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Banisia argutula (Lepidoptera: Thyrididae) is the dominant sapodilla borer in southern Florida

Jose I. Martinez^{1,2,3}, James E. Hayden⁴, John B. Heppner³, Jorge E. Peña^{1,2}, Lei Xiao³, and Daniel Carrillo^{1,2,*}

Abstract

Banisia myrsusalis Walker (Lepidoptera: Thyrididae) has been regarded as the most damaging pest of sapodilla, Manilkara zapota (L.) van Royen (Sapotaceae), in Florida, where it causes extensive damage to blooms. Two commercial sapodilla groves were surveyed for lepidopteran eggs and larvae affecting floral buds and mature flowers. All collected specimens were raised in the laboratory until adult emergence. A careful revision of reared individuals and specimens deposited in institutional collections since 1990 brought into question the identity of Banisia species affecting sapodilla in southern Florida. Specimens initially identified as B. myrsusalis are herein re-identified as Banisia argutula Whalley, based on female and male genitalic characters. Total DNA was extracted from 2 specimens raised in the survey and the COI gene sequence determined and deposited in GenBank. Larvae of B. argutula were observed chewing holes in the base of floral buds or mature flowers, entering the flower, and consuming all floral structures except the sepals. A single larva would tend to clump several flowers and floral buds together by using silk. Larvae could also fold leaves or bore into the fruit and could complete their immature development feeding on folded leaves, flowers, or inside a fruit. In contrast, B. myrsusalis is reported as strictly a leaf-folder. Banisia myrsusalis was found to be a minor part of the sapodilla pest complex. Banisia argutula was the dominant sapodilla borer in southern Florida.

Key Words: Manilkara zapota; flower-borer; fruit-borer; Banisia myrsusalis; leaf-roller

Resumen

Banisia myrsusalis Walker (Lepidoptera: Thyrididae) ha sido considerado como la plaga más dañina del zapote Manilkara zapota (L.) van Royen (Sapotaceae) en Florida, causando un excesivo daño a las florez. Dos huertos comerciales de zapote fueron monitoreados debido a que huevos y larvas de lepidópteros afectaban los brotes florales y las flores maduras. Todos los especímenes colectados fueron criados en el laboratorio hasta la emergencia del adulto. Una revisión minuciosa de los especímenes criados y de los especímenes depositados en colecciones institucionales desde 1990, puso en cuestión la identidad de la especie de Banisia que afectaba al zapote en el sur de Florida. Especímenes identificados inicialmente como B. myrsusalis son aquí re-identificados como Banisia argutula Whalley basado en las características de la genitalia del macho y la hembra. El ADN total fue extraído de dos especímenes criados en el estudio y la secuencia de COI fue determinada y depositada en GeneBank. Las larvas de B. argutula fueron observadas masticando agujeros en la base de los brotes florales y flores maduras, entrando a las flores y consumiendo todas las estructuras florales excepto los sépalos. Una simple larva tiende a agrupar varias flores y brotes florales usando seda. Las larvas pueden incluso enrollar las hojas o perforar dentro de los frutos, y pueden completar su desarrollo inmaduro alimentándose de hojas dobladas, flores o dentro de un fruto. En contraste, B. mysusalis está reportada estrictamente como enrollador de hojas. Banisia myrsusalis fue encontrada como una parte minoritaria del complejo de plagas de zapote. Banisia argutula es el perforador de zapote común en el Sur de Florida.

Palabras Clave: Manilkara zapota; perforador de flores; perforador de frutos; Banisia myrsusalis; enrollador de hojas

Sapodilla (*Manilkara zapota* [L.] van Royen; Sapotaceae) is a Neotropical fruit tree that is native from southern Mexico to Central America. Sapodilla was introduced as a food crop to southern Florida, some Caribbean Islands, and Asia through the Philippines during the Spanish colonization (Morton 1987; Mickelbart 1996; Kamala et al. 2006; Balerdi et al. 2013; Venkateswara et al. 2014). Currently, the largest producers of sapodilla are India and Mexico, followed by Sri Lanka, the Philippines, Venezuela and Guatemala (Morton 1987; Mickelbart 1996; Balerdi et al. 2013). In south Florida, sapodilla is considered a

specialty crop with a significant increase in cultivated acreage in the past few years from 8.9 ha (22 acres) in 2006 (Steel & Crane 2006) to approximately 80.9 ha (200 acres) in 2015 (J. H. Crane, personal communication).

