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Identification of *Dysmicoccus brevipes* and its association with PMWaV-1, -2, and -3 in Hawaiiana cultivar and MD-2 hybrid pineapple in Peru

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Research Paper

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Abstract

Pineapple cultivation is of economic importance for farmers; however, pineapple production can be affected by pests and diseases. Recently, the presence of mealybugs and pineapple mealybug wilt-associated viruses (PMWaV)-1, -2, and -3 has been reported in the provinces of Satipo and Chanchamayo, in Peru's central jungle. This study aimed to molecularly identify mealybugs collected from the Hawaiiana cultivar and the MD-2 hybrid in those provinces to determine if they are indeed hosts of the PMWaV-1, -2, and -3. Through amplification and sequencing of the internal transcribed spacer ribosomal genes, the mealybugs were identified as *Dysmicoccus brevipes*. In the phylogenetic analysis of these *D. brevipes*, Peruvian isolates were associated with isolates from India, China, Taiwan, and Japan. In addition, our results confirmed the presence of PMWaV-1, -2, and -3 in all mealybug specimens collected from both the Hawaiiana cultivar and the MD-2 hybrid tested, with these PMWaVs showing a 99% sequence identity with others recently reported in Peru. Therefore, *D. brevipes* is a host and probable vector of PMWaV-1, -2, and -3 for the cultivar Hawaiiana and the hybrid pineapple MD-2 in Satipo and Chanchamayo, Peru. Based on these findings and observations of crop management strategies in these provinces, we recommend integrated management practices to control this pest.

Introduction

Worldwide, pineapple exports in 2022 were among the three most traded tropical fruits, reaching 3.1 million tons (FAO, 2023). The main consumers are the USA with 3.62 kg/capita, followed by Canada with 3.30 kg/capita and Europe with an average consumption of 1.26 kg/capita, thus accounting for 76% of the market share (CIRAD, 2022). In Peru in 2021, according to the Integrated Agricultural Statistics System (SIEA, 2021), pineapple production was 588,398 tons, with a total harvested area of 16,836 ha: a yield of 34,949 kg/ha. The main producer was the Junin region, which produced 442,629 tons, with a total harvested area of 6,668 ha and a yield of 66,381 kg/ha. Junin maintained its pineapple production dominance throughout 2022 and 2023 (January–November), generating 402,049 and 404,251 tons, respectively, showing a slight downward trend of 0.54% in production.

Unfortunately, world pineapple production is seriously threatened by mealybugs, which transmit viruses that cause mealybug wilt of pineapple (MWP) disease (Sether *et al.*, 2005; Wei *et al.*, 2020). In fact, yield decline of up to 40% due to pineapple mealybug wilt-associated virus (PMWaV)-2 infection has been reported (Borroto-Fernández *et al.*, 2007). When infection occurs in the early months of planting, it reduces average fruit weight by up to 55% and by 35% for infections in late stages (Sether and Hu, 2002). In addition, asymptomatic PMWaV-1 infection causes losses of up to 30% due to premature fruit ripening (Sipes *et al.*, 2002).

The main pests of pineapple worldwide are mealybugs, belonging to the order Hemiptera, family Pseudococcidae (Dey *et al.*, 2018). *Dysmicoccus brevipes* (pink mealybug) and *D. neobrevipes* (grey mealybug) are both native to tropical South and Central America (Beardsley, 1992). These two species, with similar morphology, are considered extremely serious invasive pests (Wei *et al.*, 2020).

The polyphagous species *D. brevipes* infests a wide range of plants (53 families) including economically important crops and ornamentals such as coconut, avocado, mango, oil palm, citrus, cotton, coffee, cocoa, banana, apple, and pineapple (Ben-Dov, 1994). Pineapple (*Ananas comosus* L. Merr.) is a monocotyledonous plant of the order *Bromeliales* and family *Bromeliaceae*. In

the provinces of Satipo and Chanchamayo, in the Junin region, the main pineapple cultivars grown are Cayena Lisa, Samba, Pucalpina, Hawaiiana, and the MD2 hybrid. In infested pineapple plants, *D. brevipes* is found at the base of the stem, crown, root, and leaves. These pests feed on the plant by sucking the sap and causing symptoms such as yellowing of the leaves, defoliation, shoot abortion, size reduction, fruit deformation, and in some cases the death of the plant (Suma *et al.*, 2015). In addition, mealybugs produce honeydew that covers the fruit generating an environment conducive to fungal infection, reducing the photosynthetic rate and consequently reducing fruit marketability (Qin *et al.*, 2011).

However, the greatest threat is that these pests act as vectors of viruses. *Dysmicoccus brevipes* and *D. neobrevipes* are known vectors that transmit MWP disease (Sether *et al.*, 2005), a disease caused by a species of viruses called PMWaV-1, -2, and -3, which are recognised by the International Committee on Taxonomy of Viruses, and the putative PMWaV-4, -5, and -6 (Green *et al.*, 2020; Larrea-Sarmiento *et al.*, 2021) belonging to the genus *Ampelovirus* of the family Closteroviridae (Sether *et al.*, 1998). PMWaV-1, -2, and -3 have linear positive strand ssRNA genomes between 13,701 and 14,861 bp, and they code for 13 proteins. The 5′ end has a methylated nucleotide cap, while the 3′ end does not have a poly(A) tail. The genomic RNA is capped by capsid proteins giving a filamentous, flexuous shape about 1400–2200 nm in length and 10–13 nm in diameter (Green *et al.*, 2020). Mealybug-transmitted wilt of pineapple attacks pineapple crops wherever they grow (Gambley *et al.*, 2008; Sether *et al.*, 2010). These viruses generally cause symptoms of reddening, yellowing, apical necrosis, and stunting (Sether and Hu, 2002). In Latin America, PMWaV-1 and -3 have been reported in Mexico (Ochoa-Martínez *et al.*, 2016), PMWaV-1, -2, and -3 in Cuba (Hernandez-Rodriguez *et al.*, 2014), PMWaV-1 in Ecuador (Alvarez *et al.*, 2015), PMWaV-2 in Brazil (Peron *et al.*, 2017), and recently PMWaV-1, -2, and -3 in Colombia (Moreno *et al.*, 2023) and Peru (Carrasco-Lozano *et al.*, 2023).

