Description and Identification of Four Species of Plant-parasitic Nematodes Associated with Forage Legumes

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Abstract

Four species of plant parasitic nematodes were present in soil samples planted with forage legumes at Alachua County, Florida, USA. The detected species *Belonolaimus longicaudatus*, *Criconemella ornate*, *Hoplolaimus galeatus*, and *Paratrichodorus minor* were described in the present study. They belong to orders Rhabditida (*Belonolaimus longicaudatus*, *Criconemella ornate*, and *Hoplolaimus galeatus*) and Triplonchida (*Paratrichodorus minor*) and to taxonomical families Dolichodoridae (*Belonolaimus longicaudatus*), Hoplolaimidae (*Hoplolaimus galeatus*) Criconematidae (*Criconemella ornate*), and Trichodoridae (*Paratrichodorus minor*). The identification of the present specimens was based on the classical taxonomy, following morphological and morphometrical characters in the species specific identification keys.

Keywords: *Belonolaimus longicaudatus*, *Criconemella ornate*, *Hoplolaimus galeatus*, *Paratrichodorus minor*, morphology, species description.

Introduction

As is widely and rightly held nematodes (round worms) are diversified multicellular animals comprising free living to plant, animal and even human-parasitic species. They represent a group of the most numerous metazoans in soil and aquatic sediments. From an environmental point of view, nematodes are part of nearly all ecosystems in their roles as bacterivores, herbivores, parasites of animals and plants, and consumers of dissolved as well as particulate organic matter **(Mekete *et al*., 2012).** Among them, plant-parasitic nematodes (PPNs) form prominent and distinguished group. These latter are mostly hidden enemy of the growers as the nematodes are generally subterranean in habitats and farmers are

unaware of losses caused by them. A great loss to crops has been reported in quantitative, qualitative and monetary terms. Considering their impact on crops, **Abd-Elgawad** and **Askary (2015**) reported an average worldwide crop loss of 12.6% which equaled $215.77 billion due to these nematodes for only the top 20 life-sustaining crops based on the 2010-2013 production figures and prices. Moreover, 14.45% or $142.47 billion was an average annual yield loss in the subsequent group of food or export crops. These figures are staggering, and the authentic figure, when more crops are counted, certainly exceeds such estimations.

Nevertheless, it is noteworthy that PPNs are among the least studied, with close to only 26,000 (estimated < 3%) species described to date **(Hugot *et al*., 2001; Mekete *et al*., 2012).** Accuracy of identification is, therefore, fundamental to our understanding and communication of the ecological role of any organism. It goes without saying that PPN identification has its strength in agricultural applications because of its economic participation in nematode management implications. Consequently, PPN species delimitation methods in the context of agricultural and health-related applications are more refined at the species and below species level than methods employed in nematode biodiversity studies. Yet, both groups of studies help in better understanding of nematology and its interaction with relevant disciplines. In fact, specimen identification are imperative not only for choosing adequate management control strategies for PPNs but also for avoiding spreading of such exotic nematodes in quarantine materials. Yet, PPNs are one of the hardest groups to be identified due to their microscopic sizes as well as the difficulties in observing key diagnostic characters/features under conventional light microscope **(Carneiro *et al*., 2017).**

On the other hand, legumes represent some of the best quality forages for livestock since they are palatable, help maintain proper functioning of the ruminant digestive processes and stimulate high production of both meat and milk. Since they possess an excellent attribute of biological nitrogen fixation in association with rhizobia, which helps in sustaining soil fertility, legumes are used as green manure by ploughing them into the soil. Considering their impact on crops, PPNs associated with common forage legumes were studied for morphometric identification in this work.

Materials and Methods

Soil of three experimental areas with established stands of fifteen adapted clover cultivars were sampled for PPNs. The experimental areas, properties of the University of Florida, were located in Alachua County near Gainesville, Florida, USA at altitude of 30 degrees north and a longitude of 82 degrees west. The three experiments, set up initially to test forage cultivars, had been established in a randomized block design with clover cultivars and controls as treatments. The sampled clover cultivars comprised *Trifolium repens* (white clover) cvs 'FL-XP1',

