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NEMATODES ASSOCIATED WITH ANDEAN PAPAYA (*Carica pubescens* L.) IN SANDIA DISTRICT, PUNO, PERU

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ABSTRACT

Plant-parasitic nematodes cause 14 % of annual losses in different crops in the world and there are more than 35 genera of phytoparasitic nematodes associated with the rhizosphere of the papaya crop (*Carica* spp.), being the most important the root-knot nematode (*Meloidogyne* spp). The present study aimed to carry out the morphological identification of the genera of nematodes associated with the cultivation of Andean papaya (*Carica pubescens*), in Sandia district, Puno, Peru. One-hundred and fifty-five soil and roots samples from eight communities were evaluated. The samples were processed by the centrifugal fluctuation method in sucrose solution and subsequent identification of the nematodes at the genus level, using the dichotomous key of Mai and Mullin. We found the genus *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Mesocriconema*, *Apelenchus*, *Dorilaymus*, *Tylenchus*, *Xiphinema*, *Mononchus*, *Discocriconemella*, *Trichodorus* and other unidentified free-living nematodes. This information may allow the design of an integrated nematode management plan in Andean papaya crops.
Additional keywords: Genus of nematodes, morphometry, plant parasitic nematodes

RESUMEN

Nematodos asociados a la papaya andina (*Carica pubescens* L.), en el distrito Sandia, Puno, Perú

Los nemátodos fitoparásitos ocasionan el 14% de las pérdidas anuales en diferentes cultivos en el mundo, y más de 35 géneros de fitoparásitos han sido referidos en la rizósfera de la papaya (*Carica* sp.), siendo el más importante el nematodo de las agallas (*Meloidogyne* spp.). El presente estudio tuvo como objetivo identificar morfológica y morfométricamente los géneros de nematodos asociados al cultivo de papaya andina (*Carica pubescens*), en Sandia, region Puno, Perú. Se evaluaron 155 muestras de suelo y raíces provenientes de ocho comunidades del distrito de Sandia. Las muestras colectadas fueron procesadas por el método de fluctuación centrífuga en solución sacarosa, y posteriormente fueron identificados a nivel de género, con la clave dicotómica de Mai y Mullin. Los géneros encontrados fueron *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Mesocriconema*, *Apelenchus*, *Dorilaymus*, *Tylenchus*, *Xiphinema*, *Mononchus*, *Discocriconemella* y *Trichodorus*, así como nematodos de vida libre. Esta información puede ser de utilidad para diseñar un plan de manejo integrado de nemátodos en cultivo de papaya andina.
Palabras clave adicionales: Géneros de nematodos, nematodos parásitos de plantas, morfometría

INTRODUCTION

In southern Perú, Andean papaya is used as food for direct consuming as fresh fruit, for agro-industry and medicinal use. Therefore, it is an important source of economic activity. Its cultivation is carried out by farm families. However, there is little technical information about agronomic management and the problems that limit its production (Gaona and Ramirez, 2013). The papaya crop is characterized by having rapid development in the field with easy propagation, but production can be affected by parasites, which are usually undetectable by the farmer in a timely manner (Ritzinger and Costa, 2000), such as plant phytoparasitic nematodes, which contribute significantly to the reduction of crop productivity.

In general, it is admitted that each fruit of economic importance is affected by various

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phytosanitary problems capable of causing significant losses. Phytopathogens can cause indirect reduction in productivity, due to the debilitating action they exert on the host plant, and they can directly reduce productivity, or even fruit quality (Dias et al., 2008). The attack of phytoparasitic nematodes can cause greater plant damage because their damage can be confused with symptoms of nutritional deficiency or attack of other soil pathogens.

Root-knot nematodes (*Meloidogyne* spp.), root lesion nematodes (*Pratylenchus* spp.) and reniform nematode (*Rotylenchus* spp.) are the most common in papaya crops worldwide (Dias et al., 2008). According to Peña et al. (2018), the most damaging genus are *Meloidogyne* spp. and *Pratylenchus* spp. being the first genus causing great losses in agricultural crops. The genus *Meloidogyne* atrophies papaya root, which does not allow the entry of water and nutrients to the plant, and ultimately causes its death (Dagatti et al., 2014). The stylet of nematodes perforates root cells and releases enzymatic secretions, causing lesions, induction of giant cells resulting in the development of root galls (Hooper, 1974).

Based on the above, limited knowledge of the proper management to obtain high yields of Andean papaya has not allowed the optimal development of the crop. Phytoparasitic nematodes associated with Andean papaya have been little studied. Therefore, the aim of this study was to identify, based on morphological and morphometric features, genus of phytoparasitic and free-living nematodes associated with Andean papaya (*Carica pubescens* L.) in Sandia district, Puno, Peru.

