Nyssomyia intermedia (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae: Phlebotominae) geographical distribution and epidemiological importance

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Nyssomyia intermedia *(Lutz & Neiva 1912) and* N. neivai *(Pinto 1926) are possible vectors of tegumentary leishmaniasis in some regions of Brazil. Further, the latter was until recently, considered a junior synonym of the former. This study has the purpose of updating our knowledge of the geographical distribution of these species, based on specimens deposited at the collection of the Centro de Pesquisas René Rachou-Fiocruz, Faculdade de Saúde Pública-Universidade de São Paulo, and on data presented by literature as also to associate this distribution with the cutaneous leishmaniasis cases reported. It has been reported that* N. intermedia *occurs in the states of the Northeastern Region, in Rio de Janeiro, Espírito Santo, on the northern coast of São Paulo, in eastern Minas Gerais, Mato Grosso do Sul, and Goiás, close to the border with Minas Gerais and Bahia.* N. neivai *occurs in the Southern Region, southern coast and in western São Paulo, southern and western Minas Gerais, southern Goiás, and southern Pará, beyond Argentina, Bolivia, and Paraguay. It is important to highlight that* N. intermedia *and* N. neivai *occur in sympatry in Minas Gerais and São Paulo.* N. intermedia *or* N. neivai *are predominant or are captured abundantly in several cutaneous leishmaniasis foci in the Southeastern and Southern regions of Brazil.*

Key words: epidemiology - geographical distribution - *Nyssomyia intermedia*- *Nyssomyia neivai* - leishmaniasis - Phlebotominae

Knowledge of the sand fly's geographical distribution is fundamental to an understanding of features related to the transmission of some diseases, mainly leishmaniases, thus indicating areas at risk of acquiring infections. An accurate identification of sand fly species and of the leishmania vectors is therefore important.

Nyssomyia intermedia (Lutz & Neiva 1912) and *N. neivai* (Pinto 1926) are captured in various parts of Brazil and show a remarkable intraspecific and intrapopulational variation gradient (Andrade Filho et al. 2006). They are morphologically close (Marcondes 1996, Andrade Filho et al. 2003), present great behavioral similarities, have been collected in both forests and anthropic environments, are anthropophilic, have already been found naturally infected with trypanosomatids, *N. neivai* (Forattini & Santos 1952, Forattini et al. 1972, Casanova et al. 1995, Córdoba-Lanus et al. 2006) and *N. intermedia* (Aragão 1922, Rangel et al. 1984, Pita-Pereira et al. 2005), and are strongly suspected of being implicated in transmission of tegumentary leishmaniasis agents (Lainson & Rangel 2003).

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Until recently, the two species were considered synonymous (Martins et al. 1978 Young & Duncan 1994), However, Marcondes (1996) considered them as valid species, though he only succeeded indistinguishing the females. The morphological distinction of the males was presented by Andrade Filho et al. (2003).

The present work aims at updating on knowledge of the geographical distribution of *N. intermedia* and *N. neivai*, and at discussing their occurrence in cutaneous leishmaniasis endemic areas.

MATERIALS AND METHODS

Adult samples of *N. intermedia* and *N. neivai* from the collection of Centro de Pesquisas René Rachou-Fiocruz and Faculdade de Saúde Pública-Universidade de São Paulo were examined in order to verify their geographical distribution. Besides these data, those given by Marcondes et al. (1998), who accurately identified some of the species involved, and also those more recently reported in the literature, were taken into consideration. These literatures had been cited in each locality where the species were identified.

The bibliographical reference for each county is presented between brackets after the county's name. When the distribution has been mentioned by another author or other authors and also in this present study, the reference is given and personal observations are added.

We have made an attempt to associate the occurrence of *N. intermedia* and/or *N. neivai* with the cutaneous leishmaniasis cases reported in the literature and also with reports sent in by the state health secretaries of Espírito Santo, Paraná, and Rio de Janeiro.

RESULTS

A total of 4000 specimens, all previously identified as *N. intermedia*, were examined.

