

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Variations in the Gonostyle of *Nyssomyia intermedia* (Lutz & Neiva)
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ABSTRACT - This paper describes sand flies similar to *Nyssomyia intermedia* (Lutz & Neiva) with variations in the number of spines at the gonostyle and tests the hypothesis whether these specimens belong or not to *N. intermedia* species. Using Principal Component Analysis and Neighbour Joining, the measurements of 15 structures of the phlebotomine with variations in number of spines were compared with measurements of 30 sand flies of *N. intermedia* species. Both analyses didn't cluster the specimens with variation in spines number in one group and provided evidence to the hypothesis that those sand flies belong to *N. intermedia*.

KEY WORDS: Anomaly, morphological variation, sand fly, taxonomy

Among Neotropical sand flies species, *Nyssomyia intermedia* (Lutz & Neiva) is pointed as the principal vector of *Leishmania (Viannia) braziliensis*, the etiologic agent of american tegumentary leishmaniasis (ATL), in modified environments in the Southeastern region of Brazil (Tolezano *et al* 1980, Falqueto *et al* 2003).

Although males of *N. intermedia* present high morphological similarities with *Nyssomyia neivai* (Pinto) males, they can be distinguished by the form of the tip of the ejaculatory ducts: "deep spoon" form in *N. intermedia* and "shallow" form in *N. neivai* (Andrade Filho *et al* 2003). In the Southeastern region the two species occur in sympatry only in the states of Minas Gerais and São Paulo (Andrade Filho *et al* 2007).

The right taxonomic identification of *N. intermedia* is fundamental in the elaboration of ATL prevention measures based on the geographic distribution of this vector. However, the occurrence of morphological variations might cause confusion resulting in failure in the taxonomic position of a particular specimen, as it occurred with *Martinsmyia alphabetica* (Fonseca) male (Coutinho & Barretto 1940).

Some authors have reported variations in the genitalia of sand flies (Sherlock *et al* 1958, Ximenes *et al* 2002, Andrade Filho *et al* 2004), including *N. intermedia* (Marcondes 1999). However, most of these variations are not symmetrical and can occur in the antennae, palps, cibarium, female spermathecae, and male terminalia. As these structures are bilateral, the comparisons between the two sides of the body allow the correct specific identification (Andrade Filho *et al* 2004). However, with the occurrence of bilateral variations, such comparisons are often not precise being necessary the

inclusion of comparisons between other structures for the correct species identification.

Material and Methods

During studies in the locality of Belém (20°47'29"S; 41°00'06"W), municipality of Vargem Alta, Espírito Santo state, 12 sand fly specimens with five spines in each gonostyle (Fig 1b) and two sand flies with three spines in each gonostyle (Fig 1c) were captured. Although the specimens were similar to *N. intermedia* and *N. neivai* on external morphology, based on the "deep spoon" form of the final portion of the ejaculatory ducts, all these individuals would be included in the *N. intermedia* species. However, *N. intermedia* (Fig 1a) has four spines in each gonostyle, so these aberrant specimens do not fit well into the normal morphology of *N. intermedia*. Thus, in the present work, these 14 specimens are described and the hypothesis whether these individuals belong to *N. intermedia* is tested.

The specimens with five and with three spines in each gonostyle were examined and measured with a microscope calibrated for morphometric exams. Following the Andrade Filho *et al* (2004) proposal, the three first flagellomeres, palpomeres, head, clypeus, ejaculatory pump, genital filaments, lateral lobe, gonocoxite and gonostyle were measured. Measurements of these structures were compared with those from 30 specimens of *N. intermedia* collected in the same locality. The nomenclature and the terminology of the characters followed Galati (2003).

