

New record and update on the geographical distribution of *Pintomyia monticola* (Costa Lima, 1932) (Diptera: Psychodidae) in South America

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Abstract: The phlebotomine sand fly *Pintomyia monticola* (Costa Lima, 1932) is recorded for the first time in Brasília, central-west Brazil. A review of the geographical distribution of the species in South America is presented, and its distribution is extended. The potential geographical distribution of *P. monticola* is predicted based on ecological niche modeling. Ecological aspects of this species are discussed.

Key words: Phlebotominae, sand flies, *Pintomyia monticola*, ecological niche models

Until 2014, 986 sand fly species were recognized in the subfamily Phlebotominae (Galati pers. comm.). In the Neotropical region, 528 species are known and 274 occur in Brazil (Andrade et al. 2013; Ladeia-Andrade et al. 2014; Sábio et al. 2014; Santos et al. 2014). The genus *Pintomyia* Costa Lima, 1932 includes 75 valid species grouped into two subgenera, *Pintomyia* (*sensu stricto*) and *Pifanomyia* Ortiz & Scorza, 1963, seven species series (*pacae* Galati 1995, *monticola* Artemiev 1991, *pia* Galati 1995, *verrucarum* Firchild 1955, *evansi* Galati 1995, *serrana* Barretto 1962, *towsendi* Galati 1995), and five actual species as *incertae sedis* (Galati 2003a, 2014). The *monticola* series is represented by two species: *Pintomyia misionensis* (Castro, 1959) (synonyms: *Lutzomyia coelhoi* Coelho, Falcão & Falcão, 1967 and *Phlebotomus* sp. Bejarano & Duret, 1950) and *Pintomyia monticola* (Costa Lima, 1932) (synonym: *Lutzomyia paulwilliamsi* Martins, Falcão & Silva, 1977).

Pintomyia monticola was described based on five females captured in the state of São Paulo (Costa Lima 1932) and the males were described using specimens from a laboratory colony (Barretto and Coutinho 1941). According to Galati (2003a), *P. monticola* occurs in Peru, Brazil, Argentina and Paraguay. In Brazil, this species

has been documented in the states of Pará, São Paulo, Minas Gerais, Rio de Janeiro, Paraná, Santa Catarina, Rio Grande do Sul, Bahia, Goiás and Mato Grosso do Sul, mainly in the Atlantic Rainforest biome (Galati et al. 1996; Aguiar and Medeiros 2003; Dias-Sversutti et al. 2007; Andrade Filho et al. 2008; Teodoro et al. 2010; Cutolo et al. 2013; Galati 2014).

Pintomyia monticola is a species highly attracted to light traps such as modified CDC (Cutolo and Von Zuben 2008; Colla-Jacques et al. 2010; Carvalho et al. 2011; Cutolo et al. 2013; Nascimento et al. 2013), Falcão (Dias-Sversutti et al. 2007; Teodoro et al. 2006, 2010), Chaniots (Dias et al. 1997), and Shannon (Galati et al. 2010; Andrade et al. 2012) traps. This phlebotomine sand fly species was also captured using a Malaise trap (Andrade Filho et al. 2008), Disney trap (Dias-Lima et al. 2003), Damasceno trap (Dias et al. 1997), and mouth aspirators (Dias et al. 1997; Galati et al. 2010). In most cases, females were captured in higher numbers than males.

This phlebotomine species has been found biting humans within forests during the day and night (canopy, ground and edge) in different biomes (Galati et al. 2010). Luz et al. (1967) studied *P. monticola* in forested areas, where the native pine-tree (*Araucaria angustifolia* [Bertol.] Kuntze 1898) predominated, and specimens were caught in nests of opossums (*Didelphis* Linnaeus, 1758), on tree-trunks, and from human bait, during the warmer months of the year. Moreover, the presence of *P. monticola* in an urban area has also been recorded (Andrade et al. 2012; Nascimento et al. 2013). Experimental infection of the Guinea Pig (*Cavia aperea* Erxleben, 1777) by *Leishmania* (*Leishmania*) *enriettii* Muniz & Medina, 1948 indicated *P. monticola* as a possible vector (Luz et al. 1967). This sand fly species has been recorded in cutaneous leishmaniasis foci in Brazil and low natural infection of dissected females by flagellates

has been reported in Mato Grosso do Sul (Galati et al. 1996). Furthermore, DNA of *Leishmania braziliensis* was detected in *P. monticola* in Minas Gerais (Margonari et al. 2010).

