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First record of the parasitoid *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) associated with pupae of *Pterourus multicaudatus* (Kirby) (Lepidoptera: Papilionidae) in Mexico

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Primer registro del parasitoide *Pteromalus puparum* L.
(Hymenoptera: Pteromalidae) sobre pupas de *Pterourus*
multicaudatus (Kirby) (Lepidoptera: Papilionidae) en México

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Abstract: *Pteromalus puparum* L. is recorded for the first time parasitizing pupae of *Pterourus multicaudatus* (Kirby) in Mexico. Preliminary behavioral and biological aspects of *P. puparum* were recorded from sentinel pupae of *P. multicaudatus*. Parasitoid wasps arrived at the pupae beginning on the second day of exposure and interacted with pupae for a period of one to five hours for seven days. A parasitism level of 39% by *P. puparum* was recorded on *P. multicaudatus* pupae. Wasps began to emerge 46.15 days after pupae were exposed to parasitism, with an average of 144 wasps emerging per pupa. The sex ratio of parasitoids was skewed toward females, with a female: male ratio of 4:1.

Keywords: Biological control, Gregarious parasitoid, Papilionid, Phytophagous, Pteromalid.

Resumen: Se registra por primera vez a *Pteromalus puparum* L. como parasitoide de pupas de *Pterourus multicaudatus* (Kirby) en México. Aspectos preliminares de comportamiento y biológicos de *P. puparum* fueron registrados sobre pupas centinela de *P. multicaudatus*. Las avispas parasitoides arribaron a las pupas a partir del segundo día de exposición e interactuaron con las pupas por un período de una a cinco horas durante siete días. Las avispas comenzaron a emerger 46.15 días después de que las pupas

fueron expuestas al parasitismo, con un promedio de 144 avispas emergentes por pupa. La proporción de sexos del parasitoide no fue proporcional, dominando las hembras, en una proporción hembra: macho de 4:1.

Palabras clave: Control biológico, Fitófago, Papilionido, Parasitoide gregario, Pteromárido.

Pterourus multicaudatus (Kirby) (Lepidoptera: Papilionidae) (Fig. 1a), is a butterfly distributed from Canada and the United States through Mexico and Guatemala (NaturaLista, CONABIO, 2020). In Mexico this papilionid was reported from Chiapas, Durango, State of Mexico, Guanajuato, Hidalgo, Morelos, Oaxaca and Puebla (Cibrián-Tovar et al., 1995). Larvae of *P. multicaudatus* feed on leaves of *Citrus aurantium* L. (Rutaceae), *Fraxinus uhdei* (Wenz.) Lingelsh., *Ligustrum japonicum* Thunb. (Oleaceae), *Salix* spp. (Salicaceae), *Prunus persicae* (L.) Batsch. and *Prunus serotina* Ehrh (Rosaceae) (Cibrián-Tovar et al., 1995; Ramos et al., 2011). In rural communities of Mexico, people use adults of *P. multicaudatus* as foodstuff (Ramos et al., 2011). There is little information about natural mortality of *P. multicaudatus* (Jiménez-Galván et al., 2017). According to Gómez & Concha (2017), knowledge of insects natural enemies is a basic requirement from a science perspective, as well as a transcendental element in economic entomology. Santhosh & Basavarajappa (2017) point out that around 95% individuals in every Lepidoptera species die before adulthood due to the direct effects of entomopathogens, predators and parasitoids.

The most common parasitoids of Papilionidae are Hymenoptera (Chalcidoidea and Ichneumonoidea) and Diptera (Tachinidae) (Krishna et al., 2014; De Souza et al., 2015; Kan & Kan, 2015). The genus *Pteromalus* Swederus (Chalcidoidea: Pteromalidae) includes 504 species, 47 of them reported in North America; the majority are parasitoids of larvae and pupae of Coleoptera, Lepidoptera and Diptera (Baur, 2015; Noyes, 2018). The biology of this group of parasitoids is not well known, except that of *P. puparum* L., which is the most well-studied species. Native to Europe, *P. puparum* has been introduced into numerous countries for biological control of cabbage butterflies (*Pieris* spp.) (Lepidoptera: Pieridae) (Barron, 2004). Currently, it is registered in Canada, the United States, New Zealand, Japan, China, Iran, Russia, Spain, India, and Egypt (Abbas & El-Dakroury, 1985; Barron, 2004; Razmi et al., 2011; Liu et al., 2014), parasitizing pupae of 17 families of lepidopterans, including Nymphalidae, Papilionidae and Pieridae (Takagi, 1985; McDonald & Kok, 1990; Barron, 2004; Noyes, 2018).

