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Vistosa flor encontrada en los jardines del Napo Cultural Center (Parque Nacional Yasuní, bosque lluvioso amazónico), administrado en su totalidad por la comunidad Kichwa Añangu.

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CONTENIDO

EDITORIAL

Estimados lectores,

La Revista Ecuatoriana de Medicina y Ciencias Biológicas (REMCB) tiene el agrado de compartir con ustedes una edición especial, los investigadores han aportado de manera considerable información reciente para las ciencias médicas y biológicas aumentando la calidad científica de esta prestigiosa revista.

A continuación, presentamos artículos enfocados en la evaluación de diversidad de comunidades fúngicas presentes en tejidos foliares de la uvilla (*Physalis peruviana)* utilizando métodos cultivo-dependientes, los cuales han permitido categorizar los aislados basándose en características morfológicas de cultivos obtenidos in vitro.

También ponemos a disposición un artículo enfocado en haplotipos mitocondriales de aislamientos puros de *Phytophthora andina,* de los cuales se realizaron amplificaciones de las regiones P2 y P4 del ADNmt, dando resultados de patrones de bandas nuevos.

Por último, presentamos análisis genéticos poblacionales de *Rhodnius ecuadoriensis*, el cual es el principal vector de la enfermedad de Chagas en Ecuador, se probaron seis Repeticiones Cortas en Tándem (RCT) de *R. pallescens* para obtener resultados de las frecuencias alélicas en función de incrementar la vigilancia entomológica continua en la costa del Ecuador.

Esperamos que los temas incluidos en este número sean de su interés y a la vez, estimulen a las próximas generaciones de jóvenes científicos para publicar sus investigaciones en medios de difusión como lo es la REMCB.

Dr. Oscar Pérez
Editor en Jefe

ARTÍCULOS CIENTÍFICOS

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Artículo científico

Diversity of fungal communities inhabiting symptomatic leaves of Cape gooseberry (*Physalis peruviana*) in the Ecuadorian Andes

Diversidad de comunidades fúngicas que habitan en tejido foliar sintomático de la uvilla *Physalis peruviana* en los Andes Ecuatorianos

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ABSTRACT.- We used microbial culture specific methods to assess the occurrence and diversity of fungal communities inhabiting symptomatic foliar tissue of Cape gooseberry (*Physalis peruviana*) at four different field plots in the northern Ecuadorian Andes. We categorized the isolated fungi based on their morphological characteristics and subsequently identified them through rDNA sequencing of the ITS region. We recovered 75 ascomycetous isolates which represented 13 genera, revealing a total of 20 taxa. We found most isolates recovered from necrotic and straw-colored tissues to belong to the genus *Alternaria.* Other fungal taxa frequently found were *Epicoccum, Diaporthe*, and *Xylaria.* To our knowledge, this is the first study to report the mycobiota associated to *P. peruviana* plants showing disease symptoms, including 2 new genera of fungi for the first time in Ecuador. Our results are expected to provide useful information for future assessments of biological control on tropical commercial.

KEYWORDS: Ascomycota, rDNA, ITS, plant disease, uvilla

RESUMEN.- Utilizamos métodos cultivo-dependientes para evaluar la ocurrencia y diversidad de comunidades fúngicas que habitan en tejido foliar sintomático de uvilla (*Physalis peruviana)* en cuatro localidades del norte de los Andes ecuatorianos. Categorizamos los aislados basándonos en las características morfológicas de los cultivos obtenidos *in vitro*, los cuales identificamos al secuenciar la región de ADNr ITS. En total, recuperamos un total de 75 hongos ascomycetos que comprendían 13 géneros y 20 taxa. Encontramos que la mayoría de hongos recuperados de tejidos necróticos o cloróticos pertenecían al género *Alternaria*. Otros taxa que encontramos con frecuencia fueron *Epicoccum, Diaporthe* y *Xylaria*. A nuestro conocimiento, este es el primer estudio en reportar la micobiota asociada a plantas de *P. peruviana* que presenten hojas con síntomas de enfermedad, incluyendo 2 géneros de hongos nuevos para el Ecuador. Esperamos que nuestros resultados provean información útil para futuros estudios sobre control biológico aplicado a cultivos comerciales en los trópicos.

PALABRAS CLAVE: Ascomycota, ADNr, ITS, patología vegetal, uvilla

INTRODUCTION

Cape gooseberry (*Physalis peruviana* L.) is a biannual shrub native from the Andean highlands of northern South America that can grow up to 1 m high (Legge 1974; Fischer 2000). In recent years, Cape gooseberries have gained increasing attention in the markets of South and Central America, due to the plant's high tolerance to a broad variety of environments (Ramadan and Moersel 2003; Puente

et al. 2011). Given the variety of antioxidant types and high sugar content (Novoa et al. 2006), this crop is of great economic importance for industrial purposes worldwide (Morton 1987; Rehm and Espig 1991; McCain 1993; Ramadan and Moersel 2003; Mazorra 2006), as it has also been widely used for its medicinal properties to treat cancer and diabetes (see review in Puente et al. 2011).

In Ecuador, *P. peruviana*, localy known as *uvilla,* is grown in the northern region between 2 000 – 3 000 m.a.s.l., where moderately cold temperatures prevail (Puente et al. 2011; Fischer et al. 2014). The production of *P. peruviana* has increased considerably in recent years across Ecuador due to its growing export demands to European countries (Muñoz 2003; Altamirano 2010; Fischer et al. 2014). However, a wide array of selective pressures including biotic factors (herbivory, parasites) and abiotic stress (low nutrient availability, drought) may result in major production losses by inducing yellowing or necrosis in photosynthetic tissues (Douanla-Meli et al. 2013). As a result, several toxic pesticides (e.g. herbicides and fungicides) have been used in this region as agricultural practices to prevent plant diseases (Rodríguez-Amézquita et al. 2010). Yet, even though the main biological constraint to Cape gooseberry are fungi of the genera *Cladosporium, Phoma, Alternaria, Botrytis* and *Colletotrichum* (Angulo 2005; Fischer and Miranda 2012; De La-Rotta 2014), little is known on the mycobiota inhabiting symptomatic leaves of *P. peruviana,* which could provide useful information and assist crop management.

Studies based solely on morphology have failed to be conclusive at taxonomic species level for fungi associated to *P. peruviana* (Crozier et al. 2006), while fungal isolates often fail to form fruiting structures in culture (Gazis and Chaverri 2010). Thus, considering that leaves, as ecological niches, might influence the diversity and composition of fungal symbionts (Kriel et al. 2000), the present study is aimed at understanding the community structure and diversity of fungi isolated from symptomatic leaves of Cape gooseberry in the Andean highlands of northern Ecuador.

MATERIALS AND METHODS

Sample collection.- We collected unhealthy leaf samples showing necrosis symptoms or straw-colored tissues in plantations of *Physalis peruviana* during June 2015 at four different sites of the Pichincha Province in the northern Ecuadorian Andes: La Merced (00º17.635´ S, 078º24.158´ W, 2 611 m.a.s.l.), Virgen de Lourdes (00º17.311´ S, 078º24.384´ W, 2 628 m.a.s.l.), Tumbaco (00º13.539´ S, 078º23.979 W, 2 450 m.a.s.l.), and Yaruquí (00º11.596´ S, 078º19.710´ W, 2 579 m.a.s.l.). We randomly selected 14 mature *P. peruviana* (2 to 7 plants per site > 0.5 m in height) and then transported 75 leaf samples (5 x 5 mm) to the laboratory in sterile plastic bags. We stored the leaves for 2 d at -10 °C before the isolation of fungi.

