International Journal of Agricultural Technology 2021Vol. 17(6):2111-2126
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Morphological identification of plant parasitic nematodes associated with Rambutan (*Nephelium lappaceum* Linn.)

Kiunisala, P. B.[1], Panalangin, Jr. D. D.[1], Locsin, S. A.[1], Alvarez, L. V.[1], Rendon, A. O.[1], Mapanao, C. P.[1], Zurbano, L. Y.[2] and Bellere, A. D.[3*]

[1]Department of Biological Sciences, Polytechnic University of the Philippines, Sta Mesa, Philippines; [2]Polytechnic University of the Philippines, Lopez Quezon Branch; [3]College of Education, Central Bicol State University of Agriculture – Sipocot, Coloy – Coloy Impig, Sipcot Camarines Sur, Philippines.

Kiunisala, P. B., Panalangin, Jr. D. D., Locsin, S. A., Alvarez, L.V., Rendon, A. O., Mapanao, C. P., Zurbano, L. Y. and Bellere, A. D. (2021). Morphological identification of plant parasitic nematodes associated with Rambutan (*Nephelium Lappaceum* Linn.). International Journal of Agricultural Technology 17(6):2111-2126.

Abstract Plant-parasitic nematodes are one of the significant crop problems affecting various crop productions worldwide. Rambutan (*Nephelium lappaceum)* is one of the most economically important crops in the Philippines which its association with plant parasites is not well known. This paper aims to identify the species of plant-parasitic nematodes associated with rambutan as well as the manifestation of these plant parasites related to this fruit tree. The nematodes from soil and roots samples were extracted using the Baermann tray method and were identified under the light microscope based on their morphological characters. Four species of plant-parasitic nematodes were identified, namely: *Discocriconemella* spp., *Hemicycliophora* spp., *Helicotylenchus* spp. and *Heterodera*/*Globodera* spp. Where in *Discocriconemella* spp. was the most frequently occurring species in the soil with a total of 40%. The prevalence of *Discocriconemella* spp. indicates that the soil environment in the study area is favorable for these organisms but also suggest that abiotic factors such as Physico-chemical properties do have a relationship with the frequency of these plant-parasitic nematodes. Further studies, including molecular analysis and a vast number of samples, were recommended for accurate and specific identification of the species of the nematodes as well as utilizing multiple parameters to allow more statistical analysis about its morphometrics.

Keywords: *Discocriconemella* spp., *Globodera* spp., *Helicotylenchus* spp., *Hemicycliophora* spp., *Heterodera* spp., Physico-Chemical

Introduction

Nematodes are considered significant pests on most economical crops in the Philippines, particularly on pineapple, banana, sugar cane, ramie, tomato, and citrus. These nematodes were identified in many studies that cause serious threat in most of our essential crops, namely: *Radopholus similis* on

* **Corresponding Author**: Bellere, A. D.; **Email**: arce.bellere@cbsua.edu.ph

banana, *Tylenchulus semipenetrans* on citrus while *Meloidogyn*e spp. are more severe on various vegetable crops such as tomato, okra, and celery (Davide, 1988). Ubiquitous in nature, therefore, these PPNs (Plant-parasitic nematodes) are associated with nearly every essential agricultural crop and represent a significant constraint on food security.

According to the World Bank (2008), the population by 2050 might increase by 35% that eventually increases the food demand by 75% due to economic development and changes in food preferences. Thus, the full spectrum of crop production limitations must be considered appropriately, including the often overlooked nematode constraints (Nicol *et al.,* 2011). For example, in a recent review of unmanageable biotic constraints in Africa, not a single mention of nematodes was made (Gressel, 2004), while for the potato crops in the UK alone, it is estimated that the cyst nematodes, *Globodera* spp., account for an estimated 70 million dollars per annum or 9% of UK production (Nicol *et al.,* 2011). PPNs are among the most widespread pests and are frequently one of the most insidious and costly. However, studies' regarding this pest are minimal.

Assessing nematode impact damage resulting from PPNs infection is often less evident than pests or diseases. However, previous studies proved that the interaction between nematodes and other plant pathogens, these PPNs remain neglected and semi rooted discipline that is why lack of knowledge of PPNs lead to misdiagnosing of plant disease (Nicol *et al.,* 2011; Coyne *et al.,* 2018).

Rambutan (*N. lappaceum* Linn.) is a medium-sized tropical evergreen fruit tree belongs under the Sapindaceae family or known as the soapberry family. The name rambutan is derived from the Malay-Indonesian word for "rambut" which means hair, a denotation to the numerous hairy protuberances present on its exocarp (Morton, 1987). It is a famous fruit tree that widely distributed in the humid tropics of South East Asia, especially Malaysia, Indonesia, and Thailand. The species may not be endemic here in the Philippines. However, it is still economically significant due to its palatability. The International Union for Conservation of Nature (IUCN) assessment on the fruit tree is categorized in the least concern status justified by the publication of Barstow (2017). Rambutan trees help the food industry, particularly to the regions which produce a significant amount of its fruits all over the Philippines. However, the study about its diseases and parasites is not well known, unlike essential fruit crops such as mango, banana, citrus, etc. Hence, in research will elucidate the parasitism of PPNs present in *N. lappaceum*.

