

## Observation of the morphological characters in slide-mounted simuliids (Diptera, Simuliidae) deteriorated by age or fungus contamination

Ana M.R. do Amaral <sup>1</sup>; Magali R. Sá <sup>2</sup> & Marilza Maia-Herzog <sup>1</sup>

<sup>1</sup> Laboratório de Referência Nacional em Simulídeos e Oncocercose, Departamento de Entomologia, Instituto Oswaldo Cruz. Avenida Brasil 4365, Manguinhos, 21045-900 Rio de Janeiro, Rio de Janeiro, Brasil. E-mail: mherzog@ioc.fiocruz.br

<sup>2</sup> Casa de Oswaldo Cruz, Fundação Oswaldo Cruz.

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**ABSTRACT.** The homogeneity of the Simuliidae family has produced several taxonomic problems along the years. The studies of Brazilian *Simulium* Latreille, 1802 date from the beginning of the 20<sup>th</sup> century, with species described by Kollar, Macquart, Goeldi and Lutz. Lutz's scientific collection is a historical relic of scientific relevance, since it congregates types described from Brazil. Besides its taxonomic importance, this collection can now be used as a bio indicator. However, a lot of these slide-mounted specimens could not support the effects of time, constant handling, fungus and climatic conditions that occurred over the years. The development of a slide-mounted restoration technique had therefore become necessary for specimen preservation and observation.

**KEY WORDS.** Blackflies; scientific collection; slide-restoration.

**RESUMO. Observação morfológica de caracteres de simulídeos (Diptera, Simuliidae) em lâminas deterioradas pelo tempo ou pela contaminação de fungos.** A homogeneidade da família Simuliidae tem resultado problemas taxonômicos ao longo dos anos. Os estudos sobre *Simulium* Latreille, 1802 no Brasil datam do início do século XX, com espécies descritas por Kollar, Macquart, Goeldi e Lutz. A coleção de Lutz possui relevância científica e histórica, possuindo exemplares tipo descritos do Brasil. Além da importância taxonômica, a coleção de simulídeos de Lutz pode ser usada como bioindicadora. No entanto, muitas lâminas não suportaram os efeitos do tempo, constante manuseio, presença de fungos e ação das condições climáticas ao longo dos anos. O desenvolvimento de uma técnica de restauração do material preservado em lâminas fez-se necessário para melhor preservação e observação do material. **PALAVRAS CHAVE.** Coleção científica; restauração de lâminas; simulídeos.

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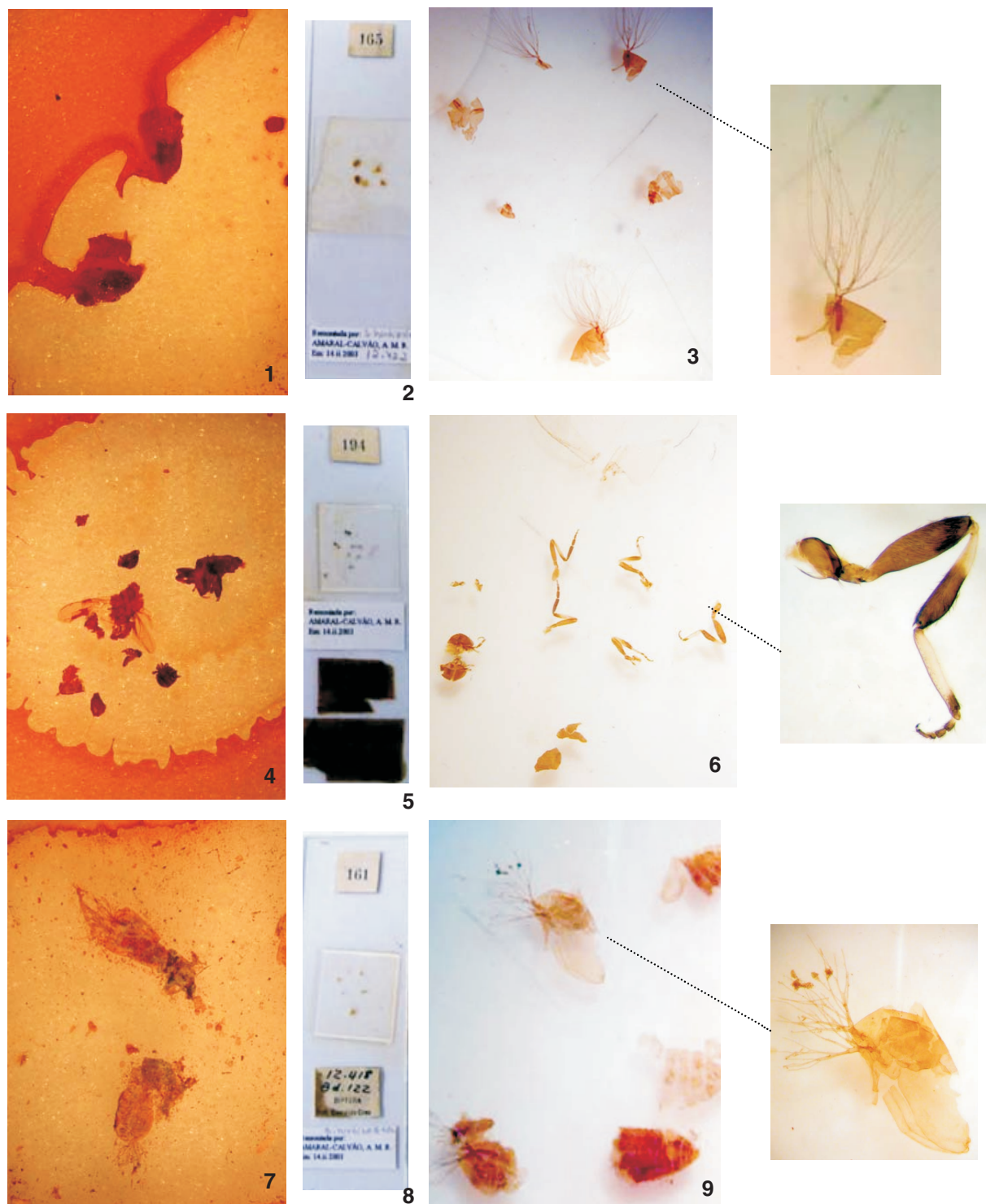
The Simuliidae family has a wide geographical distribution. They cause social-economic, agricultural and tourist damages. They carry etiological agents of disease vectors, for men and other animals. In some cases, after the rainy period, when the population of zoophilic species reaches great proportions, they cause uncomfortable attacks that affect agricultural and tourist activities.

