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## Short Communication

# Use of Kisser glycerol gelatin to prepare microscope slides of Phlebotomine sandflies (Diptera: Psychodidae)

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**Abstract**

**Introduction:** Appropriate preservation of specimens is important for taxonomic identification. In sandfly research, various methods have been used for slide preparation; however, high cost, low commercial availability, and associated hazards make their use impossible in some studies. Therefore, the efficacy of Kisser glycerol gelatin for sandfly slide preparation was tested. **Methods:** Kisser glycerol gelatin, as a substitute for Canada balsam and Berlese's fluid, was used for mounting sandflies. **Results:** Forty-two mounted specimens were created and maintained even after 14 months. **Conclusions:** Use of Kisser glycerol gelatin is simple and efficient for preparing microscope slides of sandflies.

**Keywords:** Sandfly. Taxonomy. Kisser glycerol gelatin.

Phlebotomine sandflies (Diptera: Psychodidae) are small-sized Diptera, that are light colored with a hairy body and semi-erect lanceolate wings. Their head forms a ninety-degree angle with their thorax giving them a hunchback appearance[1]. Extensive studies have been conducted on sandflies because the females are the only proven natural vector of *Leishmania* species—the causative agents of leishmaniasis[2].

Generally, sandflies are captured from biomes with differing environmental conditions. Therefore, good transport conditions and methods for preparing and conserving the specimens are important. The development of better specimen-preparation techniques is fundamental for the precise identification of taxonomic structures.

Analysis of the morphological characteristics of sandflies is the first step in species identification. Males are identified primarily by observing the characteristics of their external genitalia using slides typically prepared with Canada Balsam, a high refringence resinous medium that ensures good visualization of the morphological characters under microscopy.

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However, in females, structures such as the cibarium and spermatheca require special attention. Therefore, it is important that the slides are prepared with the specimen in the dorsoventral position to ensure that these structures can be visualized by optical microscopy[1,3]. Thus, the use of Berlese's fluid, an aqueous and low refringence medium, contributes to the visualization of the internal morphology and identification of the species, although it has a temporary character.

Currently, the procedures employed to prepare microscope slides of sandflies for taxonomic identification include the use of several chemical reagents, such as potassium hydroxide, acetic acid, and lactophenol. These chemicals promote softening of the chitin exoskeleton and visualization of morphological structures. Traditionally, to prepare permanent glass slides, Canada balsam and/or Berlese's fluid have been used[1,4-6]. However, they have some disadvantages, including high cost and toxicity. Therefore, in the present study, the use of Kisser glycerol gelatin to prepare microscope slides of sandflies to reduce cost, minimize the exposure to potentially toxic reagents, and ensure the preservation of specimens for taxonomic studies is proposed. To the best of our knowledge, this is the first to test of Kisser glycerol gelatin as an alternative to Canada balsam and Berlese's fluid.

The sandflies were collected from the urban area of Floriano, Piauí, Brazil using Hoover Pugedo (HP) light traps from 6:00

pm to 6:00 am. The sandflies were identified and diaphanized according to Vilela[7].

The preservation of sandflies in Kisser glycerol gelatin by the modified method of Salgado-Laboriau[8] has not been published in specialized literature. The method uses a solution comprised of a colorless gelatin (7 g), 21 mL of glycerin (82%), and 24.5 mL of distilled water.

Gelatin was diluted in distilled water at 50°C and then glycerin was added. Subsequently, the solution was maintained at -4°C. In the original description of Salgado-Laboriau[8], the formulation also was comprised of phenol (1 g). However, in the present study, phenol was initially not chosen for use because of its toxicity[9,10]. The sandflies were arranged on a slide, fixed using heat-liquefied Kisser glycerol gelatin having a consistency of jelly, and a coverslip was placed over it. Subsequently, the edges of the coverslip were sealed using paraffin wax, which would help preserve the specimen[8].

Forty-two slides of sandflies were prepared using the Kisser glycerol gelatin method (modified from Salgado-Labouriau[8]), of which 21 were of the species *Lutzomyia longipalpis* and 21 of *Lutzomyia* sp. (**Figure 1**). These slides were compared with those deposited in the Coleção de História Natural da Universidade Federal do Piauí (CHNUFPI), which were prepared using Canada balsam and/or Berlese's fluid.

The efficacy of the method was evaluated based on the following criteria: (1) temporal analysis, (2) coloration of the specimen, (3) visibility of morphological characteristics, (4) presence of fungi, and (5) presence of crystallized reagents and bubbles. In addition to these technical attributes, the reagent cost, hazardousness, and access to reagents were also considered for the evaluation.

The results of the comparative analysis of the slides prepared in the present study and those deposited in the CHNUFPI are presented in **Table 1**.

Earlier studies on phlebotomine taxonomy suggested that the only permanent way to assemble specimens was through the use of resinous reagents, such as Canada balsam and Berlese's fluid[1,11]. However, these reagents have certain disadvantages, such as high cost and difficulty obtaining the raw material necessary for medium preparation or the medium itself. Furthermore, it is hazardous to handle these reagents as they can cause a series of complications to humans health[10].

The results of the present study showed that Kisser glycerol gelatin preserved the staining of the specimens, maintained the visibility of the morphological characteristics, reduced slide preparation cost, and shortened the drying time when compared with those of other conventional methods, which could take months for complete hardening of the medium (**Figure 1**)[12].

