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First record of males of the invasive eucalyptus pest species *Leptocybe invasa* Fisher & LaSalle, 2004 (Hymenoptera: Eulophidae: Tetrastichinae) from South America

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Abstract. The "blue gum chalcid", *Leptocybe invasa* is a pest of eucalyptus worldwide. It has an Australian origin and has expanded into Asia, Europe, Africa and America. *L. invasa* females were reported from South America in the last decade. Thelytokous parthenogenesis is the most common reproductive mechanism of this pest. However, male adults have been reported from Asia and Southeastern Europe. In this work, *L. invasa* males are reported for the first time from South America (Argentina) and information on morphological characters of males is provided. The importance of this discovery is highlighted based on recent studies that suggested the existence of two cryptic *Leptocybe* species in invasive populations.

Key words. Blue gum chalcid, gall, forest, chalcidoidea.

The "blue gum chalcid", *Leptocybe invasa* Fisher & LaSalle, 2004 (Hymenoptera: Eulophidae: Tetrastichinae) is a global pest in *Eucalyptus* plantations. The wasp lays eggs in plant tissues causing the formation of galls on the midribs, petioles and stems of young leaves. Severely infested trees have a gnarled appearance, show stunted growth, and in young trees, this pest can cause significant injury (Mendel et al. 2004; Nyeko, 2005).

The rapid colonization after *L. invasa* spread into a novel environment can be attributed to the absence of natural enemies, the presence of large amounts of suitable host plants, the resistance to low temperatures of adults and the occurrence of two reproductive modes (Zheng et al. 2014b). This pest displays thelytokous reproduction but since the discovery of males, sexual reproduction has been considered a second reproductive strategy. Nevertheless, factors influencing reproductive strategies and the relation between reproductive strategy and population expansion are unknown (Zheng et al. 2014a). In the current study, we describe male specimens of *L. invasa* from Argentina and report the sex ratio in eucalyptus plantations in the country.

In 2017 and 2018, a survey of *L. invasa* populations and natural enemies was conducted in infested eucalyptus plantations in an extensive productive area in Buenos Aires and the Mesopotamia region (Entre Ríos, Corrientes and Misiones). Almost fifty samples of 20–30 cm long, *L. invasa* gall infested branches were collected. The plant material was kept in glass containers with voile lid and absorbent paper under controlled laboratory conditions (T: 25 ± 2ºC, HR: 50–70% and natural lighting). The insects that emerged from each sample were kept in 70% ethanol and were identified by the first author. The specimens were card- and slide-mounted following Noyes (1990) and were compared with the descriptions of the males in Doğanlar (2005), Chen et al. (2009) and Zheng et al. (2014b). Voucher specimens were deposited at the División Entomología of Museo de La Plata. Sex ratios were calculated as Σ♀/Σ(♀+♂). Measurements were taken with a Leitz Wetzlar SM/LUX; specimens were photographed using a Leica DFC295 digital camera attached to a stereomicroscope Biotraza. Series of partially focused digital images were stacked using the Helicon Focus software (Version 6.8.0 Pro) by ©Helicon Soft Ltd., 2000. Photoshop CC2018 was used to produce final images with enhanced quality.

Material examined. Argentina, Entre Ríos, Concordia en 8.iii.2018 and 5.x.2018 (sex ratio 3m:104f (0,972) and 17m:1290f (0.987)); Argentina, Corrientes, Alvear 28.ix.2018 (sex ratio 1m:24f (0,960)).

According to Doğanlar (2005), the male is similar to the female described by Fisher & LaSalle (2004), except:

