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PHYTOPARASITIC AND FREE-LIVING NEMATODES ASSOCIATED WITH THE CULTIVATION OF *Passiflora ligularis* JUSS. IN THE SANDIA VALLEY, PUNO REGION, PERU

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ABSTRACT

The identification of phytoparasitic nematodes, which is essential for the implementation of management strategies is required. The objective of this work was to identify, at the genus level, the phytoparasites and free-living nematodes associated with the cultivation of sweet granadilla (*Passiflora ligularis* Juss.) in the valley of Sandia, region Puno, Peru. For the study, 165 soil samples were evaluated, coming from eight communities from the district of Sandia, during the agricultural season 2018–2019. The samples were processed by the method of centrifugal flotation in sucrose solution, and later identified on the basis of morphological characteristics. The results showed the presence of seven genera of phytoparasitic nematodes: *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Mesocriconema*, *Xiphinema*, *Tylenchus,* and *Hemicycliophora*, and two genera of free-living nematodes, *Aphelenchus* and *Dorylaimus.* The most harmful genera were *Meloidogyne* and *Pratylenchus* because they cause damage to other crops. In the nematode community, there was variability in the density and frequency of phytoparasitic and free-living individuals in the evaluated localities.
Additional Keywords: Morphological characterization, native fruit, plant parasitic nematodes

RESUMEN

Nematodos fitoparásitos y de vida libre asociados al cultivo de granadilla (*Passiflora ligularis* Juss.) en el valle de Sandia, Región Puno, Perú

El estudio de identificación de nematodos fitoparásitos es esencial para la implementación de estrategias de manejo. El objetivo de este trabajo fue identificar, a nivel de género, los nematodos fitoparásitos y de vida libre asociados al cultivo de granadilla (*Passiflora ligularis* Juss.) en el valle de Sandia, región Puno, Perú. Para el presente estudio se evaluaron 165 muestras de suelo, provenientes de ocho comunidades del distrito de Sandia, durante la campaña agrícola 2018-2019. Las muestras fueron procesadas por el método de flotación centrífuga en solución de sacarosa, y posteriormente fueron identificadas con base en características morfológicas. Los resultados mostraron la presencia de siete géneros de nematodos fitoparásitos: *Meloidogyne, Pratylenchus, Helicotylenchus, Mesocriconema, Xiphinema, Tylenchus* y *Hemicycliophora*, y dos géneros de nematodos de vida libre identificados como *Aphelenchus* y *Dorylaimus*. Los géneros más perjudiciales fueron *Meloidogyne* y *Pratylenchus* debido a que ocasionan daños en otros cultivos agrícolas. En la comunidad de nematodos, existe variabilidad en la densidad y frecuencia de individuos fitoparásitos y de vida libre en las localidades evaluadas.
Palabras clave adicionales: Caracterización morfológica, frutal nativo, nematodos parásitos de plantas

INTRODUCTION

Sweet granadilla (*P. ligularis*) is a native fruit plant originating from the Andes region of Peru and Colombia and is currently distributed from Argentina to Mexico. It grows well in a moderately cold climate with temperatures of 14 to 24°C and humidity of 75%. It does not tolerate strong heat and requires deep and fertile soils with good aeration, a loamy or sandy loam texture, high organic matter content, and the pH value between 6 and 6.5. It can grow in areas where the elevation is between 900 and 2700 meters above sea level. The minimum required rainfall is 1500 mm per year (Suárez et al., 2015).

In Peru, the export of granadilla fruit had increased by 14.5% compared to 2017 (ADEX,

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2018). Production is concentrated in the Pasco region, with 1917 t, Junín 1083 t, Cajamarca 590 t and Huánuco 168 t. In Puno, Sandia Valley, during 2017-2018, the production of granadilla was 1200 t (DRAP, 2019).

Phytoparasitic nematodes represent an important health problem in the production of granadilla, as they reduce the root system, inhibit the normal input of nutrients, and reduce the productivity of the plant. The loss level depends fundamentally on the density of the population in the soil, crop susceptibility, and environmental conditions (such as temperature and humidity) (Sánchez, et al., 1993). The drop in production caused by root-knot nematode species in crops varies from 15 to 60 %. This means significant economic losses, although the interaction of nematodes with other pathological problems, such as fungi and bacteria, can cause even greater damage (Cornejo et al., 2021).

