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First record of a phlebotomine sand fly (Diptera: Psychodidae) on the Dutch Caribbean island of Curaçao

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ABSTRACT

Phlebotomine sand flies are known vectors of pathogens important for public and veterinary health. They are well known to transmit protozoa parasites of the genus *Leishmania*. Despite their importance as vectors, sand fly distributions remain poorly understood, particularly in the Caribbean. We present the first observation of the sand fly *Micropygomyia trinidadensis* in Parke Hatun on Curaçao, an island of the Dutch Leeward Antilles. This is the first record of sand flies in the Dutch Caribbean. We discuss the ambiguities in biting behavior, as well as the potential role of this species in the transmission of *Leishmania* parasites. This finding expands the knowledge of sand fly occurrence in the Caribbean and offers insights for public and veterinary health risk assessments. Furthermore, this discovery underscores the need for continued sand fly monitoring to understand their distribution, ecology, and impact on local health.

Keywords Phlebotomine sand flies, Dutch Caribbean, vector-borne diseases, public health, veterinary health

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INTRODUCTION

Phlebotomine sand flies are small blood-feeding insects belonging to the family Psychodidae (subfamily Phlebotominae) and are widely distributed worldwide in tropical and subtropical regions, and to a lesser degree in temperate regions. Sand flies are opportunistic blood feeders that feed on various vertebrate hosts (Cotteaux-Lautard *et al.* 2016, Pérez-Cutillas *et al.* 2020). While the vast majority of species prefer to feed on mammalian hosts, other species, such as those from the Old World genus *Sergentomyia*, prefer reptiles (Maia and Depaquit 2016).

A subset of sand fly species has been found to transmit human pathogens, such as the phlebovirus causing Toscana fever and the bacteria *Bartonella baciliformis* causing Carion's disease, and zoonotic pathogens like *Leishmania* spp. (Costa and Souza 2018). Other sand fly species have been shown to

transmit a wide range of animal pathogens that are relevant to veterinary and wildlife health only (Jancarova *et al.* 2023). For example, some species biting reptiles can transmit specific pathogens, including *Sauroleishmania*, commonly known as "lizard *Leishmania*" (Sardaneta *et al.* 2018).

The largest diversity of sand flies can be found in the Nearctic and Neotropics (Cecílio *et al.* 2022). While in the Caribbean region, hotspots of diversity have been reported from Trinidad, Tobago, and Cuba (Tikasingh 2011, Galati 2018), no sand flies have been reported from most other Caribbean islands (Rueda *et al.* 2015, Galati 2018). For these islands, it is difficult to assess whether the absence of records is the result of a gap in the distribution or simply the result of missing or insufficient research effort. This, and the gap in our knowledge of the natural history of this species group limits our understanding of the distribution of vectors of medical and (wildlife) veterinary importance.

The Dutch Leeward Antilles, comprising the islands of Aruba, Bonaire, and Curaçao, is an example of a region with no previous reports on sand fly occurrence, despite their geographic position in the neotropical region near the above-mentioned sand fly diversity hotspots. The islands are located in the southern Caribbean Sea and are home to a diverse range of flora and fauna (Naturalis Biodiversity Centre 2017). Despite the omnipresence of vertebrate hosts, including humans, livestock, rodents and wild birds, as well as the presence of suitable habitats on these islands, no sand flies or sand fly-related diseases have previously been documented on these islands (Schaffner & Braks 2016).

In this article, we report the first observation of a single specimen of the sand fly species *Micropygomyia (Sauromyia) trinidadensis* (Newstead, 1922) on Curaçao, and discuss its medical and veterinary health relevance. This record is the first finding of phlebotomine sand flies in the Dutch Caribbean.

