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Morpho-biometric data of *Chitwoodius coffeae* and of some mononchids (Nematoda: Enoplea) from Venezuela

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ABSTRACT

Free-living nematodes in Venezuela have been poorly studied. The interest by these nematodes centers on the fact that these organisms can be used as indicators of soil quality. However, their usefulness as bioindicators depends intrinsically on the role they play within the edaphic trophic web and extrinsically on their correct identification. Recently, free-living forms were identified in rhizospheric soil samples from different plants and localities of the country. Specimens were extracted from soil samples by modified Cobb method. Nematodes were fixed in a hot (80 ºC) solution of 2.5% formaldehyde, subsequently infiltrated with glycerin using the Seinhorst's rapid method, and then observed, photographed with eye-piece camera and hand drawn with help of a camera lucid mounted on a light microscope. Raw photographs were edited using Adobe® Photoshop®. Populations of C*hitwoodius coffeae*, *Prionchulus zelli*, *Sporonchulus ibitiensis* and C*rassibucca* were detected for the first time in Venezuela. Comments on the morpho-biometric characteristics of the recorded all nematodes are presented here.

Key words: Biodiversity, Bioindicators, Invertebrate, Nematodes, Taxonomy, Zoology.

Datos morfo-biométricos de *Chitwoodius coffeae* y de algunos monónquidos (Nematoda: Enoplea) provenientes de Venezuela

RESUMEN

Los nematodos de vida libre en Venezuela han sido poco estudiados. El interés por esos nematodos se centra en el hecho de que estos organismos pueden utilizarse como indicadores de la calidad del suelo. Sin embargo, su utilidad como bioindicadores depende intrínsecamente del papel que desempeñan dentro de la red trófica edáfica y extrínsecamente de su correcta identificación. Recientemente, se identificaron formas de vida libre en muestras de suelo rizosférico de diferentes plantas y localidades del país. Los ejemplares, se extrajeron de muestras de suelo mediante el método Cobb modificado. Los nematodos se fijaron en una solución caliente (80 ºC) de formaldehído al 2,5%, posteriormente se infiltraron con glicerina utilizando el método rápido de Seinhorst, y luego se observaron, fotografiaron con una cámara ocular y se dibujaron

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a mano con ayuda de una cámara lúcida montada en un microscopio óptico. Las fotografías sin procesar se editaron con Adobe® Photoshop®. Poblaciones de *Chitwoodius coffeae*, *Prionchulus zelli*, *Sporonchulus ibitiensis* y *Crassibucca* fueron detectadas por primera vez en Venezuela. Aquí se presentan los comentarios sobre las características morfo-biométricas de todos los nematodos registrados.

Palabras clave: Biodiversidad, Bioindicadores, Invertebrado, Nematodos, Taxonomía, Zoología.

INTRODUCTION

Venezuela is among the ten countries with the highest biodiversity in the world. The most important animal groups within the invertebrates are: insects (> 14000 species), crustaceans (1266 species), mollusks (1157 species) and nematodes with 596 parasitic species of animals, plants and free-living of the venezuelan marine environments not including free-living edaphic nematodes (Grande, 2019).

The little existing taxonomic literature in the country related to free-living soil nematodes is due to several reasons: a. Traditional morpho-anatomical and biometric characterization is an arduous and difficult work, especially when morphological and biological aspects of a particular species are unknown; b. Study of these organisms has focused only on the parasitic forms of plants and animals; c. Little interest in free-living nematodes and ignorance of their importance; d. Low numbers of nematologists and e. Technical-scientific limitations, especially the impossibility of accessing molecular tools for identification and use of scanning electron microscopy (SEM) to reveal morphological details of interest.

Currently, it has been demonstrated that for identification of plant-parasitic and free-living nematodes, DNA based studies are quicker than the classical strategy (morphology and biometrics). However, morphological identification is still a prerequisite for DNA based characterization because the descriptions of the animal species are still done mainly based on morphological and biometric characters. The morphological and biometric distinction are two essential component of any higher level classification of nematodes, and in many cases, provides rapid, and, most of the time, unambiguous diagnosis of species (Kumari, 2009; Decraemer and Backeljau, 2015; Shah and Mir, 2015; Bogale *et al*., 2020).

Recently, new populations of *Chitwoodius coffeae* Hoang, Chu, Nguyen, Trinh, Abolafia & Peña-Santiago, 2019 (Dorylaimida: Tylencholaimidae), *Prionchulus zelli* Winiszewska & Susulovsky, 2003 (Mononchida: Mononchidae), *Sporonchulus ibitiensis* (Carvalho, 1951) Andrassy, 1958 (Mononchida: Mylonchulidae) and *Crassibucca* Mulvey & Jensen, 1967 (Mononchida: Anatonchidae) were recovered in soil samples from different locations in the country. The interest now in free-living nematodes centers on the fact that these organisms can be used as indicators of soil quality. Therefore, this research note provides morpho-biometric data and illustration (light microscopy pictures) of these species for Venezuela.

MATERIALS AND METHODS

Material examined

The nematodes were recovered of soil samples around the roots of different cultivated and not cultivated plants from some localities of Venezuela (Table 1).

Extraction, morpho-biometric characterization and identification of the nematodes

Specimens were extracted in the Agricultural Nematology Laboratory, Department and Institute of Agricultural Zoology, Faculty of Agronomy, Central University of Venezuela, Maracay, Aragua state. from soil samples by modified Cobb method (Crozzoli, 2014). Nematodes were fixed in a hot (80 ºC) solution of 2.5% formaldehyde, subsequently infiltrated with glycerin using the Seinhorst's rapid method (Marais *et al*., 2017), and then observed, photographed with eye-piece camera MD-35 of AmScope® and hand drawn with help of a camera lucid mounted on a light microscope SM-LUX of Leitz-Wetzlar. Raw photographs were edited using Adobe® Photoshop®. All measurements were in

Table 1. Origin of the free-living soil nematodes described in the study.