To control the mealybug, a variety of approaches have been attempted such as destroying residues of the previous crop and alternate hosts and drowning the pests by abundant irrigation (Arellano *et al.*, 2015); however, this type of control has not been efficient. For a successful integrated pest management program, a thorough understanding of the crop production system and the ecology and biology of each pest or disease is critical (Mani and Shivaraju, 2016). Therefore, the objective of the present study was to molecularly identify the mealybugs collected from the Hawaiiana cultivar and the MD-2 hybrid in the provinces of Satipo and Chanchamayo in Peru's central jungle to determine if they are hosts of the PMWaV-1, -2, and -3 so that strategies for the integrated management of this pest could subsequently be developed.

Material and methods

Physiographic characteristics and collection of mealybug samples

We collected 136 samples of mealybug larvae in different stages and adults from symptomatic plants, specifically leaves and roots in pineapple production fields with the Hawaiiana cultivar and the MD-2 hybrid in the localities of San Martín de Pangoa, Alto Celendin, and Mazamari in the province of Satipo, and Alto Pichanaki in the province of Chanchamayo (table 1). These fields

Table 1. Collection site, altitude, pineapple cultivars, phenological stage of crop, symptoms in field, and PCR detection of PMWaV-1, -2, and -3 in mealybug samples

Collection site (locality, district, and province)	Altitude (m.a. s.l.)	Number of samples collected	Cultivar	Phenological stage of crop	Symptoms	Positive to RT-PCR		
						PMWaV1	PMWaV2	PMWaV3
Alto Celendin, Pangoa, and Satipo	880	30	MD – 2 Hybrid	Flowering	Yellowing, apical necrosis, chlorotic halos, and dwarfism	17 of 30 (57%)	14 of 30 (47%)	19 of 30 (63%)
Alto Belen, Mazamari, and Satipo	1346	32	MD – 2 Hybrid	Fruiting	Chlorotic halos and apical necrosis	19 of 32 (59%)	12 of 32 (38%)	21 of 32 (66%)
San Ramón de Pangoa, Pangoa, and Satipo	799	36	Hawaiiana	Flowering	Yellowing, reddening, apical necrosis, and dwarfism	14 of 36 (39%)	17 of 36 (47%)	16 of 36 (44%)
Alto Pichanaki, Pichanaki, and Chanchamayo	895	38	Hawaiiana	Fruiting	Chlorotic halos, yellowing, and reddening	14 of 38 (37%)	18 of 38 (47%)	24 of 38 (63)

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are in the Selva Alta, a climatic unit characterised by warm to temperate temperatures (14.5°C–25°C), rainfall ranging from 500 to 4,000 mm, and altitudes between 500 and 3,500 metres above sea level. Mealybug samples were preserved in Eppendorf tubes and liquid nitrogen for further analysis.

DNA and RNA extraction

Total genomic DNA extraction from adult mealybugs was performed using the innuPREP Blood DNA Kit (Analytik Jena) according to the manufacturer's instructions. Subsequently, total RNA was obtained using TRIzol (Invitrogen™). Briefly, a pool of 250 mg of larvae in different stages and adult mealybugs were pulverised with liquid nitrogen to which 1 mL of TRIzol reagent was added. The mixture was then incubated at room temperature for 10 min. The aqueous phase was centrifuged and recovered; chloroform was then added to remove protein residues. Isopropanol was added to precipitate total RNA and the pellet obtained was washed with 75% ethanol. The pellet was re-suspended in 50 µL of RNase-free water and stored at −70°C. RNA quality was estimated using a 1% agarose gel and quantified with a spectrophotometer (ThermoScientific™).

Polymerase chain reaction of the *Cytochrome oxidase I* gene and ribosomal internal transcribed spacer region of mealybug

To identify mealybugs at the molecular level, polymerase chain reaction (PCR) amplification of two target regions was performed. The mitochondrial *Cytochrome oxidase I* (*COI*) gene was amplified using the universal primers LCO-1490 (5′GGTCAACAAATCATAAAGATATTGG 3′) and HCO-2198 (5′TAAACTTCAGGGTGACCAAAAAAAAAATCA 3′) primers (Hebert *et al.*, 2003). Additionally, the ribosomal internal transcribed spacer (ITS) region, known to be highly variable and useful for differentiating closely related species, was amplified with primers specific for mealybugs, ITS2-M-F (5′CTCGTGACCAAAGAGAGTCCTG 3′) and ITS2-M-R (5′TGCTTAAGTTCAGCGGGTAG 3′) (Malausa *et al.*, 2011). PCR reactions were in a volume of 25 µL with 0.4 µM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1 × PCR buffer, 0.05 U/µL of Taq DNA polymerase (ThermoScientific™), and 30 ng of total genomic DNA. Amplification parameters were executed at 94°C for 5 min, followed by 35 cycles at 94°C, annealing at 46°C for LCO-1490/HCO-2198 and 58°C for ITS2-M-F/R primers, and an extension phase of 72°C for 60 s. The final extension step conducted at 72°C for 5 min.