Materials and methods

Rhizosphere and root samples

The collected rhizosphere samples of rambutan trees were from an orchard located in Ato Belen's Farm, Brgy. San Juan, San Pablo City, Laguna. The coordinates of the three trees where the soil samples collected were (1) 14°05'31.6" N, 121°17'43.9" E; (2) 14°05'31.6" N, 121°17'44.2" E; (3) 14°05'31.8" N, 121°17'43.9" E with 102 meters' elevation. The weather at San Pablo, Laguna, is usually hot to warm and windy condition with seasonal rain downpour which is ideal for Rambutan planting, with partially dry and loamy soil. This Rambutan Farm is a validly accredited nursery by the Department of Agriculture, Bureau of Plant Industry.

The collected root samples were from another orchard located in the residential area in Brgy. Talangka, Sta. Maria, Laguna. The roots that were obtained comes from the seedlings of rambutan. The coordinates of the area are 14°47'44.35" N, 121°43'33.98" E with 15 meters' elevation. The weather at Sta. Maria, Laguna, during collection, was sunny with low wind pressure. The type of soil in the orchard is loamy to clay.

Sample size

The land area where the rambutan trees are planted is approximately around 200 square meters. The collection of the rhizosphere samples in Barangay San Juan was selected from trees mature enough to bear fruit, with age ranging from five to seven years old. The collection of samples was based on the methods of Kibet *et al.* (2014). The obtained samples were 2 kg. The area of the land where the seedlings were randomly collected is 200 square meters. Collected roots samples weighed 200 g.

The acquisition of soil samples for Physico-chemical screening was done and collected from both sampling sites located at San Pablo City and Sta. Maria in Laguna province. The acquired soil samples weighing 1 kg each were submitted to the Bureau of Soils and Water Management or BSWM for Physico-chemical screening with various parameters, namely: pH, Nitrogen, Phosphorus, and Potassium content.

Sampling techniques

The collection of soil samples was based on the methods of Kibet *et al.* (2014). Soil samples weighing 1000 - 2000 g were randomly collected from the rhizosphere of three Rambutan trees using a soil auger. The device was pushed down in about 30 cm depth (Gazaway *et al.,* 1995) in the 5/5 cardinal points of the rhizosphere of the roots (5 points are 0.5 meters away from the roots, the other five are 1m away). Soil samples that were collected using the auger served as the sub-sample. All the sub-samples were mixed and placed on a labeled polyethylene bag and kept in a cooler box to prevent water loss. In'1qasxcf formation about the samples was also included in the data notebook, including the approximate age, coordinates, crop/weeds present in the area and the date.

The collection of root samples was done by cutting the secondary and tertiary roots and served as sub-samples. Then all the sub-samples were mixed and placed on a polyethylene bag and placed inside a cooler box (Kibet *et al.*, 2014). Information regarding the samples was included in the data notebook, including the approximate age of trees, coordinates, and the date.

The collection of soil samples for Physico-chemical screening was done using a soil auger. The collection was carried out in a random manner around the rambutan plantation area. Soil samples that were collected were served as the sub-samples. All the subsamples were mixed and placed on a labeled polyethylene bag and kept in a cooler box to prevent water loss. Information about the samples was also included in the data notebook, including the approximate age, coordinates, crop/weeds present in the area, and the date. The acquired soil samples weighing 1 kg each were submitted to the Bureau of Soils and Water Management or BSWM for Physico-chemical screening with various parameters, namely: pH, Nitrogen, Phosphorus, and Potassium content.

Data gathering procedure

Isolation of nematodes from soil samples

The method of extracting nematodes from the soil sample was done using the Baermann tray method for extraction (Brooks, 2004) and Cobb's sieving and decanting method to minimize the water samples. In the Baermann tray method, 100g of soil samples was covered using a facial tissue paper that served as the nematode filter and sat at a customized tray made out of PVC pipe and mesh as the wire screen, to support the tissue inside the bowl. Tap water was added in the set up until the soil sample inside the tissue is partially submerged, preventing the tissue paper from ripping up. The collection of water samples was done three days after setting up. The collected water samples were filtered on 180 µm, 45 µm, and 38 µm sieves, to remove dirt and excess water. It was then decanted into a vial for fixation.

Isolation, fixation, and mounting of nematode

Collected roots with a mass of 100g from rambutan seedlings (secondary and tertiary ones) were cut into pieces of 1 cm then subjected to maceration using a blender and were placed on a Baermann tray set-up. Water samples collected after three days passed through 180 µm, 45 µm, and 38 µm sieves and then transferred on a separate vial for fixation.

Fixative was added in the nematode suspension with the 2X strength. Nematode suspension was immersed in hot water (near boiling point) for 40 seconds, then 2x fixative was added to the vials equal to the amount of nematode suspension. 1x fixative is used for mounting.

A drop of 1x fixative is placed on a ringed glass slide. With the dissecting microscope's aid, the nematode was isolated from the suspension using a nematode picker in the center of the drop of fixative assuring that they do not float but remain on the bottom. The coverslip is applied with a pair of forceps. Using a filter paper, the excess fixative is removed then the coverslip is sealed using clear nail polish. The mounted nematode is observed morphologically using a light microscope.

