Assessing Genetic Diversity of Three Species of Potato Tuber Moths (Gelechiidae, Lepidoptera) in the Ecuadorian Highlands

Authors: Fu, Zhen, Castillo Carrillo, Carmen I., Rashed, Arash, Asaquibay, César, Aucancela, Rodrigo, et al.

Assessing genetic diversity of three species of potato tuber moths (Gelechiidae, Lepidoptera) in the Ecuadorian highlands

Zhen Fu[1,5,], Carmen I. Castillo Carrillo[2], Arash Rashed[3], César Asaquibay[2], Rodrigo Aucancela[2], José Camacho[2], Victoria López[2], Verónica Quimbiamba[2], Fausto Yumisaca[2], Nancy Panchi[4], and Claudio Velasco[4]*

Abstract

Three species of potato tuber moths, *Tecia solanivora* Povolny, *Symmetrischema tangolias* Gyen, and *Phthorimaea operculella* Zeller (all Lepidoptera: Gelechiidae), attack potato plants in the highlands of Ecuador and cause great economic losses. To understand their ecology and to develop precise integrated pest management strategies, a description of the molecular identification of each species and genetic diversity within populations is needed. In this study, we assessed the genetic diversity of a total of 112 moth samples of these 3 distinct species. Samples were collected from the 4 central provinces (Bolívar, Chimborazo, Cotopaxi, and Tungurahua) in the Ecuadorian highlands, where potato is a major crop for food security. Using polymerase chain reaction (PCR) and Sanger sequencing, we compared mitochondrial gene sequences among conspecific moth samples. Each of the 3 species exhibited different patterns regarding genetic diversity; more than 1 haplotype was present in *P. operculella* and *S. tangolias*, whereas all *T. solanivora* samples were found to be genetically identical. This initial effort of molecular characterization of the potato tuber moths will facilitate identifying incursion and potential migration route of Gelechiidae species as well as prevention of the pest outbreaks.

Key Words: *Tecia solanivora*; *Symmetrischema tangolias*; *Phthorimaea operculella*; haplotype; potato tuber moth

Resumen

Tres especies de polillas de la papa, *Tecia solanivora* Povolny, *Symmetrischema tangolias* Gyen, y *Phthorimaea operculella* Zeller (todos Lepidoptera: Gelechiidae), atacan el cultivo de la papa en la sierra ecuatoriana causando grandes pérdidas económicas. Para comprender su ecología y desarrollar estrategias precisas de manejo integrado de plagas, se necesita una descripción de la identificación molecular de cada especie y la diversidad genética de poblaciones intraespecíficas. En este estudio, realizamos una caracterización molecular de un total de 112 muestras de polillas de estas 3 especies. Se recolectaron muestras de las 4 provincias centrales de los Andes ecuatorianos (Bolívar, Chimborazo, Cotopaxi, y Tungurahua) donde la papa es un cultivo importante para la seguridad alimentaria. Utilizando la reacción en cadena de la polimerasa y secuenciación Sanger, comparamos las secuencias de los genes mitocondriales entre las muestras de las 3 especies de polillas. Cada una de las 3 especies exhibió diferentes patrones con respecto a la diversidad genética; más de 1 haplotipo estuvo presente en *P. operculella* y *S. tangolias*, mientras que todas las muestras de *T. solanivora* fueron genéticamente idénticas. Este esfuerzo inicial de caracterización molecular de las polillas del tubérculo de la papa facilitará la identificación de la incursión y la posible ruta de migración de las especies de Gelechiidae y prevención de despuntes poblacionales de las plagas.

Key Words: *Tecia solanivora*; *Symmetrischema tangolias*; *Phthorimaea operculella*; haplotipo; polilla de la papa

Potato (*Solanum tuberosum* L.; Solanaceae) is an economically important staple food in South American countries, including Ecuador. Numerous insect pests attack potato plants and tubers during the growing season and post-harvest (i.e., storage). Three species of potato tuber moths (Lepidoptera; Gelechiidae), the Guatemalan potato tuber moth, *Tecia solanivora* Povolny, the Andean potato tuber moth, *Symmetrischema tangolias* Gyen, and the common potato tuber moth, *Phthorimaea operculella* Zeller, are present in Ecuador and cause significant economic losses (Gallegos et al. 2004; Rondon & Gao 2018).

