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A New Species of *Enicospilus* Stephens, 1835 (Ichneumonidae, Ophioninae), from Southern Mexico, Parasitic on *Zanola verago* Cramer, 1777 (Lepidoptera, Apatelodidae), Feeding on *Piper neesianum* C. DC. (Piperaceae)

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Abstract

Plant–herbivore–parasitoid systems are poorly studied in the tropics. *Enicospilus carmenae* Campos and Palacio sp. nov. are described, originating from southern Mexico in the Yucatan Peninsula and establishing a new tri-trophic interaction. This species is a koinobiont larval endoparasitoid of the American silkworm moth caterpillar *Zanola verago* (Cramer) (Lepidoptera: Apatelodidae) feeding on the shrub *Piper neesianum* C.DC. (Piperaceae) in a semi-evergreen forest. The host plant *P. neesianum* had no herbivore records to date, and a single collection event yielded the rearing of a new species of *Enicospilus* (Ichneumonidae, Ophioninae). Morphological, molecular (COI), biological, ecological, and geographical data are integrated to delineate the new species.

Keywords: species description; natural history; tri-trophic interaction; Yucatán Peninsula

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1. Introduction

The discovery of new species and their interactions with other organisms is critical to developing a deeper understanding of biodiversity [1,2]. Interactions between plants, herbivores, and natural enemies involve over half of all described species [3], and documenting the natural history of these interactions contributes to understanding evolutionary and ecological outcomes, such as host specificity [4,5]. Studies of tri-trophic interactions between plants, herbivorous insects, and their natural enemies have contributed substantially to basic research in ecology [6–8], as well as applied sciences, including biological control [9] and conservation biology [10,11]. These tri-trophic studies are important for revealing ecological and evolutionary patterns that cannot be understood by separately studying each trophic level [12].

The genus *Piper* (Piperaceae), with a pantropical distribution, is one of the most diverse taxa within the angiosperms, with more than 1300 species in the Neotropics [13]. *Piper neesianum* C. DC. is native to the “ramonales” forests of the Yucatán Peninsula, which are semi-evergreen forests on calcareous soils dominated by *Brosimum alicastrum* Sw. [14]. Currently, the known distribution includes southern Mexico, Guatemala, and Belize [15]. There are no published herbivore associations for this species [16,17].

The American silkworm moth genus *Zanola* Cramer, 1777 (Bombycoidea, Apatelodidae), includes seven valid species [18]. *Zanola verago* is distributed throughout the Neotropical Region in relatively well-preserved forests [19–21]. The larva is grey-black and orange, with numerous secondary setae and distinctive subdorsal red spots. The head and tail have tufts of long black hairs with the tips widened into flat triangular scales [20]. *Zanola verago* is a polyphagous species that feeds on at least 12 plant species within 11 families across its distribution [17,20–22].

Ophioninae is an ichneumonid subfamily comprising slender wasps with orange to brown coloration. All are koinobiont solitary endoparasitoids of crepuscular and nocturnal Lepidoptera [23]. *Enicospilus* is the most species-rich genus within the subfamily, with more than 150 described species in the New World [24–28] and 61 species in Mexico [29]. The Mesoamerican species of *Enicospilus* are parasitoids of large moth caterpillars including Saturnidae, Lasiocampidae, and Erebidae, and, as far as is known, there is only one record of *Enicospilus* developing on Apatelodidae [17].

As a result of intensive surveys in semi-evergreen forests of the Yucatán Peninsula [30], a new tri-trophic interaction involving *Piper neesianum* (Piperaceae), *Zanola verago* (Lepidoptera, Apatelodidae), and a larval koinobiont endoparasitoid belonging to a new species of *Enicospilus* (Ichneumonidae, Ophioninae) is reported.

2. Materials and Methods

The parasitoid was reared from a manually collected caterpillar feeding on *P. neesianum* (Figure 1A) in a semi-evergreen forest in the southeast of Yucatán Peninsula (19.28° N–88.62° W). This locality is part of INIFAP, a forest reserve established in 1971. The caterpillar, identified as *Zanola verago* (Apatelodidae) (Figure 1B), was reared under ambient conditions (i.e., an outdoor facility, as is common for large rearing projects [4,17]) at the ECOSUR laboratory in a plastic cup covered with mesh and fed with leaves of *P. neesianum* until adult parasitoids emerged. The host remains were preserved in ethanol. A Nikon D850 digital camera attached to a Leica DMLB stereomicroscope was used to take photographs of the caterpillar.

