Biology, Ecology and Diversity

A survey of necrophagous blowflies (Diptera: Oestroidea) in the Amazonas-Negro interfluvial region (Brazilian Amazon)

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A B S T R A C T
The fauna of blowflies (Calliphoridae and Mesembrinellidae) in three localities of primary Amazon forest coverage in the Amazonas-Negro interfluvial region was assessed. A total of 5066 blowflies were collected, with Chloroprocata idiodae being the most abundant species (66.3%). A difference in species richness between the localities ZF2 and Novo Airão was observed. Comparison among sampled sites revealed no considerable variation in fauna composition, except for the species Eumesembrinella benoistii (Séguy 1925) and Hemilucilia sp., whose occurrence was observed only in a single locality. Apparently, Amazon rivers are not efficient geographical barriers to influence the current composition of necrophagous blowfly assemblages. Also, most of the blowfly species did not show a noticeable specificity for any specific forest among the interfluvial areas of the ombrophilous forest. Finally, an updated checklist of necrophagous blowfly species of the Amazonas state in Brazil is presented.

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Introduction
Necrophagous flies of the family Calliphoridae (Diptera: Callyptratae: Oestroidea) are among the first insects to detect, arrive, and colonize animal carcasses in many distinct environments (Hanski, 1987; Amendt et al., 2004). In addition, they have a very important role in the decomposition process (Keh, 1985; Smith, 1986; Catts and Haskell, 1991; Souza and Linhares, 1997; Oliveira-Costa, 2008), and species from this family are of major importance to forensic and medical issues, in the latter for being carriers of many pathologically microorganisms (Greenberg, 1971; Amendt et al., 2004; Sawabe et al., 2011). Mesembrinellidae (Diptera: Callyptratae: Oestroidea), a group historically treated as a subfamily of Calliphoridae, appears to have a different biology. The reproductive system of the females is modified; they are viviparous and larvae seem to have parasitoidal preferences. The biology of the immatures is still poorly understood; adults are strongly attracted to dung and carrion, and clearly all species show preferences toward inhabiting pristine forests (Guimarães, 1977).

In forensic and legal matters, knowledge about the distribution and the tolerance to different ecological parameters of these necrophagous species is crucial to infer the locale in which death, or at least the beginning of the decomposition, took place, as well as to estimate the post-mortem interval (PMI) (Greenberg, 1991; Amendt et al., 2004; Oliveira-Costa and Mello-Patiu, 2004; Rocha et al., 2009). Most of this knowledge, however, cannot be easily extrapolated to different localities and the species assemblages usually depend on the degree of conservation of a particular environment (Zabala et al., 2014).

Species in these families have different tolerances to environmental conditions, being affected by the proximity to human populations (synanthropy) and disturbances in primary vegetation coverage, more observable in species of Mesembrinellidae, which are absent in disturbed natural areas and urban environments (Polvony, 1971; Esposito et al., 2010). Also, different blowfly species present distinct dispersal rates and flight capabilities, with environmental elements acting as barriers to some, but not all, species (Macleod and Donnelly, 1960; Tsuda et al., 2009). In the Amazonian rainforest, large rivers constitute one of these barriers, historical or current, to dispersal in different groups of winged animals, such as birds (Hayes and Sewlal, 2004), even though the dynamics of isolated populations may present many other historical causes (Hafler, 1997). Data on flight and dispersal capacity in blowflies is scarce, but the studies conducted so far indicate that they can fly for very long distances; varying in a single flight between 100 and 700 m for Lucilia species and from 1250 to 1789 m—and as far as 3500 m/day—for Calliphora nigriceps (Tsuda et al., 2009). Moreover, rivers as wide as 182.88 m and slopes as high as 152.40 m do

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not seem to act as barriers to the dispersal of blowflies (Macleod and Donnelly, 1960).