Among the nematodes associated with the cultivation of granadilla, the genera *Pratylenchus, Helicotylenchus,* and *Meloidogyne* have been found in greater frequency and population density, prevailing by their wide dissemination, with *M. incognita* and *M. javanica* significantly damaging the yield and quality of the granadilla crop (Ortiz et al., 2013). Victoria and Vijaya (2021) associated *Meloidogyne* and *Helicotylenchus* with the cultivation of granadilla. The objective of this work was to identify, at the genus level, the phytoparasites and free-living nematodes associated with the cultivation of sweet granadilla (*P. ligularis* Juss.) in the valley of Sandia, Puno region, Peru.

MATERIALS AND METHODS

The soil sampling places were selected according to the importance of crop for the region, the area of land, similar or homogeneous by conditions of topography and drainage. A total of 165 soil samples were collected from eight communities: Apabuco, Laqueque, Tuana, Mororia, Ccapuna, Quiaca Ayllu, Aricato, and Queneque in the Sandia Valley (Figure 1).

From each field were collected five subsamples, 500 g of soil were extracted, by walking the area in a zigzag pattern and opening the soil in a V shape at a depth of 20 to 30 cm with the help of a hoe. The samples were later homogenized and conditioned in resistant, closed, and properly identified plastic bags. The soil had an intermediate texture (sandy clay loam, silt loam and loam).

The extraction of nematodes from the soil (250 cm^3) was carried out using the method of Jenkins (1964). The samples were washed and decanted in sieves with 400, 100 and 60 μm openings. The material retained in the last sieve was deposited in tubes for centrifugation, subjected to the clarification process by centrifugal flotation in sucrose, and then sifted. The suspension of the extracted nematodes was conditioned in glasses (50 mL) and kept in refrigeration (6 °C) in formalin 0.1 % to quantify and identify the nematode genera, using a microscope Velab Prime, with camera OD400UHW-P.

The identification was performed in the Entomology-Nematology Laboratory of the Professional School of Agronomic Engineering of the National University of the Altiplano Puno, Peru, between January and March 2019. To identify the nematode genera, morphometric and morphological criteria were considered, such as presence and size of the stylet (st), length of the body (L), shape of the head and tail, and the distribution of internal organs) based on the dichotomous key of Mai and Mullin (1996). The nematode community of the localities of the study was evaluated by absolute frequency (f_i) and relative frequency (fr_i).

RESULTS

Seven genera of phytoparasitic nematodes were identified in the present study: *Meloidogyne* sp., *Pratylenchus* sp., *Helicotylenchus* sp., *Mesocriconema* sp., *Xiphinema* sp., *Tylenchus* sp., and *Hemicycliophora* sp., as well as two free-living nematodes, *Aphelenchus* sp. and *Dorylaimus* sp.

The juveniles in stage 2 (J2) of the genus *Meloidogyne* sp. had a cylindrical shape and were vermiform (Figure 2A). The anterior part of the body of the J2 was pointed, and they presented a rounded labial disc, a stylet with strong basal nodules (Figure 2B), visible esophageal gland (Figure 2C), and conical tail with a hyaline ending as the main characteristics (2D). Morphometry: L = 420 μm; st = 11.8 μm.

The genus *Pratylenchus* presented the following morphological characteristics: cylindrical body (Figure 3A), angled labial region, narrower than the diameter of the body, stylet with spherical basal nodules (Figure 3B), position of the vulva closer to the end of the body (3C), and rounded conical tail (Figure 3D). Morphometry: L = 680.5 µm; st = 17.0 µm.

