ± 2.8 µm for* C. putoria *and 16.5 ± 3.5 µm for* C. macellaria. *The results of the present study may be useful in forensic entomology.*

Key words: Calliphoridae - carrion insects - decomposition - forensic entomology - puparia

The family Calliphoridae consists of carrion flies which may also feed on living tissues. These species are potentially dangerous to man and other animals since the larvae may cause myiasis and adults may transmit pathogens. The diseases transmitted by these flies cause substantial losses to the cattle industry (Norris 1959, Zumpt 1965, Greenberg 1971, 1973, Richard & Gerrish 1983). The genus *Chrysomya* was introduced in Brazil from Africa (Zumpt 1965) with the first records being from the states of Paraná (Imbiriba et al. 1977) and São Paulo (Guimarães et al. 1978). *Chrysomya* species are currently found from the southern United States to southern Brazil (Jiron 1979, Gagné 1981, Baumgartner & Greenberg 1984).

Insects are important in carcass decomposition, and calliphorids, which are among the most abundant and best studied carrion insects have been extensively used as indicators of the *post mortem* interval (death time) and of corpses translocation. These flies are therefore a valuable tool for forensic medicine (Megnin 1894, Smith 1986, Catts & Haskell 1990).

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In this study, we show that it is possible to distinguish among three species (*C. megacephala*, *C. putoria* and *Cochliomyia macellaria*) based on characteristics of their pupae and puparia.

MATERIALS AND METHODS

Cultures (F1) of each species were maintained in cages (25 cmx25 cmx30 cm) covered with mosquito nets, at 25 ± 1ºC, 60% relative humidity and a 12 h photoperiod. The flies were fed raw bovine liver for four days immediately after emergence and with water and sugar *ad libitum* thereafter. Eggs of the three species were collected from these cultures using ground raw bovine meat. Before hatching, the eggs were transferred from the meat to vials containing artificial culture medium (Leal et al. 1982) growth. The vials were kept under the same conditions of the F1 cultures. When the larvae reached the third instar, they were transferred to vials filled with sawdust for pupating.

After the F2 adults of each species had emerged, specimens of their puparia (without any cleaning) were examined with a binocular microscope to assess interspecific differences in the posterior spiracle region. In 30 puparia from each species, the distance between the two peritrema was measured. These distances were compared by analysis of variance followed by the Student-Newman-Keuls test (Zar 1996), with the critical value set at $p = 0.05$.

RESULTS

C. putoria puparia had six very conspicuous tubercles in the perispiracular region whereas in

C. megacephala they were less conspicuous and in *C. macellaria* they were inconspicuous. The tubercles were named according to Greenberg and Szyska (1984) (Figs 1-4). The three species also had four tubercles arranged in a line transversal to the puparia and next to the posterior margin of the anal plate.

Important differences were detected in the texture of the puparia surface: *C. putoria* and *C. megacephala* puparia had an unreflective, rough, irregular surface, whereas the puparia of *C. macellaria* had a very smooth surface with good reflectance (seen as bright spots).

The distances between the peritremas in the posterior spiracular regions were (mean ± SD) 18.9 ± 2.8 µm for *C. putoria*; 15.2 ± 3.1 µm for *C. megacephala* and 16.5 ± 3.5 µm for *C. macellaria*. ANOVA showed that all of these distances were significantly difference from each other (p=0.05; Table).

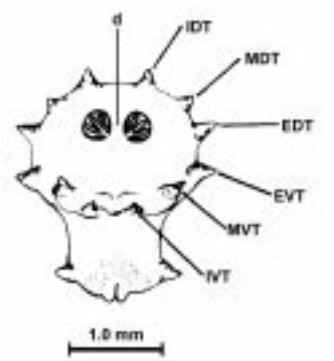

Fig. 1: diagram of the perispiracular region showing the tubercles in calliphorid flies (dorsal view) - IDT: internal dorsal tubercle; MDT: median dorsal tubercle; EDT: external dorsal tubercle; IVT: internal ventral tubercle; MVT: median ventral tubercle; EVT: external ventral tubercle; d: distance between two peritremas (according to Greenberg & Szyska 1984)

