

## Redescription of *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae)

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*The phlebotomine sand flies Nyssomyia intermedia and Nyssomyia neivai are the probable vectors of American tegumentary leishmaniasis in the Southern and Southeastern regions of Brazil. These species form a complex, being difficult to separate between either females or males of the two members based on recognized morphological characteristics. Both N. intermedia and N. neivai are redescribed here in the search for characters that facilitate their correct identification. It was possible to differentiate females by means of spermathecal characteristics. Males could be separated with confidence by the tips of the genital filaments, which have the form of a deep spoon, the angle of the concavity being well accentuated in N. intermedia and much shallower in N. neivai.*

Key words: *Nyssomyia intermedia* - *Nyssomyia neivai* - Phlebotominae - Psychodidae

*Nyssomyia intermedia* was described from adults collected at Fazenda Ouro Fino, municipality of Além Paraíba, Minas Gerais. Material from the states of São Paulo and Rio de Janeiro was also examined (Lutz & Neiva 1912). In this description these authors mainly discuss characters obtained from examination of male specimens, mention of females being limited to the phrase "the single female and two male specimens were collected at Ouro Fino". Nevertheless Barretto (1947, 1961), Martins et al. (1978), Young and Duncan (1994) and Marcondes (1997) consider this to be the first formal description of the species.

França (1920) and Marcondes (1996) redescribed *N. intermedia*, the former author from insects captured in southern Bahia and Vila Americana in the state of São Paulo, and the latter on those captured at the type locality. This description included designation of a neotype female.

Pinto (1926) described *Nyssomyia neivai* from a male captured inside a house at the Instituto Butantan, municipality of São Paulo. This was later designated as a junior synonym of *N. intermedia* by Pinto (1930) a view held by most phlebotomine workers until Marcondes (1996) revalidated the name, based on the holotype and on a female collected at Fortim Campero in the department of Tarija, Bolivia.

Distinction between the females of these two species is based principally by characters of the spermathecae (total length, number of annulations, form and length of head, and length of the common duct in relation to the individual duct) and head, i.e., number of horizontal teeth in the cibarium (Marcondes 1996). Measurement values for the wing venation (alpha length and alpha/beta ratio)

of both sexes and the male ejaculatory pump/genital filament ratio were both significantly greater in *N. neivai*. Nevertheless, Marcondes and Borges (2000) were unable to distinguish between the males of these species with confidence based on their morphology and could only separate them using morphometric studies and analyse artificial neural network.

### MATERIALS AND METHODS

The adult male and female structures of *N. intermedia* and *N. neivai* examined included some of those described by Galati (1995) based on recommendations of the CIPA Group (1991). The classification scheme used is that of Galati (1995).

The specimens of *N. intermedia* examined were captured at its type locality, Além Paraíba (21°53'S and 42°42'W), while those of *N. neivai* included the male holotype described by Pinto (1926) and specimens reared in the laboratory to the F2 generation. These were descended from insects captured in Conchal (22°18'S and 47°13'W) in the Brazilian state of São Paulo and supplied by Dr Cláudio Casanova. All insects were mounted on glass slide using Berlese liquid.

All the material was examined under an Olympus, model CH-2 optical microscope, with measurements made using an ocular micrometer, duly calibrated for this apparatus. The photographs were taken on a Zeiss microscope, and digitalised for the microcomputer using the KS300 program. The females of species were mounted in Berlese's media and the males in Canada balsam.

### RESULTS

All measurements are given in micrometers, with the mean, standard deviation, and number of specimens examined in parentheses.

Redescription of the males of *N. intermedia* and *N. neivai*  
*N. intermedia*

Head (Table I): ratio of lengths of clypeus/head 0.39 (0.41 ± 0.04; n = 19), eyes/head 0.89 (0.91 ± 0.04; n = 19). Ratio

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of lengths of labro-epipharynx (LE)/head 1.02 (1.05 ± 0.06; n = 19). Ratio between AIII/LE 0.94 (1.04 ± 0.09; n = 19) clypeus/interocular distance 1.02 (0.99 ± 0.10; n = 19). Antennal formula AIII-AXIII 2; AXIV-AXVI 0. Internal and external ascoids implanted at the same level, their posterior prolongation being long, with apex reaching very close to that of the flagellomere, not presenting basal prolongation. Pre-apical papilla present on AIII, AIV, AXIV, AXV, and AXVI. Simple bristles absent from AIII to AXIII. Segments AXV same as AXVI (n = 2) or shorter (n = 5). Newstead's spines of the 3rd palpal segment grouped in final third, in area slightly wider than the rest of the segment. Palpal formula 1.4.2.3.5 (n = 14) but formulae 1.4.3.5.2 and 1.4.2.(3.5) also occurring, each in a single specimen.