Nematode morphometrics, expert determination, and manual counting

The morphological identification of the observed nematodes was done by using identification keys references such as Identification key for agriculturally important plant-parasitic nematodes Prepared for the International Nematode Diagnosis and Identification Course 2012 by Mekete, Interactive Diagnostic Key to Plant Parasitic, Free-living and Predaceous Nematodes by Tarjan (1977) and Identification Guides for the Most Common Genera of Plant-Parasitic Nematodes by Jonathan Eisenback (2002). The morphological description and morphometric data were obtained using the Euromex BioBlue microscope provided and funded by the Commission on Higher Education or CHED.

Observed nematodes were subjected to measurement of different parameters, namely: body length and stylet length using ImageJ and ImageFocus software. The data gathered were used to suffice the identification of plant-parasitic nematodes.

The identification of plant-parasitic nematodes was also done using the expert determination method. The images were submitted to an expert nematologist from the University of the Philippines-Los Baños at Los Baños, Laguna, Philippines. Fixated nematode suspension from every 100g of soil and root samples were subjected for observation, identification, and manual counting.

Statistical treatment of data

Nematode occurrence frequency calculation

The nematodes were counted manually. With the use of obtained raw data of nematode count, the frequency percentage of each identified species was calculated by dividing the total number of observed plant-parasitic nematodes over the number of distinguished individuals and then multiplying to 100%.

$$\left(\frac{Total\ number\ of\ PPN\ in\ sample}{Number\ of\ individuals\ in\ genus}\right)100$$

Results

Physico-chemical properties of soil

As shown in Table 1, results revealed that the soil samples collected from Sta. Maria and San Pablo, Laguna, with a pH of 5.6 and 6.0, respectively, are acidic. Additionally, the soil sample obtained in Sta. Maria showed low Nitrogen content, moderately high Phosphorus, sufficient Potassium, with Medium texture (TFM). On the other hand, the soil sample collected in San Pablo elucidated medium nitrogen content, high in Phosphorus, sufficient Potassium, with a heavy texture (TFM).

Table 1. Physico-chemical properties of soil from sampling sites

Sampling site	pH	Nitrogen	Phosphorus	Potassium	Texture-Feel Method (TFM)
Sta. Maria, Laguna	5.6	Low	Moderately high	Sufficient	Medium
San Pablo, Laguna	6	Medium	High	Sufficient	Heavy

In the collected samples, the plant-parasitic nematodes under three genera, namely: *Helicotylenchus* spp., *Discocriconemella* spp. and *Hemicycliophora* spp. were observed in soil samples with 25, 40, and 35 occurrence percentage, respectively (Table 2). While the plant-parasitic nematodes identified in roots samples were *Hemicycliophora* spp. and *Heterodera/ Globodera* spp., with 73 and 27 percentage of the occurrence. The data revealed that *Discocriconemella* spp., is the most abundant PPN in soil samples while *Hemicycliophora* spp., can be observed in soil but thrives mostly on root samples.

Table 2. Occurrence Percentage of Plant Parasitic Nematodes Present in Soil and Root Samples

Plant-Parasitic Nematodes	**% Occurrence**	
	Soil	**Roots**
Helicotylenchus spp.	25	-
Discocriconemella spp.	40	-
Hemicycliophora spp.	35	73
Heterodera/Globodera spp.	-	27

Morphological characteristics

Morphological identification was done using three main processes; using identification key, image comparison supported by morphometry, and expert determination. These methods could solidly help claimed identification of PPN collected from root and soil samples of Rambutan.

***Helicotylenchus* spp.**

Class: Secernentea
Subclass: Diplogasteria
Order: Tylenchida
Superfamily: Tylenchoidea
Family: Hoplolaimidae
Subfamily: Hoplolaiminae
Genus: *Helicotylenchus* spp.

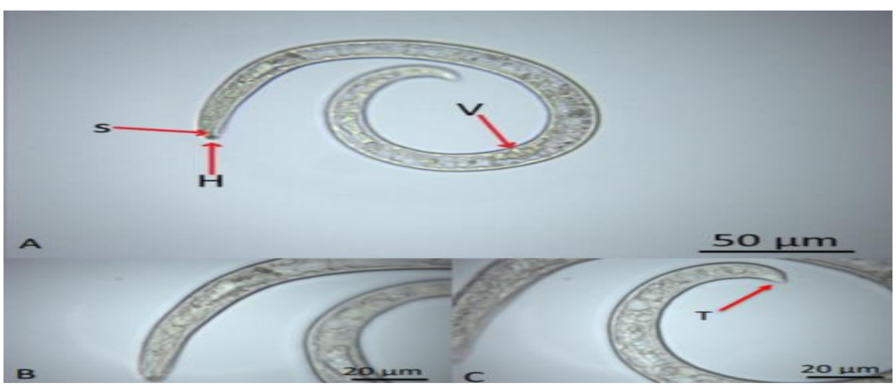

Figure 1. *Helicotylenchus* sp. A: showing the head (H), stylet (S), and Vulva (V). B; close up image of the anterior region. C; posterior region showing the tail (T)

***Discocriconemella* spp.**

Class: Secernentea
Subclass: Diplogasteria
Order: Tylenchida
Superfamily: Criconematoidea
Family: Criconematidae
Subfamily: Discocriconemellinae
Genus: *Discocriconemella* spp.

