

A SURVEY OF HYMENOPTEROUS LARVAL-PUPAL PARASITIDS
ASSOCIATED WITH *ANASTREPHA FRATERCULUS* AND *CERATITIS*
CAPITATA (DIPTERA: TEPHRITIDAE) INFESTING WILD GUAVA (*PSIDIUM*
GUAJAVA) AND PEACH (*PRUNUS PERSICA*) IN THE SOUTHERNMOST
SECTION OF THE BOLIVIAN YUNGAS FOREST

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ABSTRACT

Parasitoids of *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) infesting wild guava (*Psidium guajava* L.) and peach (*Prunus persica* (L.) Batsch) fruits in the southernmost extension of the Bolivian Yungas forest, in the Province of Tarija, were surveyed in Dec (early summer), Feb (mid summer) and Mar (late summer) 1999 and 2000. The abundance patterns and diversity of fruit fly larval-pupal parasitoid species were similar to previously published data for the southern and northern Yungas forests of Argentina. A total of 1,600 guavas and 800 peaches, weighing 57.713 kg and 24.544 kg, respectively, were collected yielding 13,080 tephritid puparia, 78.4% of which were *A. fraterculus* and 21.6% *Ceratitis capitata* (Wiedemann). We reared 712 larval-pupal parasitoids of the following species: *Doryctobracon areolatus* (Szépligeti), *D. brasiliensis* (Szépligeti), *D. crawfordi* (Viereck), *Opius bellus* Gahan, *Uetetes anastrephae* (Viereck) (all Braconidae, Opiinae), *Aganaspis pelleranoi* (Brèthes) *Odontosema anastrephae* Borgmeier, and *Lopheucoila anastrephae* (Rohwer) (all Figitidae, Eucolilinae). *Uetetes anastrephae*, *O. anastrephae*, and *L. anastrephae* are new fruit fly parasitoid reports for Bolivia, as well as *O. anastrephae* in the South American Yungas forest. A thorough sampling of other *C. capitata* and *Anastrepha* host plants, mostly native fruit species, will be necessary in the Yungas forest of Bolivia before further conclusions on abundance and composition of all fruit fly parasitoids can be reached.

Key Words: fruit flies, parasitoids, Braconidae, Figitidae, Bolivia, Yungas

RESUMEN

Se reportan los resultados de un relevamiento de parasitoides asociados con *Anastrepha fraterculus* (Wiedemann) y *Ceratitis capitata* (Wiedemann) en guayabas (*Psidium guajava* L.) y duraznos (*Prunus persica* (L.) Batsch) silvestres. Estas frutas fueron sistemáticamente colectadas en un sector del extremo más sureño de la selva de Yungas Boliviana, en la provincia de Tarija. El estudio se realizó en los meses de diciembre, febrero y marzo de 1999 y 2000. Los patrones de abundancia y la diversidad de las especies de parasitoides larvo-pupales de "moscas de la fruta" registrados en el presente estudio fueron similares a los datos previamente publicados para los sectores sur y norte de la selva de Yungas en Argentina. Se colectaron en total 1.600 guayabas y 800 duraznos, cuyos pesos totales fueron 57,713 kg y 24,544 kg respectivamente. De estos frutos, se obtuvieron 13.080 puparios de tefrítidos, de los cuales el 78,4% fueron *A. fraterculus* y el 21,6% restante fueron *Ceratitis capitata*. Se identificaron 712 parasitoides larvo-pupales pertenecientes a las especies *Doryctobracon areolatus* (Szépligeti), *D. brasiliensis* (Szépligeti), *D. crawfordi* (Viereck), *Opius bellus* Gahan, *Uetetes anastrephae* (Viereck) (Braconidae, Opiinae), *Aganaspis pelleranoi* (Brèthes) *Odontosema anastrephae* Borgmeier, y *Lopheucoila anastrephae* (Rohwer) (Figitidae, Eucolilinae). *Uetetes anastrephae*, *O. anastrephae*, y *L. anastrephae* son citadas por primera vez para Bolivia. Sin embargo, es necesario efectuar en las Yungas de Bolivia muestreos más meticulosos de otras plantas hospederas de *C. capitata* y *Anastrepha* spp., principalmente especies de frutas nativas, para llegar a conclusiones más precisas sobre la abundancia y composición de los parasitoides de "moscas de la fruta".

Translation by the authors.