In the Amazonas state (Brazil), the Amazon River and its tributaries, which probably originated circa 5 mya with the uplifting of the Andes (Hooft et al., 1995; Campbell et al., 2001), were already implicated as historical barriers for dispersal and account for the different distribution of some bird species in the region (Hafer, 1997; Hayes and Siewal, 2004). For flies, it could also be an important barrier, since in its wider portions the river can be more than 40 km wide during the rainy season. In this context, this study surveyed the blowfly fauna in the interfluvial region of the Amazonas-Negro rivers, with a comparison between the species richness and abundance of the necrophagous fly fauna in the sampled localities.

Material and methods

Van Sommeren-Rydon traps, modified to collect flies, were mounted in three localities of the interfluvial region of the Amazonas-Negro rivers between December 1st and 15th, 2013 (Fig. 1). All localities comprised regions of typical Amazonian rainforest phytophysionomy with a dense ombrophilous forest according to the classification of IBGE (2012). Sampled localities included: (1) the ZF2 biological reserve, located 50 km from the major urban center in the Manaus municipality; and private properties in the municipalities of (2) Novo Airão and (3) Careiro Castanho. In each locality, 4–7 traps were used, baited with a mixture of decomposing cow, chicken, and fish viscera. Traps were emptied after 2–3 days and all flies collected were counted and identified following the keys provided by Amat et al. (2008), Kosmann et al. (2013), Whitworth (2014), and Wolff et al. (2014).

Data from the different localities were analyzed for relative and total abundance distribution using the IBM-SPSS Statistics (2012) software. Sampling efficiency and representation were assessed using curves for species accumulation, incidence-based coverage estimator (ICE), and the Jack1 and the Chao1 non-parametric estimators of total species richness, using the Estimates 9.0.1 software (Colwell, 2013). Non-parametric Kruskal–Wallis tests and paired Mann–Whitney’s U tests were used in order to evaluate if there were significant differences in richness and abundance between the localities. The Jaccard coefficient and complementarity index was calculated as a measure of the turnover and complementarity index in species composition between localities (Colwell, 2013).

Co-occurrence and spatial assemblage structure were assessed using the C-score of Stone and Roberts (1990) using EcoSim software (Entsminger, 2014). Species reported here and a review of literature served to compile the updated checklist.

Results

A total of 6772 dipters were collected, with Calliphoridae being the most abundant (4356 specimens—64.3%), followed by the families Muscidae (715–10.5%), Mesembrinellidae (710–10.4%), Sarcophagidae (325–4.7%), and Fanniidae (203–2.9%). Other Diptera families comprised 463 individuals (6.8%). Of the 5066 blowfly specimens (Calliphoridae and Mesembrinellidae) collected, 10 were species of Calliphoridae: Chloroprocta idiodea (Robineau-Desvoidy, 1830), Chrysomya albiceps (Wiedemann, 1819), Chrysomya megacephala (Fabricius, 1794), Chrysomya putoria (Wiedemann, 1818), Cochliomyia macellaria (Fabricius, 1775), Hemilucilia segmentaria (Fabricius, 1805), Hemilucilia semidiaphana (Rondani, 1850), Hemilucilia sp. and Lucilia eximia (Wiedemann, 1819), and Paralucilia paraensis (Mello, 1969); and 3 were of Mesembrinellidae: Eumesembrinella benoisti (Séguy, 1925), Eumesembrinella randa (Walker, 1849), and Mesembrinella bellardiana (Aldrich, 1922). Both the rarefaction curve (Fig. 2) and the values of the species richness estimators, ICE, Chao1, and Jack1 (96%) (Table 1), indicated that collecting sites and the complete area assessed were well sampled.

Hemilucilia sp., a species found exclusively in the ZF2 locality, is probably a new species since the male genitalia does not match any of the described species of this genus (sensu Dear, 1985 – data not shown). Excluding this species, sampled fauna comprised 12 out of the 18 species (66.6%) currently known for the Brazilian Amazonas state (Table 2). Among these, the most abundant species was C. Idioda (66.3%), followed by E. randa (8.4%) and H. semidiaphana (5%), while C. macellaria and C. megacephala were the least abundant, represented by less than 25 individuals each (<0.36%) (Fig. 3). With the exception of the exclusive occurrence of E. benoisti in Novo Airão and Hemilucilia sp. in the ZF2 Reserve (Manaus), as well as the absence of C. megacephala in the latter, all species were found in the three localities with somewhat different abundances (Fig. 4). Comparisons between localities showed that Novo Airão is slightly richer than the other two, presenting 12 out of the 13 sampled species, a fact also observed in the rarefaction curve for all localities (Fig. 2). Non-parametrical tests showed no statistically significant differences in richness and abundance between the localities.