The "Coleção Entomológica do Instituto Oswaldo Cruz" constitutes a significant patrimony for medical, veterinarian and historical studies and to the knowledge of entomologic biodiversity. It is greatly diversified with about a million specimens. The collection started in the early XX<sup>th</sup> century, with insects collected by Instituto Oswaldo Cruz (IOC) researchers on scientific expeditions around Brazil, where they captured insects to determine their relationship with anthroponosis or zoonosis transmission (MARCHON-WHISTLES 1996).

Adolpho Lutz was one of the pioneer researchers of such expeditions. He collected materials from several Brazilian localities, mainly those related to medical entomology studies.

LUTZ (1909, 1910, 1917, 1922) started to study simuliid in Brazil at the beginning of the XX<sup>th</sup> century. In that period, he described a great part of the species known in Brazil today. Consequently, he built up an important historical-scientific collection that assembled a great number of species and specimens; it is a very important reference in dipterous study. All the material was preserved in the three traditional methods: dry pin, immersion in alcohol and slide mounting.

However, many of these slide-mounted collections could not stand the effects of time, constant handling, fungi and climatic conditions after decades. The development of a slide-mounted restoration technique was, therefore, made necessary for the observation of the specimens. The technique we have developed preserves the characteristics of Lutz simuliid collection, without any harm to the original specimen. It offers an effective way to maintain the historical slide-mounted characteristics and results in a slide-remount with the damages repaired, enhancing species identification according to modern taxonomy.



Figures 1-9. (1-3) Slide #12418, *L. hirticosta*: (1) original conservation of the pieces; (2) recuperated slide; (3) details of the restoration; (4-6) Slide #12125, *S. obesum*: (4) original conservation of the pieces; (5) recuperated slide; (6) details of the restoration; (7-9) Slide #12422, *L. hirticosta*: (7) conservação original of the pieces; (8) recuperated slide; (9) details of the restoration.