Detection by reverse transcription PCR of PMWaV-1, -2, and -3 viruses

To detect PMWaV-1, -2, and -3 in mealybug samples, cDNA was synthesised using the Maxima H Minus kit (ThermoScientific™) according to the manufacturer's recommendations. Next, a 20 µL reverse transcription PCR (RT-PCR) reaction was performed with 5 µL of the cDNA, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1× PCR buffer, 0.05 U/µL of Taq DNA polymerase (ThermoScientific™), and 0.4 µM of primer sets 225/226 for PMWaV-1, 224/223 for PMWaV-2, and 264/263 for PMWaV-3 (Sether *et al.*, 2005, 2009), which amplified a region of the *Hsp70* gene. The parameters for amplification were 94°C for 5 min, followed by 35 cycles at 94°C for 60 s, 54°C for PMWaV-1 and PMWaV-2 and 55°C for PMWaV-3 for 60 s, with an extension phase of 72°C for 60 s, and a final extension step at 72°C for 10 min.

Sequencing and bioinformatics analysis

Fragments amplified by PCR with primers LCO-1490/HCO-2198 and ITS2-M-F/ITS2-M-R (seven samples for each set of primer) and primers for PMWaV-1, -2, and -3 (two samples for each virus) were purified from agarose gels using the Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan) according to the manufacturer's instructions. The purified fragments were sequenced by the Sanger method at Macrogen (South Korea) in both directions with primers used for PCR. The sequences obtained were assembled using Geneious v8.1 software (Kearse *et al.*, 2012). For phylogenetic analysis of mealybugs and PMWaV-1, -2, and -3 viruses, a BLASTn analysis was performed, and the sequences with the highest identity percentages were downloaded for multiple alignments using the ClustalW program (Thompson *et al.*, 1994). The phylogenetic tree was constructed with the MEGA11 program (Tamura *et al.*, 2021) using the maximum likelihood method, Tamura-Nei model with 1000 bootstrap replicates for test of phylogeny. The GenBank accession numbers for the phylogenetic relationship of mealybug and PMWaV 1, -2, and -3 are listed in Supplementary Table 2. *Planococcus citri* haplotype H2 (GU134678) and Mint vein banding-associated virus isolate (KY381598) were used as out-groups.

Results

Hawaiiana pineapples cultivated in San Ramón de Pangoa and Alto Pichanaki exhibited symptoms such as yellowing, reddening, stunted growth, and necrotic leaf tips. Similarly, the MD-2 hybrid grown in Alto Celendin and Mazamari displayed comparable symptoms, accompanied by chlorotic halos (fig. 1A; table 1). Likewise, in both the cultivar Hawaiiana and hybrid MD-2, the presence of mealybug was observed mainly at the base of the leaves (fig. 1B, C), stems, and roots with characteristics described previously (Beardsley, 1992; Wei *et al.*, 2020). Adult females were on average 6 mm long and 2 mm wide, wingless, pinkish, oval in shape and covered with a layer of white wax and filaments around the insect body (fig. 1D, E). For the molecular identification of mealybugs, we used the universal primers LCO-1490/HCO-2198 (Hebert *et al.*, 2003), which generated amplicons of the mitochondrial *COI* gene with an unexpected size (image not shown). Whereas the specific primers ITS2-M-F/ITS2-M-R (Malausa *et al.*, 2011) amplified fragments of 800 bp of the ITS2 region (fig. 2A) in all samples analysed, from which at least one sample per locality and cultivar were selected for sequencing. Mealybug samples collected from the Hawaiiana cultivar in San Ramon de Pangoa (1ITS-HSMP and 3ITS-HSMP), Alto Pichanaki (6ITS-HAP), Alto Celendín (8ITS-HC), and the MD-2 hybrid from Alto Celendín (10ITS-MD2C and 12ITS-MD2C) and Mazamari (13ITS-MD2M) were positive for *D. brevipes* (Cockerell) (Beardsley, 1992; Wei *et al.*, 2020).

Nucleotide sequences were deposited in the GenBank database under the following accession numbers: PP109489 (1ITS-HSMP), PP109488 (3ITS-HSMP), PP109490 (6ITS-HAP), PP109491 (8ITS-HC), PP109492 (10ITS-MD2C), PP109493 (12ITS-MD2C), and PP109494 (13ITS-MD2M). Due to suboptimal sequencing quality, the sequences submitted to GenBank are shorter than the fragments amplified by PCR. BLASTn analysis

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Figure 1. Presence of mealybug in pineapple production fields in the province of Satipo. (a) Production field of hybrid MD-2 in the locality of San Ramon de Pangoa with yellowing, apical necrosis, and chlorotic halos symptoms; (b) presence of mealybug in plants of hybrid MD-2 and (c) cultivar Hawaiiana; (d) dorsal and (e) ventral view of the adult female body observed under a Carl Zeiss Stemi 305 stereomicroscope at 30× magnification.