Nematodes	Locality	Habitat or associated plants	Coordinates
Chitwoodius coffeae	Chabasquén, Monseñor José Vicente de Unda, Portuguesa state	*Coffea arabica* L.	9.453168, -69.973246
Mylonchulus lacustris	Maracay, Girardot, Aragua state	Tropical dry forest	10.269917, -67.608429
Prionchulus zelli	Guapa, Andrés Eloy Blanco, Lara State	*Coffea arabica* L.	Non registrated
Sporonchulus ibitiensis	El Carito, Libertad, Anzoátegui state	Tropical dry forest	9.805178, -64.744385
Sporonchulus ibitiensis	Maracay, Girardot, Aragua state	Tropical dry forest	10.269917, -67.608429
Crassibucca sp.	Aponte, Ocumare de la Costa de Oro, Aragua	*Theobroma cacao* L.	Non registrated

μm, except for body length (L) in mm, and in the form: mean ± standard deviation (range). Body dimensions, ratios and position of the pharyngeal gland nuclei (L, a, b, c, c', V, DN, S_1N_1, S_1N_2 and S_2N) were tabulated in accordance with de Man's and Loof-Coomans formulae (Nguyen *et al.*, 2016; Marais *et al.*, 2017). Permanent slides were stored at the nematological collection of Laboratorio de Nematología Agrícola, Facultad de Agronomía in Maracay, Aragua state.

Abbreviations (de Man formulae): n = number of specimens, L = total body length, a = L / maximum body diameter, b = L / pharyngeal length, b' = L / neck length, c = L / tail length, c' = tail length / anal body diameter, V = distance from anterior end to vulva x 100 / L, G_1 = length of anterior branch x 100 / total body length and G_2 = length of posterior branch x 100 / total body length.

Abbreviations (Loof-Coomans formulae) only for *C. coffeae*: DN = distance from body anterior end to the nucleus of pharyngeal dorsal gland x 100 / total neck length, S_1N_1 = distance from body anterior end to the anterior nucleus of first pair of ventro-sublateral pharyngeal glands x 100 / total neck length, S_1N_2 = distance from body anterior end to the posterior nucleus of the first pair of ventro-sublateral pharyngeal glands x 100 / total neck length and S_2N = distance from body anterior end to the nuclei of the second pair of ventro-sublateral pharyngeal glands x 100 / total neck length.

RESULTS

Description of *C. coffeae* (Figure 1A-H; Table 2)

Female. Medium sized body, ventrally curved upon fixation; lip region hemispherical, offset by a constriction; odontostyle dorylaimoid; guiding ring single and delicate, located at 18.3-21.7 μm from anterior end; odontophore rod-like, 1.1-1.2 times the odontostyle length; pharynx with a weakly muscular anterior section and expansion basal occupying 49.9-56.1% of total neck length, five pharyngeal gland nuclei located as follows: DN = 49.4-56.9%, S_1N_1 = 68-73.6%, S_1N_2 =70-75% and S_2N = 85.6-94.5%; nerve ring located at 121.4-154.2 μm from anterior end or 28.4-33.8% of total neck length; cardia conoid surrounded by intestinal tissue; vulva pore-like in lateral view, post-equatorial; vagina 18.3-27.5 μm long with three distinct parts: *pars proximalis vaginae* (14.3-22.8 μm long), *pars refringens vaginae* sclerotized visible as two small triangular pieces in optical section (8-14.3 μm wide x 1.7-3.4 μm long) and *pars distalis vaginae* (1.1-2.9 μm