The geographical distribution of the two species is present below.

Nyssomyia intermedia

BRASIL: Piauí – Teresina (Marcondes et al. 1998), Ceará – Crato (JDAF's observations); Rio Grande do Norte – Coronel João Pessoa and Dr Severiano (Ximenes et al. 2000) Alagoas – Palmeira dos Índios (JDAF's observations). Pernambuco – Jaboatão dos Guararapes, Quipapá (JDAF's observations; Marcondes et al. 1998). Bahia – Barreiras, Ilhéus (Marcondes et al. 1998), Santo Amaro (Follador et al. 1999). Espírito Santo – Afonso Cláudio (Ferreira et al. 2001), Aracruz, Colatina, Ecoporanga, Itaguaçu, Linhares, Mimoso do Sul, Santa Leolpoldina, Santa Tereza, Serra (Marcondes et al. 1998), Tancredinho (EABG's observations), Venda Nova do Imigrante (Marcondes et al. 1998), Viana (JDAF's observations; Marcondes et al. 1998), Vitória (JDAF's observations). Goiás – Sitio D'Abadia (JDAF's observation). Mato Grosso do Sul – Miranda (JDAF's observation). Minas Gerais – Aimorés, Além Paraíba (Marcondes et al. 1998, Brazil et al. 2006), Alto Caparaó (Saraiva et al. 2006), Araçuaí (Gontijo et al. 2002), Arinos, Barra Longa, Belo Horizonte, Bocaiúva, Buenópolis, Buritizeiro, Caeté, Caparaó (Saraiva et al. 2006), Caratinga, Cava Grande, Conceição de Ipanema, Conselheiro Pena, Coroací, Coronel Fabriciano, Curvelo, Corinto, Dom Cavati, Diamantina, Felipe dos Santos, Galiléia, Governador Valadares, Iapu, Itambacurí, Itanhomi, Januária, Juiz de Fora, Lagoa Santa (JDAF's observations, Marcondes et al. 1998), Lassance (JDAF's observations, Marcondes et al. 1998), Leolpoldina, Manhuaçu, Mantena, Mariana, Marliéria, Mutum, Pirapora, Pocrane, Pompeu (Marcondes et al. 1998), Porteirinha, Rio Casca, Santa Bárbara, São João do Paraíso, Silveirânea, Tarumirim, Timóteo (Andrade Filho et al. 1997), Viçosa (Santos de Marco et al. 2002), Volta Grande (JDAF's observations). Rio de Janeiro – Angra dos Reis (EABG's observation) Araruama, Cachoeiras de Macacu (Marcondes et al. 1998), Cardoso Moreira (Marcondes et al. 1998), Casimiro de Abreu (Souza et al. 2001), Cordeiro (Marcondes et al. 1998), Duque de Caxias, Ibicuí, Itaboraí, Itacurussa, Itaguaí (Marcondes et al. 1998), Itaperuna, Macaé, Magé, Muriqui, Niterói (JDAF's observations, Marcondes et al. 1998), Nova Iguaçu, Parati (Marcondes et al. 1998), Petrópolis (JDAF's observations, Souza et al. 2005), Rio Bonito (JDAF's observations, Marcondes et al. 1998), Rio de Janeiro, Silva Jardim (Souza et al. 2001), Teresópolis, Valença, Vassouras (JDAF's observations). São Paulo – Bananal (Marcondes et al. 1998), Barra do Turvo (EABG's observations), Caraguatatuba (Marcondes et al. 1998), Eldorado (EABG'observations), Ilha Bela (EABG's observations), Iporanga (JDAF's observations, Galati et al. 2002), Itariri, Juquiá (EABG's observations), Pariqüera Açu (Marcondes et al. 1998), Pedro de Toledo (EABG's observations), São Sebastião (JDAF's observations, Marcondes et al. 1998, Brito et al. 2002), Taubaté (Casanova, unpublished data), Ubatuba (Marcondes et al. 1998).