Cervix: ventro-cervical sensilla absent.

Thorax (Table II): notum and paratergite dark chestnut, pleurae clear. Bristles absent in anterior region of katepisternum. Proepimeral and superior anepisternal

bristles present. Wings measuring 554 (520 ± 28; n = 19) wide, R5 1201 (1185 ± 65; n = 19) long. Principal wing indices: α (alpha) 532 (466 ± 39; n = 19); β (beta) 238 (285 ± 35; n = 19); γ (gamma) 136 (186 ± 27; n = 19); δ (delta) 283 (229 ± 37; n = 19). Tarsomeres III and IV with thin spines being more robust in some specimens. Femur of anterior, median and posterior legs measuring 657 (647 ± 29; n = 18), 634 (628 ± 38; n = 18), and 702 (697 ± 35; n = 17) respectively. Tibia of anterior, median and posterior legs measuring 804 (818 ± 64; n = 18), 952 (988 ± 57; n = 18), and 1178 (1197 ± 81; n = 16) respectively. Tarsomere I of anterior, median and posterior legs measuring 441 (440 ± 23; n = 18), 521 (544 ± 26; n = 18), and 589 (602 ± 31; n = 16), respectively.

Abdomen (Table III): absence of papillae on abdominal tergites. Gonocoxite: 297 (292 ± 15; n = 19) long by 75 (70 ± 8; n = 19) wide in its median part, without tuft of bristles in its basal region and with some elements distributed

TABLE I

Measurements (µm) of the structures of the head and its appendages in males of *Nyssomyia intermedia* and *Nyssomyia neivai*

Structure	<i>Nyssomyia intermedia</i>					<i>Nyssomyia neivai</i>				
	X	SD	Max	Min	N	X	SD	Max	Min	N
Head length	239,95	15,37	266	211	19	242,89	9,98	261	230	9
Eye length	218,95	11,31	239	195	19	216,44	5,55	224	208	9
Interocular distance	96,53	7,92	105	75	19	99,89	6,25	109	94	9
Clypeus length	97,58	6,61	108	86	19	105,91	6,24	109	92	9
Labrum-epipharynx length	229,74	15,75	250	195	19	237,33	12,17	255	220	9
AIII	238,79	21,18	275	172	19	239,56	6,91	253	233	9
AIV	110,11	7,57	125	89	19	108,33	4,90	117	103	9
AV	109,79	6,90	122	92	19	108,78	5,52	117	97	9
P1	35,37	2,93	42	30	19	33,00	2,45	39	31	9
P2	110,05	7,69	125	94	19	109,44	4,56	117	103	9
P3	131,56	8,94	152	119	18	136,89	8,68	145	119	9
P4	52,94	3,57	61	47	18	55,44	4,28	61	47	9
P5	140,00	8,16	153	130	16	153,22	10,63	164	128	9

X: mean; SD: standard deviation; max: maximum; min: minimum; N: number of specimens examined

TABLE II

Measurements (µm) of the structures of the thorax in females of *Nyssomyia intermedia* and *Nyssomyia neivai*

Structure	<i>Nyssomyia intermedia</i>					<i>Nyssomyia neivai</i>				
	X	SD	Max	Min	N	X	SD	Max	Min	N
Wing width	519,89	28,09	578	465	19	523,67	17,80	544	487	9
R5	1185,42	64,96	1280	997	19	1132,78	27,32	1178	1087	9
Alpha	466,32	39,33	533	397	19	476,00	26,02	510	419	9
Beta	284,63	34,75	362	215	19	246,89	18,41	272	216	9
Gamma	185,89	27,35	238	125	19	187,56	18,18	227	170	9
Delta	229,00	37,18	295	159	19	240,44	27,04	272	181	9
Anterior femur	647,00	29,26	702	567	18	642,88	22,40	668	600	8
Median femur	628,06	38,27	691	521	18	623,00	23,62	646	578	7
Posterior femur	697,00	35,21	748	600	17	693,00	26,16	714	646	6
Anterior tibia	818,78	64,40	993	702	18	761,63	20,72	793	693	8
Mean tibiae	987,50	57,32	1087	838	18	933,57	24,30	963	895	7
Posterior tibia	1196,81	80,86	1341	986	16	1138,50	55,89	1201	1065	6
First anterior tarsomere	440,33	22,63	487	397	18	412,25	11,89	431	397	8
First median tarsomere	543,94	25,58	612	498	18	498,86	26,05	533	465	7
First posterior tarsomere	601,88	30,83	657	533	16	573,83	25,29	600	544	6

