

Systematics and Biology of *Cremastobombycia chromolaenae*, New Species (Gracillariidae), a Natural Enemy of *Chromolaena odorata* (L.) King and H. Robinson (Asteraceae)

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SYSTEMATICS AND BIOLOGY OF *CREMASTOBOMBYCIA CHROMOLAENAE*, NEW SPECIES
(GRACILLARIIDAE), A NATURAL ENEMY OF *CHROMOLAENA ODORATA* (L.) KING AND
H. ROBINSON (ASTERACEAE)

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ABSTRACT. A new species, *Cremastobombycia chromolaenae*, is described from Florida and Texas, USA. The larva is a leaf miner on *Chromolaena odorata* (L.) King and H. Robinson, a 2–3 m tall perennial shrub of the family Asteraceae, whose native range extends from southern Florida south to northern Argentina (Fig. 1). The plant is also known to occur in Africa, Asia, and Australia where it is considered exotic and in some countries has become a major weed. The larvae of *C. chromolaenae* are hypometamorphic and possess two distinct larval body forms and feeding behaviors—an early stage sap-feeding form with a flattened body and prognathous mouthparts and a later stage tissue-feeding form with a more cylindrical body and possessing hypognathous mouthparts. Based on head capsule counts and measurements, the larvae undergo five instars, with the first three instars being of the sap-feeding form and the last two tissue-feeding instars. The larval mine begins as a short, serpentine track which enlarges to a rounded, whitish, and eventually tentiform blotch. *Cremastobombycia chromolaenae* may have value as a biocontrol agent in those areas where the host plant has become a major problem.

Additional key words: biocontrol agent, biogeography, genital morphology, invasive plant species, larval biology.

The predominantly North American genus *Cremastobombycia* was originally proposed as a subgenus of *Lithocolletis* (= *Phyllonorycter*) by A. Braun (1908). Currently eight species are recognized in *Cremastobombycia* (Davis 1983, Davis and Miller 1984, De Prins and De Prins 2012). Although no new taxa have been reported in this genus in more than a century, at least seven new North American species are known in addition to *C. chromolaenae*, and these will be proposed in a future monograph of the North American Gracillariidae. Five of the named species are restricted to the continental United States, with *C. lantanella* Busck reported from Mexico and later introduced into Hawaii. Two new species of *Cremastobombycia* have recently been described from Africa (J. De Prins and A. Kawahara 2012b). Larvae of all species, except *lantanella* which feeds on *Lantana camara* L. (Verbenaceae), are leaf miners on various genera of Asteraceae (De Prins and De Prins 2012a).

The genus *Cremastobombycia* is most closely related to the genera *Phyllonorycter* and *Cameraria*, based on both molecular (Kawahara et al. 2011) and

morphological data. Morphologically, *Cremastobombycia* differs from these two genera in retaining vein M_2 in the forewing and CuA_1 in the hindwing (Fig. 5), which are lost in *Phyllonorycter* and *Cameraria*. The labial palpi of all three genera are similar in being greatly reduced to two short segments (Fig. 4). The elongate saccus in the male genitalia of *Cremastobombycia* is usually characteristic for this genus and is seldom developed to that extent in other gracillariid genera (Davis and De Prins 2011). Late instar, tissue-feeding larvae of *Cremastobombycia* are distinct in possessing a specialization of the hypopharynx unlike that examined in any other gracillariid genus. In addition to the dense covering of small, dorsal spines, which are present in most genera of tissue-feeding gracillariid larvae, the anterior margin of the hypopharynx of larval *Cremastobombycia* bears a transverse series of six relatively large, spinose, digitate lobes. The spines of these lobes increase in length toward the apex of each lobe. The hypopharynx of *C. chromolaenae* is identical to that of *C. ignota* (Figs. 13–15) and also to another undescribed species of

