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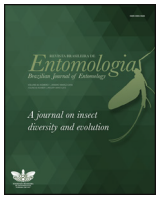

A new interaction in an invasive plant in Brazil: *Horismenus abnormicaulis* (Hymenoptera, Eulophidae) parasitizing *Acanthoscelides macrophthalmus* (Coleoptera, Chrysomelidae, Bruchinae) in seeds pods of *Leucaena leucocephala* (Fabaceae)

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ABSTRACT

The parasitism of *Acanthoscelides macrophthalmus* (Coleoptera, Chrysomelidae, Bruchinae) by the parasitoid *Horismenus abnormicaulis* (Hymenoptera: Eulophidae) is described for the first time. We harvested 90 *Leucaena leucocephala* (Fabaceae, Mimosoideae) fruit pods, in June 2019 on the campus of the Federal University of Lavras in the municipality of Lavras, Minas Gerais, Brazil. We found 21 individuals of *H. abnormicaulis* and 334 of *A. macrophthalmus*. The presence of cephalic capsule of beetle's pupa and evidence of predation into seed pods from which the wasps emerged suggested *H. abnormicaulis* is a parasitoid of *A. macrophthalmus*. *Horismenus abnormicaulis* has been added to the list of *A. macrophthalmus* parasitoids, and its occurrence has been expanded to the state of Minas Gerais. This discovery expands our knowledge of *H. abnormicaulis* biology and distribution in Brazil, as well as the tri-trophic interaction, plant-herbivore-parasitoid in invasive plant systems.

Horismenus Walker, 1843, is one of the most diverse species genera in Eulophidae (Hymenoptera), with over 420 species, many of each are known as parasitoids of immature insects of seven different orders and numerous families, as well as egg sacks from four families of arachnids (Hansson, 2009). This genus is mostly found in South and Central America (LaSalle and Schauff, 1992; Hansson, 2009), although there are also reports from North America, one in Europe (Bouček, 1965) and one in India (Narendran et al., 2011). So far, only forty *Horismenus* species have been identified in Brazil (Salgado-Neto et al., 2024).

Most species of *Horismenus* are primary parasitoids, while at least 14 of its species are secondary parasitoids of Braconidae, especially Microgastrinae (Hansson et al., 2014; Pikart et al., 2015; Salgado-Neto et al., 2024). Many Eulophidae species, including *Horismenus*, parasitize insect larvae that develop in leaves and seeds of plants (Lasalle and Schauff, 1992). Pérez-Benavides et al. (2019) provided an extensive list of bruchine beetles parasitized by *Horismenus* species.

Leucaena leucocephala (Lam.) de Wit (Fabaceae: Mimosoideae) is a leguminous plant native to southern Mexico. It is considered an invasive plant in tropical climates because of its allelopathic propensity and facility to establish its population in disturbed areas (Scherer et al., 2005). The first report of *L. leucocephala*'s introduction to Brazil was officially made for the state of São Paulo in November 1940 (Hill, 1971 *apud* Vilela and Pedreira, 1976). However, recent evidence suggests that might have been introduced in 1831 or before, and it is widespread across throughout Brazil (Machado et al., 2020). *Leucaena leucocephala* is a semi-deciduous tree that grows to 5 to 7 meters tall and can blooms during all year, but specially in September and November. When mature, the pods are thin and flat, with 15–25 seeds and vivid brown color; they have two to four fructifying cycles per year (Lorenzi et al., 2003). Due to its high nutritional value, it has been widely cultivated for the regeneration of degraded areas such as pastures and eroded landscapes, as well as a protein source

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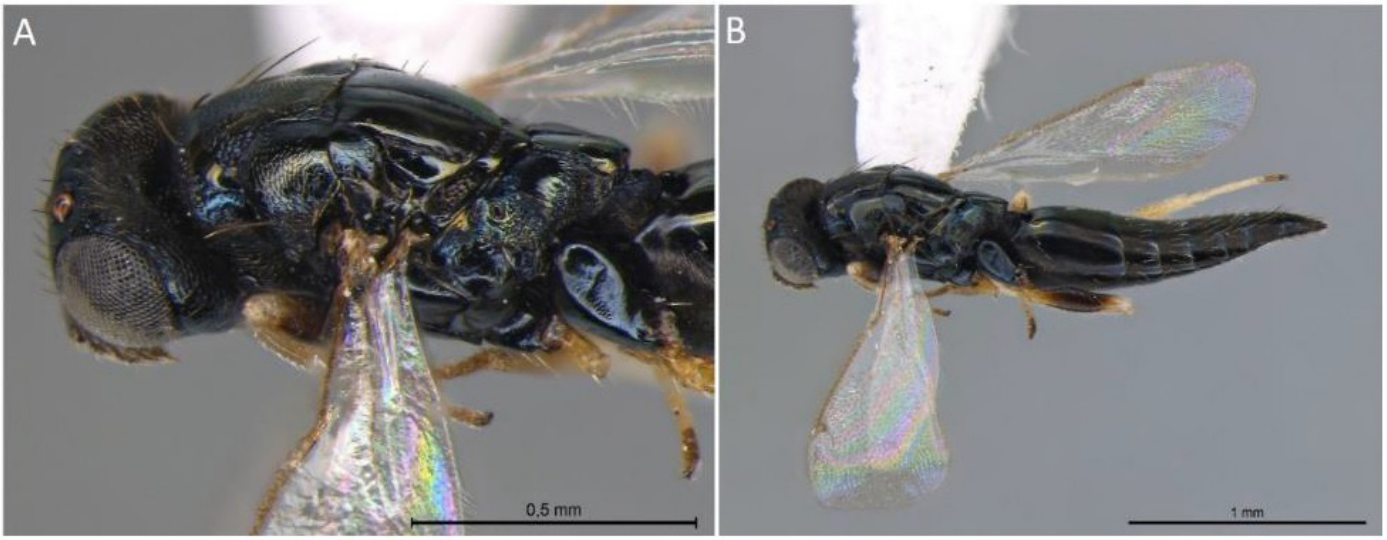