MATERIALS AND METHODS

One hundred and fifty-five samples of root and soil, were collected from thirty-one Andean papaya fields in eight communities (Apabuco, Laqueque, Tuana, Mororia, Ccapuna, Quiaca Ayllu, Aricato and Queneque) from Sandia district, Puno, Peru (Figure 1). The sampling took place in January 2019. Each papaya field was sampled in a zigzag manner. The samples consisted of 500 g of soil and 200 g of papaya roots taken from a V-shape opening in the ground 15-20 cm deep (Lima, 2018). Subsequently, the samples were taken to the Nematology Laboratory of the Professional School of Agronomic Engineering, Universidad Nacional del Altiplano, Puno, for processing.

The extraction of nematodes from samples were performed following the method of Jenkins (1964) from subsamples of 250 cm^3 of soil. After centrifugation, the suspension of the extracted nematodes was kept at 6 °C for identification. We based the nematode identification on the dichotomous key of Mai and Mullin (1996). Four main morphometric and morphological criteria were considered for identification: presence and size of the stylet, body length, head and tail shape, and the distribution of internal organs. For measurements, images were obtained using a microscope and camera Eurolab, with ImageJ IJ1.46r acquisition software.

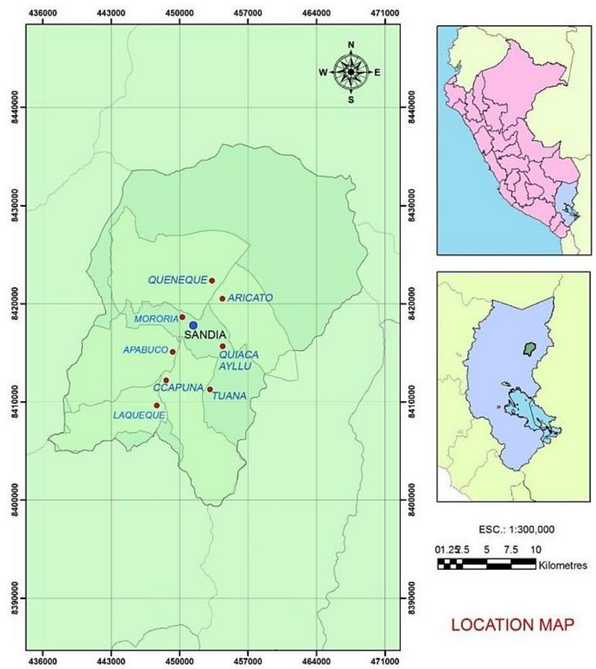

Figure 1. Map of the location and collection of samples in Sandia, Peru. Each dot represents one community

RESULTS

The genus of nematodes genus identified in crop areas of Andean papaya were *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Tylenchus*, *Trichodorus*, *Xiphinema*, *Mesocriconema*, *Discocriconemella*, *Dorilaymus*, *Aphelenchus and Mononchus*. Other free-living nematodes were found as well.

Morphological and morphometric identification. *Meloidogyne* spp. extracted from root and soil samples were analyzed in juvenile state J2; these specimens have a cylindrical and vermiform shape (Figure 2A). They have a rounded labial disc, stylet and basal nodules; the cephalic region does not bear rings (Figure 2B,C). The glands of the esophagus ventrally overlap the intestine (Figure 2D). It has a gradually tuned tail (Figure 2E), with a hyaline terminal portion at its back (Figure 2F), and a clearly visible anus (Figure 2G). Measurements of *Meloidogyne* J2 stage individuals were as follows: body length = 348.50 µm; stylet = 13.25 µm; tail length = 43.8 µm; hyaline portion = 7.75 µm (Table 1A).

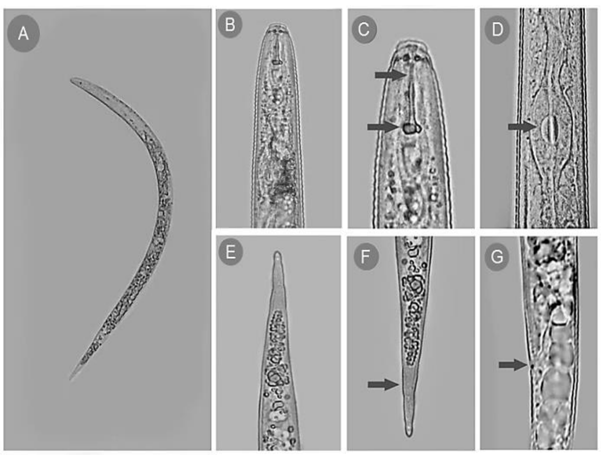

Figure 2. Genus *Meloidogyne* spp. A) Juvenile stage body length; B,C) Anterior region; rounded labial disc, stylet and basal nodules; D) Arrow showing esophagus; E) Posterior region showing tail; F) Hyaline tail region; G) Arrow showing anus

