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First report of *Pseudococcus viburni* (Hemiptera: Pseudococcidae) in Colombia: Morphometric and molecular analysis, with notes on morphological variation in specimens from Brazil and Colombia

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Abstract

The obscure mealybug *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) is recorded for the first time from Colombia based on specimens collected on *Opuntia cylindrica* (Lam.) DC., *Mammillaria* sp. (Cactaceae), *Escallonia paniculata* (Ruiz & Pav.), Roem. & Schult. (Escalloniaceae), *Ficus carica* L. (Moraceae), *Coffea arabica* L. (Rubiaceae), *Citrus* sp. (Rutaceae), *Cestrum nocturnum* L. and *Solanum betaceum* Cavanilles (Solanaceae). Multiple methods were used to identify *P. viburni* because it belongs to the "*Pseudococcus maritimus*" complex, a group composed of more than 60 species with high variation in morphological characteristics. The specimens were identified based on the morphology and morphometric analysis of third-instar nymphs and adult females. This morphological identification was corroborated by data on geographical distribution, plant hosts and a molecular identification using two different loci, CO1 (mtDNA) and the 28S ribosomal gene (nuclear genome). An updated list of species of *Pseudococcus* Westwood recorded from Colombia and information on morphological variation found in the studied specimens from Brazil and Colombia are provided.

KEYWORDS

"*Pseudococcus maritimus*" complex, Coccoidea, Coccomorpha, integrative taxonomy, Neotropical region, quarantine pest

1 | INTRODUCTION

The Pseudococcidae (Hemiptera: Coccomorpha) is the second largest family of scale insects in terms of species numbers, composed of 2033 described species in 258 genera (García Morales et al., 2016). These insects, known as mealybugs, can cause several types of damage in plants due their feeding activity: sap loss, transmission of toxins and pathogens and physical damage due to honeydew contamination and associated sooty moulds (McKenzie, 1967; Williams & Granara de Willink, 1992; Williams, 2004). Some species are considered as major agricultural pests and can cause serious problems when introduced into new geographical areas without their natural enemies (Miller et al., 2002, 2005).

Pseudococcus Westwood, 1,840 (Hemiptera: Pseudococcidae), is one of the most species-rich genera in the Pseudococcidae, composed of 168 species (García Morales et al., 2016). Several species are considered as agricultural pests because of their polyphagous habits and wide distribution (Miller et al., 2002; González, 2011; Granara de Willink & Dughetti, 2012; Correa et al., 2015). Sixteen species of *Pseudococcus* are recorded for Colombia, and many of them have been associated with crops of economic importance (Figueroa, 1946, 1952; Williams & Granara de Willink, 1992; Kondo et al., 2008; Granara de Willink & González, 2018; Caballero, 2021).

The obscure mealybug, *Pseudococcus viburni* (Signoret, 1875), belongs to a morphological complex composed of around 62 species, with high variation in features such as number of discoidal pores surrounding the eyespots, distribution and number of oral-collar and oral-rim tubular ducts, composition of cerarii, among others (Gimpel & Miller, 1996; Correa et al., 2011; von Ellenrieder & Watson, 2016; Granara de Willink & González, 2018; Pacheco da Silva et al., 2019). Morphological and molecular studies put *P. viburni* close to *Pseudococcus bryberia* Gimpel & Miller, 1996, *P. eriocerei* Williams, 1973, *P. debilis* Granara de Willink, 2018, *P. dumetum* Granara de Willink, 2018, *P. lanatii* Granara de Willink, 2018, *P. mandio* Williams, 1985, *P. maritimus* (Ehrhorn, 1900), *P. meridionalis* Prado, 2011 and *P. occultus* Granara de Willink, 2018 (Gimpel & Miller, 1996; Correa et al., 2011; Pacheco da Silva et al., 2017; Granara de Willink & González, 2018). A variety of taxonomic tools have been used to elucidate this species complex, including molecular techniques, morphological studies of postembryonic stages and biological information, i.e., host range, geographical distribution and mealybug-parasitoid relationship approaches (Gimpel & Miller, 1996; Wakgari & Giliomee, 2004; Charles, 2011; Correa et al., 2015). The *P. maritimus* complex has several species of economic and quarantine importance. Because of the difficulty of identifying species belonging to this complex, it is often necessary to use a combination of different tools in order to identify a species with accuracy.

Pseudococcus viburni is believed to be endemic to the Neotropical region and is considered a quarantine pest in many countries around the world (Charles, 2011). In the Neotropical region, it has been recorded from Argentina, Bolivia, Brazil, Chile, Costa Rica, Cuba, Ecuador, Guadalupe, Guatemala, Panama, Peru, Uruguay and Venezuela (Williams & Granara de Willink, 1992; Ben-Dov, 1994; Gimpel & Miller, 1996; Culik et al., 2007; Vera et al., 2012). In Colombia, *P. viburni* currently has the status of "Quarantine Absent Pest" (Instituto Colombiano Agropecuario ICA, 2018).

The aim of this study is to report *P. viburni* for the first time from Colombia using morphological, morphometric and molecular tools, as well as host and geographical data analysis. *Pseudococcus viburni* is diagnosed, and a list of important features that will aid in differentiating it from closely related species is provided. New information on intraspecific variation and host plant information for *P. viburni*, and an updated list of *Pseudococcus* species recorded from Colombia are also provided.

2 | MATERIALS AND METHODS

2.1 | Collecting and sample preparation

Mealybugs were collected in situ from *Mammillaria* sp. and *Opuntia cylindrica* (Lam.) DC., 1828 (Cactaceae) (Figure 1a–d), *Escallonia paniculata* (Ruiz & Pav.) Roem. & Schult., 1819 (Escalloniaceae) (Figure 1e-h), *Croton smithianus* Croizat, 1940 (Euphorbiaceae), *Ficus carica* L., 1753, (Moraceae), *Coffea arabica* L., 1753 (Rubiaceae), *Cestrum nocturnum* L. and *Solanum betaceum* Cavanilles (Solanaceae) (Figure 1i–l) in Bogotá D.C. and *Citrus* sp. (Rutaceae) in Popayan city, Colombia. Collection data are provided in Appendix S1. Mealybug specimens were collected under a collecting license "Permiso marco de recolección de especímenes de especies silvestres de la diversidad biológica con fines de investigación científica no comercial" [License framework for collecting of specimens of wild species of the biological diversity for non-commercial scientific research purposes], resolution No. 1466, expedited on December 3, 2014, by the Autoridad Nacional de Licencias Ambientales (ANLA) [Colombian National Authority Environmental Permits].