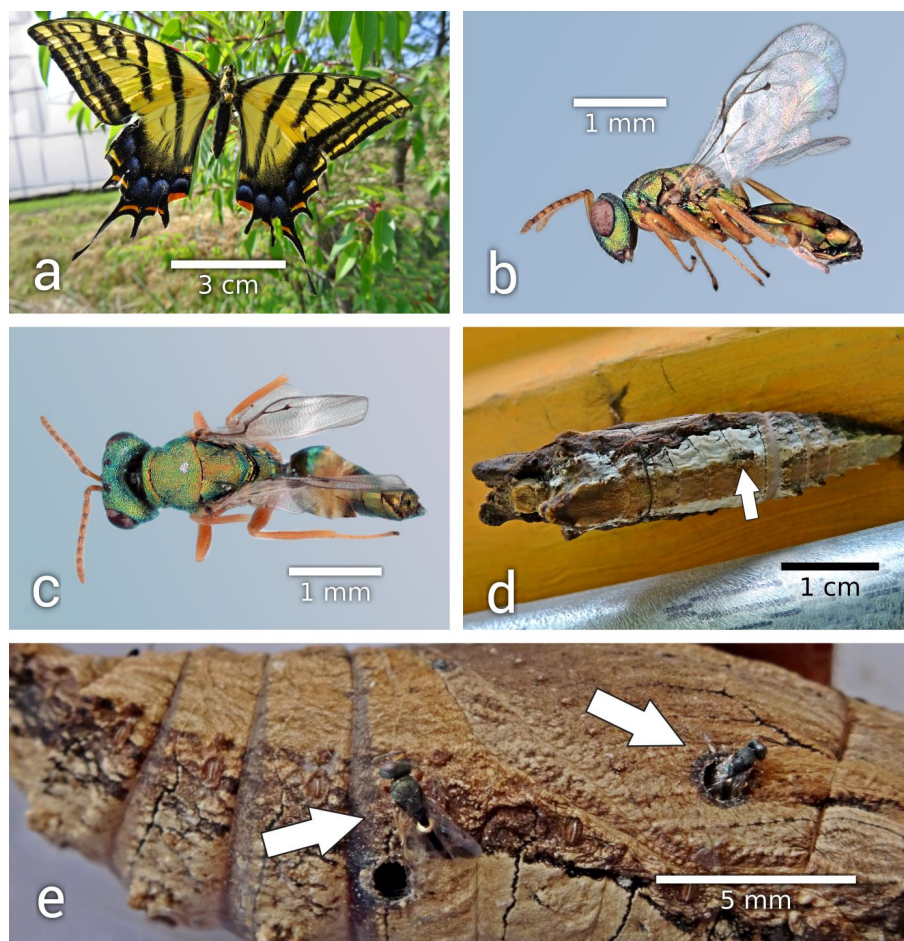


Fig. 1. *Pteromalus puparum* - *Pterourus multicaudatus* parasitoid - host complex. a. *Pterourus multicaudatus*. b. *Pteromalus puparum* male, lateral view. c. *Pteromalus puparum* female, dorsal view. d. Parasitized pupa of *P. multicaudatus*. e. Parasitoids emerging from a *P. multicaudatus* pupa.

