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First report of the stinkbug *Edessa leucogramma* (Perty) (Hemiptera: Heteroptera: Pentatomidae: Edessinae) attacking *Handroanthus chrysanthus* (Jacq.) S.O. Grose (Bignoniaceae), with descriptions of the adult and immatures and notes on associated fungi and protozoa

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Abril R., Gonzalo and Smith-Pardo, Allan H., "First report of the stinkbug *Edessa leucogramma* (Perty) (Hemiptera: Heteroptera: Pentatomidae: Edessinae) attacking *Handroanthus chrysanthus* (Jacq.) S.O. Grose (Bignoniaceae), with descriptions of the adult and immatures and notes on associated fungi and protozoa" (2023). *Insecta Mundi*. 1509.

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A journal of world insect systematics

INSECTA MUNDI

1007

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Date of issue: September 29, 2023

Center for Systematic Entomology, Inc., Gainesville, FL

Abril R G, Smith-Pardo AH. 2023. First report of the stinkbug *Edessa leucogramma* (Perty) (Hemiptera: Heteroptera: Pentatomidae: Edessinae) attacking *Handroanthus chrysanthus* (Jacq.) S.O. Grose (Bignoniaceae), with descriptions of the adult and immatures and notes on associated fungi and protozoa. *Insecta Mundi* 1007: 1–9.

Published on September 29, 2023 by
Center for Systematic Entomology, Inc.
P.O. Box 141874
Gainesville, FL 32614-1874 USA
<http://centerforsystematicentomology.org/>

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First report of the stinkbug *Edessa leucogramma* (Perty)
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Abstract. The stinkbug *Edessa leucogramma* (Perty) (Hemiptera: Pentatomidae: Edessinae) is reported as a pest of young yellow guayacán trees (*Handroanthus chrysanthus* (Jacq.) S.O. Grose, Bignoniaceae) in the metropolitan area of the Aburra Valley in Antioquia, Colombia (AMVA). We provide a short description of the adult and immature stages and report for the first-time protozoa associated with the digestive system of this species of true bug in addition to information regarding a fungus found associated with *Edessa leucogramma* in the field.

Key words. Pentatomoidea, morphology, host, life cycle.

Resumen. Se reporta el chinche *Edessa leucogramma* (Perty) (Hemiptera: Pentatomidae: Edessinae), como plaga importante de árboles jóvenes del guayacán amarillo (*Handroanthus chrysanthus* (Jacq.) S.O. Grose, Bignoniaceae) en el área metropolitana del Valle de Aburra en Antioquia, Colombia. Se presentan descripciones cortas del adulto y los estadios inmaduros, y se reporta por primera vez la presencia de protozoarios asociados con el sistema digestivo de esta especie de chinche y proveemos información acerca de un hongo encontrado en asociación con *Edessa leucogramma* en el campo.

Palabras clave. Pentatomoidea, morfología, hospedero, ciclo de vida, chinche.

ZooBank registration. urn:lsid:zoobank.org:pub:8D06E222-249D-413C-AA0A-48E34BF995C1

Introduction

There are close to 700,000 trees planted in the Metropolitan Area of the Aburra Valley (AMVA), 5,000 or more are yellow guayacáns, all registered in the Urban Tree System (Sistema de Arbol Urbano -SAU-). This tree, due to its leafiness and showy flowering, has been massively planted and propagated in the region, and nymphs and adults of the stink bug, *Edessa leucogramma* (Perty) are also spreading from nurseries, and pass unchecked and unidentified by urban tree horticultural experts.

The genus *Edessa* Fabricius is one of the most diverse genera in the second largest pentatomid subfamily Edessinae (Hemiptera), comprising about 350 species (De Albuquerque-Almeida et al. 2018). Most species are considered of quarantine importance in the United States (USDA-APHIS-PPQ pest database retrieved by A. Smith-Pardo 2022). This is partly because several species in the genus are considered crop pests. One of the most important species, *E. meditabunda* (Fabricius), commonly called the green and brown stink bug, is a severe pest in soybeans and many crops in the family Solanaceae, especially tomato and potato, as well as sunflower (Asteraceae), papaya (Caricaceae), and grapes (Vitaceae), among others (Rizzo 1971; Galileo and Heinrichs 1979; Panizzi and Machado-Neto 1992; Calizotti and Panizzi 2014).

The stink bug, *E. leucogramma* has been also hypothesized as being a vector for a pathogenic agent that is causing malformations and alterations in tree growth; up until now, these anomalies have been attributed to bacterial agents.

In this work, we report *E. leucogramma* as a pest of yellow guayacán. We also provide a morphological description of all stages of development of *E. leucogramma* in order to facilitate the identification of adults and immatures of *E. leucogramma*, in the field so that appropriate and timely decisions are made as part of the integrated management of plants; this is particularly important as they remain on trees that are obtained from nurseries.