The native South American fruit fly, *Anastrepha fraterculus* (Wiedemann), and the introduced Mediterranean fruit fly (Medfly), *Ceratitis*

capitata (Wiedemann), are some of the most important pests affecting commercial fruit production in Bolivia (Zavaleta Castro 2003). Both te-

phritid species are commonly found throughout Bolivia's southern region, including fruit-growing areas of the Bolivian provinces of Tarija and Santa Cruz, where fruit infestation levels in commercial peaches (*Prunus persica* (L.) Batsch) vary between 50 and 90% (Escalante 1995; Zavaleta Castro 2003).

The original native vegetation in this Bolivian region is a subtropical montane rainforest, locally known as "Yungas" (Kessler & Beck 2001), and is extended throughout Argentina's northwestern region (Provinces of Jujuy, Salta, Tucumán, and Catamarca) (Brown et al. 2001). This phytogeographical region is characterized by a high diversity of native and exotic fruit fly host plants growing in the remaining stands of pristine forest and in perturbed areas adjacent to commercial fruit crops and orchards (Ovruski et al. 2005).

Even though *A. fraterculus* and *C. capitata* are significant pests in the Bolivian fruit-growing regions, little information on the fruit fly parasitoids in Bolivia is available. Preliminary surveys in the Bolivian Yungas forest by Escalante (1995) revealed that native hymenopterous parasitoids commonly attack *A. fraterculus* larvae infesting mainly exotic host plants, and five *Anastrepha* parasitoid species native to the Neotropical region listed for Bolivia by Escalante (1995) and by Rogg & Camacho (2000) were *Doryctobracon brasiliensis* (Szépligeti), *D. areolatus* (Szépligeti), *D. crawfordi* (Viereck), *Opius bellus* Gahan, and *Aganaspis pelleranoi* (Brèthes). Nevertheless, little is known about their abundance, parasitization rates, host flies and host plant ranges, and distribution patterns in Bolivia.

Between 1969 and 1971, classical biological control was attempted through the introduction of *Psytalia concolor* (Szépligeti) (reported as *Biosteres concolor*), *Diachasmimorpha longicaudata* (Ashmead) (reported as *Biosteres* or *Opius longicaudatus*) (Braconidae), *Tetrastichus giffardianus* Silvestri, *Aceratoneuromyia indica* (Silvestri) (reported as *Syntomosphyrum indicum*) (Eulophidae), *Pachycrepoideus vindemiae* (Rondani) (Pteromalidae), and *Dirhinus giffardii* Silvestri (Chalcididae) into Bolivia (Pruett 1996; Ovruski et al. 2000). These parasitoid species were released in limited numbers in fruit-growing areas of the provinces of Cochabamba and La Paz (northern portion of Bolivian Yungas forest) and in the province of Santa Cruz (southern section of Bolivian Yungas forest). All parasitoid species, with the exception of *D. giffardi*, were recovered immediately following releases in the three Bolivian provinces (Pruett 1996). Later, between 1976 and 1978 new introductions of *D. longicaudata* into Bolivia and releases of thousands of this fruit fly parasitoid were made in coffee crops in the Yungas of La Paz (Rogg & Camacho 2000). Unfortunately, the failure of well planned follow-up studies made it impossible to carry out a detailed

analysis of the real effect of parasitoid releases on fruit fly populations (Rogg & Camacho 2000).

The present study reports the results of a fruit fly parasitoid survey in which wild guava (*Psidium guajava* L.) and feral peach fruits were systematically sampled in an area of the southernmost extension of the Bolivian Yungas forest. The objectives were to identify indigenous parasitoid species, determine relative abundances and variations in parasitoid and fly numbers over time, natural parasitization rates and fruit infestation levels, and parasitoid distribution patterns.

We did not try to assess the impact of parasitoids as a mortality factor in the population dynamic of *A. fraterculus*/*C. capitata* but rather to determine the natural degree of larval parasitization. Comparative studies in neighboring areas of the endangered Yungas forest in NW Argentina (Ovruski & Schliserman 2003; Ovruski et al. 2004, 2005, 2006; Schliserman et al. 2004; Schliserman 2005; Oroño & Ovruski 2007) recorded 2 exotic, 1 cosmopolitan, and 10 neotropical parasitoid species.

