RESEARCH NOTE

Influence of Preparation Methods on the Dimensions of *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera, Psychodidae, Phlebotominae)

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Morphometry was fundamental for the characterization of phlebotomine sandflies (e.g. R Newstead 1911 *Bull Ent Res 2*: 47-78). After the discovery of the importance of female cibarium, spermathecae and its ducts (S Adler & O Theodor 1926 *Ann Trop Med Parasitol 20*: 109-142), it became less used. However, the great number of sandfly species, some of them very similar, and studies of variations related to season or locality induced a need of studies of measurements.

The preparation method can have some influence on dimensions, as observed for the palpal segments (A Dampf 1947 *Ann Esc Nacc Cien Biol 4*: 423-435), but the wings were considered undeformable (AL Tonnoir 1935 *Bull Ent Res 26*: 137-147).

We compared by ANOVA 39 measurements in insects of each sex of *L. intermedia*, identified according to CB Marcondes (1996 *Mem Inst Oswaldo Cruz 91*: 457-462), mounted with Berlese's or NC (Nelson Cerqueira, Enecê in Portuguese) (NL Cerqueira 1943 *Mem Inst Oswaldo Cruz 39*: 37-41) fluids. The NC method included dehydration by a series of growing concentrations of ethanol and was detailed by CB Marcondes (1997 *Mem Inst Oswaldo Cruz 93*: in press). Insects were collected by light traps at the municipality of Venda Nova do Imigrante (20º20'23"S 41º08'05"W, 750 m a. s. l.), in the State of Espírito Santo, in December 1994.

Tables I and II show, respectively, the measurements which differed significantly in females and males. All measurements are in mm.

The preparation method significantly affected several measurements, including some in the wings. Our results indicate the need to use a factor of conversion to several measurements, before any comparison of insects prepared by different methods. LMN Passerrat de Silans et al. (1996 *Ann Trop Med Parasitol 90*: 543-550) considered that the preparation method had effect only on d and p (distance between the bifurcation of R_{2+3} and R_4 and that of M_{1+2}). They dehydrated the wings before mounting them in chloral balm or Euparal, and did not inform the preparation method for using Hoyer's fluid. This is a modification of Berlese's fluid, so the preparation would probably not include dehydration.

The fifth palpal segment, in *Phlebotomus baduelensis* Floch & Abonnenc, 1944 [=*L. trinidadensis* (Newstead, 1922), AV Martins et al. (1978 *American Sand Flies*, Acad Bras Ciências, Rio de Janeiro, 195 pp.)], had a 8 to 20% reduction, after dehydration and inclusion in Canada balsam, compared to the length in lactophenol (Dampf 1947 *loc. cit.*). In *L. intermedia* palps, only the total length in males presented smaller size in the mounting with NC, with a mean reduction of only 3.8%.

In this study, all sandflies were preserved dry; the influence of the method of preservation, dry or in 70º GL, in the measurements should be evaluated. DC Lasenby et al. (1994 *J Plankton Res 16*: 1601-1608) noted that measurements of *Chaoborus* sp. were affected by the preservative liquid.

The shrinkage of soft structures, like the spermathecae, would be more common using preparation methods involving dehydration (P Williams 1988 *Mem Inst Oswaldo Cruz 83*: 375-383). However, in this study, it was observed that shrinkage occurred much more frequently using Berlese's fluid; several of these insects had the spermathecae and ducts difficult to visualize. E Abonnenc (1972 *Les Phlébotomes de la Région Éthiopienne*, ORSTOM, Paris, 289 pp.) considered the use of chloral hydrate fluids inadequate for humid climates and RP Lane [1993 *Sandflies (Phlebotominae)*, p. 78-119. In RP Lane, RW Crosskey

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(eds) *Medical Insects and Arachnids*, Chapman & Hall, London, xv+723 pp.] recommended the use of Berlese's fluid, but considered it inadequate for long-term preservation.

Potash at 10%, used in both methods, was probably less harmful to the spermathecal ducts than at 20%, which would be bad to these ducts [DJ Lewis 1982 *Bull Brit Museum (Nat Hist) 45*: 121-209, N Léger et al. 1983 *Ann Parasitol Hum Comp 58*: 611-623]. Potash could turn invisible the membrane involving the spermathecae of *L. dasymera* (Fairchild & Hertig, 1961) and *L. shannoni* (Dyar, 1929) (GB Fairchild & M Hertig 1961 *Ann Entomol Soc America 54*: 237-255). It would be convenient to examine the flies in phenol, before using potash.

TABLE I

Dimensions of anatomical structures of female specimens of *Lutzomyia intermedia*, prepared with Berlese's or Nelson Cerqueira (NC) fluids

	Preparation method								
	Berlese				NC				
Structures	Mean	S. D.	N	C. V.	Mean	S. D.	N	C. V.	Berl./NC
Head width[b]	389	11,7	20	3	374	9,5	10	2,6	1,03
Cibarium width[a]	52,6	4,1	23	7,7	49,6	3,5	14	7,1	1,06
Eye width[a]	136	6,5	23	4,8	129	6,9	11	5,3	1,05
Mesonotum length[a]	636	3,1	24	0,49	616	13	13	2,1	1,03

S. D.: standard deviation; N: number of measured specimens; C. V.: coeficient of variation; *a*: significance at 5%; *b*: significance at 1%.

TABLE II

Dimensions of anatomical structures of male specimens of *Lutzomyia intermedia*, mounted with Berlese's or Nelson Cerqueira (NC) fluids

	Preparation method								
	Berlese				NC				
Structures	Mean	S. D.	N	C. V.	Mean	S. D.	N	C. V.	Berl./NC
Head width[b]	356	9,1	15	2,6	333	13,6	11	4,1	1,07
Distance between eyes[b]	93	4,6	15	5	100	6,7	11	6,7	0,93
Antenomere III[a]	264	11,3	13	4,3	249	15,2	8	6,1	1,06
Total length palps[b]	498	16,8	16	3,4	480	16,62	11	3,5	1,04
Length eyes[a]	225	8,7	16	3,9	213	15,1	12	7,1	1,06
Width eyes[b]	132	5	16	3,8	117	6,2	11	5,3	1,13
a[a]	480	35,8	15	7,5	511	28,3	12	5,5	0,94
d[a]	228	42,2	15	18,5	261	23,5	12	9	0,87
Length posterior femur[a]	772	35,3	15	4,6	746	27,4	12	36,7	1,04
Length genital filaments[a]	323	19,6	16	6	308	12,1	13	3,9	1,05
Maximal w. genital pump[a]	78,6	8,3	16	10,5	72,2	6,3	13	8,8	1,09

S. D.: standard deviation; N: number of measured specimens; C. V.: coeficient of variation; W: width; a: length of R_2; d: distance between the bifurcation of R_{2+3} and distal extremity of R_1; *a*: significance at 5%; *b*: significance at 1%.