Figure 1. *Horismenus abnormicaulis*, female: A) head and mesosoma lateral; B) habitus.

for animal feed in agro-silvopastoral systems (Nakamanee et al., 2019). The main seed consumer associated with *L. leucocephala* seeds in its native region is *Acanthoscelides macrophthalmus* (Schaeffer, 1907) (Coleoptera: Chrysomelidae: Bruchinae) (Kingsolver, 2004). This beetle was found consuming seeds of this plant species in Brazil (Rodrigues et al., 2012).

We collected 90 fruit pods from three individuals of *L. leucocephala* in June 2019, at the University Federal of Lavras main campus, southwestern region of the state of Minas Gerais (21°13′42.79″S; 44°59′11.04″W), Brazil. All pods were collected directly from the mother plant. In the laboratory, we individually stored each fruit in labeled PVC tubes covered with *voil* fabric. The specimens of *H. abnormicaulis* that emerged were sent to and deposited in the Oscar Monte Collection of Entomophagous Insects, Instituto Biológico, Campinas, state of São Paulo, Brazil, where they were critical-point dried (Gordh and Hall, 1979) and mounted on points (unique identifiers: IB-CBE 006619-006628) (Fig. 1A, 1B). The specimens were inspected using Leica M165C stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany), and identified using the keys provided by Schauff et al. (1997) and Pikart et al. (2015).

A total of 21 individuals of *H. abnormicaulis* and 334 of *A. macrophthalmus* emerged. The parasitism association between *H. abnormicaulis* to *A. macrophthalmus* was established when cephalic capsule of the beetle larvae was found in dissected seeds from where the wasps had emerged (following the protocol described in Morales-Silva et al., 2018), as well as the seed pattern of predation caused to it before being parasitized. The parasitoids are solitary. This is the first record of this interaction, and occurrence of *H. abnormicaulis* in the State of Minas Gerais.

Horismenus abnormicaulis was originally described from seed pods of *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) collected in November 2011 in the municipality of Patos, state of Paraíba, northeastern Brazil (Pikart et al., 2015). The authors speculated about *H. abnormicaulis* could be occurring as a hyperparasitoid of either *H. patensis* Pikart, Costa and Hansson, 2015, or *H. zuleidae* Pikart, Costa and Hansson, 2015, or both species in the same plant, but it has not been demonstrated. Pikart et al. (2015) made suggestion based on its morphological similarities with *H. distinguendus* Blanchard, 1936, which was reported to be a hyperparasitoid of Diptera (Parker et al., 1953) and Hymenoptera (Blanchard, 1936). Pikart et al. (2015) also mentioned the specimens of *Horismenus* species (including *H. abnormicaulis*) they described "were obtained from seed pods of *Pithecellobium dulce* infested with larvae of Bruchinae (Chrysomelidae) and Curculionidae (both Coleoptera)". Thus, the specimens of *H. abnormicaulis* they reared may have emerged from Bruchinae.

Despite being considered an invasive species in Brazil, studies have revealed several parasitoids are associated with *A. macrophthalmus* on *L. leucocephala* besides *Horismenus* spp., such as *Paracrias pluteus* (Hansson, 2002) (Eulophidae), *Stenocorse bruchivora* (Crawford, 1909), *Urosigalphus* sp. (Braconidae), *Eupelmus (Eupelmus) pulchriceps* (Cameron, 1904) (Eupelmidae) and *Chryseida* spp. (Eurytomidae) (Wood et al., 2017). Our findings add *H. abnormicaulis* to this list and to the understanding of tri-trophic interactions, plant-herbivore-parasitoid, in introduced/invasive plant systems, as well as the biology and distribution of *H. abnormicaulis* in Brazil. Additional rigorous collecting effort would be necessary to identify the degree to which this species may be associated with other hosts.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

TCTO conceived the study, discovered the interaction, wrote the first draft of the manuscript, revised, and approved the final version by TMS, VAC, and LDBF of this article. TMS and VAC identified and confirmed the parasitoid species.

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