Isolation of fungi.- To isolate fungi, we followed a modified protocol from Crous et al. (2009). In order to induce sporulation, we surface-disinfected the samples through immersion in 70 % ethanol for 1 min and 3 % sodium hipochlorite for 5 min. We rinsed the samples with sterile distilled water three times before transferring them to Petri dishes (90 mm) that contained a sterile Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, MI) supplemented with streptomycin to suppress bacterial proliferation. We incubated the plates at 25 °C with a 12-h photoperiod and checked them regularly for fungal growth for 7 to 15 days. After fungal growth was visible, we subcultured different colonies on new Petri dishes with the same medium for further purification and identification (Crozier et al. 2006). We categorized and divided filamentous fungi isolates based on the morphology of the colonies.

DNA extraction.- We collected about 10 mg of fresh fungal tissue using a sterile scalpel and extracted genomic DNA using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) in accordance with the manufacturer's instructions. To each tissue sample, we added 600 µl of nuclear lisis solution and vortexed the resulting solution for 15 min at 65 °C, followed by 15 min of water immersion at 37 °C. Then, we added 3 µl of RNAase solution and incubated the resulting solution at room temperature for 5 min, before we mixed it with a protein precipitation solution. We vortexed the samples for 15 s and centrifuged the protein precipitation solution at 13 000–16 000 g for 3 min. We transferred the supernatant liquid into a 1,5 mL fresh tube mixed with 600 µl ethanol at room temperature and centrifuged the columns at 13 000–16 000 g for 1 min, before we discarded the flow-through. Finally, we washed the samples as *per* manufacturer's protocol and we quantified the purified DNA concentration with a Qubit® 2.0 Quantitation Starter Kit (Invitrogen, USA) following instructions provided by the manufacturer and stored the tubes at -20 ºC.

PCR amplification.- We used PCR to amplify primer pairs of the Internal Transcribed Spacers (ITS), ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′), which are rDNA genes commonly used for fungal barcoding (White et al. 1990). We performed PCR using GoTaq Green Master mix (Promega, Madison, WI) according to manufacturer's instructions. We carried out reactions in 25 µl volume samples as follows: first initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final elongation at 72 °C for 10 min in a mixture of 22 µl GoTaq® Green Master Mix, 10 µM of primers and 2 µl of template. We analyzed PCR products by electrophoresis on a 2,5 % (w/v) agarose gel containing Sybr Safe (Invitrogen, Mulgrave, Australia) and visualized them under UV light. We purified the samples using the Wizard SV Gel and PCR Clean-up System (Promega). Reaction mixtures were sequenced by a private biotechnology company (Macrogen, Seoul, South Korea).

Fungal identification.- To identify the fungal isolates based on ITS, we compared nucleotide sequences against GenBank's database using the basic local alignment search tool (BLASTn) with the National Center for Biotechnology Information (NCBI, Bethesda, MD) database. Whenever possible, we defined fungi species considered in this study using an ITS similarity threshold of 97 % to sequences deposited in GenBank (Nilsson et al. 2008). If the ITS sequence was not discriminant at a species level, we assigned the isolates to the closest taxonomic unit. We deposited all gene sequences retrieved in this work in the GenBank database under the accession numbers: MF434350.1–MF435178.1. Fungal classification follows MycoBank (http://www.mycobank.org) database.

Statistical analysis.- We measured fungal occurrence considering both, the colonization frequency (CF) and the relative frequency of isolation (RF). We calculated CF as the total number of segments colonized by a given fungus divided by the total number of segments in that sample, expressed as percentage. We determined RF as the total number of segments colonized by a given taxon divided by the total number of taxa. We calculated fungal species dominance, diversity, and richness using Simpson's, Shannon's and Margalef's indices, respectively. We included singlets (occurrence of ≤ 2 isolates) for all analyses as they are likely to be keystone species of symptomatic foliar tissues (Gazis and Chaverri 2010). We calculated all diversity indices with PAST, version 1.9 (Paleontological Statistics) software (Hammer et al. 2001), in accordance to the following formulas:

1. Simpson's diversity (1-D) = $1-\Sigma(pi)^2$
2. Shannon-Wiener index (H') = $-\Sigma p_i (\log_2 p_i)$
3. Margalef's (D^{mg}) = (S-1) / ln N

where, pi is the proportion of colonization frequency of a given taxon, S is the total number of taxa isolated, and N is the number of species recorded.

RESULTS

Identification by ITS rDNA sequencing showed that all fungal isolates recovered in this study were Ascomycota, placed in seven orders: Pleosporales, Xylariales, Capnodiales, Glomerellales, Dothideales, Diaporthales and Hypocreales. Most isolates belonged to the Pleosporales (40 %) and Xylariales (20 %) orders. BLAST analysis revealed 13 different genera associated to symptomatic tissues of *P. peruviana*, while we were not able to identify the genus of two isolates and limited their categorization to the order level (Table 1). From a total of 20 identified taxa, the most frequently recovered fungal genera were *Alternaria*, *Epicoccum* and *Diaporthe* (Table 2), while 20 % of the isolates occurred as doubletons (occurrence of 2 isolates) and 50 % occurred only once. Fungal communities showed a low overall isolation rate of 5 ± 7.50 %, while diversity indices displayed high values for species dominance (1-D = 0,85), diversity (H' = 3,33), and richness (D^{mg} = 4,40).

DISCUSSION

To date, almost all species in the plant kingdom are known to host bacteria or fungi within the intra and inter-cellular spaces of their vegetative tissues (Petrini 1996; Arnold and Lutzoni 2007; Mondal et al. 2007; Hyde and Soytong 2008, Ghimire et al. 2011, Rocha et al. 2011, Douanla-Meli et al. 2013). Despite the fact that there is no apparent harm caused by these microorganisms on the plant's health, many fungal species have been reported to enter the plant trough stomata or wounds (Andersen and Walker 1985; Schulz and Boyle 2006; Sieber 2007), while some species may act as latent pathogens during the host's senescence (Photita et al. 2004; Promputtha et al. 2007; Rodríguez and Redman 2008; Prihastuti et al. 2009).

Tabla 1. ITS-based identification of fungal isolates recovered from symptomatic leaves of P*hysalis peruviana* in the Ecuadorian Andes.

Proposed fungal taxon	[a] GenBank Accesion No.	QC (%)	Id (%)	Sequence length (bp)	[b] Top BLAST search results (GenBank accession No.)	Reference
Alternaria sp.	MF435050.1	100	100	568	KM215624.1	Raja et al. 2015
Bipolaris cynodontis	MF435062.1	100	100	541	KJ909767.1	Manamgoda et al. 2014
Cercospora dubia	MF435168.1	99	100	537	KX287277.1	Videira et al. 2016
Colletotrichum boninense	MF435150.1	100	98.99	592	JX258799.1	Weir et al. 2012
Colletotrichum gloeosporioides	MF435164.1	100	100	571	KM257026.1	Waculicz-Andrade et al. 2017
Diaporthe helianthi	MF435054.1	98	97.73	579	AJ312356.1	Rekab et al. 2004
Diaporthe sp.	MF435071.1	100	99.65	572	KC339218.1	Panno et al. 2013
Didymella glomerata	MF435166.1	100	99.81	538	AY183371.1	Catal 2002
Dothideales sp	MF435051.1	99	99.64	553	HQ607988.1	Rodrigues et al. 2011
Epicoccum nigrum	MF435053.1	100	100	544	KX869965.1	Kernaghan et al. 2017
Fusarium equiseti	MF435058.1	100	100	547	KR094440.1	Kaur et al. 2016
Fusarium venenatum	MF435064.1	100	100	523	KP295496.1	Stefańczyk et al. 2016
Nigrospora oryzae	MF435092.1	100	100	550	EU272503.1	Miles et al. 2012
Periconia byssoides	MF435088.1	97	99.82	577	MK370654.1	Herrmann et al. 2019
Phoma sp.	MF435059.1	100	99.26	538	JN207257.1	Loro et al. 2012
Pleosporales sp.	MF435097.1	100	99.62	533	HQ631051.1	Shrestha et al. 2011
Stagonosporopsis cucurbitacearum	MF435112.1	100	100	552	GU045304.1	Ling et al. 2010
Xylaria multiplex	MF435085.1	99	99.66	588	KP133436.1	Thomas et al. 2016
Xylaria sp.	MF435158.1	100	98.45	576	FJ799949.1	Van Bael et al. 2009
Xylaria venosula	MF435102.1	97	99.65	587	EF026149.1	Hsieh et al. 2010

