

Diversity of Diptera and their parasitoids associated with *Inga vera* Willd. (1806) (Fabaceae) with new host record in Minas Gerais, Brazil

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Abstract

Diptera belonging to four different families, as well as four associated parasitoids were reared from fruits of *Inga vera* Willd. (Fabaceae) in Lavras, Minas Gerais, southeast Brazil. Four hundred and fifty fruits were collected from five trees of *I. vera* between 2020 and 2021 in various localities situated within the University Federal of Lavras. Specimens belonging to four different dipteran families were reared: *Anastrepha distincta* Greene (Tephritidae), *Asteromyia* sp. (Cecidomyiidae), *Drosophila zottii* Vilela (Drosophilidae), and *Neosilba pendula* (Bezzi) (Lonchaeidae). *Lopheucoila* sp. (Hymenoptera: Figitidae) probably is a parasitoid of *N. pendula* whereas *Eupelmus* sp. (Hymenoptera: Eupelmidae), and two species of *Sycophila* (Hymenoptera, Eurytomidae) appear to be parasitoids of *Asteromyia* sp. Additionally, the majority of the species reared in this study were characterized by DNA barcoding. This study provides valuable insights into the biology and ecology of frugivorous flies in Brazil, and sheds light on potential biological control agents, enabling better management practices.

Keywords: Flies. Host plant. Fruit flies. Lance flies. Gall midge

Introduction

Fruit flies, such as some species from the families Tephritidae and Lonchaeidae, have great economic importance because they are considered pests that affect the production of fruits and vegetables worldwide (UCHÔA, 2012). The flies belonging to species of the genus *Anastrepha* (Tephritidae) pose a significant threat to fruit growers worldwide due to the direct and indirect damage they cause to fruit crops, resulting in significant decreases in production (ALUJA, 1994).

In Brazil, numerous fly species hold economic significance, among them tephritids, such as *Anastrepha* spp. (CAMARGOS et al., 2015), *Ceratitidis capitata* (Wiedemann, 1824) (MALAVASI; ZUCCHI, 2000), *Bactrocera* spp.,

and *Rhagoletis* spp. (UCHÔA, 2012). In addition to the families Lonchaeidae with species of the genera *Dasiops*. and *Neosilba* (UCHÔA, 2012) and Drosophilidae with *Drosophila sukuzii* (Matsumura, 1931), among others species (ANDREAZZA et al., 2016). These pests not only cause direct damage to fruits, but they also hinder the export of fresh fruits. Due to the significant economic losses these pests can cause to the pomiculture industry, importing countries have strict quarantine measures in place (QUERINO et al., 2014).

Most of the research in this field has focused on commercial orchards, which neglects preserved areas and agricultural sites with native vegetation (STRIKIS; PRADO, 2013; BARROS et al., 2016). Even though comprehensive surveys of both native and exotic commercial

fruits have revealed new associations between fruit flies and their hosts (ADAIME et al., 2016; DUTRA et al., 2013), gaps on the knowledge still exist regarding native vegetation that lacks significant economic importance. Therefore, investigating non-focal fruit species provide additional information into the biology, ecology, and interaction dynamics of these insects that may inform commercial operations (ALUJA, 1999).

The emergence of new interactions among host plants, herbivorous insects, and their parasitoids, often driven by environmental changes or species introductions, can disrupt ecological dynamics (HOBBS et al., 2006). These novel interactions can lead to altered herbivore behavior and abundance, subsequently impacting the population dynamics of parasitoids (CORNELL; HAWKINS, 1995). When herbivores encounter new host plants, their feeding strategies may change, which can influence their vulnerability to parasitoids (POELMAN et al., 2011). Additionally, changes in plant chemistry in response to herbivory can affect parasitoid efficiency, thereby influencing biological control outcomes (KARBAN; BALDWIN, 1997). Habitat alterations, such as urbanization, increase the likelihood of these novel interactions, destabilizing existing ecological networks (MCKINNEY, 2002). Understanding these dynamics is crucial for maintaining biodiversity and ecosystem services like pollination and pest control (CARDINALE et al., 2012).