Specimens were stored in 75% and 95% ethanol and labelled with their respective collection data. Slide-mounted specimens were prepared according to Sirisena et al. (2013). The studied specimens are deposited in the entomological collections of "Universidad Nacional

FIGURE 1 Host plants of *Pseudococcus viburni* in Colombia: a–d, *Opuntia cylindrica* (Cactaceae) with close-up images of in situ and alcohol-preserved specimens; e–h, *Escallonia paniculata* (Escalloniaceae) with close-up images of specimens on stems and leaves; i–l, *Solanum betaceum* (Solanaceae) with close-up images of specimens on leaves, aggregation of mealybugs on branch and fruit peduncles, necrotic leaves and damage on fruits caused by *P. viburni* [Colour figure can be viewed at wileyonlinelibrary.com]

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Prepared slides of *P. viburni* collected in Brazil between 2013 and 2015 were used in this study. Brazilian samples were collected in different cities across Rio Grande do Sul State, in commercial crops, such as apple (*Malus domestica* (Suckow) Borkh, 1803), grapes (*Vitis* spp.), persimmon (*Diospyros kaki* Thunb., 1780), strawberry (*Fragaria x ananassa* Duchesne ex Rozier, 1784) and weeds (*Artemisia verlotiorum* Lamotte, 1876 and *Rumex* sp.). Collection data of samples of *P. viburni* from Brazil are listed in Pacheco da Silva et al. (2017).

2.2 | Morphological identification and morphometric analysis

Adult female specimens were identified using the taxonomic keys of Williams and Granara de Willink (1992), Gimpel and Miller (1996), Williams (2004), von Ellenrieder and Watson (2016) and Granara de Willink and González (2018). Specimens of *P. viburni* used in this study were compared with the nine most similar species of the "*P. maritimus* complex," in which *P. viburni* is included, by morphological and geographical traits to find novel intraspecific variations. Identification of nymphs was carried out based on the diagnoses by Gimpel and Miller (1996) and Wakgari and Giliomee (2004) and using the taxonomic keys of Gimpel and Miller (1996) and Gullan (2000). Terminology for morphological characters and measurement guidelines follow Gimpel and Miller (1996), numbering cerarii from anal lobes anteriorly to head (C_1 corresponds to cerarii in abdominal segment VIII and C_{17} to anterior cerarii on head). A Nikon SMZ-1 stereoscope was used for macroscopic observation. Measurement and analysis of microscopic images of Colombian samples were conducted by the first author, using a Nikon Eclipse E600 phase-contrast microscope, a Lumenera 1-C5 camera and Image Pro Insight v 8.0 software calibrated with micrometric lamina Leitz Wetzlar (0.01 mm).

The adult females of Colombian specimens were compared with seventy-eight specimens collected from the southern region of Brazil, identified as *P. viburni* by morphology and molecular methods (Pacheco da Silva et al., 2017). Measurements of Brazilian samples were taken by the second and third authors under a LEICA DM 2500 phase-contrast compound microscope. An illustration of *P. viburni* with the main variations is provided. The line drawing of *P. viburni* represents a generalized individual based on Colombian and Brazilian samples used in this study. The enlargements around the central drawing are not drawn to scale. Although translucent pores on the hind legs are mostly on the dorsal surface, they were illustrated ventrally on the main figure for convenience.

A principal components analysis (PCA) was carried out to determine whether the analysed specimens correspond to a single species. A total of 50 adult females from six hosts were selected for this process as follows: 20 specimens from *Mamillaria* sp., 20 specimens from *Opuntia cylindrica*, four specimens from *Croton smithianus* and two specimens from each of *Coffea arabica*, *Ficus carica* and *Solanum betaceum*. The data matrix was set up with 23 variables: 14 morphological traits and nine morphometric traits (Table 1). Also, a polar coordinate model was performed for exploring the possible effect of the host on the morphology of the specimens. In this case the matrix considered only the specimens from *Mamillaria* sp. and *O. cylindrica* because of the low number of specimens from the other hosts. The VARSEDIG algorithm (Guisande et al., 2016) was applied to calculate the probability of one group belonging to another by selecting those morphological variables with the highest discrimination capacity. The prioritization of variables was established using the overlap method, which calculates and contrasts the density curves of each trait in both groups and arranges them from a lower (greater discrimination capacity) to a higher overlap level (less discrimination capacity). Afterwards, the polar coordinate model was set up, estimating the Euclidean distances with all the means of X and Y values calculated in the previous step. A Monte Carlo test was performed to obtain the probability that each X and Y coordinate of one group was significantly different from the other group. The algorithm was performed under two conditions: (a) selecting all those variables with a significant *p*-value and (b) selecting only those variables with highest discrimination capacity. The VARSEDIG algorithm was performed using the R statistical software (R Development Core Team, 2019) and R Wizard open-source application and packages Candisc (Friendly, 2007; Friendly & Fox, 2015) and Ida of the MASS package (Venables & Ripley, 2002; Ripley, 2019).