MATERIALS AND METHODS

The collecting site was located between 415 and 612 meters above sea level at 22°43' to 22°39'S latitude and 64°20' to 64°19'W longitude, in the district of Bermejo, province of Tarija, southern Bolivia. The selected area covered an area of 25 km² from the southern border with the Argentinean province of Salta (district of Aguas Blancas) to next to the district of Arroales in the northern Tarija, Bolivia. The study site is part of to the environmental unit called Premontane forest (lower sector of the Yungas forest in elevation zone), which ranges from 300 to 600 m in altitude (Brown et al. 2001). The native vegetation of this Premontane forest has been almost completely eliminated and the area transformed into agricultural use and orchards (Brown & Kappelle 2001). Thus, patches of disturbed wild vegetation with high abundances of exotic fruit plants, mostly animal-dispersed fruit species, such as *Citrus* spp. (Rutaceae), *Morus* spp., *Ficus carica* L. (fig) (Moraceae), *Prunus persica* (peach), *Pirus communis* L. (pear) (Rosaceae), and *Psidium guajava* (guava) (Myrtaceae), commonly can be found in proximity to orchards. The climate is temperate-warm humid with a mean temperature of 22.5°C, and a mean annual rainfall of 1,323 mm (www.wikipedia.es/enciclopedia/Bermejo_Tarija). Approximately 92% of the annual rain falls during the summer (from Oct to Apr) (Kessler & Beck 2001).

Ten guava and 10 feral peach trees were chosen at random on each sample date in the study area. Ten fruits were harvested from each one of these guava or peach trees, and 10 fruits were also collected from the ground below each tree

canopy. Consequently, each sample had 20 guava or peach fruits originating from any 1 tree. Only ripe peach or guava fruits were collected, and fruit trees were only sampled during the peak fruiting season. Thus, peach trees were sampled every 2 weeks during Dec (early summer), 1999 and 2000, whereas guava trees were sampled every 2 weeks from Feb (mid summer) to Mar (late summer), 1999 and 2000. All peach and guava trees sampled were located in patches of secondary forests that surrounded the orchards.

Each fruit sample was placed individually into cloth bags, and fruits collected from the ground were separated from those picked from the tree canopy. The bags containing fruit samples were transported to CIRPON laboratory (Centro de Investigaciones para la Regulación de Poblaciones de Organismos Nocivos) in San Miguel de Tucumán (26°50' S, 65°13' W, 426 m), province of Tucumán, Northwestern Argentina. In the laboratory, fruit was processed as described by Ovruski et al. (2005). Fruits of each sample were weighed and rinsed with a 20% sodium benzoate solution, and placed in closed styrofoam boxes (20 × 20 × 30 cm) with damp sand in the bottom as a pupation substrate for fly larvae. Fruits were placed on a 10-mm mesh metal screen fitted about 10 cm from the bottom. Samples were kept inside a room at 26 ± 2°C, 65 ± 10% relative humidity and a photoperiod of 14:10 (L:D) h for 4 weeks, and the sand was sifted weekly to collect pupae. Afterwards, fruits were dissected to determine the presence of larvae or pupae remaining in the pulp. Live larvae were allowed to pupate and were then added to the other pupae collected from the same sample. Pupae were removed weekly and the *A. fraterculus* and *C. capitata* pupae were separated based upon external pupal characters (White & Elson-Harris 1992). After that, pupae were counted and placed in plastic glasses (300 cm³) filled with sterilized moist sand in the bottom and covered with orandy cloth over the top. These glasses were inspected twice each week and every emerging adult fly or parasitoid was removed and identified.

Fruit fly species were identified by S. Ovruski by with Zucchi's (2000) taxonomic key. Parasitoid specimens were identified to species by S. Ovruski using Wharton and Marsh's (1978) and Canal and Zucchi's (2000) keys for Braconidae, Opiinae, and the taxonomic description by Wharton et al. (1998) and the key by Guimarães et al. (2000) for Figitidae, Eucolilinae. The nomenclature for the Opiinae follows Wharton (1997) and follows Wharton et al. (1998) for the Eucolilinae. Voucher specimens were placed in the entomological collection of Fundación Miguel Lillo (FML) (San Miguel de Tucumán, Argentina).

The fruit infestation level reported was based on the number of fruit fly larvae per fruit or on the number of fruit pupae per kg of fruit. The parasitization rates were calculated on the basis of the to-

tal number of parasitoids emerged from total fruit fly pupae recovered. Means and Standard Deviation (SD) were calculated as summary statistics for the parasitism percentage and fruit infestation level data. Differences in the number of parasitoids recovered from fallen fruits versus those collected in the tree canopy were analyzed by the non-parametric Mann-Whitney *U*-test ($P < 0.05$).