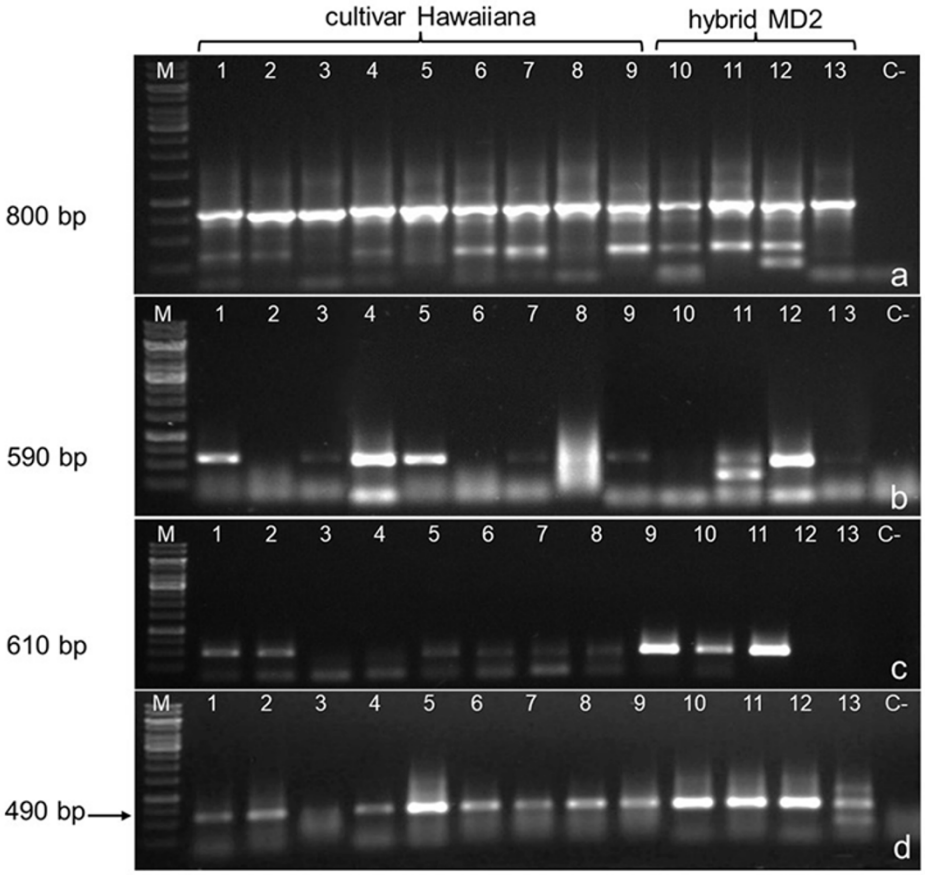

Figure 2. PCR amplification of the ITS region and RT-PCR of the *hsp70* gene of PMWaV-1, -2, and -3. (a) Amplified fragments of approximately 800 bp of the mealybug ITS region; (b) 590 bp fragments of PMWaV-1; (c) 610 bp fragments of PMWaV-2; and (d) 490 bp fragments of PMWaV-3. In a, b, c, and d, cultivar Hawaiiana wells: 1–3 San Ramon de Pangoa, 4–6 Alto Pichanaki, 7–9 Celendin; hybrid MD-2 wells: 10–12 Celendin, 13 Mazamari; M, 1 kb molecular weight marker; C, negative control.

revealed that the isolates 1ITS-HSMP, 3ITS-HSMP, 6ITS-HAP, 8ITS-HC, 10ITS-MD2C, and 12ITS-MD2C shared an average identity of 98% with isolates from India, Taiwan, and China, and 97% identity with isolates from Japan. These isolates clustered together in a single clade on the phylogenetic tree (fig. 3A). In contrast, the 13ITS-MD2M isolate displayed 95% identity with the

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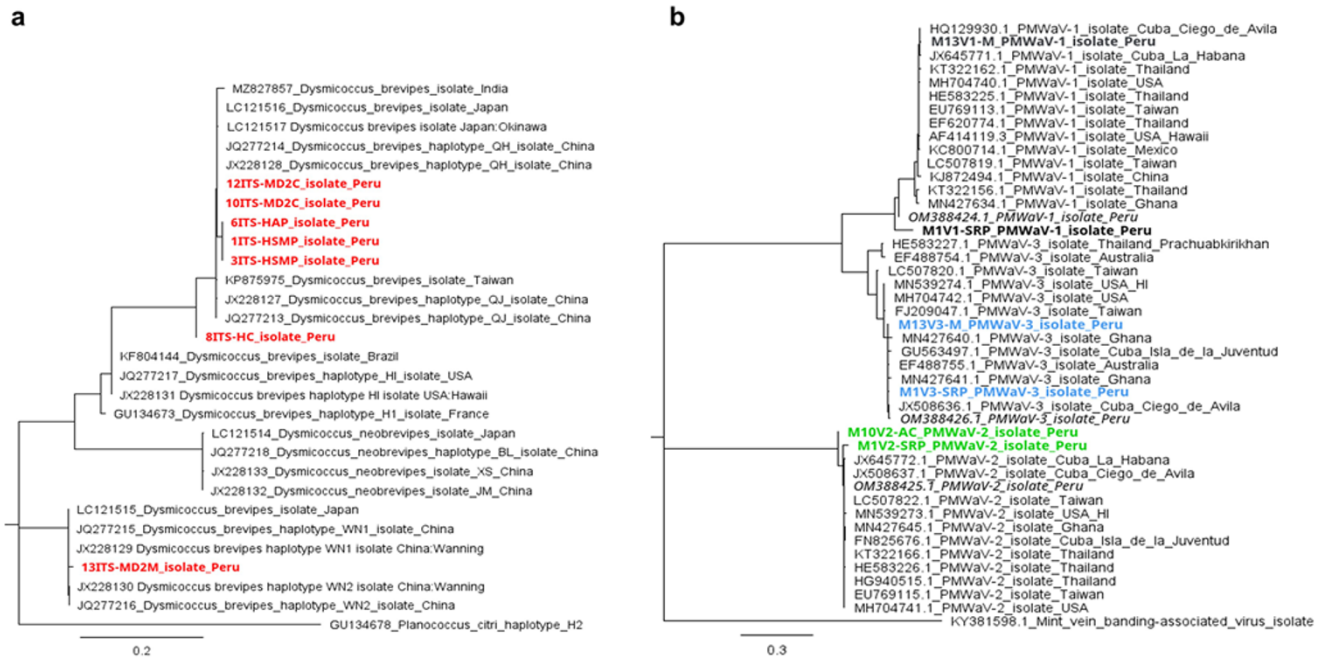