The Guatemalan potato tuber moth, *T. solanivora*, is known to have originated from Central America and is a highly diverse species in that region (Torres-Leguizamón et al. 2011). There are 60

documented haplotypes of *T. solanivora* based on the cytochrome b (cytb) gene in Guatemala, Costa Rica, and Venezuela. The distribution of this moth stretches from Mexico in the north to Ecuador on its south boundary, covering a handful of Central and South American countries such as Panama, Costa Rica, Nicaragua, El Salvador, Honduras, Guatemala, Venezuela, and Colombia (Salasar & Escalante 1984; Rincón & López-Ávila 2004; Trujillo et al. 2004; Cruz et al. 2011). Across the Atlantic, *T. solanivora* was reported from the Canary Islands, Spain, in 1999, followed by another report of the pest from Spain's mainland in 2005 (EPPO 2015). In Ecuador, *T. solanivora* was spotted for the first time in 1996 in the northern province of Carchi (Gallegos 1997). It was thought that the range of this species in the Ecuadorian Andes is confined to regions that are below 3,300 masl (Gallegos et al. 2004). However, the latest survey debunked such notation because *T. solanivora* was found in potato fields in Chimborazo where elevation reaches 3,645 masl (Castillo Carrillo et al. 2018). *Tecia solanivora* is present in Imbabura, Pichincha, Cotopaxi, Tungurahua, and Bolívar provinces. In certain areas of the province of Bolívar, *T. solanivora* is the most common potato tuber moth species (Castillo Carrillo et al. 2018). In Azuay, the presence of *T. solanivora* has not been reported. There have been informal reports of *T. solanivora* being present in Cañar province. The genetic diversity outside of its native range is low, as only 1 haplotype, H6, has been found in samples collected from Colombia, Ecuador, and the Canary Islands (Torres-Lequizamón et al. 2011). The larva of *T. solanivora* feeds exclusively on potato tubers during its development in the field and during storage. It creates galleries in the tubers as it feeds, which render tubers unmarketable and results in considerable economic losses to farmers.

A recent survey covering 4 Ecuadorian provinces determined that the Andean moth, *S. tangolias*, is more common than *T. solanivora* in Chimborazo, Cotopaxi, and Tungurahua (Castillo Carrillo et al. 2018). *Symmetrischema tangolias* may be present in large numbers; in Chagrapamba, canton Píllaro, Tungurahua, over 100 male *S. tangolias* were collected in a single pheromone trap within 24 h (Castillo Carrillo et al. 2018). *Symmetrischema tangolias* is thought to be native to the Peruvian or Bolivian Andes and is broadly distributed in the Andean zones of Colombia, Ecuador, Peru, and Bolivia. Beyond South America, *S. tangolias* is present in Australia, Tasmania, New Zealand, and Indonesia (Terauds et al. 1984; Martin 1999; Kroschel & Schaub 2013). The larva mines the potato plant stems, including tubers, both in the field and post-harvest. In addition to potato plants, *S. tangolias* can attack other solanaceous hosts, such as tomato (*Solanum lycopersicum* L.), pepino (*S. muricatum* Ait.), *S. aviculare* G. Forster, and *S. laciniatum* Ait. (all Solanaceae) (Kroschel & Schaub 2013). The first sighting of *S. tangolias* in Ecuador dates back to the early 1990s in the province of Carchi (P. Gallegos, personal communication).

Different from *T. solanivora* and *S. tangolias*, *P. operculella* is a cosmopolitan pest that causes considerable damage worldwide (Rondon & Gao 2018). *Phthorimaea operculella* can be found in over 90 countries across the Americas, Asia, Africa, and Australia (Kroschel et al. 2013; Rondon & Gao 2018). It feeds on solanaceous crops such as potatoes and tomatoes. Females oviposit on potato leaves and stems (including tubers). Their larvae mine leaves and tubers, resulting in economic damage. *Phthorimaea operculella* was reported as a significant pest of potato tubers in the early 1900s in South America (Graft 1917). In Ecuador, it was observed for the first time between 1984 and 1985 in the southern province of Cañar (P. Gallegos, personal communication). Generally, *P. operculella* is found less frequently than the other 2 moth species, *T. solanivora* and *S. tangolias*, in the potato producing regions in Ecuador, except for certain areas in Tungurahua (Castillo Carrillo et al. 2018).

