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

JIMÉNEZ-GALVÁN, Edith¹, CASTAÑEDA-VILDÓZOLA, Álvaro²,* SÁNCHEZ-PALE, Jesús R.², AGUILAR MEDEL, Sotero³, VALDEZ-CARRASCO, Jorge⁴ & CORONADO-BLANCO, Juana M.⁵

1 Posgrado en Ciencias Agropecuarias y Recursos Naturales, Universidad Autónoma del Estado de México. Toluca, Estado de México, México.
2 Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México. Toluca, Estado de México, México. * E-mail: acastanedav@uaemex.mx
3 Universidad Autónoma del Estado de México, Centro Universitario UAEMéx Tenancingo. Tenancingo, Estado de México, México.
4 Colegio de Posgraduados, Campus Montecillo. Texcoco, Estado de México, México.
5 Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas. Ciudad Victoria, Tamaulipas, México.

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

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 periodo de una a cinco horas durante siete días. Se registró una tasa de parasitismo de *P. puparum* sobre pupas de *P. multicaudatus* igual a 39%. 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. Papiliónido. Parasitoide gregario. Pteromálido.

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 rate 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.
Pteromalus 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 iudehi (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 economic entomology. Santhosh & Basavarajappa (2017) point out that around 95% individuals in every Lepidoptera enemies (Rosaceae), (Rutaceae), feed pupae of your note, we report the first record of Pteromalus multicaudatus in the state of Mexico contributing to the knowledge of the biotic agents involved in P. multicaudatus parasitism.

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 Mexico: 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 larve 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 206, were parasitized by P. puparum (Figs. 1b

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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* 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 Atzcapotzaltongo 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*. 

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

| *P. multicaudatus* | *P. puparum* |
|--------------------|--------------|
| Pupae (number)     | Emerged individuals | 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 ±          | 143.94 ±      | 74.24 ±                   | 25.87 ±                 | 4.07: 1          |
| Standard error     | 29.48         | 3.04                      | 3.06                    | -                |

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

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 *puparum* on *Pieris brassicae* (L.) with an average of 46.15 ± 7.99 days after oviposition, with a 42.36 wasps *per* pupa, but in some cases, they 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, $p > 0.01$) (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 emerged 46.15 ± 7.99 days after oviposition, with a 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.
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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