Fungus Gnat (Diptera: Sciaridae) as an Emergent Pest Associated with Berry Production in Mexico

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Fungus gnat (Diptera: Sciaridae) as an emergent pest associated with berry production in Mexico

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Abstract

Production and exportation of berries in Mexico has increased over the last decade, expanding the land area dedicated to the cultivation of these fruits, and increasing their associated pests. Recently, the proliferation of a group of flies belonging to the Sciaridae (Diptera) has become a problem for berry farming. Adults can be mechanical vectors of several species of plant pathogenic fungi. Larvae can cause indirect damage by feeding at the base of leaves, stems and roots, increasing plant susceptibility to pathogens. The aim of the present research was to identify fungus gnat species associated with berry varieties from 18 production areas in the states of Baja California, Michoacán, Jalisco, Puebla, and Tlaxcala. Sampling was carried out under multi-span polytunnels from 2018 to 2020. A total of 31 samples with 317 specimens were processed and identified using morphological characters. Four species were identified: *Pseudosciara forceps* (Pettey), *Pseudosciara neotropica* (Lane), *Lycoriella sativae* (Johannsen), and *Bradysia impatiens* (Johannsen). All species except for *B. impatiens* are first reports for Mexico but *B. impatiens* was the most frequently encountered and widely distributed.

Key Words: fungus vector; berry diseases; host distribution; *Bradysia impatiens; Pseudosciara forceps*

Resumen

La producción y exportación de berries en México se incrementó durante la última década, lo cual amplió la superficie dedicada al cultivo de las frutillas, y por consiguiente sus plagas asociadas. Recientemente la proliferación de un grupo de moscas pertenecientes a la familia Sciaridae (Diptera) se ha convertido en un problema para la producción de berries. Debido a sus hábitos, los adultos pueden ser vectores mecánicos de varias especies de hongos patógenos de plantas, dependiendo de la especie cultivada, parece ser que las larvas causan daño indirecto derivado de su actividad alimenticia en la base de las hojas, tallos y raíces, lo cual resulta en plantas susceptibles a patógenos por la presencia de conidios de hongos en el sustrato o los que puedan ser trasportados por las mismas larvas y adultos. Derivado de este problema, el objetivo del presente trabajo fue identificar las especies de moscas de los hongos asociadas con el cultivo de berries en 18 áreas de producción distribuidas en los estados de Baja California, Michoacán, Jalisco, Puebla y Tlaxcala. El muestreo se realizó bajo condiciones de macrotúnel en los años 2018 y 2020 debido a las épocas de producción en cada sitio. Un total de 31 muestras con 317 ejemplares se procesaron e identificaron usando características morfológicas. Se identificaron cuatro especies: *Pseudosciara forceps* (Pettey), *Pseudosciara neotropica* (Lane), *Lycoriella sativae* (Johannsen), y *Bradysia impatiens* (Johannsen). Todas las especies excepto *B. impatiens* representan nuevo reporte para México sin embargo la última especie fue la más ampliamente distribuida, siendo la más frecuente en los sitios de muestreo, también las cuatro especies se registran para este grupo de cultivos en este país.

Palabras Clave: vectores de hongos; enfermedades en berries; hospedantes afectados; *Bradysia impatiens; Pseudosciara forceps*

The sciarid fly family (Diptera: Sciaridae) contains more than 2,000 species (Mohrig & Menzel 2009). Adults of these flies are small (1.0 to 11.0 mm) and generally dark in color. They are characterized by compound eyes that form a bridge over the base of the antennae, and by the presence of an ocellar triangle on the dorsal portion of the head and 1 to 3 palp segments. On the wings, the strongest veins are the costa, R, R1, and R5, which also have macrotrichia, in contrast the M1, M2, M4, CuA, and CuP veins are light in appearance or may be absent (Steffan 1981; Plakidas 2019). The larvae generally feed on various types of decomposing organic matter derived from plants, such as leaf litter, wood, bark, and their associated fungi. However, some taxa of the family have adapted to the consumption of living tissues, as is the case of the genera *Phytosciara* and *Bradysia* (Shin et al. 2013). In recent years the presence of sciarids has become increasingly common in areas dedicated to agricultural, horticultural, ornamental, and forest species production (Olson et al. 2002). Furthermore, sciarids have become a serious phytosanitary problem, not just because of the mechanical damage they produce on the base of leaves, stems, and roots of plants, but because these species are frequently associated with a wide diversity of phytopathogenic fungi, including species of the genera *Pythium* (Pythiaceae), *Botrytis* (Sclerotiniaceae), *Sclerotinia* (Sclerotiniaceae), *Thielaviopsis* (Ceratocystidaceae), *Cylindrocladium* (Nectriaceae), *Fusarium* (Nectriaceae), *Phoma* (Didymellaceae), and *Verticillium* (Plectosphaerellaceae) (Radin et al. 2009; Scarlett et al. 2013; Katumanyane et al. 2018).