Figure 3. Phylogenetic trees of the ITS region for *D. brevipes* isolates (a) and the *hsp70* gene for PMWaV-1, PMWaV-2, and PMWaV-3 (b) are presented. *Dysmicoccus brevipes* isolates from Peru are marked in red, while PMWaV-1, PMWaV-2, and PMWaV-3 are indicated in black, light blue, and green, respectively. The H2 haplotype of *Planococcus citri* (GU134678) and the Mint vein banding-associated virus isolate (KY381598) served as out-group references for the respective trees.

isolate from Japan and haplotype WN1, WN2 isolates from China, forming a separate cluster (fig. 3A; Supplementary Table 2).

All mealybugs collected at the study sites were positive for the presence of PMWaV-1, -2, and -3. RT-PCR analysis using primers 225/226, 224/223, and 264/263 (Sether *et al.*, 2005, 2009) successfully amplified fragments of the expected sizes: 590 bp for PMWaV-1, 610 bp for PMWaV-2, and 490 bp for PMWaV-3 (fig. 2B–D) in mealybugs collected from the Hawaiiana cultivar and the MD-2 hybrid. Analysis of fig. 2B–D revealed the distribution of PMWaV-1, -2, and -3 in mealybugs collected from study locations. In the Hawaiiana cultivar, the San Ramón de Pangoa samples showed all three PMWaVs (sample 1), PMWaV-2 and PMWaV-3 (sample 2), and PMWaV-1 (sample 3). Alto Pichanaki samples had PMWaV-1 and PMWaV-3 (sample 4), all three PMWaVs (sample 5), and PMWaV-2 and PMWaV-3 (sample 6). Alto Celendín samples 7, 8, and 9 contained all three PMWaVs, whereas in the MD-2 hybrid, the Alto Celendín sample 10 showed PMWaV-2 and PMWaV-3, samples 11 and 12 contained all three PMWaVs, and the Mazamari sample 13 had PMWaV-1 and PMWaV-3. RT-PCR analysis revealed varying detection rates of PMWaV-1, -2, and -3 across the sampled locations. In Alto Celendin, PMWaV-3 exhibited the highest detection rate (63%), followed by PMWaV-1 (57%) and PMWaV-2 (47%). A similar trend was observed in Mazamari, with PMWaV-3 detected in 66% of samples, followed by PMWaV-1 (59%) and PMWaV-2 (38%). In Alto Pichanaki, PMWaV-3 (63%) was most prevalent, followed by PMWaV-2 (47%) and PMWaV-1 (37%). In San Ramon de Pangoa, PMWaV-2 had the highest detection rate (47%), followed by PMWaV-3 (44%) and PMWaV-1 (39%) (table 1).

For molecular identification, representative samples were selected from different locations and cultivars. Specific fragments of PMWaV-1, PMWaV-2, and PMWaV-3 amplified by RT-PCR from mealybugs collected on cultivar Hawaiiana: M1V1-SRP (PMWaV-1), M1V2-SRP (PMWaV-2), and M1V3-SRP (PMWaV-3) from San Ramón de Pangoa; hybrid MD-2: M10V2-AC (PMWaV-2) from Alto Celendín and M13V1-M (PMWaV-1) and M13V3-M (PMWaV-3) from Mazamari were sequenced. All sequenced samples were positive for PMWaV-1, -2, and -3. The samples M1V1-SRP and M13V1-M showed 94.08% identity with respect to the isolates OM388424 (PMWaV-1); M1V2-SRP and M10V2-AC showed 98.62% identity with OM388425 (PMWaV-2); and M1V3-SRP and M13V3-M showed 99.20% identity with OM388426 (PMWaV-3) (Supplementary Table 2). Phylogenetic analysis (fig. 3B) demonstrated that the isolates studied show genetic diversity associated with their geographic origin. Isolates M13V1-M and M1V1-SRP (PMWaV-1) were genetically closer to isolates from Cuba and Peru. Isolates M10V2-AC and M1V2-SRP showed a closer relationship with isolates from Cuba, while isolates M13V3-M and M1V3-SRP clustered with isolates from Cuba and Ghana.

Discussion

Mealybugs collected from the Hawaiiana cultivar and MD-2 hybrid production fields in the provinces of Satipo and Chanchamayo (Junin department, Peru) displayed morphological characteristics consistent with previous descriptions (Beardsley, 1992). However, differentiating between *D. brevipes* and *D. neobrevipes* based on morphology proved challenging due to their similar characteristics (Wei *et al.*, 2020). To distinguish closely related species within the Pseudococcidae family, several studies have employed the DNA barcoding molecular approach (Correa *et al.*, 2012; da Silva Vc *et al.*, 2014; Hebert *et al.*, 2003; Malausa *et al.*, 2011). Common DNA markers include the ribosomal 18S, 28S, EF1-α (elongation factor 1 alpha), *COI* genes (Hebert *et al.*, 2003; Rung *et al.*, 2008), and ITS1 and ITS2 regions (Malausa *et al.*, 2011). We used the *COI* gene for the molecular characterisation of mealybugs. However, the primers LCO-1490 and HCO-2198 exhibited a Tm difference