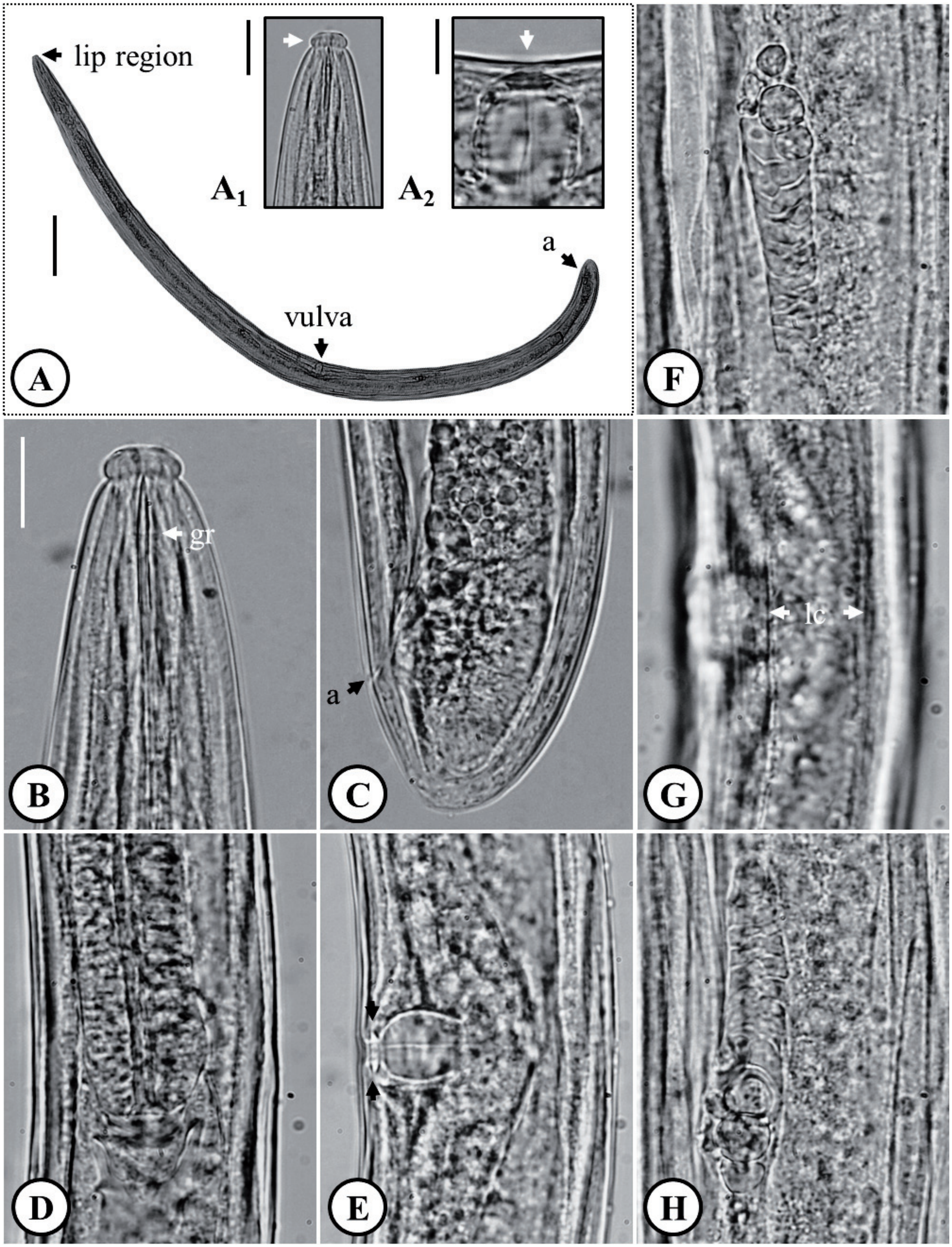

Figure 1. Female of *Chitwoodius coffeae* from Chabasquén, Portuguesa (Venezuela). A: Entire body (A_1 = lip region; A_2 = vulva indicated by arrow and vagina). B: Anterior region (gr = guiding ring). C: Posterior region (a = anus). D: Pharyngo-intestinal junction. E: Vagina (*pars refrigens vaginae*). F: Anterior ovary. G: Lateral chord at vulva level (lc = lateral chord). H: Posterior ovary. Bar A = 125 µm, Bar A_1 = 25 µm, Bar A_2 = 10 µm, Bar B-H = 20 µm.

Table 2. Female biometrics data of *Chitwoodius coffeae*, *Mylonchulus lacustris*, *Prionchulus zelli* and *Sporonchulus ibitiensis* from Venezuela.

Measured and ratios[1]	*C. coffeae*	*M. lacustris*	*P. zelli*	*S. ibitiensis*	
	Portuguesa	**Aragua**	**Lara**	**Anzoátegui**	**Aragua**
	♀♀ (n=11)	**♀♀ (n=12)**	**♀♀ (n=2)**	**♀♀ (n=5)**	**♀♀ (n=6)**
L (mm)	1.72±0.15	0.90±0.09	2.14, 2.37	1.3 ± 0.20	1.1 ± 0.14
	(1.5-1.9)	(0.76-1.1)	-	(1.0 - 1.5)	(1.0 - 1.3)
a	25.5±2.5	23.8±2.3	22.1, 24.6	28.1 ± 4.1	26.4 ± 3.4
	(21.7-29.6)	(20.6-27.4)	-	(23.8 - 33.1)	(21.9 - 29.8)
b	4.5 ± 0.30	3.4±0.2	3.7, 3.9	4.5 ± 0.6	4.1 ± 0.4
	(4.0-4.9)	(3.1-3.7)	-	(3.8 - 5.2)	(3.4 - 4.7)
b’	3.9 ± 0.24	-	-		
	(3.5-4.2)	-	-		
c	58.1 ± 4.2	31.2±3.9	13.2, 14.7	24.7 ± 2.7	22.9 ± 3.7
	(51.5-66.8)	(26.1-40.2)	-	(21.0 - 27.8)	(17.4 - 26.8)
c’	0.73 ± 0.03	1.3±0.2	2.8, 3.7	1.9 ± 0.3	1..9 ± 0.2
	(0.68-0.78)	(1.0-1.7)	-	(1.4 - 2.2)	(1.6 - 2.3)
V(%)	56.2 ± 1.2	64.6±1.4	63.9, 66.7	60.5 ± 1.6	61.5 ± 0.7
	(54.4-57.6)	(62.2-66.9)	-	(58.9 - 62.7)	(60.7 - 62.5)
Lip region diameter	15.9 ± 0.9	20.9±3.1	57.1, 60.0	28.6 ± 1.8	22.6 ± 2.9
	(14.3-17.1)	(17.1-28.6)	-	(25.7 - 30.0)	(20.0 - 26.8)
Lip region height	6.4 ± 0.8	-	12.9, 14.3	5.5 ± 0.5	5.0 ± 1.2
	(5.1-7.4)	-	-	(4.7 - 5.7)	(3.4 - 6.8)
Odontostyle length	29.7 ± 1.8	-	-	-	-
	(26.8-33.7)	-	-	-	-
Odontophore length	26.4 ± 2.5	-	-	-	-
	(21.7-29.7)	-	-	-	-
Buccal cavity length	-	25.1±1.4	51.4, 54.3	29.3 ± 2.4	27.8 ± 4.0
	-	(22.8-27.4)	-	(26.8 - 32.6)	(23.9 - 34.3)
Buccal cavity width	-	14.7±0.8	30.0, 31.4	16.2 ± 1.8	14.8 ± 2.8
	-	(13.1-15.4)	-	(14.3 - 18.8)	(10.2 - 18.3)
Position of tooth apex	-	18.8±1.0	41.4, 41.4	18.7 ± 1.2	18.4 ± 1.7
		(17.7-20.6)	-	(17.1 - 20.0)	(17.1 - 21.7)
Total neck length	441.6±17.8	-	-	-	-
	(416.9-468.4)	-	-	-	-
Body diam. at mid-body	67.8 ± 8.7	38.2±3.0	87.1, 107.1	45.1 ± 6.5	44.7 ± 1.5
	(56.4-81.4)	(35.7-44.3)	-	(38.6 - 52.8)	(42.8 - 45.7)
Body diam. at anus	40.5 ± 2.7	24.2±2.2	45.7, 57.1	28.0 ± 2.8	25.7 ± 1.8
	(37.1-45.7)	(20.7-28.6)	-	(24.3 - 30.0)	(24.3 - 28.6)
Rectum length	34.0 ± 4.4	20.1±2.3	47.1, 51.4	26.0 ± 1.2	23.0 ± 3.7
	(29.0-39.4)	(17.1-24.3)	-	(24.3 - 27.1)	(19.9 - 29.7)
Tail length	29.6 ± 2.1	29.2±3.2	-	51.4 ± 8.9	50.5 ± 4.8
	(26.3-32.6)	(24.3-34.3)	161.4, 162.8	(35.7 - 57.1)	(45.7 - 57.1)

[1]All measurements are in µm except ratios a, b, b’ c, c’ and L in mm.

long); reproductive system didelphic-amphidelphic with almost equally developed branches, anterior branch 264.5-351 µm long (G_1 = 15.7-20.2% of total body length), posterior 232.8-370.7 µm long (G_2 = 15.1-20.3% of total body length), ovaries reflected, anterior ovary 60-87.1µm long, posterior 56.5-98.5 µm long; lateral chord about 20-25.7 µm (32.6-36%) of body width at mid body; tail short and rounded; male not found.