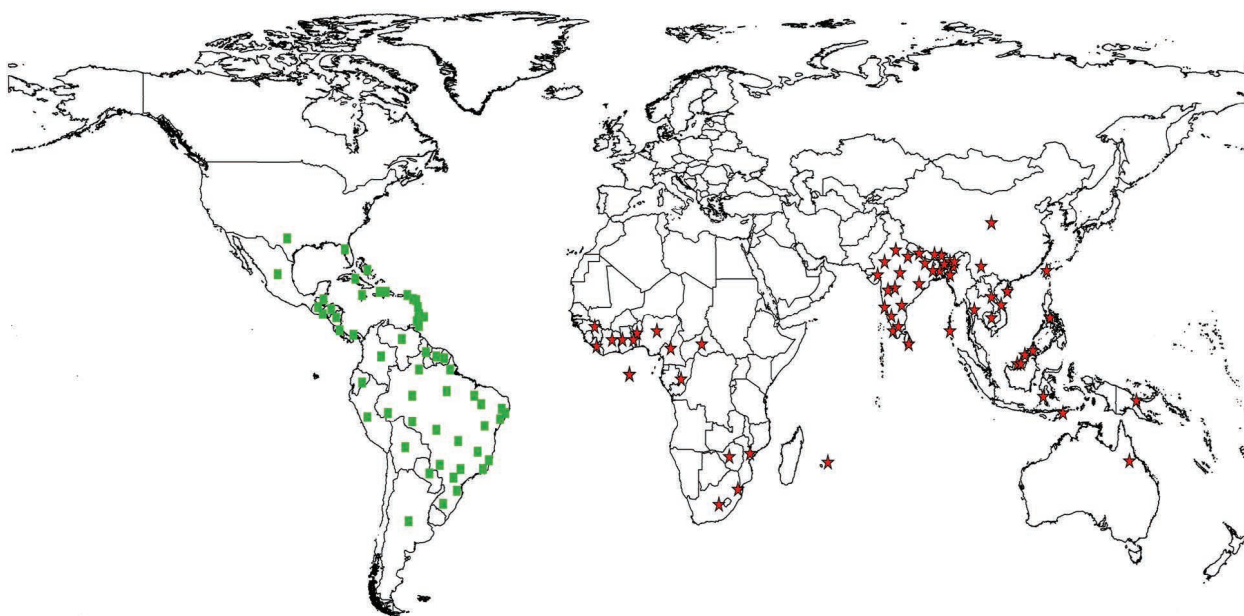
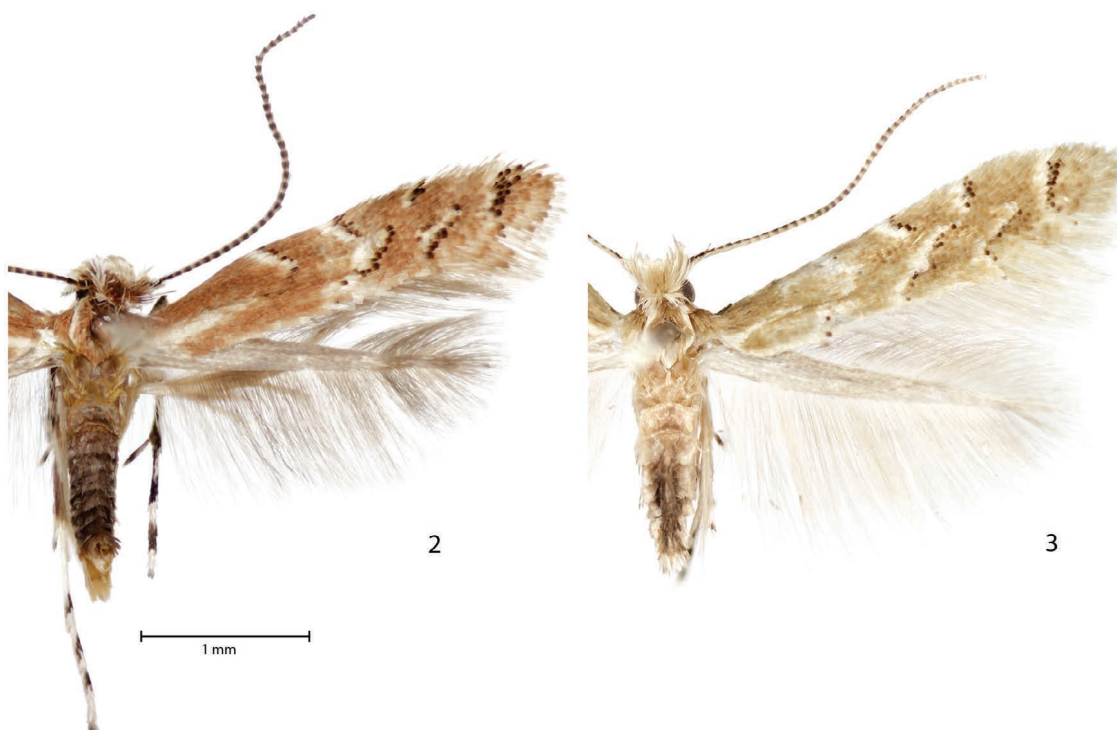