Nyssomyia neivai

BRAZIL: Pará – São Geraldo do Araguaia (Galati, unpublished data). Goiás – Corumbaíba, Itumbiara (JDAF's observations, Marcondes et al. 1998). Mato Grosso do Sul – Bataguassu (EABG's observations). Minas Gerais – Alfenas, Arinos, Bambuí (JDAF's observations, Marcondes et al. 1998), Betim, Conceição do Rio Verde, Coração de Jesus, Corinto, Divinópolis, Esmeralda, João Pinheiro (Marcondes et al. 1998), Lagoa Santa, Lassance, Paracatu, Passos, Pedro Leolpoldo, Perdões (JDAF's observations, Marcondes et al. 1998), Pirapora, Presidente Olegário, Tupaciguara, Uberlândia (JDAF's observations). São Paulo – Angatuba, Araçatuba, Araraquara, Atibaia (Marcondes et al. 1998), Barra do Turvo, Barueri, Caçapava (EABG's observations), Cajamar (Marcondes et al. 1998), Cajati (EABG's observation), Capela do Alto (Marcondes et al. 1998), Conchal (Casanova et al. 2005), Dourado, Eldorado (Marcondes et al. 1998, EABG's observations), Iporanga (Marcondes et al. 1998, Galati et al. 2002), Itariri (EABG's observations), Itupeva (Marcondes et al. 1998), Juquiá, Luis Antônio (EABG's observations), Miracatu, Monte, Natividade da Serra (Marcondes et al. 1998), Osasco, Ourinhos (EABG's observations), Pariqüera Açu, Pedro de Toledo, Pereira Barreto, Pirapora do Bom Jesus, Porto Ferreira (Marcondes et al. 1998), Ribeirão Branco (EABG's observations), São José dos Campos (EABG's observations), São Luís do Paraitinga (Marcondes et al. 1998), São Paulo (JDAF's observations, Marcondes et al. 1998), São Roque (Marcondes et al. 1998), Taubaté (Casanova, unpublished data), Teodoro Sampaio (Marcondes et al. 1998). Paraná – Bandeirantes (Massafera et al. 2005), Cianorte, Fênix, Jussara, Paiçandu, Peroba (Marcondes et al. 1998), Ribeirão Claro (EABG's observations), Santo Antônio, São Jorge do Ivaí (Marcondes et al. 1998), Terra Boa (Teodoro et al. 2001). Santa Catarina – Biguaçu, Indaial, Itajaí, Quilombo (JDAF's observations, Marcondes et al. 1998), Rancho Queimado (Marcondes et al. 1998), Santo Amaro da Imperatriz, São Carlos (JDAF's observations). Rio Grande do Sul – Campo Novo (Marcondes et al. 1998), Porto Alegre (EABG's observations), Santa Maria (Marcondes et al. 1998), Tenente Portela (JDAF's observations, Silva & Grunewald 1999). ARGENTINA: Corrientes (Salomón et al. 2006a), Entre Rios (Salomón et al. 2006b), Formosa (Salomón et al. 2002), Salta (Salomón et al. 2004), Santa Fé (Salomón et al. 2006b), Tucumán (Marcondes et al. 1998, Córdoba-Lanus & Salomón 2002, Córdoba-Lanus et al. 2006). BOLIVIA: Tarija (Marcondes et al. 1998). PARAGUAY: Alto Paraná (Marcondes et al. 1998), Itapua and Misiones (Salomón et al. 2003).

DISCUSSION

The data on geographical distribution reported by Marcondes et al. (1998) are similar to those found in this present study, which corroborates the validity and possibility of accurately identifying both species.

The geographical distribution of *N. intermedia s. l.* is very wide, occurring in Argentina, Bolivia, Paraguay, and in all Brazilian regions, in Northern, Northeastern,

and Mid-Western states, however, they are not as abundant as in the Southeastern and Southern states.