Specimens of the genus *Pratylenchus* have a cylindrical body (Figure 3A), with rounded labial region, a well-developed stylet, and large basal nodules (Figure 3B,C). It also bears defined esophagus lobes in the esophageal glands overlapping with the ventral intestine (Figure 3D). The individuals have a visible excretory pore (Figure 3E); the vulva in in the back (Figure 3F). The shape of the tail is sub-cylindrical, conical with truncated termination (Figure 3G). Measurements of *Pratylenchus* spp. were: body length = 670.35 µm; stylet = 16.9 µm; tail length = 37.6 µm (Table 1B).

Individuals of *Helicotylenchus* spp. have an arc or spiral-shaped body (Figure 4A), rounded conical head without longitudinal striations, stylet with strong basal nodules (Figure 4B,C). Regular phasmids with an excretory pore (Figure 4D). The tail is asymmetric, rounded with a ventral projection (Figure 4E,F). The arrow shows the vulva (Figure 4G). The morphological characteristics confirm the identification of the genus *Helicotylenchus* spp. (Table 1C).

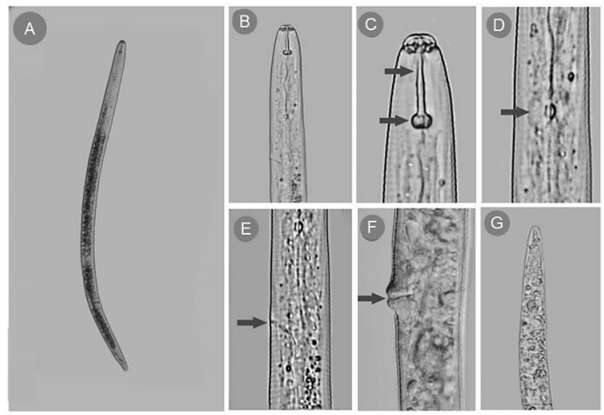

Figure 3. Genus *Pratylenchus* spp.; A) Body length; B,C) Anterior region of the body. Arrows showing stylet and basal nodules; D) Esophagus region; E) Excretory pore; F) Vulva; G) Posterior region showing tail

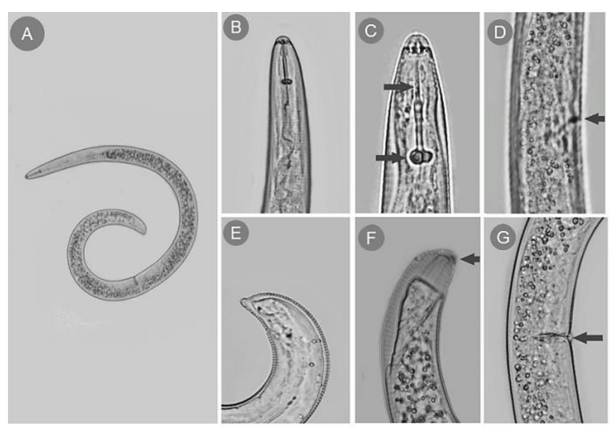

Figure 4. Genus *Helicotylenchus* spp.; A) Body length; B,C) Anterior region showing head and stylet; D) Excretory pore; E) Shape of tail and mucron; F) Tail without mucron; G) Vulva

Mesocriconema spp. individuals have a short, robust and ventrally arched body (Figure 5A). The cuticle is ringed, round and crooked with smooth margins. The labial region is not demarcated from the rest of the body (Figure 5B,C). Two small lip rings and a well-developed stylet were observed (Figure 5D). The esophagus is typical of nematodes in the Criconematidae family (Figure 5E. The vulva closed with a non-ornamented anterior lip. Pointed tail with small but clearly differentiable rings (Figure 5F,G). Average measurements of individuals of *Mesocriconema* spp. were: body length = 502.05 µm; stylet = 70.15 µm; tail length = 27.12 µm; body width = 9.16 µm (Table 1D).