FIG. 1. Native (green squares) and exotic (red stars) distribution of *Chromolaena odorata*. Data obtained from the Global Biodiversity Information Facility (www.gbif.org).



FIGS. 2–3. Adults, *Cremastobombycia chromolaenae*. 2. Holotype ♂, St. Lucie Co: Fort Pierce, Florida; Host: *Chromolaena odorata*. 3. Paratype ♂, Cameron Co: Southmost, Audubon Sabal Palm Refuge, Texas; Host: *Chromolaena odorata* “*Eupatorium odoratum*”.

Cremastobombycia examined. In lieu of these prominent hypopharyngeal lobes, the anterior margin of the hypopharynx of tissue-feeding *Phyllonorycter* larvae possess a series of over 20 much smaller, curved, smooth spines (Davis and Deschka 2001). The last two larval instars of *Cameraria* are non-feeding, silk-spinning instars with vestigial mandibles. The dorsal surface of the hypopharynx in *Cameraria* is mostly smooth and without anterior spines.

Chromolaena odorata (L.) King and H. Robinson, commonly referred to as Siam weed or Christmas bush, is a 2–3 m tall perennial shrub with white to purple inflorescences (Holm et al. 1977). It has a broad geographic distribution from the southern United States to northern Argentina (Fig. 1, Kriticos et al. 2005). This plant is also present in Africa, Asia and Australia where it is considered exotic and in some countries has become a major weed (Zachariades et al. 2011). In the exotic range, *C. odorata* invades a wide range of habitats including grasslands, open woodland and forest margins and gaps (reviewed by Zachariades et al. 2009). The plant often forms dense stands which prevent establishment of other species, both due to competition and allelopathic effects. Areas invaded by *C. odorata* are considered a fire hazard because of the large biomass accumulation and presence of oils in the stems and leaves (McFadyen 2004). Due to the widespread negative impact to human livelihood and natural areas, several countries have implemented classical biological control as a management strategy against *C. odorata* (Siebert 1989, McFadyen 2002, Zachariades et al. 2011). Herein, we describe a new potential biological control agent of *C. odorata* discovered in southern Florida and Texas.

MATERIAL AND METHODS

Specimen preparation. Genitalic dissections were cleared by heating in hot 10% KOH for ~30 minutes, and subsequently cleaned and stained with either 2% chlorazol black E or mercurochrome solutions. All genitalic illustrations were drawn from dissections temporarily stored in glycerine, which were later permanently embedded in Canada balsam. Genitalic terminology follows Klots (1970). The lengths of eggs, larvae and pupae were measured with calibrated digital images, and the cocoons and mature mines were measured using a caliper.

Specimens examined in this study are deposited in the following institutions:

DLW Collection of David L. Wagner, Storrs, CT, USA.
MGCL McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA.