2.3 | Molecular analysis

The specimens used for DNA characterization correspond to the sample collected on *Solanum betaceum*. The process was performed at the biochemistry laboratory of Facultad de Agronomía (UdelaR), using the non-destructive method described by Malausa et al. (2011). The DNA was extracted using DNEasy Blood and Tissue kit (QIAGEN, Valencia, CA), following the manufacturer's recommendations. Voucher cuticles were kept in 70% ethyl alcohol for later slide-mounting and morphological identification. DNA was amplified from two different loci, the cytochrome oxidase subunit 1 (mtDNA) and the 28S ribosomal gene (nuclear genome). For amplification, the primers (forward, reverse) used were PcoF1 5′CCTTCAACTAATCATAAAAATATYAG3′ and LepR1 5′TAAACTTCTGGATGTCCAAAAAATCA3′ for the COI gene region (Park et al., 2010) and C-28SLong-F 5′GAGAGTTMAASAGTACGTGAAAC3′ and C-28SLong-R 5′TCGGARGGAACCAGCTACTA3′ (28S-D2) for 28S (see method in Sequeira et al., 2000). Polymerase chain reactions (PCRs) were carried out using Taq DNA Polymerase (Thermo Fisher Scientific™), with a 20 ml reaction mixture and 5 ml of diluted DNA. PCR conditions were as follows: initial denaturation for 15 min at 95°C, 35 cycles of denaturation at 95°C for 30 s, hybridization for 90 s at 55°C for 28S and 46°C for COI, elongation at

TABLE 1 Code and description of the morphological and morphometric characters used in the principal component analysis and polar coordinate model

Code	Name of the variable
ch_1	Number of oral-rim tubular ducts on dorsal abdomen
ch_2	Number of oral-rim tubular ducts on dorsal body surface
ch_3	Number of oral-rim tubular ducts on submedial dorsum of abdominal segment III
ch_4	Number of ventral oral-collar tubular ducts associated with cerarius 10 and cerarius 11
ch_5	Number of trilocular pores in cerarius 12
ch_6	Number of ventral oral-collar tubular ducts associated with submargin of cerarius 14
ch_7	Number of ventral oral-collar tubular ducts associated with cerarius 12
ch_8	Number of ventral multilocular disc pores on abdominal segment VIII + IX
ch_9	Number of ventral multilocular disc pores on abdominal segment VII
ch_10	Number of ventral multilocular disc pores on abdominal segment VI
ch_11	Number of ventral multilocular disc pores on abdominal segment V
ch_12	Number of ventral multilocular disc pores on abdominal segment IV
ch_13	Number of ventral multilocular disc pores on abdominal segment III
ch_14	Length of labium
ch_15	Length of anal lobe setae
ch_16	Length of metafemur
ch_17	Length of metatibia
ch_18	Length of metatarsus
ch_19	Length of trochanter + femur on hind legs
ch_20	Length of tibia + tarsus on hind legs
ch_21	Ratio of tibia/ tarsus of hind legs
ch_22	Transverse diameter of circulus
ch_23	Number of ventral oral-collar tubular ducts on each side of head

72°C for 60 s and a final extension for 10 min at 72°C. PCR-amplified fragments were analysed in 1% agarose gel under UV light. For bidirectional sequencing, PCR products were sent to Institut Pasteur (Montevideo, Uruguay) for sequencing. Consensus sequences and alignments were generated and manually edited in Bioedit v.7.02. BLAST searches (MEGABLAST method) were carried out on the NCBI GenBank database (http://www.ncbi.nlm.gov/BLAST).

3 | RESULTS

Large populations of *P. viburni* were found on the stems and modified leaves of *Mammillaria* sp. and *O. cylindrica*, also on leaves, twigs and fruit of *E. paniculata* and *S. betaceum*, forming large aggregations associated with sooty mould-causing fungi (species not determined). The latter three species (i.e., *O. cylindrica*, *E. paniculata* and *S. betaceum*) are new plant host records for *P. viburni*. On *S. betaceum* the mealybugs were found on leaves, twigs and fruit, where the mealybug populations developed until the tissues showed chlorosis and necrosis symptoms. It was observed that fruit of *S. betaceum* affected by the mealybugs had a slower development compared to non-affected fruits. The highest mealybug populations occurred on the peduncles of leaves and fruits (Figure 1i–l).

3.1 | Taxonomic identification

After sequencing, clean DNA fragments of 585 bp for 28S and 310 bp for COI were obtained. BLAST hits with sequence similarity of 100% were obtained for taxonomically verified 28S (AY427309 from Hardy et al., 2008) and COI (KU499447 from Malausa et al., 2016) sequences assigned to *Pseudococcus viburni* (Hemiptera: Pseudococcidae) and the morphological information corroborated this identification.

3.1.1 | Adult female

The adult female of *P. viburni* has the following traits: discoidal pores associated with eyes, not within a sclerotized rim (Figure 2a); cerarius 12 (C_{12}) with 15–23 trilocular pores and

