ABSTRACT


Potato is an important crop for thousands of small-holder farmers in Bolivia. The potato cyst nematodes (Globodera rostochiensis and G. pallida) have been reported in most cultivated areas of Bolivia and are a significant constraint to the production of potato in the country. A survey was conducted in 2022 to provide a current understanding of the identity, distribution, and occurrence of Globodera spp. in Bolivia. Soil samples were collected from 144 potato fields and Globodera spp. cysts were found in 82% of the samples. There were significantly ($P < 0.001$) higher population densities of Globodera spp. in the departments of La Paz, Cochabamba, and Chuquisaca (297 to 1,008 cysts/250 cm$^3$ soil) compared to Potosí and Tarija (~85 cysts/250 cm$^3$ soil). Based upon molecular identification, both G. rostochiensis and G. pallida were found, with G. rostochiensis being the most prevalent species in Bolivia found in 87% of infested fields. These findings provide an updated assessment of the current status of Globodera spp. in Bolivia.

Key words: Potato, potato cyst nematode, South America

RESUMEN


La papa es un cultivo importante para miles de pequeños agricultores en Bolivia. Los nematodos del quiste de la papa (Globodera rostochiensis y G. pallida) han sido reportados en la mayoría de las áreas cultivadas de Bolivia y son una limitante importante para la producción de papa en el país. Se realizó una encuesta en el 2022 para proporcionar una comprensión actual de la identidad, distribución y ocurrencia de Globodera spp. en Bolivia. Se recolectaron muestras de suelo en 144 fincas de papa y se encontraron quistes de Globodera spp. en el 82% de las muestras. Hubo densidades poblacionales significativamente ($P < 0.001$) más altas de Globodera spp. en los departamentos de La Paz, Cochabamba y Chuquisaca (297 a
INTRODUCTION

Potato is Bolivia’s most important food crop (Coca-Morante, 2019). It is grown on approximately 180,000 ha by thousands of smallholder farmers. Bolivia’s potato production is important because it is at the center of domestication and genetic diversity of potato (Solanum tuberosum) in the Andean Region of the country (Hawkes, 1990). Over time, potato production has expanded from the Andean Region of Bolivia to the valleys and plains at lower elevations. Potato is grown in six departments in Bolivia (La Paz, Cochabamba, Potosí, Oruro, and parts of Chuquisaca and Tarija) (Coca-Morante, 2019). The most important departments for potato production in Bolivia are La Paz, Cochabamba, and Potosí. Native (Solanum andigena) and adapted potatoes (S. tuberosum) are grown in Bolivia including Waych’a, Désirée, and Imillas-type. Similar to potato-growing regions around the world, potato production is affected by many biotic and abiotic factors, including potato cyst nematodes (Globodera spp.).

Potato cyst nematodes (Globodera rostochiensis and G. pallida) have been reported in most cultivated areas in Bolivia (Silvestre et al., 2021). These nematodes are globally recognized parasites of potatoes and have been reported to cause up to 80% yield loss of potato (Brodie and Mai, 1989). In addition to the loss in yield caused by Globodera spp., indirect losses such as rejection of seed potatoes from infested fields in Bolivia have also been reported (Pacajes et al., 2002). The earliest assessment of Globodera spp. in Bolivia occurred in 1974 (CIP, 1976). Globodera rostochiensis was the predominant species found south of Lake Titicaca in Bolivia. In 1975, both G. pallida and G. rostochiensis were found in Bolivia (Evans et al., 1975). Available data from diagnostic laboratories and publications on Globodera spp. in Bolivia were summarized by Franco and González (2011). In this study, five departments were considered: Cochabamba, Potosí, La Paz, Tarija, and Chuquisaca. Globodera spp. were reported in these five departments with an incidence of 78%. Population densities of Globodera spp. varied across the departments with high densities observed in the departments of Cochabamba and La Paz. Both G. pallida and G. rostochiensis were found in Bolivia, with G. pallida more commonly found in Chuquisaca, La Paz, and Cochabamba. Whereas G. rostochiensis was more commonly found in La Paz and Tarija. The last time that Globodera spp. occurrence and distribution was considered in Bolivia was more than 10 years ago (Franco and González, 2011). The objective of this research was to provide an updated assessment of the distribution and occurrence of Globodera spp. in Bolivia.