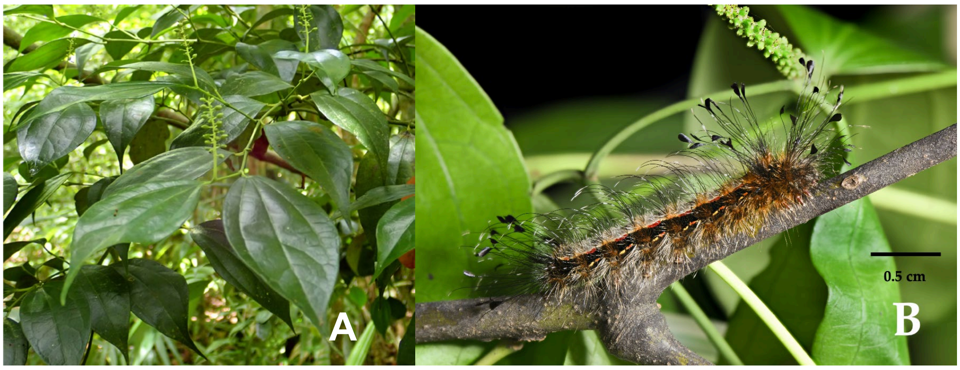

Figure 1. Habit of the host plant and host larva. (**A**) *Piper neesianum* (photo by E. Tepe); (**B**) *Zanola verago* (photo by D. Campos).

Morphological terminology, measurements, and indices for the species description follow [23,24]. Layer photos of the new species were taken at the Instituto Colombiano Agropecuario (ICA) using a LEICA MC170 HD camera attached to a LEICA S8APO stereomicroscope. The female holotype was compared with layer photos of *E. gamezi* taken at NHMUK using a Canon SLR EOS 5DSR with a 65 mm macro lens mounted on a copy stand with an automated Z-stepper; images were aligned using Helicon Focus software, version 6.6.1. The holotype is preserved at the Arthropod Collection of ECOSUR, Chetumal (ECO-CH-AR).

DNA Protocols

DNA extraction and PCR were carried out at El Colegio de la Frontera Sur (ECOSUR) Chetumal, using standardized protocols. DNA was extracted (from a single EtOH-preserved leg) according to the protocol in [31]. PCR amplification of the COI gene used primers LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ [32]. The faster rate of evolution of the mitochondrial genes is sometimes helpful for separating closely related species, and they are frequently used for the species delimitation of parasitoids [33,34]. We sent PCR products to the High Throughput Genomics Center in Seattle, USA, for sequencing. Newly generated sequences were deposited in GenBank database under accession number MZ960307. Additionally, sequences of species closely related (BLAST best hit) to new *Enicospilus* species were obtained from the Bold system [35,36]. Sequences were aligned in MEGA 10 [37,38]. Interspecific genetic distances were calculated using the Kimura-2-parameter model (K2P) [37] to produce the neighbour-joining (NJ) tree to display divergences [39], using *Thyreodon maculipennis* and *Cryptophion inaequalis* as outgroups.

3. Results

Enicospilus carmenae Campos & Palacio sp. nov.

ZooBank 965D83BA-07AF-4E22-AB69-157EE838792E (http://zoobank.org/965D83BA-07AF-4E22-AB69-157EE838792E, accessed on 21 April 2025).

3.1. Description

Holotype:

Zoobank: 3C55513F-C906-416F-927F-52CE8C57BA51;

Locality: 19.28° N; 88.62° W semi-evergreen forest in the southeast of Yucatán Peninsula; Mexico; female; adult; Catalog Code: AR730

Female: Length of body 21.0 mm. Fore wing length 16 mm. AI = 1.285; CI = 0.421; ICI = 0.633; SDI = 1.254.