RESULTS

Altogether 1,600 guavas and 800 peaches, weighing 57.713 kg and 24.544 kg respectively, were processed during this study (Table 1). Out of these, 800 (27.868 kg) guavas and 400 (11.828 kg) peaches were fallen fruit collected from the ground. Mean (± SD) individual weight of guava and peach fruit per sample was 36.8 ± 5.6 g and 32.2 ± 4.7 g, respectively ($n = 160$ guava samples and $n = 80$ peach samples).

Only 2 fruit fly species, *A. fraterculus* and *C. capitata*, were recovered from the all fruits collected during 1999 and 2000. Eight species of parasitoids, all native to the Neotropical region, were recovered from *A. fraterculus* pupae obtained mainly from guavas. These were *Doryctobracon areolatus*, *D. brasiliensis*, *D. crawfordi*, *Utetes anastrephae* (Viereck), *Opius bellus* (all Braconidae, Opiinae), *Aganaspis pelleranoi*, *Odonosema anastrephae* Borgmeier, and *Lopheucoila anastrephae* (Rohwer) (all Figitidae, Eucolilinae). The species *D. areolatus*, *D. brasiliensis*, *U. anastrephae*, and *A. pelleranoi* were also recovered from *A. fraterculus* pupae stemmed from peaches. Only 1 parasitoid species, *A. pelleranoi*, was recovered from *C. capitata* pupae obtained from peaches during both 1999 and 2000.

The abundance of each fruit fly and parasitoid species recovered per month and year in association with the fruit species is summarized in Table 1. Of all parasitoids recovered during the study, opiine and eucoilinae species represented 55.3% and 44.7%, respectively.

The fruit infestation levels by *A. fraterculus* and *C. capitata*, and parasitization rates on both tephritid species per collecting month and year are shown in Table 2. A significantly higher number of eucoilinae parasitoids were obtained from guava and peach samples collected from the ground than from the tree canopy ($U = 671.0$, $Z = 8.6$, $P < 0.0001$, $n = 80$ for guava, and $U = 370.0$, $Z = 4.1$, $P < 0.0001$, $n = 40$ for peach) (Fig. 1). On the contrary, no significant differences were found in opiine parasitoids ($U = 2674.0$, $Z = 1.8$, $P = 0.07$, $n = 80$ for guava, and $U = 683.0$, $Z = 1.1$, $P = 0.26$, $n = 40$ for peach).

DISCUSSION

The high levels of infestation in wild guava and in feral peach recorded in this study reveal

TABLE 1. TOTAL NUMBERS OF *A. FRATERCULUS* AND *C. CAPITATA* PUPAE AND ADULTS, AND PARASITOID SPECIES RECOVERED FROM WILD GUAVA AND PEACH FRUITS COLLECTED IN BERMEJO, PROVINCE OF TARLJA, SOUTHERN BOLIVIA (1999 AND 2000).

Sample Date (mo and yr)	Fruit species	Fruit (kg)	Total No.												
			Cc pupae ¹	Cc adults	Af pupae	Af Adults ²	Parasitoid species ³								All parasitoids
							Da	Db	Dc	Ua	Ob	Ap	Oa	La	
Feb/1999	Guava	400 (14,251)	1	1	1,706	712	37	27	1	3	0	53	1	0	122
Mar/1999	Guava	400 (14,339)	2	2	2,462	955	62	33	0	19	0	72	0	0	186
Dec/1999	Peach	400 (13,115)	1,394	623	206	82	6	5	0	1	0	22	0	0	34
Total/1999		1,200 (41,705)	1,397	626	4,374	1,749	105	65	1	23	0	147	1	0	342
Feb/2000	Guava	400 (12,931)	10	2	2,397	902	35	21	0	10	1	63	0	0	130
Mar/2000	Guava	400 (16,192)	2	2	3,669	1,209	83	32	0	18	0	91	0	1	225
Dec/2000	Peach	400 (11,633)	1,067	451	164	65	0	0	0	0	0	15	0	0	15
Total/2000		1,200 (40,756)	1,079	455	6,230	2,176	118	53	0	28	1	169	0	1	370
Total		2,400 (82,461)	2,476	1,081	10,604	3,925	223	118	1	51	1	316	1	1	712

¹Cc, *Ceratitidis capitata*.