USNM Collections of the former United States National Museum, now deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

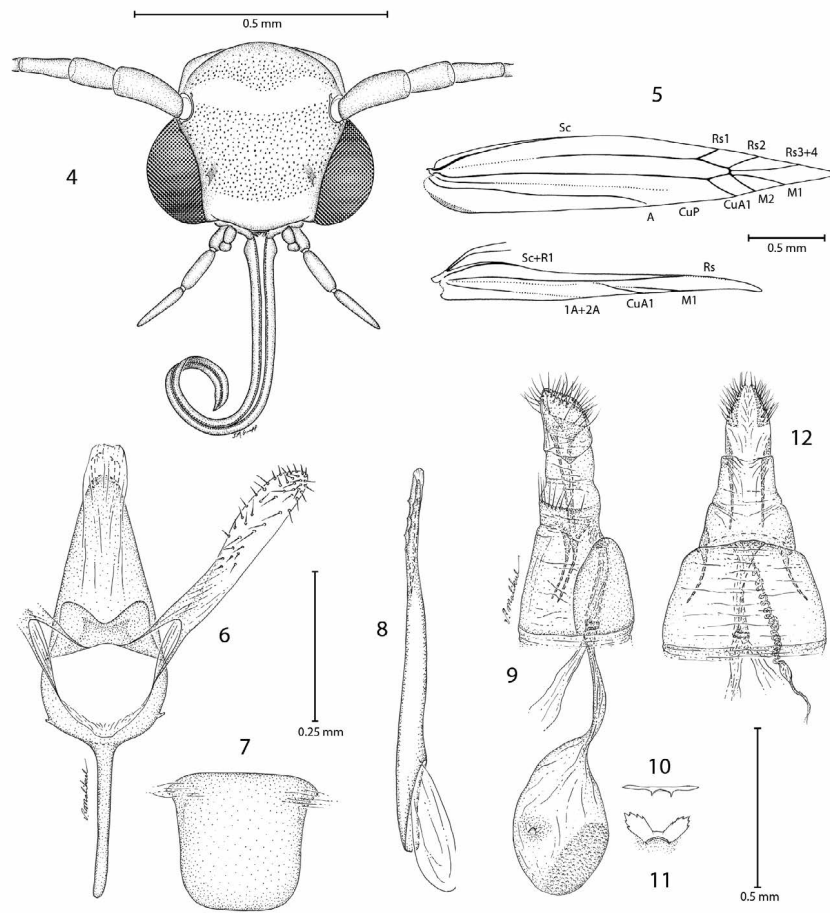
Cremastobombycia chromolaenae Davis, new species

(Figs. 2–3, 6–12, 17–24)

Diagnosis. The forewing pattern of *C. chromolaenae* most closely resembles that of *C. solidaginis* (Frey and Boll) in possessing four mostly white costal and two white dorsal strigulae, but differs slightly in having the basal first and second costal strigulae more developed in *chromolaenae*. In particular, the second costal strigula typically continues with the first dorsal one to form a complete fascia across the forewing of the latter. The valva of the male genitalia of *chromolaenae* terminates in a more rounded apex than does that of *solidaginis*. The signum of the female genitalia differs from that of all other *Cremastobombycia* in possessing a pair of minute, serrated alate lobes, with a melanized, rugose, oval disk opposite the signum. The caudal margin of the male eighth abdominal sternum of *chromolaenae* (Fig. 7) is distinct in being more truncate, compared to sharply triangular in *solidaginis*.

Description. Adult (Figs. 2–3). Forewing length 2.5 – 3.0 mm.