Comparison among the measurements of sand flies

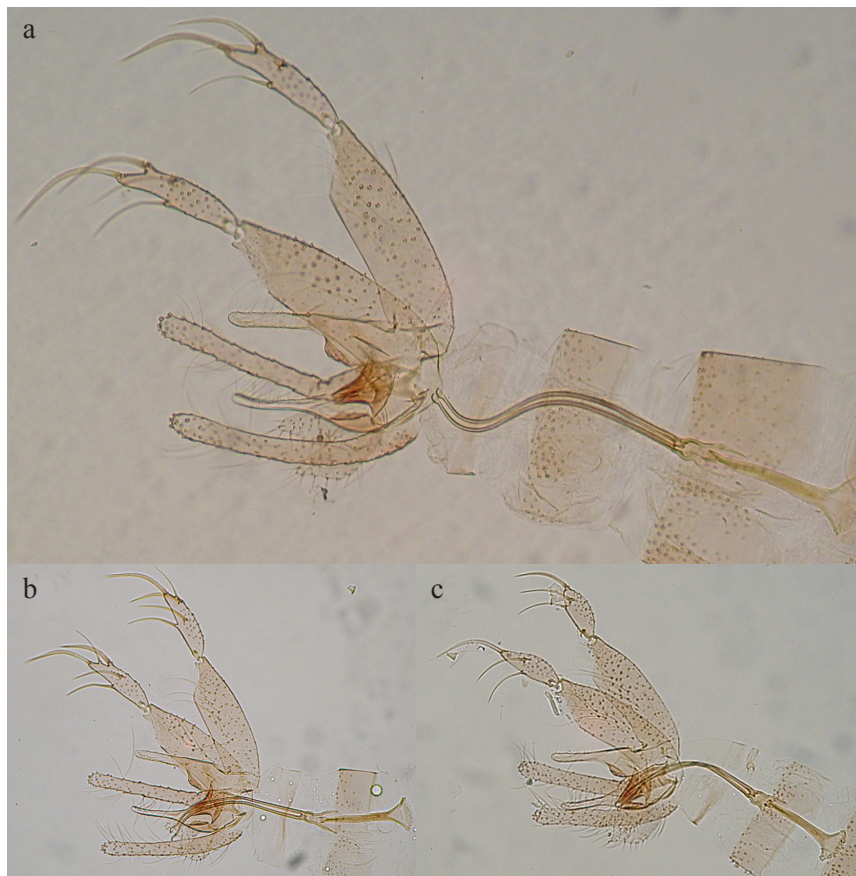


Fig 1a) Terminalia of *Nyssomyia intermedia*; b) terminalia of specimen similar to *N. intermedia* with five spines in each gonostyle, c) terminalia of specimen similar to *N. intermedia* with three spines in each gonostyle.

with five and with three spines in each gonostyle and the measures of the 30 specimens of *N. intermedia* was done by multivariate analysis of Principal Components (PCA) and by Neighbour Joining (NJ) analysis. The analyses were performed using the PAST program (Hammer *et al* 2001). A sand fly sample with five spines in the gonostyle was not used in the analyses due to the absence of some structures that could have been lost during collection and/or handling for identification.

The voucher specimens of sand flies with variations in the number of spines in the gonostyle are deposited in the Phlebotomine Collection of the Instituto de Pesquisas René Rachou-Fiocruz, Belo Horizonte, Minas Gerais, Brazil.

Results and Discussion

The relation of the measured structures, their mean and their standard deviations for the sand fly samples with five and three spines in the gonostyle and for the samples of *N. intermedia* are plotted in the Table 1. The measurements of the structures of the sand flies with five and with three spines in each gonostyle do not show significant difference in relation to the measurements of the specimens of *N. intermedia* studied (Fig 2) and neither with distinct groups (Fig 3).

Nyssomyia intermedia shows four spines in the gonostyle,

being one apical, one external subapical, one external proximal and one internal proximal. The proximal spines are implanted at the same level (Fig 1a). In the sand flies specimens with five spines in each gonostyle, except for the presence of a spine between the apical and the external subapical (Fig 1b), the implantation of the spines follows the same pattern as in *N. intermedia*. In the same way, the specimens with three spines in each gonostyle also present the same pattern of implantation of spines in the gonostyles as *N. intermedia*, except for the absence of the subterminal external spine (Fig 1c). This corroborates the hypothesis that we are dealing with individuals with intraspecific variation.

The analysis of Principal Components grouped individuals with and without variation in the number of gonostyle spines, offering evidence that favors the hypothesis that all the analyzed sand flies belong to the species *N. intermedia* (Fig 2). This hypothesis was also corroborated by the Neighbour Joining analysis that did not group in the same cluster all the individuals with the same number of spines in each gonostyle (Fig 3).

Therefore, the association between the morphological similarity and the evidences obtained by PCA and NJ similarity support the hypothesis that the examined sand fly samples with symmetric bilateral variations in the number of gonostyle spines belong to *N. intermedia* species. Furthermore, adding molecular data could explain the significance of the variation

Table 1 Mean and standard deviations, in micrometers, of the 15 measured structures of sand flies morphologically similar to *Nyssomyia intermedia* with five and with three spines in each gonostyle, and of samples of *N. intermedia*.