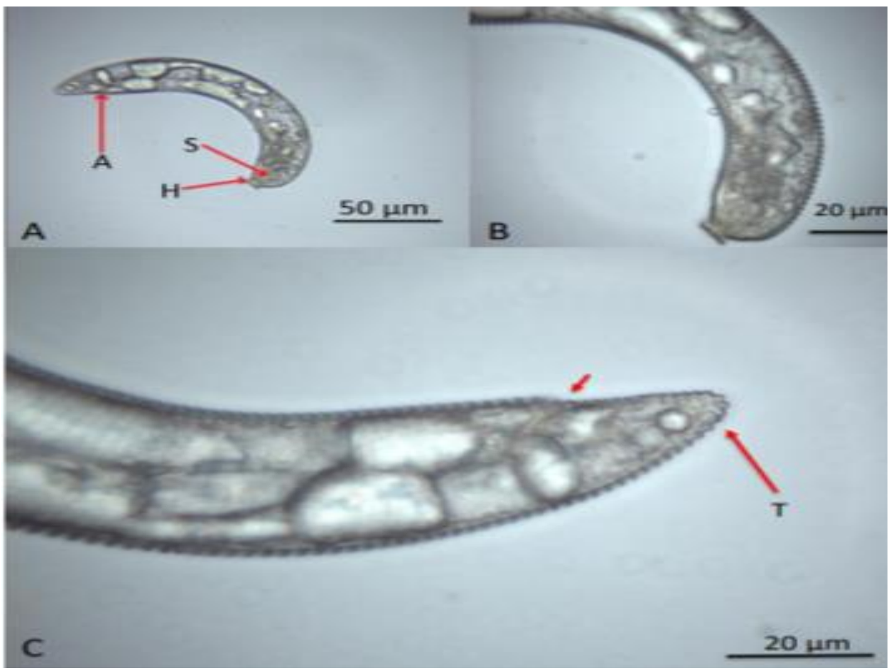

Figure 2. *Discocriconemella* sp. Whole body image (A), Anterior region (B), Posterior region (C). Legend: A- anus, S- stylet, H- head, T- tail

***Hemicycliophora* spp.**
Class: Secernentea
Subclass: Diplogasteria
Order: Tylenchida
Superfamily: Criconematoidea
Family: Criconematidae
Genus: *Hemicycliophora* spp.

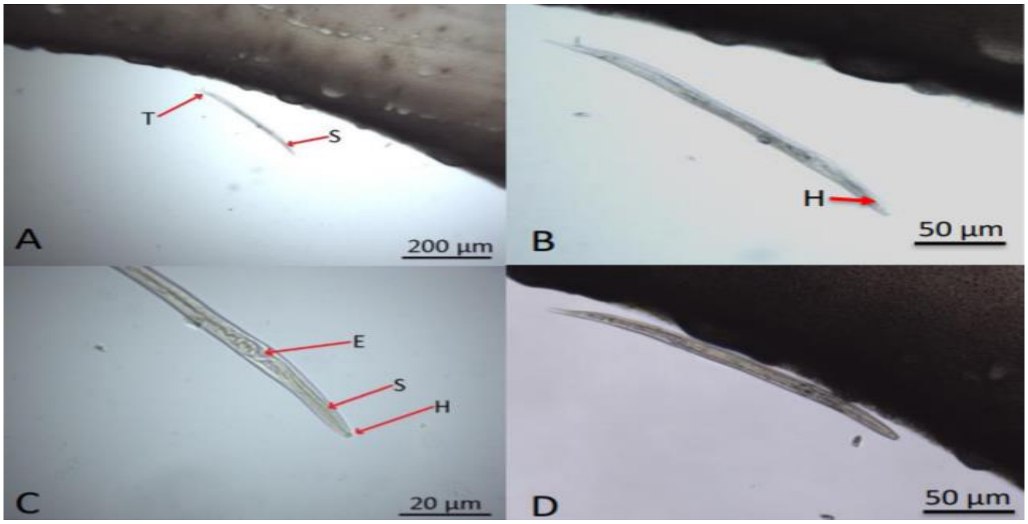

Figure 3. *Hemicycliophora* sp. from soil samples whole image (A) showing the tail (T) and stylet (S). Image B shows the close up image. Image C shows the zoomed in image of the anterior region showing the esophagus (E), while the image in D is a whole image of the individual (Legend: H- head, S- stylet, E- esophagus)

