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First record of *Monalonion velezangeli* (Hemiptera: Miridae) affecting avocado and cherimoya (*Annona cherimola*) as new hosts in Ecuador

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Abstract

Monalonion velezangeli is a polyphagous hemipteran reported as one of the main pests of economic importance in avocado and coffee crops. This is the first report of *M. velezangeli* affecting avocado (*Persea americana* (Mill)) in Ecuador and the first report of cherimoya (*Annona cherimola*) as a new host plant. The species was identified through classical taxonomy based on morphology, with parallel molecular identification targeting the cytochrome oxidase I region.

Key words: *Persea americana,* pest, coffee, chamusquina bug, diversity, morphology

Introduction

Avocado (*Persea americana*) is planted in low valleys of the Ecuadorian highlands. The expansion of the area of this crop for export has brought with it the emergence of new pests and diseases. One such pest is plant bug *Monalonion velezangeli* (Carvalho & Costa) (Miridae: Bryocorinae: Monaloniini) has been found affecting Fuerte and Hass varieties.

Monalonion is a genus with an endemic distribution limited to the Neotropics, includes 33 associated taxa, of which 16 are recognized as valid species (Gamboa *et al.*, 2020; Namyatova & Cassis, 2016; Carvalho & Costa, 1988). Three species of this are reported in Ecuador. *M. annulipes* and *M. dissimulatum* have been identified affecting cocoa (*Theobroma cacao*), and *M. atratum* with no plant host reported (Froeschner 1981, Carvalho & Costa 1988; Schuh 2002; Schuh 2013; Giraldo *et al.* 2010).

Monalonion velezangeli is a mirid of twilight habits with a wide range of plant hosts. In Colombia, it was first recorded in 1984 on avocado (Carvalho & Costa 1988). Subsequently, it was recorded feeding on guava (*Psidium guajaba*), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), coffee (*Coffea arabica*), eucalyptus (*Eucalyptus spp.*), and blackberry (*Rubus glaucus*) (Giraldo *et al.* 2010; Rodas *et al.* 2014). Its presence is only reported in Colombia in the departments of Cauca, Huila, Antioquia, Caldas, and Quindío (Laiton-Jímenez *et al.* 2020; Carabalí-Muñoz *et al.* 2021; Gamboa *et al.* 2020). Its geographic distribution limited to Colombia and Ecuador. This is likely due to a lack of sampling in other countries in the region, where it does not represent a phytosanitary problem.

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Here, we report for the first time *M. velezangeli* affecting avocado in Ecuador in the provinces of Pichincha and Imbabura. Furthermore, this is the first report of *M. velezangeli* on cherimoya (*Annona cherimola*) as a feeding host. The damage caused by the mirid is usually confused with a fungal disease by farmers and technicians. The identification and early detection of this pest species in avocado and cherimoya orchards will lead to the development of proper management strategies and prevent yield losses in Ecuador.

Materials and methods

Study Area. Adults of *M. velezangeli* were found in three avocado orchards in Ecuador between July and September 2023. Orchard A, located in Imantag in Pichincha province, (coordinates 0°09'52" N 78°25'09.8" W) at 2,448 masl. Planted in 2009, occupying an area of four hectares with Fuerte variety in a density of 270 plants per hectare. The ecological formation corresponds to humid montane scrub of the western Andean Mountain range (Sierra *et al.* 1999) with a mesothermal semi-humid equatorial climate associated with the lower, sheltered inter-Andean valleys. The average of the annual temperatures ranges between 12 and 20°C, with annual rainfall ranging between 800 and 2000 mm (Porrout *et al.* 1985). Orchard B, located in Perucho in Pichincha (coordinates 0°8'42.42" N 78°25'54.44" W) at 2,267 masl; planted in 2013, occupying an area of three hectares with Fuerte variety, in a density of 330 plants per hectare. The ecological formation corresponds to montane dry scrub (Sierra *et al.* 1999), with an annual precipitation range from 500 to 900 mm. Orchard C, located in Imantag in Imbabura province (coordinates 0°19'33.35" N 78°15'20.47" W) at 2,176 masl; planted in 2012, with an area of six hectares with Hass variety, in a density of 400 plants per hectare. Also located in an ecological formation corresponding to montane dry scrub (Sierra *et al.* 1999), with an annual precipitation range from 500 to 1000 mm. All of them, within two defined seasons: warm and dry (from May to September) and cool and rainy (from October to April).