TABLE

Comparison of the distances between the peritrema in the perispiracular region among *Chrysomya* and *Cochliomyia*. ANOVA followed by the the Student-Newman-Keuls test

Comparison	Q statistic	p
C. putoria vs. C. megacephala	5.315	<0.05
C. putoria vs. C. macellaria	5.143	<0.05
C. megacephala vs. C. macellaria	2.807	<0.05

DISCUSSION

The family Calliphoridae contains species that develop on or in corpses and may serve as indicators of the death time. These species are the most abundant components in the corpse-associated fauna around Campinas in the State of São Paulo, Brazil (Carvalho et al. 2000).

C. putoria, *C. megacephala* and *C. macellaria* were examined to distinguish the pupae of these species when bred in the same recipient in the laboratory or on corpses. Other important calliphorid species will be examined later.

The differences in the shape and volume of the tubercles in the posterior region of the puparia make this a very conspicuous character for distinguishing among the three species. This approach provides a rapid means of identifying each species since the characters are qualitative and not expressed as exact measures. The distance between peritremas (Fig. 1) was also useful for differentiating among *C. putoria*, *C. megacephala* and *C. macellaria* (Table).

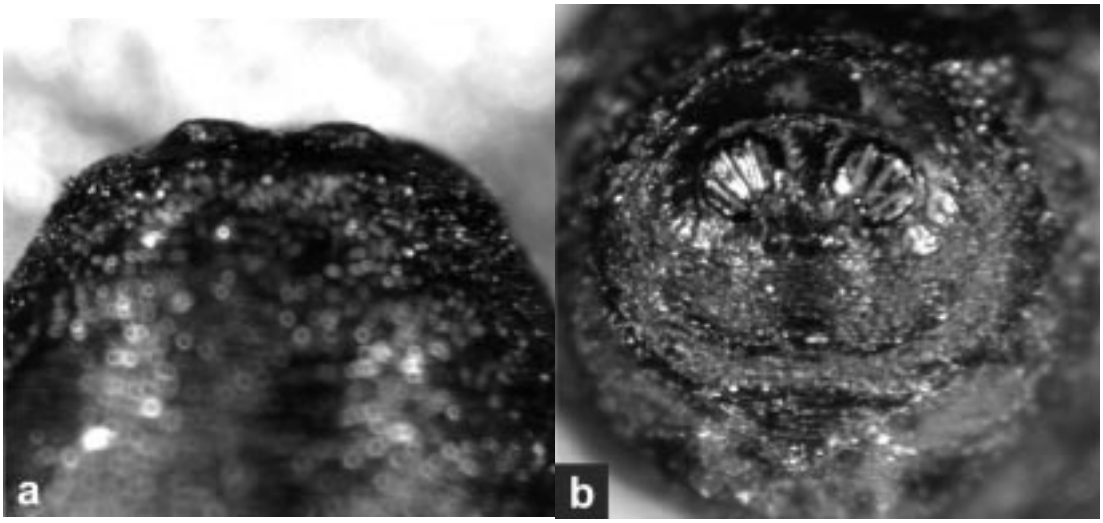

Fig. 2: the inconspicuous tubercles of the perispiracular region in *Cochliomyia macellaria* - a: dorsal view; b: posterior view