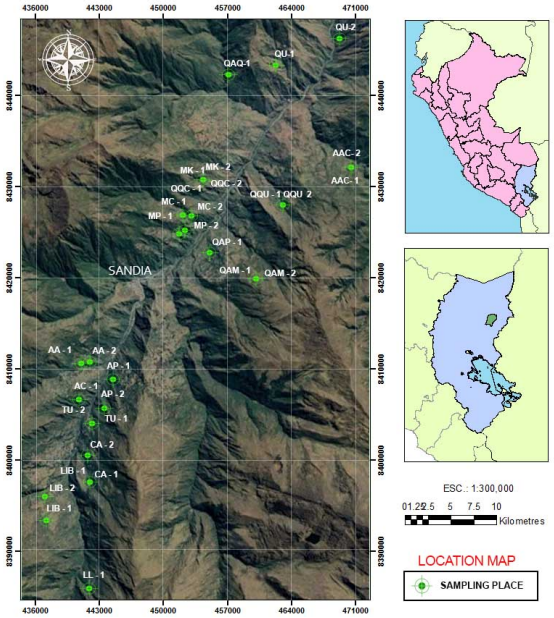

Figure 1. Location map of evaluated communities (Apabuco, Laqueque, Tuana, Mororia, Ccapuna, Quiaca, Aricato and Queneque) of Puno region

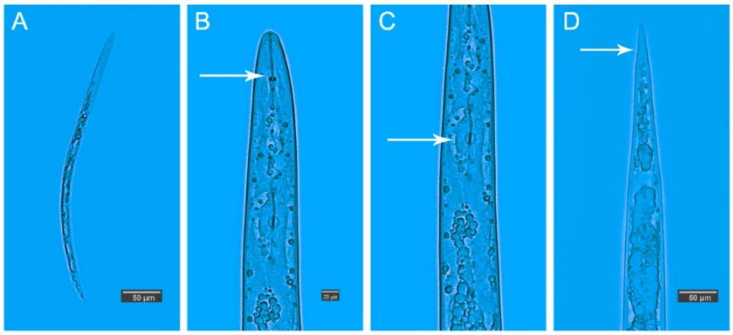

Figure 2. A: Whole body of the genus *Meloidogyne* sp. B: Anterior part of *Meloidogyne* sp. (J2) showing the labial part, stylet and bulb. C: Esophagus D: Posterior part, showing the tail and hyaline zone

The genus *Helicotylenchus* presented the following characteristics: body in a state of rest acquires a spiral position or the number "6" (Figure 4A), a well-developed cephalic structure, a high, rounded conical head region with a robust stylet (Figure 4B), and generally curved tail termination with a slight ventral projection (Figure 4C), and vulva (Figure 4D). Morphometry: L = 548.5 µm; st = 21 µm.

Mesocriconema sp. presented a short body with an intensely ringed thick cuticle (Figure 5A), showing a large and strong stylet, well-defined basal nodules (Figure 5B), the end of the tail in a conical shape (Figure 5C). Body with visible esophageal glands (Figure 5D). Morphometry: L = 490 µm; st = 59 µm.

Individuals of the genus *Xiphinema* presented a fairly long body (Figure 6A), and head with a hollow and bifurcated stylet at the point of attachment with the odontophore, known as the odontostyle, with three basal extensions and a guide ring (Figure 6B). The tail was slightly bent

to the ventral part (Figure 6C), and vulva with a superimposed lip (Figure 6D). Morphometry: L = 2050 µm; st = 152.8 µm.

Tylenchus sp. specimens were thin, with the particularity that the body at death had a straight or slightly curved shape (Figure 7A). The stylet had small basal knobs (Figure 7B). The esophagus had a medium muscular bulb. The long cone-shaped tail had a pencil-shaped termination and a bent tip (Figure 7C). The vulva is well visible (Figure 7D). Morphometry: L = 1040 µm; st = 12 µm.