X: mean; SD: standard deviation; max: maximum; min: minimum; N: number of specimens examined

sparingly over the median region. Gonostyle: 147 (141 ± 7; n = 19) long, with four well developed spines, one being apical, one external superior implanted on the apical fourth, one external inferior and one internal. These two last implanted at the same level, a little further on than the middle of the structure. Pre-apical bristle absent. Paramere digitiform, wide at base and middle of the structure with a sharp point on the ventral region ventral. Groups of fine bristles present on the dorsal margin of the apical region and also the ventral margin, implanted in the widest region of the paramere. Lateral lobe length approximately equivalent to that of the gonocoxite, with ratio gonocoxite/lateral lobe 1.05 (1.04 ± 0.04; n = 19). Aedeagus conical. Ejaculatory pump with wide body. Genital filament short, ratio genital filament/ejaculatory pump 1.40 (1.34 ± 0.11; n = 19). Tip of genital filament in form of deep spoon, with angle of concavity highly accentuated (Fig. 1).

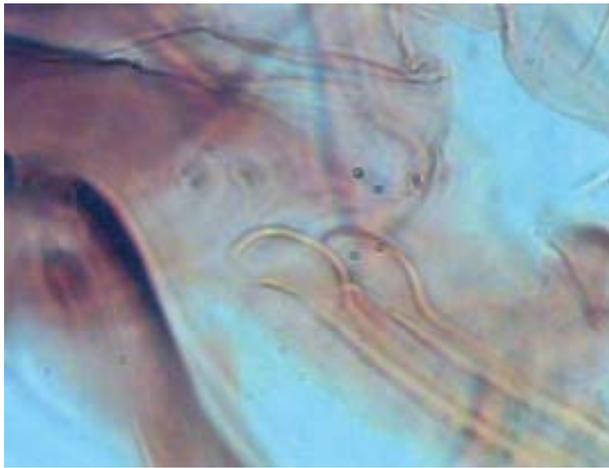


Fig. 1: *Nyssomyia intermedia* ♂: tip of the genital filaments (1000X)

*N. neivai*

Holotype. Head (Table I): ratio of lengths clypeus/head 0.37 (0.41 ± 0.02; n = 9) and eyes/head 0.86 (0.89 ± 0.03; n = 9). Labrum-epipharynx (LE) long, ratio of LE/head 0.90 (0.98 ± 0.06; n = 9). Ratio between AIII/LE 1.09 (1.01 ± 0.06; n = 9). Head of holotype lying in its side, it not being

possible to measure the interocular distance. Ratio clypeus/interocular distance in remaining insects 1.00 ± 0.06 (n = 9). Antennal formula AIII-AXIII 2; AXIV-AXVI 0. Ascoids long, their apex being close to the end of the flagellomere. Internal and external ascoids implanted at the same level. Pre-apical papilla present on AIII, AIV AXIV, AXV and AXVI. Simple bristles absent from AIII to AXIII. Two final antennal segments of variable size, AXV being equal to AXVI (n = 1) or shorter (n = 4). Newstead's spines of 3rd palpal segment grouped on median third in area wider than rest of segment. Palpal formula 1.4.2.5.3, that in the remaining specimens being 1.4.2.3.5 (n = 9).

Cervix: ventro-cervical sensilla absent.