Despite the economic importance and coexistence of all 3 species in the same region (Dangles et al. 2009; Castillo Carrillo et al. 2018), information regarding the identification and genetic diversity is largely missing except for *T. solanivora* (Torres-Leguizamón et al. 2011). This lack of accurate identification and knowledge of their biology and distribution contributes in part to inefficient management of these species. To establish a clear genetic description of these 3 insect species, we characterized intraspecific genetic diversity of the potato tuber moth samples collected from 4 Ecuadorian provinces using previously published primers that target the cytochrome oxidase I (COI) gene for *P. operculella* and *S. tangolias* and the cybt gene for *T. solanivora*.
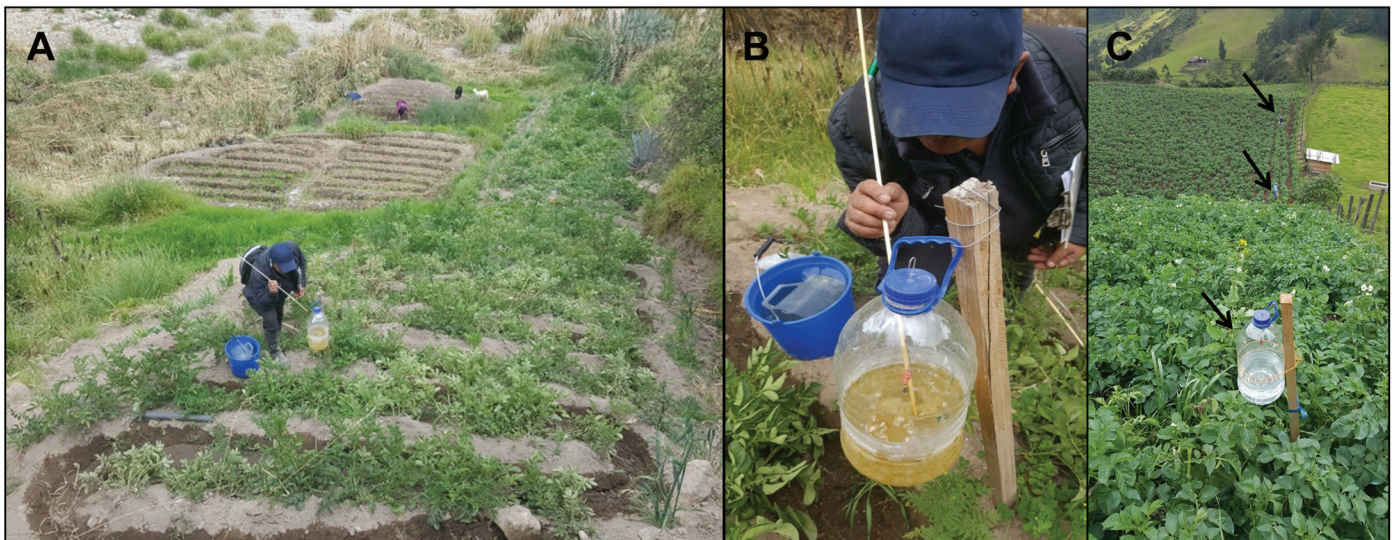
Fig. 1. Use of pheromone traps to collect potato tuber moths in Cacha, Chimborazo, and Tishinguirí, Bolívar: (A) potato crops are grown in mountainous regions of Ecuador where smallholder terrace farming is predominant; (B) low-cost pheromone traps were made of plastic bottles; a species-specific lure is fixed to the bottle cap using a string, and thumb-sized holes were carved to allow moths to fly into the bottle that was filled with soapy water; (C) 3 different traps were placed (pointed by arrows) in each field at least 100 m apart to catch different moths.

Table 1. Primers used in this study.

| Targeting species	Primer name	Sequences (5' to 3')	Product size (base pairs)	Reference
Symmetrischema tangolias	Sym-tan-S369	GCTGAATTAGGTAACCCAGGC	431	Sint et al. 2016
	Lep-gen-A309	GGTATTTGGTCAAATGAAAGTCC		Sint et al. 2016
Phthorimaea operculella	Pht-ope-S349	AGGAATAGTTGGAACCTCTCTTAGT	469	Sint et al. 2016
	Lep-gen-A309	GGTATTTGGTCAAATGAAAGTCC		Sint et al. 2016
Tecia solanivora	TsF1-re	GCTAACATTGAGTTAGCTTTTTAT	963	This study
	TsR-re	AAAAATTAGAGTATCTCAAAAT		This study