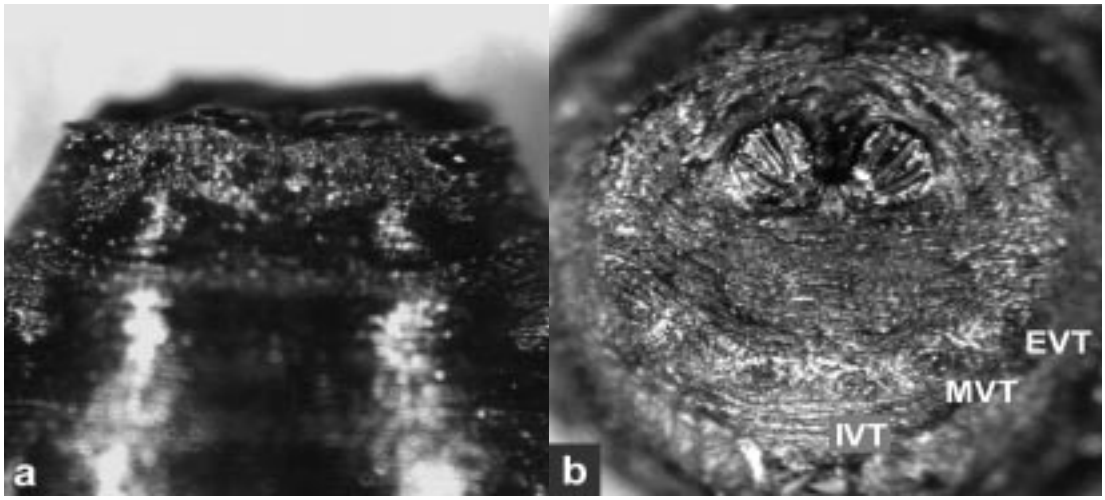

Fig. 3: the tubercles in the perispiracular region of *Chrysomya megacephala* - a: dorsal view; b: posterior view; IVT: internal ventral tubercle; MVT: median ventral tubercle; EVT: external ventral tubercle (according to Greenberg & Szyska 1984)

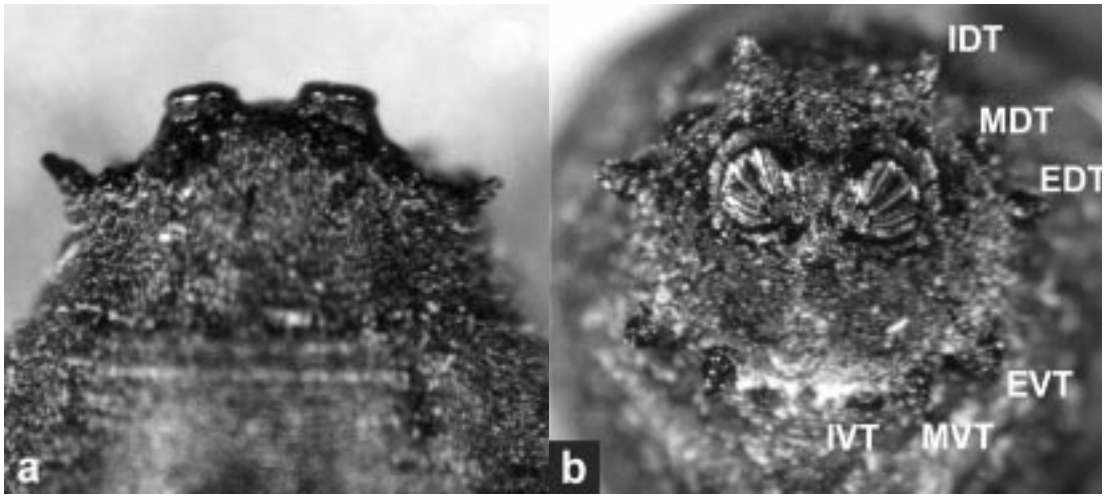

Fig. 4: the tubercles in the perispiracular region of *Chrysomya putoria* - a: dorsal view; b: posterior view; IDT: internal dorsal tubercle; MDT: median dorsal tubercle; EDT: external dorsal tubercle; IVT: internal ventral tubercle; MVT: median ventral tubercle; EVT: external ventral tubercle (according to Greenberg & Szyska 1984)

Considering the difficulty in determining the species involved in the primary stages of succession in a carcass in advanced decomposition, the puparia left by insects may provide an alternative means of identification. In addition the results described here may be applicable to investigative procedures and techniques used to determine the *post mortem* interval.

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