We also provide valuable information concerning the internal morphology of the digestive tract highlighting salivary glands and the ventricle, which are the reservoirs of protozoans that may have a role in the malformations, overgrowth, and premature yellowing of the host tree. This agent, a flagellate protozoan, has not been recorded in yellow guayacán at this time.

In addition, we also provide information and the first record of a possible entomopathogenic fungus colonizing *E. leucogramma* in natural conditions.

Materials and Methods

Adults, eggs, and nymphal instars used for this study were collected the months of January to July of 2021 on young yellow guayacán in Envigado, Departamento of Antioquia, Colombia (Lat. 6.167780°, Long. 75.586135°) at an altitude of 1576 m and on the campus of the Universidad Nacional de Colombia, Medellín Branch (Lat. 6.260644°, Long. 75.577559°) at an altitude of 1466 m.

Dissections were performed using a Zeiss Stemi 305 stereomicroscope with an ocular adapted to the camera of a Huawei YP8. Dissected structures were slide mounted in glycerin and observed and photographed using a Zeiss Primo Star ocular microscope. Illustrations of the internal and external anatomy were produced using Adobe Illustrator CS6. The terminology used to describe the different structures follows Snodgrass (1993) and Chapman (2013).

Observations on biology were made at the Entomology Laboratory of the Universidad Nacional de Colombia, Medellín Branch. The specimens studied in this work were identified as *Edessa leucogramma* Stål by Dr. T. J. Henry and 4♂♂, 2♀♀ voucher specimens were deposited in the Francisco Luis Gallego Entomological Museum MEFLG of the Universidad Nacional de Colombia, Medellín (specimen label data: Envigado: Antioquia: COLOMBIA. on *H. chrysanthus*. March 1995. Hand collected by G. Abril R).

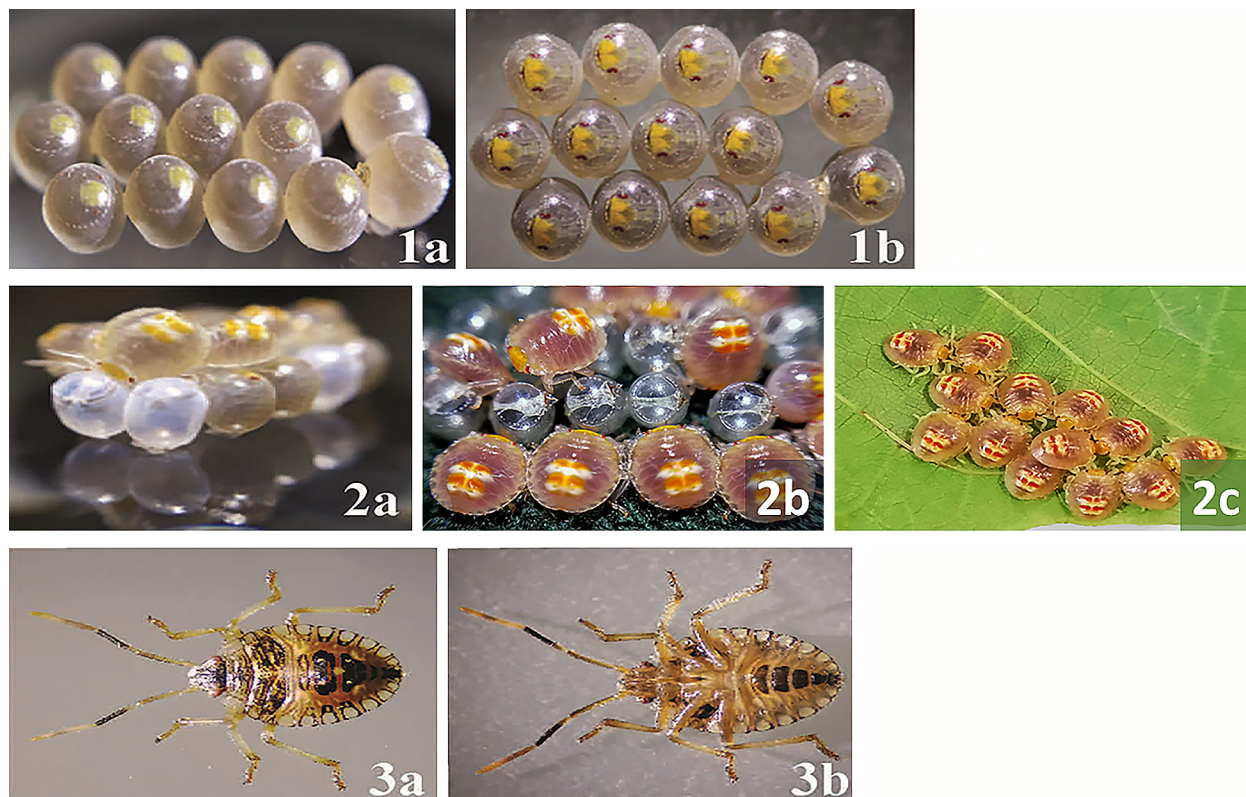
Results

Description of Life Stages

Eggs and oviposition. Eggs usually are laid on the underside of leaves, but also on branches and stems and are firmly attached to the plant tissue by a viscous substance that is initially liquid but solidifies on contact with the air. Each female can lay up to 14 eggs at a time (Fig. 1a) and no maternal care was observed. Newly laid eggs have a creamy coloration and are barrel-shaped, with ornamentations around the operculum. Eggs have a mean ($n = 14$) diameter of 1.5 mm and height of 2.0 mm. Development of the embryos is easily observed through the chorion during development. Immatures inside the eggs change from a grayish-rose to cream when the eye spots and yellow frons of the nymphs become evident (Fig. 1b).