Materials and Methods

COLLECTION OF MOTHS

Live moths were collected using pheromone traps (ChemTica Internacional, Heredia, Costa Rica). These traps were specific to each species, and they were set up in potato fields in provinces of Bolívar, Chimborazo, Cotopaxi, and Tungurahua (Fig. 1, Table S1). Moths that were attracted to each species-specific pheromone trap were verified to be the correct species based on wing patterns (Sporleder et al. 2016a, b; Rondon & Gao 2018). Individual samples were placed in 1.5 mL micro-
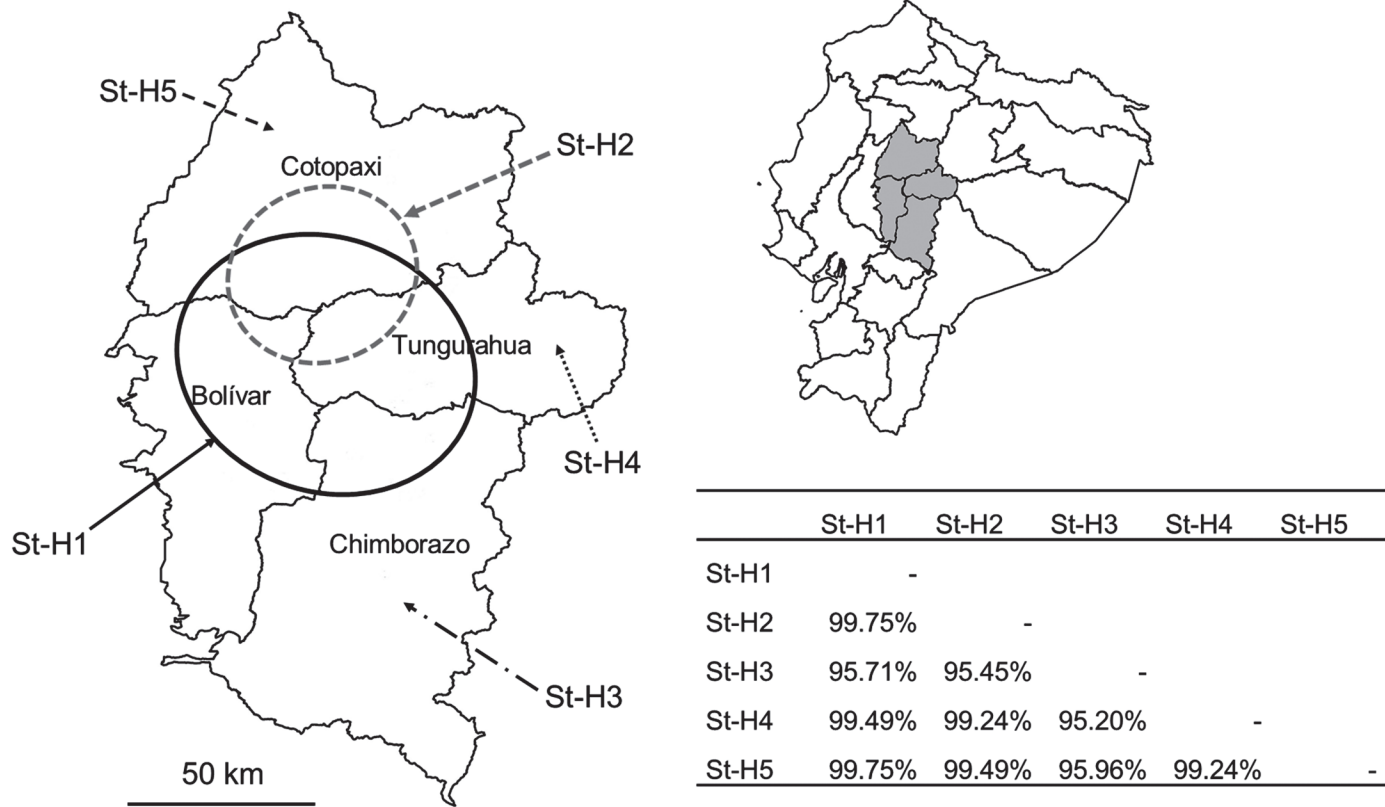
centrifuge tubes (Hauppauge, New York, USA) and stored in 95% ethanol at 17 to 18 °C upon arrival at the laboratory. A total of 112 moths, including 47 *T. solanivora*, 37 *S. tangolias*, and 28 *P. operculella* samples that represented moth populations from 4 provinces were processed for DNA extractions and characterized with polymerase chain reaction (PCR) and Sanger sequencing.

