RESEARCH/INVESTIGACIÓN

OCCURRENCE AND DISTRIBUTION OF *GLOBODERA* SPP. IN BOLIVIA

C. Sainz[1†], C. L. Villarroe[1†], I. A. Zasada[2]*, L. M. Dandurand[3], J. Kuhl[3], R. Silvestre[3], C. Hesse[4], A. B. Peetz[1], T. Bendetti[5], H. V. Baker[1], and S. A. Subbotin[6]

[1]Biointegra Consulting, Cochabamba, Bolivia; [2]USDA-ARS Horticultural Crops Disease and Pest Management Research Unit, Corvallis, OR; [3]University of Idaho, Moscow, ID USA; [4]USDA-ARS Horticultural Crops Production and Genetic Improvement Research Unit, Corvallis, OR USA; [5]Oregon State University, Corvallis, OR USA; [6]California Department of Food and Agriculture, Plant Pest Diagnostic Center, Sacramento, CA USA; †Co-first authors; *Corresponding author: inga.zasada@usda.gov

ABSTRACT

Sainz, C., C. L. Villarroe, I. A. Zasada, L. M. Dandurand, J. Kuhl, R. Silvestre, C. Hesse, A. B. Peetz, T. Bendetti, H. V. Baker, and S. A. Subbotin. 2023. Occurrence and distribution of *Globodera* spp. in Bolivia. Nematropica 53:82-88.

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^3soil). 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

Sainz, C., C. L. Villarroe, I. A. Zasada, L. M. Dandurand, J. Kuhl, R. Silvestre, C. Hesse, A. B. Peetz, T. Bendetti, H. V. Baker, and S. A. Subbotin. 2023. Ocurrencia y distribución de *Globodera* spp. en Bolivia. Nematropica 53:82-88.

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

1008 quistes/250 cm^3de suelo) en comparación con Potosí y Tarija (~85 quistes/250 cm^3 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

INTRODUCTION

Potato is Bolivia's most important food crop (Coca-Morante, 2019). It is grown on approximately 180,000 ha by thousands of small-holder 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, Boliva), 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 cm^3 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

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 (5′ – ACA ACA GCA ATC GTC GAG - 3′), PITSr3 (5′ – AGC GCA GAC ATG CCG CAA - 3'), and ITS5 (5′ – GGA AGT AAA AGT CGT AAC AAG G - 3′) 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 transluminator (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 cm^3 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.

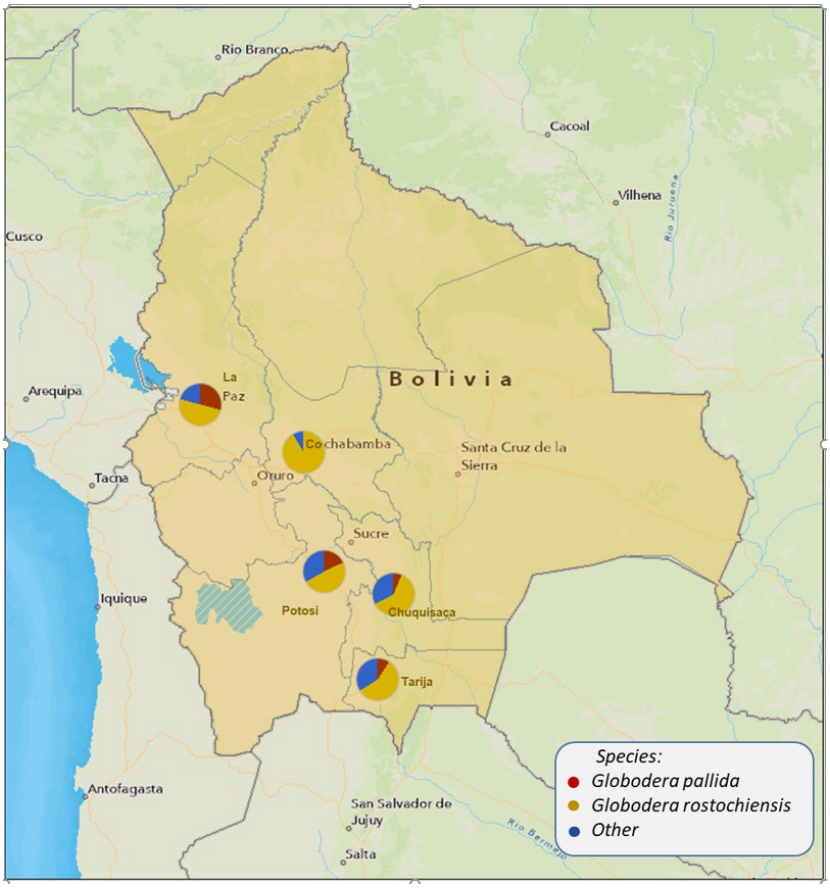

Figure 1. Distribution of *Globodera* spp. in Bolivia (n = 765 cysts from 102 fields). Circles in each department represent the percentage of populations identified as *G. rostochiensis*, *G. pallida*, and other/no amplification.