Immature stages. Nymphs from the same clutch emerged in synchrony. Emergence was aided by the presence of an air bubble inside the egg, which breaks the operculum by pressure. The average time for a nymph to hatch was about six minutes. Newly emerged nymphs are shiny orange (Fig. 2a).

First instar ($n = 14$). Length 2.5 mm, width 3.0 mm. Ovoid; coloration mostly pinkish brown, with head yellow and eyes red; dorsal glands visible between abdominal segments 3/4 and 4/5 as two orange spots (Plate 1-2a,2b,2c) through which they secrete an orangish substance when disturbed. Once hatched, nymphs congregate around empty eggshells. (Fig. 2b).

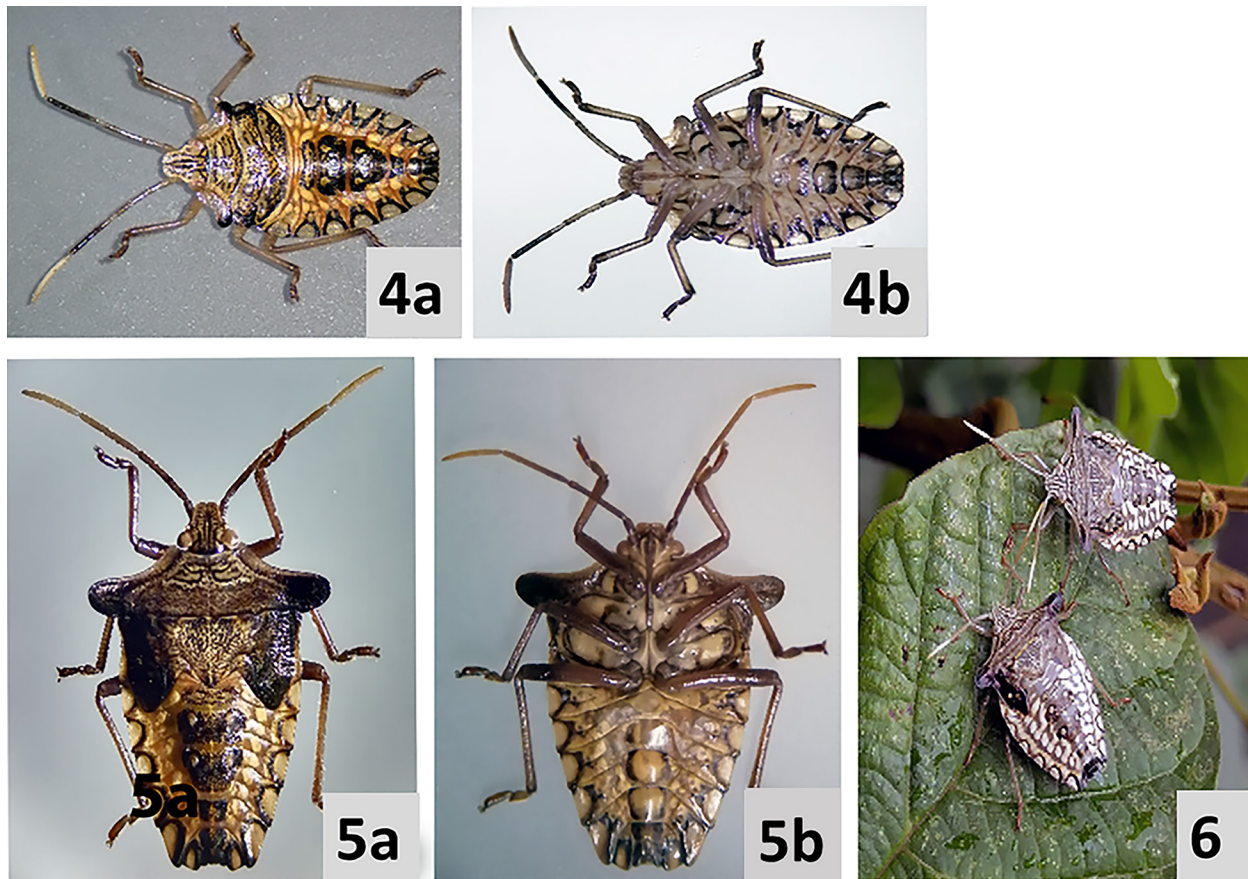


Figures 1–3. Immature stages of *Edessa leucogramma*. **1)** Eggs and oviposition of *Edessa leucogramma*. **a)** Number of eggs per clutch. **b)** Nymphs developed inside the egg. **2)** First-instar nymphs of *Edessa leucogramma*. **a)** Newly emerged nymphs. **b)** Nymphs congregating around the egg clutches. **c)** Clustering nymphs showing dorsal-posterior orange spots on the abdomen. **3)** Second-instar nymphs of *Edessa leucogramma*. **a)** Dorsal habitus. **b)** Ventral habitus.