MATERIALS AND METHODS

Soil samples (N = 144 fields) were collected between April and June 2022. Upon entering a field, if potato plants were present, several plants were uprooted and roots examined for cysts. The root system was then shaken into a bag to remove adhering soil and cysts. Soil samples were then collected from multiple locations (5 to 10) in a field to a depth of 15 cm using a trowel, combined, and placed in a paper bag. At the laboratory of PROINPA Foundation (Fundación para la Promoción e Investigación de Productos Andinos; Quillacollo, Bolivia), bags were opened, and the soil was air dried for at least two weeks. Cysts were extracted from the soil using the flotation and sieve method (Fenwick, 1940). Extracted cysts were enumerated using a dissecting microscope and picked. Cyst densities are expressed as number of cysts/250 cm³ soil. A portion of the picked cysts were placed in 70% ethanol for 20 min, rinsed with water and shipped to USDA-ARS in Corvallis, OR, for molecular identification (Subbotin et al., 2020). When available (102 fields out of the original 144 fields), up to 8 single cysts were selected for DNA extraction; a total of 765 DNA samples were considered. Using aseptic precautions, eggs within

1008 quistes/250 cm³ de suelo) en comparación con Potosí y Tarija (~85 quistes/250 cm³ de suelo). Con base en la identificación molecular, se encontraron tanto G. rostochiensis como G. pallida, siendo G. rostochiensis la especie más prevalente en Bolivia, encontrado en el 87% de los campos infestados. Estas investigaciones brindaron una evaluación actualizada del estado actual de Globodera spp. en Bolivia.

Palabras clave: Papa, nematodo del quiste de la papa, Suramérica
an individual cyst were freed into 20 μl of nematode extraction buffer. Freed eggs were then transferred via pipet into a 1.7 ml tube and physically disrupted with a total of three freeze-thaw events. Briefly, the tube was immersed in liquid nitrogen for 30 s followed by immersion in water in a heat block set to 60°C for 30 s. Samples were centrifuged briefly at high speed and then pulverized using a micro pestle for 30 s. Another 20 μl of NEB was used to rinse the micro pestle before samples were centrifuged briefly at high speed. Lysate was transferred to a 200 μl tube and 4 μl of proteinase K was added before incubating at 60°C for 1 hr, then 95°C for 15 min in a thermocycler. Samples were held at -20°C until further use.

DNA for each sample was analyzed using published primers targeting rDNA via conventional multiplex PCR (Skantar et al., 2007). Briefly, 0.2 μM each of primers PITSp4 (5ʹ–ACA ACA GCA ATC GTC GAG - 3ʹ), PITSr3 (5ʹ–AGC GCA GAC ATG CCG CAA - 3ʹ), and ITS5 (5ʹ–GGA AGT AAA AGT CGT AAC AAG G - 3ʹ) were combined with 12.5 μl Accustart ToughMix (Quantabio, Beverly, MA), 1 μl template DNA, and 8.5 μl molecular grade water in a total volume of 25 μl. Cycling conditions included an initial denaturation step of 94°C for 3 min followed by 40 cycles of 94°C for 30 s, 60°C for 15 s, and 72°C for 30 s, and then a final elongation step of 72°C for 7 min. For each PCR reaction, 10 μl of product was analyzed on a 1% agarose gel stained with ethidium bromide (1 μg/ml) and visualized by UV illumination. Images of each gel were captured with an AlphaImager 2200 transilluminator (Alph Innotech, San Leandro, CA). Positive controls were run in parallel from DNA extracted from G. pallida and G. rostochiensis and water was used as a non-template negative control.