The present study reports the presence of *P. monticola* in Brasília, central-west Brazil, and discusses the finding of this species in Peru in Pará state. It also updates the distribution of *P. monticola* and presents a potential geographical distribution model for this species.

In November 2013, sand fly specimens were captured in a gallery forest (15°57'41" S, 047°56'38" W) in the Fazenda Água Limpa (FAL) of the University of Brasília, located in a protected area (Área de Proteção Ambiental [APA] Gama e Cabeça de Veado). Two Shannon traps were set up for three hours and specimens were captured using a Castro aspirator. Sand flies were cleared (Forattini 1973), mounted and identified (Galati 2003b).

Distributional data for *P. monticola* were obtained from Martins et al. (1978), Luz et al. (1967), Rangel et al. (1986), Hermeto et al. (1994), Dias et al. (1997), Silva and Grunewald (1999), Teodoro et al. (1999, 2006, 2010), Luz et al. (2000), Ferreira et al. (2001), Souza et al. (2001), Dias-Lima et al. (2003), Souza et al. (2004), Resende et al. (2006), Saraiva et al. (2006), Afonso et al. (2007), Dias-Sversutti et al. (2007), Loiola et al. (2007), Andrade Filho et al. (2008), Carreira-Alves (2008), Colla-Jacques et al. (2010), Cutolo and Von Zuben (2008), Galati et al. (2010), Carvalho et al. (2011), Shimabukuro and Galati (2011), Andrade et al. (2012), Pinto et al. (2012), Reinhold-Castro et al. (2012), Carvalho et al. (2013), Cutolo et al. (2013), Nascimento et al. (2013) and Paula et al. (2013). Previous lists report *P. monticola* in Peru (Cáceres et al. 2000; Cáceres and Galati 2001) and in the Brazilian state of Pará (Ryan 1986), but these findings need confirmation (E. Galati pers. comm.; T.V. Santos pers. comm.) and therefore were excluded from the present analyses. Distributional data in the collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP/USP) and in other museums provided by Species Link (<http://splink.cria.org.br/>) were accessed and were georeferenced using Global Gazetteer (<http://www.fallingrain.com/world/>) with an uncertainty of ≤ 5 km and precision of 0.01°.

To characterize climate variation across the distribution of *P. monticola*, we used eight climatic variables: annual mean temperature, diurnal temperature range, temperature seasonality, maximum temperature in the warmest month, minimum temperature in the coldest month, annual precipitation, and precipitation in the wettest and driest months. We obtained these variables from the WorldClim project (Hijmans et al. 2005). All environmental databases used in this study covered areas of South America, resampled to a spatial resolution of 2.5' (~5 km) (Almeida et al. 2013).

Ecological niche models were produced using Maxent version 3.2.1 (Phillips et al. 2006). We used a random seed to generate 10 replicate analyses based on bootstrap subsampling. We used median output grids as a hypothesis of suitability, and imported the results into ArcView 3.3 for assessment and analysis. Distributional data were separated into two sets: one for model calibration (75% of points) and one for model evaluation (25% of points). We assessed model accuracy by examining omission rate associated with test points (Anderson et al. 2002) and also used Maxent's jackknife test to identify variables that most influenced model predictions (Phillips et al. 2006).