Head: Clypeus in profile weakly convex, in frontal view 1.6× as broad as long, margin subtruncate (Figure 2A). Lower face as broad as long, polished, punctate. Head in dorsal view with genae rounded behind eyes; posterior ocellus very close to eye; FI = 0.651 (65%); occipital carina mediodorsally distinct, not joining hypostomal carina. Antenna with 61 flagellomeres, central flagellomeres at least 2.0× as long as broad.

Mesosoma: Mesoscutum polished, with fine shallow punctures, in profile evenly rounded, with anterior margin slightly turned forwards, notauli vestigial. Mesopleuron polished, the upper and lower parts punctostriate; epicnemial carina abruptly curved towards anterior margin of pleuron. Scutellum, in profile weakly convex, laterally carinate entire length; in dorsal view 1.11× as long as anteriorly wide, virtually smooth. Metapleuron strongly convex, with fine diagonal striation; submetapleural carina broadened towards base; posterior transverse carina of mesosternum complete. Propodeum in profile declivous; anterior transverse carina complete, strong (Figure 3A); posterior transverse carina absent; anterior area coriaceous to striate; spiracular area short, slightly rugulose; posterior area strongly rugose; metapleural carina complete, not joined to spiracular margin by a short carina. Fore tibia slightly flattened, with scattered spines on outer surface. Mid-leg with longer tibial spur 1.40× the length of the shorter. Hind leg with tarsomere IV

3.3× as long as broad; claws evenly down-curved on apical quarter (Figure 3C), with long stout pecten.

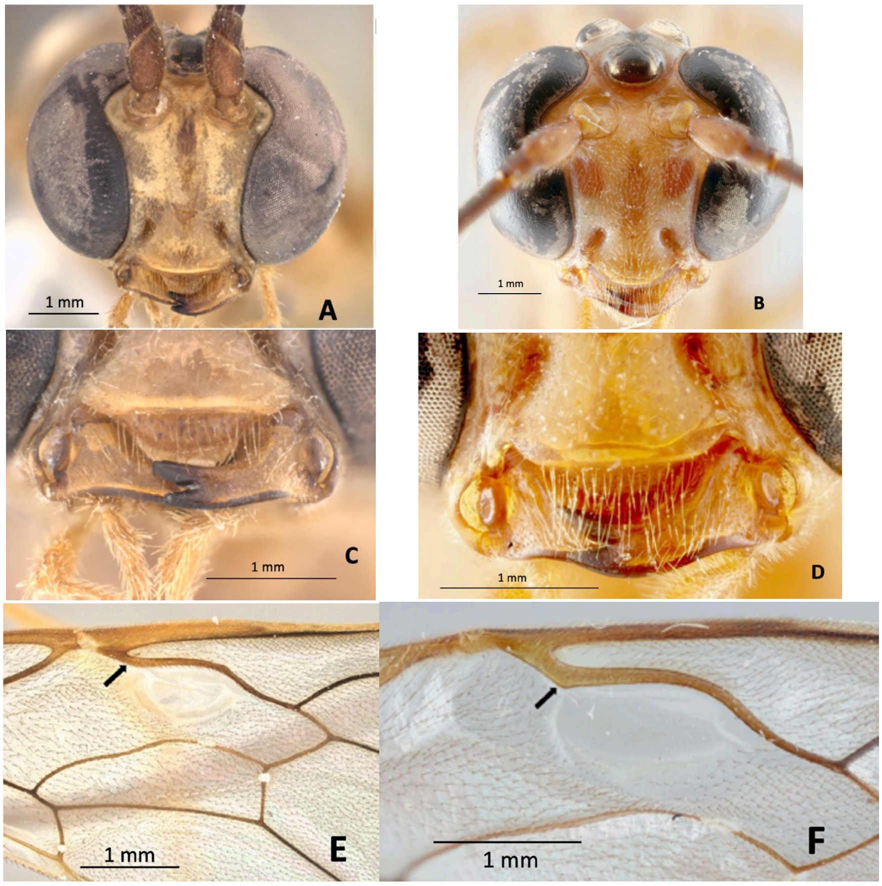

Figure 2. Holotype of *Enicospilus carmenae* (**A**,**C**,**E**) (photos by E. Palacio); holotype of *Enicospilus gamezi* (**B**,**D**,**F**) (photos by G. Broad): (**A**,**B**) head in frontal view; (**C**,**D**) mandibles; (**E**,**F**) fore wing; arrow points to the basal section of vein *Rs* + 2*r*.