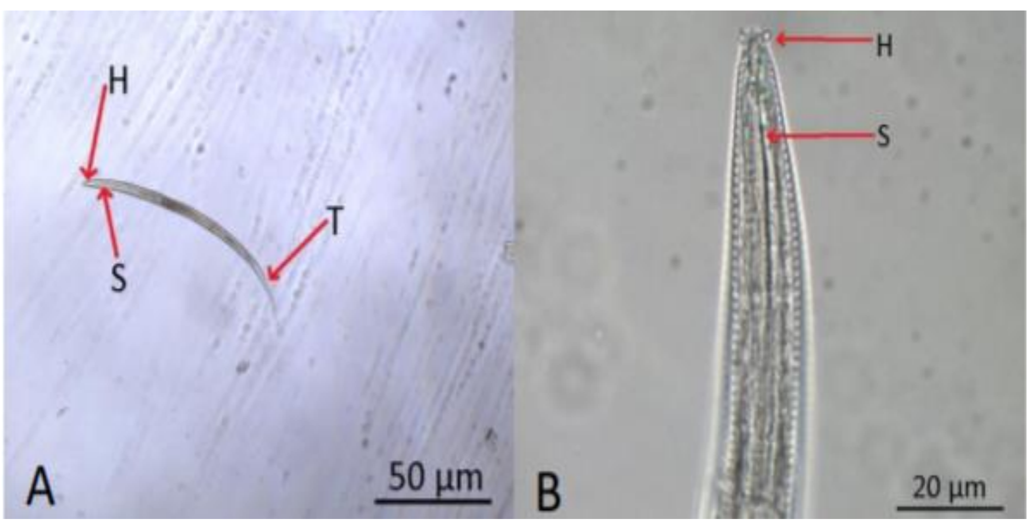

Figure 4. *Hemicycliophora* sp. from root samples whole image (A) and head region (B) (Legend: H- head, S- stylet, T- tail)

***Heterodera* and *Globodera* spp.**

Class: Secernentea
Subclass: Diplogasteria
Order: Tylenchida
Superfamily: Tylenchoidea
Family: Heteroderidae
Subfamily: Heteroderinae
Genus: *Heterodera* spp.

Class: Chromadorea
Order: Rhabditida
Family: Heteroderidae
Subfamily: Heteroderinae
Genus: *Globodera* spp.

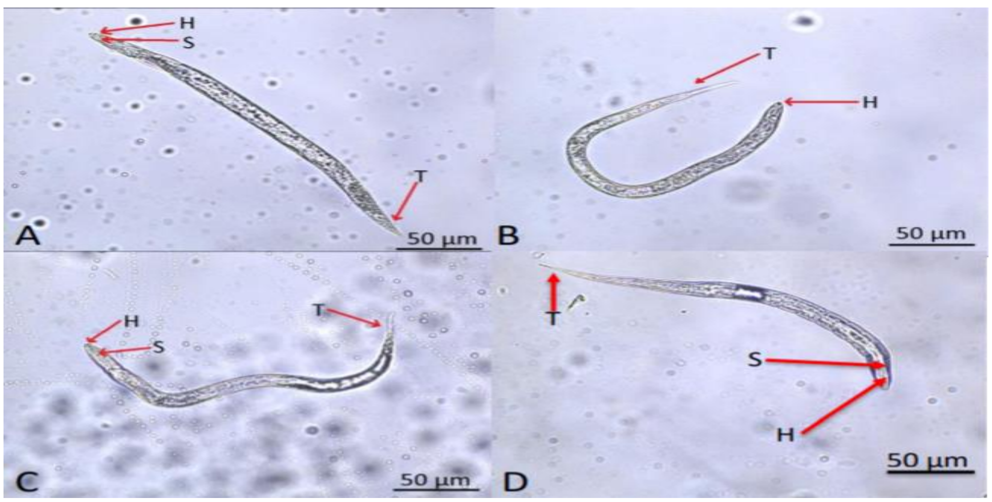

Figure 5. Four different individuals under *Heterodera*/*Globodera* spp. (Legend: H- head, S- stylet, T- tail)

Discussion

Helicotylenchus species have a moderately long body, conical head but weak framework, its vulva is near mid-body, the stylet is short to slightly long. The tail is usually offset. Morphometric data shows its average overall body length 570-730 µm, and its stylet 22- 26 µm (Kashi and Karegar, 2014). Species under the genus have a 25% percentage occurrence.

Helicotylenchus spp. is found in warm and temperate climates (Crow, 2014) such as the Philippines. They have a broad preference in soil types, from heavy to sandy and organic soils. Eggs contain a single juvenile embryo that would hatch and find its first host at J2 stage. Most species tend to migrate as they are generally identified as ectoparasites; some act as semi-endoparasites (Crow, 2014).

The symptoms of these nematodes stated by Crow (2014) were reduced root length, decreased yield, and stunted growth. However, these symptoms could only be seen in severely infested trees and subtler than other economically significant PPN's, like Meloidogyne spp. In fact, despite being able to parasitize many plants, including those that are financially important, it is rarely considered a major pest.

Discocriconemella spp. was first described by De Grisse and Loof (1965), and was known to have short bodies, deep annulations with smooth edges, long and thick stylets and anchor-shaped knobs, aside from its cephalic disc which is also used to identify the species (Vovlas, 1992). The overall body length around 440-595 µm, while stylet length can be short or exceptionally long, from 33-66 µm to 99-133 µm. (Siddiqi, 2000). Discocriconemella spp. have a 40% percentage occurrence in samples.