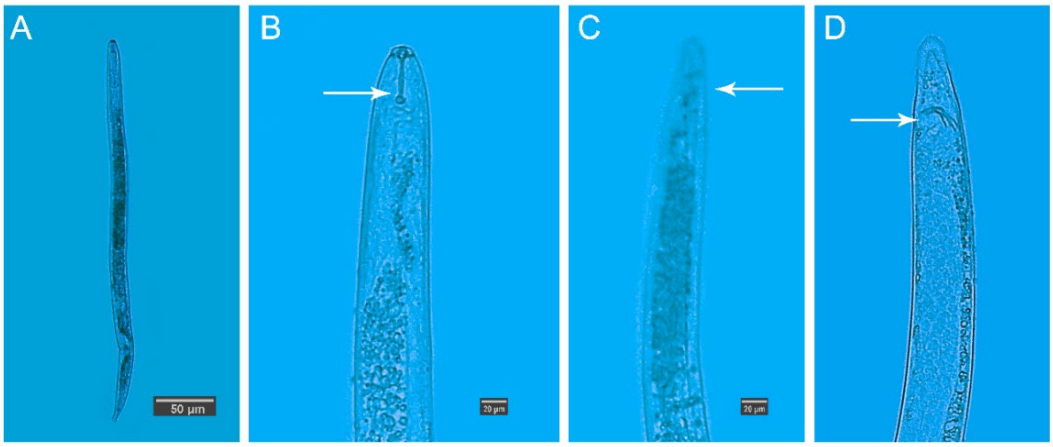

Figure 3. A: Complete body of the genus *Pratylenchus* sp. B: Anterior part of *Pratylenchus* sp. showing the labial part, stylet and bulb. C: Posterior part, showing the shape of the tail. D: Spicule of the male. D:

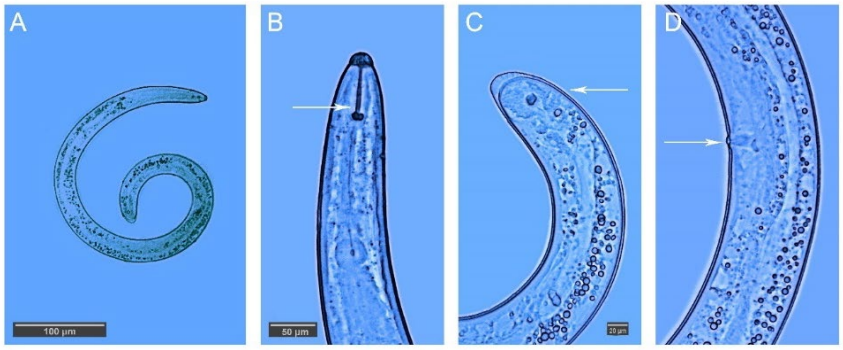

Figure 4. A: Whole body of the genus *Helicotylenchus* sp. B: anterior part of *Helicotylenchus* sp. showing the labial part, stylet and bulb. C: Posterior part, showing the shape of the tail (without mucron). D: Vulva

Hemicycliophora sp. had a body with a kind of double cuticle; the second one was loose with a long stylet (Figure 8A, B). The vulva was notably visible (Figure 8C). These individuals also had a blunt-shaped tail with a short stinger at the tip (Figure 8D). Morphometry: L = 1510 µm; st = 110 µm.

Aphelenchus sp. presented the following characteristics: a more or less straight cylindrical body, slightly arched (Figure 9A), thin visible stylet without nodules (Figure 9B), visible esophagus (Figure 9C), and a rounded tail (Figure 9D). Morphometry: L = 638 µm; st = 13.9 µm.

Dorylaimus sp. had a fairly long body (Figure 10A), bottle-shaped lips and odontostyle (Figure 10B, C), and slightly curved and blunt tail end (Figure 10D). Morphometry: L = 2010 µm; st = 180 µm (odontostyle).

Additionally, others free-living nematodes associated with the cultivation of sweet granadilla in the Sandia Valley in the Puno region, were unidentified for having morphological characters not previously described.

In the evaluated localities, there was variability in the nematode density with high values of specimens in the phytoparasitic *Helicotylenchus* and *Mesocriconema* and lower quantities in the genus *Dorylaimus*, and variability in the frequency of phytoparasitic and free-living nematodes. Nematodes with a high population frequency were free-living (48.10%), followed by phytoparasites *Helicotylenchus* (23.9%), *Mesocriconema* (18.5%), and *Pratylenchus* (3.5%); the other genera were found at low percentages (Table 1).

DISCUSSION

These results were related to those mentioned by Perry and Moens (2014) and Peraza (2021), who indicated that the genus *Meloidogyne* in its juvenile infective state (J2) is vermiform in shape, with a cone-shaped anterior part rounded with a narrow and rounded termination, varying its body length between 250 and 600 µm and stylet length of 6 to 16 µm.