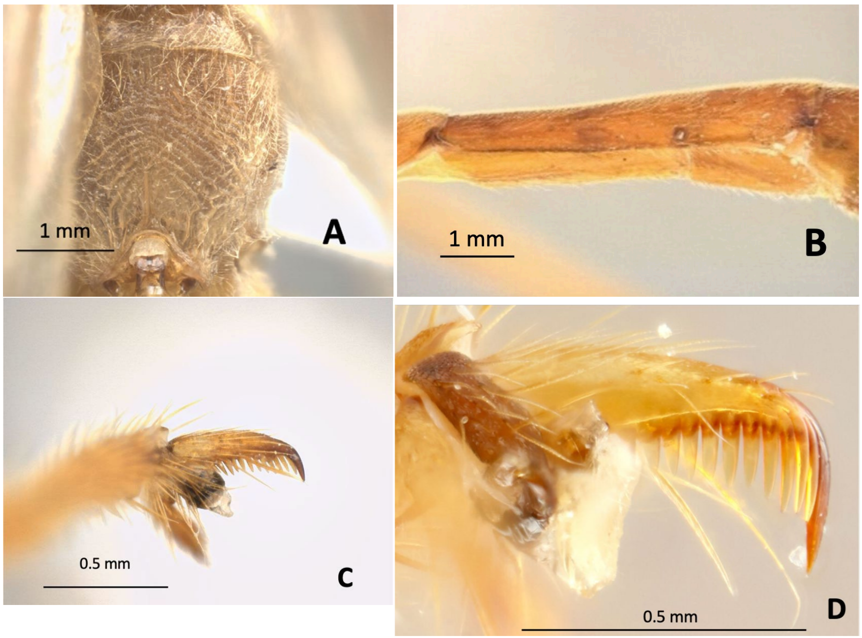

Figure 3. *Enicospilus carmenae* (**A**–**C**) (photos by E. Palacio); *E. gamezi* (**D**) (photo by G. Broad from Holotype in NHMUK): (**A**) propodeum in dorsal view; (**B**) metasomal segment II in lateral view; (**C**) metatarsal claw of *E. carmenae;* (**D**) metatarsal claw of *E. gamezi*.

Wings: Fore wing with vein *Rs* + 2*r* strongly sinuate, not basally angulate below (Figure 2E) fenestra without distinct sclerites, basally not extending to level of base of pterostigma, its apex clearly separated from base of vein *Rs*; *cu-a* proximal to base of *RS* and *M* by about 0.27× its own length; marginal cell proximally evenly hirsute; first subdiscal cell with anterior 0.4 sparsely hirsute; second discal cell 3.29× as long as wide. Hind wing with eight hamuli on R1, first abscissa of *Rs* straight, second abscissa slightly curved.

Metasoma: Slender, tergite 2 in profile 4.4× as long as posteriorly deep, laterotegite not pendant, thyridia oval and separated from anterior margin of tergite by about 3.9× its own length (Figure 3B). Ovipositor slender, its sheath narrow.

Colour: Pale yellowish-brown, with basal antennomeres and metasomal segment V darker (Figure 4A); interocellar area with black markings bordering the ocelli. Pterostigma golden, wings hyaline.