Second instar ($n = 7$). Length 5 mm, width 3.8 mm; interocular width 1.0 mm; combined antennal segment lengths 4.5 mm. Labium and antennae are well developed. Antennal segments I and II are pale brown; III brown, pale at the base, and segment IV is largely white (Plate I- 3a, 3b). Pronotum lacking lateral projections. Overall body coloration is pale brown, with dark brown spots on the thoracic segments and quadrate translucent areas on connexiva along abdominal margins Dorsal abdominal scent glands openings between segments 3/4, and 4/5 widely black-bordered and 5/6 more narrowly bordered with black (Fig. 3a, b).

Third instar ($n = 4$). Length 8.1 mm, width 5.8 mm interocular width 1.4 mm; combined antennal segment lengths 6.2 mm. Antennal segments I and II are pale brown; segment III is dark brown, with a base paler; segment IV is white. Lateral projections on the pronotum are apically black, distinctly visible laterally with a weak indication of wing pads. Body slightly more convex ventrally than in the second instar; sternites clearly separated, with some spiracles visible. Overall body coloration is pale yellowish brown; thoracic segments finely black spotted; abdominal connexiva with translucent pale areas bordered in black. Dorsal abdominal scent gland openings are white, bordered in black between segments 3/4, 4/5, and 5/6 (Fig. 4a, b).

Fourth instar ($n = 5$). Length 14 mm, width 8.0 mm; interocular distance 1.6 mm; combined antennal lengths 9.80 mm. Antennal segments I–III are pale brown, segment IV is white. Lateral projections on the pronotum are well developed, apically black. Overall body coloration is pale cream ventrally, and pale brown dorsally; thoracic segments are densely black spotted. Wing pad length approximately 3.3 mm, extending to abdominal segment III, black, with two small white spots. The abdominal dorsum is brown, with a yellow spot on each side of the segment, and each connexivum is narrowly bordered in black. Dorsal abdominal scent gland openings are yellow, bordered with black (Fig. 5a). Metathoracic scent-gland opening is visible between meso- and metacoxae (Fig. 5b).



Figures 4–6. Immature states of *Edessa leucogramma*. 4) Third-instar nymphs of *Edessa leucogramma*. a) Dorsal habitus. b) Ventral habitus. 5) Fourth-instar nymphs of *Edessa leucogramma*. a) Dorsal habitus. b) Ventral habitus. 6) Fifth-instar nymphs of *Edessa leucogramma* on host.

Fifth instar ($n = 7$). Length of 16 mm, width 9.3 mm; interocular distance 3.0 mm; antennal length of 9.8 mm. Antennal segments I–III brown, segment IV white. Lateral projections on the pronotum are pronounced, black. Scutellum extending to second abdominal tergum. Sternites are clearly separated, with visible spiracles. Wing pads large, extending to nearly to abdominal tergite IV; black, with several white spots, their mean length about 3.4 mm. Abdomen brown, with a yellow spot on each side and each connectivum narrowly bordered in black. Scent-gland openings are white and bordered with black (Fig. 6).

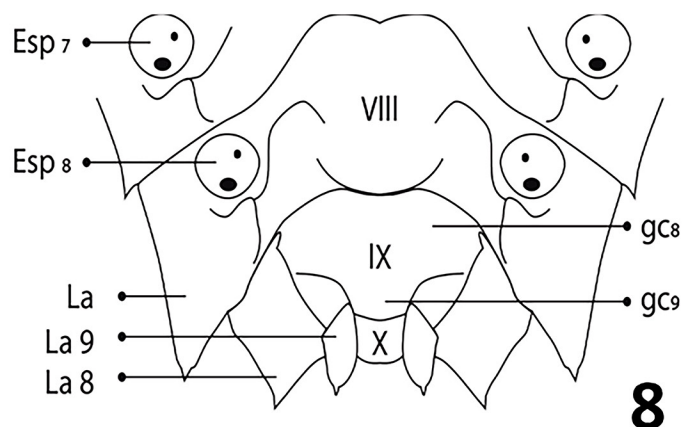
Adults

Male. Length 17 mm, width 9.0 mm; interocular width 3.0 mm. Ocelli red. Antennal color is light brown. Pronotum with long, apically black, lateral projections. Scutellum large, triangular, with white margins. General body appearance is smooth and shiny; wings grayish, with white veins (Fig. 7).