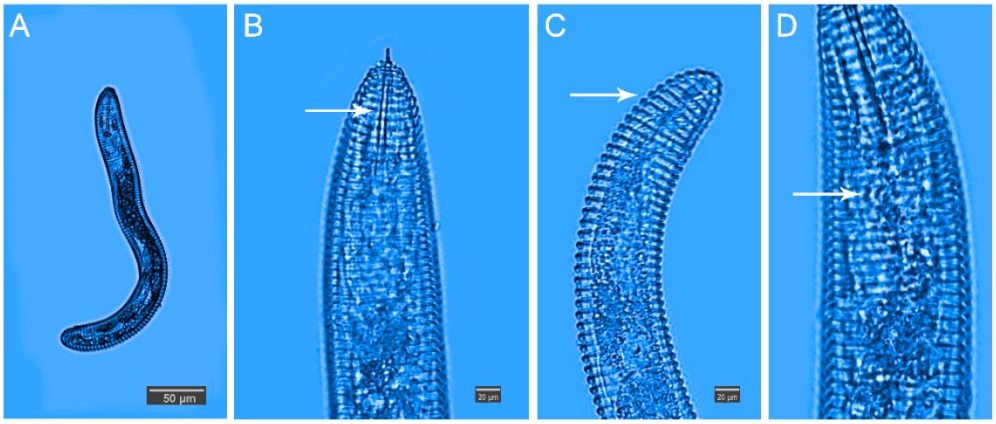

Figure 5. A: Complete body of the genus *Mesocriconema* sp. B: Anterior part of the nematode showing the labial part and stylet. C: Posterior part, showing the shape of the tail. D: Esophagial gland

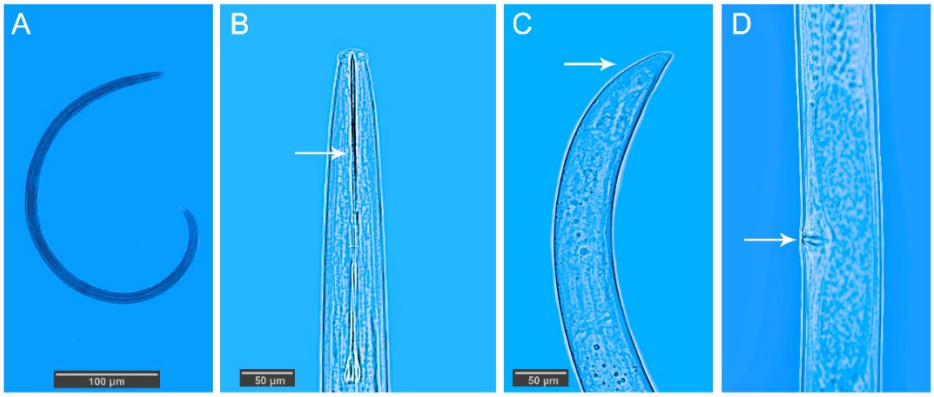

Figure 6. A: Complete body of *Xiphinema* sp. B: Anterior part showing the labial part, with odontostyle. C: Posterior part, showing the shape of the tail. D: Vulva

Jesus et al. (2020) described *Pratylenchus* as individuals with a cylindrical and robust body with a rounded tail, the latter being a characteristic that may change according to the species of the genus, with lengths ranging from 400 to 700 µm. The data confirm the results obtained in the present study.

Castillo and Vovlas (2008), and Uzma et al. (2015) described that in the inactive state (rest), *Helicotylenchus* spp. acquires a spiral position. Our results coincide with Uzma et al. (2015) and Cornejo et al. (2021), who indicated that the body of *Helicotylenchus* can measure between 3500 and 1200 µm and that they also present characteristics

such as a rounded head, a robust stylet approximately 3 to 4 times the width of the head region, and a typical shape wrapped in a spiral.

The morphological characteristics of *Mesocriconema* sp. resembled those mentioned by Peraza (2014), who stated that they are thick, cuticle nematodes with a short, robust body. Likewise, Divers et al. (2019) and Forge et al. (2020) indicated that *Mesocriconema* sp. have an intensely ringed thick cuticle, with an average body length of 241.12 to 619.75 µm and average stylet length of 42.4 to 91.62 µm.