[a] All sequences were deposited in the NCBI GenBank (Accession No. column). QC: query cover, Id: identity. [b]

Sequences available in GenBank used for comparisons with sequences obtained in this study via BLAST software.

Tabla 2. Total number of isolates, isolation rate (%) and relative frequency of ascomycetous fungi recovered from symptomatic foliar tissues of *Physalis peruviana* in the Ecuadorian Andes.

Taxa	No. Isolates	Isolation rate (%)	Relative frequency
Capnodiales			
Cercospora malayensis	1	0.775	0.0244
Diaporthales			
Diaporthe helianthi	3	2.326	0.0732
Diaporthe sp.	11	8.527	0.2683
Dothideales			
Dothideales sp.	1	0.775	0.0244
Glomerellales			
Colletotrichum boninense	1	0.775	0.0244
Colletotrichum gloeosporioides	1	0.775	0.0244
Hypocreales			
Fusarium equiseti	2	1.550	0.0488
Fusarium venenatum	1	0.775	0.0244
Pleosporales			
Alternaria sp.	22	17.054	0.5366
Bipolaris cynodontis	2	1.550	0.0488
Didymella glomerata	2	1.550	0.0488
Epicoccum nigrum	15	11.628	0.3659
Periconia byssoides	1	0.775	0.0244
Phoma sp.	2	1.550	0.0488
Pleosporales *sp.*	1	0.775	0.0244
Stagonosporopsis cucurbitacearum	1	0.775	0.0244
Xylariales			
Nigrospora oryzae	3	2.326	0.7320
Xylaria multiplex	3	2.326	0.0732
Xylaria sp.	1	0.775	0.0244
Xylaria venosula	1	0.775	0.0244

In the present study, we used ITS sequencing to identify fungal communities inhabiting symptomatic leaves of *P. peruviana*. Our results are consistent with previous studies that have also shown a dominance by Ascomycota members within fungal assemblages of tropical plants (Arnold and Lutzoni 2007; Gonzaga et al. 2015). Previous studies on fungal assemblages of commercial crops have reported *Alternaria, Colletotrichum, Epicoccum, Fusarium, Nigrospora, Phoma,* and *Xylaria* taxa inhabiting foliar tissues (Crous et al. 1995; Gazis and Chaverri 2010; Parsa et al. 2016). However, most studies have focused on isolating fungal endophytes inhabiting asymptomatic aerial organs, while very few of them have investigated the fungal species from symptomatic tissues (Maher et al. 2012; Bruez et al. 2014; Dávila et al. 2018). This work constitutes the first report of mycobiota on Cape gooseberry, from which *Alternaria, Bipolaris, Cercospora, Colletotrichum, Didymella, Epicoccum, Fusarium, Nigrospora, Phoma* and *Xylaria* taxa have been previously reported in Ecuador (Evans and Reeder 2000; Pacin et al. 2003; Ramírez et al. 2006; Thomas et al. 2008; Cornejo-Espinoza 2014; Moya-Maldonado 2016; Dávila et al. 2018), whereas *Periconia* and *Stagonosporopsis* represent new records for the mycoflora of continental Ecuador and are reported for the first time in *P. peruviana* within its center of origin.

The overall colonization of fungal species screened in the present study is low when compared to that reported in asymptomatic tissues of other plant species (Arnold and Lutzoni 2007; Sun et al. 2008). However, the diversity and richness of species is

similar to other studies of fungal communities inhabiting symptomatic tissues in tropical crops (Dávila et al. 2018). Furthermore, the most dominant genera isolated in the current study were *Alternaria* and *Epicoccum*, which coincides with other fungal communities from tropical angiosperms (Parsa et al. 2016). Within the fungal isolates obtained here, we detected common Cape gooseberry pathogens belonging to the genera *Alternaria, Fusarium, Colletotrichum, Phoma* and *Cercospora* (Rao and Subramonian 1976; Zapata et al. 2002; Angulo 2005; Fischer and Miranda 2012; De La-Rotta 2014) although only the *Alternaria* genus was isolated considerably in this study. As we did not conduct Koch's postulates for the isolates obtained in this study, our results do not imply that the isolated taxa are pathogenic.

The great diversity of fungal species reported in this study is expected to provide novel information for the crop management and biological control on the Ecuadorian Cape gooseberry, when dealing with necrosis symptoms in leaves. The current inventory of fungal species in *P. peruviana* suggests potential sources of culturable secondary metabolites isolated from decaying foliar tissues (Paparu et al. 2008), which may help to establish healthier agricultural practices, while it also contributes to the ecological understanding of specificity patterns shown by fungal communities. Further research of fungal interactions in Cape gooseberry is needed to comprehend the role that the most abundant species isolated in this study play within the fungal assemblages of symptomatic leaf tissues.

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Artículo científico

Rhodnius pallescens microsatellite markers for population genetic analysis in *Rhodnius ecuadoriensis*: preliminary assessment

Marcadores de microsatélite de *Rhodnius pallescens* para análisis genéticos poblacionales en *Rhodnius ecuadoriensis*: Evaluación preliminar

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RESUMEN.- *Rhodnius ecuadoriensis* Lent & León (Hemiptera: Reduviidae) es el prinicipal vector de la enfermedad de Chagas en Ecuador, donde la estructura genética de sus poblaciones es poco conocida. Nosotros probamos seis Repeticiones Cortas en Tamdem (RCT) de *R. pallescens* Barber en poblaciones selváticas y domésticas de R. ecuadoriensis. Dos microsatelites fueron monomórficos, dos dieron resultados ambiguos y dos fueron polimórficos (16 y 19 alelos) y fueron utilizados para análisis. Los resultados de las frecuencias alélicas, AMOVA y los pruebas Bayesianas para genética favorecen la teoría de la existencia de una sola población. Estos resultados preliminares sugieren que las poblaciones selváticas y domésticas de *R. ecuadoriensis* intercambian frecuentemente migrantes. Por consiguiente el control de la Enfermedad de Chagas requiere vigilancia entomológica continua en la costa del Ecuador.

PALABRAS CLAVES: Enfermedad de Chagas, Ecuador, microsatelites, *Rhodnius*

ABSTRACT.- *Rhodnius ecuadoriensis* Lent & León (Hemiptera: Reduviidae) is a major Chagas disease (CD) vector in Ecuador, but little is known about its population genetic structure. We tested six Short Tandem Repeats (STRs) from *R. pallescens* Barber in wild and synanthropic *R. ecuadoriensis* populations. Two STRs were monomorphic, two yielded ambiguous alleles, and two were polymorphic (16 and 9 alleles) and used for analysis. Allele frequencies, AMOVA, and a Bayesian population structure test all favored a single-population hypothesis. These preliminary results suggest that local wild andsynanthropic R. ecuadoriensis populations frequently exchange migrants; long-term CD control will therefore require continuous entomological surveillance in coastal Ecuador.