Therefore, *Inga vera* Willd was selected as the focal species due to its abundance and the high number of insects associated with its fruits (DE OLIVEIRA et al., 2023a), making it an interesting option as a natural reservoir of natural enemies near crops of interest. *Inga vera* belongs to the family Fabaceae and the subfamily Caesalpinioideae. It is an evergreen tree of medium size, reaching 5–10 meters in height, and is commonly found along riversides in South and Central America. *Inga vera* has great potential for

reforestation (ANTUNES et al., 2019) and this genus has been shown to have pharmacological and antioxidant properties (leaves), as well as being highly valued as food in Northern Brazil (POMPEU et al., 2012). Additionally, the species plays a crucial role in agroforestry systems by improving soil fertility through nitrogen fixation, which benefits surrounding crops. Its multiple ecological and economic benefits make *Inga vera* a valuable species for sustainable agricultural practices (KEBEDE, 2021). This study was developed with the objective of recording the flies (Diptera) and their hymenopteran parasitoids associated with fruits of the native plant species *Inga vera* (Fabaceae).

Material and methods

Study area

The study was carried out in forest fragments located in the vicinity of the Federal University of Lavras (UFLA), located in Lavras, state of Minas Gerais, Brazil (21° 13' 42.79" S, 44° 59' 11.04" W). The region has a highland tropical climate, with moderate summers featuring precipitation and dry winters. The average annual temperature is 19.4 degrees Celsius, and the yearly rainfall is 1,529.5 millimeters (SPAROVEK et al., 2007).

Host plant – *Inga vera*

According to Lorenzi (1992), the flowers of *I. vera* typically bloom during August and September, and the fruits mature in December and January. In this study, 450 mature fruits attached to five trees of *I. vera* were gathered over two years (2020, 2021) during the fruiting season. These trees were located within the campus of the Federal University of Lavras in the southwest region of Minas Gerais, at the following coordinates with minimum distance of 400 meters apart: Tree-I1 (21°14'6.9" S 044°58'28.22" W), Tree-I2 (21° 13'46.17" S,

044°58'58.87" W), Tree-I3 (21° 13'53.39" S, 044° 58'47.47" W), Tree-I4 (21°13'51.55" S, 044°59'3.71" W), and Tree-I5 (21°13'39.18" S, 044° 58'.58.47" W).

Insect rearing

The sampled fruits were then kept within rearing chambers constructed from PVC tubes and covered with "veil" fabric to trap the insects inside them. Chambers were inside the laboratory, but the room was not climate controlled. These chambers were weekly checked for the emergence of insect herbivores and parasitoids. During the fruit sorting process, adult insects found outside of the seeds were designated as "emerged". While those remaining inside the seeds after three months of sorting were removed and considered "non-emerged". To facilitate their storage and organization, all insects were placed in microtubes filled with 98 % ethanol and labeled them according to the plant and fruit source.

The key by Gibson *et al.* (1997) was used as a reference for identifying Hymenoptera, specifically the Chalcidoidea (Table SM1). Meanwhile, a broader range of literature was consulted to identify the Diptera host species (ARIAS *et al.*, 2014; MCALPINE; STEYSKAL, 1982; VILELA, 1983) and this research has uncovered some novelty interactions between these insects and the host plant. Finally, voucher specimens were deposited in the Colección Nacional de Insectos (CNIN), Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM) and Laboratory of Ecology and Complexity, Universidade Federal de Lavras (UFLA).