Remarks. According to the description and key published by Hoang *et al.* (2019) our specimens comes close to C. *coffeae*. The measurements (Table 2) and description of venezuelan specimens (female) correspond well to the holotype and paratypes described by these authors except for the c value (58.1 vs 68) and tail length (29.6 µm vs 25 µm). Differences between population from Venezuela and the type population of C. *coffeae* may be considered as intraspecific variation. The genus C*hitwoodius* has been reported in South America only Brazil and Colombia (Peña-Santiago, 2002) and C. *coffeae*, is a nematode described in Vietnam from soil samples of C*offea canephora* Pierre ex A. Froehner.

Description of *M. lacustris* (Figure 2A-J; Table 2)

Female. Small to medium sized body, ventrally curved upon fixation and J or spiral-shaped; cuticle smooth 1.4-3 µm thick at various body regions; buccal cavity in V-shaped, dorsal tooth anterior, its apex at 76% (71-81%) from base of stoma, opposed by 6-7 transverse rows of denticles, submedian teeth present; pharynx cylindrical and muscular, 273 µm long (206-314 µm), pharyngo-intestinal junction non-tuberculate; nerve ring and excretory pore inconspicuous; vulva transverse, *pars refringens vaginae* sclerotized visible as two small pieces in optical section, vulva-anus distance 171-361 µm; reproductive system didelphic-amphidelphic with almost equally developed branches, anterior branch 91-113 µm long (G_1 = 10-13% of total body length), posterior 93-114 µm long (G_2 = 10-13% of total body length), ovaries reflected; rectum straight; tail conoid with truncated terminus, ventrally bent, three caudal glands and terminal spinneret opening.

Remarks. Compared with other populations of *M. lacustris*, the biometric and allometric values of females of the venezuelan population agree well with populations from India (Jana *et al*., 2010; Tahseen *et al*., 2013) but differs in having gonad (G_1 = 12-19% vs 10-13% and G_2 = 10-19% vs 10-13%) comparatively short. This nematode has a wide worldwide distribution and has been reported in Australia, Canada, India, Japan, Mexico, Nigeria, Panama, South Africa, USA and Venezuela (Loof, 1964; Ahmad and Jairajpuri, 2010).

Description of *P. zelli* (Figure 3A-I; Table 2)

Female. Medium sized body, ventrally curved upon fixation, J-shaped; cuticle smooth 4.8-5.2 µm thick at mid-body; lip region hemispherical with conical cephalic papillae, slightly offset by a depression; amphid oval its aperture 5.1 µm wide, located 18.8 µm from anterior end; buccal cavity in V-shaped, dorsal tooth apex directed forward (situated at 76-81% of buccal cavity length from its base) and opposed by 11-12 denticles arranged in two parallel longitudinal rows; pharynx cylindrical and muscular, 543-634 µm long, pharyngo-intestinal junction non-tuberculate; nerve ring located at 150.0-189.9 µm from anterior end; vulva transverse with asymmetrical lips, post-equatorial; vagina 32.8-37.1 µm long, *pars proximalis vaginae* 27.1-31.4 µm long, *pars refringens vaginae* sclerotized visible as two triangular pieces in optical section (5.7 µm long) and *pars distalis vaginae* absent; reproductive system didelphic-amphidelphic, asymmetric branches, anterior branch 537.0 µm long (G_1 = 22.7% of total body length), posterior 656.9 µm long (G_2 = 27.7% of total body length), ovaries reflected, anterior ovary 232.8 µm long, posterior 284.2 µm long; intrauterine egg no equinulated 92.8 µm long x 84.3 µm wide; rectum straight; tail elongate-conoid, ventrally bent, caudal glands and spinneret opening absent; male not found.

Remarks. The measurements (Table 2) and description of venezuelan specimens (female) correspond well to the holotype and paratypes described by Winiszewska and Susulovsky (2003) but differs in having gonad (G_1 = 22.7% vs 8.3-13.9% and G_2 = 27.7% vs 7.6-15.2%) and ovaries comparatively long. This species has been only reported in Czech Republic, Germany, Poland and Ukraine (Winiszewska and Susulovsky, 2003).