In the Northern Region, *N. neivai* may be limited to southern of Pará where it has been captured in São Geraldo do Araguaia, on the Serra das Andorinhas (Galati, unpublished data). In Tocantins, neither of the two species was captured (Andrade Filho et al. 2001), nevertheless, Carvalho et al. (1989) mentioned the presence of *N. intermedia* in other areas. However, accurate identification of this taxon is necessary, having in mind that this area may be a dispersion corridor for *N. neivai* from the Southern Region, western São Paulo and the Triângulo Mineiro as far as the state of Pará.

In the Mid-Western Region, both species were found. In Goiás, *N. intermedia* has been reported in Sítio D'Abadia county and *N. neivai* in Corumbaíba and Itumbiara. Its presence in this latter county has also been reported by Marcondes et al. (1998). The presence of *N. neivai* and *N. intermedia* is also confirmed In the state of Mato Grosso do Sul, the former in Bataguassu and the second in Miranda counties; both species however seem to be rare in this state, as no other authors have reported it (Galati et al. 1996, 2006 Oliveira et al. 2003), as well as in the state of Mato Grosso (Biancardi & Bermudéz 2000, Azevedo et al. 2002).

Only *N. intermedia* has been reported in the Northeastern Region. In Piauí, it have reported in Teresina and in the state of Ceará this species has occurred only in Crato county. In the state of Rio Grande do Norte, Ximenes et al. (2000) report it in two municipalities but no specimen has been examined in the present study. In the state of Paraíba, this species has already reported (Martins et al. 1978), however no specimen has been examined in this study and Marcondes et al. (1998) considered it to be *N. intermedia s.l.* In Pernambuco, has been possible to confirm the presence of *N. intermedia* in two counties. In this latter state, however, its frequency is also very low. In Alagoas *N. intermedia* was captured in July 1999 in Palmeira dos Índios county, and it corresponded to about 10% of all the insects captured (JDAF's observation). In Sergipe, Marcondes et al. (1998) reported *N. intermedia s.l.*, though no specimen from this state has been examined in.

In Bahia, *N. intermedia* presented a wider distribution; however, its frequency is low in the majority of the counties (Vexenat et al. 1986, Sherlock et al. 1996), except in Santo Amaro and "Fazenda Vovo" (Follador et al. 1999, Pereira & Hoch 1990), where this species was prevalent, thus differing from the general tegumentary leishmaniasis pattern, in other areas of the state where *Nyssomyia whitmani* (Antunes & Coutinho 1939) is the main vector species (Vasconcelos et al. 1994, Azevedo et al. 1996). Pereira and Hoch (1990) do not reported in which county the "Fazenda Vovo" is located.

In the Southeastern Region, both species occur in Minas Gerais and São Paulo as previously reported by Marcondes et al. (1998). In Rio de Janeiro and Espírito Santo, only *N. intermedia* has been found.

In Espírito Santo, *N. intermedia* is quite abundant and is suspected of transmitting leishmaniases in some regions of the state (Falcão et al. 1991). In Afonso Cláudio county, this species occurs with higher frequency in regions below 750 m a.s.l. It is at these altitudes that the highest indices of tegumentary leishmaniasis cases are found (Ferreira et al. 2001). This state is composed of 77 counties, in 13 of which the presence of *N. intermedia* has been reported. It was in those 13 counties that 43% of all the cases of tegumentary leishmaniasis in the state notified from 1986 to 1996 occurred. This figure attained 46% in 1997; with a gradual fall to 25% by the first semester of 2002 (Fundação Nacional de Saúde and Secretaria do Estado da Saúde do Espírito Santo).