Fig. 1. Collecting sites and sampling points in the Amazonas state, Brazil.
significant differences in species abundance (Kruskal–Wallis; df = 2, \( p = 0.649 \)); nevertheless, a statistically significant difference in richness was found (Kruskal–Wallis; df = 2, \( p = 0.047 \)). A post hoc paired test using Mann–Whitney tests showed significant differences only between the localities of Novo Airão and ZF2 \( (p < 0.05, r = 0.030) \). Although these statistical differences were found, high values of the Jaccard similarity coefficient and low values of complementarity were recorded in all localities. Comparisons given as (Jaccard

<table>
<thead>
<tr>
<th>Site</th>
<th>Nt</th>
<th>Ns</th>
<th>Ni</th>
<th>ICE</th>
<th>Chao1</th>
<th>Jack1</th>
<th>Mean ± SD</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Careiro Castanho</td>
<td>4</td>
<td>11</td>
<td>550</td>
<td>11.43</td>
<td>11</td>
<td>11.75</td>
<td>11.4 ± 0.4</td>
<td>96.5</td>
</tr>
<tr>
<td>Novo Airão</td>
<td>6</td>
<td>12</td>
<td>1031</td>
<td>12.41</td>
<td>12</td>
<td>12.83</td>
<td>12.4 ± 0.4</td>
<td>96.7</td>
</tr>
<tr>
<td>ZF2</td>
<td>7</td>
<td>11</td>
<td>3479</td>
<td>11.43</td>
<td>11</td>
<td>11.86</td>
<td>11.4 ± 0.4</td>
<td>96.2</td>
</tr>
<tr>
<td>Complete area</td>
<td>17</td>
<td>13</td>
<td>5060</td>
<td>13.32</td>
<td>13</td>
<td>13.94</td>
<td>13.4 ± 0.5</td>
<td>96.9</td>
</tr>
</tbody>
</table>

Fig. 2. Rarefaction curve for necrophagous blowflies in the three interfluvial collecting sites.