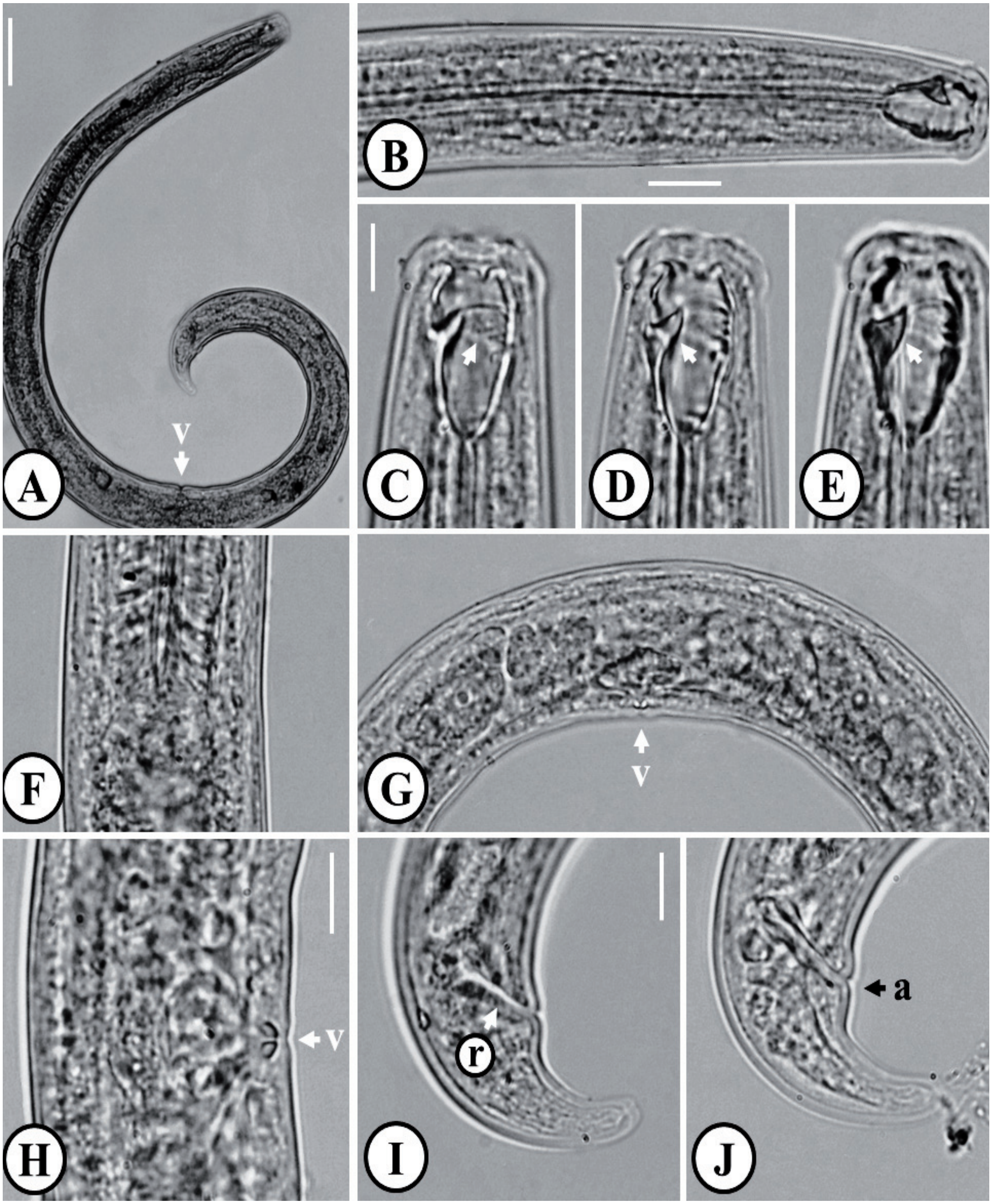

Figure 2. Female of *Mylonchulus lacustris* from Maracay, Aragua (Venezuela). A: Body entire (v = vulva). B: Anterior región. C-E: Buccal cavity (rows of denticles and dorsal tooth indicated by arrows). F: Pharyngo-intestinal junction. G: Reproductive system. H: Vagina (*pars refrigens vaginae*). I-J: Tail (a= anus; r = rectum). Bar A = 50 µm, Bar B = 20 µm in G, Bar C = 10 µm in D-F, Bar H = 10 µm, Bar I = 10 µm in J.

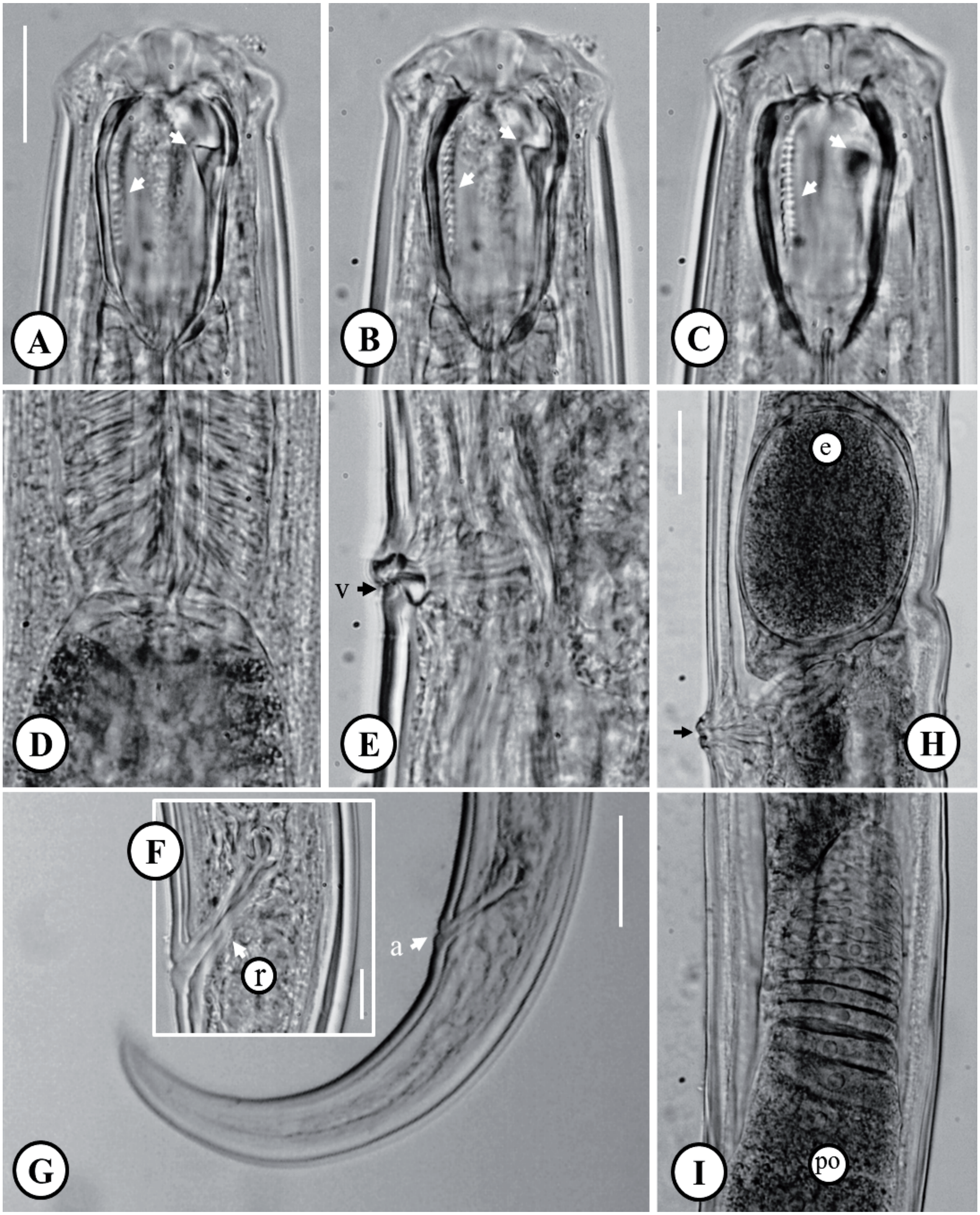

Figure 3. Female of *Prionchulus zelli* from Guapa, Lara (Venezuela). A-C: Buccal cavity. D: Pharyngo-intestinal junction. E: Vagina (*pars refrigens vaginae*). F: Anal region (r = rectum). G: Tail (a = anus). H-I: Reproductive system (vulva indicated by arrow; e = egg and po = posterior ovary). Bar A-E = 25 µm, Bar F = 15 µm, Bar G = 50 µm, Bar H-I = 35 µm.