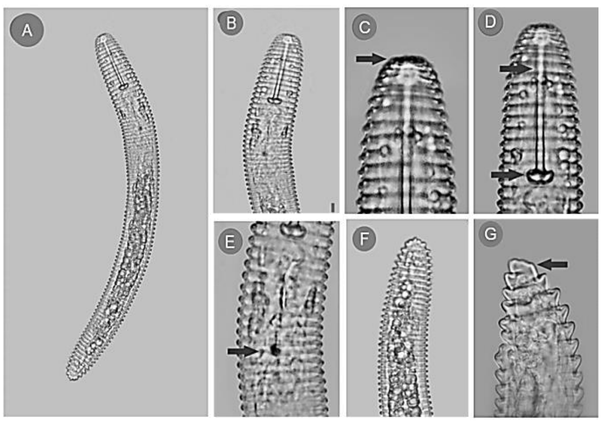

Figure 5. Genus *Mesocriconema* spp. A) Body length; B) Anterior region showing stylet and cuticle; C) Labial region; D) Two small lip rings and well-developed stylet; E) Arrow showing esophagus; F) Tail; G) Tail end

Observed specimens of *Discocriconemella* sp. have a ventrally curved body (Figure 6A). These specimens have an enlarged head ring forming a flattened disc clearly separated from the second ring. The stylet is long and rigid, the knobs have an anchor shape (Figure 6B,C). The esophagus is typical of the genus (Figure 6D). The vulva is closed and cone-shaped (Figure 6E). The posterior edge of the body bears smooth and finely closed rings (Figure 6F,G). The average measurement of *Discocriconemella* spp. were: body length = 328 µm; stylet = 52.8 µm. These characteristics confirm the presence of this genus in soils of Andean papaya crops.

Aphelenchus spp. individuals have a somewhat straight cylindrical body, slightly arched (Figure 7A). The head is round or slightly rounded, with visible and thin stylet, lacking nodules (Figure 7B,C). The esophageal bulb is visible and well-developed; it occupies three quarters of the width of the body (Figure 7D). The tail is rounded (Figure 7E). The picture shows the vulva and male spicule (Figure 7F,G). Average measurements of individuals of *Aphelenchus* spp. were: body legth = 638 µm; stylet = 13.9 µm. These characteristics correspond to genus *Aphelenchus* spp.

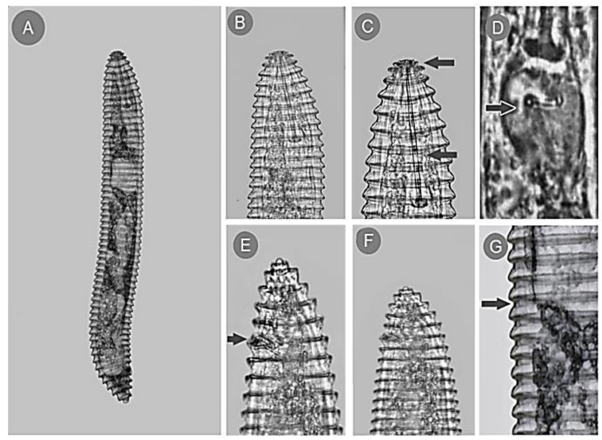

Figure 6. *Discocriconemella* spp. A) Body length; B) Anterior region showing head and stylet; C) Arrow showing disc-shaped cephalic ring and stylet; D) Esophagus region; E) Arrow showing vulva; (F) Posterior region showing tail; G) Arrow showing annulus

Individuals of the genus *Dorylaimus* spp. have an elongated body (Figure 8A) and a false stylet that in the apical part is chamfered (Figure 8B,C). They have a tooth in the cephalic region (Figure 8C). The lip region is slightly deviated from body contour; lips, slightly apart (Figure 8D). The esophagus has a gradual expansion in the form of a bottle, and a pointed tail (Figure 8E,F). The female genital system is amphidelfic, and the vulva has transverse cleft (Figure 8G). Average measurements of *Dorylaimus* spp. were: body length = 790.5 µm; stylet = 174.1 µm. These features correspond to the genus *Dorylaimus* spp.

Xiphinema spp. individuals have a spear and arched body, narrowing towards the extremities (Figure 9A). The esophageal bulb is developed and occupies approximately three quarters of the width of the body. The stylet lacks nodules and is hollow and bifurcated at the junction point with the odontophore, also known as odontostyle (Figure 9B,C,D). Smooth body cuticle. Lip region 10-12 μm in diameter, slightly compensated with the body profile. Vulvar region (Figure 9E) and reproductive system is monodelphic. Thick-walled vagina, hat occupy up to 50 % of the body width, and cone-shaped tail (Figure 9F,G).

The measurements were: body length = 2120 μm; stylet = 150.2 μm; tail length = 80.2 μm. (Table 1E). These characteristics correspond to genus *Xiphinena*.