Male internal structures. A dissection of the male genitalia is given in Figure 9a and a line drawing of the different structures observed is given in Figure 9b. Overall, the reproductive system comprises a pair of testes, each with four spermathecal tubes and a pair of deferent ducts that end on the accessory glands as well as a spermathecal bulb that ends at the ejaculating duct. The gonads are red, and the accessory glands and spermathecal bulb are paler pink. The aedeagus is highly sclerotized and slightly convex; coloration is dark brown and with a ventral sulcus (Fig. 10a, b).



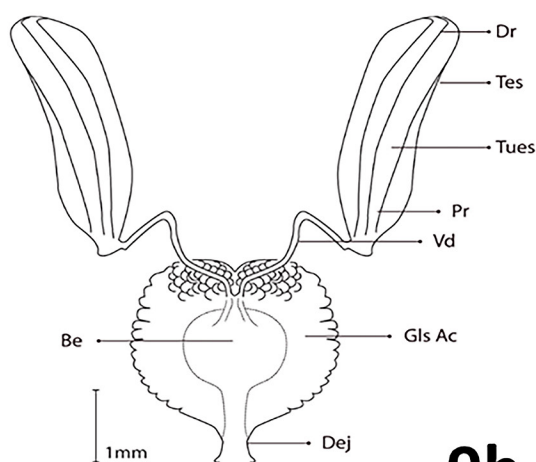
7



8



9a



9b

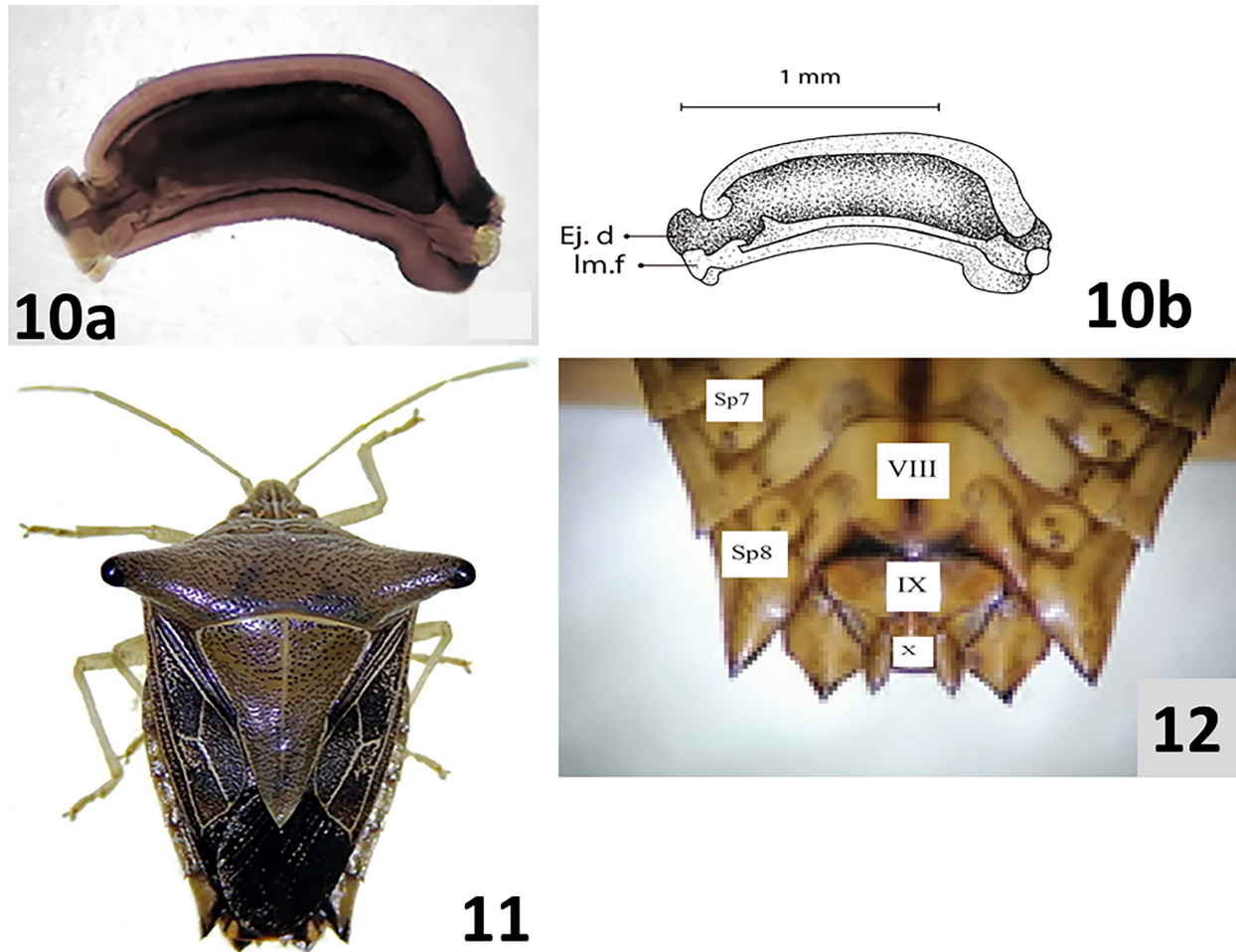
Figures 7–9. Adults of *Edessa leucogramma*. **7)** Adult male of *Edessa leucogramma*. **8)** Female external genitalia and sternites. **9)** Internal reproductive system of the male. **a)** Dissected genitalia. **b)** Illustration of male genitalia: (Be.) ejaculatory bulb; (Dej.) ejaculatory duct; (Dr.) distal region; (GlsAc.) accessory glands; (Vd.) deferent ducts; (Tes.) testis exhibiting four testicular follicles; (Tues.) testicular follicle; (Pr.) proximal region.