Data was analyzed in JMP v. 14 (SAS Institute, Cary, NC). Difference in population densities of Globodera spp. in the Bolivian departments was determined by the Kruskal-Wallis rank sums test followed by Dunn’s multiple comparison test (P < 0.05). The map shown in Fig. 1 was made using ArcGIS online software (Esri, Redlands, CA).

RESULTS

Globodera spp. cysts were found in 82% of the sampled fields across the country (N = 144; Table 1). The highest percentage occurrence was in Chuquisaca followed by Potosí and Cochabamba, with the lowest percentage occurrence in Tarija. In Bolivian fields where Globodera spp. cyst were found, population densities averaged 410 cysts/250 cm³ soil (Table 1). There were significantly (P < 0.001) higher population densities of Globodera spp. in the departments of La Paz, Cochabamba, and Chuquisaca compared to Potosí and Tarija (P < 0.001).

Molecular identification of the Globodera spp. populations (N = 756 cysts) based on results of conventional multiplex PCR revealed that both G. rostochiensis and G. pallida were confirmed in Bolivia (Fig. 1; Table 2). The presence of the species-specific 265 bp and/or 434 bp bands on a gel indicated that a sample was positive for G. pallida and/or G. rostochiensis, respectively. When data from departments were combined, G. rostochiensis was the most prevalent species (Table 2). Globodera pallida was found in all departments but at a lower percentage compared to G. rostochiensis. Of the cysts considered in the molecular analysis, approximately one quarter were not identified to species with the primers used in this study.

Across the fields in Bolivia where cysts were found, G. rostochiensis was the dominant species present (Table 3). In the department of La Paz near Lake Titicaca, 44% of the fields had mixed populations of G. pallida/G. rostochiensis (Table 3). Only 2 out of 18 fields had G. pallida as the only Globodera spp. present in La Paz. The percentage of fields comprised of G. rostochiensis increased from 78% in La Paz to >86% in departments located south of La Paz. Globodera pallida was not found in Cochabamba but was found either alone or in mixed populations with G. rostochiensis further south in the departments of Potosí, Chuquisaca, and Tarija.

DISCUSSION

Similar to the last effort that was undertaken in Bolivia to determine the prevalence of Globodera spp. (Franco and González, 2011), our survey found a high occurrence of Globodera spp. in the country with greater than 80% of fields infested. This finding was not unexpected considering that Bolivia is part of the Andean region in South America where Globodera spp.
originated (Grenier et al., 2010; Subbotin et al., 2020). Until the 1950s and 1960s, potatoes were grown in the High Andes of Bolivia (Coca-Morante, 2019). Then, cultivation started to spread to the valleys and the plains of the country. As native potatoes moved from the points of origin, it is likely that *Globodera* spp. moved as well.

In this survey, the departments of La Paz, Cochabamba, and Chuquisaca had higher densities of cysts/250 cm$^3$ soil than the departments of Potosí and Tarija. These results are similar to those of Franco and González (2011) who found the highest *Globodera* spp. egg densities (15 to $> 35$ eggs/g soil) in La Paz and Cochabamba. The majority of the fields considered in Tarija by Franco and González (2011) were either free of *Globodera* spp. ($\sim$40%) or had low to medium egg densities (1 to 15 eggs/g soil; 45%), similar to our findings of lower densities in this department. Contrary to our findings, 70% of fields in Chuquisaca had low egg densities reported by Franco and González (2011); while in our survey, Chuquisaca grouped with Cochabamba and La Paz as having the highest density of cysts/250 cm$^3$ soil.