Measured structures	With five spines in the gonostyle (n = 11)		With three spines in the gonostyle (n = 2)		<i>N. intermedia</i> (n = 30)	
	Mean	St. deviation	Mean	St. deviation	Mean	St. deviation
Clypeus	101.56	8.87	109.00	7.70	96.82	9.12
Ejaculatory pump	231.87	9.55	226.17	3.85	237.07	5.85
Flagellomere I	265.07	13.18	264.33	3.85	263.05	9.80
Flagellomere II	118.91	6.81	117.18	3.85	121.90	3.91
Flagellomere III	118.91	6.81	117.18	3.85	121.89	3.90
Genital filaments	336.41	9.14	340.62	11.56	340.08	3.38
Gonocoxite	320.55	11.12	318.82	11.56	319.00	5.30
Gonostyle	154.58	7.01	149.87	19.26	155.32	4.46
Head	271.01	3.52	272.50	0.00	274.49	4.16
Lateral lobe	312.63	11.24	313.37	3.85	315.01	8.14
Palpomere I	29.23	2.74	29.97	3.85	30.15	2.76
Palpomere II	129.31	5.49	130.80	0.00	129.52	4.21
Palpomere III	142.69	5.34	141.7	0.00	150.78	5.41
Palpomere IV	57.96	3.67	57.22	3.85	56.49	2.67
Palpomere V	150.12	6.14	149.87	11.56	141.88	5.05

in number of spines, as could examination of females of *N. intermedia* from the same geographic region.

Finally, the present study reports the finding of variations in the gonostyle of *N. intermedia*, and given the rarity of this occurrence in sand flies, the above observations are important to avoid taxonomic misidentification and erroneous descriptions of new species.

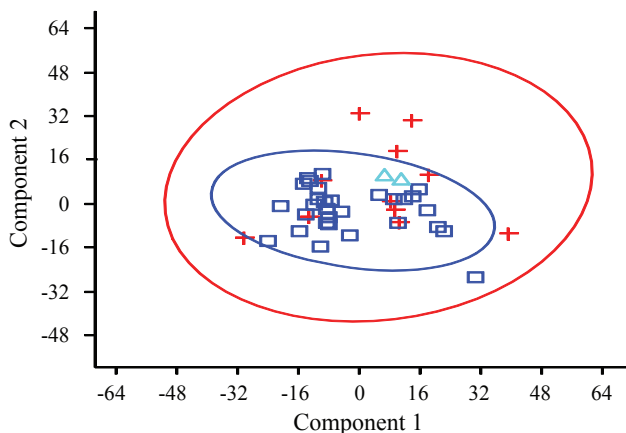


Fig 2 Analysis of principal components “1” and “2” using matrix of covariance obtained with the program PAST using measures of 15 morphological structures of 30 *Nyssomyia intermedia* samples (“□”), 11 sand flies samples similar to *N. intermedia* with five spines in each gonostyle (“+”) and two samples similar to *N. intermedia* with three spines in each gonostyle (“▲”).

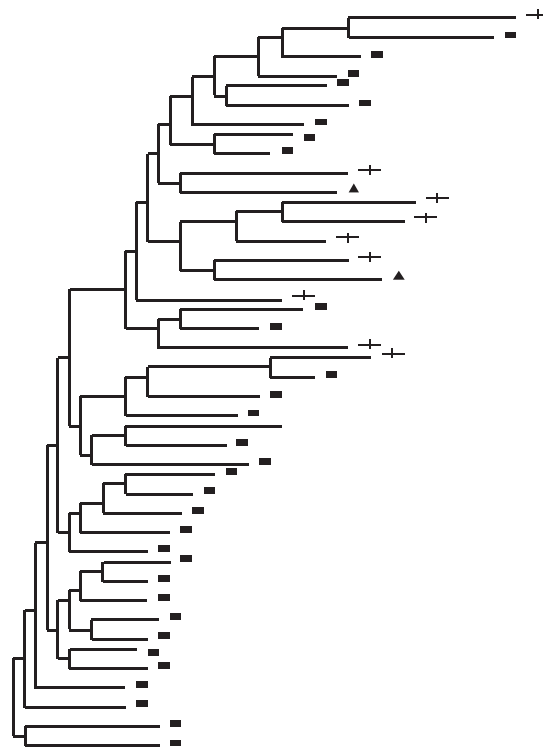


Fig 3 Neighbour Joining tree obtained with the program PAST using measures of 15 morphological structures of *Nyssomyia intermedia* (“□”) (n = 30) and of samples of sand flies similar to *N. intermedia* with five spines (“+”) (N = 11) and with three spines in each gonostyle (“▲”) (n = 2).

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