Female. Body length 19 mm, width 10 mm. Ocelli red. Antennal segments I–III yellowish white, and segment IV is white (Fig. 11). Rest of body as in the male. Pygidium and external genitalia as shown in Figures 8 and 12.

Female internal structures. The reproductive system of the female consists of two ovaries, each formed by seven ovarioles united at the apex by a terminal filament (Fig. 13a). Ovaries are panoistic, with a single egg visible inside each; lateral oviducts are well differentiated, ending on a common oviduct that ends at the genital chamber (Fig. 13b,c). The spermatheca (next to the genital chamber) is well developed, with three well-defined parts: the seminal receptacle with lateral cushions, the ring or middle cup-shaped structure, and the pouch-shaped spermathecal bulb, containing an internally sclerotized channel (Fig. 13c).

Relations with the host, related microbial fauna, and potential biocontrol

Feeding by *E. leucogramma* on the terminal meristems of young *H. chrysanthus* trees causes multiple side branches to develop and the loss of apical dominance (Fig. 14). Damage is particularly severe on juvenile trees of between two and three years old. When the damage is severe, the tree responds with the production of new buds anywhere on the adjacent branches. In addition, the young trees show accelerated defoliation, terminal bud destruction,



Figures 10–12. Adults of *Edessa leucogramma*. **10)** Male reproductive system of *Edessa leucogramma*, aedeagus: **a)** photograph of the male aedeagus in ventral view, **b)** illustration of the male aedeagus in ventral view: (Ej.d.) ejaculatory duct, (lm.f.) medial lobe of the phallus. **11)** Dorsal habitus of the female. **12)** Photograph of pygidium and external genitalia of the female: (VIII, IX, X) sternites 8, 9, and 10 respectively; (Sp7 and Sp8) spiracles 7 and 8.

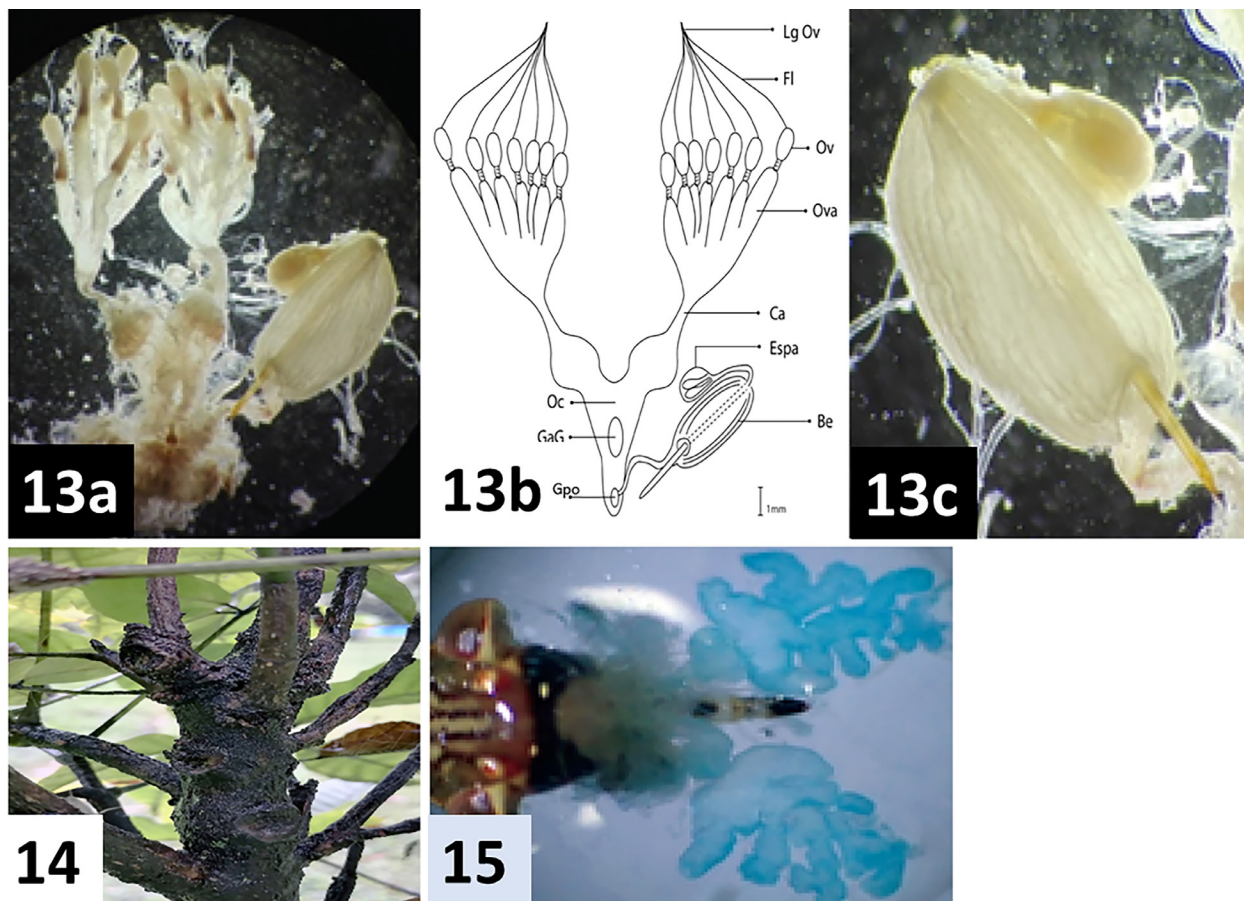
and splitting and cracking of the bark. Once the terminal branches begin to dry out and die the damage is irreversible, even after eliminating the bugs from the tree.