Discocriconemella spp. are also called sheathoid nematodes due to the distinct characteristic exhibited by the females in its body being enclosed by a loosened cuticular sheath, and its disc-shaped annule in the Cephalic region separated from other annules in the body (De Grisse and Loof, 1965; Siddiqi, 2000, Geraert, 2010). They occur in areas with warm temperatures (Inserra *et al.*, 2014). Morphological identification is a challenge because some characteristics and diagnostic value overlap in some species. Molecular information of the species is limited, and other species of different areas differ in DNA sequences than in the earlier described (Inserra *et al.,* 2014).

Like *Helicotylenchus* spp., *Discocriconemella* spp., *juvenile* phases must reach the J2 stage before hatching and feeding on roots (Dasgupta *et al.*, 1969). These nematodes have been suggested as obligate migratory ectoparasitic nematodes feeding on roots (Loos, 1949 and Whitlock and Steele 1960).

It was noted that *D. aquatica* was found in various plant hosts such as *Parkia javanica*, *Meyna laxiflora*, *Pinus insularis,* and *Mangifera indica* (Vovlas, 1992). This can be the first research to discover the genus being present in Rambutan.

Symptoms that can be observed in plants infested with nematodes under Criconematidae are stunted, chlorotic with root malformation, and signs of nutrient deficiencies; However, there is no significant effect usually seen in plants and crops that are being infested by most species of *Discocriconemella spp*. as they are not considered aggressive plant parasites (McSorley *et al.*, 1980; Inserra *et al.,* 2014; Vovlas, 1992).

Hemicycliophora spp. have very long, slightly curved stylets, thick but moderately long, heavily annulated body, and pointed tails, especially in females (Eisenback, 2002). Its overall body length extents for about 460-760 µm, while its stylet 49.3-90 µm (Subbotin *et al.,* 2014), percentage occurrence is 35% in soil samples, while 73% in root samples.

The key features of species under the genus *Hemicycliophora* are long stylet, rounded basal knobs, and a cuticular sheath (Subbotin *et al.*, 2014). Like the two previous species, *Hemicycliophora spp.* can parasitize a large variety of hosts, from crops to trees (Siddiqi, 2000), only some species are reported by Subbotin *et al.* (2014) as significant parasites among plants like *H. arenaria* (Van Gundy and Rackham, 1961), *H. parvana* (Tarjan, 1952), *H. similis* (Riffle, 1962), *H. typical* (Thorne, 1961*), H. conida* (Spaull and Newton, 1982) which causes root-tip galls, stubby roots, and stunting.

Morphological identification is a challenge in researches since phenotypic plasticity is apparent even between the same species (Siddiqi, 2000). Molecular approaches could reveal that single species can be considered cryptic; that is, even if morphological data shows they are the same, they have different genetic and phylogenetic makeup (Gutiérrez-Gutiérrez *et al.,* 2010; Cantalapiedra-Navarrete *et al.*, 2013).

Heterodera and *Globodera* spp. are known for their tail terminus, which is made of a hyaline, short stylet with distinct bulbs, and rounded sclerotized, head region (Eisenback, 2002). Morphometric reference shows an average of 412-600 µm in its overall body length and 19.6-26.5 µm stylet length. It was recorded with a 27% occurrence in collected root samples.

Heterodera spp. and *Globodera* spp. belong to the most economically significant plant-parasitic nematodes worldwide (Subbotin *et al.,* 2010). Some species under one genus is even synonymous to the other species of different genus as the two genera are almost the same, differing mainly in their hosts, other than genetic makeup, like *Globodera rostochiensis* (Mackesy *et al.*, 2014). Observed individuals may be at their juvenile stage as the body's swelling is a distinct characteristic among the parasitic females.

Symptoms include wilting, stunting, and root malformation in heavy infestations, especially in potato, in which it is considered a major parasite (Bert *et al.,* 2006). According to a research conducted by Sabaratnam (2012), these symptoms are not diagnostic for the said species but may also be influenced by other factors, like soil quality, drought, herbicide injury, nutrient deficiency, and other environmental factors. Other PPN present in the area is also possible to induce symptoms (Sabaratnam, 2012). There are no particular symptoms recorded aboveground that are caused by cyst nematodes (CIP, n.d.)

Observed PPN and their role in infestation in Rambutan

Leaf blight disease is evident in most trees, especially in leaves at the lowest part of the crown. It complements with chlorosis and necrosis of some leaves, which goes up to the center of crown, occurring in clusters. Lack of leaves is also apparent in the top region of the crown in all rambutan trees in the area, which is quite strange since the other trees of different species around the area, "Pomelo" (*Citrus maxima*) trees are healthy and does not show any of the diseases exhibited in Rambutan. Overall, these diseases are not significant since the majority of the leaves and other plant parts are healthy. There was no isolate being caused exclusively by nematodes, as these symptoms can also be exhibited by plants affected by different factors, such as nutrient deficiencies, and bacterial, viral, and fungal infections (McCauley *et al.*, 2011).