Mitochondrial DNA barcoding

In addition to the morphological information, species identification was confirmed by DNA barcoding of a portion of the cytochrome oxidase I (COI) gene of the mtDNA. This approach

facilitates future identification of the species collected herein for future researchers with limited taxonomic expertise. DNA was extracted from the entire specimens (around 3 individuals per species) using the EZ-10 spin column animal genomic DNA miniprep kit (Bio Basic) according to the manufacturer's protocol, but with the inclusion of a pestle crushing step before incubation. PCR reactions included 12,5 μ L of Apex Taq RED Master Mix 2X (Apex Bio Research Products), 0.4 μ M of each primer (Supplementary material; Table SM2), and 2,0 μ L of extracted DNA for a final reaction volume of 25 μ L. The cycler performed the following PCR protocol: 2:00 minutes at 95°C for initial denaturation, followed by 35 cycles of 00:15 s at 94°C for denaturation, 00:30 s at the corresponding annealing temperature (Table SM2), and 00:45 s at 72°C for extension, followed by a final extension of 05:00 s at 72°C, and infinite hold at 4.0°. PCR products were Sanger sequenced by the University of Arizona Genetics Core, and sequences were manually edited and aligned using MEGA 10 software (KUMAR *et al.* 2018).

Establishing interaction

To know from which host insect a particular parasitoid emerged, we examined all the seeds from which parasitoids had emerged checking for cephalic capsules, oral apparatus, and remnants of the host which could aid in host determination. The parasitoid identify was confirmed by the dimensions and shape of the emergence hole they left behind (MORALES-SILVA *et al.*, 2019; OLIVEIRA *et al.*, 2022, DE OLIVEIRA *et al.*, 2023b). The potential host-parasitoid interactions were also informed by the relevant literature (MAIA; AZEVEDO, 2009; PARANHOS; NAVA; MALAVASI, 2019).

Results and discussion

This represents the first record of the dipteran community and their parasitoids associated with

I. vera. A total of 328 specimens belonging to four different Diptera families were observed consuming *I. vera* fruit pulp, and 307 specimens belonging to four morphospecies of hymenopteran parasitoids associated with them. Twenty five individuals of *Anastrepha distincta* Greene, 1934, 44 of *Neosilba pendula* (Bezzi, 1919), 60 of *Drosophila zottii* Vilela, 1983 and 199 of *Asteromyia* spp. (197 larvae) also emerged. The specific and potential interactions among Diptera species and their parasitoids are shown in **Figure 1**.

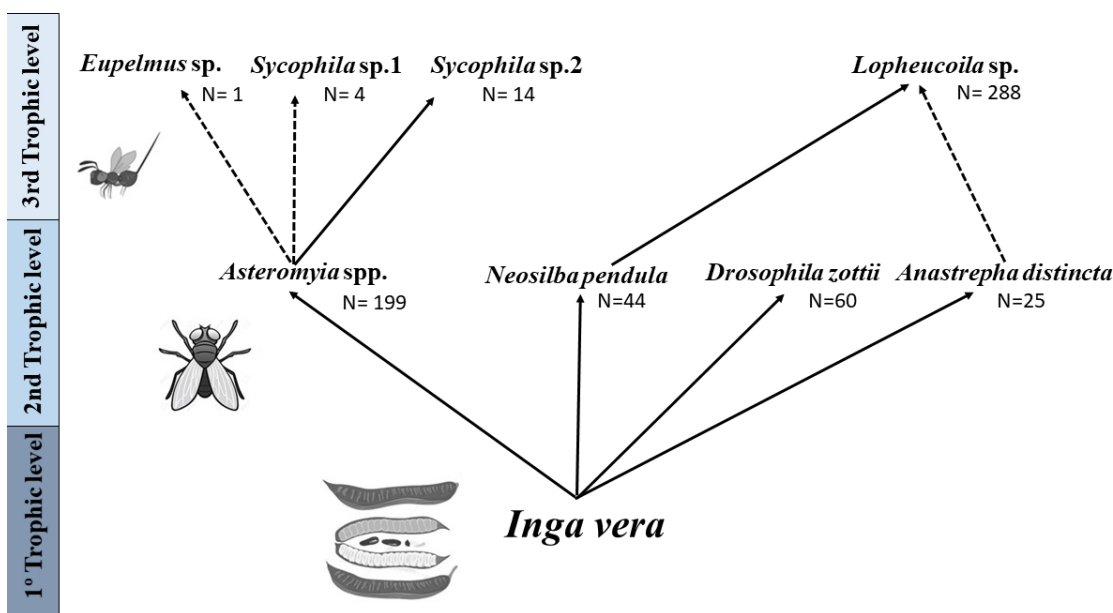
Anastrepha distincta Greene, 1934 - (Figure 2A)