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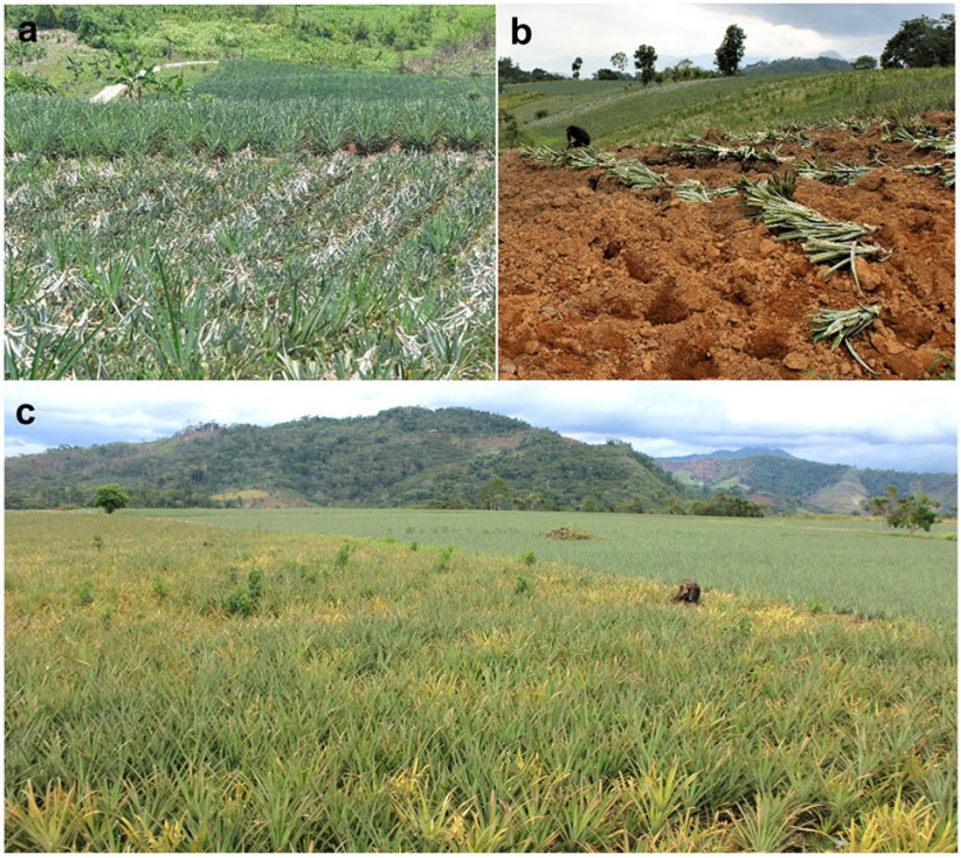

Figure 4. Vegetative propagation of the pineapple crop cultivar Hawaiiana in the province of Satipo. (a) Sun-drying of the suckers to heal the wound generated when removing the shoot; (b) planting of the suckers in new fields; (c) crop fields of healthy cultivar Hawaiiana with symptoms of yellowing, reddening, apical necrosis, and dwarfism in the locality of Alto Pichanaki, province of Chanchamayo.

exceeding 5°C, which is not recommended. This discrepancy made it difficult to obtain fragments of the expected size, resulting in low-quality sequencing for bioinformatic analyses. Amplification using the ITS region primers ITS2-M-F/ITS2-M-R (Malausa *et al.*, 2011) was subsequently used, which identified the mealybugs as *D. brevipes*. This finding is further supported by the phylogenetic analysis (fig. 3A). Phylogenetic analyses revealed that isolates 12ITS-MD2C, 10ITS-MD2C, 6ITS-HAP, 1ITS-HSMP, and 3ITS-HSMP clustered closely with isolates from China belonging to haplotype QH. Isolate 8ITS-HC was most closely associated with a Chinese isolate of haplotype QJ. On the other hand, isolate 13ITS-MD2M clustered with a Chinese isolate of haplotype WN1. Taken together, these results suggest a diversity of mealybugs in the study areas.

Although pineapple cultivation in Peru's central jungle faces a variety of pests, mealybugs are the most problematic (Arellano *et al.*, 2015; Chellappan *et al.*, 2022). Our results provide evidence that mealybugs can harbour multiple strains of PMWaV, suggesting their potential role as vectors of the MWP complex (Gambley *et al.*, 2008; Sether and Hu, 2002). Our results also agree with those reported recently in which PMWaV-1, -2, and -3 and mixed infections were detected in both plant samples and mealybugs collected in the Department of Valle del Cauca, Colombia (Moreno *et al.*, 2023).

While some samples lacked detectable PMWaV-1, -2, and -3, the mealybug wilt disease (MWP) complex in pineapple can involve other PMWaVs. Putative PMWaV-4, PMWaV-5 (Green *et al.*, 2020), and PMWaV-6 (Larrea-Sarmiento *et al.*, 2021) cause similar symptoms to PMWaV-1, -2, and -3. Additionally, Pineapple bacilliform CO virus-HI1 (PBCOV-HI1) and its nine genomic variants (A through H) (genus *Badnavirus*, family Caulimoviridae) have been reported, transmitted by *D. brevipes* (Sether *et al.*, 2012). Considering the symptoms observed in the fields (figs. 1A and 4C) and the presence of PMWaV-1, -2, and -3 in the study localities, we hypothesise that pineapple production in these areas could also be affected by other variants of the PMWaV complex (PMWaV-4, -5, and -6) or Badnaviruses. Therefore, we recommend further studies to identify these potential pathogens.