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. (~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

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

Department	Fields (#)	Occurrence (%)	Mean (#) cysts/250 cm^3 soil when present	Max (#) cysts/250 cm^3 detected
Chuquisaca	29	96.7	297 a[z]	1,375
Cochabamba	48	81.3	444 a	2,835
La Paz	25	76.0	1,008 a	5,158
Potosí	18	88.9	84 b	180
Tarija	24	66.7	85 b	253
Total Bolivia	144	82.1	410	5,158

[z]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 2. Identification of cysts of *Globodera* spp. in the Bolivian departments.

Department	DNA samples (#)[z]	*Globodera pallida* (%)	*Globodera rostochiensis* (%)	NA (%)
Chuquisaca	236	6.4	61.0	32.6
Cochabamba	224	0.0	91.5	8.5
La Paz	140	29.3	50.0	20.7
Potosí	112	17.9	49.1	33.0
Tarija	53	9.4	56.6	34.0
Bolivia total	765	10.6	65.9	23.5

[z]DNA was extracted from single cysts.

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 3. Distribution of *Globodera* spp. in potato fields in Bolivian departments.

Department	Fields (#)	*G. pallida* (%)	*G. rostochiensis* (%)	*G. pallida* + *G. rostochiensis* (%)	NA (%)
Chuquisaca	29	10.3	75.9	10.3	3.4
Cochabamba	33	0	93.9	0	6.1
La Paz	18	11.1	33.3	44.4	11.1
Potosi	14	14.3	57.1	28.6	0
Tarija	8	0	62.5	25.0	12.5
Bolivia total	102	6.9	70.6	16.7	5.9

in 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: AP20PPQS&T00C079 (agreement no. 20-0255-000-FR). We also appreciate the Bolivian famers who allowed us access to their fields.

LITERATURE CITED

Bulman S. R., and J. W. Marshall. 1997. Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). New Zealand Journal of Crop and Horticultural Science 25:123–129.

Brodie B. B., and W. F. Mai. 1989. Control of the golden nematode in the United States. Annual Review Phytopathology 27:443–61.

Canto, M., and M. Scurrah. 1977. Races of the potato cyst nematode in the Andean region and a new system of classification. Nematologica 23:340-349.

CIP-International Potato Center. 1976. CIP Annual Report 1975. Lima, Peru. https://hdl.handle.net/10568/109465.

Coca-Morante, M. 2019. Potato production system in the Andean region of Bolivia: Modern seed potato production, the use of agricultural technology, and genetic erosion. Research & Reviews: Journal of Agriculture and Allied Sciences 8:86-95.

Evans, K., J. Franco, and M. De Scurrah. 1975. Distribution of species of potato cyst nematodes in South America. Nematologica 21:365-369.

Fenwick, D. W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. Journal of Helminthology 18:155-172.

Franco, J., and A. González. 2011. Pérdidas causadas por el nemátodo quiste de la papa (*Globodera* sp.) en Bolivia y Perú. Revista Latinoamericana de la Papa 16:233-249.

Grenier, E., S. Fournet, E. Petit, and G. Anthoine. 2010. A cyst nematode "species factory" called the Andes. Nematology 12:163–69.

Hawkes, J. G. 1990. The Potato: Evolution, Biodiversity and Genetic Resources. Belhaven Press: London, England.

Hesse, C. N., I. Moreno, O. Acevedo Pardo, H. Pacheco Fuentes, E. Grenier, L. M. Dandurand and I. A. Zasada. 2021. Characterization of *Globodera ellingtonae* populations form Chile utilizing whole genome sequencing. Journal of Nematology 53:e2021-88.

Lax, P., J. C. Rondan Dueñas, J. Franco-Ponce, C. N. Gardenai, and M. E. Doucet. 2014. Morphology and DNA sequence data reveal the presence of *Globodera ellingtonae* in the

Andean region. Contributions to Zoology 83:227–43.

Pacajes, G., J. Franco, R. Esprella, and G. Main. 2002. Efecto de diferentes cultivos y prácticas culturales sobre la multiplicación del nemátodo quiste de la papa (*Globodera* spp.) en Bolivia. Revista Latinoamericana de la Papa 13:52-65.

Plantard, O., D. Picard, S. Valette, M. Scurrah E. Grenier, and D. Mugniéry. 2008. Origin and genetic diversity of Western European populations of the potato cyst nematode (*Globodera pallida*) inferred from mitochondrial sequences and microsatellite loci. Molecular Ecology 17:2208–2218.

Silvestre, R., L. M. Dandurand, I. A. Zasada, J. Franco, and J. C. Kuhl. 2021. An assessment of potato cyst nematode (*Globodera* spp.) research from the Andean Region of South America. Part 1: Occurrence and impact. Nematropica 51:85-105.

Skantar, A. M., Z. A. Handoo, L. K. Carta, and D. J. Chitwood. 2007. Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. Journal of Nematology 39:133-144.

Subbotin, S. A., J. Franco, R. Knoetze, T. V. Roubtsova, R. M. Bostock, and I. Cid del Prado Vera. 2020. DNA barcoding, phylogeny and phylogeography of the cyst nematode species from the genus *Globodera* (Tylenchida: Heteroderidae). Nematology 22:269-297.

Received: *Accepted for publication:*

21/V/2023 *3/VIII/2023*

Recibido: *Aceptado para publicación:*