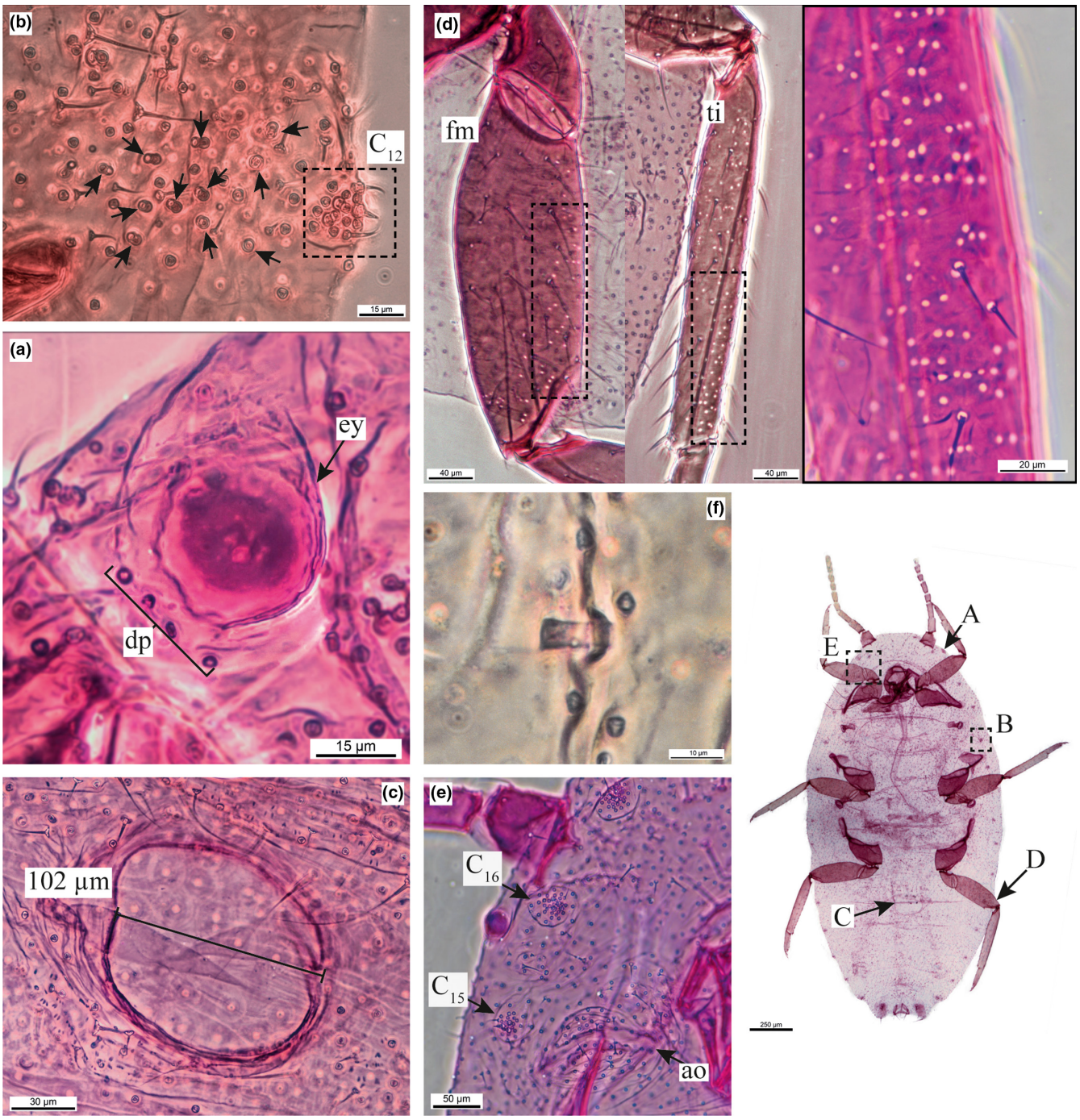

FIGURE 2 Microphotographs of the adult female of *Pseudococcus viburni* (Signoret) with close-ups of diagnostic features. a, Discoidal pores (dp) associated with eyes (ey) not set in a sclerotized rim (indicated by arrows). b, Cerarius 12 (C_{12}) with more than seven associated oral-collar tubular ducts (indicated by arrows) and more than 14 trilocular pores. c, Circulus greater than 70 µm wide. d, Translucent pores on hind femur (fm) and hind tibia (ti). e, Dorsal section delimitated by cerario 15 (C_{15}), cerarius 16 (C_{16}) and anterior ostiole (ao) without oral rim tubular ducts. f, Oral rim tubular duct in lateral view [Colour figure can be viewed at wileyonlinelibrary.com]

more than 7 associated oral-collar tubular ducts (Figure 2b); circulus present, more than 70 µm wide, with an intersegmental line (Figure 2c); translucent pores absent from hind coxa and trochanter, present on both hind femur and tibia (Figure 2d); ventral multilocular pores present on abdominal segment IV and posterior segments; dorsal multilocular pores absent; dorsal oral-rim tubular ducts absent from area delimited by C_{15}, C_{16} and anterior ostiole (Figure 2e); ventral oral-collar tubular ducts present on submargin at level of C_{14}; ventral oral-collar tubular ducts present on submargin at level of mid-coxa, numbering 0–2 (adapted from Williams & Granara de Willink, 1992; Gimpel & Miller, 1996).

The intraspecific variation of the characters found in the Colombian specimens ($n = 50$) is presented in Table 2. The morphometric traits related to the hind leg fall towards the lower limit of the variability range, whereas circulus diameter falls towards the variations in the upper limit. Gimpel and Miller (1996) reported that ventral multilocular pores on abdominal segment III are "usually absent in *P. viburni*." In general, the specimens herein studied fit well the descriptions of *P. viburni* by authors (i.e., Williams & Granara de Willink, 1992; Gimpel & Miller, 1996; Williams, 2004; Granara de Willink & González, 2018). However, some specimens presented unusual morphology that appear to be due to deformations of rather stable characters: three specimens had one partially developed cerarius on pair C_4, C_{14} and C_{17}; one specimen had just one dorsal oral-collar tubular duct on submarginal area mesad of C_3; and another specimen had just one duct in the submarginal area mesad to C_2. Furthermore, in 13 out of the 50 studied specimens the number of ventral multilocular disc pores on abdominal segment III varied between 1 and 5.

3.1.2 | Third-instar female

The third-instar nymphs were compared with the descriptions of that instar by Gimpel and Miller (1996) and Wakgari and Giliomee (2004) for *P. viburni*. Novel morphological variations were recorded as a mean with its standard deviation, followed by the range in parenthesis; the ranges reported in the above-mentioned descriptions are given in square brackets: length of hind femur 187.4 ± 11 (161–208) μm [110–202 μm], length of labium 117.7 ± 7.5 (104–127) μm [105–169 μm], length of longest anal lobe setae 92.1 ± 9.4 (64–109) μm [93–127 μm], transversal diameter of anal ring 66.9 ± 3.7 (60–74) μm [57–70 μm], number of ventral oral-collar tubular ducts in submarginal region of abdominal segments 0 ± 0.5 (0–2) [0–1] and number of dorsal oral-rim tubular ducts on thoracic area between C_9 and C_{14} 3 ± 1 (1–4) [0].