Material examined: Brazil. Minas Gerais. Lavras, UFLA, 16.i.2020, 21°13'51.55"S; 44°59'3.71"W, collected in *Inga vera* fruit (I4-Fr8), T. C. T. Oliveira, 1 female (CNIN); same label information, except: (I4-Fr26), 1 female (CNIN) (Figure 2A); 23 specimens (11 females, 12 males) (UFLA-Laboratory of Ecology and Complexity).

Distribution: United States, Mexico, Guatemala, Nicaragua (BORGE; BASEDOW, 1997); Costa Rica, Panamá, Colombia. British Guiana; Brazil: Roraima, Amapá, Amazonas, Acre, Rondônia, Pará, Tocantins, Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia, Mato Grosso, Goiás, Distrito Federal, Mato Grosso do Sul, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul (UCHOA, 2023); Bolivia (RAMOS et al., 2021), Ecuador, Peru, Trinidad (STONE, 1942); Paraguay (ARIAS et al., 2014; CLAVIJO et al., 2020);

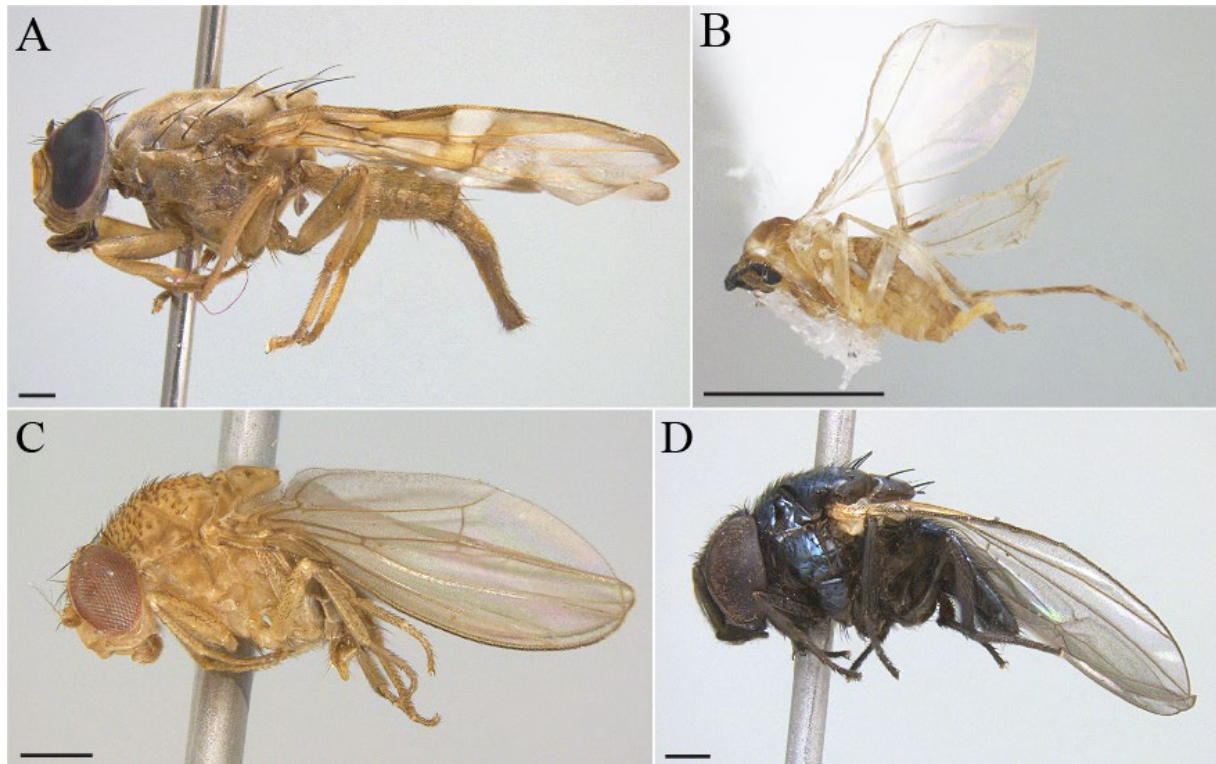
Previous known hosts: *Annona muricata* L. (Annonaceae), *Inga cinnamomea* Spruce ex Benth., *I. edulis* Mart., *I. feuillei* DC., *I. laurina* (Sw.) Willd., *I. goldmanii* Pittier, *I. vera*, *I. hayesii* Benth., *I. lushnathiana* Benth., *I. panamensis* Benth., *I. punctata* Willd., *I. spuria* Humb. & Bonpl. ex Willd., *I. setifera* DC. (Fabaceae), *Psidium guajava* L., *Eugenia*