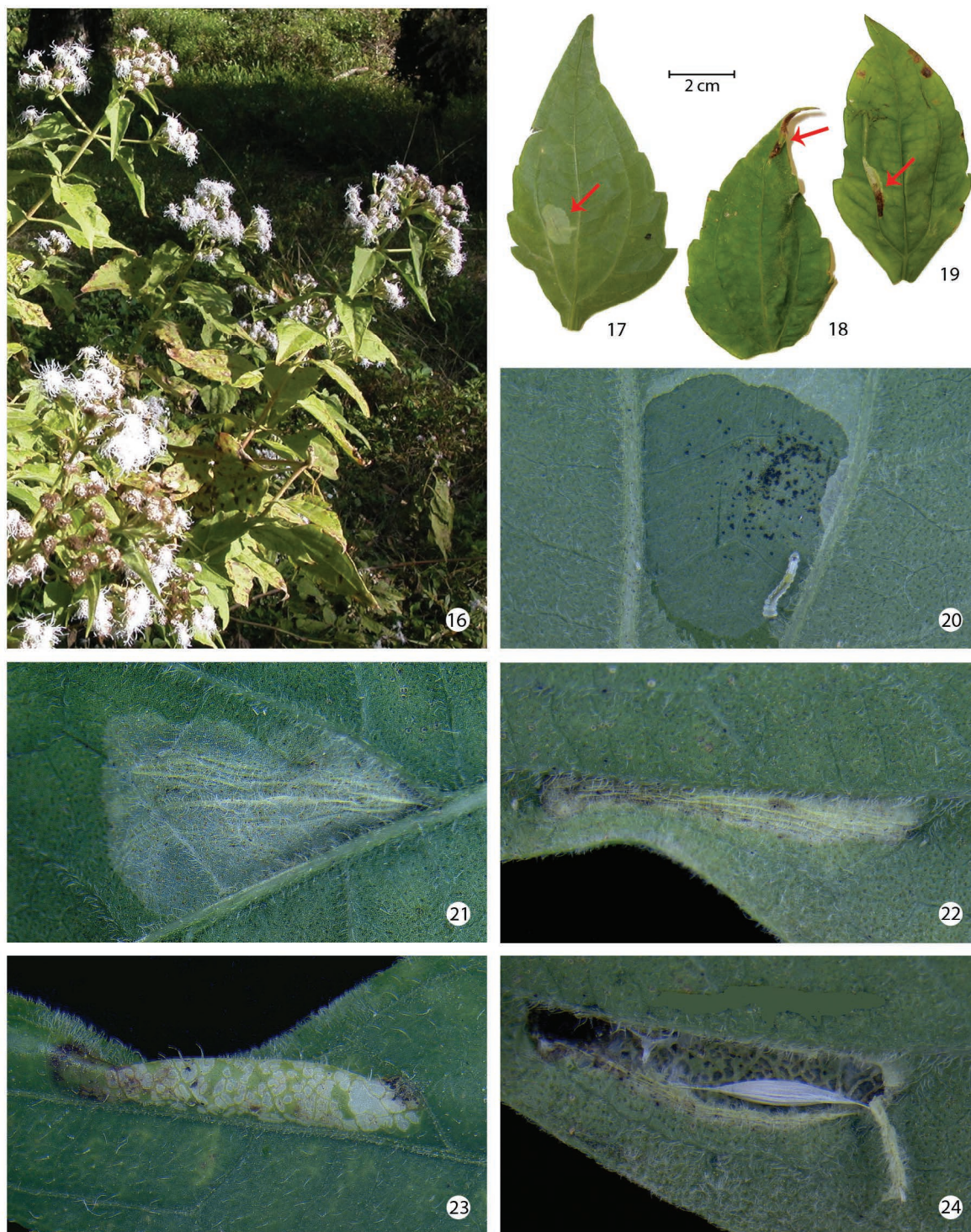
Head: Frons silvery white, becoming slightly suffused with pale brown toward vertex; vertex brownish-orange with prominent, raised tufts of elongate, piliform setae along occiput; anterior and medial occipital setae brownish-orange; lateral occipital setae white. Labial palpus straight, directed ventrally, silvery white. Antenna ~ 0.9× the length of forewing; flagellomeres uniformly silvery white ventrally, dorsally with basal half of entire antenna dark fuscous, with basal half of each flagellomere gradually becoming more gray over distal half of antenna. **Thorax:** Most of pronotum and tegulae brownish orange, with a lateral pair of white streaks extending from white occipital tufts of head to mesonotum; venter of thorax white. **Forewing:** brownish orange with a conspicuous white streak along basal third of anal vein and four mostly white costal and two white dorsal strigulae, each partially bordered distally by a few black scales; second costal and first dorsal striae usually contiguous near middle of wing, forming a sharp angle; fringe white along dorsal margin, becoming pale brown toward apex. **Hindwing:** uniformly gray. Foreleg with femur black dorsally and silvery white ventrally; tibia black dorsally with two silvery bands ventrally; tarsomeres black, banded with silvery white; midleg with coxa pale brownish dorsally and streaked diagonally with black and white ventrally; tibia paler, brownish dorsally, mostly white ventrally; tarsomeres white with more narrow black bands; hindleg pale brownish with whitish diagonal stripes dorsally, mostly white ventrally with narrow black bands around tarsomeres. **Abdomen:** Gray dorsally, dull white ventrally with lateral diagonal streak of pale brown. Eighth abdominal sternite (Fig. 7) well developed; caudal margin broadly rounded to subtruncate. **Male genitalia** (Figs. 6, 8): Tegumen triangular, elongate, ~ 0.8 × length of valva. Valva elongate, slender with smoothly rounded apex. Transtilla broad, with anterior margin (caudal margin when reversed as in Fig. 6) prominently bilobed. Vinculum a narrow ventral ring terminating in an elongate, slender saccus ~ 0.6 × length of valva. Aedeagus a relatively straight, slender cylinder, gradually tapering to caudal apex, ~ 1.5 × length of valva; apex narrowly divided with 3–4 minute dentations along each border of cleft. **Female genitalia** (Figs. 9–12): Anterior apophyses ~ 0.55 × length of caudal apophyses. Ostium a simple, slightly enlarged