Tylenchus spp. Individuals have a small and ventrally curved body (Figure 10A), a very long tail with filiform termination (Figure 10B), lateral fields with jagged edges, striated labial region separated from the body region, by a short and narrow space. Small stylet with basal nodules (Figure 10C,D); the esophagus is muscular (Figure 10E); these individuals present the vulva in the middle of the body (Figure 10F). Average measurements of *Tylenchus* spp. were: body length = 528 μm; style = 10.2 μm.

Table 1. Morphometric comparison between the most harmful nematodes collected from Andean papaya (*Carica pubescens* L.) plantations and other studies

	Variable (μm)	*Carica pubescens* L.	Powers et al. (2018)	Uysal et al. (2017)	Perry and Moens (2013)
A*	L	348.5	339 – 576	328 - 465.6	250 - 600
	St	13.25	10.2 - 16.6	11.2 - 14.4	06 - 16
	T	43.8	31.6 – 62	44.8 - 68.8	-
	H	7.75	7 - 13.5	6.4 - 17.6	-
B	Variable (μm)	*Carica pubescens* L.	De Sousa (2018)	Lira, et al. (2014)	De Jesus et al. (2020)
	L	670.35	441.64 - 759.85	482.05 - 750	514.7 - 778.1
	St	16.9	15.89 - 25.32	16.68 – 23.95	14.1 – 21.6
	T	37.6	-	20.39 -37.08	21.3 – 45.1
	Ac	-	-	19.16 – 27.81	21.1 – 37.0
C	Variable (μm)	*Carica pubescens* L.	Uzma et al. (2015)	Kirsch et al. (2016)	Schereck et al. (2010)
	L	610.01	350 – 1110	532.8 - 778.9	510 - 890
	St	24.6	16 – 38	22.5 - 27.0	20 - 26
	T	39.1	-	32.5 - 49.7	-
D	Variable (μm)	*Carica pubescens* L.	Bernardo (2017)	Divers et al. (2019)	Kuhn (2015)
	L	502.05	462,8 – 619,75	241.12 - 588.22	410 - 560
	St	70.15	71,1 - 85,9	42.64 - 91.62	73.0 - 85.0
	T	27.12	16,2 – 28,1	12.47 - 28.45	23.15 - 47.12
	Ac	9.16	-	7,88 -13.99	8.6 - 11.98
E	Variable (μm)	*Carica pubescens* L.	Archidona-Yuste (2016)	Mayta (2017)	Rossi and Ferraz (2005)
	L	2120	1300 – 4300	2050	1712 - 2341
	St	150.2	142 – 218	152.8	148 - 191
	T	80.2	17 – 100	-	40.7 - 86.7

*A: *Meloidogyne* spp., *B: Pratylenchus* spp., *C: Helicotylenchus spp., D: Mesocriconema* spp, *E: Xiphinema* spp.
L (body lenght); St (Stylet length); T (Tail length); H (hyaline portion); Ac (body width)

Mononchus spp. individuals have an elongated body that narrows slightly in the anterior part and considerably in the posterior part (Figure 11A). They have a truncated head, sub-oval oral cavity, two to three times as long as wide, with a sturdy dorsal tooth (Figure 11B,C) and esophagus region (Figure 11D); females have an amphithematic genital system (Figure 11E); the caudal region is elongated, cylindrical or filiform with a rounded end (Figure 11F,G). Average measurement of

Mononchus spp. were: body length = 919 µm; tail length = 63.2 µm.

We also found free-living nematodes which were present in various forms and sizes. They did not have a stylet (Figure 13A through H).

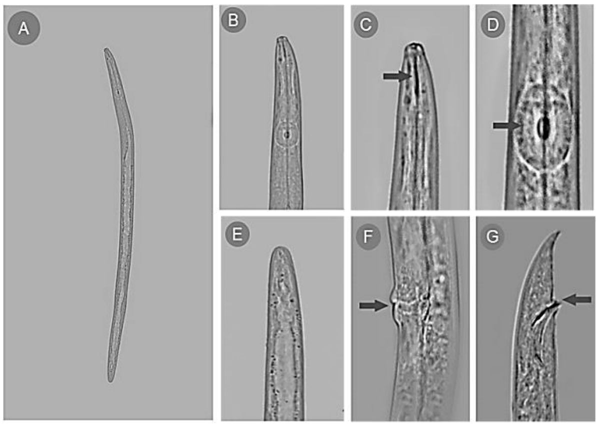

Figure 7. Genus *Aphelenchus* spp. A) Body length; B) Anterior region showing stylet and esophageal region; C) Head shape and stylet; D) Esophageal bulb; E) Tail; F) Arrow showing vulva; G) Posterior region of male showing spicule