Damage Assessment of *M. velezangeli* To confirm the damage caused by the insect on both hosts, orchards showing signs of the pest were monitored, and confirmation tests were conducted. Specimens and healthy fruits were confined in cages (mesh / dimensions of 20 x 20 x 20 cm) in situ. Symptoms of damage in the fruit and peduncle were observed after three and seven days. The number of punctures was counted, and the process was documented with photographic records. This experiment was conducted with ten replicates for avocado and six replicates for cherimoya.

Insect Sampling A total of 14 adults and three nymphs were collected, avocado and five in cherimoya. They were stored in vials with 70% ethyl alcohol. Identification was carried out following a taxonomic key for adults (Carvalho & Costa 1988) and observing the external morphological structures and male genitalia previously prepared (Hardwick 1950). The identified specimens were deposited in the Gustavo Orcés Natural History Museum of the National Polytechnic School of Ecuador.

Photography and Image Editing Microphotographs were captured with a Nikon D7500 camera (Nikon USA) mounted on an automated Stackshot macro rail (Cognysis Inc., Traverse City, MI). Additionally, a Nikon PB4 bellows, MEIKE extension tubes, Rainox DCR-250 tube lens, and Olympus 4X Plan C N infinity achromatic NA 0.10 microscope objective were used. Between 50 and 175 images were stacked using Zerene Stacker V.1.04. Photomicrographs were captured with a Better Scientific trinocular microscope with a 10X infinity achromatic NA 0.25 lens and an AMScope 5 MPX camera with a pixel size of 2.2µm x 2.2µm (BSI). Series of 20 to 25 photographs were taken and stacked with Toup View V. 4.11 software. Image editing for both types of photographs was done with Photoshop CC V. 2017.0.0 software.

DNA Extraction and Sequencing Simultaneously, five specimens (including two dark morphotypes, two light morphotypes, and one fourth instar nymph) were simultaneously sent to the Id Gen laboratory for identification. DNA was extracted from 100 mg of the insect leg samples using 400 µl of extraction buffer (Tris-HCl pH=8, 200 mM; EDTA pH=8, 25 mM; NaCl, 200 mM; 0.5% SDS) with the addition of 2 µl of β-mercaptoethanol. To eliminate RNA contamination, 1 µl of RNAse enzyme was added, and the samples were incubated at 37°C for 30 minutes. The integrity and quality of the DNA were assessed through microvolume spectrophotometry and agarose gel visualization. DNA samples were then diluted to a concentration of approximately 30 ng/µL for amplification via polymerase chain reaction (PCR) using COI primers LCO1490/HCO2198 (Klinbunga, 2003). The extracted DNA was of high quality, suitable for the amplification process.

PCR products were subsequently purified and sequenced using the Sanger method (Sanger and Coulson, 1977). Raw sequences were processed and assembled using Geneious R11 software.

Results

Damage and Host Plants Nymphs and adults of *M. velezangeli* were found on the leaves (Fig. 1A) and fruits of avocado trees (Fig. 1B). Punctures from the insect caused welt-like lesions on the branches, commonly concentrated at the tip (Fig. 1C). Lesions on the fruits reached sizes of two to five mm in diameter. Initially, they were welts, then depressions formed in the pericarp of the fruit (Fig. 1B). When the puncture is recent or fresh, there is oxidation of perseitol, a seven-carbon alcoholic compound released in the injured areas of the avocado (Hoddle & Hoddle 2008), generating a solid white exudate in the form of splashes (Fig. 1D). When the puncture is older than 10 days, it turns into a dark circular spot with necrotic tissue and a depression is observed.