FIGS. 4–12. Adult morphology. **4.** *Cremastobombycia solidaginis*, head, anterior view. **5.** *C. solidaginis*, wing venation. **6–12.** *C. chromolaenae*, genitalia. **6.** Male genitalia, ventral view. **7.** Eighth abdominal sternite. **8.** Aedeagus. **9.** Female genitalia, lateral view. **10.** Signum, enlarged anterior view. **11.** Signum, enlarged lateral view. **12.** Female genitalia, ventral view.



FIGS 13–15. *Cremastobombycia ignota*, head morphology of last instar larva. **13.** Mouthparts, ventral view. **14.** Labrum and hypopharynx, anterior view. **15.** Hypopharynx (enlarged from Fig. 13), ventral view.



FIGS. 16–24. Leaf mines on *Chromolaena odorata* and larval biology of *Cremastobombycia chromolaenae*. **16.** *Chromolaena odorata*, photographed October 2011, Ft. Pierce, Florida. **17.** Early instar abaxial blotch mine (arrow). **18.** Adaxial leaf mine near center of leaf (arrow). **19.** Subapical adaxial leaf mine (arrow). **20.** Opened blotch mine with sapfeeding larva. **21.** Abaxial leaf mine showing the beginning of cuticle contraction (tentiform). **22.** Abaxial tentiform mine. **23.** Adaxial view of Fig. 22. **24.** Opened mine with single cocoon.

opening at caudal edge of eighth sternum. Ductus bursae $\sim 1.75 \times$ length of caudal apophyses; duct of accessory bursa arising midway along ductus bursae; corpus bursae with a small, strongly bilobed signum about midway along length of corpus bursae; apical margins of lobes minutely serrate; a single, relatively large, melanized, rugose disk midway along opposite wall of corpus. *Larval mine* (Figs. 17–24): Length: 12.9 ± 2.0 mm, width: 3.6 ± 0.8 mm (mean \pm S.D., $n = 81$). The mine begins as a short, serpentine track which enlarges to a rounded, whitish blotch visible from the abaxial side of the leaf (Figs. 17, 20). Eventually the abaxial surface of the blotch contracts (Figs. 21–22) and the mine becomes narrow and tentiform (Figs. 22–23).