The *Prionchulus* Cobb, 1916 genus was recorded in the country by Torrealba (1969) but not supported by descriptions, measurements, ratios or illustrations of the specimens.

Description of *S. ibitiensis* (Figure 4A-H; Table 2 and 3)

Female. Medium sized body, ventrally curved upon fixation, J-shaped; cuticle smooth; dorsal tooth apex directed forward (situated at 59-74% of buccal cavity length from its base) and opposed by numerous denticles arranged in four irregular rows; pharynx cylindrical and muscular, 241-314 μm long, pharyngo-intestinal junction non-tuberculate; nerve ring located at 83-136 μm from anterior end; vulva transverse and post-equatorial with pars *refringens vaginae* visible as two rectangular pieces in optical section, vulva-anus distance 269-543 μm; reproductive system didelphic-amphidelphic with almost equally developed branches, anterior branch 98-179 μm long (G_1 = 9-13% of total body length), posterior 109-174 μm long (G_2 = 9-13% of total body length), ovaries reflected; tail conoid, ventrally arcuate, caudal glands and spinneret opening absent; male not found.

Juvenile stages. All four juvenile stages were separated using morphological characters such as body length, position of dorsal tooth apex and tail length (Table 3). Its resembles to female in general morphology except for smaller size. Specifically, J1 was characterized by subventral denticles absent with except subsequent stages (J2, J3 and J4).

Remarks. The morpho-anatomical and biometric characteristics of the females (venezuelan populations) of *S. ibitiensis* do not differ from those indicated by Carvalho (1951), Chaves (1990) and Loof (2006) except for the length of the tail which is shorter in our specimens (Table 1). Regarding the juvenile stages, the literature consulted does not indicate allometric measurements and relationships. Reports of *S. ibitiensis* in Argentina (South America), Brazil (South America), Cameroon (Africa), India (Asia), Malaysia (Asia), Nigeria (Africa), Sri Lanka (Asia) and Zaire (Africa) suggest a distribution essentially tropical and subtropical (Andrássy, 1993; Ahmad and Jairajpuri, 2010). In Venezuela, the only species mentioned is *S. dentatus*; However, its description, measurements and allometrics relations were based on the study of a single specimen from San Felipe, Yaracuy state (Mulvey, 1963). The other known record of *Sporonchulus* was made by Torrealba (1969) without presenting a description, measurements, allometric relations and illustrations of the nematode.

Description of *Crassibucca* sp. (Figure 5A-E; Table 3)

Female. Cylindrical body and small length, ventrally curved upon fixation, C-shaped; cuticle smooth; conical papillae; dorsal tooth anterior, its apex at 80% (80-81%) from base of stoma, four ventrosublateral denticles: one pair in posterior half [9.8 μm (9.1-10 μm) from base of stoma] and one pair in anterior of stoma [21 μm (21-22 μm) from base of stoma]; cylindrical pharynx, 205 μm long (171-246 μm); nerve ring and excretory pore inconspicuous; vulva transverse, post-equatorial, vulva-anus distance 161 μm (143-194 μm); reproductive system apparently prodelphic; tail conoid-arcuate, caudal glands and spinneret absent; juveniles stages and male not found.

Remarks. C*rassibucca*, is a genus of predatory nematodes extremely rare in the world; only four species are known: C. *asterocaudata* Jairajpuri, Ahmad & Sturhan, 1998, C. *colombica* Siddiqi, 1984, C. *macrocauda* Mulvey & Jensen, 1967 and C. *penicula* Mulvey & Jensen, 1967 (Ahmad and Jairajpuri, 2010). It is important to highlight that the morpho-anatomical characteristics, especially those related to the buccal cavity, correspond to those of the genus. However, the separation at the species level in this case is complicated, because the venezuelan population presents values and biometric ranges lower than those indicated for C. *penicula* despite the fact that our specimens present a remarkable morphological similarity with this species. In addition, the length and shape of the tail in the venezuelan specimens differ from those indicated for the rest of the species. The presence of two aciculiform structures at the end of the tail was observed in a single specimen (Figure 4E). Representatives of this genus have been reported in Cameroon (Africa), Colombia (South America), Santa Lucia Island (South America), Nicaragua