In Rio de Janeiro, *N. intermedia* is very frequent in several regions, being the predominant species in several areas (Rangel et al. 1990, Brazil et al. 1991, Oliveira et al. 1995). Aguiar et al. (1987) found a large number of specimens of this species in Itaguaí, being most frequently captured biting humans. In Petrópolis county, this species predominated on the human bait (80.3%), has been collected in larger numbers in the hottest months of the year (Souza et al. 2002) and presented significant positive correlations with the intensity of the moonlight when captured with human bait and negative one with CDC light traps (Souza et al. 2005). In this state, though it has been possible to confirm the presence of *N. intermedia* in 25 of the 82 counties, in four of them (Cardoso Moreira, Ibicuí, Itacurussa, and Muriqui) no tegumentary leishmaniasis have been reported. In the state of Rio de Janeiro, 4212 cases of this disease were registered between 1985 and 2001; 57% of these cases occurred in those counties in which *N. intermedia* has been identified (Secretaria de Estado de Saúde do Rio de Janeiro).

In Minas Gerais, the distribution of *N. intermedia* and *N. neivai* basically follows that reported by Marcondes et al. (1998). *N. intermedia* occurs in the following regions: Jequitinhonha, Vale do Rio Doce, Metallurgic, and Zona da Mata, whereas *N. neivai* occurs in the Triângulo Mineiro and southern region. Both are found in Alto São Francisco and the northeast regions.

Both species are found sympatrically in Arinos, Pirapora, Lassance, and Corinto counties and with the exception of the first, where only one specimen of *N. intermedia* and two of *N. neivai* were analyzed, a high predominance was observed for one or the other. Interestingly, all these counties lie along large rivers (Urucaia river in Arinos, São Francisco river in Pirapora, and Velhas river in Lassance and Corinto), which may be serving as geographical barriers to separate the two species, and the few specimens of one or other species that could reach the opposite bank are not numerous enough to compete with the prevailing species. It is important, however, to carry out systematized captures in these counties in order to corroborate this point of view, since the captures were carried out on the left bank of the Velhas river in Lassance and Corinto, and it was not possible to carry them out on the other bank to verify if there was any difference in the species composition and predominance.

In Lagoa Santa, in the metropolitan region of Belo Horizonte, both species seem to have occurred; they were collected with low frequency in Lapinha's cave, though in different periods between 1957 and 1960. From 1993

to 1995, only *N. intermedia* was collected in this cave (Andrade Filho et al. 1998).

In other counties of the metropolitan region of Belo Horizonte, both species have been observed, *N. intermedia* in the capital and *N. neivai* in Betim, Esmeraldas, and Pedro Leopoldo.

N. intermedia seems to be the tegumentary leishmaniasis vector in several areas of Minas Gerais, such as Araçuaí, where, in a survey of an outbreak of this disease, 99% of the sand flies collected were of this species (Gontijo et al. 2002). A similar result has been reported in Alto Caparaó and Caparaó, where it is also implicated as the likely vector (Saraiva et al. 2006).

In Corinto, it was possible to investigate some human cases of the disease, and *N. neivai* is thought to be the vector, as it represented about 90% of all sand flies captured, there being frequent inside homes, causing bothersome to the inhabitants as the insects bite frequently inside residences.

In São Paulo, both species were found to be sympatric in the region of the Vale do Ribeira, in Pariqüera Açu (Marcondes et al. 1998), Iporanga (Galati et al. 2002), Barra do Turvo, Eldorado, Itariri, Juquiá, and Pedro de Toledo (EABG's observations), as also in Taubaté county, in the Paraíba Valley (Casanova, unpublished data). In the present study, *N. intermedia* was captured on the northern coastline of the state and *N. neivai*, in the countryside, which is in accordance with Marcondes et al. (1998). The deforestation of the area previously covered with Atlantic forest seems to be benefiting *N. intermedia s.l.* to the detriment of other species such as *N. whitmani* and *Pintomyia pessoai* (Coutinho & Barretto 1940, Gomes et al. 1989, 1995).

On the northern coastline of São Paulo, *N. intermedia* was the most abundant species accounting for virtually 95% of the total sand flies captured in the intradomicile, peridomicile, and the woods (Brito et al. 2002). On the southern coastline, in Cananéia county, *N. intermedia s.l.* occurs infrequently whether in the woods or in the domicile, whereas in Iguape county, it was the only taxon reported (Gomes & Galati 1989, Gomes et al. 1990).