²Af, *Anastrepha fraterculus*.

³Da, *Doryctobracon areaolatus*; Db, *D. brasiliensis*; Dc, *D. crawfordi*; Ua, *Utetes anastrephae*; Ob, *Opius bellus*; Ap, *Aganaspis pelleranoi*; Oa, *Odontosema anastrephae*; La, *Lopheucoila anastrephae*.

TABLE 2. PARASITIZATION RATES ON *A. FRATERCULUS* AND *C. CAPITATA* AND INFESTATION LEVELS IN WILD GUAVA AND PEACH FRUITS COLLECTED IN BERMEJO, PROVINCE OF TARIJA, SOUTHERN BOLIVIA (1999 AND 2000).

Sample date (month and year)	Fruit Species (and No. samples)	Infestation level by Af ¹			Infestation level by Cc ¹		
		Larvae per fruit	Pupae per kg fruit	% Parasitism ¹ on Af	Larvae per fruit	Pupae per kg fruit	% Parasitism ¹
Feb/1999	Guava (40)	4.27 ± 1.68	119.84 ± 11.24	7.61 ± 5.60	0.01 ± 0.02	0.06 ± 0.13	0.00 ± 0.00
Mar/1999	Guava (40)	6.16 ± 2.06	170.21 ± 29.25	8.40 ± 5.52	0.01 ± 0.03	0.13 ± 0.26	0.00 ± 0.00
Dec/1999	Peach (40)	0.52 ± 0.71	15.92 ± 4.24	1.36 ± 2.51	3.50 ± 1.21	108.03 ± 20.76	1.38 ± 2.24
Feb/2000	Guava (40)	5.99 ± 1.81	185.58 ± 23.83	5.59 ± 3.78	0.03 ± 0.14	0.79 ± 1.52	0.00 ± 0.00
Mar/2000	Guava (40)	9.17 ± 3.07	225.99 ± 24.12	6.83 ± 3.57	0.01 ± 0.03	0.12 ± 0.27	0.00 ± 0.00
Dec/2000	Peach (40)	0.42 ± 0.56	14.35 ± 7.23	0.43 ± 1.94	2.71 ± 1.22	91.79 ± 16.09	1.43 ± 3.04

¹Values are presented as Mean ± Standard Deviation.

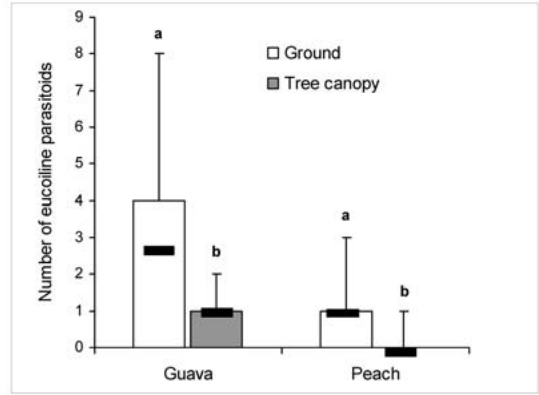


Fig. 1. Results of the Mann-Whitney U-test comparing eucoiline parasitoid number obtained from guava and peach fruits collected from the ground and from the tree canopy. The small black bar represents Median value, white and gray boxes represent Quartiles values (25-75 percentiles), and whisker represents Maximum value. Bars with different letters differ significantly (U -test, $P < 0.05$).

that these fruits are important reservoirs of *A. fraterculus* and *C. capitata*, respectively, in perturbed Yungas forests growing along the mountains of Tarija, southern Bolivia, and probably contribute to their high populations in agricultural environments, in accordance to that reported by Ovruski et al. (2003, 2004, 2005) in the Argentinean Yungas forests.

About 93% of all parasitoids were recovered from *P. guajava*. Seemingly, wild guava fruits not only would be a reservoir from which *A. fraterculus* would spread to orchards, but also as a valuable native parasitoid source in the southern Yungas forest of Tarija, Bolivia. Similar observations were made by López et al. (1999) and Aluja et al. (1998) in a tropical rainforest in Veracruz, México, and by Ovruski et al. (2004, 2005) in the Yungas rainforests of NW Argentina.