Thorax (Table II): notum and paratergite dark chestnut, pleurae pale. Proepimeral and superior anepisternal bristles present. Anterior region of katepisternum without bristles. Wings measuring 600 (524 ± 18; n = 9) long, R5 1348 (1133 ± 27; n = 9) wide. Principal wing indices: α (alpha) 544 (476 ± 26; n = 9); β (beta) 283 (247 ± 18; n = 9); γ (gamma) 238 (188 ± 18; n = 9); δ (delta) 295 (240 ± 27; n = 9). Tarsomeres III and IV with thin spines, implanted at two levels, one apical and the other median. Anterior leg lost in the holotype, anterior, median and posterior femur in the other examples measuring 643 ± 22 (n = 8), 680 (623 ± 24; n = 7), and 725 (693 ± 26; n = 7) respectively. Tibia of anterior, median and posterior leg measuring 762 ± 21 (n = 8), 1042 (934 ± 24; n = 7), and 1258 (1139 ± 56; n = 6) respectively. Tarsomere I of anterior, median and posterior leg 412 ± 12 (n = 8), 589 (499 ± 26; n = 7) and 680 (574 ± 25; n = 6) respectively.

Abdomen (Table III): tergal papillae absent. Gonocoxite: 289 (279 ± 13; n = 9) long by 50 (64 ± 5; n = 9) wide. Basal tuft of bristles absent, only few sparse bristles in median area. Gonostyle measuring 139 (134 ± 5; n = 9) long, presenting four strong spines, one being apical, one external superior implanted on the apical fourth, one external and one internal. Latter two are implanted at same level, on the apical half of the structure. Pre-apical bristle absent. Paramere digitiform, wide at base and with a sharp edge to ventral region, with groups of fine bristles in the dorsal margin of the apical dorsal region and ventral margin, implanted on the widest part of the paramere. Lateral lobe 269 (267 ± 12; n = 9) long, with ratio gonocoxite/lateral lobe 1.07 (1.05 ± 0.07; n = 9). Aedeagus conical. Ejaculatory pump with wide body. Genital filament

TABLE III

Measurements (µm) of the structures of the abdomen in males of *Nyssomyia intermedia* and *Nyssomyia neivai*

Structure	<i>Nyssomyia intermedia</i>					<i>Nyssomyia neivai</i>				
	X	SD	Max	Min	N	X	SD	Max	Min	N
Gonocoxite length	292,42	15,20	316	260	19	279,13	12,62	302	266	9
Gonocoxite width	70,37	7,90	84	55	19	63,56	4,95	69	53	9
Gonostyle length	140,74	7,12	153	122	19	134,22	5,26	141	127	9
Lateral lobe length	281,26	12,78	306	258	19	267,33	11,76	288	252	9
Lateral lobe width	25,11	3,61	33	22	19	26,56	1,94	30	25	9
Ejaculatory pump	219,05	7,31	233	203	19	195,56	8,14	211	186	9
Genital filament	297,79	19,78	325	255	19	291,89	10,58	313	277	9

X: mean; SD: standard deviation; max: maximum; min: minimum; N: number of specimens examined

short, ratio genital filament/ejaculatory pump 1.60 ( $1.49 \pm 0.08$ ;  $n = 9$ ). Tip of genital filament distended, in form of shallow spoon (Fig. 2).



Fig. 2: *Nyssomyia neivai* ♂: tip of the genital filaments (1000X)

#### Redescription of females of *N. intermedia* and *N. neivai* *N. intermedia*

Head (Table IV): ratio between lengths of clypeus/head 0.49 ( $0.48 \pm 0.04$ ;  $n = 19$ ), and eyes/head 0.88 ( $0.88 \pm 0.03$ ;  $n = 19$ ). Labro-epipharynx long, ratio labro-epipharynx/head 1.36 ( $1.37 \pm 0.07$ ;  $n = 19$ ) and AIII/labro-epipharynx 0.65 ( $0.64 \pm 0.03$ ;  $n = 19$ ). Ascoids implanted at same level, without basal prolongation basal, distal prolongation being long, reaching apex of subsequent segment. Antennal formula AIII-AXIII 2; AXIV-AXVI 0. Simple bristles absent from AIII to AXIII. AXV shorter than AXVI ( $n = 11$ ), may be equal ( $n = 3$ ) or shorter ( $n = 2$ ). Palpal formula highly variable, most common being 1.4.5.2.3 ( $n = 11$ ), but 1.4.2.5.3 ( $n = 5$ ), and 1.4.(2.5).3 ( $n = 4$ ) also occurring. Newstead's spines grouped in median region

of 3th palpal segment, or also implanted on apical fourth in small groups, close to each other. Lacinia of maxilla presents two longitudinal groups of 8-10 external teeth ( $n = 19$ ). Cibarium with 8-10 (usually 8) horizontal teeth. Vertical teeth strong and numerous, number varying between 16 and 24. Cibarial arch complete and pigment patch well defined. Pharynx without teeth, with small rugosities in its apical region.