Many insects have been reported in association with sapodilla in Mexico, Florida (USA), French West Indies, Puerto Rico, Venezuela, Brazil, India, China, Indonesia, Pakistan, Malaysia, Philippines, and Thailand (Clarke 1954; Rubio-Espina 1968; Butani 1975; Bradley 1981; Medina-Goud et al. 1987; Witethom & Silawatchananai 1990; Ibrahim

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1992; Iruegas et al. 2002; Peiris 2010). The most damaging pests are Lepidoptera that feed on leaves, flowers, and fruits. Among them, *Banisia myrsusalis* Walker (Thyrididae), *Eustalodes achrasella* Bradley (Gelechiidae), and *Nephopteryx eugraphella* Ragonot (Pyralidae) are considered to be key pests (Parvathi & Belavadi 1994; Patange et al. 1997; Soares-da Silva et al. 2003). In southern Florida, *B. myrsusalis* has been reported as the most damaging pest causing extensive damage to blooms that affects fruit production (Myers et al. 2008; Balerdi et al. 2013). During 2015, sapodilla growers from southern Florida suffered significant losses (about 80%) of the fruit crop, which were attributed largely to lepidopteran pests. This situation motivated a monitoring effort by the authors to verify the identity of insects causing damage as the basis for future management programs. A careful revision of the collected specimens brought into question the identity of *Banisia* species affecting sapodilla in southern Florida.

The genus *Banisia* Walker includes 4 described species in the New World: *B. myrsusalis*, *B. furva* Warren subsp. *fracta* Whalley, *B. extravagans* Warren, and *B. argutula* Whalley (Whalley & Heppner 1995). *Banisia myrsusalis* is pantropical, including the Caribbean and southern Florida. *Banisia furva* occurs in the Florida Keys and occasionally on the mainland, but it is not treated here because it was not found in the survey of sapodilla. In Florida, *B. furva fracta* has been reared on wild dilly (*Manilkara jaimiqui* subsp. *emarginata* [L.] Cronquist; Sapotaceae), but has not been recorded on sapodilla to any extent (one rearing from Upper Matecumbe Key was on sapodilla in 1974; J. B. H. unpublished data). *Banisia extravagans* and *B. argutula* are native to northern South America (Whalley 1976).

Banisia myrsusalis was recorded from the Florida Keys by Kimball (1965, under the genus Rhodoneura Guenée), who cited a few specimens collected in 1955. Whalley (1976) examined specimens of B. myrsusalis from Key Largo in Kimball's collection. The next citation of B. myrsusalis in Florida is by Peña (1994), who described the larval damage as observed in Homestead. Heppner (2003, 2009) also noted B. myrsusalis and B. furva fracta on sapodilla, but the latter has not been an economically important pest in Florida. Whalley (1976) described B. argutula from 8 male specimens collected in French Guiana, Guyana, and Surinam in the early 1900s. He illustrated the male genitalia and gave diagnostic characters. There is no other literature on its biology and behavior. The female of B. argutula has not been described previously, which initially complicated the identification effort in Florida.