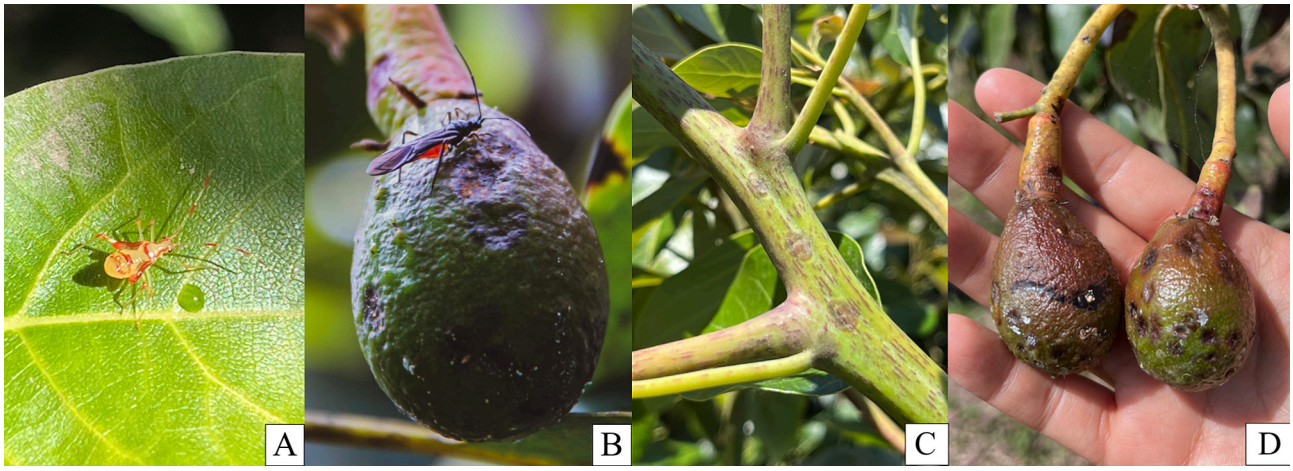

FIGURE 1. A. Nymph of *M. velezangeli* on an avocado plant. B. Adult of *M. velezangeli* on an avocado fruit. C. *M. velezangeli* bites on young avocado branches. D. Damage to small fruit of 2.2 and 2.4 cm in polar diameter.

The attack occurs both on small fruits of two cm in equatorial diameter and large fruits over five cm in diameter. Analyzing the lesions caused by *M. velezangeli*, it was quantified that in seven days, an adult makes an average of 45.5 lesions or punctures per fruit. Consequently, the damage that a single insect can cause is of high impact on the productivity of the orchard. In the physiologically mature fruit, there is no evidence of pulp damage; however, the scarred lesions on the skin are evident as defects, affecting the commercial value of the fruit.

The damage caused by *M. velezangeli* on the fruit of cherimoya (*A. cherimola*) is characterized by dark depressions of one to two mm in diameter (Fig. 2E). The appearance of the damage varies when the puncture is fresh or old (Fig. 2F). The bug's puncture likely facilitates the colonization of black saprophytic fungi by causing wounds in the fruit's epidermis.