Fig. 3. Species abundance distribution of necrophagous blowflies in the Amazonas-Negro interfluvial region.
Table 2
Updated checklist of necrophagous blowflies (Calliphoridae and Mesembrinellidae) of Brazilian Amazonas province. RFAD, Reserva Florestal Adolfo Ducke; Ca, Careiro Castanho; Co, Coari; Mao, Manaus; NvA, Novo Airão.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sites sampled in this study</th>
<th>Municipality</th>
<th>Locality</th>
<th>Bait</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calliphoridae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysomyiinae</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Chloroprocotidae (Robineau-Desvoidy 1830)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, RFAD, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi (1996), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Chrysomyia albiceps (Wiedemann 1819)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, RFAD, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi (1996), Barros-Souza et al. (2012), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Chrysomyia putoria (Wiedemann 1818)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Mauana city, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi and Castellón (1993), Paraluppi (1996), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Cuchliomyia macellaria (Fabricius 1775)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Mauana city, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi (1996), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Hemilucilia segmentaria (Fabricius 1805)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Mauana city, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi (1996), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Hemilucilia semidiapana (Rondani 1850)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Mauana city, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi (1996), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Hemilucilia souavalopesi (Mello 1972)</td>
<td>–</td>
<td>Mao</td>
<td>Mauana city</td>
<td>Pig</td>
<td>Ururahy-Rodrigues et al. (2013)</td>
</tr>
<tr>
<td>Hemilucilia sp.</td>
<td>ZF2</td>
<td>Mao</td>
<td>ZF2</td>
<td>Fish and chicken viscera</td>
<td>This study</td>
</tr>
<tr>
<td>Paralucilia paraensis (Mello 1969)</td>
<td>All</td>
<td>Mao, Co</td>
<td>Mauana city, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Esposito et al. (2010), Barros-Souza et al. (2012)</td>
</tr>
<tr>
<td>Paralucilia sp.</td>
<td>–</td>
<td>Mao</td>
<td>RFAD</td>
<td>Pig</td>
<td>Ururahy-Rodrigues et al. (2013)</td>
</tr>
<tr>
<td>Lucilinae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lucilia albofusca (Whitworth 2014)</td>
<td>–</td>
<td>Mao</td>
<td>RFAD</td>
<td>Flight trap</td>
<td>Whitworth (2014)</td>
</tr>
<tr>
<td>Lucilia eximia (Wiedemann 1819)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Mauana city, RFAD, Urucu</td>
<td>Cow, pig, fish and chicken viscera</td>
<td>Paraluppi (1996), Esposito et al. (2010), Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td><strong>Mesembrinellidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euemembrinella benoisti (Séguy, 1925)</td>
<td>Novo Airão</td>
<td>Ca, Co, Mao, NvA, RFAD</td>
<td>Pig, fish and chicken viscera</td>
<td>Ururahy-Rodrigues et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Euemembrinella quadrilineata (Fabricius 1805)</td>
<td>–</td>
<td>Co</td>
<td>Urucu</td>
<td>Cow viscera</td>
<td>Esposito et al. (2010)</td>
</tr>
<tr>
<td>Euemembrinella randa (Walker 1849)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Urucu</td>
<td>Cow, fish and chicken viscera</td>
<td>Esposito et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Mesembrinella batesi (Aldrich 1922)</td>
<td>–</td>
<td>Co</td>
<td>Urucu</td>
<td>Cow viscera</td>
<td>Esposito et al. (2010)</td>
</tr>
<tr>
<td>Mesembrinella bellardi (Aldrich 1922)</td>
<td>All</td>
<td>Ca, Co, Mao, NvA, Urucu</td>
<td>Cow, fish and chicken viscera</td>
<td>Esposito et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Mesembrinella bicolor (Fabricius 1805)</td>
<td>–</td>
<td>Co</td>
<td>Urucu</td>
<td>Cow viscera</td>
<td>Esposito et al. (2010)</td>
</tr>
</tbody>
</table>

Coefficient; Complementarity index) are ZF2-Novoo Airão: (0.769; 0.23); Careirazo-ZF2: (0.833; 0.166); and Novoo Airão-Careiro (0.916; 0.083). The estimated C-score for co-occurrence (0.03845) being significantly smaller than the critical simulated index (0.04157) indicates that necrophagous blowfly species are randomly spatially distributed. This is coherent with the composition and distribution on most of the species assemblage.

**Discussion**

Diversity patterns of the blowfly assemblage are consistent with the trends found in previous studies for the Amazon region (Amat, 2010; Esposito et al., 2010). The number of species reported here was lower than that reported by Esposito et al. (2010) during a collection of three months in an undisturbed Amazon area. Our study also corroborates the dominance of C. idiodea, E. randa, and H. semidiaphana in the Amazonian necrophagous blowfly assemblages; these findings are in agreement with those of Esposito et al. (2010). The faunistic composition was almost the same except for three species of Mesembrinellidae not found in this study: Euemembrinella quadrilineata (Fabricius, 1805), Mesembrinella batesi (Aldrich, 1922), and Mesembrinella bicolor (Fabricius, 1805).

The composition and the significant difference in richness between the localities of Novoo Airão and ZF2 may be explained as the probable effect of the closer proximity to urban centers, as shown by the absence in ZF2 of C. megacephala in the sampling, an introduced species with high dispersion capacity and highly synanthropic habit (Prado and Guimarães, 1982; Baumgartner and Greenberg, 1983; Paraluppi and Castellón, 1993; Paraluppi, 1996), and for the exclusive presence of E. benoisti and Hemilucilia sp. in each of these two locations, respectively. Unfortunately no detailed biological data are available about these two species to suggest
preliminary inferences about this spatial distribution pattern, although some rare species from the genus *Hemilucilia* and all of Mesembrinellidae used to be highly asynanthropic (Guimarães, 1977; Baumgartner and Greenberg, 1985).