'Regal', 'FL-XP2', 'LA-SI', and 'Tillman'; *T. pretense* (red clover) cv. 'Kenstar'; *T. vesiculosum* (arrowleaf clover) cvs 'Amclo', 'REPS-5' and 'Yuchi'; *T. incarnatum* (crimson clover) 'Dixie'; *T. subterraneum* (subterranean clover) cvs 'Mt. Barker' and 'Woogenellup'; *T. alexandrinum* (Egyptian clover) cv. 'Bigbee'; *Medicago sativa* (alfalfa) cv. 'FL-77'; and *Melilotus alba* var *annua* (sweet clover) cv. 'Hubam'. Phytonematodes associated with the forage legumes of these experiments were reported to the generic level **(Abd-Elgawad *et al*., 2017).** About five 2.5-cm diameter cores were taken randomly within the root zone (upper 15-20 cm) from each plot and then mixed thoroughly to form one sample representing the plot. Each sample was placed in a plastic bag, stored in an ice chest, and transported to the nematology laboratory. Samples not processed immediately were stored at 10 ± 1 ^{0}C until processed. Nematodes were extracted from 100 cm^{3} soil from each sample using a centrifugal-flotation technique **(Caveness and Jensen, 1955),** placed in vials, and stored in a refrigerator at 4°C for no more than three days until identified to genera and counted. Thereafter, some of the nematodes extracted from the study **(Abd-Elgawad *et al*., 2017)** were taken and processed for identification to the species level as originally described by **Goodey (1963).** Three percent formalin was used for making temporary mounts of nematodes. One percent water agar was used (for en face mounts) and Zut® slide-ringing compound was used to seal the slides. Drawings were made with the camera lucida technique equipped to the microscope. All measurements except ratios are expressed in µm as means ± standard deviation. Key references of **Rau (1963), Luc and Raski (1981), Jairajpuri and Baqri (1973)**, and **Decraemer (1980)** were consulted to identify species of *Belonolaimus*, *Criconernella*, *Hoplolaimus*, and *Paratrichodorus.* A magnification of 1000 x was used to identify all nematodes. Throughout these identifications, we adopted the systematic scheme of **De Ley and Blaxter (2002)** for the higher classification which has been updated where appropriate by **Decraemer and Hunt (2013)** to reflect new taxa proposals.

Results and Discussions

Species of four genera of plant-parasitic nematodes associated with all the above-mentioned clover cultivars were identified as *Belonolaimus longicaudatus*, *Criconemella ornate*, *Hoplolaimus galeatus*, and *Paratrichodorus minor* (Tables 1-4; Figs. 1-4). Measurements and drawings of nematode species are presented in Tables 1 through 4 and Figures 1 through 4; respectively. **Golden (1971**) classified the genera and higher categories of the order Tylenchida in which all of these nematodes are placed except *Paratrichodorus minor* which is in the order Dorylamida and published by **Siddiqi (1973).** Nevertheless, family Trichodoridae is no longer part of Dorylaimida and Tylenchida doesn't exist (now it is Rhabditida). In fact, recent molecular phylogenetic analyses recognize 12 clades within the Nematoda, with plant-parasitic taxa located in the basic clade I (Trichodoridae) and clade II (Longidoridae) and in the more advanced clade 12 with the

Tylenchomorpha **(Holterman *et al.*, 2006; Decraemer and Hunt, 2013).** Accordingly, the species identified herein belong to orders Rhabditida (*Belonolaimus longicaudatus*, *Criconemella ornate*, and *Hoplolaimus galeatus*) and Triplonchida (*Paratrichodorus minor*) and to taxonomical families Dolichodoridae (*Belonolaimus longicaudatus*), Hoplolaimidae (*Hoplolaimus galeatus*) Criconematidae (*Criconemella ornate*), and Trichodoridae (*Paratrichodorus minor*). The morphometric identification carried out herein neither negates the need for molecular tools for its documentation nor declines the presence of other PPN species associated with such forages **(Abd-Elgawad *et al.*, 2018).** In fact, PPNs have been receiving sufficient attention regarding their taxonomy and evolution and therefore they are currently undergoing continuous modifications in their classification, phylogeny and taxonomy **(**e.g**. Sun *et al.*, 2014; Guesmi-Mzoughi *et al.*, 2016).**

Belonolaimus longicaudatus (Table1; Fig.1) was identified using the dichotomous key of **Rau (1963).** He stated that this species is different from *B. euthychilus*, *B. gracilis*, and *B. maritimus* to which it is related in ratio of stylet length to tail length. Sixty to 100% of *B. longicaudatus* populations have stylets shorter than tails while the opposite is true with the other species. *B. longicaudatus* differs from *B. nortoni* in that *B. longicaudatus* has a relatively longer stylet and lips of vulva are not protruding.