KEY WORDS: *Rhodnius*, Chagas disease, Ecuador, STRs.

INTRODUCTION

Chagas Disease (CD), caused by *Trypanosoma* cruzi and transmitted via the feces of infected triatomine bugs, is endemic in western Ecuador *Rhodnius ecuadoriensis* is the main vector in the coastal region (Grijalva et al., 2010, 2012, 2014), where it occupies both artificial and sylvatic ecotopes – mainly *Phytelephas aequatorialis* palm trees and squirrel nests (Abad-Franch et al. 2001, 2005; Grijalva et al. 2012, 2014; Suárez-Dávalos et al. 2010). Quantitative phenotypic analyses suggest that wild populations are involved in the re-infestation of insecticide-treated households (Villacís et al. 2010, Grijalva et al. 2014), which is common in central coastal Ecuador (Grijalva et al. 2011; 2014). Yet, the population dynamics of this vector species remain largely unknown; in particular, powerful molecular genetics tools have never been used for the study of inter-population relationships in *R. ecuadoriensis* as they have in other important CD vectors (e.g., Fitzpatrick et al. 2008). Here we address *R. ecuadoriensis* population connectivity in central coastal Ecuador by testing the utility of Short Tandem Repeat (STR) markers developed for the closely-related species, *R. pallescens* (Harry et al. 1998, Abad-Franch et al. 2009).

MATERIAL AND METHODS

Fieldwork was conducted in 2009-2010 in the province of Manabí, where CD is endemic and insecticide-based vector control strategies perform poorly (Grijalva et al. 2011). Triatomines were manually collected in domestic-peridomestic structures and nearby sylvatic habitats in eight localities (Fig. 1).

All collections were conducted following protocols approved by the Institutional Review Boards of Catholic University of Ecuador and Ohio University. Overall, 270 specimens were studied, 150 collected in sylvatic habitats and 120 in artificial structures. Sampling included 100 individual ecotopes (1 to 16 bugs/ecotope, median=2, interquartile range 2-3), including squirrel nests (*Sciurus stramineus*), hen nests, houses, bird nests (*Campylorrynchus fasciatus* and one *Synallaxis* sp.), mouse and rat nests, guinea pig enclosures, and timber piles. Specimens included all stages except first-instar nymphs, with adults representing about 67 % of the sample and evenly distributed in relation to sex. Hence, we are confident that our sample fairly represents local population diversity.

Figure 1. Map of Manabí province indicating the location of the sampled communities. Insert shows a map of Ecuador with the location of Manabí.

DNA was isolated and purified using DNeasy® extraction kits (Qiagen) following the manufacturer's protocol for animal tissue. Microsatellite amplification followed a protocol modified from Harry et al. (1998), using primers designed for six loci (L3, L9, L13, L25, L43, L47) of *R. pallescens*, a species phylogenetically close to *R. ecuadoriensis* (Abad-Franch et al. 2009). For PCR, we used the GoTaq® Colorless Master Mix kit (Promega), adding 6.4 µl of $MgCl_2$ 25mM, 0.4pM of each fluorochrome-labeled primer, and 10ng/µl of DNA for a final reaction volume of 25µl. PCR was carried out in a PTC-100® thermal cycler (MJ Research), with a denaturation step (95 ºC for 5 min) followed by 40 cycles of denaturation (95 ºC, 30 sec), annealing (50 ºC, 30 sec) and extension (72 ºC, 30 sec), and a final extension step (72 ºC, 5 min). For loci L13 and L43, a nested PCR was carried out using the product of the first PCR asthe template and the protocol above – except that no additional $MgCl_2$ was used.

We first screened the full microsatellite panel with 30 randomly selected bug samples to check for polymorphism over all loci. This scan revealed that two loci were monomorphic (L9 and L25) and two (L3 and L43) yielded ambiguous allele signatures that precluded confident scoring. These four loci were therefore disregarded in subsequent analyses, and only the two polymorphic markers yielding unambiguous alleles (L13 and L47) were used to analyze the full sample of triatomines. Genotyping was performed by capillary electrophoresis in an ABI 313xl with a standard G500 LIZ ladder (Applied Biosystems); .fsa files from capillary runs were then analyzed with Peak Scanner™ 1.0 for manual allele scoring. A total of 93 samples yielded no PCR product; results below are therefore based on 177 bugs, 82 from sylvatic and 95 from artificial ecotopes. Different analyses were conducted in Excel® spreadsheets, Arlequin 3.1 (Excoffier et al. 2005), and STRUCTURE 2.2 (Pritchard et al. 2000).

RESULTS

Overall, 16 alleles were identified for L13 and nine for L43; in both cases, allele frequencies were similar in wild and synanthropic populations, with only one L47 allele found at higher frequency among wild bugs (Fig. 2 and Table).

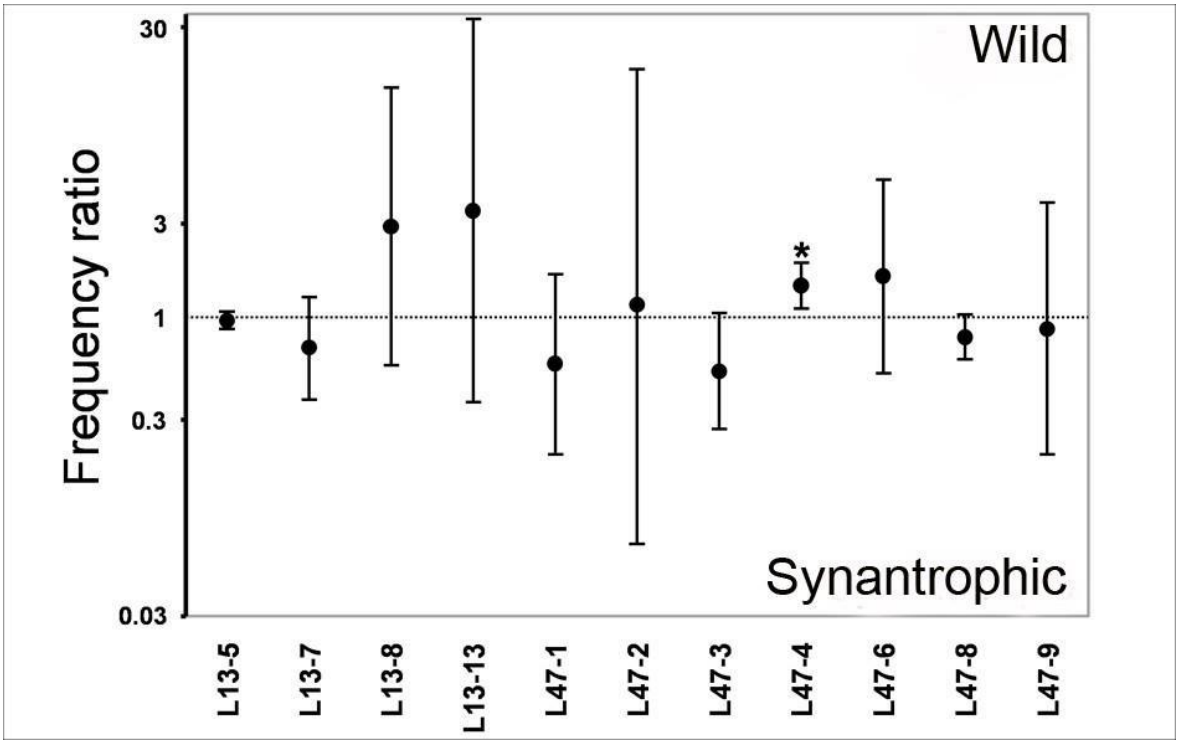

Figure 2. Allele frequency *(f)* ratios, estimated as *f*(wild)/f(synanthropic), for 11 shared alleles on two microsatellite loci (L13 and L47) in *Rhodnius ecuadoriensis* populations. Point estimates (solid circles) and 95% confidence intervals (CIs, between short horizontal lines) are shown, with allele codes given on the x-axis. The asterisk indicates the only allele (L47-4) for which we found evidence of a higher frequency in one of the populations (wild); for the rest, the fact that CIs include 1 (horizontal dotted line) indicates that allele frequencies are not significantly different at the 5% level.