DNA EXTRACTION

Wings of a single moth were separated from the body, and the whole body was placed in a 1.5 mL microcentrifuge tube. The moth body was homogenized using a Mini-BeadBeater homogenizer (GlenMills, Clifton,
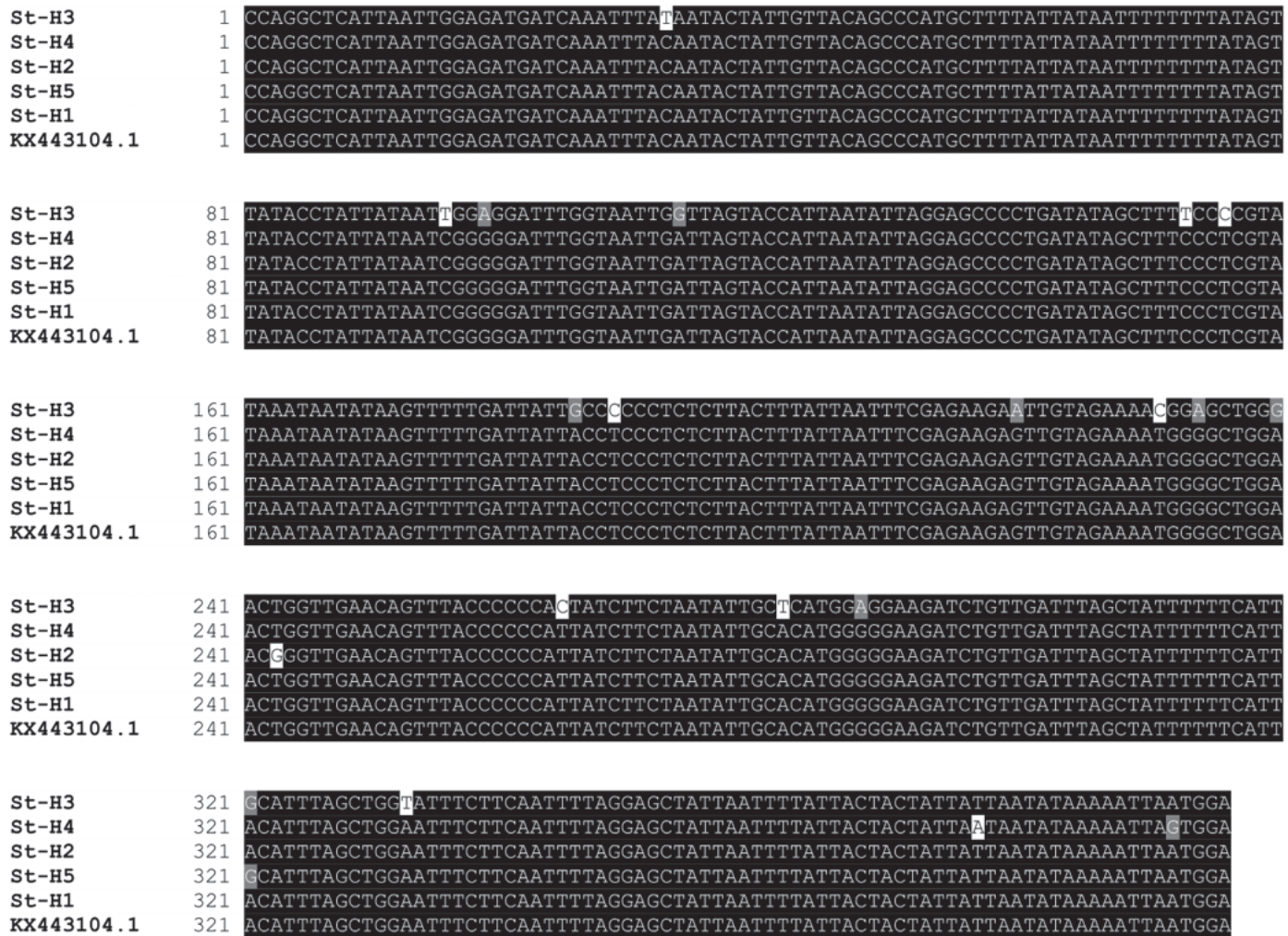
Fig. 2. Alignment of 5 COI haplotype sequences of *Symmetrischema tangolias* samples. KX443104.1 is a reference sequence from GenBank. Sequences of these 5 haplotypes were deposited in GenBank with accession numbers MN223391 to MN223395.

	St-H1	St-H2	St-H3	St-H4	St-H5
St-H1	-				
St-H2	99.75%	-			
St-H3	95.71%	95.45%	-		
St-H4	99.49%	99.24%	95.20%	-	
St-H5	99.75%	99.49%	95.96%	99.24%	-

Fig. 3. Distribution of 5 haplotypes and sequence identity between each pair of *Symmetrischema tangolias* haplotypes. St-H1 was present in all 4 provinces; St-H2 was present in 3 provinces; the other 3 haplotypes were present only in 1 province for each haplotype. Arrows point to province(s) instead of a specific sampling location.

New Jersey, USA) for 2 min in 180 μL of ATL buffer (Qiagen DNeasy Blood & Tissue Kit, Qiagen, Germantown, Maryland, USA), with 5 glass beads (3 mm diam) added. The tissue homogenate was incubated overnight at 56 °C. The DNA extraction process was continued using a Qiagen DNeasy Blood & Tissue Kit. The DNA concentration was quantified using a Nano-Drop 2000 (ThermoFisher Scientific, Frederick, Maryland, USA).