3.2 | Host effect on morphological variation

The principal component analysis (PCA) generated three tentative clusters. Most specimens fell within the "*Mammillaria* group" as follows: *Mammillaria* sp. (14 specimens) and part of *O. cylindrica* (6 specimens), *C. smithianus* (3 specimens) and *S. betaceum* (1 specimen). The second cluster, the "*Opuntia* group," included most of the specimens from *O. cylindrica* (14 specimens), and part of *Mammillaria* sp. (6 specimens), *C. arabica* (2 specimens), *C. smithianus* (1 specimen) and *S. betaceum* (1 specimen). The third cluster was composed by specimens from *Ficus carica* and one of *O. cylindrica* (Figure 3a). The analysis generated 22 axes and the first seven explain 75.9% of the variation (Table 3). In general, vectors of most traits are directed toward the "*Opuntia* group," whereas the vectors of morphological traits related to oral-rim tubular ducts and ventral oral collar tubular ducts associated with cerarius 12 are directed towards the "*Mammillaria* group." The longest vectors correspond to the number of oral-rim tubular ducts (ch_1 and ch_2) and measurements from the hind tibia and tarsus (ch_17, ch_18 and ch_21).

The scatterplot of the polar coordinates that compares the specimens from a group versus other groups is shown in Figure 3b. The characters with the highest discriminatory capacity are the number of ventral oral-collar tubular ducts associated with C_{10} and C_{11} (ch_4), length of metafemur (ch_16) and circulus width (ch_22). Although the groups are not completely separated, the arrows show that the vector of the variables ch_4 and cha_16 tends to increase and decrease, respectively, in the *Mammillaria* group. The model calculated the highest probability of a specimen of a host group belonging to another host group (Figure 3c,d). In both cases, the *p*-value in both X and Y polar coordinates was higher than 0.05, so the null hypothesis was accepted (H_0 = all specimens belong to a single group). This means that the most variable specimens collected on *Mammillaria* sp. are morphologically similar to specimens collected from any other hosts (Figure 3c, X and Y *p*-value = 0.286); in the same way, the most variable specimen from other plant hosts is similar to those specimens collected on *Mammillaria* sp. (Figure 3d, X *p*-value = 0.48, Y *p*-value = 0.38). Mealybugs feeding on the two cacti species were not morphologically different from mealybug specimens collected on other hosts. Thus, we can conclude that there is only one single species group.

3.3 | Host plants and geographical distribution

Host range and geographical distribution provide additional information that supports the identification of *P. viburni*. Host plants of specimens found in this study correspond to *O. cylindrica* and *Mammillaria* sp. (Cactaceae), *E. paniculata* (Escalloniaceae), *F. carica* (Moraceae), *C. arabica* (Rubiaceae), *Citrus* sp. (Rutaceae), *C. nocturnum* and *S. betaceum* (Solanaceae). The species closely related to *P. viburni* were analysed, except for *P. mandio* and *P. bryberia*. *Pseudococcus meridionalis* has been recorded on *Conyza bonariensis* (L.) Cronquist, 1943 (Asteraceae), *Diospyros kaki* Thunb., 1780 (Ebenaceae), *Punica granatum* L., 1753 (Lythraceae), *Pyrus* sp. (Rosaceae) and *Vitis vinifera*

TABLE 2 Morphological comparison between specimens from Colombia and closely related species of the *Pseudococcus maritimus* complex

Character		Colombian specimens ($n = 50$)	*Pseudococcus*								
			viburni	*meridionalis*	*maritimus*	*eriocerei*	*mandio*	*bryberia*	*debilis*	*dumetum*	*lanatii*
Number of pairs of cerarii		16–17	16–17	17	16–17	16–17	16–17	17	14	17	15–17
Number of dorsal oral-collar tubular ducts in submarginal area mesad of cerarii ($C_{\#}$)	C_2	0	0	0	0	0	0–2	0	0	1	0
	C_3	0	0	0	0	0	0–1	0	0	0	0
	C_4	0	0	0	0	0	1–3	0	0	2	0
	C_5	0	0	0	0	0	0–3	Present (no data)	0	1	0
	C_6	0	0	0	0	0	0–3	Present (no data)	Present (no data)	1	0
	C_7	0	0	0	0	0	0–1	Present (no data)	Present (no data)	3	0
Number of oral-rim tubular ducts on dorsal abdomen		14 ± 2.8 (6–20)*	10–18	2–44	19–35	9–22	0–4	12	27–36	± 22	23–39
Ventral oral-collar tubular ducts on each side of head		3 ± 2 (0–9)*	0–6	6–16	3–25	1–5	1–5	0–2	0	3–4	±12
Number of ventral oral-collar ducts associated with C_{10} and C_{11}, opposite of each mid-coxa		0.4 ± 0.7 (0–3)*	0–2	3–17	6–20	0–5	0–6	0–9	Present (no data)	Present (no data)	7–8
Number of dorsal oral-rim tubular ducts between C_{15} and C_{16} and anterior ostiole		0	0	0	1	0	0	1	1	0	0
Number of ventral oral-collar tubular ducts associated with C_{12}		13 ± 3.3 (5–21)*	8–16	7–27	10–25	1–4	0–7	2–11	Present (no data)	Present (no data)	11–14
Length of labium (μm)		162 ± 8.8 (148–189)	146–207	117–183	154–207	168–180	183–200	124–143	122–135	220	183–210
Length of longest anal lobe setae (μm)		122 ± 9.7 (94–146)*	109–136	78–120	136–168	101–119	111–168	69–118	100	no data	no data
Length of hind femur (μm)		312 ± 19.1 (267–349)	263–356	no data	239–395	217–267	247–301	180–242	179–203	285	288–320
Length of hind tibia (μm)		328 ± 19.4 (256–370)*	268–381	no data	261–473	237–284	279–358	190–267	171–210	359	308–320
Length of hind tarsus (μm)		124 ± 7.4 (92–140)*	114–142	no data	100–132	79–104	89–114	99–109	83–86	117	113–117
Ratio of tibia/tarsus of hind legs		2.2–3.4*	2.2–3.0	2–2.9	2.2–3.6	2.4–3.1	2.7–3.5	1.8–2.5	2–2.2	3.1	2.8
Transversal diameter of circulus (μm)		128 ± 15.4 (95–159)*	71–148	158–176	98–220	32–60	no data	71–131	66–86	147	117–148

Note: Data for analysed specimens include mean, standard deviation and range of variation in parentheses. Values marked with an asterisk (*) indicate new information for intraspecific variation within *P. viburni*. Information of listed species was taken from Correa et al. (2011), Gimpel and Miller (1996), González (2011), Granara de Willink and González (2018) and Williams and Granara de Willink (1992).