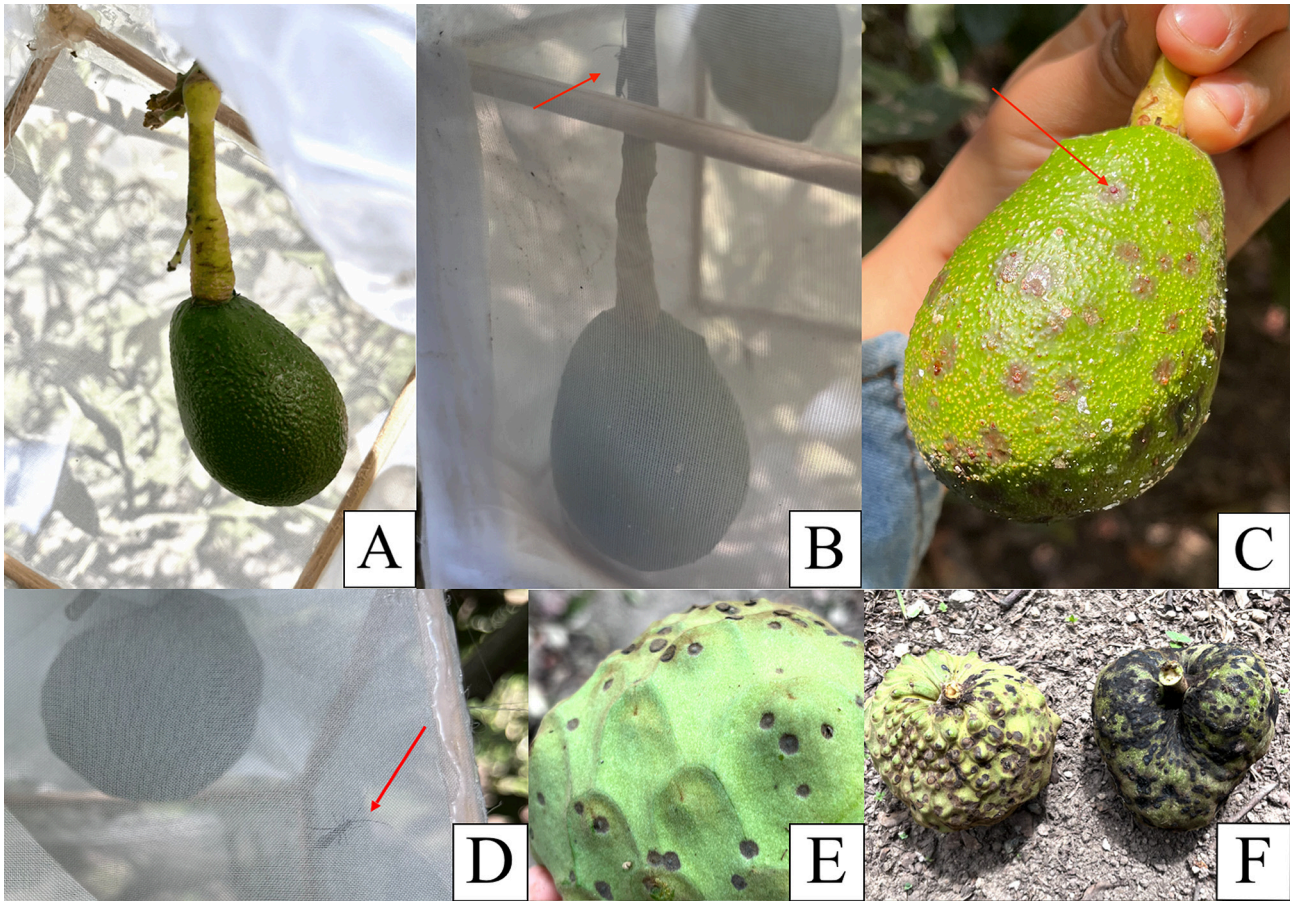

FIGURE 2. Confirmation test for *M. velezangeli* damage in avocado and cherimoya. A. Healthy fruit. B. Fruit confined with an adult *M. velezangeli*. C. Fruit of 6.3 cm in polar diameter with damage due to the bug's puncture. D. Fruit confined with an adult *M. velezangeli*. E. Fruit affected by *M. velezangeli*. F. Fruit with recent pitting (left) and fruit with old pitting (right) and beginnings of a saprophytic fungal infection.

Taxonomic Description of *M. velezangeli* Adults of *M. velezangeli* (Fig. 3A) have a shiny black head with segmented antennae covered with small black hairs and divided into four antennomeres. The first is glabrous compared to the other three (Fig. 3B). The rostrum extends beyond the first pair of legs and has four segments (Fig. 3C).

The thorax and abdomen are variable in color from black to orange. The pronotum and scutellum also show a diversity of tones (Fig. 3D, 3E). Intraspecific variability shows that colors vary among specimens of the same species (Carvalho 1972). There is also variability in coloration among adults depending on sex, with variations between black and red (Giraldo *et al.* 2010). For this reason, color is not a good descriptor for species determination (Gamboa *et al.* 2020). The hind legs are toned from chestnut to black, with femurs thickened toward the distal part, a creamy white band toward the middle, and densely hairy tibiae (Fig. 3F, 3G).

The hindwings are membranous in texture, light cream to transparent in color; the hemelytra (forewings) are variably colored from ochre to black in the corium; depending on the individual's morphotype, they present one or two spots inside the cell with the main vein near the cuneus and two outside the cell near the red vein (Fig. 3H, 3I), which matches the description by Giraldo *et al.* (2010) and differs from what Carvalho & Costa (1998) reported: two spots inside the cell near the cuneus and another outside the cell near the vein.