Materials and Methods

Two sapodilla orchards we surveyed weekly for 6 mo starting in Mar 2015. The first study site was a 0.4 ha sapodilla grove (105 sapodilla trees) at the University of Florida Tropical Research and Education Center (UF-TREC). The second site was a commercial orchard, "Finca 6 Palmas. Inc.," with 192 sapodilla trees. Both study sites were located in Miami-Dade County in Homestead, Florida. Ten percent of the sapodilla trees were randomly sampled every month at each study site. Each tree was observed for 15 min, and 10 to 12 inflorescences and leaves per plant were collected, placed in labeled plastic bags, and transported to the UF-TREC Tropical Fruit Entomology Laboratory for observation under a stereomicroscope (20× magnification). Floral buds and mature flowers were inspected for lepidopteran eggs and larvae. Eggs were placed individually in Petri dishes until eclosion. Neonate larvae were transferred to 50 mL plastic vials with wet filter paper holding a small bouquet of fresh flowers that served as food for the larvae. Flower bouquets were replaced every 3 d until larvae reached the pupal stage. Pupae were transferred to small Petri dishes with wet tissue paper on the bottom. Emerged adults were pinned, labeled, and sent for identification to the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI, Gainesville, Florida). Specimens previously sent from UF-TREC to FDACS-DPI since 1990 that had previously been identified as *B. myrsusalis* were also examined.

Dissection of genitalia followed Robinson (1976). Abdomens were macerated in 10% aqueous KOH at 100 °C, cleaned in water, stained with Chlorazol black and Eosin Y, and slide mounted in Euparal or stored in glycerin. Papillae anales were dissected along the dorsal midline. Wings were measured in millimeter increments. Photographs were taken with the Auto-Montage Pro 5.01 system (Syncroscopy, Synoptics Ltd., Cambridge, United Kingdom) using a JVC KY-F75U digital camera (Victor Co. of Japan Ltd.) and Leica Z16APO lens. The habitus images were photographed over standard 18% gray cards under tungsten lights. Further processing included stacking images with Auto-Montage and auto-adjusting white and gray background levels with Adobe Photoshop Elements 11 (Adobe Systems Inc. 2012).

Specimens were identified with the key, diagnoses, and illustrations in Whalley (1976). Morphological terms follow Klots (1970) and Whalley (1976). Specimens were deposited in the Florida State Collection of Arthropods (FSCA, housed in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, Florida; MGCL), in the personal collection of Terhune S. Dickel (Anthony, Florida; TSDC), and in the reference collection of the Tropical Fruit Entomology Laboratory (UF-TREC, Homestead, Florida).

DNA EXTRACTION AND PCR AMPLIFICATION

Total DNA was extracted from 2 legs removed from 2 specimens raised in the survey by using a QIAGEN DNeasy Blood & Tissue kit and eluted in 50 µL Buffer AE. A 687 bp region of the mitochondrial cytochrome c oxidase subunit I gene (the COI barcode) was amplified with a pair of primers LepF1 (5' ATTCAACCAATCATAAAGATAT) and LepR1 (5' TAAACTTCTGGATGTCCAAAAA) (Hebert et al. 2004) using the following polymerase chain reaction (PCR) conditions: 94 °C for 1 min; 5 cycles of 94 °C for 30 s, 45 °C for 40 s, and 72 °C for 1 min; 35 cycles of 94 °C for 30 s, 51 °C for 40 s, and 72 °C for 1 min; and 72 °C for 5 min. Each PCR contained 2 µL DNA templates, 1× PCR buffer, 2 mM MgCl₂, 0.1 mM dNTPs, 0.2 μM of each primer, and 0.2 μL Platinum Taq DNA polymerase (Invitrogen) in a total volume of 20 µL. The PCR products were analyzed by gel electrophoresis followed by Sanger sequencing. Alignment and editing of the COI barcode sequences were performed in Geneious Pro 5.5.3. Sequences were verified to be free of stop codons and gaps.