What is obvious from our study is that *D. brevipes* is a threat to pineapple production in Satipo and Chanchamayo provinces. Under the current climate change scenario, rising temperatures associated with climate change may influence *D. brevipes* by (1) shortening life cycle: warmer temperatures can potentially accelerate development, leading to more generations per year; (2) increasing population growth rates: warmer temperatures might favour faster reproduction and population growth; and (3) enhancing dispersal: climate change could influence wind patterns and weather events, potentially impacting the spread of *D. brevipes* (Chellappan *et al.*, 2022; Wei *et al.*, 2020).

The geographical variations, thermal characteristics, and ecological conditions across locations like Alto Celendin, Mazamari, San Ramon de Pangoa, and Alto Pichanaki (Satipo and Chanchamayo) could further influence *D. brevipes* life cycle

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(Honêk, 2013). For instance, a study of table grapes (Bertin *et al.*, 2019) showed *D. brevipes* thriving between 8°C and 35°C, with an optimum at 30°C. This could accelerate their life cycle and potentially increase generations from three to five per year, potentially explaining the high mealybug incidence observed in the sampled fields. Furthermore, *D. brevipes* can survive on alternative hosts like citrus, banana, cacao, coffee, ginger, and mango (Ben-Dov, 1994) when pineapple is not available. These alternative hosts are widely cultivated in Satipo and Chanchamayo, providing a refuge for *D. brevipes* populations between pineapple growing seasons.

Our observations at the sampled locations indicate another potential factor contributing to the spread of PMWaVs. Farmers commonly use suckers obtained from mother plants in other production fields, often after a 1-month solarisation process (fig. 4A, B). This practice can inadvertently spread PMWaVs because these viruses are systemic within infected plants (Sether *et al.*, 1998). Suckers, stems, leaves, fruits, and roots can all serve as sources of inoculum for neighbouring fields, potentially explaining the observed symptoms (fig. 4C). To mitigate this problem and ensure the sustainability of the Hawaiiana cultivar and MD-2 hybrid pineapple production in Junin, Peru, we recommend the following: (1) apply stricter quarantine measures to prevent and minimise the spread of *D. brevipes* to other pineapple growing regions (Paini *et al.*, 2016); (2) encourage farmers to obtain vegetative seeds (suckers) from certified virus-free production fields; and (3) promote the use of seedlings derived from *in vitro* meristem culture, a technique known to produce virus-free plants (Delgado-Haya, 2020). These combined approaches can significantly reduce the dispersal of PMWaVs and ensure the health of pineapple crops.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S000748532510014X.

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Competing interests. The authors declare no conflict of interest.

References

Alvarez RA, Martin RR and Quito-Avila DF (2015) First report of *Pineapple mealybug wilt associated virus-1* in Ecuador. *New Disease Reports* **31**, 2044–2588.

Arellano G, Vergara C and Bello S (2015) Plagas entomológicas y otros artrópodos en el cultivo de la piña (*Ananas comosus* var. comosus (L.) Merr., Coppens & Leal) en Chanchamayo y Satipo, Departamento de Junin, Perú. *Ecología Aplicada* **14**, 175–189.

Beardsley JW (1992) The pineapple mealybug complex; taxonomy, distribution, and host relationships. In I International Pineapple Symposium. **334**, 383–386.

Ben-Dov Y (1994) *A Systematic Catalogue of the Mealybugs of the World (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with Data on Geographical Distribution, Host Plants, Biology and Economic Importance*. Andover, UK: Intercept Limited, pp. 686.

Bertin A, Lerin S, Botton M and Parra JRP (2019) Temperature thresholds and thermal requirements for development and survival of *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) on table grapes. *Neotropical Entomology* **48**, 71–77.

Borroto-Fernández EG, Torres-Acosta JA and Laimer M (2007) RT-PCR detection and protein-protein interaction of viral components of Pineapple mealybug wilt-associated virus 2 in Cuba. *Journal of Plant Pathology* **89**, 435–439.

Carrasco-Lozano EC, Carrillo-Ordóñez GA, Gamarra-Gamarra D, Torres-Suarez G, Villar CM, Stange C and Valverde RA (2023) Pineapple mealybug wilt-associated viruses 1, 2, and 3 are associated with mealybug wilt disease of pineapple in Peru. *Journal of Plant Pathology* **105**, 581–586.

Chellappan M, Viswanathan A and Mohan LK (2022) Pests and their management in pineapple. In Mani M (ed.), *Trends in Horticultural Entomology*, 689–699.

CIRAD (2022) Análisis de mercado de las frutas tropicales en Europa: Mercado mundial de la Piña. Internet Resource: https://fruttmac.com/wp-content/uploads/2022/11/Mercado-mundial-de-la-pina.pdf (accessed 12 July 2023).

Correa MCG, Germain JF, Malausa T and Zaviezo T (2012) Molecular and morphological characterization of mealybugs (Hemiptera: Pseudococcidae) from *Chilean vineyards*. *Bulletin of Entomological Research* **102**, 524–530.

da Silva Vc P, Bertin A, Blin A, Germain JF, Bernardi D, Rignol G and Malausa T (2014) Molecular and morphological identification of mealybug species (Hemiptera: Pseudococcidae) *in Brazilian vineyards*. *PLoS One* **9**, e103267.

Delgado-Haya H (2020) Optimización de sistemas de inmersión temporal para la producción masiva de plántulas de piña (*Ananas comosus* L. Merr.) en la región San Martín.