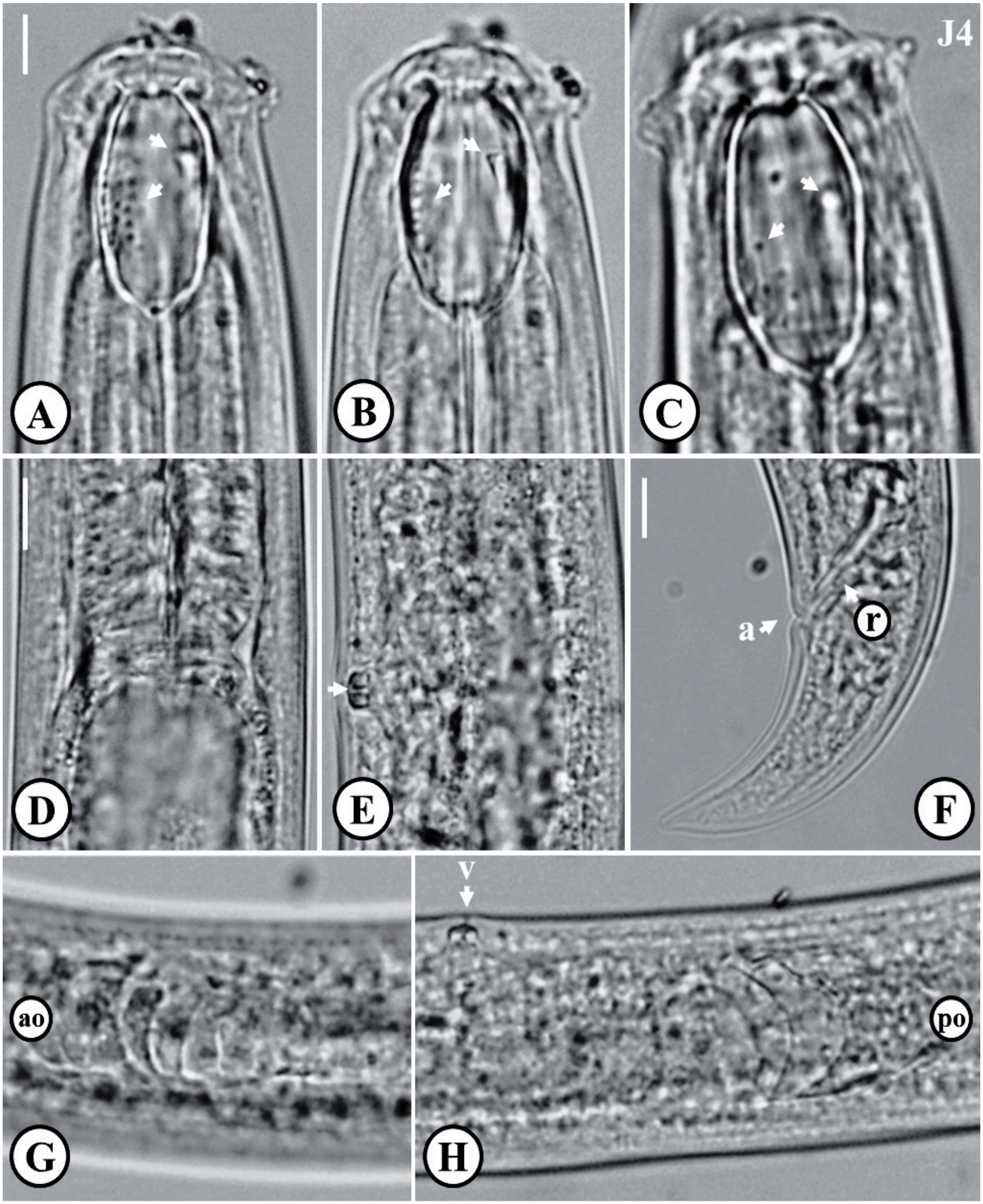

Figure 4. Female and J4 of *Sporonchulus ibitiensis* from Maracay, Aragua (Venezuela). A-B: Buccal cavity (dorsal tooh and denticles indicated by arrows). C: Buccal cavity of J4. D: Pharyngo-intestinal junction. E: Vagina (pars refrigens vaginae). Tail (a = anus and r = rectum). G-H: Reproductive system (vulva indicated by arrow; ao = anterior ovary and po = posterior ovary). Bar A= 5 µm in B-C, Bar D = 10 µm in E, Bar F = 10 µm in G-H.

Table 3. Juveniles biometrics data of *Sporonchulus ibitiensis* and females biometrics data of C*rassibucca* sp. from Venezuela.

Measured and ratios[1]	*S. ibitiensis* juveniles stages				***Crassibucca* sp.**
	J1 (n=1)	**J2 (n=3)**	**J3 (n=1)**	**J4 (n=4)**	**♀♀ (n=5)**
L (mm)	0.32	0.54	0.87	0.98 ± 0.07	0.74 ± 0.11
	-	(0.48-0.67)	-	(0.92 - 1.07)	(0.66 - 0.90)
a	19.0	18.8 ± 2.8	25.0	26.6 ± 2.2	22.7±1.7
	-	(15.6 - 20.5)	-	(23.8 - 28.9)	(21.5-25.4)
b	3.1	3.7 ± 0.2	4.1	4.3 ± 0.3	3.7±0.3
	-	(3.5 - 3.8)	-	(3.9 - 4.6)	(3.2-3.9)
b'	-	-	-	-	-
	-	-	-	-	-
c	11.9	19.0 ± 4.7	25.1	22.7 ± 1.0	19.0±2.8
	-	(16.0 - 24.5)	-	(21.5 - 23.5)	(16.0-23.5)
c'	2.0	1.5 ± 0.1	1.5	1.8 ± 0.08	1.9±0.2
	-	(1.4 - 1.6)	-	(1.7 - 1.9)	(1.6-2.1)
V(%)	-	-	-	-	72.8±1.6
	-	-	-	-	(70.3-74.3)
Lip region diameter	14.9	16.8 ± 1.3	18.3	24.0 ± 4.0	22.7±3.2
	-	(16.0 - 18.3)	-	(18.8 - 28.6)	(18.8-26.8)
Lip region height	2.9	3.5 ± 0.5	3.0	4.7 ± 0.3	-
	-	(3.0 - 4.0)	-	(4.6 - 5.1)	-
Buccal cavity length	15.4	20.9 ± 1.6	21.1	23.0 ± 2.0	26.0±0.5
	-	(20.0 - 22.8)	-	(21.1 - 25.7)	(25.1-26.3)
Buccal cavity width	8.0	11.3 ± 1.3	9.7	12.6 ± 1.0	14.8±0.7
	-	(10.0 - 12.6)	-	(11.4 -13.7)	(14.3-16.0)
Position of tooth apex	10.9	12.2 ± 1.4	16.0	16.2 ± 1.4	20.9±0.5
	-	(10.9 - 13.7)	-	(14.3 - 17.7)	(20.0-21.1)
Body diam. at mid-body	17	28.7 ± 4.8	34.3	36.8 ± 1.4	32.6±3.1
		(23.4-32.8)	-	(35.7-38.6)	(28.6-35.7)
Body diam. at anus	13.7	18.9 ± 1.2	22.8	24.3 ± 1.2	21.1±2.3
	-	(17.7 - 20.0)	-	(22.8 - 25.7)	(18.6-24.3)
Rectum length	12.0	15.1 ± 0.9	26.3	22.8 ± 1.0	16.0±2.1
	-	(14.3 - 16.0)	-	(21.4 - 24.0)	(12.9-18.6)
Tail length	26.8	28.7 ± 1.3	34.8	43.2 ± 2.4	38.9±1.9
	-	(27.4 - 30.0)	-	(40.0 - 45.7)	(37.1-41.4)

[1]All measurements are in μm except ratios a, b, b' c, c' and L in mm.