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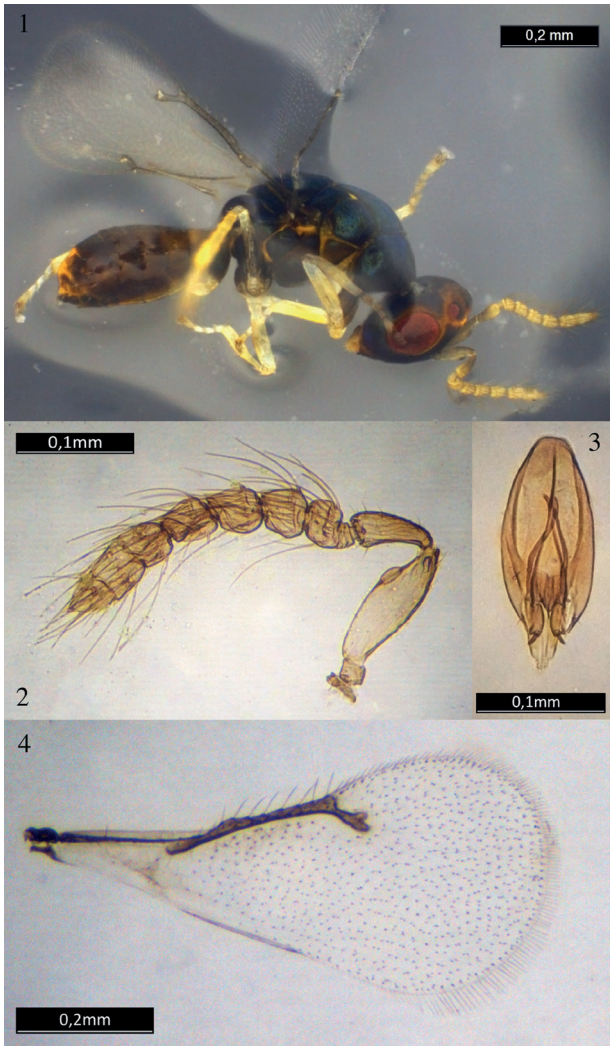

Figs. 1–4. *Leptocybe invasa* male from Argentina. **1**. lateral habitus; **2**. antenna; **3**. genitalia; **4**. forewing.

head and mesosoma brown with distinct blue to green metallic shine (Fig. 1); ocellar triangle and mouth margin light brown; eyes and ocellus red; malar sulcus dark brown; metasoma brown; legs almost white, except mid and hind coxae same color of the body and hind femur light brown. Antenna with scape and pedicel light brown darkened dorsally and apically; funicle and clava light brown to almost white. Wings hyaline, veins light brown (Fig. 4). Genitalia (Fig. 3) with digitus having one claw. The specimens collected in this study are similar to those described by Doğanlar (2005) except for the following measurements and ratios: Head 1.28 times as broad as high. Antenna (Fig. 2) with scape 3.13 times as long as broad, ventral plaque 0.18–0.24x length of scape; pedicel 1.9–2.8x as long as broad, longer than anelli plus F1; F1 shorter than F2 (in figure 1b in Doğanlar (2005) it is observable that F1 is shorter than F2 although he mentions that "*F1 is 1.46x longer than F2*"), about 0.75x length of F2, as long as broad to 0.7 times as long as broad, F2 to F4 subequal in length, each slightly longer than broad; clava about 2.75 times as long as broad, i.e., slightly longer than that described by Doğanlar (2005), with C1–C3 decreasing in length, except in one specimen in which C1 is shorter than C2 and C3.

Leptocybe invasa males were reported from Turkey, China, India, Taiwan and Thailand, and populations of this pest around the world are known to have different sex ratios over their geographic distribution (Nugnes et al. 2015). Males are rare in Turkey (sex ratio: 0,992, n= 125) (Doğanlar 2005) and India (sex ratio: 0,992, n=141) (Akhtar et al. 2012). In China a low proportion of males was reported from Fujian, Guangdong, Hainan, Guangxi, Jiangxi and Sichuan (sex ratio ≥ 0,95), but a high proportion of males was reported from other regions (males proportion ranging from 18–48%) and in Thailand (sex ratio: 0,663) (Liang et al. 2010; Santongprow et al. 2011; Zheng et al. 2018).

In this work, in only two out of twenty sampling sites males were found (in Entre Ríos and in Corrientes) and a clearly female-biased sex ratio was found, with values ≥ 0,96.

While *L. invasa* females were recorded from several countries in America since 2007 (Brazil (2007), Argentina (2009), Paraguay (2012), Uruguay (2013), Chile (2014), Mexico (2014) and USA (2008) (Costa et al. 2008; Wiley and Skelley, 2008; Aquino et al. 2011; SAG 2014; Benítez et al. 2014; Vanegas et al. 2015; Jorge et al. 2016)), this is the first report of *L. invasa* males from America. Recent molecular analyses suggested that *L. invasa* is, in fact, a complex of at least two cryptic species involved in the rapid and efficient spread of the wasp (Nugnes et al. 2015; Dittrich-Schröder et al. 2018). Nugnes et al. (2015) proposed that there is a link between the presence of males in the invasive range and lineage identity. Further molecular analyses are necessary to understand the invasion pathways and to characterize the populations of *Leptocybe* species in Argentina and other countries in America.

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