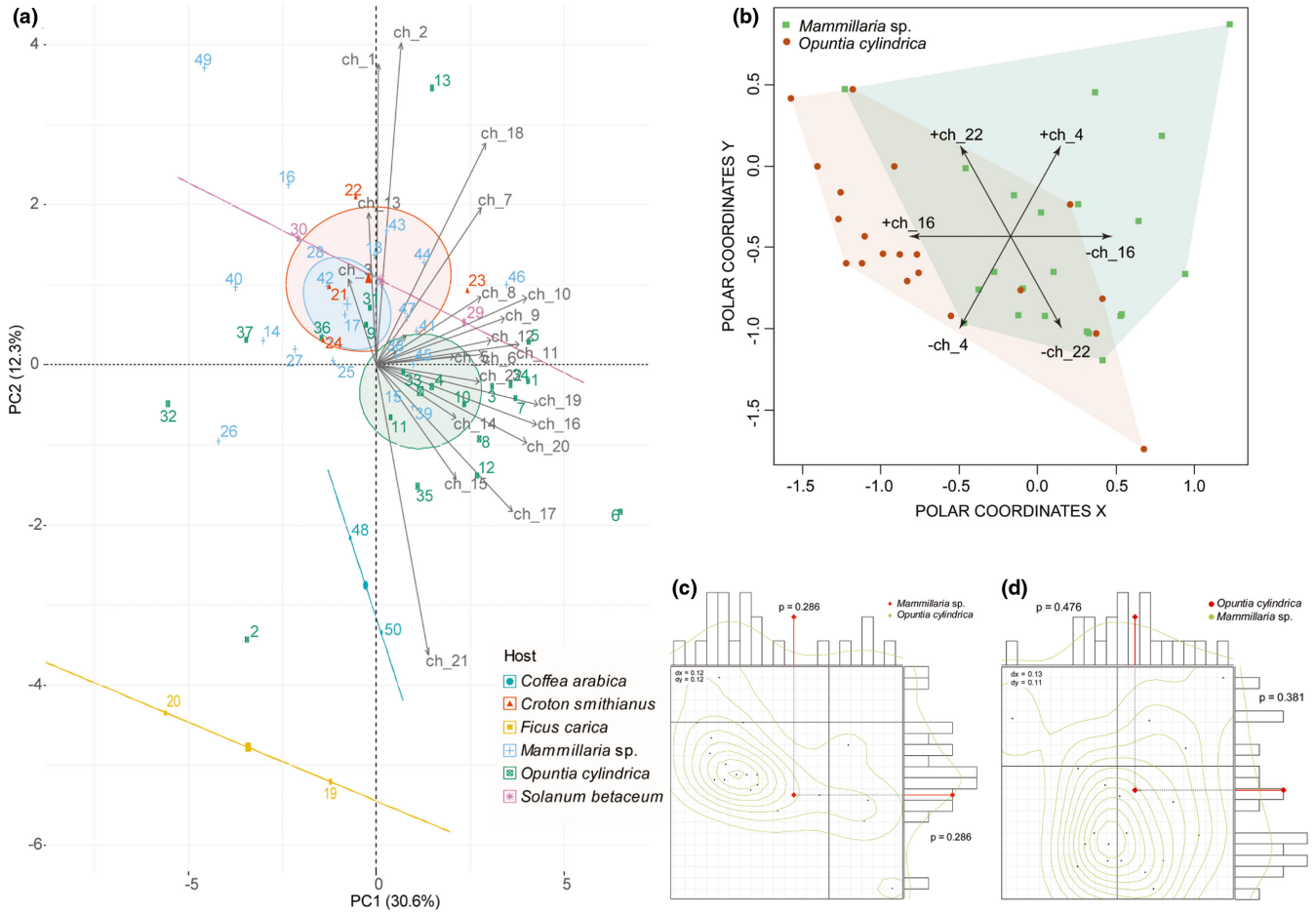

FIGURE 3 a, Principal component analysis plot of specimens of *Pseudococcus viburni* (Signoret) grouped by host. Variable codes listed in Table 1. b, Scatterplot of the polar coordinates for *Mammillaria* sp. versus *Opuntia cylindrica* (Lam.) DC. using the variables: number of ventral oral-collar tubular ducts associated with C_{10} and C_{11} opposite each midcoxa (ch_4), length of metafemur (ch_16) and circulus width (ch_22). The inner and outer ellipses show significance levels of 0.05 and 0.95, respectively. c, Bivariate randomization test with polar coordinate model. The green line indicates the contours of the distribution of randomized values. The two marginal histograms indicate the univariate test on each axis with its corresponding *p*-values. The red point shows the specimen collected on *Mammillaria* sp. with the highest probability of belonging to the specimens' group collected on *O. cylindrica*. d, Same bivariate randomization test of Fig. c, but showing the specimen from *O. cylindrica* with highest probability of belonging to the specimens' group collected on *Mammillaria* sp [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Details for each component including eigenvalues, individual and accumulated percentages of variance and associated variables

Principal component	Eigenvalues	Percentage individual variance	Percentage accumulated variance	Associated variables
PC1	7.04	30.592	30.6	ch_19, ch_16, ch_20, ch_10, ch_11, ch_17
PC2	2.82	12.276	42.9	ch_2, ch_1, ch_21
PC3	2.48	10.789	53.7	*
PC4	1.65	7.155	60.8	ch_5
PC5	1.24	5.397	66.2	*
PC6	1.19	5.158	71.4	*
PC7	1.05	4.581	75.9	ch_3
PC8	0.96	4.175	80.1	*