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Despite the emerging phytosanitary problem represented by fungus gnats in different production systems at the global level, research in Mexico on the economic impacts of these insects is very scarce, and presence of the 26 species in the family has been rarely reported (Ibáñez-Bernal 2017). *Lycoriella ingenua* (Dufour), *Bradysia impatiens* (Johannsen), and *B. difformis* Frey (considered a minor synonym of *B. impatiens* according to Mohrig et al. [2012]) have been reported as pests of economic importance in forest nurseries of pine tree (*Pinus* spp.; Pinaceae) and eucalyptus (*Eucalyptus* spp.; Myrtaceae) from Temamatla, México state, and Morelia, Michoacán. Similarly, fungus gnats have been recorded in poinsettia greenhouses (*Euphorbia pulcherrima*; Euphorbiaceae) located in San Lorenzo Tlacotepec, México state; San Gregorio Atlapulco, Ciudad de México; Tenango de las Flores, Puebla; and Zacatepec and Tetela del Monte, Morelos (Cibrían-Tovar et al. 2008; López-Pérez et al. 2009; Villanueva-Sánchez et al. 2013; Marín-Cruz et al. 2015).

Some of the crops affected by fungus gnats are berries or red fruits, such as *Rubus fruticosus* (blackberry; Rosaceae), *R. idaeus* (raspberry; Rosaceae), *Vaccinium corymbosum* (blueberry; Ericaceae), and *Fragaria* x *ananassa* (strawberry; Rosaceae). In 2022, berries were the most exported agri-food product from Mexico, surpassing beer and avocado. In accordance with FAOSTAT (2023) the world demand for berries has increased production all over the world from 10.5 (2020) to 12.1 (2021) million tons; an important portion of these fruits are destined to supply the demand of the United States, the main consumer of these berries. The growing importance of the crop has led Mexico to position itself in the international market as one of the main producers of blackberries, strawberries, and raspberries (González-Ramírez et al. 2020). To date, it is estimated that the area dedicated to these crops in Mexico is around 55,000 hectares, distributed in 22 states, of which Baja California, Jalisco, Guanajuato, and Michoacan have the highest production (Aneberries 2022).

As is common in large productive areas, the activity of certain pest insects has increased, especially during peak berry production. Fungus gnats are now one of the most reported problems for these crops. According to the activity report of the National Association of Berry Exporters in 2021, losses related to the proliferation of fungus gnats in strawberry multi-span polytunnels were around 30% to 50% of plants on the production site (Aneberries 2022). Therefore, the present work aimed to identify the fungus gnat species associated with the production of blackberry, raspberry, blueberry, and strawberry in 18 productive areas of the country.

Materials and Methods

Fungus gnat samples were obtained in 2018 and 2020 from 18 production areas distributed in 5 states: 6 farms were sampled in Jalisco (Aug to Oct), 4 farms were sampled in both Baja California (Sep and Dec) and Michoacán (Feb, Aug, and Sep), 3 in Puebla (May) and 1 in Tlaxcala (May). The altitudinal range of the sampling was from 18 to 2,450 meters above sea level (m.a.s.l.) (Table 1) where farming is carried out in multi-span polytunnels (6.6 m height, 6 m wide and variable length made of steel frame covered with polyethylene). Blackberry, raspberry, blueberry, and strawberry crops were examined. The survey was carried out according to phenology of each commodity by randomly sampling 1 ha for 30 mins at the soil level and around the plant crown. Adult flies were aspirated directly with conventional mouth operated entomological aspirators. Captured specimens were preserved in vials with 70% alcohol, and later, were transported to the Entomology Laboratory of the Faculty of Natural Sciences of the Autonomous University of Queretaro (Queretaro city, Queretaro, Mexico).

Identification at the species level was carried out based on the taxonomic criteria proposed by Steffan (1981), Mohrig et al. (2012), Moh-

Table 1. Sample locations, crop commodities, species detected, and meters above sea level (m.a.s.l.) for a survey of fungus gnats in berry production in Mexico.