We obtained 144 records for *P. monticola* and its updated geographical distribution map presents the *bona fide* records in South America and the new record (seven females) reported in the present study (Figure 1). All test points were included in the predicted suitable area (0% omission). Highest climate suitability for

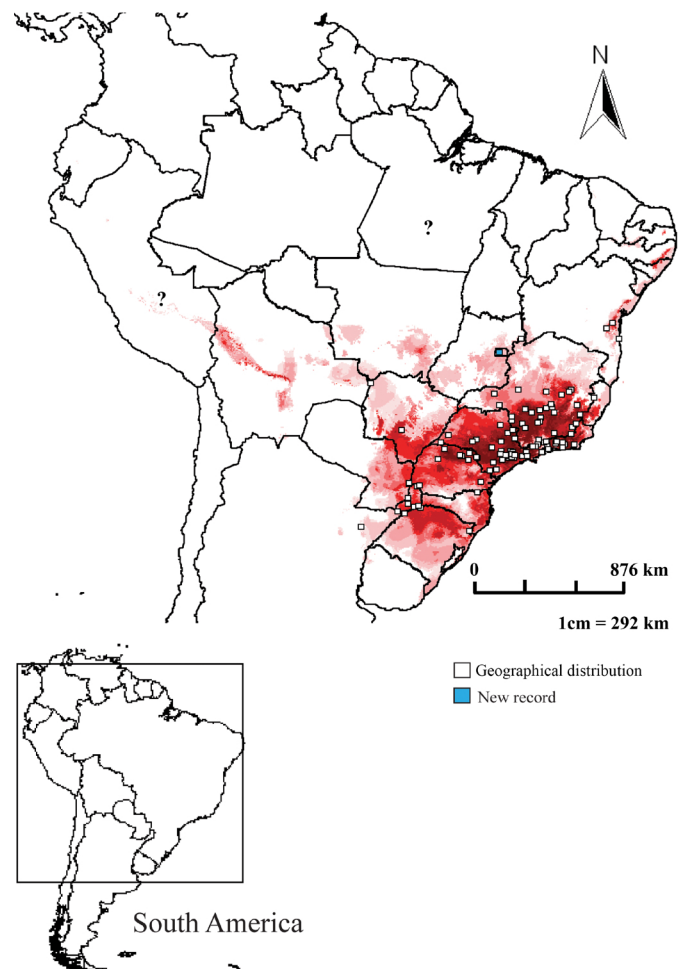


Figure 1. Geographical distribution of *Pintomyia monticola* in South America. White squares represent the known records. The blue square represents the new record from Brasília, central-west Brazil. Non-confirmed record in Peru and in the Brazilian state of Pará are represented by a question mark (see text for details). Areas identified as suitable based on climatic variables using ecological niche modeling (Maxent method) are shown on a scale of red: light red (low suitability) to dark red (high suitability).

P. monticola was observed along the Brazilian Atlantic coast in the Atlantic Forest (Figure 1). Annual mean temperature, temperature seasonality, and minimum temperature in the coldest month were the variables that most influenced models of *P. monticola*.

Our study updates the occurrence map of *P. monticola* in South America and predicts a wide potential geographical distribution that possibly includes other Brazilian states, such as Alagoas, Sergipe, Paraíba, Pernambuco and Mato Grosso where the species have not yet been recorded. The same was observed in relation to Bolivia. The species is also probably present in the southern region of Peru. The sand fly fauna is well known in Pernambuco and Mato Grosso (Dantas-Torres et al. 2010; Missawa and Maciel, 2007). The *Pintomyia* species that occurs in Pernambuco is *Pintomyia fisheri* (Costa Lima, 1932) and three have been recorded in Mato Grosso, but no species of the *monticola* series. The absence of *P. monticola* in these climatically suitable areas could represent situations where the species has not yet dispersed or where the species has not become established due to competition with species previously present in that area. In addition, it is possible that human action, such as deforestation, could hinder the maintenance of *P. monticola* in forest remnants. Future studies using different trapping methods, with higher capture efforts, in the Atlantic Forest and Cerrado areas in these states should confirm the predictions presented herein.

In the state of São Paulo, *P. monticola* was the most frequent and abundant species in the Atlantic Forest and Cerrado biomes (Cutolo and Von Zuben 2008; Galati et al. 2010; Cutolo et al. 2013). In the 'Parque Estadual Intervalles' (São Paulo), *P. monticola* was found inside and near caves. Moreover, one specimen was infected with unidentified microfilariae (Galati et al. 2010). A previous study found *P. monticola* females naturally infected by flagellates in a cutaneous leishmaniasis focus (Galati et al. 1996). Aspirations in armadillo burrows detected *P. monticola* females with *Brumptomyia* species indicating this ecotope as a possible breeding site (Forattini 1973; Galati et al. 2010).