Figure 1. Food web of Diptera species and their parasitoids associated with *Inga vera* fruits in Minas Gerais, Brazil. The first level of this food web is represented by the plant, the second by the Diptera species, and the third by the hymenopteran parasitoid species. Interactions were established through direct observation of host remains and relevant literature records. Dotted lines represent potential interactions, solid lines represent well-established interactions. The numbers below the species' names are their relative abundances.



Source: Drawn by the authors (2024).

Figure 2. Lateral habitus of Diptera associated with *I. vera* fruits: (A.) *Anastrepha distincta* Greene, 1934; (B.) *Asteromyia* sp.; (C.) *Drosophila zottii* Vilela 1983; (D.) *Neosilba pendula* (Bezzi, 1919). Scale bar: 0.5 mm.



Source: Elaborated by the authors (2024).

nesiotica Standl. (Myrtaceae), *Mangifera indica* L. (Anacardiaceae), *Passiflora nitida* Kunth. (Passifloraceae), *Chrysophyllum cainito* L. (Sapotaceae) (DUTRA et al., 2013; LAMPERT et al., 2020; DO NASCIMENTO et al., 2017; SOUZA et al., 2018; STONE, 1942; UCHÔA; NICÁCIO, 2010).

Parasitoids recorded in Brazil:

Doryctobracon areolatus (Szépligeti, 1911) (MARSARO JÚNIOR et al., 2010), *Opius bellus* Gahan, 1930 (PARANHOS; NAVA; MALAVASI, 2019), *Opius* sp. (CANAL; ZUCCHI, 2000; PARANHOS; NAVA; MALAVASI, 2019).

GenBank accession number: OQ160348.

Remarks: This is the first record of this species consuming *Inga vera* in Minas Gerais. *Anastrepha distincta* is recorded for several host species and shows a preference towards fruits of the Fabaceae, especially the ice-cream-bean (*Inga edulis*) (DO NASCIMENTO et al., 2017).

Asteromyia sp. - (Figure 2B)

Material examined: Brazil. Minas Gerais. Lavras, Universidade Federal de Lavras (UFLA), 16.i.2020, 21°13'51.55" S; 44°59'3.71" W, collected in *Inga vera* fruit (I4-extra), T. C. T. Oliveira, 1 male and 1 female (CNIN) (Figure 2B); 197 immature specimens (UFLA- Laboratory of Ecology and Complexity).

GenBank accession number: OQ749899; OQ749900.

Remarks: Cecidomyiidae is a taxonomically difficult family that is rarely identified at species level, either due to taxonomic uncertainties or because they have not been described. Several species still need to be formally described, particularly in Brazilian biomes (ARAÚJO; FERNANDES; SANTOS, 2019). The two barcode sequences of *Asteromyia* sp. have important variation, therefore indicating that they represent more than one species present in *I. vera* fruits.

The identification of the adult individuals was not possible due to poor condition of the samples, and the larval stage required a taxonomic expertise that we were unable to find.