L., 1753 (Vitaceae) (Correa et al., 2011; Pacheco da Silva et al., 2017). *Pseudococcus eriocerei* has been recorded only on *Cleistocactus* sp. (Cactaceae) and *Zingiber* sp. (Zingiberaceae) (Gimpel & Miller, 1996). *Pseudococcus maritimus* has been recorded in association with around 83 plant species but none in the Cactaceae; the records of *P. maritimus* from Rubiaceae correspond to *Spermacoce* sp. and those from Solanaceae correspond to *Cestrum* sp. and *Solanum melongena* L., 1753 (Matile-Ferrero, 1978; Ben-Dov, 1994; Gimpel & Miller, 1996). On the other hand, *P. viburni* has been recorded on more than 250 plant species, including *Mammillaria* sp., *Opuntia* sp., *F. carica*, *Coffea* sp., *C. nocturnum*, and five species of *Solanum* (Williams, 1985, 2004; Ben-Dov, 1994; Gimpel & Miller, 1996). In this respect, specimens of *P. viburni* identified in this study fit well with the recorded host range of the species.

The known geographical distribution of the species also provides further support when defining the identity of the studied specimens. *Pseudococcus meridionalis* is currently restricted to the central region of Chile and southern Brazil, and *P. eriocerei* is only known from Argentina (Williams & Granara de Willink, 1992; Ben-Dov, 1994; Gimpel & Miller, 1996; Correa et al., 2011; Pacheco da Silva et al., 2017). *Pseudococcus maritimus* was recorded from Colombia by Figueroa (1952), but Kondo et al. (2008) indicated that this species had not been recorded in Colombia since the report by Figueroa (1952), and there are no voucher specimens available to verify this record. Furthermore, *P. viburni* is recorded in all neighbouring countries of Colombia (i.e., Bolivia, Brazil, Ecuador, Panama, Peru and Venezuela); thus, the presence of *P. viburni* in Colombia was to be expected. With the addition of *P. viburni*, the number of species belonging to the genus *Pseudococcus* in Colombia is increased to 17 (Table 4). Excluding the dubious record of *P. maritimus*, among the *Pseudococcus* species recorded from Colombia, *P. viburni* is closest to *Pseudococcus elisae* Borchsenius and *Pseudococcus jackbeardsleyi* Gimpel & Miller.

3.4 | Remarks on the morphological variation in specimens from Brazil and Colombia

The ranges of morphological variation found in specimens from Brazil and Colombia include: (a) number of

TABLE 4 *Pseudococcus* Signoret (Hemiptera: Pseudococcidae) species recorded from Colombia

Species	References
Pseudococcus calceolariae (Maskell, 1879)	Caballero et al. (2020), Kondo et al. (2008)
Pseudococcus colombiensis Granara de Willink, 2018	Granara de Willink and González (2018)
Pseudococcus comstocki (Kuwana, 1902)	Figueroa (1952)
Pseudococcus elisae Borchsenius, 1948	Borchsenius (1948), Caballero et al. (2019), Caballero et al. (2020), Gimpel and Miller (1996), Williams and Granara de Willink (1992)
Pseudococcus espeletiae Williams & Granara de Willink, 1992	Ben-Dov (1994), Williams and Granara de Willink (1992)
Pseudococcus importatus McKenzie, 1960	Gimpel and Miller (1996)
Pseudococcus insuetus Granara de Willink, 2018	Granara de Willink and González (2018)
Pseudococcus jackbeardsleyi Gimpel & Miller, 1996	Caballero et al. (2019), Caballero et al. (2020), Gimpel and Miller (1996), Kondo and Muñoz (2016)
Pseudococcus landoi (Balachowsky, 1959)	Balachowsky (1959), Caballero et al. (2019, 2020), Gimpel and Miller (1996), Kondo and Muñoz (2016), Williams and Granara de Willink (1992)
Pseudococcus longispinus (Targioni Tozzetti, 1867)	Ben-Dov (1994), Caballero et al. (2020), Figueroa (1952), Kondo et al. (2008), Williams and Granara de Willink (1992)
Pseudococcus luciae Caballero, 2021	Caballero (2021)
Pseudococcus maritimus Ehrhorn, 1900*	Figueroa (1952)
Pseudococcus microcirculus McKenzie, 1960	Ben-Dov (1994), Gimpel and Miller (1996), Williams and Granara de Willink (1992)
Pseudococcus peregrinabundus Borchsenius, 1947	Ben-Dov (1994), Borchsenius (1948), Gimpel and Miller (1996)
Pseudococcus salazari Granara de Willink, 2018	Granara de Willink and González (2018)
Pseudococcus sociabilis Hambleton, 1935	Kondo et al. (2008), Williams and Granara de Willink (1992)
Pseudococcus viburni (Signoret, 1875)	Present study

Note: The species marked with an asterisk (*) is considered a dubious record.