Evidence indicates that *P. monticola* is attracted to humans, as demonstrated in the states of São Paulo (Galati et al. 2010), Minas Gerais (Hermeto et al. 1994), Rio de Janeiro (Afonso et al. 2007), and Rio Grande do Sul (Dias et al. 1997). In Espírito Santo, *P. monticola* was found in a hen house (Pinto et al. 2012). In Minas Gerais, *P. monticola* was found near caves, and in remnants of a deforested area (Carvalho et al. 2013). In Bahia, this species occurred in areas deforested for cocoa plantations and pasture (Dias-Lima et al. 2003). The presence of *P. monticola* in urban areas was recorded in the states of São Paulo (Colla-Jacques et al. 2010), Mato Grosso do Sul and in Pedro Juan Caballero, Paraguay (Andrade et al. 2012).

In Rio Grande do Sul, *P. monticola* was captured in the Atlantic Forest by different methods such as Chaniots and

Damasceno traps and by using mouth aspirators (Dias et al. 1997). Alternative collection methods, such as the Malaise trap, can be used to collect this species and apparently is the only method that permits the capture of more males than females (Andrade Filho et al. 2008).

Pintomyia monticola has been recorded from 60–1,200 m above sea level (a.s.l.) (Galati et al. 2010; Colla-Jacques et al. 2010; Carvalho et al. 2013; Nascimento et al. 2013), with temperatures between 16°C–23.5°C (Sherlock and Santos, 1964; Dias-Lima et al. 2003; Teodoro et al. 1999, 2010; Dias-Sversutti et al. 2007; Galati et al. 2010; Andrade et al. 2012) and precipitation ranging from 1,100–1,700 mm (Teodoro et al. 1999, 2010; Dias-Sversutti et al. 2007). The record of *P. monticola* in Peru was published in two checklists. In the first, the species was reported in Calca province, Cusco department (Cáceres et al. 2000) and, in the second, no locality record was provided (Cáceres and Galati 2001). Although Calca has average temperatures close to those recorded in Brazil (15–20°C), the altitude is much higher (2,928 m a.s.l.) and average rainfall is lower (600–880 mm) than areas where *P. monticola* is recorded. *Pintomyia monticola* has been recorded in the Atlantic Forest and Cerrado biomes of Brazil, as well as in Argentina and Paraguay. However, no suitable area passing through Bolivia and reaching Peru was observed in the predictive distribution model (Figure 1). Nonetheless, future studies are necessary to confirm the presence of the species in these countries.

The record of *P. monticola* in Pará is doubtful. Ryan (1986) recorded the species along km 12 of the Trans-Amazonian Highway between Marabá and Itaituba municipalities. The specimens were deposited in the 'Coleção de Flebotômíneos do Instituto Evandro Chagas' (COLFlebIEC) and the vouchers also include two males mounted in Berlese medium from Serra dos Carajás and Jari, both noted as needing confirmation (T.V. Santos pers. comm.). The male genitalia of *P. monticola* present bristles on the median region of the gonocoxite and in those males, these setae are absent.

Information about the biology, ecology and distribution of *P. monticola* is limited. Based on data available, *P. monticola* seems to be less abundant when compared with other sand flies present in the same areas. The sex ratio in all studies was female-biased and this species presents diurnal activity in forested areas with a higher occurrence during the crepuscular period between 6:00 and 7:00 pm or between 9:00 pm and 3:00 am in the coldest areas. It is a suspected vector of *L. enriettii*, the causative agent of cutaneous leishmaniasis in guinea pigs, in southern Brazil. Males and females of *P. monticola* are attracted to light sources and animal baits used in traps. The species can be found in the forest canopy, on the ground and at the edge, but also inside caves and in anthropized areas, occurring in the Atlantic Forest and in the Brazilian Cerrado. Armadillo burrows and opossum nests are potential breeding sites for *P. monticola*. Further research is needed to better understand the ecology and vector role of this species.

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