Dey KK, Green JC, Melzer M, Borth W and Hu JS (2018) Mealybug wilt of pineapple and associated viruses. *Horticulturae* **4**, 52.

FAO (2023) Principales frutas tropicales. Análisis del mercado 2022. Internet Resource: https://www.fao.org/3/cc7108es/cc7108es.pdf (accessed 12 July 2023).

Gambley CF, Steele V, Geering ADW and Thomas JE (2008) The genetic diversity of ampeloviruses in Australian pineapples and their association with mealybug wilt disease. *Australasian Plant Pathology* **37**, 95–105.

Green JC, Rwahnih MA, Olmedo-Velarde A, Melzer MJ, Hamim I, Borth WB and Hu JS (2020) Further genomic characterization of pineapple mealybug wilt-associated viruses using high-throughput sequencing. *Tropical Plant Pathology* **45**, 64–72.

Hebert PD, Cywinska A, Ball SL and DeWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270, 313–321.

Hernandez-Rodriguez L, Ramos-Gonzalez PL, Garcia-Garcia G, Zamora V, Peralta-Martin AM, Peña I and Ferriol X (2014) Geographic distribution of mealybug wilt disease of pineapple and genetic diversity of viruses infecting pineapple in Cuba. *Crop Protection* **65**, 43–50.

Honêk A (2013) Geographical variation in thermal requirements for insect development. *European Journal of Entomology* **93**, 303–312.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S and Drummond A (2012) Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649.

Larrea-Sarmiento A, Olmedo-Velarde A, Wang X, Borth W, Matsumoto TK, Suzuki JY and Hu J (2021) A novel ampelovirus associated with mealybug wilt of pineapple (*Ananas comosus*). *Virus Genes* **57**, 464–468.

Malausa T, Fenis A, Warot S, Germain JF, Ris N, Prado E and Kreiter P (2011) DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). *Journal of Applied Entomology* **135**, 142–155.

Mani M and Shivaraju C (2016) Biology. In Mani M, and Shivaraju C (eds.) *Mealybugs and Their Management in Agricultural and Horticultural Crops*. Berlin, Germany: Springer **9**, 87–106.

Moreno I, Rodríguez-Arévalo KA, Tarazona-Velasquez R and Kondo T (2023) Occurrence and distribution of pineapple mealybug wilt-associated viruses (PMWaVs) in MD2 pineapple fields in the Valle del Cauca Department, Colombia. *Tropical Plant Pathology* **48**, 217–225.

Ochoa-Martínez DL, Uriza-Ávila DE, Rojas-Martínez RI and Rodríguez-Martínez D (2016) Detection of Pineapple mealybug wilt-associated virus 1 and 3 in Mexico. *Revista Mexicana de Fitopatología* **34**, 131–141.

Paini DR, Sheppard AW, Cook DC, De Barro PJ, Worner SP and Thomas MB (2016) Global threat to agriculture from invasive species. *Proceedings of the National Academy of Sciences* **113**, 7575–7579.

Peron FN, Calloni R, Ventura JA and Fernandes PMB (2017) Bioinformatics approach to the study of the molecular behaviour of mealybug wilt of pineapple. In *IX International Pineapple* Symposium **1239**, 177–184.

Qin Z, Wu J, Qiu BL, Ren S and Ali S (2011) Effects of host plant on the development, survivorship and reproduction of *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudoccocidae). *Crop Protection* **30**, 1124–1128.

Rung A, Scheffer SJ, Evans G and Douglass M (2008) Molecular identification of two closely related species of mealybugs of the genus *Planococcus* (Homoptera: Pseudococcidae). *Annals of the Entomological Society of America* **101**, 525–532.

Sether DM, Borth WB, Melzer MJ and Hu J (2010) Spatial and temporal incidences of pineapple mealybug wilt-associated viruses in pineapple planting blocks. *Plant Disease* **94**, 196–200.

Sether DM and Hu JS (2002) Yield impact and spread of Pineapple mealybug wilt associated virus-2 and mealybug wilt of pineapple in Hawaii. *Plant Disease* **86**, 867–874.

Sether DM, Melzer MJ, Borth WB and Hu JS (2009) Genome organization and phylogenetic relationship of Pineapple mealybug wilt associated virus-3 with family Closteroviridae members. *Virus Genes* **38**, 414–420.

Sether DM, Melzer MJ, Borth WB and Hu JS (2012) Pineapple bacilliform CO virus: Diversity, detection, distribution, and transmission. *Plant Disease* **96**, 1798–1804.

Sether DM, Melzer MJ, Busto J, Zee F and Hu JS (2005) Diversity and mealybug transmissibility of ampeloviruses in pineapple. *Plant Disease* **89**, 450–456.

Sether DM, Ullman DE and Hu JS (1998) Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* **88**, 1224–1230.

SIEA (2021) Anuarios de Producción Agrícola. Internet Resource: https://siea.midagri.gob.pe/portal/publicacion/boletines-anuales/4-agricola (accessed 12 July 2023).

Sipes BS, Sether DM and Hu JS (2002) Interactions between *Rotylenchus reniformis* and *Pineapple mealybug wilt associated virus*-1 in pineapple. *Plant Disease* **86**, 933–938.

Suma P, Mazzeo G, La Pergola A, Nucifora S and Russo A (2015) Establishment of the pineapple mealybug *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) in Italy. *EPPO Bulletin* **45**, 218–220.

Tamura K, Stecher G and Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* **38**, 3022–3027.

Thompson JD, Higgins DG and Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Wei J, Peng L, He Z, Lu Y and Wang F (2020) Potential distribution of two invasive pineapple pests under climate change. *Pest Management Science* **76**, 1652–1663.

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