Twelve rare L13 alleles, each with an overall frequency < 0.012, were only found in either wild (six alleles) or synanthropic bugs (six alleles); this was the case for just two L47 alleles, found only in wild specimens (Table). A total number of 70 synanthropic (73, 68 %) and 48 wild (58, 54 %) individuals were homozygotic for the L13 locus, with an overall observed heterozygosity HO = 33,33 %. Homozygote figures for L47 were 71, 58 % (68/95) for synanthropic and 70,73 % (58/82) for wild populations; overall observed heterozygosity was therefore HO = 28,81 %. L47 allele frequencies departed significantly from expectations under Hardy-Weinberg equilibrium among both synanthropic (expected heterozygosity HE = 72,41 %, vs. HO = 28,42 %, p < 0,0001) and wild bugs (HE = 65,89 %, vs. HO = 29,27 %, p < 0,0001), with exact p-values (Guo and Thompson 1992) estimated via a Markov chain of 100 000 steps (1 000 discarded as burn-in) implemented in Arlequin 3.1. This heterozygote deficit is likely due to allelic dropout caused by poor primer specificity, although selection acting on genes located in the same chromosome region as L47 or random drift might also contribute to this finding; our preliminary dataset does not allow for a detailed appraisal of these alternatives.

Gene diversity was slightly higher in the wild (0,799 ± 0,023) than in the synanthropic population (0,783 ± 0,021). An analysis of molecular variance (AMOVA) suggested that an overwhelming proportion of genetic variation (98,7 %) lies among individuals within populations. Both AMOVA and an exact test of population differentiation suggest that allele frequencies in both populations are

Table 1. Allele frequencies of two microsatellite loci (L13 and L47) in wild and synanthropic populations of *Rhodnius ecuadoriensis* from coastal Ecuador

Locus-allele	Wild (*N*=82)		Synanthropic (*N*=95)		Overall
	Count	Frequency	Count	Frequency	
L13-1	1	0.0061	0	0	0.0028
L13-2	2	0.0122	0	0	0.0056
L13-3	0	0	2	0.0105	0.0056
L13-4	0	0	1	0.0053	0.0028
L13-5	128	0.7805	154	0.8105	0.7966
L13-6	0	0	2	0.0105	0.0056
L13-7	15	0.0915	25	0.1316	0.1130
L13-8	5	0.0305	2	0.0105	0.0198
L13-9	0	0	1	0.0053	0.0028
L13-10	2	0.0122	0	0	0.0056
L13-11	4	0.0244	0	0	0.0113
L13-12	0	0	1	0.0053	0.0028
L13-13	3	0.0183	1	0.0053	0.0113
L13-14	1	0.0061	0	0	0.0028
L13-15	3	0.0183	0	0	0.0085
L13-16	0	0	1	0.0053	0.0028
L47-1	5	0.0305	10	0.0526	0.0424
L47-2	1	0.0061	1	0.0053	0.0056
L47-3	11	0.0671	24	0.1263	0.0989
L47-4	75	0.4573	60	0.3158	0.3814
L47-5	2	0.0122	0	0	0.0056
L47-6	7	0.0427	5	0.0263	0.0339
L47-7	1	0.0061	0	0	0.0028
L47-8	59	0.3598	86	0.4526	0.4096
L47-9	3	0.0183	4	0.0211	0.0198

comparable (F_{ST} = 0,013, exact p = 0, 09); an F_{ST}-based estimate of between-population migration suggests that about 20 reproductive individuals are exchanged per generation.

Taken together, these results all suggest that both wild and synanthropic populations belong to a single meta-population occurring across the study region. To further test this possibility, we conducted a Bayesian test of population subdivision using STRUCTURE 2.2. The analysis estimates and compares the likelihood of the data over a set of possible numbers of subdivisions (K); we run 20 iterations for each K value between 1 and 9, without a *priori* assignment of individuals to putative clusters. ΔK was then calculated following Evanno et al. (2005): the most likely number of subdivisions corresponds to the K value that maximizes ΔK. In our case, as shown in Fig. 3, there was strong support for the existence of a single genetic cluster (K=1).

DISCUSSION

We have presented the first population-level analysis of microsatellite loci in *R. ecuadoriensis*, a locally important CD vector that frequently re-invades and re-colonizes insecticide-treated households. The present study is a first step towards more detailed appraisals, which should include further polymorphic loci and the design of species-specific primer pairs (e.g., Fitzpatrick et al. 2008). The development of specific markers for R. ecuadoriensis is needed to improve our understanding of the vector's population dynamics, especially as wild populations seem to play a key role in CD transmission (Grijalva et al. 2014).

Even if still preliminary, the results of our analyses are suggestive of a single meta-population scenario in which *R. ecuadoriensis* migrants frequently move between sylvatic and artificial ecotopes – at a frequency high enough to homogenize both gene