Table (1): Measurements and ratios of females and males of *Belonolaimus longicaudatus* associated with forage legumes.

Body regions and organs	Dimensions (µm) and ratios					
	Females (n= 10)			Males (n= 10)		
	Minimum	Maximum	Average±S.D.	Minimum	Maximum	Average±S.D.
Body length	2085	2871	2395 ± 121	1987	2193	2031 ± 136
Body diameter	36	44	41	38	43	41
Tail length	120	172	150 ± 14.8	110	169	141 ±16
Anal body diameter	32	38	34	29	34	31
Stylet length	118	129	123 ± 11.3	112	119	115 ± 11
Esophagus length	268	290	276	314	335	325
Distance from head to vulva	1028	1520	1278	--	-	-
Spicule length	-	-	-	39	47	43
A ratio	62	67	-	61	65	-
B ratio	7.8	8.3	-	6.6	7.3	-
C ratio	14	19	-	13	15	-
Ć ratio	3.8	4.5	-	3.8	3.9	-
V%	49%	55%	—	-	-	-

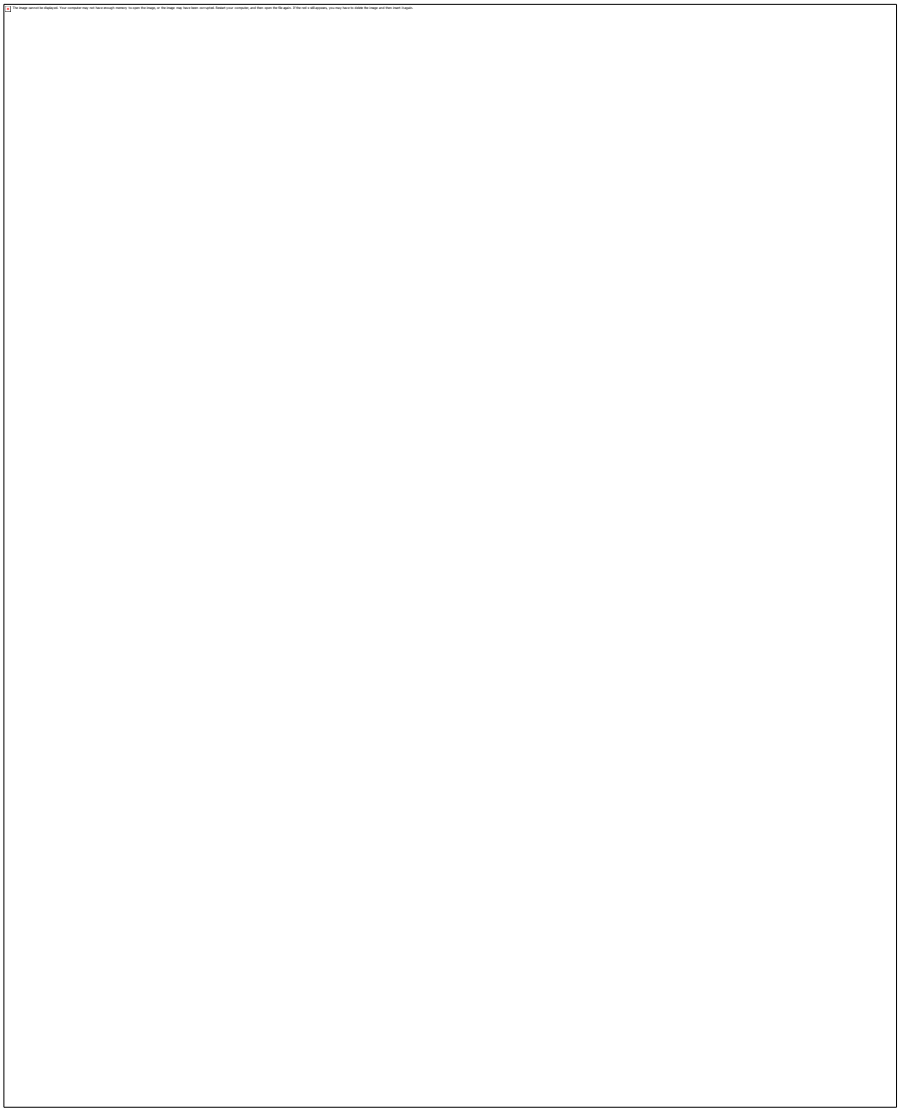

Fig. (1): *Belonolaimus longicaudatus* **(Rau, 1958)**.