Drosophila zottii Vilela 1983 - (Figure 2C)

Material examined: Brazil. Minas Gerais. Lavras, UFLA, 16.i.2020, 21°13'51.55"S; 44°59'3.71" W, collected in *Inga vera* fruit (I4-Fr27), T. C. T. Oliveira, 4 males and 15 females (CNIN) (Figure 2C); 41 specimens (UFLA-Laboratory of Ecology and Complexity).

Distribution: Brazil: Minas Gerais, São Paulo (VILELA, 1983), Santa Catarina and Rio Grande do Sul (GOTTSCHALK et al., 2008).

Previous known hosts: *Passiflora alata* Curtis (Passifloraceae), *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae) (VILELA, 1983), *Phaseolus vulgaris* L. (Fabaceae) (GOTTSCHALK et al., 2008).

GenBank accession number: OQ727408.

Remarks. This species belongs to the *Drosophila repleta* group (VILELA, 1983), which includes endemic species from the Americas. Some species are geographically widespread due to human activities. The species in this group are morphologically similar, and male terminalia analysis is necessary for its proper identification (VILELA, 1983). This study represents the first record of *D. zottii* consuming *I. vera* fruits.

Neosilba pendula (Bezzi, 1919) - (Figure 2D)

Material examined: Brazil. Minas Gerais. Lavras, UFLA, 13.i.2021, 21°13'46.17" S; 44°58'58.87" W, collected in *Inga vera* fruit (I2-Fr10), T. C. T. Oliveira, 4 females (CNIN); same label information, except: (I2-Fr13), 2 males and 1 female (CNIN); (I2-Fr13), 3 females (CNIN) (Figure 2D); 34 specimens (UFLA-Laboratory of Ecology and Complexity).

Distribution: Venezuela (PEÑA; BENNETT, 1995). Brazil: Pará, Rio Grande do Norte,

Alagoas, Roraima, Mato Grosso do Sul, Bahia, Minas Gerais, São Paulo, Rio de Janeiro (UCHOA, 2023).

Previous known hosts: *Spondias mombin* L., *S. purpurea* L. (Anacardiaceae), *Caryocar brasiliense* Camb. (Caryocaceae), *Inga vera* (Fabaceae), *Malpighia emarginata* DC. (Malpighiaceae), *Eugenia brasiliensis* Lam., *E. dysenterica* DC., *E. involucrata* DC., *E. neonitida* Sobral, *E. stipitata* McVaugh, *E. tomentosa* Berg., *Psidium cattleianum* Sabine, *P. guajava* L., *P. guineensis* Swartz. (Myrtaceae), *Ziziphus joazeiro* Mart. (Rhamnaceae), *Eryobotria japonica* (Thunb.) Lindley, *Rubus urticaefolius* Poir (Rosaceae), *Coffea arabica* L. (Rubiaceae) (MCALPINE; STEYSKAL, 1982; ARAÚJO; ZUCCHI, 2002; STRIKIS; PRADO, 2009; GISLOTI et al., 2017).