In the Ribeira Valley, an endemic area for tegumentary leishmaniasis, in Pariqüera Açu county, *N. intermedia s.l.* seems to be more adapted to the modified environment, being considered a tegumentary leishmaniasis vector (Gomes et al. 1980), with the vector-human contact occurring in the domicile (Gomes et al. 1983). This taxon still prevails in residual woodland, on the border of the woods and on open land (Gomes & Galati 1987). In the county of Pedro de Toledo, *N. intermedia s.l.* prevailed, mainly in intra and peridomicile (Domingos et al. 1998).

In the mid-western of São Paulo, in Araraquara and São João da Boa Vista counties, a few specimens of *N. intermedia s.l.* were captured in the woods, whereas in two other counties with larger prevalence of this species, Dourado and Mogi-Guaçu, the inverse occurred, though this woods there were not so well conserved as in the former (Gomes et al. 1989). This taxon also prevailed in the western São Paulo, in the county of Teodoro Sampaio (Condino et al. 1998). According to the distribution reported by Marcondes et al. (1998), *N. neivai* is likely to prevail in these counties.

In São Paulo, a correlation of 88% was found in the counties investigated between the presence of cases of tegumentary leishmaniasis and the presence of *N. intermedia s.l.*, being this taxon the most frequent among the species colleted, with the widest distribution and prevailing, together with *Migonemyia migonei* (França, 1920) in all kinds of vegetation and in all topographical regions; both being considered the principal vectors (Camargo-Neves et al. 2002).

In Paraná, *N. neivai* occurs in the greater part of the state (Marcondes et al. 1998), there being no report of *N. intermedia*, though it may occur in the northeastern region close to the border with São Paulo, where *N. intermedia* has been reported. Jussara county, where *N. neivai* (mentioned as *N. intermedia)* prevailed (Aguiar et al. 1989), presented an incidence of 422.80 cases of tegumentary leishmaniasis per 100,000 inhabitants in 2000, the highest incidence in Paraná (data from "Secretaria Estadual de Saúde").

In Santa Catarina and Rio Grande do Sul, only the occurrence of *N. neivai* was reported (Marcondes et al. 1998), it being widely prevalent in Santa Catarina, mainly in modified areas. In Rio Grande do Sul, as few studies have been carried out, its prevalence has been reported only in the "Parque Estadual do Turvo", in Tenente Portela county (Dias et al. 1997) and Derrubadas county (Silva et al. 2004).

According to these data, *N. intermedia* or *N. neivai* prevail or are abundant in several Brazilian areas and *N. neivai* in Paraguayan and Argentinean areas in which tegumentary leishmaniasis occurs. According to Killick-Kendrick (1988), in order to confirm the disease-vector species, it is important to meet the following criteria: (1) the species should be abundant in the leishmaniasis focus; (2) it should be highly anthropophilic; (3) it should present the development of the parasite in the absence of blood in the gut; (4) it should show the highest natural infection rate among the sand flies observed; and (5) the parasites isolated from sand flies should be indistinguishable from those isolated from human cases.

N. intermedia and *N. neivai* presented characteristics that meet the first and second criteria above. As for the third and fourth criteria, several studies have already shown natural infection among sand flies of these two species, with natural infection by flagellates indices, varying from 0.1 - at least 0.85% (Forattini & Santos 1952, Forattini et al. 1972, Rangel et al. 1984, Casanova et al. 1995, Pita-Pereira et al. 2005, Cordoba-Lanus et al. 2006). Only the last criterion for these species has not been met, as date, sand fly parasites have not been isolated, characterized or compared with those obtained from human patients.

Experiments have shown that these species may be easily infected with several strains and species of *Leishmania* (Chagas 1940, Coelho et al. 1967, Rangel et al. 1992, 1993, Silva & Gomes 2001), which shows that these species are susceptible to infection by *Leishmania*. In the light of these data, there seems to be little doubt as to the vectorial capacity of *N. intermedia* and *N. neivai*.

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