Cervix: ventro-cervical sensilla absent.

Thorax (Table V): colour and presence of bristles as in male. Wing 623 ( $621 \pm 24$ ;  $n = 19$ ) long, R5 1394 ( $1350 \pm 54$ ;  $n = 19$ ) long. Principal wing indices:  $\alpha$  (alpha) 544 ( $557 \pm 45$ ;  $n = 19$ );  $\beta$  (beta) 295 ( $310 \pm 32$ ;  $n = 19$ );  $\gamma$  (gamma) 192 ( $215 \pm 21$ ;  $n = 19$ );  $\delta$  (delta) 306 ( $298 \pm 51$ ;  $n = 19$ ). Tarsomeres III and IV with thin spines, implanted at two levels, one apical and the other median. Femur of anterior, median and posterior leg measuring 702 ( $683 \pm 26$ ;  $n = 19$ ), 714 ( $686 \pm 26$ ;  $n = 19$ ), and 770 ( $750 \pm 32$ ;  $n = 19$ ) long respectively. Tibia of anterior, median and posterior leg measuring 816 ( $805 \pm 40$ ;  $n = 19$ ), 1031 ( $1017 \pm 45$ ;  $n = 18$ ), and 1280 ( $1252 \pm 75$ ;  $n = 19$ ) long respectively. Tarsomere I of anterior, median and posterior leg measuring 442 ( $429 \pm 24$ ;  $n = 19$ ), 555 ( $548 \pm 31$ ;  $n = 18$ ), and 634 ( $624 \pm 33$ ;  $n = 19$ ) long, respectively.

Abdomen (Table VI): spermathecae annulated (Fig. 3), measuring 47 ( $56 \pm 6$ ;  $n = 19$ ) long by 17 ( $17 \pm 1$ ;  $n = 19$ ) wide (Fig. 3). Number of annulations varying between 8-15, usually 10 or 11. Sum of annulations in two spermathecae varying from 17-33, usually 22. Head of spermatheca about 11 ( $11 \pm 2$ ;  $n = 19$ ) long, 12 ( $12 \pm 2$ ;  $n = 19$ ) broad at its apex and 10 ( $10 \pm 2$ ;  $n = 19$ ) at base, simple form predominating but simple/bilobed and bilobed forms also occurring. Individual ducts striated, with some excrescences in its insertion with the spermatheca, measuring 97 ( $99 \pm 12$ ;  $n = 19$ ) long. Common duct 47 ( $48 \pm 10$ ;  $n = 19$ ) long (Fig. 4). Ratio individual/common ducts 2.06, varying between 1.20 and 3.05 ( $2.14 \pm 0.52$ ;  $n = 19$ ) (Fig. 5). Bristles without special characteristics, measuring 144 ( $144 \pm 10$ ;  $n = 19$ ) long.

TABLE IV

Measurements ( $\mu\text{m}$ ) of the structures of the head and its appendages in females of *Nyssomyia intermedia* and *Nyssomyia neivai*

Structure	<i>Nyssomyia intermedia</i>					<i>Nyssomyia neivai</i>				
	X	SD	Max	Min	N	X	SD	Max	Min	N
Head length	251,37	11,46	280	230	19	266,89	7,54	280	255	9
Eye length	222,89	10,20	250	211	19	234,22	8,23	249	224	9
Interocular distance	119,95	7,27	138	105	19	117,00	10,31	139	100	9
Clypeus length	120,37	9,53	142	109	19	121,78	5,83	130	116	9
Labrum-epipharynx length	346,89	20,69	402	302	19	350,56	20,34	369	316	9
AIII	221,79	13,59	253	200	19	226,00	10,31	241	208	8
AIV	95,47	3,99	103	89	19	96,00	4,28	103	89	8
AV	95,47	4,36	105	89	19	96,63	2,50	100	94	8
P1	42,21	3,12	47	36	19	42,25	3,01	44	39	8
P2	154,11	8,45	169	139	19	158,00	3,21	161	152	8
P3	169,16	9,78	191	153	19	175,63	3,78	177	172	8
P4	59,84	5,05	69	50	19	67,75	5,28	72	58	8
P5	148,74	6,64	161	139	19	153,38	5,88	161	147	8