Holotype: ♂, UNITED STATES: FLORIDA: St. Lucie Co: Fort Pierce, near University of Florida Campus, 27.430° N, 80.410° W: 5 Dec 2011, R. Diaz, J. McClurg, Host: DRD 2710, *Chromolaena odorata* (L.), em. 12–19 Dec 2011. (USNM).

Paratypes: FLORIDA: Monroe Co: Key Largo: J Pennecamp St Pk, 10 ♂, 14–16 Nov 1986, DLW Lot 86L38, Host composite (*Eupatorium*?), em. 17 Nov 1986, 1 ♂, em. 20 Nov 1986, 1 ♂, 1 ♀, 1 ♀ digital image captured, em. 22 Nov 1986, 1 ♀, em. 24 Nov 1986, 2 ♂, 1 ♀, em. 30 Nov 1986, 1 ♂, 1 ♀, genitalia slide 34375, em. 1 Dec 1986, 1 ♀, em. 3 Dec 1986, 1 ♂, 1 ♂, genitalia slide 34374, 4 ♀, em. 8 Dec 1986, 1 ♀, em. 12 Dec 1986, 2 ♂, 1 ♀, em. 20 Dec 1986, 1 ♀, em. 3 Jan 1987, 1 ♂, em. 23 Jan 1987. St. Lucie Co: Fort Pierce: Same data as holotype; 16 ♂, 15 ♀, 5 ♂, 6 ♀; Fort Pierce: Selvitz Road: 27.404° N, 80.368° W: 3 ♂, 2 ♀, 11 Aug 2011, J. McClurg, K. Jordan, Host: *Chromolaena odorata* (L.), slide 34352; Fort Pierce: Weatherbee Road: 27.382° N, 80.330° W, 6 ♂, 6 ♀, 24 Oct 2011, Host: DRD 2708, *Chromolaena odorata* (L.), em. 28 Oct 2011, 2 ♂, 1 ♀, DOA 7 Nov 2011, R. Diaz, J. McClurg, K. Jordan, slides USNM 34344, 34347–34349, 34351, R. Diaz, J. McClurg, K. Jordan. TEXAS: Cameron Co: Southmost: Audubon Sabal Palm Refuge, 1 ♂, 1 ♀, 27–29 Dec 2000, em. 20 Jan 2001, DLW Lot 2000M12, Host *Eupatorium odoratum*. Hidalgo Co: Santa Ana Nat. Wildlife Refuge, vicinity Visitor's Center, 2 ♀, 30 Dec 1986, D. L. Wagner No: 86M39, 2 ♀, 11–14 Jan 1987, Host *Eupatorium odoratum*, slide USNM 34383. (Paratypes deposited in DLW, MGCL, USNM).

Distribution. Currently known from the general type locality, Fort Pierce, St. Lucie County, Florida, USA, south to Key Largo, Monroe County, Florida; also occurring in Cameron and Hidalgo Counties in southern Texas. Considering the broad, native range of its host, the actual distribution of the moth may be much greater than currently documented.

Etymology. The specific name is derived from the generic name of its plant host and is considered an adjective in the nominative singular.

Host. Asteraceae: *Chromolaena odorata* (L.) King and H. Robins. Leaf mines containing *Cremastobombycia chromolaenae* were collected from three suburban sites (27.3816°N, -80.3297°W; 27.4036°N, -80.3672°W; 27.3913°N, -80.3495°W) in the city of Fort Pierce, St. Lucie County, Florida. All collection sites were highly disturbed areas located in open or partially wooded lots. Leaf mines on *C. odorata* were also found common by D. W. Wagner at J Pennecamp State Park, Key Largo, Florida and less so at two sites in southern Texas. Field observations taken at the same time that *C. chromolaenae* were collected in central (St. Lucie Co.) and south Florida (Miami-Dade Co.) revealed that it does not form mines on *Mikania scandens* (L.) Willd., *Mikania micrantha* Kunth or *Mikania cordifolia* (L. f.) Willd. (R. Diaz personal observations). Because *Mikania* is included within the same tribe (Eupatorieae) as *C. odorata*, these

observations suggest that *C. chromolaenae* may have a narrow host range.