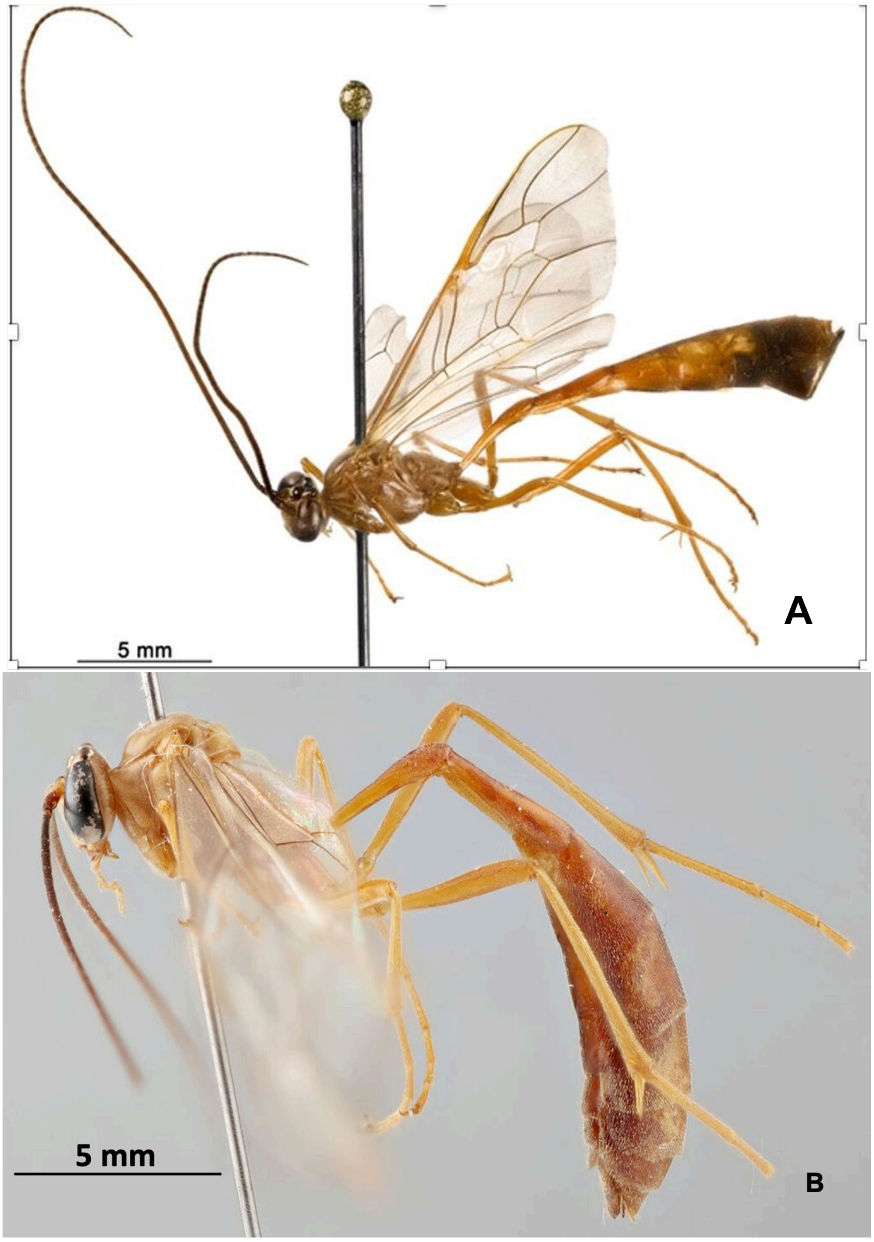

Figure 4. Habitus of *Enicospilus:* (**A**) holotype *E. carmenae* (photo by E. Palacio); (**B**) holotype *Enicospilus gamezi* (photo by G. Broad from Holotype in NHMUK).

Male: Unknown.

3.2. Diagnosis

E. carmenae can be distinguished from other species of *Enicospilus* by the combination of the following characteristics: fore wing with vein *Rs* + 2r tubular, sinuous, not basally angulate below; fenestra without distinctive sclerites (Figure 3E); apex of fenestra detached from *Rs* vein base; second discal cell 3.29× longer than high; anterior transverse carina of propodeum complete, strong (Figure 4A).

Remarks: *E. carmenae* sp. nov. links to *E. gamezi* in couplet 20 of Gauld's [23] key to *Enicospilus* species. As in *E. gamezi* (Figure 4B), the new species has central flagellar segments 2 or more times as long as broad and a propodeum without posterior transverse carina, but differs in the more slender mandibles with a subtruncated upper tooth (Figure 3C) (thicker mandibles, with the upper tooth pointed in *E. gamezi*—Figure 2D), the clypeus 1.6× as broad as long (Figure 2A) (1.4–1.5× in *E. gamezi*—Figure 3B), the vein Rs + 2r of the fore wing not basally angulated below (Figure 2E) (clearly angulated below in *E. gamezi*—Figure 3F), and the hind leg tarsal claw evenly down-curved on its apical quarter (Figure 3C) (abruptly down-curved in *E. gamezi*—Figure 3D).