The parasitoid survey revealed 8 parasitoid species (*D. areolatus*, *D. brasiliensis*, *D. crawfordi*, *U. anastrephae*, *O. bellus*, *A. pelleranoi*, *O. anastrephae*, and *L. anastrephae*) attacking *A. fraterculus*, and 1 parasitoid species (*A. pelleranoi*) attacking *C. capitata* for Tarija, Bolivia. This survey is also provides the first records of *U. anastrephae*, *O. anastrephae*, and *L. anastrephae* for Bolivia, and the first evidence of *O. anastrephae* in the South American Yungas forest. All of these opiine and eucoiline species belong to the fruit fly parasitoid guild number 2 described by Ovruski et al. (2000), which is characterized by koinobiont, solitary, larval-pupal endoparasitoids of *Anastrepha* spp.

Before this study, *D. areolatus*, *D. brasiliensis*, *D. crawfordi*, *O. bellus*, and *A. pelleranoi* were the

only native parasitoid species recorded from *A. fraterculus* in the Yungas forest of Santa Cruz, a province in southern Bolivia (Escalante 1995). The exotic parasitoid *D. longicaudata*, which was recovered 6 years after its last release in the northern Yungas forest of La Paz, Bolivia, (Rogg & Camacho 2000) was not found in this southern survey.

All fruit fly parasitoid species recovered in this survey, with the exception of *O. anastrephae*, had been previously recorded from *A. fraterculus* in the Argentinean Yungas forests (Wharton et al. 1998; Ovruski et al. 2004, 2005). However, *D. crawfordi*, which is a widespread neotropical opiine species (Ovruski et al. 2000), is yet to be recorded in the southernmost portion of the Yungas forest of Argentina. Probably, the natural distribution in Yungas forest of this species includes only the northernmost portion of the Argentinean Yungas (Ovruski et al. 2005), which spreads as far as southern Bolivia embracing Tarija and Santa Cruz.

The temporal variations in parasitoid abundance and parasitization rates recorded in this study throughout the 3 summer months (Dec, Feb, and Mar) in 1999 and 2000 were closely related to the changes that occurred over time in the fruit infestation levels caused by the most important fly host, *A. fraterculus*, in wild guava fruits. Thus, higher parasitism rates on *A. fraterculus* and numbers of the 4 most commonly collected parasitoid in this study occurred in late summer (Mar). For example, *A. pelleranoi*, *D. areolatus*, *D. brasiliensis*, and *U. anastrephae*, increased between 1.4- and 6.3-times in abundance from Dec to Mar in both 1999 and 2000. This is consistent with data reported by Ovruski et al. (2005) for the northernmost sector of the Argentinean Yungas forest. Probably the increasing abundance of parasitoids from Dec to Mar in association with *A. fraterculus* population, has been influenced by environmental factors, such as heavier rainfall toward the end of the summer, a climatic characteristic of the Yungas forest phytogeographical region (Brown et al. 2001; Kessler & Beck 2001).

The eucoilid *A. pelleranoi* and the opiine *D. areolatus* were the most abundant parasitoid species attacking *A. fraterculus* larvae in wild guavas and feral peaches in the southern section of Yungas forest in SW Bolivia. Apparently, these 2 native parasitoid species exhibit a less competitive interaction in a host plant (Sivinski et al. 1997). These authors found differences in the foraging patterns between *A. pelleranoi* and *D. areolatus*. Thus, *D. areolatus*, like others fruit fly opiine parasitoids, stays on the fruit surface searching for host larvae, while *A. pelleranoi* mainly attacks larvae in fallen fruit by entering through holes in the fruit. The comparative analysis between eucoiline and opiine parasitoid numbers recovered

from fruit samples collected from the ground and those from the tree canopy for this study, corroborated previous reports by Sivinski et al. (1997) for Mexico, Ovruski et al. (2004) for Argentina, and Guimarães & Zucchi (2004) for Brazil, indicating that eucoiline parasitoids predominantly parasitize fruit fly larvae in fallen fruit.

The results of this study showed for first time several parasitoid species associated with *A. fraterculus* and only 1 with *C. capitata* in the southernmost portion of the Bolivian Yungas forest. However, these considerations are preliminary because only 2 fruit fly host plants were sampled in this extensive phytogeographical region of Bolivia. More exhaustive sampling of other *C. capitata* and *Anastrepha* host plants, mostly native fruit species, are therefore needed in this distinctive type of Bolivian subtropical forest before more definitive conclusions on parasitoid abundance patterns and diversity of fruit fly parasitoids species can be reached.

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