SHORT COMMUNICATION

Isolation and Identification of 9-methylgermacrene-B as the Putative Sex Pheromone of *Lutzomyia cruzi* (Mangabeira, 1938) (Diptera: Psychodidae)

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Lutzomyia (Lutzomyia) cruzi *has been named as a probable vector of* Leishmania chagasi *in Corumbá, Mato Grosso do Sul, Brazil. Taxonomically* L. cruzi *is closely related to the* L. longipalpis *species complex. Females of* L. cruzi *and* L. longipalpis *are morphologically indistinguishable and associated males must be examined carefully to confirm identifications. Chemical analysis hexane extracts of male* L. cruzi *has revealed the presence of a 9-methylgermacrene-B (C16), a homosesquiterpene (mw 218) previously shown to be the sex pheromone of one of the members of the* L. longipalpis *species complex.*

Key words: Phlebotominae - *Lutzomyia cruzi* - sex pheromone - 9-methylgermacrene-B

The sand fly *Lutzomyia cruzi* (Mangabeira) has been named as a probable vector of *Leishmania chagasi* (Kinetoplastida: Trypanosomatidae), the causative agent of visceral leishmaniasis in the State of Mato Grosso do Sul in central Brazil (Santos et al. 1998). *L. cruzi* is closely related to the *L. longipalpis* (Lutz & Neiva) species complex, both belong to the subgenus *Lutzomyia* and are members of the same series (longipalpis) (Martins et al. 1978, Young & Duncan 1994). The females of *L. cruzi* and *L. longipalpis* are morphologically indistinguishable and associated males should be examined carefully to provide accurate species identification.

Field and laboratory observations have shown that prior to copulation *L. cruzi* males wing flutter, behaviour that may possibly be associated with pheromone release in male sand flies of other species. Previous studies on male *L. cruzi* have shown that potential pheromone-disseminating structures are visible, as a pair of pale patches, on the fourth tergite (Spiegel et al. 1998) and are similar in appearance to those seen in the *L. longipalpis* complex (Mangabeira 1969). The patches are typically characterised by numerous small papules associated with underlying secretory tissue. They are the confirmed site of sex pheromone production in the *L. longipalpis* complex (Morton & Ward 1989, Ward et al.1993, Hamilton et al.1994) and a suspected site of sex pheromone production in other Neotropical sand flies (Hamilton & Ward 1994, Hamilton et al. 1999a).

The sex pheromones of the *L. longipalpis* species complex have been shown to be novel homosesquiterpenes (C16) or a diterpene (C20). The homosesquiterpenes have been characterised as 3-methyl-α-himachalene (found in *L. longipalpis* from Jacobina, State of Bahia, Brazil) and (*S*)-9-methylgermacrene-B (found in *L. longipalpis* from Lapinha Cave, State of Minas Gerais, Brazil) (Hamilton et al. 1996a,b, 1999b,c). The diterpene is a cembrene and is found typically in *L. longipalpis* from Sobral (State of Ceará, Brazil) (JCG Hamilton unpublished). These compounds act as sex pheromones and are attractants for conspecific females and may help to maintain species isolation (Roelofs & Comeau 1969, Hamilton et al. 1994). The objective of this study was to collect preliminary information on the putative structure of the sex pheromone of *L. cruzi*.

L. cruzi were collected with CDC light traps in a chicken coop in Corumbá, Mato Grosso do Sul (18º59'44"S and 57º39'16W). After separating males from females and checking species identities, males of *L. cruzi* were placed in glass ampoules prepared from Pasteur pipettes with n-hexane (20 µl) (spectroscopic grade, Sigma Co.) and flame sealed. Prior to analysis, extracts were removed from the Pasteur pipette vials, filtered through glass wool to remove the flies and fly hairs, and the volume reduced under N_2 to 1 µl. All the chemical analysis was done according to the procedures of Hamilton et al. (1999a). Six individual males were examined. Mass spectra and gas chromatography retention times were compared with authentic 9-methylgermacrene-B. Peak enhancement studies were performed by coinjecting extracts of *L. longipalpis* from Lapinha and *L. cruzi*. GC-MS analysis was carried out on a Hewlett Packard 5890 II+ gas chromatograph with an HP-5MS capillary column, 30 m x 0.25 mm i.d., 0.25 mm film thickness, directly coupled to a Hewlett Packard 5972A benchtop mass spectrometer, EI, 70eV, 165ºC. Sample was

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introduced via an on-column injector (40°C). The gas chromatograph (GC) was temperature programmed with an initial 2 min at 40°C, then an increase of 10°C min^{-1} to a final isothermal period at 250°C (10 min).

The mass spectral, retention time and peak enhancement results showed that *L. cruzi* males produce 9-methylgermacrene-B. The finding of the presence of 9-methylgermacrene-B in *L. cruzi* males confirms the close taxonomic relationship between *L. cruzi* and *L. longipaplis*. However 9-methylgermacrene-B can occur in number of different isomeric forms and in *L. longipalpis* from Lapinha it occurs as the (*S*) form. The isomeric form of 9-methyl-germacrene-B from *L. cruzi* has not yet been determined.

L. cruzi is found only in central Brazil and as far as we are aware does not occur sympatrically with *L. longipalpis* for the majority of its distribution. It would be interesting in the future to determine the role of 9-methylgermacrene-B as a sex pheromone and how it maintains species isolation in *L. cruzi.*

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