State	County	Crops	Fungus gnat	m.a.s.l.
Baja California	Costa Brava	*Fragaria* sp.	*P. forceps* *B. impatiens*	60
	Ensenada	*V. corymbosum*		17
	Eréndira	*R. fruticosus*		130
	Vicente Guerrero	*R. idaeus*		36
Jalisco	Chapala	*V. corymbosum* *Fragaria* sp. *R. idaeus* *R. fruticosus*	*P. forceps* *B. impatiens* *P. neotropica*	1,543
	Ciudad Guzmán			1,572
	Tala			1,252
	Tapalpa			2,345
	Zapopan			2,240
Michoacán	Jacona	*R. fruticosus*	*P. forceps*	1,560
	Lagunillas	*V. corymbosum*	*L. sativae*	2,020
	Tangamandapio	*Fragaria* sp.	*B. impatiens*	1,715
	Tangancícuaro	*R. idaeus*	*P. neotropica*	1,652
Puebla	El Seco	*Fragaria* sp.	*B. impatiens*	2,397
	Tlachichuca	*R. idaeus*		2,590
	Libres	*R. fruticosus*		2,450
Tlaxcala	Huamantla	*Fragaria* sp. *R. idaeus*	*B. impatiens*	2,450

Crop species: *Fragaria* x *ananassa*., *Vaccinium corymbosum*, *Rubus fruticosus* and *Rubus idaeus*.
Fungus gnat species: *Bradysia impatiens*, *Lycoriela sativae*, *Pseudosciara forceps* and *Pseudosciara neotropica*.

rig and Menzel (2014), and Plakidas (2019). The abdomens of males were removed at the 6th tergite. The anterior leg, antennae, palps, and wings also were dissected with the use of a Leica® S9i dissection microscope. Slides were prepared with a semi-permanent mounting medium (CMCP9; Stehr, 1987). Subsequently, the slides were placed in a drying oven at 45 °C for 5 d to clarify the structures and dehydrate the fixing medium. Finally, the slides were observed in a Leica® DM500 compound microscope equipped with a Leica® ICC50W photographic camera. The preserved material was deposited in the entomological collection of the Faculty of Natural Sciences of the Autonomous University of Querétaro (Queretaro city, Queretaro, Mexico).

To determine if the occurrence of the species of fungus gnats was correlated with the type of berry and the geographic location, the number of specimens of each species found by locality and species of berry were considered. With these data, an abundance matrix was created and a χ^2 test was performed using the PAST® 4.0 statistical package (Hammer 2021) in order to determine the relationship of fungus gnat species to berry host, as well as fungus gnat and sampled locality.

Results

The fungus gnat species identified were *Bradysia impatiens* (Johannsen), *Pseudosciara forceps* (Pettey), *Pseudosciara neotropica* (Lane), and *Lycoriella sativae* (Johannsen) (Figs. 1 to 4). The distribution of each species is presented in Table 1. As the taxonomy is based only on males, a total of 317 specimens were obtained, of which 63.49% and 30.15% were *B. impatiens* and *P. forceps*, respectively, and the remaining 6.36% was represented by 16 *L. sativae* and 4 *P. neotropica* specimens. The last 2 species were not considered in the χ^2 test due to their low representation in the samples. The distributions of *L. sativae* and *P. neotropica* were restricted to production areas in Michoacán and Jalisco. The representation of *B. impatiens* and *P. forceps* in the sampled sites was determined from the analysis of χ^2 and adjusted residues. The collection of *B. impatiens* significantly exceeded the expected frequencies in the multi-span polytunnels of Jalisco at Chapala (5.84%); Baja California at Ensenada (6.49%), Eréndira (20.13%) and Vicente Guerrero (18.83%); Puebla at Tlachichuca (12.34%), and Tlaxcala at Huamantla (25.32%). For *P. forceps*, the collections that exceeded the expected frequencies were obtained from Michoacán at Lagunillas (16.84%), Santiago Tangamandapio (6.32%) and Tangancícuaro (25.26%); Jalisco at Ciudad Guzmán (20.00%) and Zapopan (12.35%), with (χ^2 = 201.61, df = 17, $p < 0.05$). No fungus gnat males were collected at localities in Los Reyes, Michoacán, and Sayula, Jalisco, therefore, these locations were not considered in the analyses and so finally a total of 17 production areas with sampled males were used.

Regarding fly presence by berry species, *P. neotropica* was obtained from blackberry, raspberry, and strawberry multi-span polytunnels, yet *L. sativae* was found only in samples from blackberry and raspberry multi-span polytunnels. In contrast, *B. impatiens* and *P. forceps* were