X: mean; SD: standard deviation; max: maximum; min: minimum; N: number of specimens examined

TABLE V  
Measurements (µm) of the structures of the thorax in females of *Nyssomyia intermedia* and *Nyssomyia neivai*

Structure	<i>Nyssomyia intermedia</i>					<i>Nyssomyia neivai</i>				
	X	SD	Max	Min	N	X	SD	Max	Min	N
Wing width	621,21	24,00	657	578	19	633,00	21,64	657	600	8
R5	1349,84	53,60	1427	1235	19	1321,38	15,21	1337	1292	8
Alpha	557,32	45,46	646	435	19	579,25	34,26	634	533	8
Beta	310,05	32,21	374	238	19	285,00	18,47	306	261	8
Gamma	215,26	20,88	260	181	19	223,75	21,39	249	193	8
Delta	297,53	50,75	408	204	19	313,00	16,46	351	306	8
Anterior femur	682,58	26,49	736	646	19	696,83	20,49	714	668	6
Median femur	686,16	25,93	736	657	19	690,80	14,31	702	668	5
Posterior femur	750,00	32,14	827	691	19	759,00	18,94	793	736	8
Anterior tibia	805,47	39,73	884	725	19	779,83	15,28	793	759	6
Mean tibiae	1016,78	45,42	1099	929	19	997,00	37,15	1042	952	5
Posterior tibia	1252,16	74,98	1427	1110	19	1215,13	28,33	1269	1190	8
First anterior tarsomere	429,32	23,77	487	397	19	413,50	6,35	419	408	4
First median tarsomere	548,22	30,64	612	510	19	524,50	19,63	533	499	4
First posterior tarsomere	623,58	32,55	691	578	19	600,29	14,39	623	589	7

X: mean; SD: standard deviation; max: maximum; min: minimum; N: number of specimens examined

TABLE VI  
Measurements (µm) of the structures of the abdomen in males of *Nyssomyia intermedia* and *Nyssomyia neivai*

Structure	<i>Nyssomyia intermedia</i>					<i>Nyssomyia neivai</i>				
	X	SD	Max	Min	N	X	SD	Max	Min	N
Spermatheca length	56,26	5,74	67	47	19	41,70	2,50	44	36	10
Spermatheca width	16,89	1,20	19	14	19	13,70	0,95	14	11	10
Head of spermatheca length	11,16	2,12	14	8	19	8,30	0,95	11	8	10
Head of spermatheca width in the apex	11,79	2,42	14	8	19	7,90	1,37	89	55	6
Head of spermatheca width in the base	10,25	1,65	14	8	19	6,21	0,63	8	6	18
Individual duct length	98,89	11,71	128	86	19	71,33	11,34	89	55	6
Common duct length	48,21	10,16	72	33	19	21,60	4,67	28	17	5
cerca length	143,53	10,18	161	125	19	148,10	10,52	166	130	10

X: mean; SD: standard deviation; max: maximum; min: minimum; N: number of specimens examined

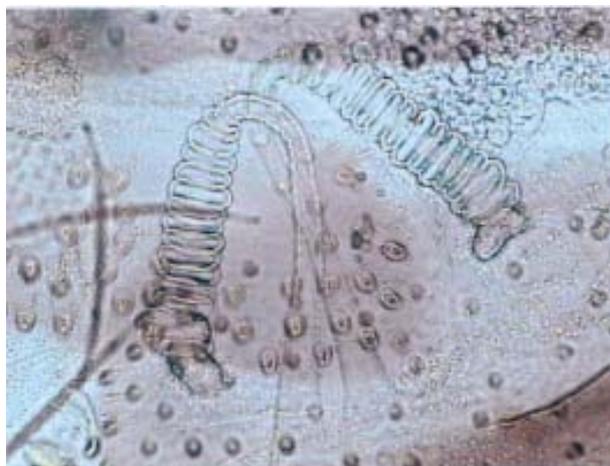


Fig. 3: *Nyssomyia intermedia* ♀: spermatheca (400X)