PCR AMPLIFICATION AND PURIFICATION

The total volume of all PCR reactions was 25 μL, which included 2.5 μL 10× PCR buffer, 1.5 μL 25mM MgCl$_2$, 1.25 μL dNTPs, 0.5 μL of each 10 μM forward and reverse primer (Table 1), 0.25 μL Takara Ex Taq (Takara, Mountain View, California, USA), 1 μL extracted moth DNA, and 17.5 μL of molecular grade water. PCR buffer, MgCl$_2$, and dNTPs were supplied with Takara Ex Taq; all concentrations listed above were the concentration prior to adding into the mix. The program for PCR was 94 °C for 2 min, then 35 cycles of 94 °C for 15 s, 55 °C for 60 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 5 min, in a S1000 Thermal Cycler (Bio-Rad, Hercules, California, USA). The PCR products were visualized on 1.5% agarose gels.

For *T. solanivora*, we initially used primers TsF1 and TsR, according to Torres-Leguizamón et al. (2011). However, the gel electrophoresis indicated that there were multiple amplicons present. As a result, the sequences appeared to be poor quality, and the background noise was high. Thus, we redesigned primers TsF1-re and TsR-re (Table 1), which were used to amplify a 963 base pair section of the *T. solanivora* cytb gene. The PCR condition for redesigned primers was the same as described above, except the annealing temperature was raised from 55 °C to 59 °C.

For samples that formed a single clear band, 5 μL PCR product was purified using ExoSAP-IT (ThermoFisher Scientific, Frederick, Maryland, USA) according to the manufacturer protocol. Since ExoSAP-IT purified DNA was too concentrated for Sanger sequencing, we diluted them 10 times with molecular grade water. Two μL of the diluted DNA and 0.8 μL of either forward or reverse primers (10 μM) were added to a 0.2 mL microcentrifuge tube with 12.2 μL of molecular grade water to reach a total volume of 15 μL. Sanger sequencing with forward and reverse primer were performed separately for each DNA sample.

DATA ANALYSES AND ARCHIVING

Raw sequences were processed using the program BioEdit (vers. 7.0.5; Hall 1999). Consensus sequences were generated from the alignment of reverse and forward reads. Sequences from the same moth species were aligned using the program MUSCLE (Edgar 2004). Five sequences representing 5 unique haplotype of *S. tangolias* were deposited in GenBank with accession numbers MN223391 to MN223395. Three sequences representing 3 unique haplotypes of *P. operculella* were deposited in GenBank with accession numbers MN205567 to MN205569. The sequence of the only haplotype discovered within *T. solanivora* samples was archived in GenBank with accession number MN205566.

Results

We successfully amplified 42 of 47 *T. solanivora* samples with the newly designed primers TsF1-re and TsR-re. A single and clear band was seen on the gel for each successful amplification. All of the sequences

Fig. 4. Alignment of 3 unique COI haplotype sequences of *Phthorimaea operculella*. MF121882 is a reference sequence from GenBank. Sequences of these 3 haplotypes were deposited in GenBank with accession numbers MN205567 to MN205569.

were 100% identical to each other, with 100% similarity to several sequences that previously had been deposited in GenBank, including a mitochondrial genome of *T. solanivora* from Colombia (KT326187.1) and several previously reported *T. solanivora* samples from Ecuador (EF212202.1 and DQ780487.1). Lastly, the cybt sequence of *T. solanivora* in our study was 100% identical to H6, the only haplotype discovered from Ecuador, according to Torres-Leguizamón et al. (2011).

Out of 37 *S. tangolias* samples, 35 had a distinct band of the expected size of 431 base pairs. Five haplotypes were identified within these 35 samples. Sequence St-H1 was the dominant haplotype, representing over 75% of the collected samples in the 4 provinces (Figs. 2, 3). Sequence St-H2 was present in 4 samples from 3 provinces (Tungurahua, Bolívar, and Cotopaxi). Sequence St-H2 was only one nucleotide different from sequence St-H1 (Fig. 2). Sequence St-H3 was the most distant haplotype in relation to the other 4 haplotypes. The identity between St-H3 and any haplotype was less than 96% (Fig. 3). Only 2 samples collected from Chimborazo were characterized as St-H3 haplotype. One sample from Tungurahua and 1 sample from Cotopaxi were characterized as St-H4 and St-H5, respectively. These 2 haplotypes were 99% identical with 3 bases different from each other (Fig. 2).

We processed 28 samples of *P. operculella*, of which 25 showed a distinct band at the expected 469 base pairs. Three haplotypes were present within 25 samples (Figs. 4, 5). The first haplotype, Po-H1, was the most dominant haplotype; 19 out of 25 *P. operculella* samples spanning 4 provinces were characterized as Po-H1. The sequence of Po-H1 was 100% identical to that of MF121882, which was deposited in

GenBank, with only 1 nucleotide different from the other 2 haplotypes, Po-H2 and Po-H3 (Fig. 4). Six samples were characterized as Po-H2 and Po-H3, the 2 haplotypes that are differentiated by only 2 nucleotides (Fig. 4). The 2 samples which were characterized as Po-H2 were present only in Tungurahua. Four samples that belong to Po-H3 were present in Cotopaxi and Chimborazo.