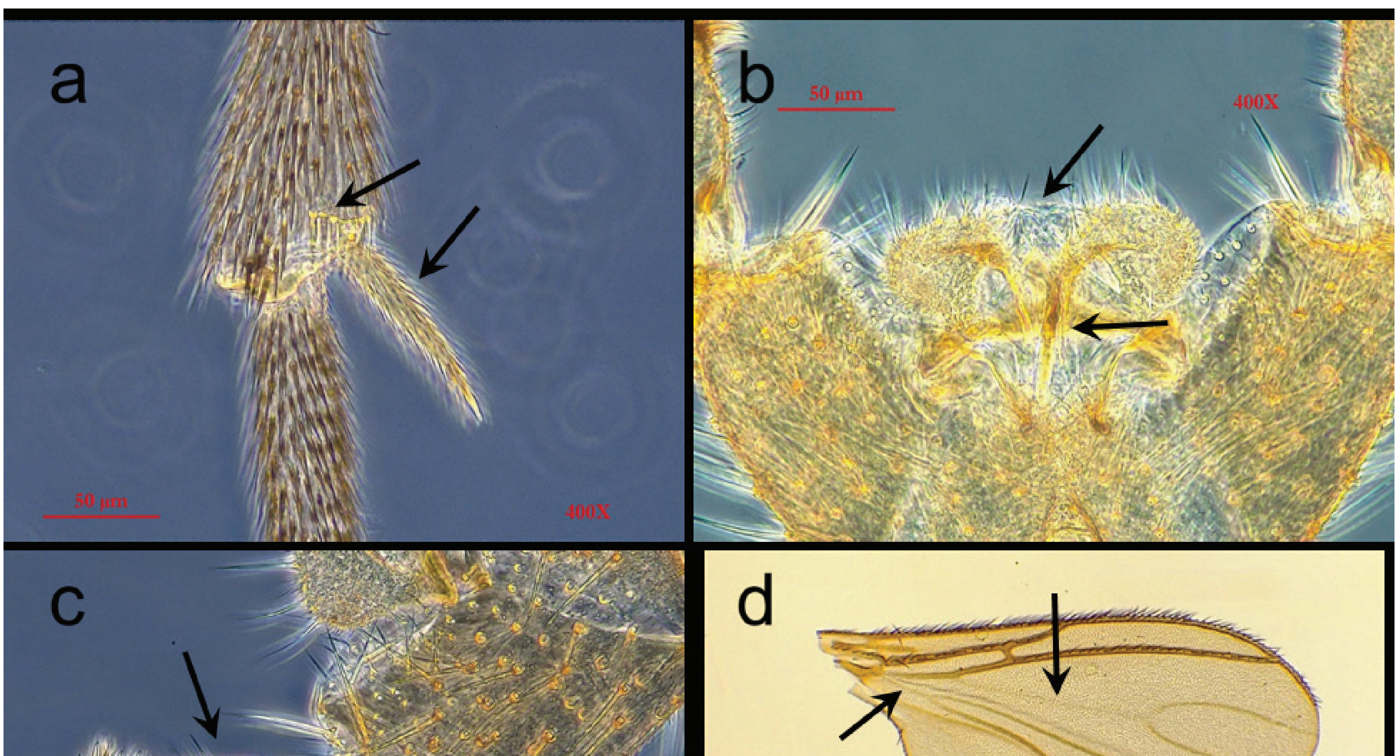

Fig. 1. Morphological characteristics of *Bradysia impatiens*. a) Apex of the anterior tibia; b) ventral view of the hypopygium and aedeagus; c) gonostylus and gonocoxite; d) wing. Arrows indicate taxonomic features.