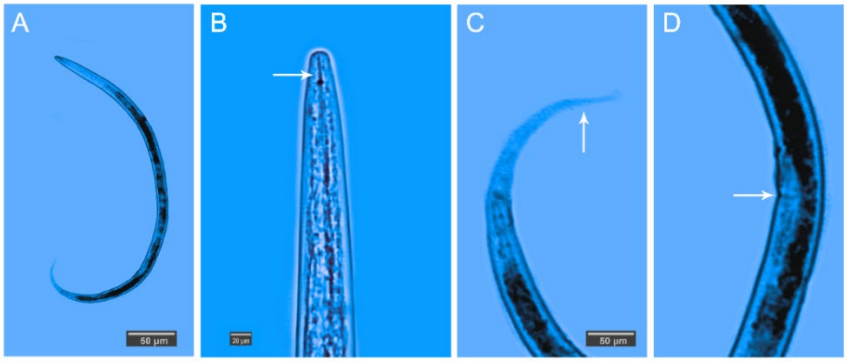

Figure 7. A: Complete body of the genus *Tylenchus* sp. B: Anterior part showing the lip and stylet. C: Posterior part, showing the shape of the tail. D: Vulva

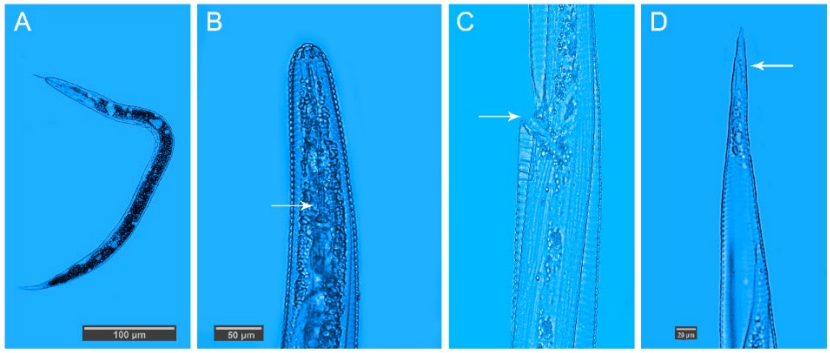

Figure 8. A: Whole body of the genus *Hemicycliophora* sp. B: Anterior part showing the lip and stylet. C: Vulva. D: Posterior part, showing the shape of the tail

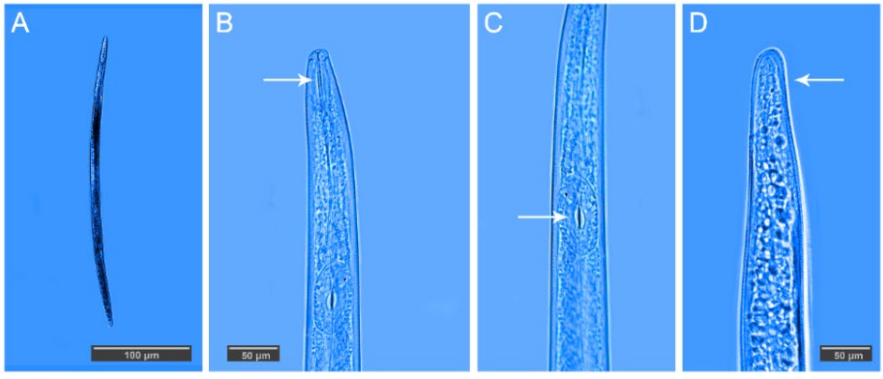

Figure 9. A: Whole body of the genus *Aphelenchus* sp. B: Anterior part showing the lip and stylet. C: esophagus. D: posterior part, showing the shape of the tail

As for *Xiphinema* sp., the results agree with Coomans et al. (2001), Ravichandra (2014), and García et al. (2015). Similarly, the characteristics coincide with the descriptions made by Lamberti

et al. (2004), who indicated that the length of the body is 1.2 to 3.0 µm, the shape of the body varies from a "C" more or less open to a spiral in the state of relaxation induced by heat, rarely a continuous labial region, usually delimited by a smooth depression or deep constriction, robust odontostyle rarely greater than 150 µm in length, and esophageal bulb usually with thickening of the cuticular layer that covers the lumen wall. Similar studies showed a value of 158.2 µm for a stylet of this genus (Garambel et al., 2022).