METHODS

From 13 November 2022 to 6 December 2022, a mosquito biodiversity survey was carried out on the Dutch Caribbean islands of Aruba, Curaçao, and Bonaire. The aim of the survey was to obtain an overview of disease vectors and design new identification tools. The survey was a joint enterprise of Dutch and French entomologists, public health officials, local public health agencies, vector control units, and local nature conservation agencies. The primary focus of the survey was the sampling of the local mosquito fauna (Diptera: Culicidae), but other disease vectors were also collected albeit with lower sampling effort, including biting midges (Diptera: Ceratopogonidae), kissing bugs (Hemiptera: Reduviidae), hard ticks (Arachnida: Ixodidae), and a single phlebotomine sand fly. The results of the sampling of mosquitoes and other disease vectors will be reported in separate articles.

During the survey, CO_2-baited BG-Pro® traps (Biogents, Regensburg, Germany) were used to trap mosquitoes at a total

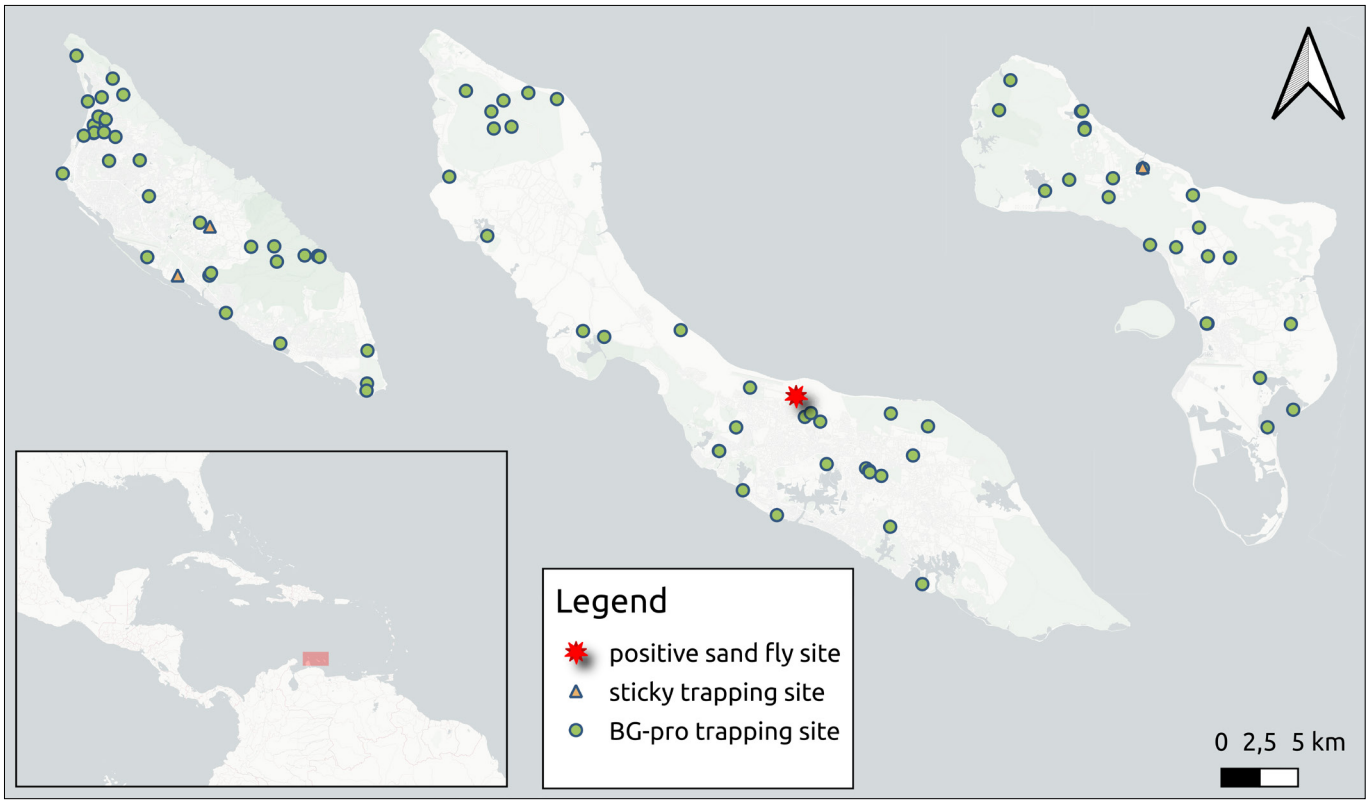

Figure 1: Map depicting the trapping sites across the Dutch Leeward Antilles (Aruba, Curaçao, and Bonaire) during the mosquito biodiversity survey. Traps included CO_2-baited BG-Pro traps (circles) and sticky traps (triangles). The location of the sand fly species *Micropygomyia trinidadensis* (Newstead, 1922) discovery site on Curaçao is marked by a red star.

of 95 locations (Aruba: 34, Curaçao: 33, and Bonaire: 28) (Fig. 1). The traps were set up for 24 h, with the exception of a few traps (n = 9) that remained in the field for 48 h. The traps were used as EVS-style traps and were hung on a tree branch within the vegetation, approximately 100 cm above the ground (Johnston *et al.* 2014). Carbon dioxide was produced by sugar fermentation using a mixture of 150 g beet sugar, 5 g active-dry yeast for fruit fermentation (*Saccharomyces bayanus,* SafSpirit™ FD-3), 0.5 g yeast nutrient salt, and 1200 ml tap water. The samples were stored in a freezer (-20°C) until identification. After identification, specimens were stored in 70% ethanol.