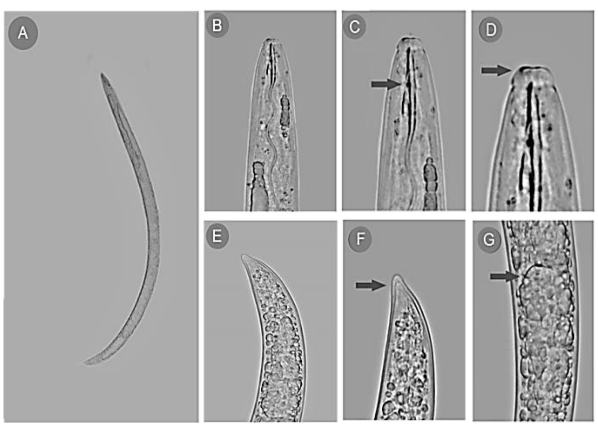

Figure 8. *Dorylaimus* spp. A) Body length; B) Anterior region of the body; C) Arrow showing false stylet; D) Labial region; E) Tail; F) Tail end; G) Vulvar region

Trichodorus spp. individuals have a cylindrical body in the shape of a cigar, somewhat round and truncated in the extremities. These specimens presented the body arched ventrally (Figure 12A); the region of the lips, rounded, the lips are together, with a relatively long, onchostylet like a hard-dorsal tooth, stiletto-shaped (Figure 12B). The female reproductive tract is paired, the vagina is barrel-shaped and sclerotized. Rounded tail end (Figure 12C). Average measurement of the body length of *Trichodorus* spp. was 594 µm.

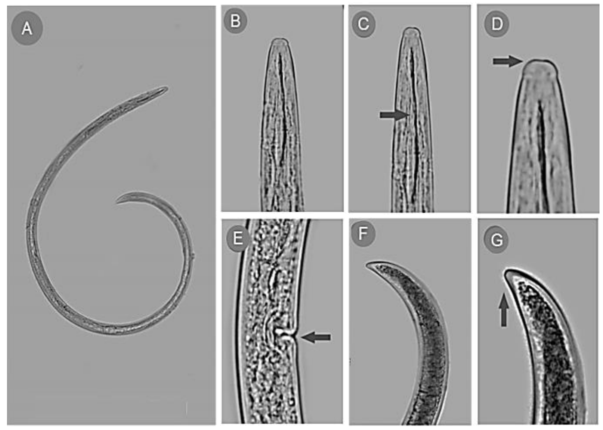

Figure 9. *Xiphinema* spp. A) Body length; B) Anterior region of the body; C) Stylet; D) Labial region; E) Vulvar region; F) Tail, G) Tail end

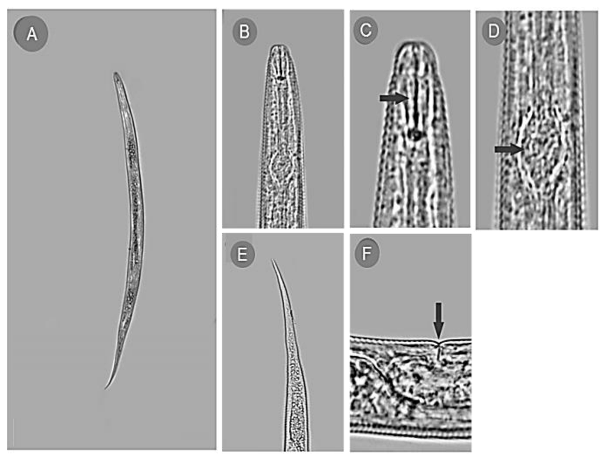

Figure 10. Genus *Tylenchus* spp. A) Body length; B) Anterior region of the body; C) Arrow showing stylet; D) Esophageal region; E) Tail; F) Vulva

DISCUSSION

The morphological characteristics of the specimens identified as *Meloidogyne* spp. coincide with the descriptions for the J2 (Eisenback, 1985; Uysal et al., 2017; Powers et al., 2018).

Likewise, morphometric characteristics (Table 1A) were similar with data from other populations performed by Perry and Moens (2013), Uysal et al. (2017) and Powers et al. (2018), and the body length averages obtained are within the measurements observed for this genus. De Sousa (2018) states that there is the possibility of finding variability in morphometric parameters within the species of the genus, so the body length variation with the populations studied can be considered common, since they come from different places and crops.