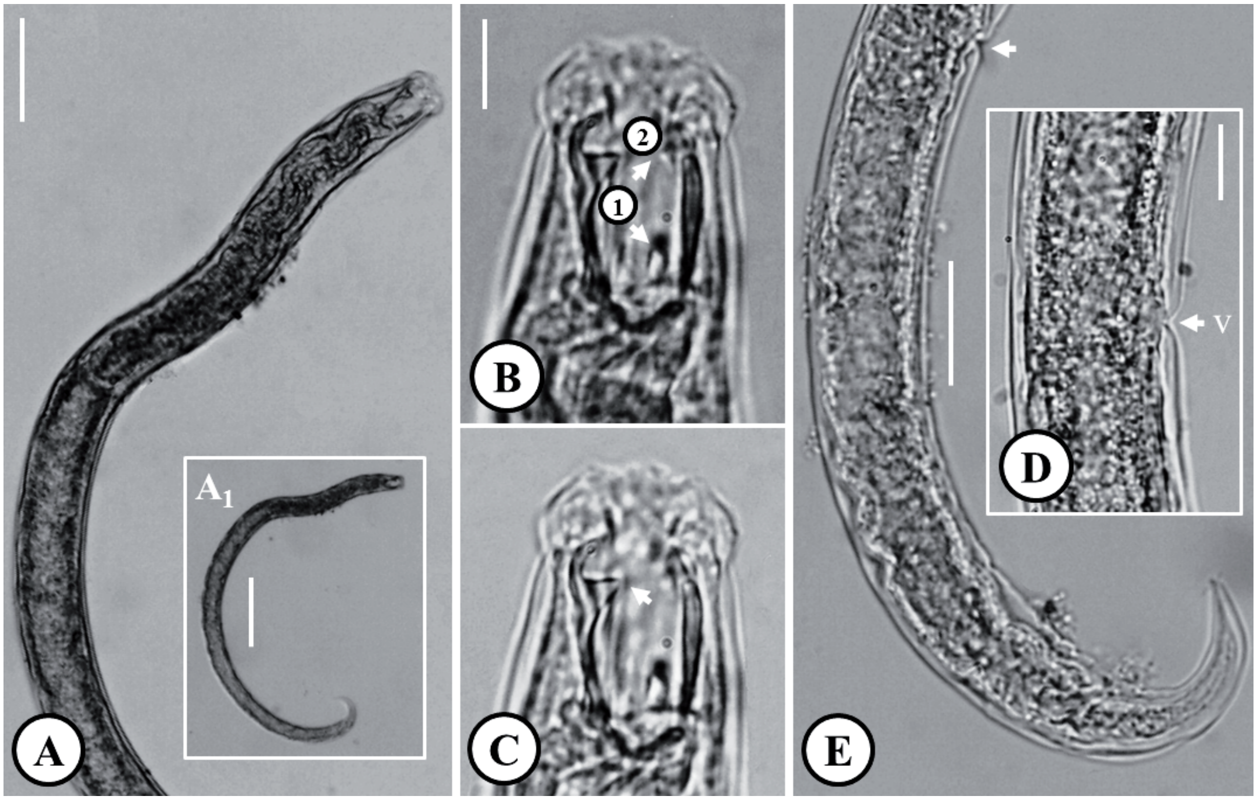

Figure 5. Female of *Crassibucca* sp. from Ocumare de la Costa de Oro, Aragua (Venezuela). A: Anterior region. A_1: Entire body. B-C: Lip region and buccal cavity (1 = first subventral teeth, 2 = second subventral teeth and dorsal tooth indicated by arrow). D: Body at vulva level (v = vulva). E: Posterior region (vulva position indicated by arrow). Bar A = 50 µm, Bar A_1 = 110 µm, Bar B = 10 µm in C, Bar D = 15 µm. Bar E = 30 µm.

(Central America) and Nigeria (Africa) (Mulvey and Jensen, 1967; Hunt, 1978; Ahmad, 2000; Ahmad and Jairajpuri, 2010).

DISCUSSION

Free-living soil nematodes in Venezuela have been poorly studied, but, apparently are very rich in genera and species due to the great variety of terrestrial and coastal environments present in the country. The only references available are those of McBeth (1956), Mulvey (1963), Loof (1964), Torrealba (1969), Dao (1970) and Coomans *et al*. (1990). No other data available except for Perichi *et al.* (2008) and Morales-Montero and San Blas (2014) on predatory nematodes (Mononchida and Dorylaimida, respectively).

This is the first record of *C. coffea* in Venezuela, and it is the first time it has been cited outside of Vietnam. In the country, up to now, studies on free-living soil nematodes of the order Dorylaimida have revealed *ca* 60 species, which belong to twenty-five genera of five families (Loof, 1964; Coomans *et al*., 1990; Morales-Montero and San Blas, 2014). The genus *Chitwoodius* Furstenberg & Heyns, 1966 is cited for the first time in Venezuela. Molecular analyses presented by Hoang *et al*. (2019), show in a close evolutionary relationship among *Chitwoodius* and *Tylencholaimus* de Man, 1876. The dorylaimids of this last genus are considered strict fungivorus nematodes (Okada *et al*., 2005).

Regarding the mononchids, only few species have been reported from in the country till date viz, *Bathyodontus cylindricus* Fielding, 1950, *Clarkus papillatus* (Bastian, 1865) Jairajpuri, 1970, *Coomansus parvus* (de Man, 1880) Jairjapuri & Khan, 1977, *C. venezolanus* Loof, 1964, *Minconchus digiturus* (Cobb, 1893) Andrasy, 1958, *Mononchus truncatus* Bastian, 1865, *Mylonchulus contractus* Jairajpuri, 1970, *M. lacustris* (Cobb, 1915) Cobb, 1917, *M. minor* (Cobb, 1893) Andrasy, 1958 and *M. sigmaturus* (Cobb, 1917) Altherr, 1953 (Loof, 1964; Perichi *et al*., 2008). In this paper, the genus C*rassibucca* and the species *P. zelli* and *S. ibitienis* are cited for the first time in Venezuela. The predaceous nematodes play a significant role in regulating the population of plant-parasitic nematodes (Devi and George, 2018).

All these nematodes are an integral part of the interlocking chain of nutrient conversions that occur in terrestrial environments. They function in the recycling of carbon-containing substances, mineral nutrients and nitrogenous components. Likewise, they control explosions of microflora and microfauna and maintain the stability of life forms that constitute the delicate balance of nature (Swart *et al*., 2017; Yadav *et al*., 2018).

In natural and agricultural soils, essential research on nematodes is required to know their biodiversity, synchronize the release and availability of nutrients in relation to the needs of plants, to test ecological hypotheses and monitor the environment. However, their usefulness as bioindicators depends intrinsically on the role they play within the edaphic trophic web and extrinsically on their correct identification. Therefore, species-level discrimination is necessary to permit further advances in understanding the role of nematodes in soil processes and thus in ecosystem resilience (Yeates, 2003; Neher, 2010; Ahmed *et al*., 2016; Lazarova *et al*., 2021).

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