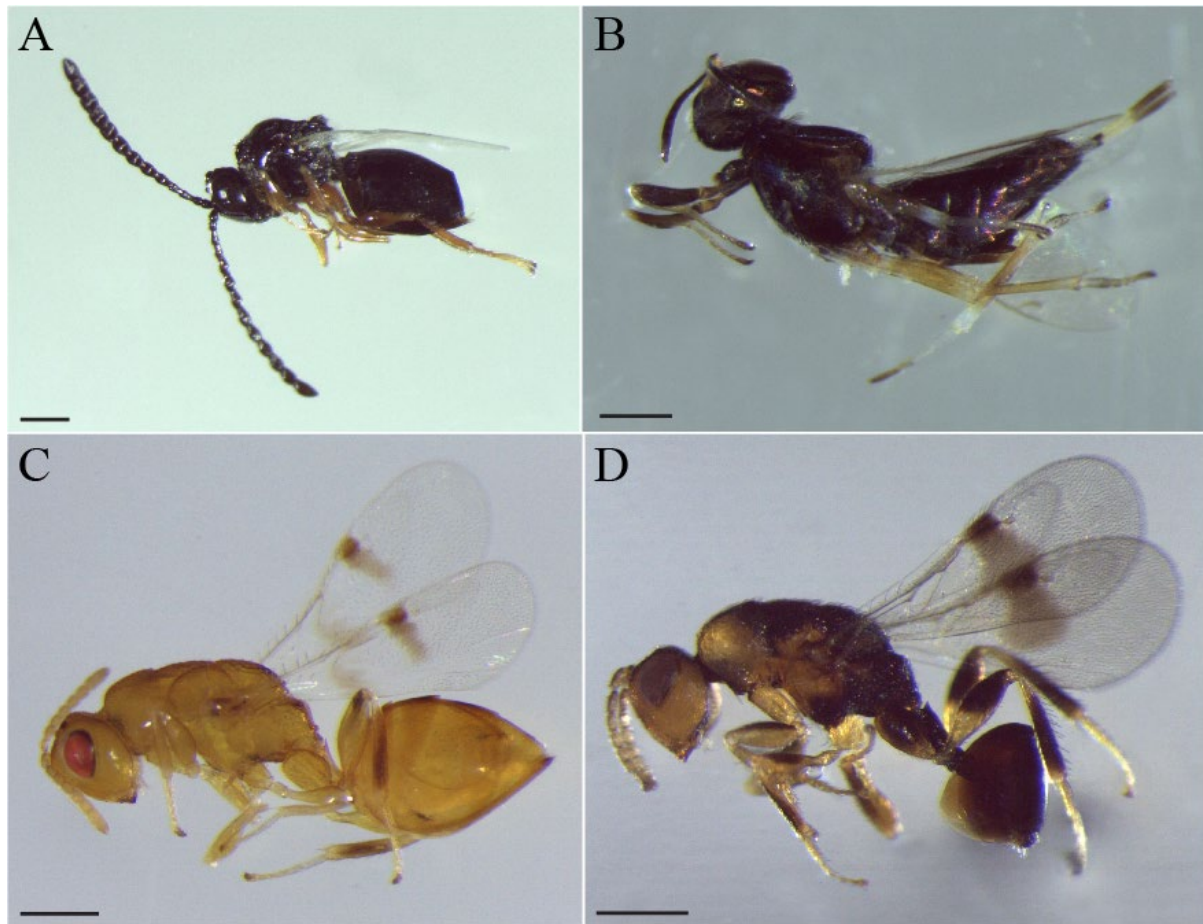
GenBank accession number: OQ160349.

Parasitoids recorded in Brazil: *Microcrasis lonchaeae* (Costa Lima, 1937) (PARANHOS; NAVA; MALAVASI, 2019), *Aganaspis pelleranoi* (Brèthes, 1924) (DUTRA et al., 2013; PARANHOS; NAVA; MALAVASI, 2019), *Tropideucoila weldi* Costa Lima, 1940 (GUIMARÃES; DIAZ; ZUCCHI, 2000; PARANHOS; NAVA; MALAVASI, 2019).

Remarks: This species is probably a secondary invader associated with damage caused by Tephritidae or other primary pests (MCALPINE; STEYSKAL, 1982). *Neosilba pendula* was previously reared from various fruits infested with *Ceratitidis capitata* (Wiedemann, 1824) and *Anastrepha fraterculus* Wiedemann, 1830 in Campinas, Brazil (MCALPINE; STEYSKAL, 1982). Araújo and Zucchi (2002) observed that, under certain conditions, *N. pendula* could become a primary invader of *Malpighia emarginata* (Wied.).

The most abundant parasitoid in this study was *Lopheucoila* sp. (Figitidae) (Figure 3A). The species of Figitidae are well-described

Figure 3. Lateral habitus of Hymenopteran parasitoids of Diptera associated with *I. vera* fruits: (A.) *Lopheucoila* sp.; (B.) *Eupelmus* sp.; (C.) *Sycophila* sp. 1; (D.) *Sycophila* sp. 2. Scale bar: 0.5 mm.