Life history. Eggs of *C. chromolaenae* (diameter: 0.13mm, $n = 2$) are laid externally on the abaxial leaf surface near the mid vein. Larvae are hypometamorphic and possess two distinct larval body forms and feeding behaviors—an early stage sap-feeding form with a flattened body and prognathous mouthparts and a later stage tissue-feeding form with a more cylindrical body and possessing hypognathous mouthparts (Davis, 1987). As is typical of other *Cremastobombycia* studied, five larval instars were observed for *C. chromolaenae*, consisting of three sap-feeding instars and two later tissue-feeding instars. Head capsule widths were 0.16 ± 0.01 , 0.18 ± 0.01 , 0.24 ± 0.04 , 0.30 ± 0.01 and 0.34 ± 0.01 mm for larval instars 1–5, respectively (mean \pm S.D.). First instar larvae initiate a narrow, short serpentine mine that in the later instars becomes visible from both sides of the leaf. Eventually the mine is expanded into an oval, whitish blotch (Fig. 17) which is formed under the abaxial cuticle. Observations suggest that the total leaf area of the mine is formed by the sap-feeding larval stages (Fig. 20). The tissue-feeding instars remove large amounts of the mesophyll layer of the leaf and the resulting injury becomes visible from the adaxial leaf surface as contiguous, whitish blotches (Fig. 23). As the tissue-feeding larvae mature, they lay down silken strands on the inner surface of the mine. Contraction of the blotch mine by the combination of this silk and the empty mesophyll cells creates the strongly cylindrical, tentiform leaf mines observed in the late instars (Figs. 21–23). Pupation occurs inside an elongate, spindle-shaped, silken cocoon (length: 6.1 ± 0.7 mm, $n = 31$) which is attached to the inner surface of the mine at both ends (Fig. 24). Before adult eclosion, the mature pupa (length: 3.9 ± 0.2 mm, $n = 9$) pierces the cocoon and leaf cuticle using the pointed frontal process of its head. Empty pupal cases were often found attached to the leaf cuticle. During the first two weeks of October 2011, leaves of *C. odorata* containing *C. chromolaenae* mines were collected from the field sites mentioned above. Leaves were placed in plastic bags, and subsequently examined in the laboratory to assess *C. chromolaenae* survival and sources of mortality. Upon arrival to the laboratory, each mine was inspected under the microscope for signs of holes. Mines with holes were dissected and mines without holes were placed individually in plastic containers and monitored for three weeks. The following criteria were used to assign sources of mortality; 1) death by parasitism: indicated by the presence of live immatures or pupal cases of parasitoids inside the mine or the emergence of parasitoid adults, 2) death by predation of sap-feeding

larva: indicated by a large entry hole on the leaf cuticle and the absence of mesophyll feeding damage in the inner surface of the mine, 3) death by predation of tissue-feeding larva or pupa: indicated by a large entry hole on the leaf cuticle and the presence of mesophyll feeding damage in the inner surface of the mine, 4) emergence of *C. chromolaenae* adult: indicated by the presence of a pupal case or the emergence of an adult, 5) unknown larval mortality: indicated by the presence of a decayed larva inside the mine. A total of 106 *C. chromolaenae* mines were collected. The fate of individuals were characterized as follows: 29% were parasitized, 2% were predated in the sap-feeding larval stage, 26% were predated in the tissue-feeding larval or pupal stages, 6% were dead due to unknown causes, and moths emerged from 29%. These results indicate that biotic mortality plays a major role in the population dynamics of *C. chromolaenae* within its native range.

Natural enemies. Braconidae: Microgastinae (unidentified to genus). Eulophidae: *Elasmus albicoxa* Howard; *Baryscapus* species. Approximately four species of parasitoid Hymenoptera were recovered from *Cremastobombycia chromolaenae* leaf mines and deposited in the USNM. Dissections of the leaf mines revealed that all parasitoids emerged from larvae of *C. chromolaenae*.

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