*N. neivai*

Head (Table IV): ratio between lengths of clypeus and head 0.47 (0.46 ± 0.03; n = 9), eyes/head 0.91 (0.88 ± 0.02; n = 9). Labro-epipharynx long, ratio labro-epipharynx/head

1.31 (1.31 ± 0.09; n = 9) and AIII/labro-epipharynx 0.67 (0.64 ± 0.04; n = 9). Ascoids implanted at same level in all segments, without presenting basal prolongation, apical prolongation reaching to apex of next segment. Antennal formula AIII-AXIII 2; AXIV-AXVI 0. Simple bristles absent on AIII-AXIII; pre-apical papillae present on AIII, AIV, AXIV, AXV and AXVI. AXV shorter than AXVI (n = 4). Two palpal formulae encountered, i.e., 1.4.5.2.3 (n = 7) and 1.4.(2.5).3 (n = 2). Newstead's spines found dispersed over median region of 3rd palpomere, or in two or three small groups. Lacinea of maxilla presenting two longitudinal groups of 7-10 external teeth (n = 9). Cibarium with 8-10 horizontal teeth, usually 10. Vertical teeth strong, number varying between 17-24. Cibarial arch complete and pigment patch well defined. Pharynx without teeth, with small rugosities in apical region.

Cervix: ventro-cervical sensilla absent.

Thorax (Table V): colour and presence of bristles as in male. Wings measuring 567 (633 ± 22; n = 8) wide, R5 1178 (1321 ± 15; n = 8) long. Principal wing indices: α (alpha) 465 (579 ± 35; n = 8); β (beta) 329 (285 ± 18; n = 8); γ (gamma) 204 (224 ± 21; n = 8); δ (delta) 215 (313 ± 16; n = 8). Tarsomeres III and IV with thin spines, implanted at two

levels, one apical and other median. Femur of anterior, median and posterior leg measuring  $634 (697 \pm 20; n = 6)$ ,  $634 (691 \pm 14; n = 5)$  and  $748 (759 \pm 19; n = 8)$  long respectively. Tibia of anterior, median and posterior leg measuring  $668 (780 \pm 15; n = 6)$ ,  $884 (997 \pm 37; n = 5)$ , and  $1190 (1215 \pm 28; n = 8)$  long respectively. Tarsomere I of anterior, median and posterior leg measuring  $363 (440 \pm 6; n = 4)$ ,  $442 (525 \pm 20; n = 4)$ , and  $589 (600 \pm 14; n = 7)$  long, respectively.

Abdomen (Table VI): spermatheca formed by 7-10 (usually 8 or 9) annulations (Fig 4), measuring  $39 (42 \pm 3; n = 10)$  long by  $14 (14 \pm 1; n = 10)$  wide (Fig. 5). Sum of annulations of two spermathecae varying between 13-22, usually 17. Head of spermatheca simple in the majority of examples, measuring  $8 (8 \pm 1; n = 10)$  long and  $8 (8 \pm 1; n = 10)$  at its apex, and six ( $6 \pm 1; n = 18$ ) at its base. Individual ducts lightly striated, measuring  $64 (71 \pm 11; n = 6)$  long. Common duct  $22 (22 \pm 5; n = 5)$  long (Fig. 6). Ratio individual/common duct 2.91, varying from 2.68-4.41, ( $3.42 \pm 0.73; n = 5$ ) (Fig. 6). Cercæ without special characteristics, measuring  $152 (148 \pm 11; n = 10)$  long.



Fig. 4: *Nyssomyia neivai* ♀: spermatheca (400X)

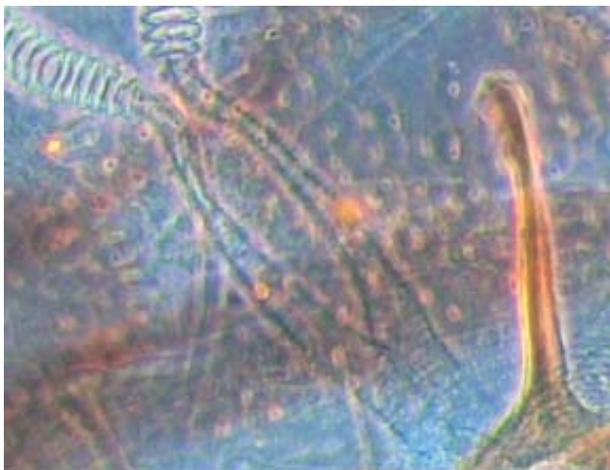


Fig. 5: *Nyssomyia intermedia* ♀: individual and common ducts of the spermathecae (400X)