(A) Anterior body region, (B) Head region, (C) Vulval region, (D) Female tail, E - Male tail.

Criconemella ornate (Table 2; Fig. 2) is related to *C. curvatum* but differs from the latter by the absence of labial plates and by the pointed outline of the anterior vulvar flap **(Raski, 1952).** Additional important features of *C*. ornate is the presence of the sub-lateral lobes and the relatively few annules, i.e., 87 to 92 annules **(Raski, 1958).**

Table (2): Measurements and ratios of females of *Criconemella ornate* associated with forage legumes.

Body regions and organs	Dimensions (µm) and ratios(n= 13)		
	Minimum	Maximum	Average ± S.D.
Body length	340	460	404 ± 34
Body diameter	28	46	37
Tail length	14	27	21 ± 5.4
Anal body diameter	13	24	19
Stylet length	44	61	52 ± 4.5
Esophagus length	85	127	103
Distance from head to vulva	310	395	366
Spicule length	-	--	--
A ratio	9	16	--
B ratio	3	4	--
C ratio	16	27	--
Ć ratio	1.1	1.1	--
V%	86%	96%	91%

Jairajpuri and Baqri (1973) presented a key for *Hoplolaimus* spp. They differentiated between *H. Tylenchiformis*, having three annules in the lip region and female tails usually bluntly rounded, and *H. galeatus* (Table 3; Fig. 3), having four or more annules and female tails rounded. Also, they used the following characteristics of *H. galeatus* to differentiate it from other *Hoplolaimus* spp.: four incisures on the body, excretory pore located below the hemizonid, one phasmid in anterior part of the body and one phasmid in the posterior part of it, and spicules 40 - 50 µm long.

Table (3): Measurements and ratios of females and males of *Hoplolaimus galeatus* associated with forage legumes.

Body regions and organs	Dimensions (µm) and ratios					
	Females (n= 10)			Males (n= 4)		
	Minimum	Maximum	Average ± S.D.	Minimum	Maximum	Average ± S.D.
Body length	1283	1643	1464 ± 119	983	1195	1072 ± 99
Body diameter	40	55	47	35	42	39
Tail length	15	37	26 ± 8	20	32	26 ± 5
Anal body diameter	31	39	35	25	33	28
Stylet length	48	60	5.2 ± 4.6	42	50	45 ± 3.6
Esophagus length	178	220	200	165	190	180
Distance from head to vulva	736	853	807	--	--	--
Spicule length	--	--	--	51	59	55
A ratio	25.8	37.8	--	28.1	28.5	--
B ratio	6.7	8.4	--	6	6.3	--
C ratio	38.4	97.6	--	37.3	49.2	--
Ć ratio	0.5	1.0	--	0.8	1.0	--
V%	52%	58%	--	--	--	--