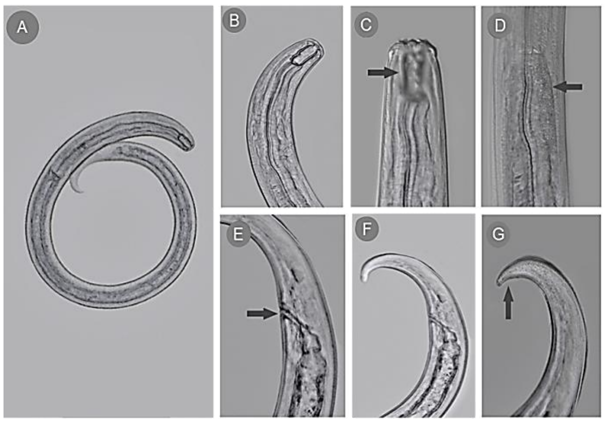

Figure 11. *Mononchus* spp. A) Body length; B) Anterior region of the body; C) Truncate head showing dorsal tooth; D) Esophageal region; E) Vulva region; F) Tail; G) Tail end

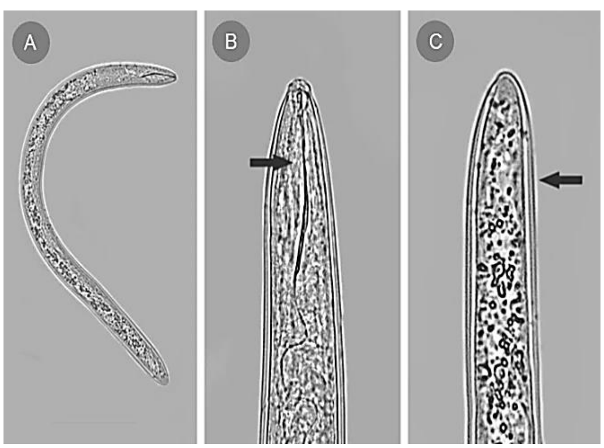

Figure 12. *Trichodorus* spp. A) Body length; B) Anterior region of the body showing the onchostylet; C) Tail

The morphological characteristics of the specimens of *Pratylenchus* spp. resemble the descriptions and measurements made in previous studies (Loof, 1990; Mai and Mullin, 1996; Jesus et al., 2020), and in general, they are within the range (Lira et al., 2014; De Souza, 2018; De Jesus et al., 2020) (Table 1B). However, body length, stylet and tail morphometric features differ from others studies. In crops of coffee in the Sandia district, *Pratylenchus* body length is shorter (Mayta, 2017). On the other hand, in samples from potato and corn it has been found body lengths of 682 and 555 µm, respectively (Flores et al., 2017; Lima et al., 2017); those figures are close to ours and inside the range described by Lira et al. (2014), De Souza (2018) and De Jesus et al. (2020). Morphometric studies that detected variations between populations of *Pratylenchus* attributed to origin (geographical area or host plant) the cause of differences (Olowe and Corbett, 1984).

The morphological characteristics of the specimens identified as *Helicotylenchus* spp. coincide with the characteristics reported by other authors (Uzma et al., 2015; Rybarczyk et al., 2019). They indicate that the labial region is truncated and ringed, the stylet has a length of 22 µm and specimens have well-defined basal nodules. Females do not have mucron (Figure 4H). Small phasmids are present at the level of the anus. Characteristics such as body length, tail and stylet size are within the range of some known measurements (Schreck et al., 2010; Uzma et al., 2015; Kirsch et al., 2016).

The specimens identified as *Discocriconemella* spp. showed morphological characteristics that are consistent with the descriptions proper of the genus: enlarged and round ring or head disc with continuous margins, the disc has deep dorsal and ventral grooves (Vovlas, 1992), the body size is 180-490 µm long, the stylet has a variable length, 33-113 µm, and can be rigid or flexible (Vovlas and Sharma, 1989; Powers et al., 2010).

The morphological characteristics of the specimens identified as *Aphelenchus* spp. are within the descriptions of Chatuverdi and Khera (1979): females have body lengths from 580 to 740 µm. The stylet can be from 14 to 18 µm.

The specimens identified as *Dorylaimus* spp. have morphological characteristics that correspond to the descriptions made by Gusakov and Gagarin (2015). On the other hand, Sen et al. (2011), point out that in males the upper end is ventrally more curved than in females, with blunt termination, with body length between 1145 to 2010 µm, which is much longer than our findings. The stylet ranges from 174 to 210 µm.

The morphological characteristics of specimens identified as *Xiphinema* coincide with the ranges of the genus (Evans et al., 1993; Orlando et al., 2016). The morphometric characteristics are within the measures observed by Rossi y Ferraz (2005), Archidona (2016), and Mayta (2017).