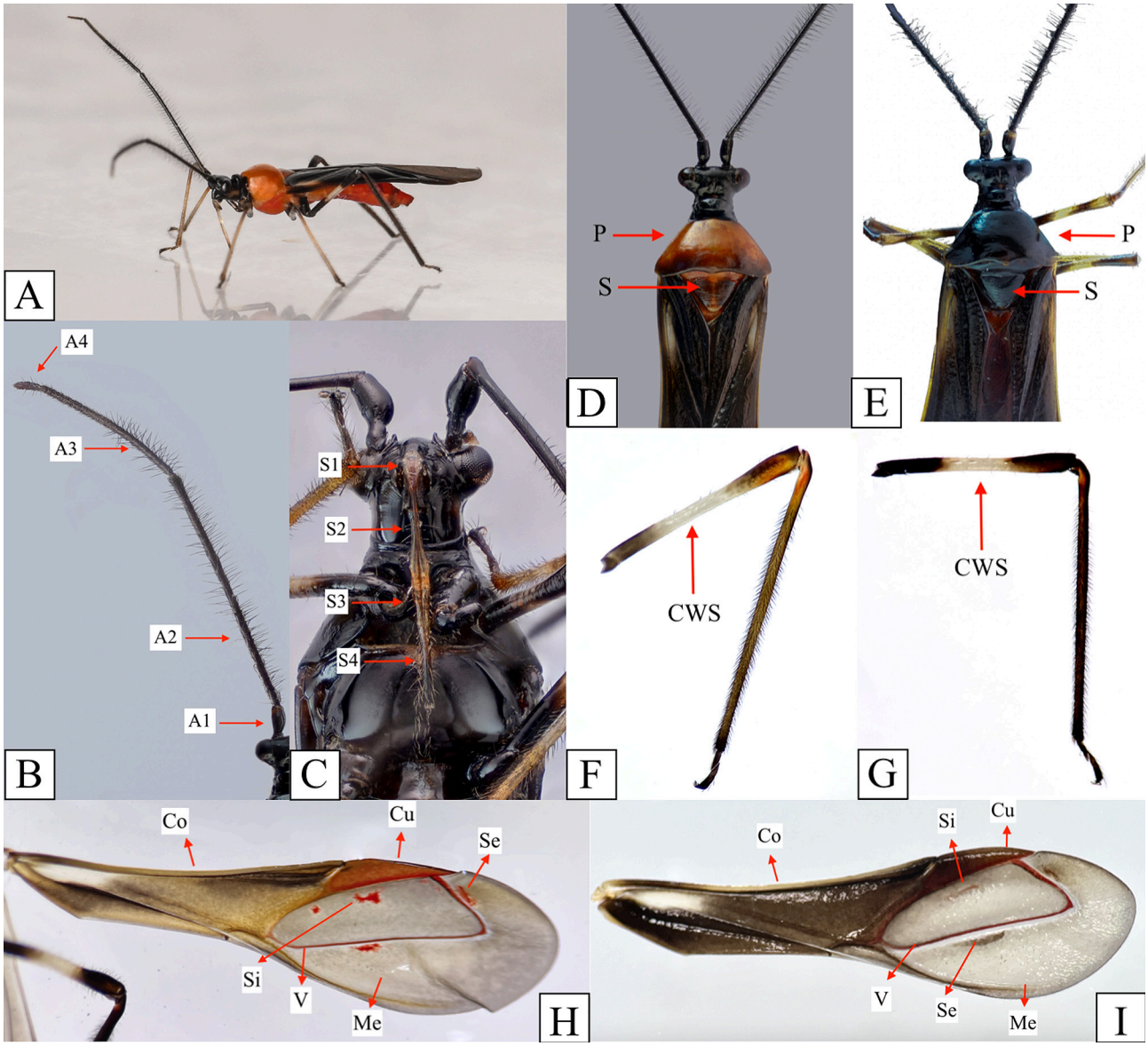

FIGURE 3. A. *M. velezangeli* adult in vivo. B. Antennae with four segments. C. Rostrum of *M. velezangeli* with its four segments. D and E. Coloration diversity in the *M. velezangeli* pronotum (P), scutellum (S). F and G. Coloration diversity in the legs of *M. velezangeli* and creamy white strip in the middle of the femurs (CWS). H and I. Coloration diversity in forewings in cuneus (Cu) and corium (Co), spots inside (Si) and outside the vein in the membrane (Me).