Results

Banisia argutula has been raised from fruit of *M. zapota* in southern Florida since 1990, with additional specimens from 1991, 2002, and 2013 to present. Female specimens sent from UF-TREC to FDACS-DPI in 2013 were initially identified as *B. myrsusalis* because their genitalic differences were not obvious to cursory examination. On the other hand, the male genitalia of specimens reared in this survey (Hayden 2015) were very different and matched Whalley's Figure 287 (1976). Subsequent dissection of specimens reared on sapodilla since 1990 at the TREC indicated that *B. argutula* has been present in Homestead since at least that year. Dissection of more female specimens revealed that the differences between the 2 species are clear and consistent. The specimens raised in flowers and fruit since 1990 were herein reidentified as *B. argutula*.

Larvae of *B. argutula* were observed chewing holes in the base of floral buds or mature flowers, entering the flower, and consuming all

floral structures except the sepals. A single larva would tend to clump together several flowers and floral buds by using silk, presumably to avoid exposure to predators. Larvae could also fold leaves or bore into the fruit and could complete immature development feeding on folded leaves, flowers, or inside fruit. *Banisia argutula* infested flowers turned reddish brown and showed larval excrement before drying completely.

Banisia myrsusalis was also present in southern mainland Florida as a minor part of the sapodilla pest complex. Thus far, we have raised 1 specimen on sapodilla leaves in Homestead. Specimens have been caught at lights in Fuchs Hammock near Homestead (in 1982 and 1985) and in Key Largo (in 1995; TSDC). Specimens have also been caught in suction, Malaise, and Jackson traps at the United States Department of Agriculture/Agricultural Research Service/Subtropical Horticulture Research Station (Coral Gables, Florida) and in Broward County, Florida.

Figures 1–8 illustrate the habitus, Figs. 9–16 the genitalia of *Banisia* species. Whalley (1976) described the male genitalia of *B. argutula* (Fig. 9). Diagnostic characters (restated from Whalley 1976) include the round, spatulate, non-bifid uncus (u, a unique shape in the genus), a gnathos (g) with 2 small patches of spines near the center, the mesal margin of the sacculus with 1 tooth (msp), and 5 or 6 large cornuti (c) in the phallus (p). The valva has a reduced fibula (f) consisting of a low, smooth-margined ridge without protrusions.

Description of female habitus and genitalia of B. argutula (Figs. 3, 4, 10, 11, 15).—The female frenulum is triple. In the genitalia, the ostium bursae (ob) has very short spinules and is not modified, lacking a protruded lamella antevaginalis. An accessory sac (as) is attached to the ductus bursae (db) near the corpus bursae (cb). The corpus bursae does not have an appendix bursae. The single oval, granulose signum (si) is about three-fifths the length of the corpus bursae.

 The GenBank accession numbers are KU530228 and KU530229.

DIAGNOSIS FROM B. MYRSUSALIS

Male (Fig. 12)—In *B. myrsusalis*, the uncus is narrowly pointed and bifid. The gnathos has broad, lateral fringes of spines. The mesal saccular process (*msp*) bears a leaf-shaped process with multiple teeth. The sacculus (*sl*) is about one-third the length of the valva, whereas it is about half the length in *B. argutula*. The well-developed fibula of the valva is serrate and bears a basal triangular process that *B. argutula* lacks. The phallus of *B. myrsusalis* has 1 serrate cornutus.

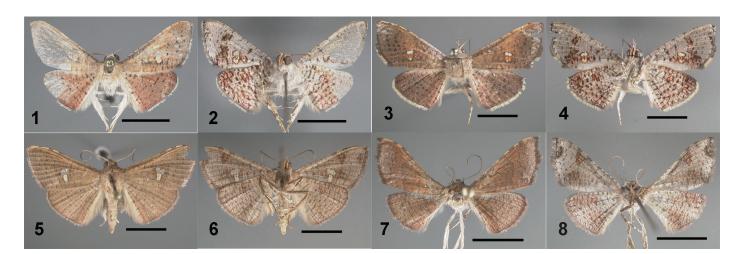
Female (Figs. 13, 14, 16)—In *B. myrsusalis*, the 8th abdominal segment (AVIII) is rougher in texture and has a modified ostium bursae consisting of a fishtail-shaped lamella antevaginalis (*Ia*) and a medial longitudinal ridge (*r*) posterior of the ostium bursae. In *B. argutula*, AVIII is less rough and lacks any modifications of the ostium bursae. The papillae anales (*pa*) are more elongate in *B. myrsusalis*. The ductus bursae of *B. myrsusalis* is shorter than in *B. argutula*. The corpus bursae of *B. myrsusalis* has a larger signum that extends almost the whole length, and an appendix bursae (*ab*), which is absent in *B. argutula*, emerges from the corpus bursae.