There are several factors needed to evaluate before diagnosing the cause of plant diseases, as causes between different pathogens may reveal similarities in conditions. For instance, leaf blight disease can be exhibited by a plant infected with bacteria or fungi. However, the probability of nematode influence in the observed diseases is still possible since the presence of plant-parasitic nematodes was detected. Symptoms detected in Rambutan trees has been hypothesized being caused by two probable origins; its direct infestation with the plant-parasitic nematodes present, and the outcome of disease complexes induced by the combination of some microorganisms other than PPN's. Previous researchers have discovered PPN behavior as plant pathogens being a vector for fungal parasites and vice versa. PPN's can also help some species of bacteria infect the same host (Davis *et al.*, 1984).

The information gathered from other researches reveals that even with the presence of PPN around the sampling area, they may not impose a high impact on the infestation. Studies confirmed that PPN's under the genus *Helicotylenchus*, *Discocriconemella*, *Hemicycliophora*, *Globodera*, and *Heterodera* are not overly aggressive parasites Rambutan, and it not inconsistent to claimed that the presence of these plant-parasitic nematodes has nothing to do or not associated with Rambutan. More studies in exploring their association in Rambutan must be conducted to identify the genetic makeup, a detailed view on morphological plasticity displayed by the PPN's, its association with other microorganisms in forming various disease-complexes and the effect of infestation in Rambutan by occupying a larger area, and higher sample size.

Acknowledgments

The authors are grateful to Asst Prof. Romnick A. Latina of Plant Pathology Department at University of the Philippines, Los Baños, Laguna, their consultant in the study and for letting them use the Nematology Laboratory in UPLB. They are also grateful to Commission on Higher Education (CHED) through K to 12 Research – DARE To Grant for the funding assistance given to this research.

References

Barstow, M. (2017). *Nephelium lappaceum*. The IUCN Red List of Threatened Species 2017: e.T33266A67808476. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.20173.RLTS.T33266A67808476.en

Bert, W., Leliaert, F., Vierstraete, A. R., Vanfleteren, J. R. and Borgonie, G. (2008) Molecular phylogeny of the Tylenchina and evolution of the female gonoduct (Nema-toda: Rhabditida). Molecular Phylogenetics, 48:728-744.

Brooks, F. E. (2004). Plant-parasitic nematodes of banana in American Samoa. Nematropica, 34: 65-72.

Cantalapiedra-Navarrete, C., Navas-Cortés, J. A., Liébanas, G., Vovlas, N., Subbotin, S. A., Palomares-Rius, J. E. and Castillo, P. (2013). Comparative molecular and morphological characterisations in the nematode genus Rotylenchus: Rotylenchus paravitis n. sp., an example of cryptic speciation. Zoologischer Anzeiger, 252:246-268.

CIP (n.d.) “Nematodes”. Centro Internacional de la Papa. Retrieved from http://www.cipotato.org/potato/Pests_ Disease/IPM/ Pests/nema.html

Coyne D. L., Cortada, L., Dalzell, J. J., Claudius-Cole, S. G., Luambano, N. and Talwana, H. (2018). Plant-parasitic nematodes and food security in sub-saharan Africa. Annual Review of Phytopathology, 56:381-403.

Crow, W. T. (2014). Spiral Nematode Helicotylenchus spp. (Nematoda: Tylenchida: Hoplolaimidae). IFAS Extension, University of Florida.

Davide, R. G. (1988). Nematode Problems Affecting Agriculture in the Philippines. The Journal of Nematology, 20:214-218.

Davis, M. J., Gillaspie, A. G. Jr., Vidaver, A. K. and Harris, R. W. (1984). Clavibacter: A new genus containing some phytopathogenic coryneform bacteria including Clavibacter xyli subsp. xyli sp. nov.; subsp. nov. and Clavibacter xyli subsp. cynodontis subsp. nov.; pathogens that cause ratoon stunting disease of sugarcane and Bermudagrass stunting disease. International Journal of Systematic Bacteriology, 34:107-117.

Dasgupta, D. R., Raski, D. J. and Van Gundy, S. D. (1969). Revision of the genus Hemicriconemoides Chitwood and Birchfield, 1957. (Nematoda: Criconematidae). Journal of Nematology, 1:126-145.

De Grisse, A. and Loof, P. A. A. (1965). Revision of the genus Criconemoides (Nematoda: *Tylenchida*). Mededelingen van de Landbouwhogeschool en de Opzoekingsstations van de Staat te Gent, 38:1303-1328.

Eisenback, J. D. (2002). Identification guides for the most common genera of plant-parasitic nematodes. Blacksburg, Virginia: Mactode Publications.

Gazaway, W., Hagan, A. K., Mullen, J. M. and Sikora, A. J. (1995). Collecting Soil and Root Samples for Nematode Analysis. The Alabama Cooperative Extension System (Alabama A&M University and Auburn University).

Geraert, E. (2010). The Criconematidae of the World. Identification of the Family Criconematidae (Nematoda). Ghent, Academia Press. pp.615.

Gressel, J., Hanafi, A., Head, G., Marasas, W., Obilana, A. B, Ochanda, J., Souissi, T. and Tzotzos, G. (2004). Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. Crop Protection, 23:661-689.

Gutiérrez-Gutiérrez, C., Palomares-Rius, J. E., Cantalapiedra-Navarrete, C., Landa, B. B., Esmenjaud, D. and Castillo, P. (2010). Molecular analysis and comparative morphology to resolve a complex of cryptic Xiphinema species. Zoologica Scripta, 39:483-498.