Pteromalus puparum L. has been reported as parasitoid of 11 species of Papilionidae (Noyes, 2018). To our knowledge, the genus has got comparatively little attention in Mexico. Until now the only known record for *P. puparum* in Mexico was for the state of Guanajuato parasitizing *Pieris rapae* L. pupae (Marín-Jarillo & Bujanos-Muñiz, 2001). A preliminary biological study about *P. multicaudatus* published by Jiménez-Galván et al. (2017) reported a wasp of genus *Pteromalus* associated with pupae of this papilionid in the state of Mexico, but the authors were not able to determine the species. In an attempt to its identification, we received help from Dr. Hannes Baur (expert taxonomist in the genus) and, in this note, we report the first record of *P. puparum* parasitizing pupae of *P. multicaudatus* in the state of Mexico contributing to the knowledge of the biotic agents involved in *P. multicaudatus* mortality.

From September to November 2015, 13 *P. multicaudatus* pupae were collected from Santa Cruz Atzacapotzaltongo (19.1908° N, 99.3916° W; 2,621 m.a.s.l.), Toluca, State of Mexico. Pupae were transported

to the laboratory, where they were placed individually in Petri dishes (9 cm diameter) under laboratory conditions. The pupae were checked daily until the emergence of the parasitoids. Adult parasitoids were collected and preserved in 70% ethanol and sent to Dr. H. Baur (Institute of Ecology and Evolution, University of Bern, Switzerland), who provided species determination. Voucher specimens were deposited in the Insectarium of the Facultad de Ciencias Agrícolas at the Universidad Autónoma del Estado de Mexico.

A search for natural enemies of immature stages of *P. multicaudatus* was carried from March to September 2016; 48 eggs and 397 larvae in different stages of development were collected from leaves of *P. serotina* trees growing in private yards from two localities of the State of México: 1) El Cerrillo, Piedras Blancas (19.2432° N, 99.4120° W; 2,614 m.a.s.l.), municipality of Toluca, and 2) Agua Bendita (19.5230° N, 99.4406° W; 2,658 m.a.s.l.), municipality of Timilpan. In the laboratory, eggs were individually placed in Petri dishes (5.5 cm diameter) with absorbent paper moistened with distilled water as an incubation substrate. Larvae were placed in groups of three in plastic jars (14.0 × 10.5 cm diameter) with *P. serotina* leaves as a food source and they were replaced every three days. The jars were covered with cheesecloth to avoid larvae escape. Eggs and larvae were incubated at 26 ± 1 °C, 70% relative humidity, photoperiod of 10:14 h, and were checked daily to determine the presence of parasitoids.

From the larvae maintained under the aforementioned conditions, a sample of 28 “sentinel” pupae were exposed to parasitism. In three private yards (PY), pupae were adhered at a height of 1.75 m to the exterior walls of houses that had a *P. serotina* tree nearby. Eight pupae were evaluated in El Cerrillo, Piedras Blancas (June 25th, 2016; PY 1), ten pupae in Santa Cruz Atzacapotzaltongo (August 9th, 2016; PY 2) and ten in Timilpan (September 8th, 2016; PY 3). The behavior of parasitoids toward exposed pupae was observed daily during ten days from 08:00 to 20:00 h in Santa Cruz Atzacapotzaltongo (PY 2). Fifteen days later, pupae were removed and placed in individual Petri dishes to await the emergence of parasitoids or butterflies. The measured variables were the number of pupae parasitized and the number of parasitoids emerging *per* pupa, and a X^2 test was used to determine sexual proportion of the parasitoids. Statistical test was conducted using the software SAS (SAS, 2009).

No parasitoids were recorded on eggs or larvae in the sampled localities during 2016. Instead, five of the 13 pupae collected in 2015 in Santa Cruz Atzacapotzaltongo, all (ten) of the exposed pupae in Santa Cruz Atzacapotzaltongo, and one of the ten exposed pupae in Timilpan in 2016, were parasitized by *P. puparum* (Figs. 1b and 1c). No pupae parasitoids were recorded in El Cerrillo, Piedras Blancas. The distinctive morphological characters of *P. puparum* are mentioned by Askew & Shaw (1997) and Baur (2015).