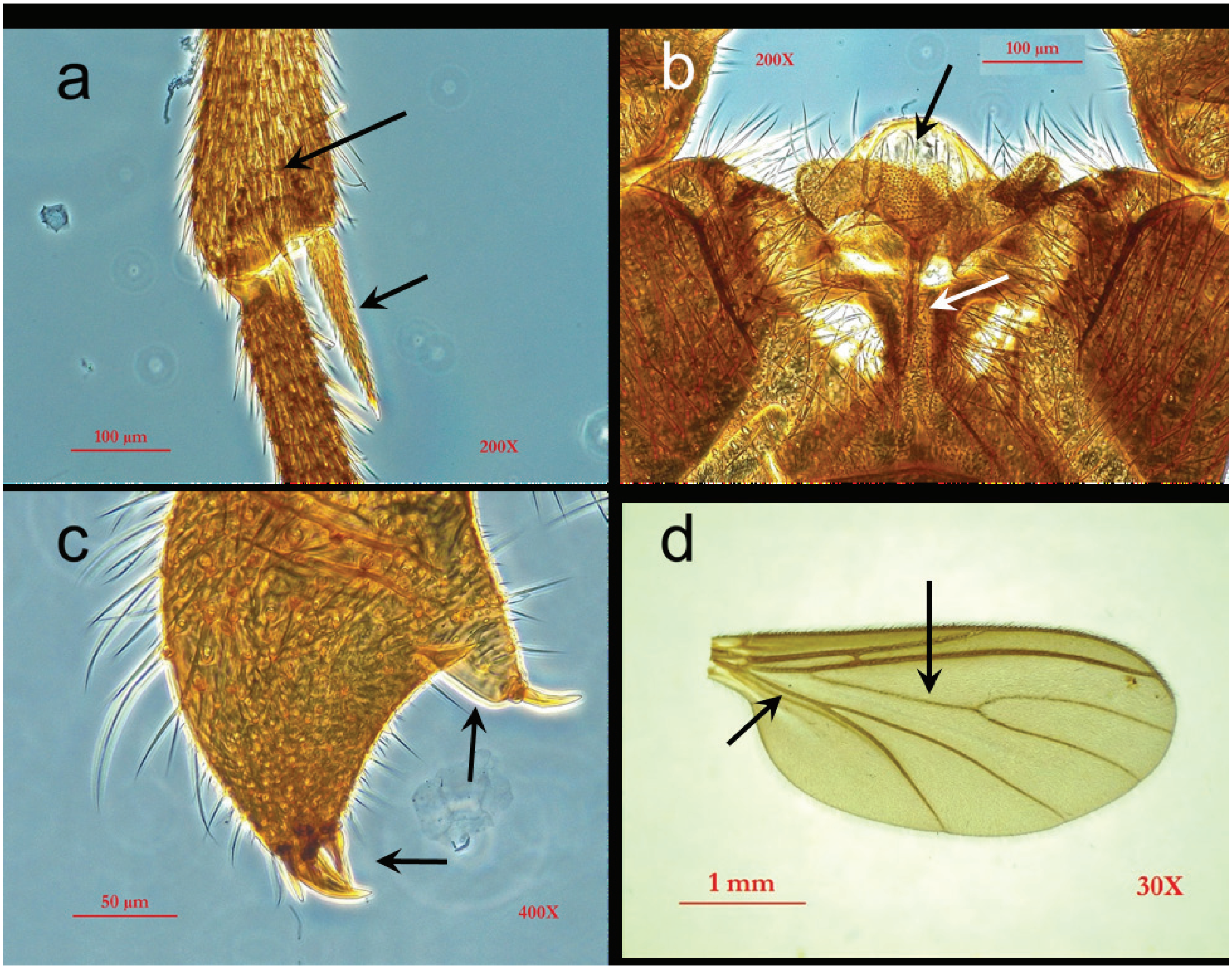

Fig. 2. Morphological characteristics of *Pseudosciara forceps*. a) Apex of the anterior tibia; b) ventral view of the hypopygium and aedeagus; c) apical and subapical teeth of the gonostylus; d) wing. Arrows indicate taxonomic features.

obtained from all 4 berry crops. *Bradysia impatiens* was more abundant in blackberry (33.77%), followed by raspberries (24.02%), strawberry (22.08%), and blueberry (20.13%), and *P*. *forceps* was most abundant on blackberry (47.36%), followed by blueberry (36.84%), raspberry (13.70%), and strawberry (2.10%). For both *B*. *impatiens* and *P*. *forceps*, the discrepancies between the observed and expected frequencies were significant for at least 1 of the 4 berry species evaluated ($X^2 = 28.322$, df = 3, $p < 0.05$). According to the adjusted residue analysis, *B*. *impatiens* was particularly associated with strawberry crops, with a difference of 7.62% between the observed and expected frequencies. The association was also significant for blackberry and blueberry crops, and the observed frequencies were lower than expected, by a difference of 5.19% and 6.18%, respectively. In contrast, the count of individuals in raspberry crops was within the expected range. For *P*. *forceps*, a significant association was obtained for blackberry and blueberry crops, with a difference of 8.41% and 10.32% between observed frequencies, respectively. There was no relevant association for samples from strawberry crops, from which there were 12.35% fewer individuals obtained than expected; the frequency in raspberry was within the expected range.

Discussion

Although 4 fungus gnat species were identified, the most common species *B. impatiens* had the greatest distribution and was found in the production areas of Baja California, Michoacán, Jalisco, Puebla, and Tlaxcala. In contrast, *P. forceps* was collected only in Baja California, Michoacán, and Jalisco, ranging from 17 to 2,450 m.a.s.l.

In contrast to *B. impatiens*, an association was found for *P. forceps* with respect to the host berry species. This may be explained by the geographical distribution of the species, the representativeness of each berry variety in the set of samples studied, or infestation management in each multi-span polytunnel. However, the list of host species for fungus gnats of economic importance is considerably extensive; for example, *B*. *impatiens* alone has been reported as a pest in 36 commodity species including ornamentals, vegetables, grains, and berries, indicating the great adaptability of these insects. If fungus gnat were associated with particular hosts, this information could be useful in preventing and containing crop infestations (Villanueva-Sánchez et al. 2013; Shin et al. 2014; Marín-Cruz et al. 2015; Sueyoshi & Yoshimatsu 2019; Katumanyane et al. 2020).