In addition, sticky traps on A5 format white copy paper (148 × 210 mm) coated with castor oil were hung at two sites on Aruba and a single site on Bonaire (adapted from Cazan *et al.* 2021) (Fig. 1). This passive trapping method intercepts rather than attracts sand flies and is used to sample habitats showing high potential for sand fly breeding (i.e., rocks and stone walls with a good layer of old debris at the base and in cavities). Each site was equipped with 5-10 paper sheets that were hung close to the potential breeding sites and left in the field for two or three days equaling a sampling effort of 0.15-0.31 m^2/night.

Identification of the sand fly collection was performed by DNA barcoding of a 658 bp long fragment of the mitochondrial cytochrome oxidase I (COI) gene (Folmer *et al.* 1994). To extract DNA, three legs were removed from the sand fly, washed in ddH2O for 2x 5 min to remove any traces of ethanol, and then fully dried before being placed in 20 µl of QuickExtract DNA Extraction Solution for DNA extraction, following the manufacturer's protocol. PCR amplification was performed in a 35 µl volume with 1x AceTaq® DNA buffer with Mg2+ (Vazyme Biotech Co. Ltd, Nanjing, China), 0.2 mM of each dNTP, 0.4 µM LCO1490/HCO2198 primer pair (Folmer *et al.* 1994), 1 U AceTaq® DNA polymerase, and 2 µl of DNA extract. The amplification protocol included an initial denaturation step for 4 min. at 95°C, followed by 35 cycles of each for 30 s denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, followed by a final extension for 7 min. at 72°C. The amplification products were checked on a 1% agarose gel and then sent for PCR purification and Sanger sequencing at Macrogen Europe BV (Amsterdam, the Netherlands).

The generated forward and reverse reads were cleaned and aligned using Unipro UGENE software (version 41.0) (Okonechnikov *et al.* 2012). The resulting consensus sequence was blasted with a 'blastn' search against the GenBank database, employing default settings for identification (Altschul *et al.* 1990). The obtained results were then aligned with our newly acquired sequence using the MUSCLE algorithm (Edgar 2004), followed by the construction of an unrooted maximum likelihood phylogenetic tree utilizing PhyML (Guindon *et al.* 2010) with the default settings in Unipro UGENE. Visualization of the phylogenetic tree was performed using the online Iroki tool (Moore *et al.* 2020).