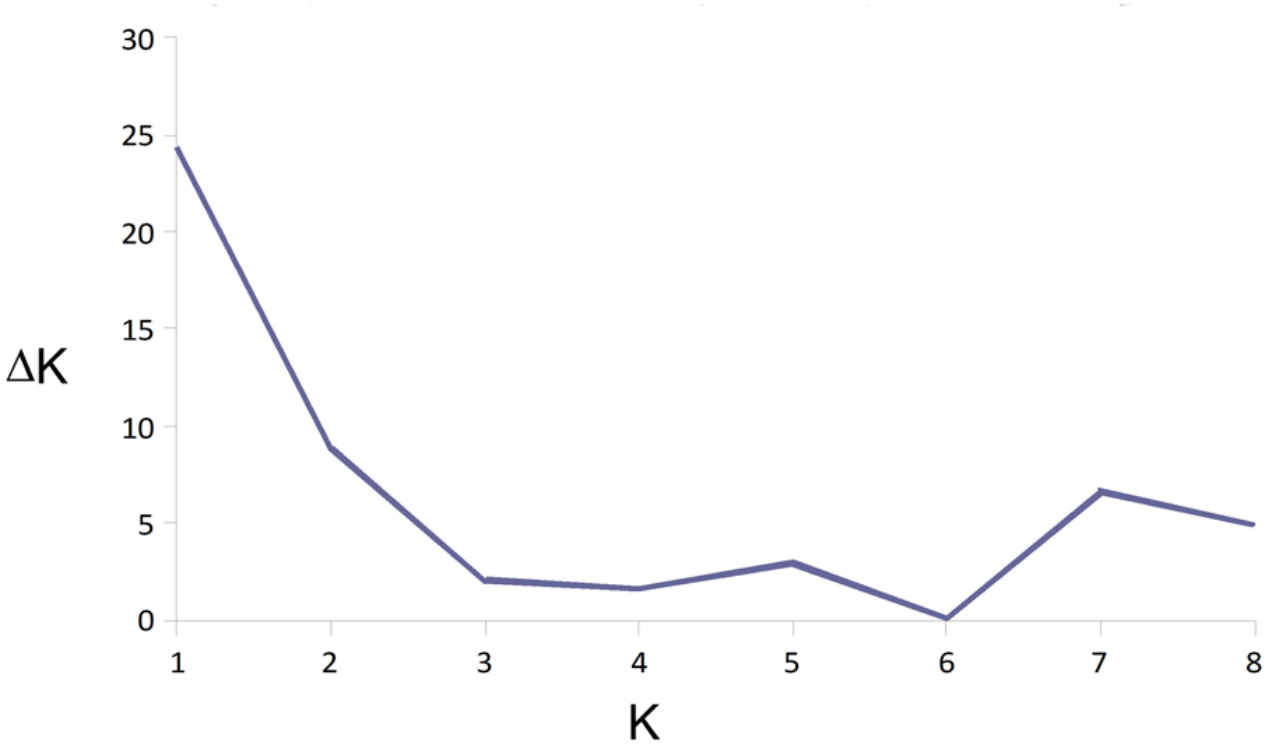

Figure 3. Estimation of the number of genetic subdivisions in *Rhodnius ecuadoriensis* populations from central western Ecuador. *K*, putative number of subdivisions; Δ*K*, Bayesian statistic derived from the rate of change in the log-probability of the data, given each *K* value. The peak value of Δ*K* at *K*=1 suggests that all specimens probably belong to a single meta-population.

pools. This agrees with ecological and quantitative phenotypic assessments showing both frequent re-infestation of treated households (Grijalva et al., 2011, 2014) and a lack of morphological or morphometric differentiation of wild and synanthropic specimens (Villacís et al. 2010).

In line with previous findings (Grijalva et al. 2011), our data thus suggest that longitudinal surveillance will be a key requirement of long-term CD control in central coastal Ecuador. Entomological surveillance usually performs better when the community takes on an active role in reporting infestation (Abad-Franch et al. 2011); however, a timely, professional response of vector control services is also needed, and this should include not only insecticide application but also environmental management of peridomestic ecotopes prone to harbor R. ecuadoriensis breeding colonies – particularlypalm trees and chicken coops in our study setting (Abad-Franch et al. 2005, Grijalva et al. 2011).

CONCLUSIONS

- Only two of six microsatellites developed for *R. pallescens* showed polymorphic loci in *R. ecuadoriensis* populations.

- Results of our analyses are suggesting only one genetic cluster is present in populations of *R. ecuadoriensis* in the province of Manabi, where migrants appear to move between sylvatic and artificial ecotopes.

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Artículo científico

Haplotipos mitocondriales de *Phytophthora andina* de tomate de árbol en el Ecuador

Mitochondrial DNA haplotypes of *Phytophthora andina* from tree tomato in Ecuador

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RESUMEN.- Se determinó el haplotipo mitocondrial de 131 aislamientos puros de *Phytophthora andina* colectados de tomate de árbol (*Solanum betaceum*) en varias provincias del Ecuador. Se amplificaron las regiones P2 y P4 del ADNmt, y los productos de PCR obtenidos fueron digeridos con las enzimas de restricción MspI y EcoRI. Todos los aislamientos analizados mostraron un patrón de bandas correspondiente al haplotipo Ia para *P. infestans*, es decir, bandas de tamaño 720 y 350 pb con MspI (región P2) y bandas de tamaño 394, 361 y 309 pb con EcoRI (región P4). Sin embargo, también se observó de forma consistente en todos los aislamientos analizados bandas adicionales que no han sido reportadas anteriormente. Para la región P2 se obtuvieron bandas adicionales de 400 y 500 pb y un fragmento no digerido de 1070 pb, y para la región P4 bandas adicionales de 603 y 755 pb además de un fragmento no digerido de 964 pb. El encontrar un patrón nuevo de bandas de manera consistente para todos los aislamientos de *P. andina* analizados sugiere que la descripción del haplotipo Ia asignado a *P. andina* de tomate de árbol debería ser redefinido.

PALABRAS CLAVE: ADN mitocondrial, enzimas de restricción, *Solanum betaceum*, tizón.

ABSTRACT.- The mitochondrial DNA haplotype of 131 pure cultures of *Phytophthora andina* collected from tree tomato (*Solanum betaceum*) in several provinces in Ecuador was determined. The P2 and P4 regions of the mtDNA were amplified, and the PCR products obtained were digested with MspI and EcoRI restriction enzymes. All isolates analyzed showed a band pattern corresponding to the Ia haplotype for *P. infestans*, that is, bands of sizes 720 and 350 bp with MspI (P2 region) and bands of sizes 394, 361 and 309 bp with EcoRI (P4 region). However, additional bands that have not been reported previously were also consistently observed in all isolates analyzed. For the P2 region, additional bands of 400 and 500 bp and an undigested fragment of 1070 bp were obtained, and for the P4 region, additional bands of 603 and 755 bp in addition to an undigested fragment of 964 bp. Finding new band patterns in a consistent manner for all analyzed isolates of *P. andina* suggests that the description of haplotype Ia assigned to *P. andina* from tree tomato should be redefined.

KEYWORDS: Blight, mitochondrial DNA, *Solanum betaceum*, restriction enzymes.

INTRODUCCIÓN

El tomate de árbol (*Solanum betaceum*) es un cultivo nativo del Sudamérica, bastante extendido en todas las provincias de la Sierra y Amazonía del Ecuador, de alta rentabilidad y con propiedades organolépticas deseables(Bohs 2007; Morales 2002; ESPAC 2016). Este cultivo es de gran importancia económica para pequeños y medianos agricultores del Ecuador, con una producción en el 2016 de 28 500 t (ESPAC 2016). Tradicionalmente, la mayor producción se ha concentrado en las provincias de Tungurahua e Imbabura.

Una de las principales enfermedades del cultivo es el tizón, presente en todas las zonas tomateras del Ecuador (Revelo et al. 2004a, Revelo et al. 2004b). El agente causal es *Phytophthora andina,* que ha sido reportada únicamente en los Andes y fue descrita en base a colecciones y estudios realizados en el Ecuador (Ordoñez et al. 2000; Adler et al. 2004; Chacón Acosta 2007; Oliva et al. 2010). También puede atacar al pepino dulce *(S. muricatum)* y la naranjilla (*S. quitoense*), así como a especies silvestres de la sección *Anarrhichomenum* de *Solanum* (Oliva et al. 2010; Forbes et al. 2013, 2016). *Phytophthora andina* ataca los tejidos del ápice, hojas y tallos de las plantas jóvenes, y follaje y ramillas en las plantas adultas (Revelo et al. 2004b), ocasiona defoliación severa y afecta seriamente la producción si no se toman medidas de control (Revelo et al. 2004a). En un principio, el patógeno fue identificado como *P. infestans* (el tizón tardío de la papa), pero análisis genéticos determinaron que era una especie nueva resultado de la posible hibridación entre *P. infestans* y otra especie aún no identificada de *Phytophthora* (Kroon et al. 2004; Gómez-Alpizar et al. 2008; Oliva et al. 2010; Goss et al. 2011; Martin et al. 2014). Recientemente, el agente causal de tizón en tomate de árbol en Colombia ha sido descritocomo *P. betacei* (Mideros et al. 2018), mientras que en Perú se ha determinado que es *P. andina*. (Forbes et al. 2016).