Although Canada balsam seldom crystallizes or incorporates air bubbles during slide preparations,[1] this reagent polymerizes very quickly, which hinders the microscope slide preparation of the specimens. The air bubbles temporarily observed in Kisser glycerol gelatin were due to heating, which preserves the original staining of the morphological structures of the specimens[13].

The absence of phenol in the initial composition did not prevent the application of Kisser glycerol gelatin for the preparation of microscope slides of sandflies. However, it might have contributed to the appearance of fungi nine months after slide preparation. It is noteworthy that the fungal hyphae were visualized at the lateral edges of the coverslip and never at the center of the slide. To evaluate the importance of phenol in the chemical composition, nine slides were prepared by adding phenol to the Kisser glycerol gelatin solution. The results revealed that phenol inhibited fungal growth, thus guaranteeing the conservation of the specimens. The use of phenol while preparing slides allowed for the conservation of the specimens and maintenance of transparency of the tissues, without altering their structures[13].

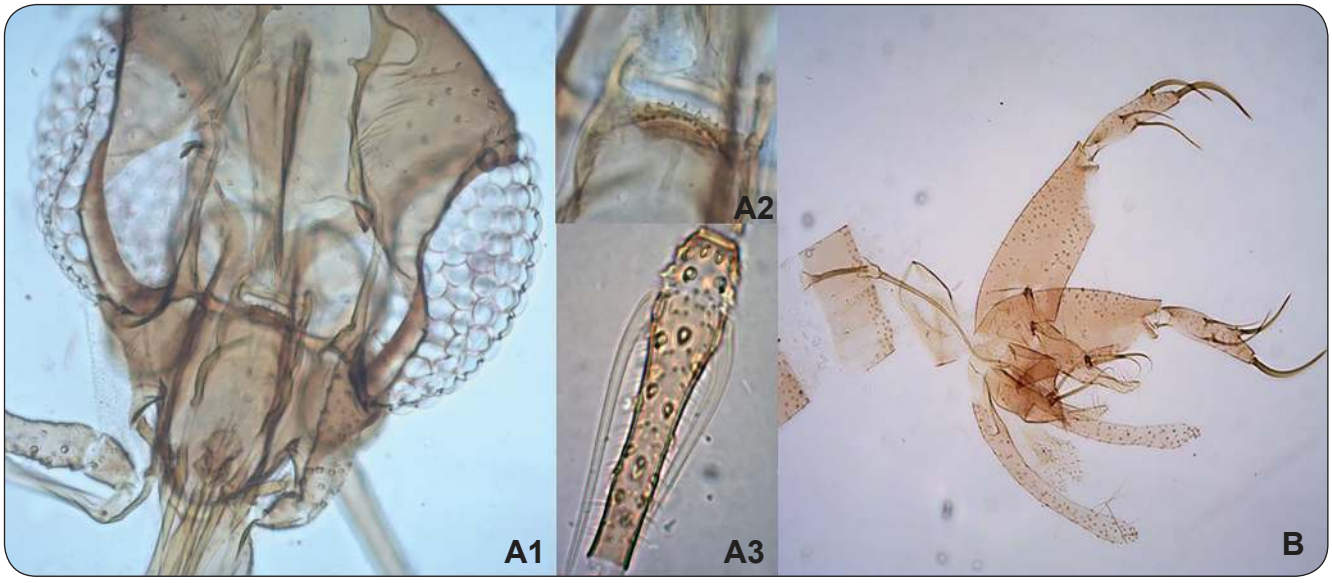


**FIGURE 1:** Specimens of *Lutzomyia longipalpis* 14 months after preparation using Kisser glycerin gelatin. **(A1).** Female head showing cibarium (200X), **(A2).** Cibarium (1000X), and **(A3)**. Flagellomere (1000X); **(B).** Male specimen, external genitalia (100X).

**TABLE 1:** Criteria for the analysis of microscopic preparations using different preparation methods.

| Criteria | Berlese's fluid | Canada balsam | Kisser glycerol gelatin |
|---|---|---|---|
| Temporal analysis (durability and conservation) | 14 months | 14 months | 14 months |
| Coloration | Light yellow | Light Yellow | Light yellow |
| Presence of fungi over time | No | No | Yes |
| Visibility of morphological characters | Yes | Yes | Yes |
| Presence of crystallized reagents | No | No | No |
| Presence of bubbles | No | No | Yes |
| **Total cost (R$) for 100 mL of solution** | **612.08** | **40.96** | **8.47** |

Martins[14] used glycerinated gelatin to prepare glass slides of eggs and oocysts of endoparasites to permanently conserve and preserve the integrity of samples. Huber and Reis[10] used colorless stained glass varnish (Acrilex®), xylol, and immersion oil as an alternative medium to prepare microscope slides of *Ctenocephalides* sp., *Aedes aegypti*, and *Pediculus* sp. to permanently conserve the samples and maintain the transparency of tissues necessary for visualization of the morphological structures under a microscope.

The advantages of using Kisser glycerol gelatin for preparing microscopic glass slide of sandflies are: (1) it enhances the visibility of the morphological structures of the specimens; (2) it enables quick drying; (3) it maintains the integrity of the sample, in addition to having low cost and easy acquisition of the reagents; and (4) it maintains the fundamental characteristics for the taxonomic studies of this group of insects.

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**Conflict of interest:** The authors declare that there is no conflict of interest.

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