Both *G. pallida* and *G. rostochiensis* have been reported in Bolivia. The occurrence of these nematodes was first investigated in the 1970s. The International Potato Center found *G. rostochiensis* south of Lake Titicaca (CIP, 1976). Evans et al. (1976) considered five populations from Bolivia, two near Lake Titicaca in the department of La Paz and three from department of Cochabamba. *Globodera pallida* was found in the two samples from northern Bolivia, while *G. rostochiensis* was the species found in Cochabamba. At this time, and based upon sampling in Colombia, Ecuador, Peru, and Bolivia, the demarcation line between the areas in which *G. pallida* and *G. rostochiensis* were found was 15.6˚S latitude, with one *G. pallida* population at 16.3˚S (Subbotin et al., 2020). The results suggested that the distribution of the *Globodera* species depended on latitude, different
temperature regimes, or day length, as well as the movement of potato tubers (Evans et al., 1976).

Our molecular analysis to determine the identity of *G. pallida* and *G. rostochiensis* in the collected populations showed that *G. rostochiensis* was the most frequently encountered species in Bolivia. This is contrary to the findings of Franco and González (2011), who reported that *G. pallida* slightly exceeded the incidence of *G. rostochiensis* in the country. They also reported that the departments of Chuquisaca, La Paz, and Cochabamba were the areas most affected by *G. pallida*, while the departments of Tarija and La Paz were most affected by *G. rostochiensis*. Our analysis also showed that *G. rostochiensis* was the most frequently encountered species in all of the departments considered; *G. pallida* was not detected in Cochabamba. The department of La Paz had the highest incidence of *G. pallida*. Franco and González (2011) did not report mixed populations in their study. In our survey, mixed populations were found in 10 to 44% of the fields where samples were collected in the departments of Chuquisaca, La Paz, Potosí, and Tarija.

We utilized primers that were developed by Skantar et al. (2007) and Bulman and Marshall (1997) for molecular identification of *G. pallida* and *G. rostochiensis*. These primers were developed using populations from Idaho and New Zealand. While the origin of *G. pallida* and *G. rostochiensis* is likely the Andean region of South America, it is hypothesized that population in Europe originated in southern Peru and were then distributed from Europe to other regions of the world (Plantard et al., 2008). Therefore, there was a bottleneck in diversity and primers developed for the identification of non-South American *Globodera* spp. populations are likely limited in their utility for the identification of diverse *Globodera* spp. populations present in Bolivia. The primers we used either resulted in an “other” or “no amplification” outcome in 23% of the cysts evaluated. There is another *Globodera* spp. that has been found infecting potato in South America, *G. ellingtonae* (Lax et al., 2014; Hesse et al., 2021). So far, this nematode has been reported in regions of Chile and Argentina that are geographically not far from Bolivia. Subbotin et al. (2020) found through phylogenetic analysis that the south Andes might be considered as another and more recent center of origin for *G. rostochiensis*, *G. ellingtonae*, and *G. tabacum*. The primers utilized

### Table 1. Occurrence and population densities of *Globodera* spp. in the Bolivian departments.

<table>
<thead>
<tr>
<th>Department</th>
<th>Fields (#)</th>
<th>Occurrence (%)</th>
<th>Mean (#) cysts/250 cm³ soil when present</th>
<th>Max (#) cysts/250 cm³ detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chuquisaca</td>
<td>29</td>
<td>96.7</td>
<td>297 a²</td>
<td>1,375</td>
</tr>
<tr>
<td>Cochabamba</td>
<td>48</td>
<td>81.3</td>
<td>444 a</td>
<td>2,835</td>
</tr>
<tr>
<td>La Paz</td>
<td>25</td>
<td>76.0</td>
<td>1,008 a²</td>
<td>5,158</td>
</tr>
<tr>
<td>Potosí</td>
<td>18</td>
<td>88.9</td>
<td>84 b</td>
<td>180</td>
</tr>
<tr>
<td>Tarija</td>
<td>24</td>
<td>66.7</td>
<td>85 b</td>
<td>253</td>
</tr>
<tr>
<td>Total Bolivia</td>
<td>144</td>
<td>82.1</td>
<td>410</td>
<td>5,158</td>
</tr>
</tbody>
</table>

*Values followed by same letter are not significantly different according to Kruskal-Wallis rank sums test followed by Dunn’s multiple comparisons test (*P* < 0.05).