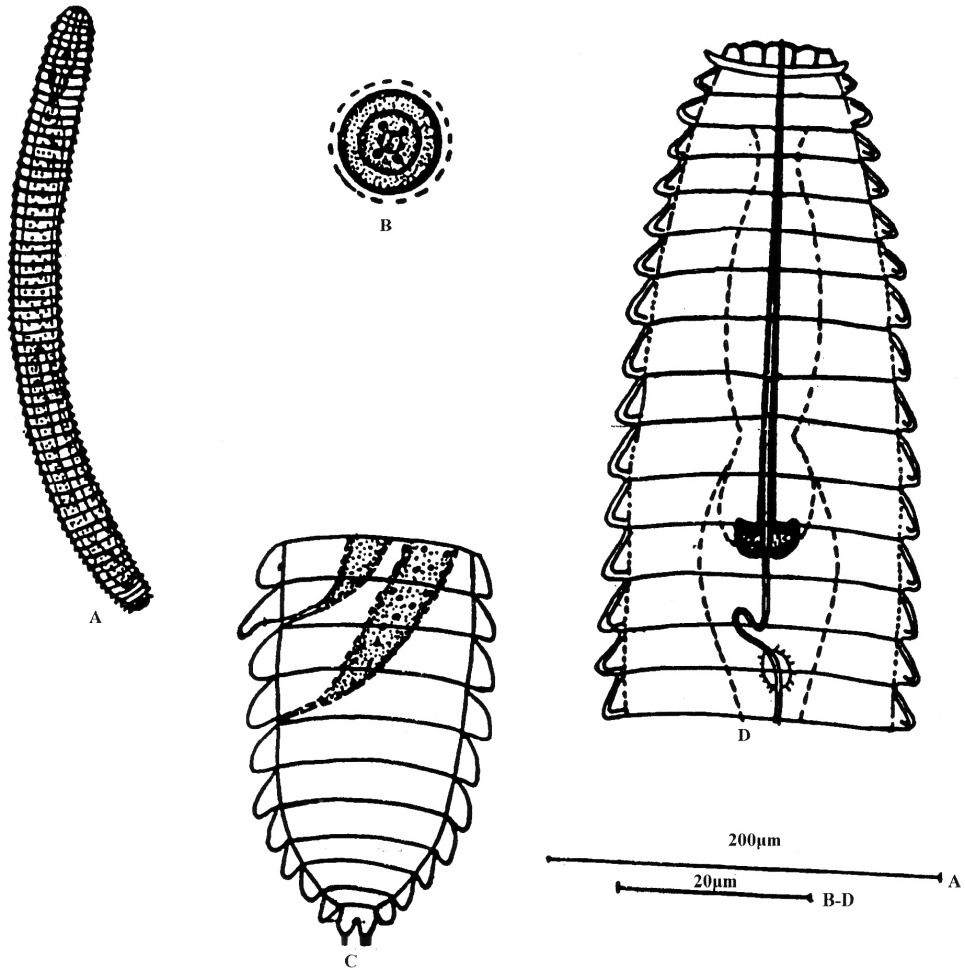

Fig. (2): *Criconemella ornate* **(Raaki, 1958, Luc & Raski, 1981).**

(A) Adult female, (B) Head en face view, (C) Female tail, (D) Anterior body region.