Source: Elaborated by the authors (2024)

natural enemy of *N. pendula* (PARANHOS; NAVA; MALAVASI, 2019) and *A. distincta* (CANAL; ZUCCHI, 2000; PARANHOS; NAVA; MALAVASI, 2019). In Brazil, the only record for this genus is, *L. anastrophae*, which is parasitoid of *Anastrepha* spp., and *Neosilba* in the family Lonchaeidae (GARCIA; RICALDE, 2013; GUIMARÃES; DIAZ; ZUCCHI, 2000; NICÁCIO et al., 2011; WHARTON; MARSH; SHARKEY, 1997). Here direct evidence was found for connecting *Lopheucoila* sp. to *N. pendula*, but based on literature it can also be parasitoid of *A. distincta*. This is the first record of the genus *Lopheucoila* associated with dipterans consuming fruits of the Fabaceae plant. The following most abundant parasitoid species, *Sycophila* sp. 1 and *Sycophila* sp. 2,

belong to the hymenopteran family Eurytomidae (Figure 3C; 3D). This genus is associated with the galls of various insects, such as gall wasps and gall midges (e.g., Cecidomyiidae spp.) (BALDUF, 1932; MAIA; AZEVEDO, 2009). These individuals were found within fruits infested with *Asteromyia* sp. larvae, but there is only enough evidence to establish a connection between *Asteromyia* sp. and *Sycophila* sp. 2 since *Sycophila* sp. 1 and *Eupelmus* sp. (*Eupelmidae*) (Figure 3B) were in low abundance. However, the three hymenopteran species were associated with fruits infested with *Asteromyia* sp.

The discovery of native parasitoids is particularly valuable as they are well-adapted to local ecosystems and can offer long-term control of pest populations (DE CLERCQ;

MASON; BABENDREIER, 2011). Moreover, they are less likely to cause unintended harm to non-target species, reducing the risk of disrupting the ecological balance of the locally ecosystem, therefore being more often accepted by farmers, policy makers, and the general population (COLMENAREZ et al., 2018). Here, four interactions among four native parasitoid species and their Dipteran hosts were found, all of which could be used as potential biological control agents in the future.

Both morphological and molecular approaches were employed to identify the reared species. Despite our efforts, parasitoids are one of the most diverse (FORBES et al., 2018) and challenging groups to identify in the insect world (GADELHA; PENTEADO-DIAS; SILVA, 2012). Therefore, we believe the parasitoid herein presented are potentially undescribed species. Further studies should focus on their identification and a better understanding of their biology, which are essential to developing effective biological control strategies. For future comparisons and phylogenetic hypotheses, we present the mtDNA barcode sequences of three of these parasitoids: *Lopheucoilas* sp. (OQ817698); *Sycophila* sp. 1 (OQ817696); and *Sycophila* sp. 2 (OQ817697). We were not able to sequence the barcode of *Eupelmus* sp. due to the low quality of the DNA extracted from specimens, despite numerous primers used.

Conclusion

This study sheds light on the intricate interactions between four Diptera species and their host plant, *I. vera*. This study provides the first records of *D. zottii* consuming *I. vera* fruits. These discoveries highlight the understudied role that native fruiting species may serve as reservoirs of pest insects that can attack agricultural crops. Our findings also uncover the presence of four potentially undetermined species of native parasitoid genera associated

with these agriculturally Dipteran pests, and show a complex and dynamic ecological relationship between these species, remarking the importance of understanding the intricate balance between pests and their natural enemies for effective pest management.

Given the potential of these parasitoids as effective biological control agents, it is strongly recommend prioritizing future research to investigate their biology and identity. To achieve this, increased investments in the training of taxonomists and their research are recommended. The use of parasitoids as biological control agents offers several advantages over traditional chemical pesticides, including reduced environmental contamination and the prevention of pest resistance. Overall, identifying and utilizing native parasitoids can provide a sustainable solution to controlling fruit pests while preserving the ecological balance of the area.

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