Inserra, R. N., Stanley, J. D., Ochoa, A., Schubert, T. S., Subbotin, S. A., Crow, W. T. and McSorley, R. (2014). Hemicriconemoides Species as Crop Damaging Parasitic Nematodes in Florida. Nematology Circular No. 223.

Kashi, L. and Karegar, A. (2014): Description of *Helicotylenchus persiaensis* sp. n. (Nematoda: *Hoplolaimidae*) from Iran. Zootaxa, 3785:575-588.

Kibet, T. K., Kinyua, J., Kariuki, D., Mamati, E. G., Onguso, J. and Wafula, E. (2014). Occurrence of Fungivorous Nematodes in Ngere Tea Catchment Area, Murang'a County, Kenya. International Journal of Life Sciences Research, 2:89-96.

Loos, C. A. (1949). Notes on free-living and plant-parasitic nematodes of Ceylon – No.4. Journal of the Zoological Society of India, 1:17-22.

Mackesy, D., Molet, T. and Sullivan, M. (2014). CPHST Pest Datasheet for *Globodera rostochiensis*, USDA-APHIS-PPQ-CPHST

Mekete, T., Dababat, A., Sekora, N., Akyazi, F. and Abebe, E. (comps). (2012). Identification key for agriculturally important plant-parasitic nematodes Prepared for the International Nematode Diagnosis and Identification Course 2012 - A manual for nematology. Mexico, D.F.: CIMMYT.

McCauley, A., Jones, C. and Jacobsen, J. (2011). Nutrient Management Module. Plant Nutrient Functions and Deficiency and Toxicity Symptoms, 9.

McSorley, R., Campbell, C. W. and Goldweber, S. (1980). Observations on a mango decline in south Florida. Proceedings of Florida State Horticultural Society, 93:132-133.

Morton, J. (1987). In: Fruits of warm climates. Julia F. Morton, Miami, FL. Rambutan. pp.262-265.

Nicol, J. M., Turner, S. J., Coyne, D. L., den Nijs, L., Hockland, S. and Tahna Maafi, Z. (2011) Current nema-tode threats to world agriculture. In: Jones, J., Gheysen, G. and Fenoll, C. (eds) Genomics and Molecular Genetics of Plant-Nematode Interactions. Springer, Dordrecht, the Netherlands, pp.21-43.

Riffle, J. W. (1962). Nematodes associated with maple dieback and maple blight. Phytopathology, 52:749.

Sabaratnam, S. (2012). Potato Cyst Nematodes. British Columbia Ministry of Agriculture. Accessed from: http://www.agf.gov.bc.ca/cropprot/goldennema.html

Siddiqi, M. R. (2000). Tylenchida parasites of plants and insects, 2nd edn. Wallingford, UK: CABI Publishing.

Spaull, A. M. and Newton, P. G. (1982). Numbers of root-ectoparasitic nematodes under some forage crops. Nematologica, 28:450-452.

Subbotin, S. A., Mundo-Ocampo, M. and Baldwin, J. G. (2010). Systematics of cyst nematodes. (Nematoda: Heteroderinae). Hunt, D. J. and Perry, R. N. (Eds.), Nematology Monographs and Perspectives 8B, the Netherlands: Leiden pp. 512.

Subbotin, S. A., Chitambar, J. J., Chizhov, V. N., Stanley, J. D., Inserra, R. N., Doucet, M. E., McClure, M., Ye, W., Yeates, G. W., Mollov, D. S., Cantalapiedra-Navarette, C., Volvas, N., Van Den Berg, E. and Castillo, P. (2014). Zoological Journal of the Linean

Society. Molecular Phylogeny, Diagnostics, and diversity of plant-parasitic nematodes of the genus *Hemicycliophora* (Nematoda: Hemicycliophoridae)

Tarjan, A. C. (1952). The nematode genus *Hemicycliophora* de Man, 1921 (Criconematidae) with a description of a new plantparasitic species. Proceedings of the Helminthological Society of Washington, 19:65-77.

Tarjan, A. C. (1977). Application of municipal solid waste compost to nematode-infected citrus was an attempt to put to good use solid waste compost, otherwise known as "garbage". The material applied was "supersoil", a product made of thoroughly digested and pulverized municipal waste. Results indicated a slight beneficial effect as measured by growth response, plsnt appearance and fruit yield. Nematropica, 7:53-56.

Thorne, G. (1961). Principles of nematology. New York: McGraw-Hill Book Company.

Van Gundy, S. D. and Rackham, R. L. (1961). Studies on the biology and pathogenicity of *Hemicycliophora arenaria*. Phytopathology, 51:393-397.

Vovlas, N. (1992). Taxonomy of *Discocriconemella* (Nematoda: Criconematoidea) with a Redescription of D. mauritiensis. J. Nematol, 24:391-398.

Whitlock, L. S. and Steele, A. E. (1960). Notes on Hemicriconemoides gaddi from camellias in Louisiana and Georgia. Plant Disease Reporter, 44:446-447.

World Bank (2008). World Development Report 2008: Agriculture for Development. The World Bank, Washington.

(Received: 14 August 2020, accepted: 30 October 2021)