After habitus photographs were taken, we tried to mount the specimen in Euparal as described in Ciliberti (2019), the only difference being the clearing step. We cleared the specimen in a solution of cold KOH 10% for 24 hours at room temperature, instead of heating it in KOH 10% in a water bath for seven minutes. Unfortunately, the specimen was lost while transferring the specimen between the clearing and washing step, making morphological identification impossible.

The identified sand fly was registered in the collection of Naturalis Biodiversity Center (Leiden, the Netherlands) under collection number RMNH.INS.1565650, and the sequence is stored in the Barcode of Life Data System (BOLD) under process ID CAMOZ007-23.

RESULTS

This expedition resulted in the collection of over 4,400 mosquitoes and over 1,000 biting midges. Within the 95 BG-Pro trap samples, only one sand fly was found (Fig. 2). The sticky traps did not result in the collection of additional specimens.

Based on the GenBank BLAST results of the COI barcode, the specimen was identified as *Mi. trinidadensis,* with a similarity between 98.7% and 96.2% with the available fourteen Columbian sequences (Gutiérrez *et al.* 2014, Romero-Ricardo *et al.* 2016, Duque Granda *et al.* 2023, Posada-López *et al.* 2023) and one sequence from Panama (Azpurua *et al.* 2010). Our sequence fell within the cluster of available sequences (Supplementary Fig. S1). The closest available sequence of other species is *Mi. (Sau.) peresi* (Mangabeira, 1942) from Brazil, with 87.7% similarity (Pinto *et al.* 2015). Morphological confirmation was not possible due to the destruction of the specimen during slide mounting.

The sand fly was retrieved (12.178083, -68.936944, 26 November 2022) from Parke Hatun (Fig. 3), a forest patch in the Seru Fortuna neighbourhood on the island of Curaçao. Parke Hatun is a relatively old forest patch with little understory and relatively tall trees. The patch is dominated by manchineel trees ('manzaliña' - *Hippomane mancinella*), along with only a few other trees in low numbers, such as Bahama strongbark ('watakeli' - *Bourreria succulenta*), seagrape ('dreifi di laman' - *Coccoloba uvifera*), and great morinda or Indian mulberry ('noni' - *Morinda citrifolia*). The trap was placed close to a 25m wide pond between a group of low manchineel trees next to a fallen tree, in an area where the forest floor was covered in a layer of dry litter consisting of leaves, twigs, and other organic matter (Figs. 3b,c).

DISCUSSION AND CONCLUSION

Our study provides the first record of sand flies in the Dutch Leeward Antilles, specifically the sand fly species *Mi. trinidadensis.* The relevance of this finding, particularly in the light of veterinary and public health, is further discussed below.

The new location on Curaçao falls within the wide distribution range of this species, which extends from southern Mexico to southern Brazil (Young and Duran 1994), suggesting the