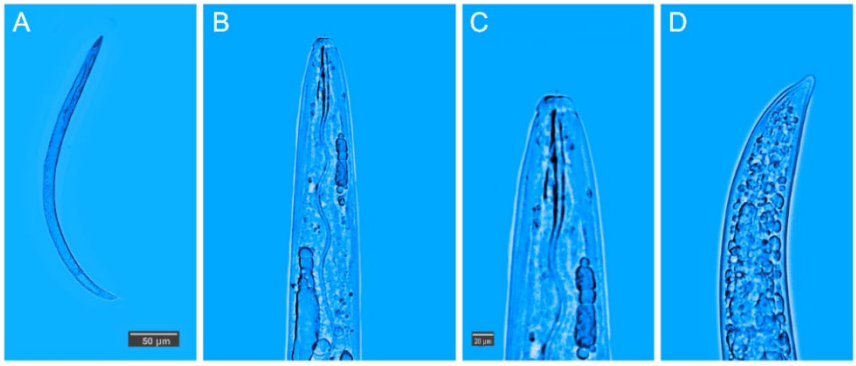

Figure 10. A: Complete body of the genus *Dorylaimus* sp. B: Anterior part of the nematode. C: Labial part and odontostyle. D: Posterior end, showing the shape of the tail

Table 1. Mean number of nematode specimens per sample, absolute frequency (fi) and relative frequency (fr) of plant-parasitic and free-living nematodes found in sweet granadilla crops

	Apabuco	Laqueque	Tuana	Mororia	Ccapuna	Quiaca Ayllu	Aricato	Queneque	**fi**	**fr**
Meloidogyne sp	1.1	0.0	2.7	0.4	1.2	1.0	1.6	1.4	9.4	1.2
Pratylenchus	2.6	0.8	4.3	3.2	6.3	1.5	3.1	5.5	27.3	3.5
Helicotylenchus	19.9	22.3	24.4	20.0	20.5	32.0	23.3	26.1	188.5	23.9
Mesocriconema	20.8	17.1	24.4	13.9	19.5	18.0	11.0	20.9	145.6	18.5
Aphelenchus	1.7	1.1	0.0	0.0	0.0	0.2	3.9	1.3	8.2	1.0
Dorylaimus	1.3	0.8	1.2	1.3	0.0	0.0	0.0	0.5	5.1	0.6
Xiphinema	3.7	0.0	0.0	0.6	3.2	0.1	0.4	0.0	8.0	1.0
Tylenchus	1.5	2.0	0.0	0.1	1.2	0.2	0.3	4.5	9.8	1.24
Hemicicliophora	2.0	1.7	0.0	0.6	0.0	1.2	1.0	0.3	6.8	0.9
Free-living	49.5	40.3	50.5	40.8	46.9	41.9	48.3	60.6	378.8	48.10
Total									787.5	100.0

Hajizadeh et al. (2015) and Siddiqi (2000) stated that the genus *Tylenchus* is thin and has a straight or slightly curved shape that measures an average of 1043 µm when dying. The stylet has small basal knobs. The tail is long and cone-shaped with a pencil point ending. These features are corroborated by Bert and Geraert (2000). The hooked or curved tip of the tail is a particular characteristic of *Tylenchus*. These descriptions are within the range of the details described in this work.

Hoyos and Moya (2010) indicated that the most outstanding characteristics for identifying the genus *Hemicycliophora* are the length of the stylet, the presence of the filled spermatheca, and the shape of the tail. Likewise, Raski and Luc (1987) indicated that nematodes of this genus are characterized by a fairly soft epidermal layer, which is always present, generally loose, and not membranized. The length of the stylet is 108–120 µm, sloping back with a labial ring, without modification or separation. The vulva has a transverse fissure, which is half the diameter of the body in females. Caudal wings cover less than one-third of the tail. This indicates that it resembles the specimens found in the present investigation.