Fig. 6: *Nyssomyia neivai* ♀: individual and common ducts of the spermathecae (400X)

#### MATERIALEXAMINED

*N. intermedia* - Material collected at Além Paraíba, Minas Gerais by JD Andrade Filho, with Falcão light trap, on 27/08/2002, deposited in the collection of the Centro de Pesquisas René Rachou-Fiocruz, total number still not determined, with entry number 3143/02.

*N. neivai* - Holotype male, collected at Instituto Butantan, São Paulo, by R Fischer, on 02/07/1926, deposited in collection of Instituto Oswaldo Cruz-Fiocruz, with accession number 2267. The other examples used were laboratory-reared examples of this species (F2), collected in the municipality of Conchal, São Paulo, by C Casanova.

#### DISCUSSION

Based on morphological characteristics such as absence of ventro-cervical sensilla, and pilosity in the anterior region of the catapisternum, only one apical papilla present on AIII, 4th + 5th palpomeres longer than the 3rd and Newstead's spines absent on the 2nd palpomere, the two species can be placed with confidence within the genus *Nyssomyia*.

The males of the two species can be separated from the other species of the genus by the combination of the following characters: absence of simple bristles on the basal segments of the antennae and short genital filaments, the genital filament/ejaculatory pump ratio being less than 1.60 with the body of the pump very much wider than in the other species. Females may be separated from the other species by absence of simple bristles on the basal segments of the antennae, by number of cibarial teeth, by the shape and number of annulations of the spermathecae and by the length of the ducts. The coloration of the thorax also

facilitates separation of *N. intermedia* and *N. neivai* from some species.

Without doubt *N. intermedia* and *N. neivai* are valid species that can be separated from each other. Although *Phlebotomus mazzai* Paterson, 1926 was described from material from Argentina (Paterson 1926) in the same year as *N. neivai* was recognized, there is no record of the date and month the description was made. According to the International Code for Zoological Nomenclature, chapter 5, article 21, when this occurs the last day of the year of publication should be taken as the description date (ICZN 1999), i.e., 31 December 1926 in this case. We thus conclude that *N. neivai*, whose formal description was published on 30 June 1926, is the senior synonym of *P. mazzai*. This synonymy also was verified by Marcondes and Lozovei (1999).

No morphological features of the head or thorax were found that permitted separation of the two species. The genital filament tips were distinct, however. In *N. intermedia* the tip has the form of a deep spoon, while in *N. neivai* it is markedly shallower.

The great variation found in the number of horizontal cibarial teeth do not permit separation of the females (Andrade Filho 2003). The number of spermathecal annulations may aid in the correct identification, but this feature is inadequate in the majority of cases, given that values for the two species overlap. Other aspects of this structure may be useful, however. The spermatheca of *N. intermedia* frequently present their maximum width in the apical annulations, diminishing gradually to the basal annulations, while in *N. neivai* the central annulations are normally broadest. Another important feature of the spermathecae of *N. intermedia* is that they may present some irregular, asymmetric annulations, which rarely occurs in *N. neivai*. The sum of the annulations of the spermathecae may also be useful, almost half of the examples studied presenting less than 16 annulations in *N. neivai* and more than 23 in *N. intermedia*.

The shape of the head of the spermatheca may assist in the correct separation of the species, given that it is rarely bilobed in *N. neivai*. In addition, the spermathecal head of *N. intermedia* is always wider both at the apex and the base, giving it a more robust aspect than that of *N. neivai*.

The individual and common ducts of the spermatheca may also be useful. The common duct is always longer in *N. intermedia* while the individual ducts are more striated than those of *N. neivai* and show excrescences where they join with the spermathecae.

The group of characters described above or the presence of at least some of them permit females of *N. intermedia* and *N. neivai* to be separated with complete confidence. Based solely on morphology it was impossible to determine whether a third species was also present. Although unlikely to be the case, this question could be resolved using molecular techniques.

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