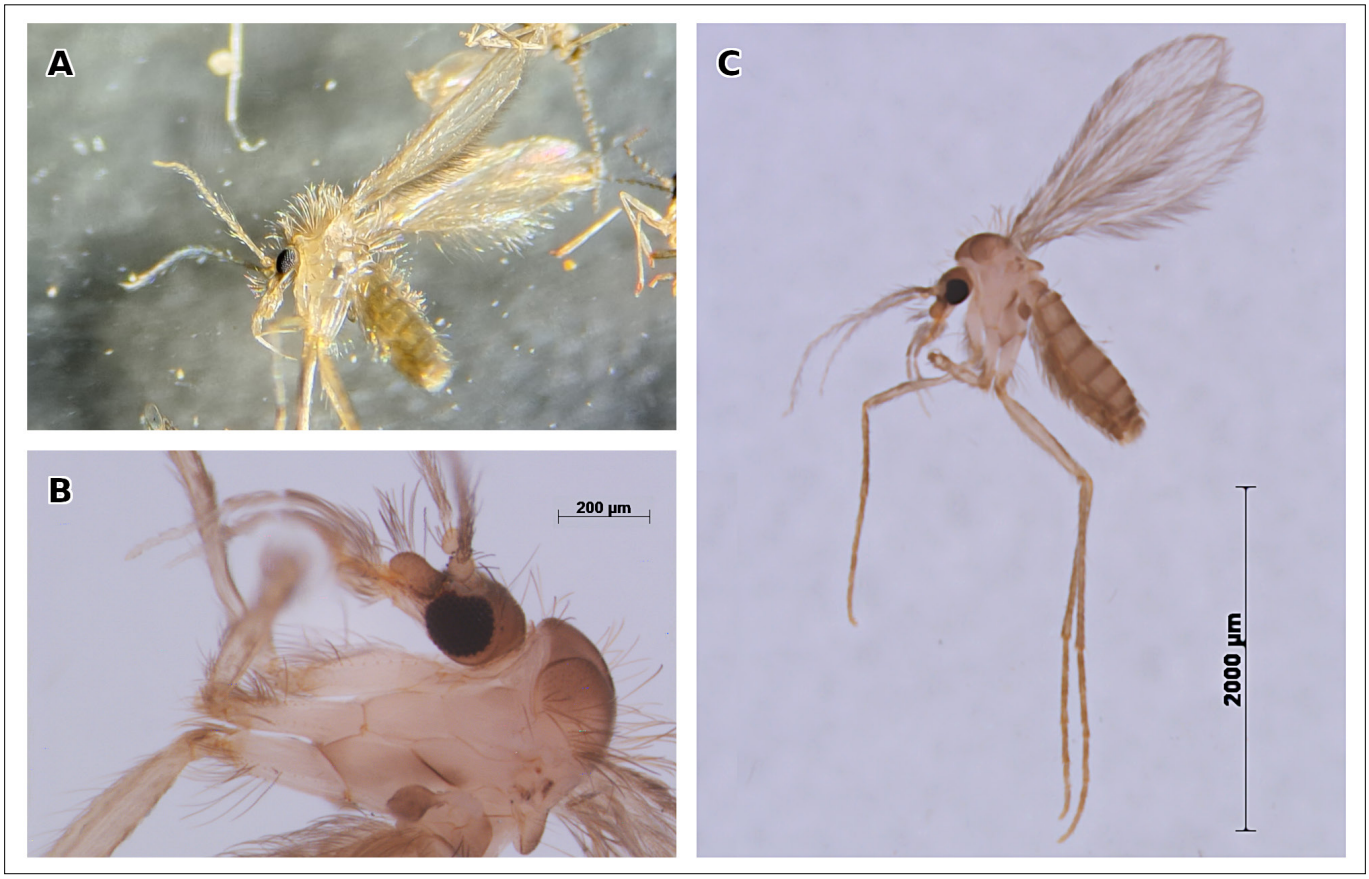

Figure 2: Photographs of the *Micropygomyia trinidadensis* (Newstead, 1922) sand fly specimen collected on Curaçao during the expedition: (A) Scales and hairs on dry specimen; (B) Close-up of thorax and head; (C) Habitus of wet specimen. Photograph courtesy Figure 2A: Jordy van der Beek and Figure 2B,C: Pasquale Ciliberti.

species is native to Curaçao and potentially to the other Dutch Leeward Antilles. Although Parke Hatun is located close to an airport (approximately 1 km away), it is unlikely that the specimen was introduced by human-mediated transport. Sand flies are weak flyers, typically using hopping flights and dispersing only a few hundred meters (Service 2008). The short flight distance, combined with the specific habitat needs of many sand fly species, explains why the subfamily of phlebotomine sand flies lacks invasive species (ECDC 2017); these insects do not colonize distant areas through passive transport.

The sampling effort during the expedition did not specifically target sand flies, except with the occasional setting of interception sticky papers. Due to the specific behaviour and specific trapping requirement, sand flies might therefore have been overlooked at certain trapping sites. More targeted research is needed to evaluate the distribution and abundance of sand flies on the island of Curaçao.