Table I. Material of the Adolpho Lutz Simuliid Collection that were selected and submitted to the Restoration.

| Simuliid species                                   | Stage                         | Identification Number | Restorator | Date        |
|--|-------------------------------|-----------------------|------------|-------------|
| <i>Simulium</i> Latreille, 1802                    |                               |                       |            |             |
| <i>S. amazonicum</i> Goeldi, 1905                  | larva                         | 12009                 | MM-Herzog  | ?           |
|  | larva                         | 12009                 | MM-Herzog  | ?           |
|  | larva                         | 12009                 | MM-Herzog  | ?           |
|  | larva                         | 12009                 | MM-Herzog  | ?           |
| <i>S. auristriatum</i> Lutz, 1910                  | female parts                  | 12017                 | MM-Herzog  | 1986        |
|  | female                        | 12064                 | MM-Herzog  | 29.XII.1982 |
|  | female                        | 12065                 | MM-Herzog  | 29.XII.1982 |
| <i>S. distinctum</i> Lutz, 1910                    | female                        | 12066                 | MM-Herzog  | 29.XII.1982 |
|  | female                        | 12070                 | MM-Herzog  | 29.XII.1982 |
|  | female                        | 12503                 | MM-Herzog  | 29.XII.1982 |
| <i>S. diversibranchium</i> Lutz, 1910              | pupa                          | 12097                 | R Malaguti | ?           |
|  | female parts                  | 12221                 | MM-Herzog  | ?           |
|  | female parts                  | 12221                 | M M-Herzog | ?           |
| <i>S. exiguum</i> Roubaud, 1913                    | female parts                  | 12520                 | MM-Herzog  | ?           |
|  | female parts                  | 12520                 | MM-Herzog  | ?           |
|  | female parts                  | 12520                 | MM-Herzog  | ?           |
| <i>S. hirtipupa</i> Lutz, 1910                     | slide with female neotype     | ?                     | MM-Herzog  | ?           |
| <i>S. incrustatum</i> Lutz, 1910                   | exuvia parts                  | 12.136                | MM-Herzog  | 25.II.1984  |
|  | male and exuvia parts         | 12.148                | MM-Herzog  | 1983        |
| <i>S. minusculum</i> Lutz, 1910                    | larva                         | 12.009                | MM-Herzog  | ?           |
| <i>S. nigri-manum</i> Macquart, 1838               | exuviae and larva parts       | 12.296                | AMRAmaral  | I.2002      |
|  | exuvia abdomen and larva head | 12.294                | AMRAmaral  | 14.II.2003  |
|  | exuviae parts                 | 12.295                | AMRAmaral  | 14.II.2003  |
|  | exuviae parts                 | 12.297                | AMRAmaral  | I.2002      |
|  | female                        | 12.307                | MM-Herzog  | ?           |
| <i>S. obesum</i> Vulcano, 1959                     | female                        | 12.126                | AMRAmaral  | I.2002      |
|  | female parts                  | 12.125                | AMRAmaral  | 14.II.2003  |
| <i>S. paraguayense</i> Schrottky, 1909             | pupae parts                   | 12.227                | TC Leite   | 1983        |
| <i>S. pertinax</i> Kollar, 1832                    | exuviae gill                  | 12.258                | AMRAmaral  | I.2002      |
| <i>S. rubrithorax</i> Lutz, 1909                   | larva head                    | 12.321                | MM-Herzog  | 1983        |
|  | larva                         | 12.357                | MM-Herzog  | 1983        |
|  | larva                         | 12.358                | MM-Herzog  | 1983        |
| <i>S. scutistriatum</i> Lutz, 1909                 | female parts                  | 12.628                | AMRAmaral  | 14.II.2003  |
| <i>S. spinibranchium</i> Lutz, 1910                | male parts exuvia Lectotype   | 12.337                | AMRAmaral  | I.2002      |
| <i>S. subnigrum</i> Lutz, 1910                     | exuviae parts                 | 12.110                | AMRAmaral  | I.2002      |
| <i>Lutzsimulium</i> d'Andretta & d' Andretta, 1947 |                               |                       |            |             |
| <i>L. flavopubescens</i> Lutz, 1910                | female                        | 12118                 | AMRAmaral  | I.2002      |
| <i>L. hirticosta</i> Lutz, 1909                    | exuvia parts                  | 12.420                | AMRAmaral  | I.2002      |
|  | exuvia parts                  | 12.420                | AMRAmaral  | 14.II.2003  |
|  | exuvia parts                  | 12.420                | AMRAmaral  | 14.II.2003  |
| <i>L. simplicicolor</i> Lutz, 1910                 | female parts and exuviae      | 12.323                | AMRAmaral  | I.2002      |
| Total  | 40 remounts                   |                       |            |             |

In order to clarify any questions about the possibility of identification of the material from Adolpho Lutz slide-mounted collection, 40 damaged slide-mounted pieces of simuliids were selected and submitted to the restoration technique (Tab. I).

In order to avoid damaging any historical 19<sup>th</sup> century material by using chemical reagents and handling, the slide-mounting restoration technique was first evaluated.