Discussion

Pest management for potato production in the Andean region of Ecuador is important because potato is a major staple food and is of significant economic value. Our study made the initial effort to document and characterize 2 species of potato tuber moths, *S. tangolias* and *P. operculella*, in Ecuador using PCR and sequencing based on the COI gene. Additionally, we compared our results of *T. solanivora* cybt sequences to a previous study which scrutinized haplotypes of *T. solanivora* in Central and South American countries. Five haplotypes were discovered from the 35 sequenced Andean potato tuber moth *S. tangolias*. The presence of 3 haplotypes was detected in the collected *P. operculella* moth samples. All of the collected Guatemalan potato tuber moths, *T. solanivora*, were genetically identical for the cybt gene sequenced. Mitochondrial gene sequences presented here and archived in GenBank will facilitate further development of molecular markers for diagnostic purposes as well as for scrutinizing phenotypic variation among haplotypes within a species.
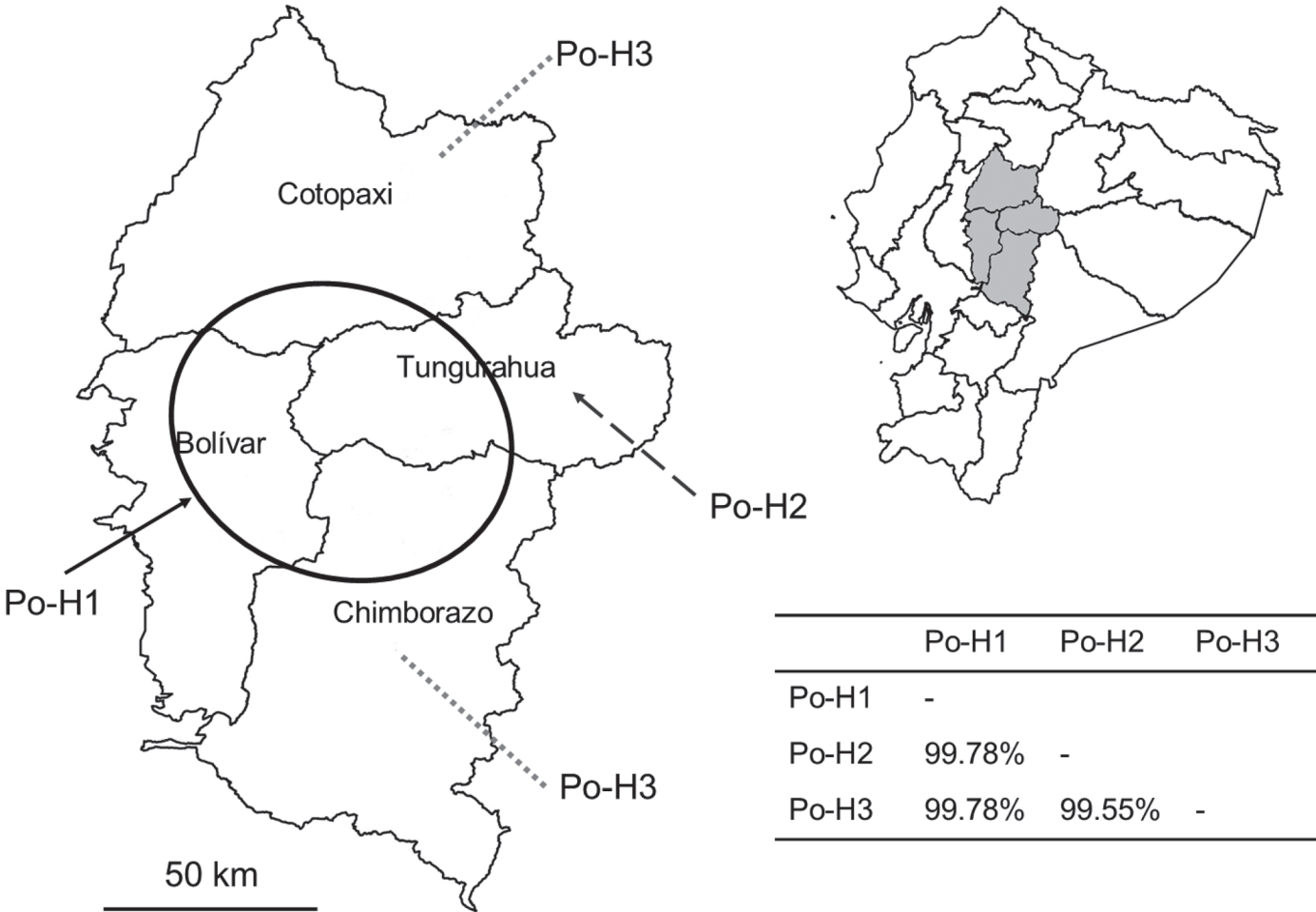
Fig. 5. Distribution of 3 haplotypes and sequence identity between each pair of *Phthorimaea operculella* haplotypes. Po-H1 was present in all 4 provinces, Po-H3 was present only in Cotopaxi and Chimborazo; Po-H2 was limited to Tungurahua.