The specimen collected in the trap was not blood fed. Literature from other areas within its range has been inconclusive regarding the blood-feeding preferences of *Mi. trinidadensis*. A preference for cold-blooded animals has been hypothesized based on the overlapping species of *Leishmania* flagellates between forest geckoes and *Mi. trinidadensis* in Panama (Christensen & Telford 1972). This was confirmed by bloodmeal analyses, in which at least 75% of the bloodmeals originated from reptiles (Tesh *et al.* 1971). In contrast, more recently blood-feeding of this species on mammals, including humans, was observed in urban environments in Venezuela (Scorza *et al.* 1979) and genetic bloodmeal analyses showed feeding on humans, dogs, and rodents in Brazil (Guimarães-e-Silva *et al.* 2017), anteaters in Columbia (Paternina *et al.* 2016), and chickens in Ecuador (Anaguano *et al.* 2015). Intraspecific preference for feeding on both reptiles as well as mammals is considered unusual for most hematophagous insects. In that context, Williams (1988) questioned whether the reptile feeding in Central America (Tesh *et al.* 1971, Christensen & Telford 1972) and the urban mammal feeding from Venezuela (Scorza *et al.* 1979) arise from the existence of two or more distinct species under the same name. This might have originated from the problematic taxonomic history of *Mi. trinidadensis*, resulting from misidentification of this species both in the present and the past, among others, with *Mi. (Micropygomyia) cayennensis* (Floch & Abonnenc, 1941), *Lutzomyia (Tricholateralis) gomezi* (Nitzulescu, 1931), and *Lu. (Lutzomyia) longipalpis* (Lutz & Neiva, 1912) (Williams 1988). It remains unclear whether the *Mi. trinidadensis* found on Curaçao is of the 'mammalophilic type' or the 'herpetophilic type'.

Although the vector competence of *Mi. trinidadensis* for *Leishmania* spp. is unclear, specimens, probably of the

Figure 3: Habitat and collection site of the forested area on Curaçao where the *Micropygomyia trinidadensis* (Newstead, 1922) sand fly specimen was collected: (A) Overview; (B) Abundant leaf litter covering the forest floor; (C) BG-Pro trap placement. Photograph courtesy Figures 3A,C: Jordy van der Beek and Figure 3B: Carel de Haseth.

mammalophilic type, have been found infected with unknown *Leishmania* promastigotes in Brazil (Gil *et al.* 2003, Lainson 2010, Guimarães-e-Silva *et al.* 2017) and Venezuela (Bonfante-Garrido *et al.* 1990). The Venezuelan sand fly promastigotes were indistinguishable from the promastigotes of *Leishmania venezuelensis* isolated from humans and produced a similar tumor-like inflammation in a hamster (Bonfante-Garrido *et al.* 1990).

Given the ambiguities in the host preference of specimen called *Mi. trinidadensis*, the potential risk for the public and (wildlife) veterinary health caused by sand flies on Curaçao is unknown, and difficult to assess based on our findings. For a good indication, more information on the blood-feeding preference and its local distribution needs to be collected. So far, there have been no reports of human or animal leishmaniasis cases on the islands. For now, we assume that based on the absence of any reports of sand fly-borne diseases and the low number of sand flies that have been encountered, there is currently no reason to assume that there is a major risk for public or veterinary health. Overall, our findings indicate that further monitoring of sand flies is required to improve our knowledge on the geographical distribution of this species and its relevance to human, veterinary, and wildlife health.