Wing pattern (Figs. 1–8) varies in both species and is not as reliable as the genitalia for identification. The best character is that the hind wings' ventral side has more distinct mottling in *B. argutula* (Figs. 2, 4) than in *B. myrsusalis* (Figs. 6, 8): the dark gray spots are larger and surrounded by pink. Other pattern elements are not reliable; in particular, the hyaline spots on the forewing may be present, reduced, or absent in both sexes of *B. argutula*.

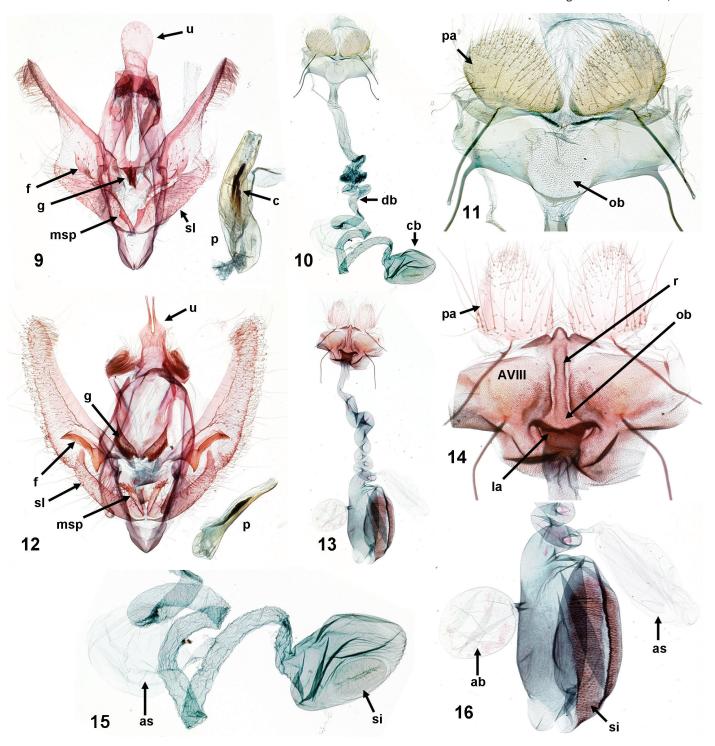
Although *B. argutula* is a relatively small species (Whalley 1976), size is not diagnostic for Florida specimens. For *B. argutula*, the mean forewing length (base to apex of costa) is 9.0 mm, range 8.0-11.0 mm (n=10), which fits with Whalley's 8.5-11.0 mm. However, the few available *B. myrsusalis* specimens are 8.0-8.5 mm, smaller than the 11.5-12.0 mm cited by Whalley (1976). It is possible that variable nutrition and the effects of control influence the size.

MATERIAL EXAMINED

Banisia argutula: USA, Florida: 1M: Dade Co., 12-VI-1990, J. Peña, Host: sapodilla; 1M: Dade Co., Homestead, 28-V-1991, J. Peña, collected from sapodilla; 1M: same data except 29-VIII-1990 (abdomen lost);



Figs. 1–8. Habitus of *Banisia* species. 1–4: *Banisia argutula*; 1, male, dorsal aspect (wings worn) (Florida, Homestead, TREC, 16-VI-2015, FSCA); 2, same, ventral; 3, female, dorsal aspect (Florida, Homestead, TREC, 11-VIII-2015, FSCA); 4, same, ventral. 5–8: *Banisia myrsusalis*; 5, male, dorsal aspect (Florida, Homestead, Fuchs Hammock, 11-XII-1985, TSDC); 6, same, ventral; 7, small female, dorsal aspect (Florida, Miami, USDA ARS SHRS, 19–26-X-2015, FSCA); 8, same, ventral. Scale bars = 5 mm.