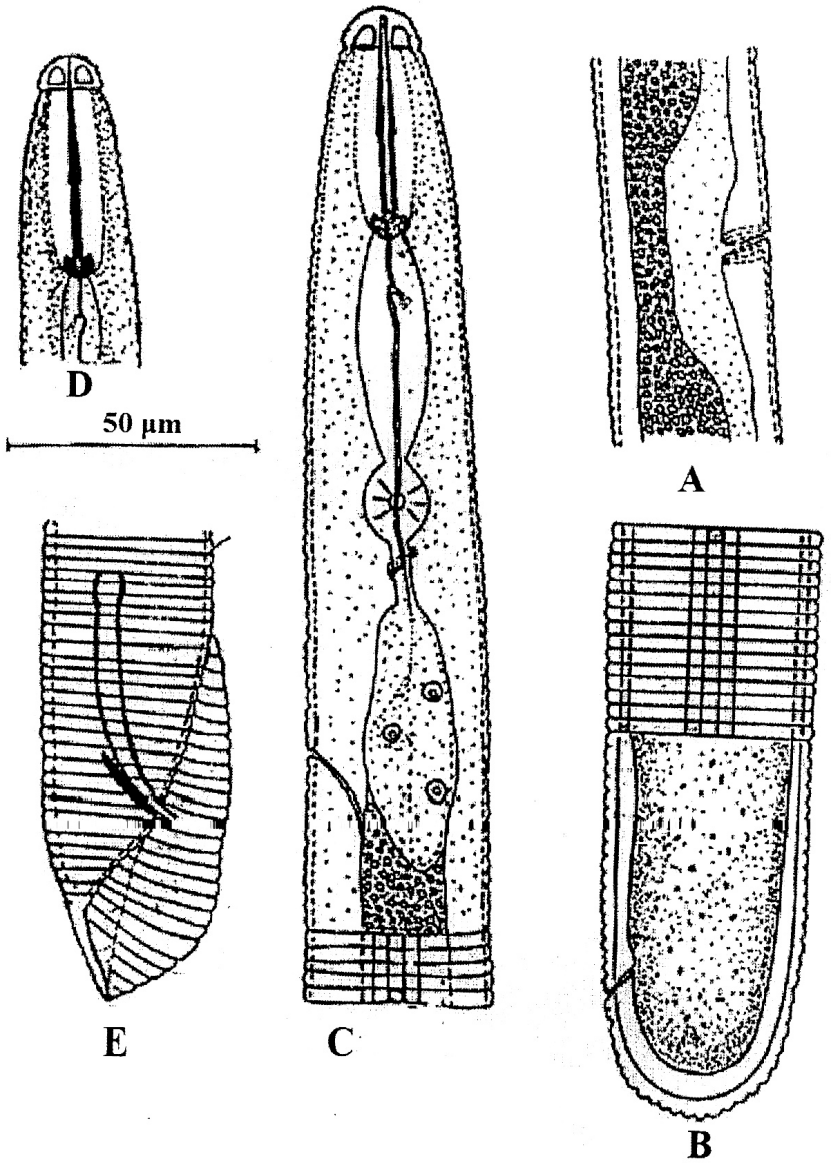

Fig. (3): *Hoplolabnus galeatus* **(Cobb, 1913, Thorne, 1935).**

(A) Vulval region, (B) Female tail, (C) Anterior body region, (D) Male, head region, (E) Male, tail region.

After **Siddiqi's division (1973)** of the genus *Trichodorus* into *Paratrichodorus* and *Trichodorus*, there had been considerable confusion in the literature because some authors apparently had not recognized *Paratrichodorus* spp. as a valid genus, whereas others had **(Perry and Rhoades, 1982).** Now, both genera are well established (e.g., **Decraemer and Hunt, 2013).** In our specimens (Table 4; Fig. 4) the two characters used for separating the genus, *i.e.* male with bursa and female with weak and indistinct sclerotization between the vulva and vagina were recognizable and it keys to *P. christiei*. Yet, our specimens agreed with the synonomy of *P. christiei* and *P. minor* since the lengths of the onchiostyle and spicules of our specimens overlap those of both species **(Loof, 1975; Decraemer, 1980).**

Table (4): Measurements and ratios of females and males of *Paratrichodorus minor* associated with forage legumes.

Body regions and organs	Dimensions (µm) and ratios					
	Females (n= 10)			Males (n= 4)		
	Minimum	Maximum	Average± S.D.	Minimum	Maximum	Average ± S.D.
Body length	462	735	610 ±117	589	692	633 ± 4
Body diameter	30	49	35	34	38	37
Tail length	--	--	--	19	23	20 ± 2
Anal body diameter	--	--	--	9	12	10
Stylet length	30	48	41 ± 8	32	40	36 ± 4
Esophagus length	96	122	106	85	90	87
Distance from head to vulva	240	370	310	--	--	--
Spicule length	--	--	--	58	66	61
A ratio	15	15.4	15.2	17.3	18.2	--
B ratio	4.8	6	5.4	6.9	7.7	--
C ratio	--	--	--	30	31	--
Ć ratio	--	--	--	1.9	2.1	--
V%	50%	52%	51%	--	--	=