Interestingly, one of the *S. tangolias* haplotypes, St-H3, displayed a much greater divergence compared to the other intraspecific haplotypes (> 3%). Both samples that were characterized as St-H3 were from the same province, Chimborazo. When we queried the sequences of these 2 samples in GenBank, the closest homologs (96.5% identity) were 2 *S. tangolias* samples collected from northern Chile, KY951811.1 and KY951905.1. The sequence divergence of 3% also is much higher than what was reported in 3 Lepidopteran families. Hajibabaei et al. (2006) examined several specimens of Hesperiidae, Sphingidae, and Saturniidae in a tropical region, and the reported average within-species divergences of COI sequences were 0.17%, 0.43%, and 0.46%, respectively. Contrarily, Cognato (2006) detected a greater range of intraspecific divergence, i.e., 0.4% to 4.8% from Prodoxidae (yucca moth), with an average of 2.2% based on the COI gene. Cognato (2006) also showed overlap between intraspecific and interspecific sequence divergence within Prodoxidae. The present survey also brought our attention to the issues associated with species boundaries and cryptic species despite examining the same region of the COI gene, i.e., the section of COI gene amplified in Hajibabaei et al. (2006) overlapped with our study. Although delimiting species boundaries of *S. tangolias* samples is beyond the scope of this work, we still need to interpret the results with caution. We suggest future work could focus on broader sampling efforts followed by detailed morphological characterization and molecular identification.

Genetic variation and diversity reflect the evolutionary history and ecological adaptation of insect pests, which are vital to implementing effective pest management programs. Many lepidopteran pests are highly mobile and have a great propensity to invade new agricultural regions. Using COI genetic marker, Naik et al. (2020) found 2 common haplotypes of cotton pink ballworms, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), across cotton-growing regions of India. The star-shaped haplotype network, with the most common haplotype at the center surrounded by rare haplotypes with a few mutational steps, suggested a recent population expansion of this Gelechiidae moth. In a similar study based on genetic sequences of 2 mitochondrial genes (COII and Nad4), Liu et al. (2010) discovered similar star-shaped phylogeny of *P. gossypiella* across multiple provinces in China. Overall, low genetic diversity of *P. gossypiella* population was attributed to invasion bottlenecks (Liu et al. 2010). The haplotype prevalence in both *S. tangolias* and *P. operculella* in our study were similar to what was described in Liu et al. (2010) and Naik et al. (2020), and that was the presence of 1 dominant haplotype along with multiple less common haplotypes. It is possible that these 2 species underwent population expansion, rapid growth from a small-sized founder population (Grapputo et al. 2005; Saw et al. 2006). In order to elucidate the evolutionary history of *S. tangolias* and *P. operculella*, future work is needed to evaluate the population genetics based on both historical samples and samples from broader geographic regions, including those from their native ranges. Genetic variations within species also have been linked

to some behavioral differences. Examples are the observed differences in host preference by 2 fall armyworm strains (Pashley 1986) and the presence of allochronic populations of processionary moth (Santos et al. 2013; Leblois et al. 2018). There were only a handful of studies that examined genetic diversity and variation of Gelechiidae (Liu et al 2010; Medina et al. 2010; Torres-Leguizamón et al. 2011; Naik et al 2020). It is in our future interest to test whether haplotypes of each potato tuber moth species show differences in host preference, behavior, and life-history traits.

Multiple potato tuber moth species may co-exist in potato fields, as demonstrated in our survey. Due to large-scale cultivation of plants in agroecosystems, the presence of multiple insect pest species attacking a single crop becomes a likely scenario (Daamen & Stol 1994; Davidson et al. 2007; Dangles et al. 2008). This could bring bad news for the farmers because the presence of multiple pests, with their species-specific ecology/biology, may exacerbate the effectiveness of implemented management practices. It has been shown recently that the presence of *T. solanivora*, *S. tangolias*, and *P. operculella* together can lead to greater damage to potato crops than when each pest is present alone (Dangles et al. 2009). The underlying mechanisms of this additive effect of multiples pest species remain elusive. Complete resource use and interaction of insect pests may contribute to losses in field crops. Further research is warranted to determine the extent of the additive effect of crop loss due to the coexistence of 3 potato tuber moths in Ecuador.

Acknowledgments

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