Se han desarrollado varios marcadores fenotípicos y genéticos para caracterizar e identificar las diferentes poblaciones de *P. infestans*, como son polimorfismos de longitud de fragmentos de restricción (RFLP) del genoma nuclear, tipos de apareamiento, haplotipos mitocondriales (ADNmt), patrones enzimáticos y más recientemente microsatélites (Waterhouse 1963; Galindo y Hohl 1985; Griffith y Shaw 1998; Ordoñez et al. 2000; Flier et al. 2002; Adler et al. 2004; Gómez-Alpizar et al. 2008; Oliva et al. 2010; Goss et al. 2011; Blair et al. 2012; Forbes et al. 2012, 2016; Mideros et al. 2018; Martin et al. 2019). En base a estos mismos marcadores se han caracterizado a las poblaciones de *Phytophthora* en tomate de árbol. Así, se ha reportado en Colombia para *P. betacei* el linaje EC-3 en base a RFLPs, tipo de apareamiento A1 y haplotipo mitocondrial Ia (Mideros et al. 2018), para *P. andina* en Perú el linaje PE-8, tipo de apareamiento A2 y haplotipo mitocondrial Ic, (Forbes et al. 2016) y para *P. andina* en Ecuador el linaje EC-3, tipo de apareamiento A1 y haplotipo mitocondrial Ia (Oliva et al. 2010).

El empleo de marcadores moleculares de fácil uso, bajo costo y eficaces en distinguir las poblaciones del patógeno son indispensables para el estudio y manejo de la enfermedad. La población ecuatoriana de *P. andina* de tomate de árbol fue descrita por última vez en el 2010 (Oliva et al., 2010), y desde entonces no se ha realizado un análisis exhaustivo de la población. En esta investigación se reporta un posible nuevo haplotipo mitocondrial para *P. andina* en base a muestras colectadas en tomate de árbol en el 2018 en el Ecuador, obtenido tras la amplificación de las regiones P2 y P4 de ADN mitocondrial, y la respectiva digestión con las enzimas de restricción MspI y EcoR.

MATERIALES Y MÉTODOS

Cultivos puros de 131 aislamientos de *P. andina* obtenidos de hojas infectadas de *S. betaceum* de 21 localidades en el Ecuador fueron multiplicados en cajas petri con agar centeno A (Caten y Jinks, 1968). El micelio fue recogido de la superficie de la caja con un asa bacteriológica previamente esterilizada y, se colocó en microtubos de 1.5 mL. El ADN genómico total fue extraído usando el Wizard® Genomic DNA Purification Kit (Promega, Cat. # A1125), segúnlasespecificaciones delfabricante. La concentración de ADN (ng/µL) de cada muestra fue cuantificada usando el espectrofotómetro Nanodrop 1000 (Thermo Fisher Scientific) y el programa ND-1000 V3.7.1 (Thermo Fisher Scientific).

Dos regiones específicas del ADN mitocondrial denominadas P2 (1070 pb) y P4 (964 pb) (Griffith y Shaw, 1998) fueron amplificadas por separado, utilizando 15 ng de ADN y las siguientes condiciones (concentraciones finales): 1X PCR Buffer, MgCl2 3 mM, dNTPs 0.2 mM, 1 U de Platinum® Taq DNA Polymerase (Thermo Fisher Scientific, Cat. # 10966-030 y # R0181) y 0.325 µM por cada cebador en volúmenes de 20

Tabla 1.-Aislamineto ecuatoriano de *Phytophtora andina* de tomate de arbol

PROVINCIA	LOCALIDAD	NÚMERO DE AISLAMIENTOS
Azuay	Bulán, Don Julo, Tomebamba	25
Imbabura	San Isidro, Zuleta	10
Napo	Baeza, Conduija, Linares	15
Pichincha	Canchacoto, Guayllabamba, Illolán1, Illolán2, Nanegalito, Tandapi	42
Tungurahua	Izamba, Píllaro, Runtún1, Runtún2, Runtún4, Runtún5, San Francisco	39

µL. Los cebadores empleados fueron, en sentido 5'-3': P2, F2: TTCCCTTTGTCCTCTACCGAT, R2: TTACGGCGGTTTAGCACATACA; P4, F4: TGGTCATCCAGAGGTTTATGTT, R4: CCGATACCGATACCAGCACCAA) (Griffith y Shaw 1998). Las reacciones de PCR fueron llevadas a cabo en el termociclador SureCycler 8800 (Agilent Technologies), con los siguientes parámetros: 94 ºC por 3 minutos, seguido de 27 ciclos de 92 ºC por 30 segundos, 60 ºC por 50 segundos, 72 ºC por 1 minuto y 10 segundos y, finalmente, 72 ºC por 5 minutos. Se confirmó la amplificación de las regiones P2 y P4 mediante electroforesis de los productos en un gel de agarosa al 1.5 % preparado en TBE 0.5X y teñido con Syber® Safe DNA Gel Stain (Thermo Fisher Scientific, Cat. # S33102). Luego, los productos de PCR (16 µL) de la región P2 fueron digeridos con 2 U de enzima de restricción Anza™ 65_Msp1 (Thermo Fisher Scientific, Cat. # IVGN0656), mientras que los productos de la región P4 (16 µL) fueron digeridos con 2 U de enzima de restricción Anza™ 11_EcoR1 (Thermo Fisher Scientific, Cat. # IVGN0116) según las especificaciones del fabricante, en un termociclador MultiGene ™ OptiMax (Labnet International) a 37 ºC por 2 horas. Los productos de digestión fueron visualizados en un gel de agarosa al 2 % preparado en TBE 0.5X y teñido con Syber® Safe DNA Gel Stain, según las especificaciones del fabricante. La electroforesis de los productos de digestión sellevó a cabo en una cámara horizontal (Enduro™ 96, Labnet International) a 100 V, 50 mA por 1 hora 30 min en buffer TBE 0.5X. Se documentó el tamaño de banda de los productos de digestión con el equipo Molecular Imager® Gel Doc™ XR System (Bio-Rad). Los tamaños de banda se calcularon según el marcador TrackIt ™ 100 bp DNA ladder (Thermo Fisher Scientific, Cat. # 10488058) y el programa Image Lab 6.0.1 (Bio-Rad). Los resultados fueron comparados con lo reportado para los haplotipos mitocondriales Ia, Ib, IIa, IIb (Carter et al. 1990; Griffith y Shaw 1998) y Ic (Ordoñez 2000; Flier et al. 2002; Oliva et al. 2002).

RESULTADOS Y DISCUSIÓN

Los 131 aislamientos de *P. andina* analizados presentaron fragmentos de digestión cuyo tamaño concuerda con lo reportado para el haplotipo mitocondrial Ia de *P. infestans* en las regiones P2 y P4 del ADNmt (Griffith y Shaw 1998): 720 y 350 pb para P2, y 394, 361 y 209 pb para P4 (Figura 1). Sin embargo, los productos de PCR de la región P2 digeridos con la enzima MspI, presentaron de forma consistente fragmentos adicionales de 400 pb y 500 pb y un fragmento no digerido de 1070 pb (Figura 1A). Así mismo, los productos de PCR de la región P4 digeridos con la enzima EcoRI presentaron fragmentos adicionales de 603 pb y 755 pb, así como un fragmento no digerido de 964 pb (Figura 1B).