Flagellate protozoa associated with the digestive tract of *E. leucogramma*

During the dissections of the salivary glands (Fig. 15). we found multiple protozoa at different levels of development, which are possibly species of *Phytomonas* Donovan (Trypanosomatidae); these protozoans are being investigated as possible causative agents for the damage seen in yellow guayacáns. Parasites of this genus previously have been associated with other species of the genus *Edessa*, for example, *E. loxdali* Westwood, while feeding and damaging *Cecropia palmata* Willd. (Urticaceae) (Kastelein 1985; Camargo and Wallace 1994).

The amastigote and promastigote forms of the protozoa (Fig. 16a–c) found in salivary glands were also found in the ventriculus of the digestive system (Fig. 17). Similar protozoans have been associated with many sucking insects and is known to cause foliar lysis, visible as yellowing leaves.

During this study, we also found an adult female of *E. leucogramma* colonized by a fungus. The specimen was dead (mummified and covered with sporulated fungus) and grasping the cortex on a branch of a host seedling. This first record of an assumed entomopathogenic fungus associated with *E. leucogramma*. The identity of

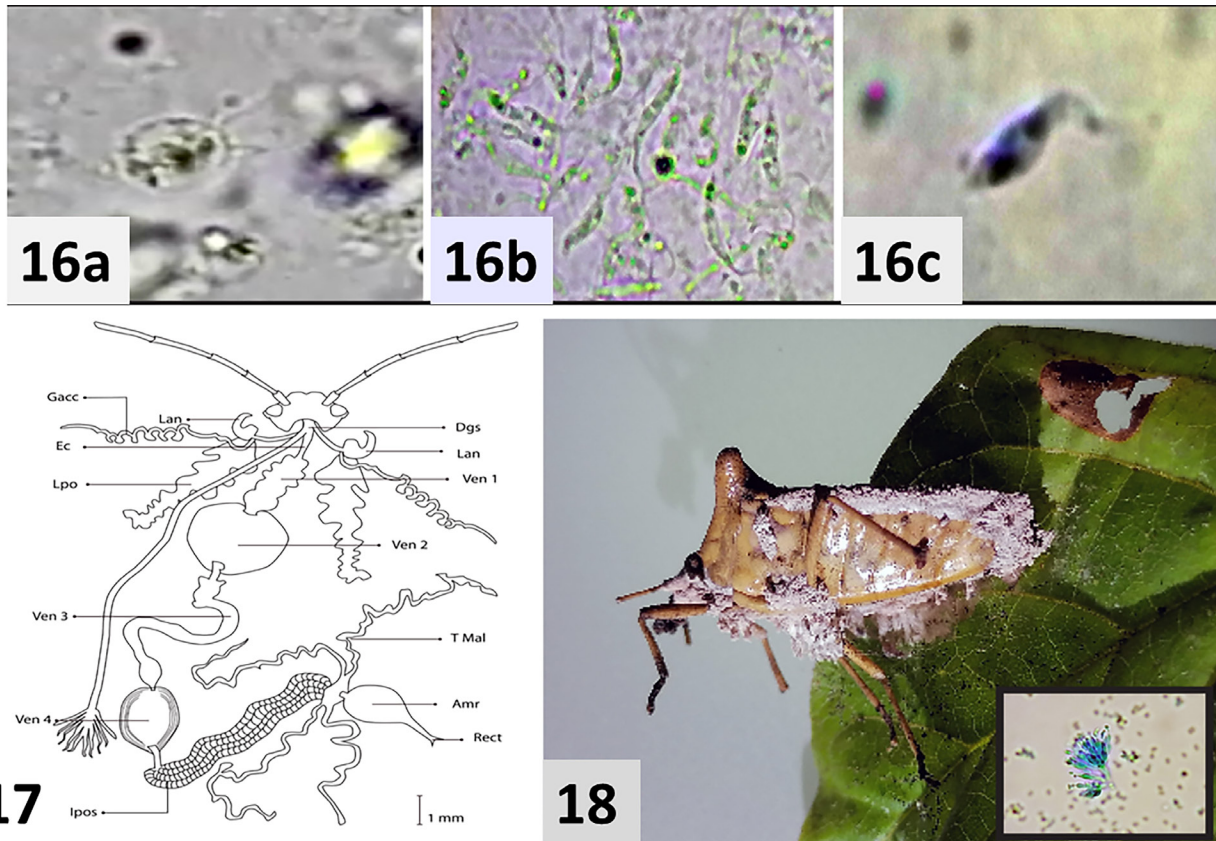


Figures 13–15. *Edessa leucogramma* biology and natural history. **13)** Reproductive system of the female of *Edessa leucogramma*. **a)** Photograph of female’s reproductive system. **b)** Illustration of the female’s reproductive system: (Be.) spermatheca; (CaG.) genital? chamber; (Espa.) spermathecal bulb; (Fl.) lateral filament; (Gop.) gonopore; (Lg.) ovarian ligament; (Oc.) common oviduct (Ola.) lateral oviduct; (Ov.) ovarium; (Ova.) ovarioles. **c)** Photograph of the spermatheca and associated parts. **14)** Damage produced by the feeding of *E. leucogramma* on *H. chrysanthus*. **15)** Dissection of the salivary glands of *E. leucogramma* (Organ where the live protozoa were found).

this fungus is being investigated for an upcoming paper. Images are given of the parasitized stinkbug (Fig. 18), as well as the mycelia and spores of the parasitic fungus (Fig. 18, inset square).

Discussion

The genus *Edessa* is a highly speciose group, that has been poorly studied, especially in regard to the life cycle of the different species, their general biology, and the internal morphology of individual species. Furthermore, research in this genus has mainly concentrated on *E. mediatubunda* because it has been considered a pest of different crops of economic importance such as cotton and alfalfa. Whereas *E. leucogramma* (pest referred to in this document) has not been studied at all, in part because until recently it was only occasionally found in pink guayacan [*Tabebuia rosea* (Bertol) Bignoniaceae], and totumo [*Crescentia cujete* L. Bignoniaceae]. Nonetheless, with recent mass planting campaigns of yellow guayacáns in the AMVA, population densities of the stink bug *E. leucogramma* have increased to the point that today they are a pest and nuisance in the cities and the yellow guayacán has become its main host; additionally, with the increase in the populations of the stink bug, severe damage