Figs. 9–16. Genitalia. 9, Banisia argutula male genitalia (Florida, Homestead, MGCL slide 3040); 10, B. argutula female genitalia (Florida, Homestead, MGCL slide 3074); 11, same, detail of ostium bursae and papillae anales; 12, B. myrsusalis male genitalia (Florida, Cutler Bay USDA Station, MGCL slide 3125); 13, B. myrsusalis female genitalia (same locality, MGCL slide 3124); 14, same, detail of ostium bursae and papillae anales; 15, B. argutula, detail of corpus bursae and accessory sac; 16, B. myrsusalis, detail of corpus bursae and accessory sac. ab, appendix bursae; as, accessory sac; AVIII, 8th abdominal segment; c, cornuti, cb, corpus bursae; db, ductus bursae; f, fibula; g, gnathos; la, lamella antevaginalis; msp, medial saccular process; ob, ostium bursae; p, phallus; pa, papillae anales; si, signum; sl, sacculus; u, uncus.

2M, 2F: Dade Co., Homestead, VI-1991, J. Peña, TREC sapodilla fruits; 2M: Homestead, VI-2002, *Manilkara zapota*, J. Peña, 38-02; 1F: Miami-Dade Co., Homestead, TREC, 21-VI-2002, L.R. Myers., ref #824-02 (abdomen lost; associated by maculation); 2F: Miami-Dade Co., Homestead, 14381 SW 182 Ave., ex fruit of *Manilkara zapota*, 9-VIII-2013,

J. Peña & J. Wasielewski, DPI #E2013-6731, MGCL slide 1403, 128-13; 1M: Miami-Dade Co. Homestead, 18905 SW 280 St., ex flowers of *Manilkara zapota*, 16-VI-2015, J. Martinez, DPI #E2015-3272, MGCL slide 2975; 1F: same data except: 21-V-2015, MGCL slide 2976, DNA JEH 2015122B; 2F: same data except: 11-VIII-2015, (1) MGCL slide 3074,

DNA JEH20151122A; 1F: same data except: 14-VIII-2015; 1M: same data except: ex leaves of *Manilkara zapota*, 11-VIII-2015, MGCL slide 3040.

Banisia myrsusalis: USA, Florida: 1M:Dade Co., Fuchs Hammock near Homestead, 11-XII-1985, Terhune S. Dickel (TSDC); 1F: same data except 13-X-1982 (TSDC); 1M: Monroe Co., Key Largo Hammock State Botanical Site, 24-II-1995, MV light, Terhune S. Dickel (TSDC); 1M: Miami-Dade Co., Homestead, 18905 SW 280 St., ex leaves of Manilkara zapota, 14-VIII-2015, J. Martinez, MGCL slide 3073; 2M, 1F: Miami-Dade Co., Miami, Cutler Bay USDA ARS SHRS, 25.642653°N, 80.297022°W, malaise trap ca. (19–26)-X-2015, J. Hayden (MGCL slides 3124, 3125); 1F: same data except: suction trap, 25.6429°N, 80.2946°W, (9–16)-XI-2015, S. Halbert (FSCA); 1F: Broward Co., Davie, 26.1071°N, 80.3411°W, Jackson TML trap, 3-V-2016, A. Demien, DPI E2016-2116 (FSCA). Puerto Rico: 3M, 3F: Caribbean National Forest, El Yunque, Sierra Palma (22-26)-V-1987, L.C. Dow, (1 M) MGCL slide 2980, (1 F) MGCL slide 2981 (MGCL). Belize: 1M, 2F: Cayo, Pine Ridge, (9-16)-V-1990, L.C. Dow & M.S. Adams (MGCL).