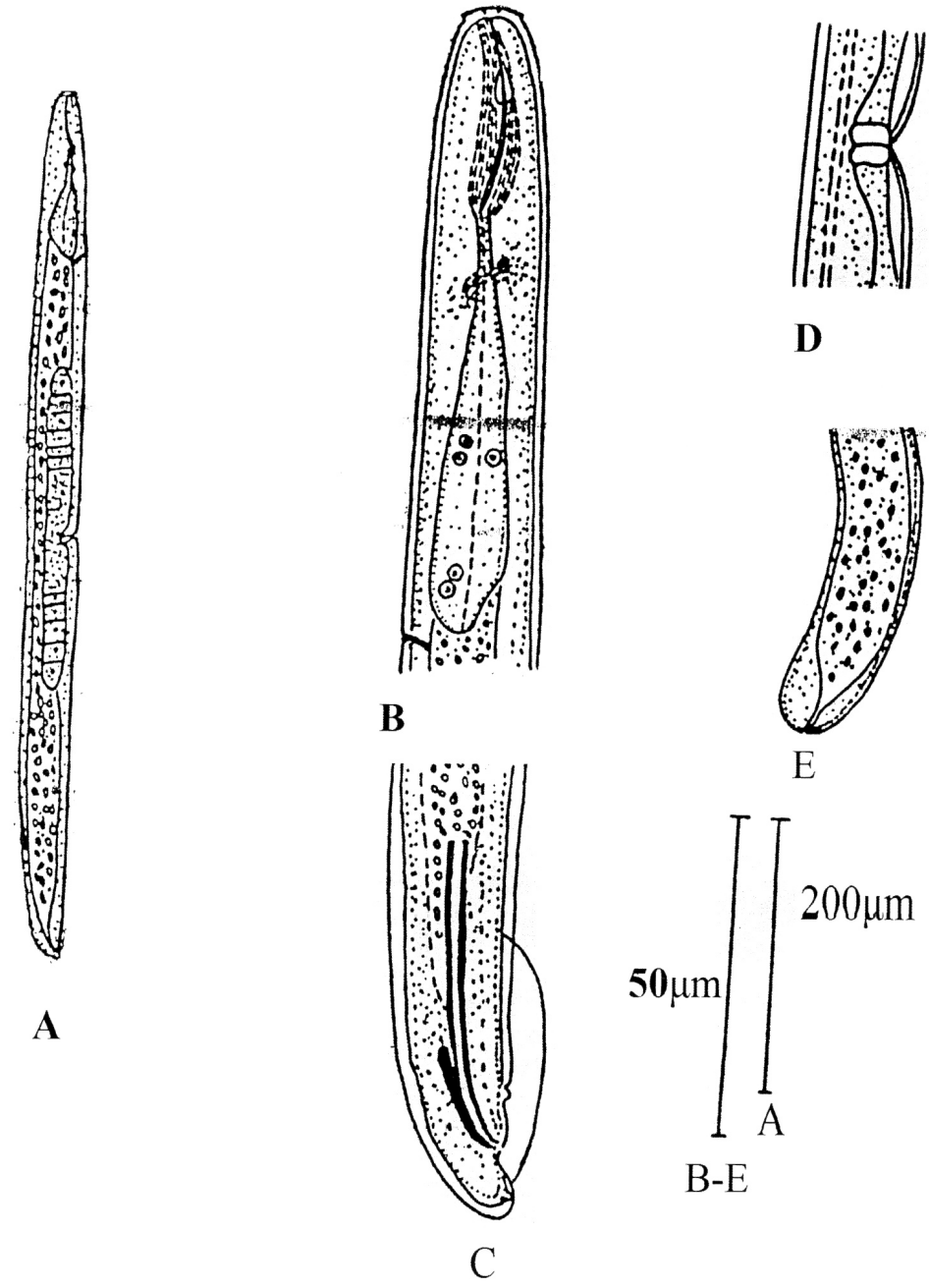

Fig. (4) *Paratrichodorus minor* **(Allen,1957, Siddiqi, 1973).**

(A) Adult female, (B) Female, anterior body region, (C) Male tail, (D) Vulval region, E- Female tail.

Acknowledgement

This study was supported in part by the NRC In-house project entitled 'Pesticide alternatives against soilborne pathogens attacking legume cultivation in Egypt'.

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وصف وتعريف أربعة أنواع من النيماتودا المتطفلة نباتيًا المرتبطة بالبقوليات العلفية

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الملخص العربي

تم وصف وتعريف أربعة أنواع من النيماتودا المتطفلة نباتيا المرتبطة بالبقوليات العلفية في مقاطعة الاشوا في ولاية فلوريدا بالولايات المتحدة الأمريكية. الأنواع المكتشفة في هذه الدراسة هي *بيلونولايموس لونجيكوداتوس*، *كريكونيميلا أورنات*، *هوبلولايموس جالياتوس*، و*باراتريكودوروس مينور*، وهي تنتمي إلي الرتب ربديتيدا (*بيلونولايموس لونجيكوداتوس*، *كريكونيميلا اورنات*، و*هوبلوليموس جالياتوس*) وتريبلونشيدا (*باراترايكودوروس مينور*) والعائلات التصنيفية دوليكودوريداي (*بيلونولايموس لونجيكوداتوس*)، هوبولولايميداي (*هوبلولايموس جاليتوس*) كريكونماتيدا (*كريكونيميلا اورنات*)، وتريكودوريداي (*باراتريكودوروس مينور*). استند التعريف الحالي لهذه الأنواع إلى التصنيف الكلاسيكي باستخدام مفاتيح تحديد الأنواع المحددة بناء على الصفات والقياسات المورفولوجية (الخاصة بالشكل الظاهري).

الكلمات الدالة: *بيلونولايموس لونجيكوداتوس– كريكونيميلا اورنات– هوبلولايموس جالياتوس– باراتريكودوروس مينور*– الشكل الظاهري – وصف الأنواع.