| <i>P. multicaudatus</i> | <i>P. puparum</i> | | | |
|-----------------------------|-------------------------------|--------------------------------|------------------------------|-----------------------|
| Pupae (number) | Emerged individuals (n) | Emerged females (% ± SE) | Emerged males (% ± SE) | Female: Male Ratio |
| 1 | 19 | 57.89 | 42.10 | 1.37: 1 |
| 2 | 105 | 81.90 | 18.09 | 4.52: 1 |
| 3 | 7 | 71.42 | 28.57 | 2.50: 1 |
| 4 | 42 | 69.04 | 30.95 | 2.23: 1 |
| 5 | 96 | 90.62 | 9.37 | 9.66: 1 |
| 6 | 108 | 65.74 | 34.25 | 1.91: 1 |
| 7 | 202 | 76.23 | 23.76 | 3.20: 1 |
| 8 | 179 | 84.91 | 15.08 | 5.62: 1 |
| 9 | 173 | 59.53 | 40.46 | 1.47: 1 |
| 10 | 167 | 88.02 | 11.97 | 7.35: 1 |
| 11 | 498 | 68.07 | 33.93 | 2.00: 1 |
| 12 | 58 | 72.41 | 27.58 | 2.66: 1 |
| 13 | 74 | 87.83 | 12.16 | 7.22: 1 |
| 14 | 142 | 90.84 | 9.15 | 9.92: 1 |
| 15 | 265 | 52.07 | 47.95 | 1.08: 1 |
| 16 | 168 | 71.42 | 28.57 | 2.50: 1 |
| Average ± Standard error | 143.94 ± 29.48 | 74.24 ± 3.04 | 25.87 ± 3.06 | 4.07: 1 - |

Table I. Detail of *Pteromalus puparum* emerged from *Pterourus multicaudatus* pupae.

Pteromalus puparum wasps arrived at the pupae beginning on the second day of exposure, usually one female, though at times up to three, interacted with a pupa for a period of one to five hours. In Santa Cruz Atzacapotzaltongo a prepupa of *P. multicaudatus* of wild origin not belonging to our “sentinel” sample was observed near to our experimental sentinel pupae. Three females of *P. puparum* were observed moving along its body for eight hours; probably these females may have been waiting for the individual to transition to the pupal stage in order to parasitize it. Takagi (1985) reported similar behavior in *P. puparum* on prepupae of *Papilio xuthus* L. (Lepidoptera: Papilionidae), though it was described there as a case of phoresis. In this study, *P. puparum* oviposited in the non-sclerotic intersegmental areas between the thorax and abdomen of the pupae (Fig. 1d). Finally, on the seventh day of exposure, no more *P. puparum* wasps were observed on pupae of *P. multicaudatus*.

Pteromalid larvae consumed the entire internal tissues and organs of the host. The wasps chewed from the interior of the pupal wall outward and they exited the pupae over a period of one to seven days through one to three holes (Fig. 1e). In this study, we recorded sixteen *P. multicaudatus* pupae parasitized, out of 41, representing a parasitism

rate of 39%. In average, adult parasitoids emerged 46.15 ± 7.99 days after oviposition, with a range of 39-53 days (N = 10 pupae) during October and November. The number of parasitoids that emerged varied from seven to 498 wasps *per* pupa. Females outnumbered males at a ratio of 4:1 ($\chi^2 = 172.16$, DF = 15, $p \neq 0.01$) (Table I). Lasota & Kok (1986) have reported a rate of parasitism of 48.3% in pupae of *P. rapae* attributed to *P. puparum*, with an average of 52.3 wasps *per* pupa and a 1:1 female: male ratio. Razmi et al. (2011), calculated a 47.89% rate of parasitism by *P. puparum* on *Pieris brassicae* (L.) with an average of 42.36 wasps *per* pupa, but in some cases, they recorded up to 200 wasps *per* pupa. According to the importance of *P. puparum* as a cause of mortality in pupae of *P. multicaudatus*, additional studies are needed to determine its influence on population dynamics of this lepidopteran associated to *P. serotina*, that according to Cibrián-Tovar et al. (1995), is classified as a pest of forestry interest in Mexico.

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