Discussion

Sapodilla was introduced to the southern part of Florida prior to Colonial times (Popenoe 1920; Morton 1987). Until recently, it was considered a minor crop, but the commercial acreage has increased significantly in the past 10 yr. Campbell (1971) reported 6 ha, and Steele & Crane (2006) 3.2 ha (8 acres). However, during the last 10 to 15 yr, the number of Hispanic- and Asian-American producers has increased along with demand for the fruit. This, along with growers seeking alternative fruit crops to citrus, resulted in acreage increasing to an estimated 21 ha in 2009, 29 ha in 2012, 48 ha in 2013 to 80 ha in 2015 (J. H. Crane, personal communication). Most of this commercial acreage is in Miami-Dade County, but small groves exist in Lee and Palm Beach counties as well. The increasing importance of this crop and the recent pest outbreaks indicate the need for a sustainable, biologically based pest management strategy for sapodilla.

Proper identification is the first step towards establishment of a management program for sapodilla pests. This effort includes the deposition of voucher specimens in institutional collections. Our study indicates that *B. argutula*, and not *B. myrsusalis*, is the dominant sapodilla borer in south Florida. Corrected identifications can indicate the right region in which to search for biological control agents (Herren & Neuenschwander 1991). For instance, a search for natural enemies of *B. argutula* should start by focusing on northern lowland South America, where this species seems to be restricted, whereas the search for natural enemies of *B. myrsusalis* may be harder due to its pantropical distribution (Whalley 1976). Moreover, behavioral differences between *B. myrsusalis* and *B. argutula* may lead to different management strategies.

Larvae of *B. argutula* are primarily borers in buds and flowers, and they may also enter fruit or fold leaves. In contrast, *B. myrsusalis* in India, Africa, and South America is described as strictly a leaf-folder (Alibert 1946; Patel et al. 1993; Soares-da Silva et al. 2003; Shukla & Patel 2012; Sathish et al. 2015). First-instar larvae consume epidermis near the leaf midrib, preferentially attacking young leaves. Subsequent instars fold leaves into purses and scrape the epidermis inside these shelters (Sathish et al. 2015). The specimen of *B. myrsusalis* that we reared on leaves in this survey corroborates this behavior.

We did not find any biological control agents for *B. argutula* and *B. myrsusalis* in the survey. In Costa Rica, species of *Dolichogenidea* Viereck (Hymenoptera: Braconidae) and *Pristomerus* Curtis (Hymenoptera: Ichneumonidae) have been reared from *B. myrsusalis* (Janzen &

Hallwachs 2009). *Chelonus noyesi* Huddleston & Walker (Hymenoptera: Braconidae) is associated with *Banisia myrtaea* Drury in Indonesia (Huddleston & Walker 1994).

Although B. argutula has the diagnostic characters of Banisia, its relationships within the genus were unclear to Whalley: "[I]n the absence of the female, the exact position of this species is uncertain" (Whalley 1976: 145). Knowledge of the female morphology does little to improve its classification at present. Drawing from Whalley (1971, 1976), the closest relatives of B. argutula should have an ostium bursae without folds or protrusions, an accessory sac present, the appendix bursae absent, and a relatively small, oval signum. The photographs and descriptions in Whalley (1971, 1976) are too vague to establish which species have these structures. Whalley placed B. argutula in the "myrsusalis-group" based on external similarity to Banisia aldabrana Fryer, although the male genitalia differ. The females also differ in that B. aldabrana has an appendix bursae but not an accessory sac (Whalley 1971). The relationships of B. argutula should be sought by reexamination of dissections and consideration of immature morphology and genetic evidence.

We considered that *B. extravagans* could be conspecific, because it is known only from female specimens from the same region as *B. argutula*. However, the female genitalia of *B. argutula* differ in having a short oval signum (rather than elongate), a less spinulose ostium bursae, and no longitudinal ridge posterior of the ostium.

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