According to the values of the similarity coefficient and the complementarity indices, the three localities are similar in species composition (10 species shared). These findings may indicate that Amazon river are not efficient barriers for all species assemblage dispersion, at least in a historical perspective. The three localities sampled are relatively similar in terms of forest physiognomy; the differences found in this context do not seem to be a significant factor influencing this faunal assemblage except for a few species with possible geographical restricted ranges or intrinsic biological factors mentioned in the last paragraph.

A similar level in abundance among interfluvial sites and the randomly spatially distribution jointly with the long dispersal capability of blowflies (Tsuda et al., 2009) allow us to suggest that this interfluvial region acts as a unique area of blowfly populations interactions. Differences in abundance may be more noticeable at even larger areas than those studied here (mean: 11 km between localities) and more evident in highly fragmented landscapes (Zabala et al., 2014). The remarkably low densities and abundance for the native species *C. macellaria*—which is considered a highly synanthropic species in the Peruvian Amazon (Baumgartner and Greenberg, 1985)—may be explained as a consequence of the relatively low human impact on the surveyed areas or may reflect the effects of ecological competition with the introduction of the *Chrysomyia* species to the Americas, since *C. macellaria* was one of the most frequent species and the most abundant fly in the Peruvian rainforest from 1979 to 1981 (Baumgartner and Greenberg, 1985). It is noteworthy to mention the importance of assessing the effects of these invasive species on the native carrion fly communities in the Amazonian region.

Although there seems to be no evidence supporting the rivers as historical barriers affecting necrophagous dipteran assemblages distribution in this region, it is still possible that these rivers may constitute current barriers to gene flow, leading to significant differences in populations among interfluvial localities. An assessment of population structure and differentiation based on molecular data is being conducted and should provide further insights on this matter and also in the forensic context. Spatial distributions of blowflies are strongly affected by synanthropic effects, dispersal capability, and the local and specialized breeding sites of the immature stages (Norris, 1965; Polvony, 1971). These aspects must be taken into consideration in biogeographical inferences and probably account for a large extent of the patterns found in this study for the Amazonian forest.

The checklist presented here may serve as a baseline for future ecological studies and applications in the forensic entomology framework of the Amazon region, since a local fauna inventory is essential in this context. Since a comprehensive study must include a broad temporal dimension and consider seasonal variations and temporal activities of flies, which vary due to intrinsic factors (such as life history, population dynamics, reproductive cycles etc.) and extrinsic factors (temperature, humidity, resources availability etc.) (Hwang and Turner, 2005), the diversity patterns of the necrophagous blowfly assemblage found here must be interpreted with caution due to the short time of field collection. In this sense they are probably underestimated compared to a long term study. These findings indicate the lack of monitoring and intensive collecting efforts and also the little knowledge currently available about the carrion feeding insect fauna of one of the world’s major megadiversity hot-spots.

**Conflicts of interest**

The authors declare no conflicts of interest.
Acknowledgments

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References

Kossmann, C., Pinto de Mello, R., Harterreiten-Souza, E.S., Pujol-Luz, J.R., 2013. A list of current valid blow fly names (Diptera: Calliphoridae) in the Americas South of Mexico with key to the Brazilian species. Entomol. Res. 6, 74–85.
Sawabe, K., Hoshino, K., Isawa, H., Sasaki, T., Kim, K.S., Hayashi, T., Tsuda, Y., Kurohashi, H., Kobayashi, M., 2011. Blow flies were one of the possible candidates for transmission of highly pathogenic H5N1 Avian Influenza virus during the 2004 outbreaks in Japan. Influenza Res. Treat., 8, Article ID 652652.