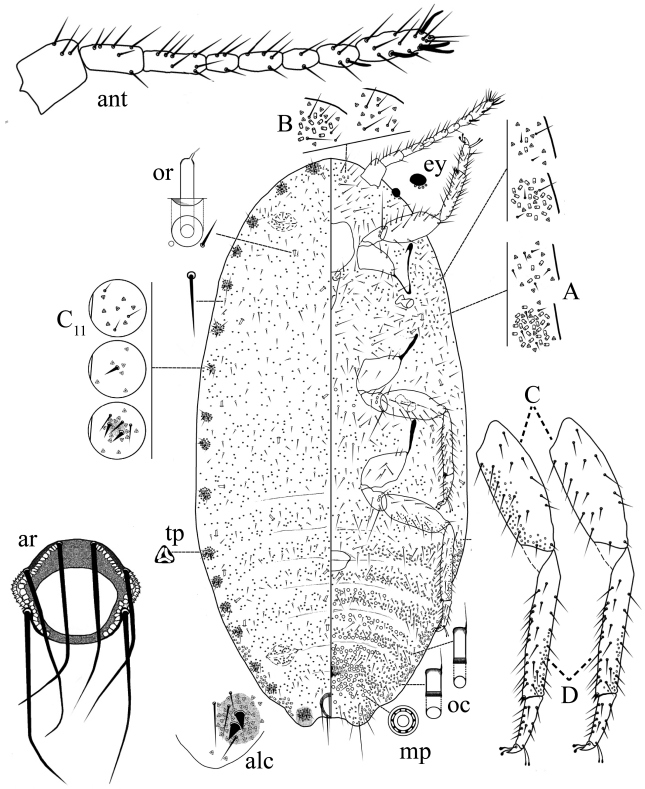

FIGURE 4 Adult female of *Pseudococcus viburni* (Signoret) showing the morphological variation present in the Neotropical region based on Colombian and Brazilian specimens: a, ventral oral-collar tubular ducts at level of cerarius 12; b, oral-collar tubular ducts on each side of the head; c, translucent pores on hind femur and d, translucent pores on hind tibia. Other anatomical structures: antenna (ant), oral-rim tubular duct (or), eyespot with associated discoidal pores (ey), variation of cerarius 11 (C_{11}), trilocular pore (tp), anal ring (ar), anal lobe cerarius (alc), oral-collar tubular ducts (oc) and multilocular disc pore (mp)

ventral oral-collar tubular ducts at level of cerarius 12 (C_{12}): 5–21 (Colombia) and 7–23 (Brazil) (Figure 4a); (b) number of oral-collar tubular ducts on each side of head: 0–9 (Colombia) and 1–6 (Brazil) (Figure 4b); (c) number of dorsal oral-rim tubular ducts on abdomen: 6–18 (Colombia) and 5–21 (Brazil); (d) number of translucent pores on dorsal surface of hind femur: 28–95 (Colombia) and 0–105 (Brazil) (Figure 4c); and (e) number of conspicuous translucent pores on dorsal surface of hind tibia: 48–126 (Colombia) and 0–104 (Brazil) (Figure 4d).

4 | DISCUSSION

The mealybugs herein studied fit well the published descriptions of *P. viburni*; however, some characters show some intraspecific variation (Table 2, characters indicated with an asterisk (*)). Morphologically, *P. meridionalis* is the closest species to *P. viburni* (Correa et al., 2011). However, they differ in the number of ventral oral-collar tubular ducts on each side of head (0–6 in *P. viburni*; 6–16 in *P. meridionalis*) and ventral oral-collar tubular ducts between cerarii 10 and 11 (0–2 in *P. viburni*; 3–17 in *P. meridionalis*). Based on the character states provided by Correa et al. (2011), the studied specimens were identified as *P. viburni*. Regarding the number of ventral oral-collar ducts on each side of the head, specimens from this study overlap with *P. meridionalis*, but the mean is 3 ducts per head on each side with standard deviation of 2; the extreme values (>6) were found in only three specimens. Other species closest to *P. viburni* are *Pseudococcus elisae* and *Pseudococcus jackbeardsleyi*; however, *P. viburni* can be differentiated from these two species by the absence of a sclerotized rim around the eyes (sclerotized rim around eyes present in *P. elisae* and *P. jackbeardsleyi*). *Pseudococcus viburni* also resembles *P. peregrinabundus*, but the two species can be differentiated by the absence of multilocular pores on the dorsum in *P. viburni* (multilocular pores present on dorsum in *P. peregrinabundus*) and also ventrally on the head (present on head on area anterior to clypeus in *P. peregrinabundus*). *Pseudococcus maritimus* is another species similar to *P. viburni*, and a detailed discussion to differentiate these two species was given by Gimpel and Miller (1996, p. 135–136). The analysis of discrimination capacity for each trait shows no preference between morphological and morphometric characters. However, in the PCA, the morphological traits related to the number and distribution of oral-rim tubular ducts are associated with the "*Mammillaria* group," whereas the morphometric traits are associated with the "*Opuntia* group."

The host-effect analysis did not show a phenotypic plasticity effect in female mealybugs that were evaluated for the family host plant, in this case, Cactaceae. Nevertheless, the PCA showed that specimens collected from *C. arabica* and *F. carica*, respectively, are farther separated from the rest of the specimens. The effects of the host plant on the morphological characters of mealybugs are linked to environmental factors, among them, temperature (Cox, 1983; Correa et al., 2011), which was not evaluated here. However, we consider it is worthwhile to mention these preliminary results as they may serve as a baseline for future studies that should evaluate not just the effect of the host plant, but also temperature, location, altitude, among other factors. Nevertheless, Colombian and the Brazilian specimens did not show marked morphological differences, except for the number of translucent pores on the hind femur and tibia, despite the differences in mean annual temperature of sampling locations (Bogotá~ 15–20°C; Popayán and Rio Grande do Sul ~18–23°C). Further statistical analysis should be conducted to determine differences between

populations found at different geographical regions and how the environmental factors may affect the morphology of *P. viburni*.

5 | CONCLUSIONS

The high morphological variation of *Pseudococcus viburni* makes this species hard to identify. However, we were able to confirm the presence of *P. viburni* from Colombia, associated with eight host plant species based on a combination of morphological character states, molecular sequence data and ecological information. With this new record, *P. viburni* is currently known from all countries of South America except for Paraguay. Although the distribution of *P. viburni* in Colombia is restricted to urban areas (cities of Bogotá and Popayán), the specimens were collected on plants of economic importance, i.e., coffee, fruit trees and ornamental plants. The distance between the sampling localities, and the wide range of hosts are factors to keep in mind when designing monitoring plans for the species.

Newly found morphological differences in Neotropical specimens of *P. viburni* indicate that the taxonomic characters currently used for separating species, such as the presence of translucent pores on hind femur and tibia should be revaluated. The morphometric analysis did not show differences between specimens from different hosts, nor marked differences between morphometric and morphological traits in terms of discriminatory capacity. The effect of abiotic factors such as altitude, host plant species, plant tissue and temperature on the morphology of *P. viburni* must be investigated in order to improve our knowledge on species delimitation and better understand the intraspecific variation of the species.

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