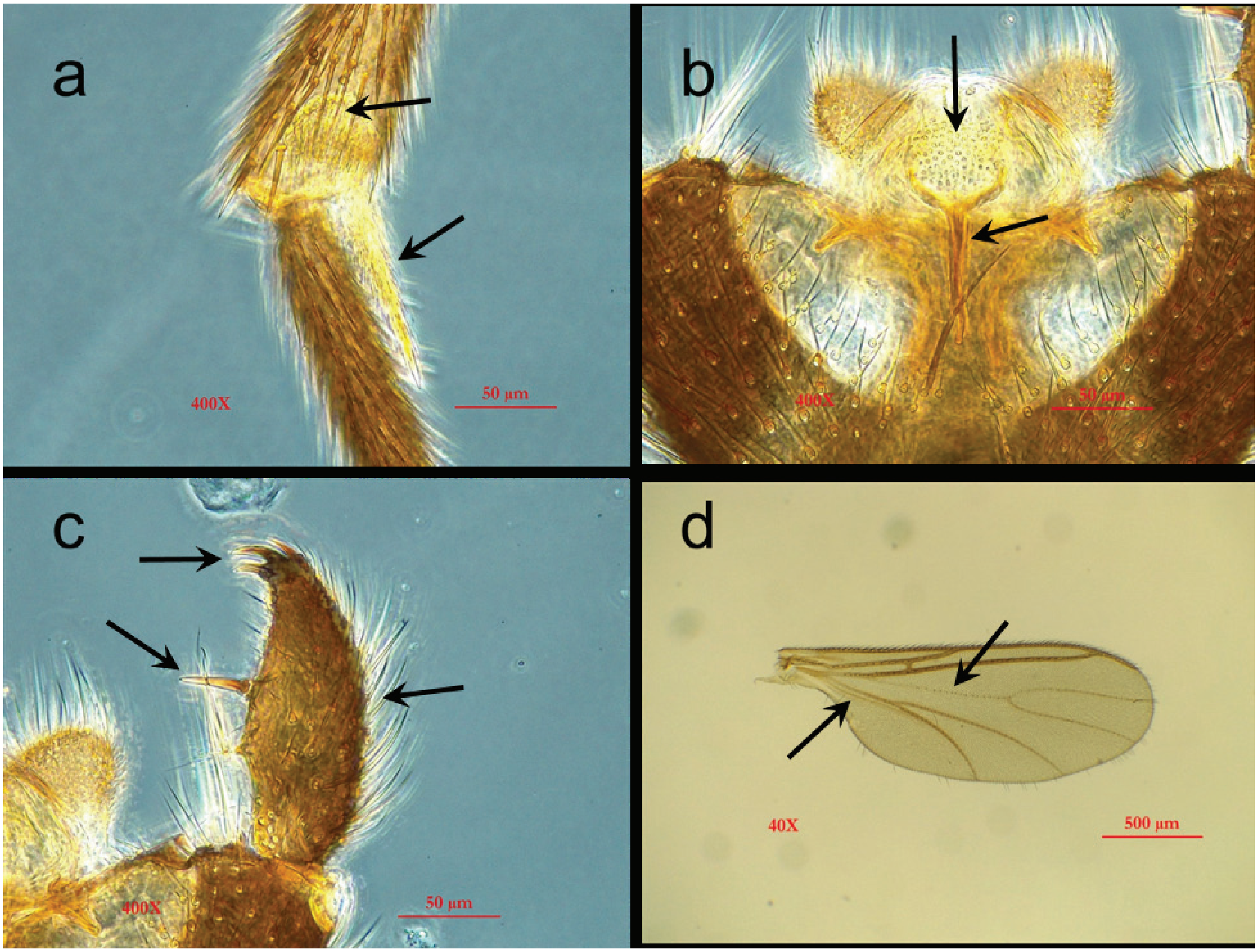

Fig. 3. Morphological characteristics of *Pseudosciara neotropica*. a) apex of anterior tibia; b) ventral view of the hypopygidium and aedeagus; c) apical and subapical teeth of the gonostylus; d) wing. Arrows indicate taxonomic features.

Regarding the distribution of the species, at least *B. impatiens* and *L. sativae* are cosmopolitan species, and their presence is well characterized for some European countries. In America, knowledge about the distribution of fungus gnat species in general is very limited. According to reviews made for the continent, *B. impatiens* has been found in the United States and Brazil (Menzel & Smith 2003; Mohrig et al. 2012). *Pseudosciara forceps* has been recorded in the United States, Dominican Republic, Costa Rica, and Trinidad and Tobago (Mohrig et al. 2012; Mohrig & Menzel 2014). *Lycoriella sativae* has been observed in the United States and Central America (Mohrig et al. 2012; Menzel et al. 2013), and *P. neotropica* is known only in Brazil and Honduras (Amorim 1992; Mohrig et al. 2004).