The male genitalia of the analyzed specimens match the description by Carvalho & Costa (1988) presenting a generic type aedeagus with a large membranous portion (Fig. 4A) and an elongated basal plate. The left paramere is approximately twice the size of the right one, with a rounded apex (Fig. 4B). The right paramere is small, simple, and curved with a sharp apex (Fig. 4C). The genitalia description in the reference is brief, and it seems the variations of this structure within the genus *Monalonion* are subtle and sometimes nonexistent, with no taxonomic descriptions clearly separating the species; however, we recognize that visually they resemble those described by Carvalho & Costa (1988) and Giraldo *et al.* (2010). To clarify the taxonomic status of the genus *Monalonion*, Gamboa *et al.* (2020) proposed a nomenclature for the male genitalia as a taxonomic tool, detailing the structures that compose this reproductive organ (Fig. 4A), but no differentiated descriptions for each species are presented, only a general one for the genus *Monalonion*.

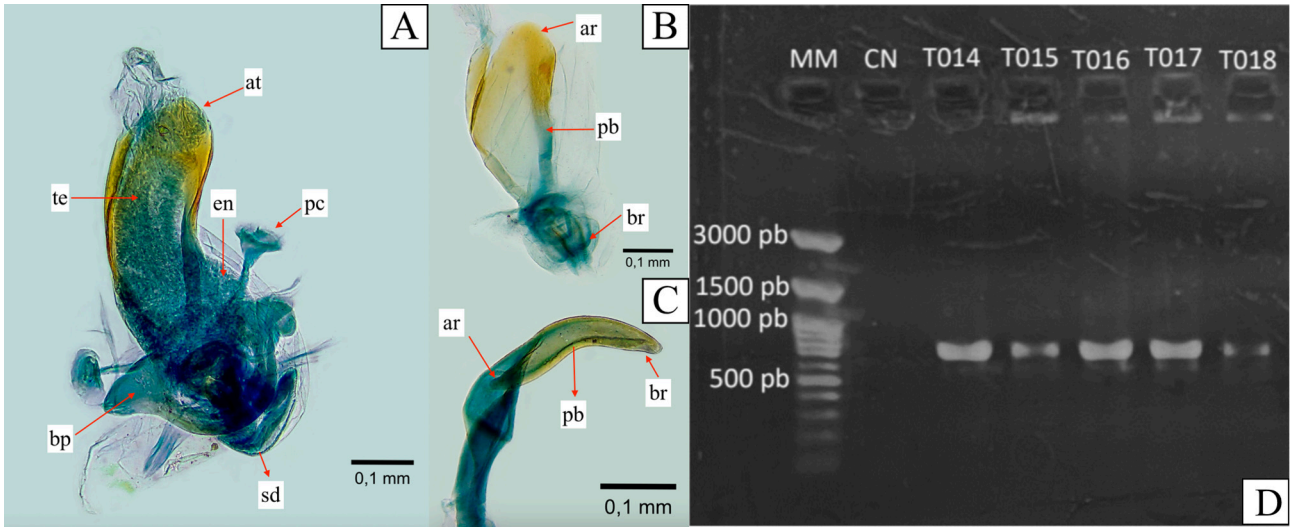

FIGURE 4. Structures of the male genitalia of *M. velezangeli*. A. Aedeagus: teak opening (at), teak (te), endosoma (en), seminal duct (sd), phallus base (bp), captured process. B. Left paramere: basal region (br), paramere body (pb), apical region (ar). C. Right paramere: basal region (br), paramere body (pb), apical region (ar). D. Agarose gel with PCR products for the COI fragment of five of the collected samples (MM= molecular weight marker, CN = negative control).

The identification of *Monalonion* species lacks sufficient taxonomic tools, and studies for understanding the interspecific and intraspecific morphological variations within the genus are scarce (Gamboa *et al.*, 2020). Consequently, molecular studies of all described species and their holotypes are necessary to clarify the variation of the morphological characteristics to determine whether they are one or more species and understand the phylogeny of the genus.

Regarding the molecular results of the present study, all specimens were similar. Amplicons of approximately 700 bp were observed (Fig. 4D), suggesting that coloration is not an indicator of genetic differences, at least for the cytochrome oxidase I (COI) gene region. These assembled sequences were compared with the NCBI GenBank nucleotide database, and the matches were null for this species; therefore, this report constitutes the first molecular reference of *Monalonion velezangeli* in the GenBank (Accession numbers: PQ046647.1, PQ046646.1 PQ046645.1, PQ046644.1), which can be used for future research.

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The authors declare that they have no conflict of interest.

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