3.3. Etymology

It is named after Carmen Pozo, who has dedicated most of her life to the study of the taxonomy, biogeography, and ecology of Lepidoptera and the conservation of biodiversity in southeastern Mexico.

3.4. Distribution

Southern Mexico, Yucatan Peninsula.

3.5. Ecology

Habitat: Known in semi-evergreen tropical forests.

3.6. Biology

All species of Ophioninae are known as koinobiont endoparasitoids, and this is also the case for *E. carmenae*, which was parasitizing the *Zanola verago* larva while it was still actively feeding on *Piper* leaves. The exact moment at which the host caterpillar was parasitized is unknown, but it became a pupa eight days after being collected and the female parasitoid wasp emerged as an adult 18 days later.

3.7. DNA Barcode

A single 584 bp sequence was recovered from the holotype (AR730, Bold no. DFCM CH752B07, NCBI MZ960307). The closest related species was *E. gamezi,* with a variation p-distance of 1.5% (Figure 5), with differences in nucleotide positions 238G, 268A, 340C, 373G, 442C, 460C, 562C, and 629A. These differences are sufficient to separate many morphologically and ecologically distinctive species [40–42]. Interspecific variations with other related species (*E. cameronii*, *E. bozai*, *E. chiriquensis*) show p-distances of 6–8%.

Figure 5. Phenogram based on K2P distances for COI for *E. carmenae* sp. nov. (NCBI MZ960307) and related sequences of the genus *Enicospilus* downloaded from the BOLD system and International Nucleotide Sequences Database. The black circle indicates holotype.

4. Discussion

During the 40 years of intensive rearing of caterpillar parasitoids carried out by Janzen and Hallwachs and their team in Costa Rica, only one species of *Enicospilus*, *E. gamezi*, has been recorded as a parasitoid of apatelodid larvae [17]. It is striking that *E. gamezi* is the most closely related described species to *E. carmenae* according to the current study, suggesting a consistent pattern of interactions between *Enicospilus* and Apatelodidae hosts. Similar caterpillar-rearing projects, carried out for over 35 years in Costa Rica [42] and 25 years in Ecuador [43], have not reported apatelodid caterpillars parasitized by *Enicospilus*, so these interactions are likely to be rare. Rare ecological interactions and rare species are key components of the functional and taxonomic diversity of tropical ecosystems, often contributing disproportionately to ecosystem stability and resilience [4,44]. These rare interactions may reflect specialized evolutionary histories or ecological constraints that are an important part of understanding the full complexity of tropical food webs [4]. We acknowledge that this description is based on a single specimen, and while the evidence supports its designation as a new species, further research on this genus is necessary, especially for exploring genetic variation among closely related species.

Taking into account that Lasiocampidae, Erebidae, and Saturniidae are the Lepidopteran families most commonly reported as hosts of Mesoamerican *Enicospilus* [16,45], the recording of Apatelodidae is not entirely unexpected. However, it is interesting that the host plant, *P. neesianum*, had no herbivore records to date [16,17] and that a single collection event yielded the rearing of a new species of *Enicospilus* from a host belonging to a family of Lepidoptera that had only been reported once as a host of Ophioninae [17]. There is much to be done in the study of food webs, especially with respect to the identification of tropical species involved in these poorly studied plant–herbivore–parasitoid systems [4,5], and much more research is required to understand patterns of specialization and speciation in Ophioninae [44,46].

5. Conclusions

In conclusion, this new plant–herbivore–parasitoid system contributes to the continued documentation of tri-trophic interaction diversity [1]. The fact that this is a singleton is relevant to questions about assessing specialization and interaction diversity in tri-trophic interaction webs that are dominated by rare species. It is necessary to continue documenting trophic interaction diversity [1] while contributing to resolving taxonomic and systematic issues for the taxa that comprise these interactions.

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Conflicts of Interest: The authors declare no conflict of interest.

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