To date, in Mexico, the presence of *B. impatiens* and *L. ingenua* has been reported from forest nurseries of *Pinus montezumae*, *P. michoacana*, and *P. pseudostrobus*, and in ornamental greenhouses of *E. pulcherrima* in central portions of the country (Cibrían-Tovar et al. 2008; López-Pérez et al. 2009; Villanueva-Sánchez et al. 2013; Marín-Cruz et al. 2015). Therefore, with this study, the list of species is expanded by registering the presence *P. forceps*, *P. neotropica*, and *L. sativae* for the first time in Mexico. Further studies will clarify if they are vectors of fungal conidia. This report is also the first finding of *B. impatiens* and *P. forceps* on blackberry, raspberry, strawberry, and blueberry, for Mexico. On the other hand, since there are no published records of *P. neotropica* on blackberry, raspberry, and strawberry, and *L. sativae* on blackberry and raspberry, it could represent the first documentation for this commodities worldwide. However, fungus gnats are poorly studied due to the systematic complexity of the family and complicated taxonomic determination of the species (Shin et al. 2014). Therefore, it is expected that as the richness and diversity of fungus gnats present in the country's berry production areas increases and as the inclusion of molecular methods aimed at integrative taxonomy become known, there will be more elements to interpret their distribution and occurrence.

Due to the complications in the production of various crops because of the proliferation of phytopathogenic fungi, it is necessary to make a review of the fungus gnat species and their association with fungal diseases in berries. Some of the most common phytopathogenic fungi in the berry production systems in Mexico are *Fusarium oxysporum*, *F. subglutinans*, *F. incarnatum*, *F. seuedograminearum*, *Botrytis cinerea*, *B. caroliniana*, *Lasiodiplodia parva* (Botryosphaeriaceae),