In relation to the genus *Aphelenchus*, Chaturvedi and Khera (1979) and Cornejo et al. (2021) described the morphometry of females with lengths varying from 580 to 740 µm, with a

stylet of 14–18 µm. There was a minimal difference in the length of the stylet, indicating the diversity that could exist within the same population.

Cornejo et al. (2021) indicated that the *Dorylaimus* genus nematodes have a labial region sitting in a deep constriction with a 196.7 µm long stylet and 4030 µm body length, and the tail is slightly curved and rounded. The latter characteristic may fluctuate due to abiotic conditions. These characteristics are similar to those indicated by Sen et al. (2011), who reported that in males, the upper end is ventrally more curved than in females, with blunt termination. The length of the body in males and females ranged from 3900 to 4200 µm, and the length of the stylet can vary from 174 to 210 µm, while in the smallest species, the length of the body measures from 1100 µm in all its stages (Islam and Ahmad, 2021), both authors stated that this genus is found in soils with high humidity and / or organic matter. This confirms that these characteristics belong to the genus *Dorylaimus*, which was found in our investigation.

Neher (2010) mentioned that free-living nematodes have fairly long body lengths with a cylindrical body and thick cuticle and do not have a stylet but a pseudo- stylet. These results coincide with those mentioned by Tihohod (2000), who stated that they do not have a stylet but rather an odontostyle (odontophore) and that the body has a cylindrical and sometimes round shape. Cornejo et al. (2021) confirmed that these organisms do not have a stylet but rather an odontostyle and that the body has a cylindrical and sometimes round shape. These characteristics coincide with those found in the present study.

For the community of nematodes, Yeates and Bongers (1999) indicated that monocultures tend to favor certain genera of phytonematodes. The results of this study confirm this. For the cultivation of granadilla, there was a high frequency of the phytoparasites *Helicotylenchus* and *Mesocriconema*. In relation to the other genera, Sikandar et al. (2021) reported that the phytoparasites *Heterodera*, *Tylenchorhynchus,* and *Helicotylenchus* were the most prevalent and had higher density than other genera. The high density of free-living nematodes found in this study allows us to infer that they may act in processes such as biological control, reducing the populations of phytoparasitic nematodes that were found at low frequency.

Meloidogyne and Pratylenchus are considered to have a wide geographical distribution and cause damage of economic importance to various crops; however, according to their relative frequency (Table 1), they are currently not a problem for sweet granadilla farmers in the Sandia district. Nevertheless, these nematodes and others must be identified, because over the years those genera can cause productivity losses in agricultural crops (Salazar and Guzmán, 2013). The low population of nematodes found may be due to the fact that remains of organic matter are frequently added in crops in these localities, because it is a region with organic production system. In other crops, the incorporation of organic matter and cultural management in addition to the use of resistant varieties are factors that allow reducing populations of plant-parasitic nematodes in field soils (Curay et al., 2021). Free-living nematodes allow reducing populations of phytoparasitic nematodes (Khan and Kim, 2007). In the present study, the population density of free-living nematodes is high, which may be contributing to the decrease in phytoparasitic nematodes.

The genera *Helicotylenchus* and *Mesocriconema* have considerable population density, those genera have been causing serious problems in others agricultural crops around the world, including bananas, where *Helicotylenchus* reduces production by between 19 and 34% (Guzmán, 2011). After *Meloidogyne*, the *Helicotylenchus* genus was the second most prevalent plant-parasitic nematodes, in studies carried out in the cultivation of greenhouse vegetables, plant diseases caused by *Helicotylenchus* were present 81.6% of all crops examined (Tileubayeva et al., 2021). The plant-parasitic nematodes, *Helicotylenchus* and *Mesocriconema* widely occur in Korean turfgrass fields causing damage (Kabir et al., 2021). These findings serve for monitoring and management of plant-parasitic nematodes in fields of sweet granadilla crops.

CONCLUSIONS

Seven genera of phytoparasitic nematodes, namely *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Mesocriconema*, *Xiphinema*, *Tylenchus*, and *Hemicycliophora*, and free-living

nematodes, *Aphelenchus* (mycophage) and *Dorylaimus* (omnivore) were identified in fields of sweet granadilla. Variability in the density and frequency of phytoparasitic a free-living nematode were also found.

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