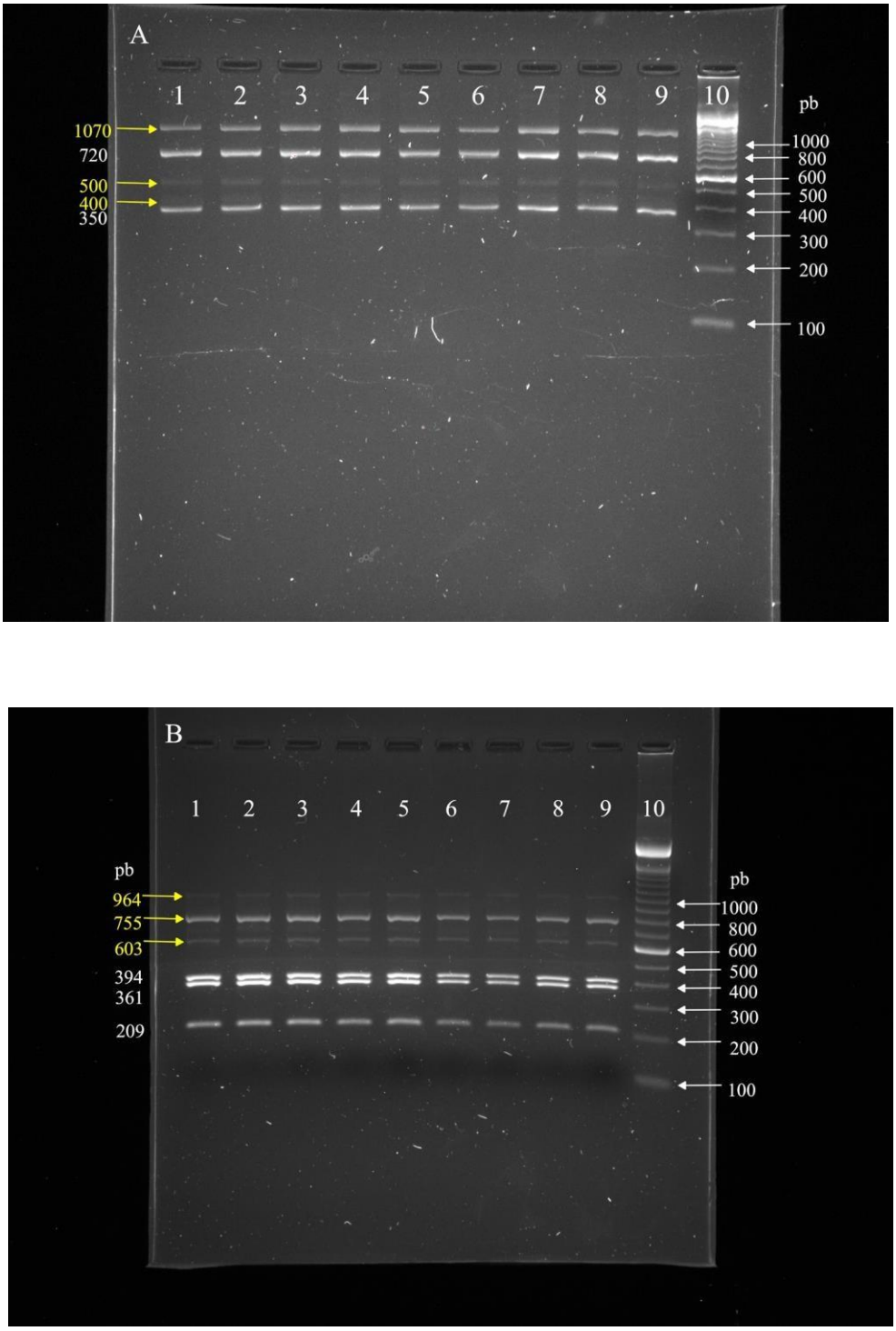

Figura 1.. Haplotipos mitocondriales de las regiones P2 y P4 de *P. andina* de tomate de árbol. 1A. Fragmentos de digestión de la región P2 con MspI. 1B. Fragmentos de digestión de la región P4 con EcoRI. El orden de las muestras es la misma para 1A y 1B, para cada columna se indica la provincia y localidad. Columna 1, 2 y 3: Napo, Linares; Columna 4 y 5: Tungurahua, San Francisco; Columna 6 y 7: Pichincha, Nanegalito; Columna 8: Azuay, Tomebamba; Columna 9: Azuay, Don Julo; Columna 10: TrackIt ™ 100 bp DNA ladder. Bandas adicionales en amarillo.

La determinación de los haplotipos mitocondriales en *P. infestans* y *P. andina* tiene su base en la metodología de Griffith y Shaw (1998), en el que los cebadores utilizados fueron desarrollados para la caracterización de *P. infestans*. La mayor parte de estudios sobre la caracterización de *P. andina* no incluyen imágenes de los distintos haplotipos mitocondriales encontrados ni se reportan los tamaños de bandas obtenidos, a excepción de la investigación de Forbes et al. (2016), que en la información suplementaria muestra el patrón de bandas característico del haplotipo mitocondrial Ia de *P. andina* de tomate de árbol con MspI. Allí también se observan bandas adicionales leves de 400 y 1070 pb, similares a las encontradas en el presente trabajo. Estas bandas adicionales también se las puede observar en Ordoñez et al. (2000), donde se documenta el patrón de bandas característico de P2 con MspI para los haplotipos mitocondriales Ia de *P. infestans* y Ic de aislamientos que en ese entonces fueron considerados como *P. infestans* sensu lato, pero que posteriormente fueron clasificados como *P. andina* (Adler et al. 2004; Oliva et al. 2010).

Hasta donde se tiene conocimiento, no existen trabajos publicados sobre *P. andina* proveniente de tomate de árbol en los que se reporte los haplotipos mitocondriales en la región P4 del ADNmt utilizando EcoRI. Este trabajo sería el primer reporte de este tipo, y que documenta bandas adicionales a lo previamente descrito para este patógeno de *S. betaceum* (Adler et al. 2004; Oliva et al 2010; Forbes et al. 2016; Mideros et al. 2018).

La presencia de bandas adicionales en los haplotipos de *P. andina* de tomate de árbol, además de los registrados para *P. infestans*, se podría explicar por la existencia de sitios de restricción adicionales en las regiones P2 y P4 del ADNmt. Esto tendría coherencia con la postulación de que *P. andina* es un producto de hibridación entre *P. infestans* y otra especie desconocida de *Phytophthora* (Goss et al. 2011, Martin et al. 2015). Así, el sitio de anclaje de los cebadores y los sitios de restricción de las enzimas pudieran variar, ya que originalmente éstos fueron diseñados para *P. infestans.*

Análisis recientes de cinco loci del genoma mitocondrial de *P. infestans* (Martin et al. 2019), donde se incluyó en el estudio una muestra de *P. andina* de tomate de árbol del Ecuador, evidenció la presencia de 37 diferentes haplotipos mitocondriales en *P. infestans* y un haplotipo único para la muestra de *P. andina*. Esos autores reconocen que su técnica no es práctica para estudios poblacionales con un número grande de muestras, ya que el tiempo y los costos son elevados. El hecho de que bandas adicionales se presenten de manera consistente en todos los aislamientos de *P. andina* de tomate de árbol analizados en este estudio, mediante técnicas rápidas y económicas, sugiere que estas bandas deberían ser consideradas para la descripción de un nuevo haplotipo mitocondrial en *P. andina* de tomate de árbol que difiere del Ia de *P. infestans.*

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