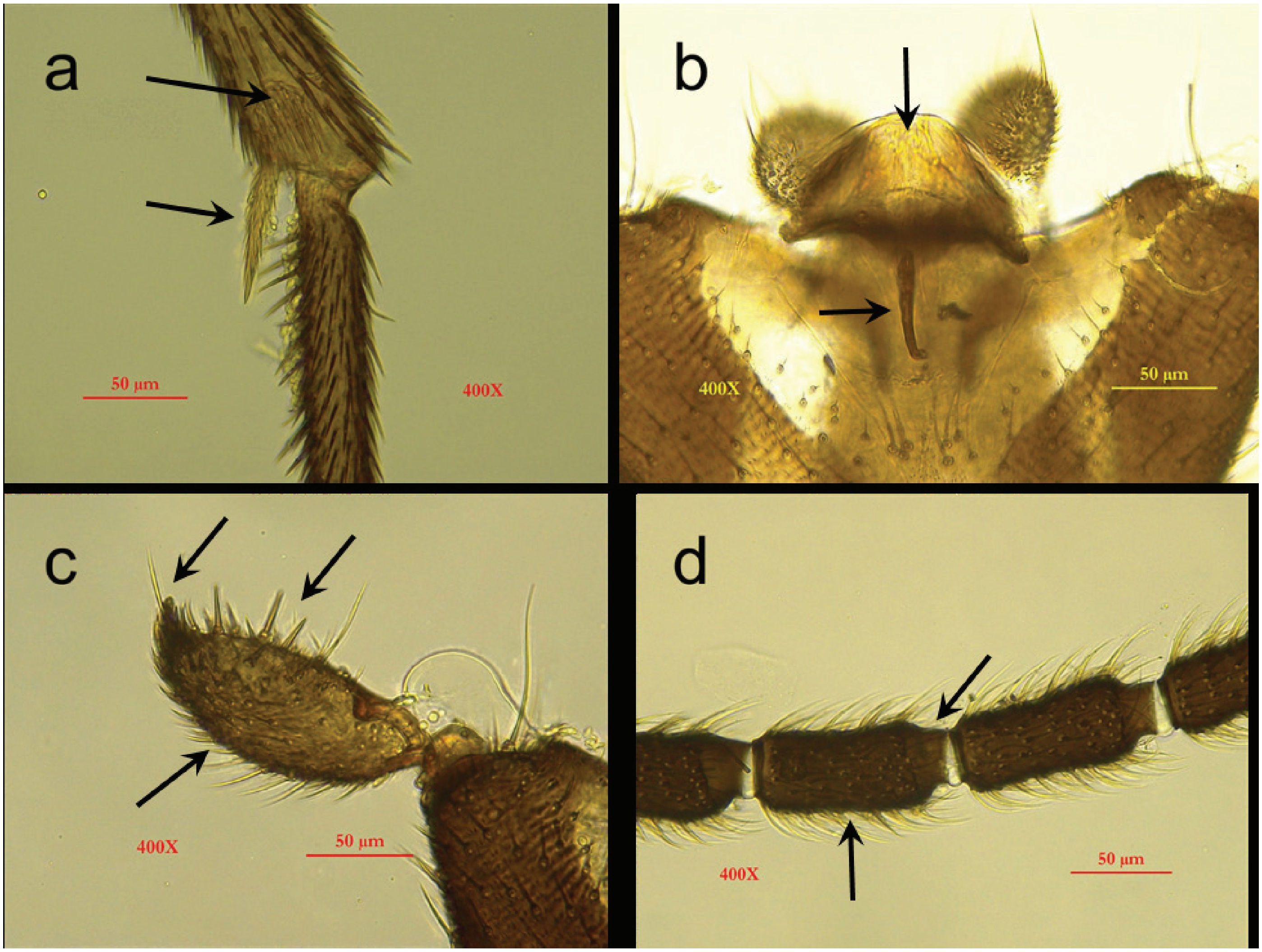

Fig. 4. Morphological characteristics of *Lycoriella sativae*. a) Apex of anterior tibia; b) ventral view of the hypopygidium and aedeagus; c) apical and subapical teeth of the gonostylus; d) flagelomeres 4 and 5. Arrows indicate taxonomic features.

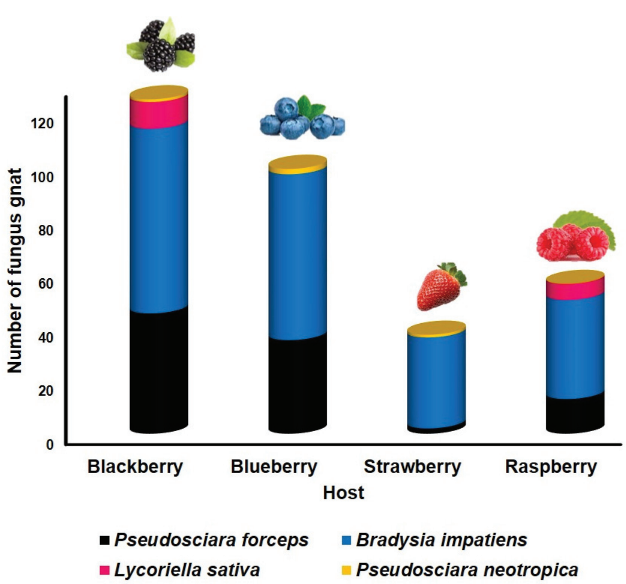

Fig. 5. Number of fungus gnats of each species collected per host crop. Note that *Bradysia impatiens* was the most frequently collected species in all crops surveyed.

Penicillium spp. (Aspergillaceae), and *Alternaria* spp. (Pleosporaceae) (Contreras-Pérez et al. 2019; Morales-Cedeño et al. 2019; Nieto-Cortez et al. 2019; Rebollar-Alviter et al. 2019; Zacarias-Conejo et al. 2019; Terrones-Salgado et al. 2020). Some of these pathogens have been found in specimens of *Bradysia* and *Lycoriella* (Radin et al. 2009; Scarlett et al. 2013), findings that need to be studied in relation to the bioecology for each species of fungus gnat and berry.

Bradysia impatiens and *P. forceps* were found in greatest abundance and were present in all 4 berry crops studied. In contrast, *L. sativae* was only collected from blackberry and raspberry, and *P. neotropica* was only collected from blackberry, raspberry, and strawberry (Fig. 5). *Bradysia impatiens* had the greatest distribution and was found in the producing areas of Baja California, Michoacán, Jalisco, Puebla, and Tlaxcala. However, it was especially abundant in the localities of Baja California, Puebla, and Tlaxcala, where 83.76% of the specimens were collected. *Pseudosciara forceps* was found in Baja California, Michoacán, and Jalisco, but 86.32% of the collection occurred in the localities of the latter 2 states. *Lycoriella sativae* and *P. netropica* were collected only in Michoacán and Jalisco, ranging from 1,652 to 2,345 m.a.s.l. In reference to the altitude above sea level, it seems not to be a barrier for some fungus gnat species like the most widely distributed in the present study, but more research is needed for the less common species. As fungus gnat infestation is favored by plant overwatering conditions with shorter life cycles at relatively high environmental temperatures, optimal of 30 °C (Olson et al. 2002; Villanueva-Sánchez et al. 2013; Katumanyane et al. 2020), monitoring irrigation could be important for its management. Also due to the importance of these species in the spread of fungal diseases in multi-span polytunnels, it is necessary to determine the set of phytopathogenic fungi of berries that are being transported by fungus gnat larvae and adults.

The task of defining management strategies for the Sciaridae flies initially depends on the timely identification of the species, for which the present research is focused. Further study is needed to apply the knowledge about the fungus gnat species present in the country, with new data concerning their life cycles, population dynamics, habitats, and ecological associations.

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