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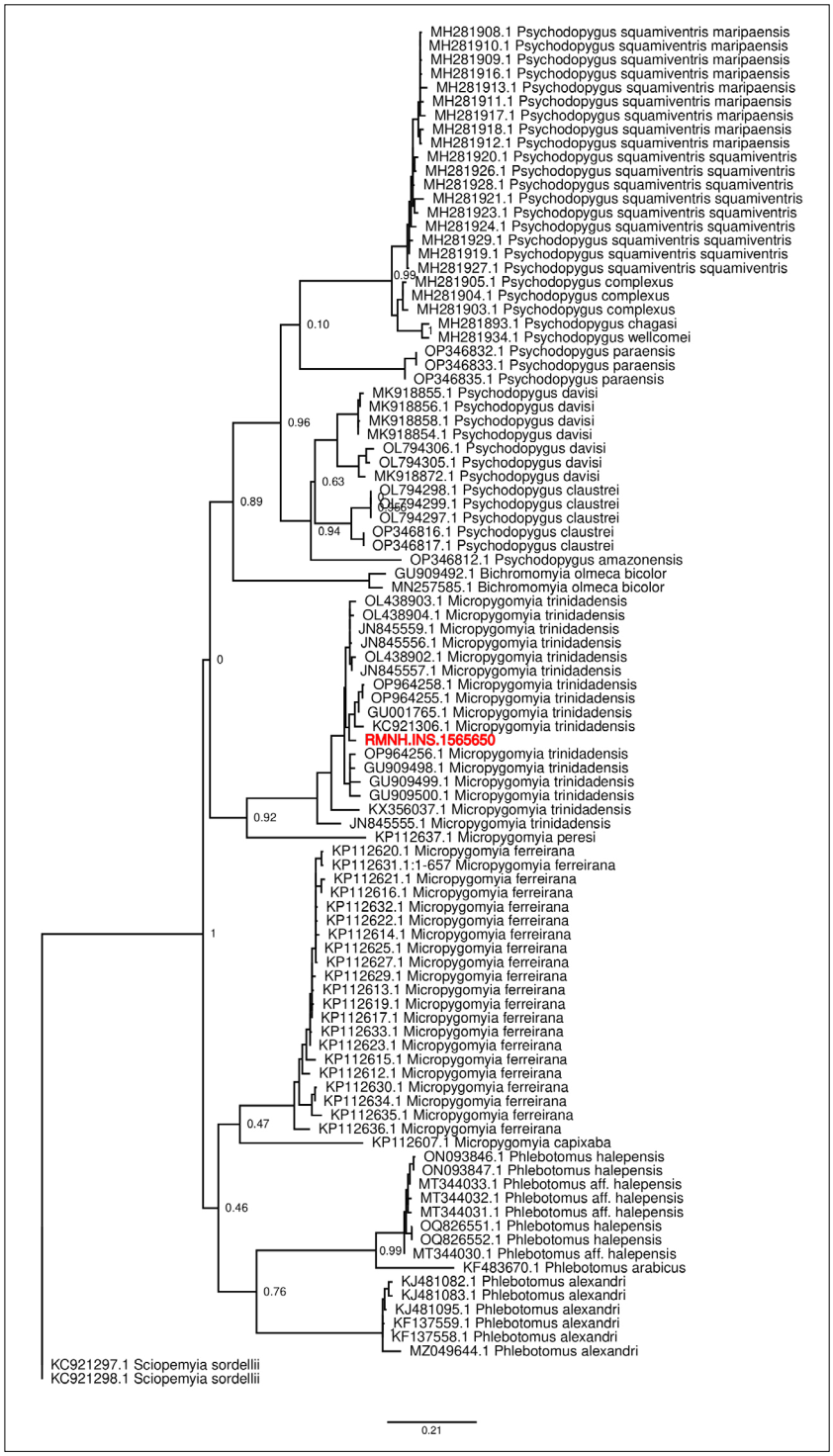

SUPPLEMENTARY MATERIAL

Supplementary figure 1: Genetic maximum-likelihood tree illustrating the relationship of the collected *Micropygomyia trinidadensis* (Newstead, 1922) sand fly specimen COI barcode sequence with existing sequences from Genbank. The figure shows that the Curaçao specimen (in red) falls within a cluster of sequences identified as *Mi. trinidadesis* from multiple sources. The taxonomy has been updated according to Shimabukuro *et al.* (2017). (Jordy van der Beek)

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