### Slide-mounted restoration technique

The slide-mounted specimen should be submerged in tap water for 24 hours – or until the labels come off – and then removed very carefully to avoid damage. The labels are kept in an appropriate place until they dry up. By means of exhaustion, the slide is submerged in xylem (100%) in a properly sealed recipient for 24 hours, until the glass cover and the dissected structures detach from the slide. After the glass cover is removed, dissected structures are removed without damage and can be transferred to a Petri plate containing 100% alcohol, where it lies for 10 minutes. After this stage, everything is transferred again to acetic acid (10%), for 10 minutes. The next step is to wash the dissected structures, leaving them submerged in tap water for five minutes. Everything is then transferred to potassium (10%) and taken to the microwave at low potency of 30 seconds to one minute, depending on the amount of restoration needed. The structures are checked. If they clarified properly, with the straight micro-dissecting needle, vestiges of muscular bundle or tissue and fat are removed. Everything is transferred to berlese (Gum Chloral Mountant) and more structures other than those of taxonomic interest can be further dissected, as it did not happen to Lutz, who usually did not mount some structures, like the genitalia, for example. The structures are arranged on the original (previously) cleaned slide. It is repaired in berlese, distributing the structures in the central portion of the slide, and covering it with glass cover. After the slide has dried, the original labels are reused enclosing the current data of the remount, such as locality, date and name of the person responsible for the restoration (Figs 1-8).

Lutz Collection comprehends larvae, pupae and adults Diptera, collected from different places in Brazil, mainly in the Southeast area, more specifically in Serra da Bocaina Mountains in São Paulo. The material collected from 1903 to 1925 contains approximately 1300 samples preserved in alcohol, entomological pins, ampoules and 477 slides-mounted specimens. This is a collection of historical and scientific relevance, since it congregates samples collected by Oswaldo Cruz and Carlos Chagas, as well (AMARAL-CALVÃO & MAIA-HERZOG 2003).

This technique allowed the restoration of 40 slides of 20 different species from Lutz simuliid collection, including a new type to *Simulium hirtipupa* Lutz, 1910. It is a simple and easy method that removes the fungi and recovers the specimen, restoring the visualization of the slides, without damaging them or depriving them of the characteristics of the historical collections of the beginning of the last century.

Restoration work of Lutz Collection was already made by MAIA-HERZOG *et al.* (1985) that analyzed specimens of *Simulium incrustatum* Lutz, 1910. The slide-mounted material was repaired when necessary. This technique has already been used to eliminate doubts in relation to the species *S. incrustatum* and *S. hirtipupa*.

LUTZ (1920a) substituted the clarifying agent, employed creosote for phenol, passing the material in terebentene essence or carnation essence before placing it in balsam. Lutz mentions the inconveniences of using phenol, as it tends to change the color of the material after a long exposition to active light. This effect can be delayed by the use of thymol, but not avoided after one century.

LUTZ (1920b) referred his method of conserving small organisms for microscopic exam in capillary glass ampoules, whose rotation would allow one to examine them in any direction. The material contained in capillary ampoules was conserved in pure phenol, or by similar portions of water, glycerin and alcohol, added to a percentage of carbolic acid, or still in pure glycerin. Some times, Lutz can also substitute phenol for Guaicol, which does not have the tendency to become red in light exposition. The whole material mounted by Lutz with guaicol became totally black after decades. Specimens contained in ampoule are not advisable to restoration; due to the difficulty of putting the material back into similar ampoules. These are eventually substituted by slide-mountings, which, unfortunately, will be uncharacterized parts of Lutz collection. The substitution and remount of specimens in ampoule only takes place to explain significant taxonomy doubts pertaining to the specimen, and when there is not any other available specimen conserved on mounted slides, as it happened with *S. hirtipupa*.

LUTZ (1922, 1924) described several conservation and preparation methods for insects, including a preparation technique for microscopic study of simuliid pupae and culicoide. Ever since, several preparation methods for slide-mounting insects were created or improved for identification and long-term permanent storage (VOCKEROTH 1966, JONES 1967, MARTIN 1977). Now more effective methods are in use, according to CALVÃO-BRITO & MAIA-HERZOG (2003).

There is still controversy today with relationship to the taxonomic situation of several simuliid species in Brazil; this group is very homogeneous and there are scarce identification keys, appropriate descriptions or monographs on the subject. For a more complete study, cytogenetic and biological methods are now used besides other traditional techniques.

The use of Adolpho Lutz collection is fundamental to clarify complexes of species among simuliids in Brazil and the morphological identification of this exemplary collection.

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