<table>
<thead>
<tr>
<th>Department</th>
<th>DNA samples (#)</th>
<th><em>Globodera pallida</em> (%)</th>
<th><em>Globodera rostochiensis</em> (%)</th>
<th>NA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chuquisaca</td>
<td>236</td>
<td>6.4</td>
<td>61.0</td>
<td>32.6</td>
</tr>
<tr>
<td>Cochabamba</td>
<td>224</td>
<td>0.0</td>
<td>91.5</td>
<td>8.5</td>
</tr>
<tr>
<td>La Paz</td>
<td>140</td>
<td>29.3</td>
<td>50.0</td>
<td>20.7</td>
</tr>
<tr>
<td>Potosí</td>
<td>112</td>
<td>17.9</td>
<td>49.1</td>
<td>33.0</td>
</tr>
<tr>
<td>Tarija</td>
<td>53</td>
<td>9.4</td>
<td>56.6</td>
<td>34.0</td>
</tr>
<tr>
<td>Bolivia total</td>
<td>765</td>
<td>10.6</td>
<td>65.9</td>
<td>23.5</td>
</tr>
</tbody>
</table>

*DNA was extracted from single cysts.*
Globodera spp. in Bolivia: Sainz et al.

Our study would not have identified *G. ellingtonae* or other diverse *Globodera* spp. These findings provide a starting place for additional research in Bolivia on *Globodera* spp. It is important to know the *Globodera* species and pathotype present to properly deploy resistance. While we did not determine the pathotype of the populations we collected, several pathotypes of *G. rostochiensis* and *G. pallida* have been reported from around Lake Titicaca in Bolivia (Canto and Schurrah, 1977). Pacajes et al. (2002) considered how rotation crops and potato clones impacted *Globodera* spp. population densities, this effort should be continued. Finally, the presence and distribution of *G. ellingtonae* and other *Globodera* spp. should be determined in Bolivia to facilitate the management of these nematodes in potato.

**ACKNOWLEDGMENTS**

We thank Fundación para la Promoción e Investigación de Productos Andinos (PROINPA) for graciously allowing us to use their facilities to extract nematodes. This effort was partially funded by the USDA APHIS FarmBill grants: AP20PQS&T00C079 (agreement no. 20-0255-000-FR). We also appreciate the Bolivian famers who allowed us access to their fields.

**LITERATURE CITED**


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<table>
<thead>
<tr>
<th>Department</th>
<th>Fields (#)</th>
<th><em>G. pallida</em> (%)</th>
<th><em>G. rostochiensis</em> (%)</th>
<th><em>G. pallida</em> + <em>G. rostochiensis</em> (%)</th>
<th><em>G. pallida</em> (%)</th>
<th><em>G. rostochiensis</em> (%)</th>
<th>NA (%)</th>
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</thead>
<tbody>
<tr>
<td>Chuquisaca</td>
<td>29</td>
<td>10.3</td>
<td>75.9</td>
<td>10.3</td>
<td>3.4</td>
<td>10.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Cochabamba</td>
<td>33</td>
<td>0</td>
<td>93.9</td>
<td>0</td>
<td>6.1</td>
<td>0</td>
<td>6.1</td>
</tr>
<tr>
<td>La Paz</td>
<td>18</td>
<td>11.1</td>
<td>33.3</td>
<td>44.4</td>
<td>11.1</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Potosi</td>
<td>14</td>
<td>14.3</td>
<td>57.1</td>
<td>28.6</td>
<td>0</td>
<td>28.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Tarija</td>
<td>8</td>
<td>0</td>
<td>62.5</td>
<td>25.0</td>
<td>12.5</td>
<td>25.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Bolivia total</td>
<td>102</td>
<td>6.9</td>
<td>70.6</td>
<td>16.7</td>
<td>5.9</td>
<td>16.7</td>
<td>5.9</td>
</tr>
</tbody>
</table>
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