Figures 16–18. *Edessa leucogramma* biology and natural history. **16)** Flagellate protozoa associated with *E. leucogramma*. **a–c)** Different types of flagellate protozoa found in the digestive tract (i.e., salivary glands and ventriculus) of *E. leucogramma*. Photographs at a magnification of 1000 times the actual size (1000×). **17)** Illustration of the digestive system of *E. leucogramma*: (Amr.) rectal ampulla; (Ec.) esophagus; (Dgs.) salivary gland's duct; (Gacc.) accessory gland; (Ipos) hindgut; (Lan.) anterior lobule of the salivary gland; (Lpo.) posterior lobule of salivary gland; (Ven 1–4) ventriculus 1–4 (Tmal) Malpighian tubes (Amr) rectal ampulla. **18)** Dead adult female of *E. leucogramma*, mummified presumably by the attack of an entomopathogenic fungus, insert (square, lower right) shows the sporangium and spores of the potential entomopathogenic fungus.

in young trees have become more common due to the feeding of the stink bugs, to the point that the AMVA has decided to temporarily restrict the planting of yellow guayacáns.

This work may be the first step in future work determining, among other things, that a bacterium may not be the causal agent of the damage observed in yellow guayacán trees as had been speculated. We also record with field material the presence of a possible entomopathogenic fungus, which should be explored in the future as a potential biological control agent for *E. leucogramma*.

Acknowledgments

We would like to thank Dr. Thomas Henry for his help with the identifications and for going through the draft of the manuscript multiple times, his comments and suggestions were of paramount importance for this work; We are also grateful to Eduardo Amat for also helping with the review of the manuscript and for his comments; to Catalina Cossio Sanchez for the illustrations and to Dr. Carlos A. Palacios Bejarano of the Instituto Colombiano Agropecuario (ICA) for helping with the identification of the entomopathogenic fungus and the reviewers:

Thomas Henry, Irina Morales-Castaño and Joe Eger for their insightful comments which contributed to making this a better paper. We would also like to thank the Faculty of Sciences of the Universidad Nacional de Colombia in Medellín for its support to the authors and AHSP will also like to thank USDA-APHIS- PPQ Science and Technology for allowing time to work on this project.

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Received June 29, 2023; accepted August 23, 2023.

Review editor Joe Eger.