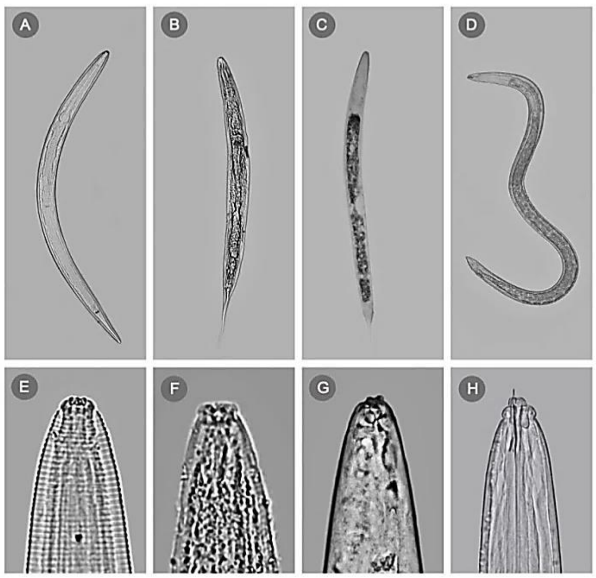

Figure 13. Unidentified free-living nematodes: A) Body length of nematode; E) Anterior region of the body; B) Body length; F) Anterior region of the body; C) Body length; G) Anterior region of the body; D) Body length; H) Anterior region of the body

The specimens identified as *Tylenchus* spp. have the morphometric and morphological characteristcs of the genus (Sidiqui, 2000; Hajizadeh et al., 2015), small or medium in size from 400 µm to 1300 µm, and stylet from 8 to 21 µm long.

In the morphological and morphometric analysis of *Mononchus* spp., these individuals resemble the characteristics reported previously (Lordello, 1958): body and tail length of 788-924 µm and 53.5-68.8 µm, respectively. However, other descriptions of the genus indicate different sizes for body length: 1000-2950 µm (Siddiqi et al., 2015), values higher than the ones reported here. Sohlenius and Boström (2001) refer that these differences can be attributed to environmental, geographical or competition factors that exist in the soil.

The morphological characteristics of the specimens identified as *Trichodorus* spp. show that the body length is in the range of 0.35 to 1.8 mm, and resemble those observed by Almeida and Decraemer (2015), and Elshishka et al. (2016). Similarly, Carta and Skantar (2014) indicate that the stylet has 42-45 µm length.

Regarding to the free-living nematodes, they are large, approximately 1,00 mm of length (Sánchez and Talavera, 2013) and are abundant within the soil microfauna. They have a sclerotized oral cavity with a cylindrical stoma, and some have an odontophore. These characteristics coincide with the individuals observed in the present work (Figure 13). The size and shape of the nematodes are due to important adaptations in order to live. Most of the free-living nematodes are less than 2500 µm long.

According to Hickman et al. (1998), the body of these organisms is perfectly cylindrical, hence the name of round worms. Males tend to be smaller than females and their posterior region is usually curved in the form of a hook where they carry a pair of copulatory spines. The fertilization is internal, and the eggs are usually stored in the uterus until laid.

According to Musarrat et al. (2016), free-living nematodes are beneficial in decomposition of organic matter and recycling of nutrients from the soil. Their diet recycles minerals and other nutrients, and returns them to the soil, thus being accessible to the roots of the plants. Under field conditions, bacterivorous and predatory nematodes contribute directly and indirectly to nitrogen mineralization in agricultural systems (Yadav et al., 2018).

The present study shows that phytoparasitic nematodes are widely distributed in all Andean papaya fields. The genus *Meloidogyne* and *Pratylenchus* are the most dangerous for the crop. Different authors describe these nematodes responsible for most damage on several plant species world-wide (Dias et al., 2008; Jones et al., 2013; Peña et al., 2018). However, the other genus of pathogenic nematodes we found may cause damage to the Andean papaya crop as well. The diagnosis of plant-parasitic nematodes and the knowledge of the distribution, population density of nematodes is an information essential for planning control measure strategies.

CONCLUSIONS

Nematodes associated with Andean papaya fields in Sandia district are *Meloidogyne, Pratylenchus, Helicotylenchus, Mesocriconema, Apelenchus, Dorilaymus, Tylenchus, Xiphinema, Mononchus, Discocriconemella, Trichodorus* and other free-living nematodes.

The identification of nematodes associated with Andean papaya is considered essential to understand their distribution and dissemination, and so implement management strategies.

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