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Morphological and Molecular Characterization of Prevalent Plant-Parasitic Nematodes from Turfgrasses in Guangdong, China

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Abstract: The turfgrass industry has undergone a rapid development in Guangdong province, China, which has the largest number of golf courses in the country. Recent surveys of turfgrasses in the province revealed five plant-parasitic nematodes that are prevalent: *Helicotylenchus dihystera*, *Mesocriconema xenoplax*, *Meloidogyne graminis*, *Hemicriconemoides rosae* and *Tylenchorhynchus leviterminalis*. The most prevalent species are *M. xenoplax* and *M. graminis*, found in 60.6% and 27.3% of locations, respectively. These five species are morphologically and morphometrically described. Molecular characterization and phylogenetic analyses using 18S rRNA and the D2-D3 expansion segments of 28S rRNA sequences are provided. This is the first report on molecular characterization and phylogenetic relationships of plant-parasitic nematodes associated with turfgrasses in Guangdong, China. This work was a first step for future study including pathogenicity assay, relationship examination with other pathogens and development of control measures of these turf nematodes to provide more precise and effective management options to turf superintendents.

Keywords: taxonomy; morphology; morphometrics; plant-parasitic nematode; turfgrass; taxonomy; 18S rRNA; 28S rRNA D2-D3; phylogeny

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1. Introduction

With rapid urbanization and growing demand for high-quality life, the turfgrass industry has undergone a rapid expansion in Guangdong province, China. In 2019, Guangdong province ranked first in the country for green space, with an estimated 502,400 hectares [1]. Guangdong has always been at the forefront of golf course development in China. It currently has 97 golf courses [2]. These golf courses are mainly distributed throughout the economically developed Pearl River Delta region, including Guangzhou, Zhongshan, Foshan, Dongguan, Huizhou, Zhuhai and Shenzhen. To maintain a high-quality turfgrass, especially putting greens, turfgrass needs to be intensely managed with pesticides, fertilizer and irrigation to prevent diseases and pest insects, and to minimize deleterious effects due to extremes in environmental conditions [3]. However, due to economic pressures, restrictions on the application of pesticides and damage by pests, it is often difficult for management inputs to maintain a desirable turfgrass surface.

Of the pests that impact turfgrass, plant-parasitic nematodes are an important pathogen; however, they are often overlooked because their microscopic size makes it difficult to see them with the naked eye and because the symptoms they cause are similar to those caused by drought stress, nutrient deficiency and fungal root diseases, making it hard to diagnose

them. Furthermore, there are no turfgrass cultivars resistant to nematodes at the present time, and few effective measures can be taken to manage the nematodes in turfgrass once their infestation is established. Soil fumigation is effective for controlling nematodes before planting, but it can't be used easily once turf is established, and some effective fumigants, for example, Dichloropropene, were restricted due to expensive cost (about $16,000 for 35 acres of fairway) and their being potentially phytotoxic to turfgrass [4]. Therefore, nematodes have become a serious problem in turfgrass worldwide, especially in subtropical and tropical regions like Guangdong province. Nematode problems highlight the need for a greater understanding of nematodes infecting turfgrasses; this includes species identification so that targeted and sustainable management strategies can be developed.

Several surveys of nematodes associated with turfgrasses have been conducted in the USA and other countries, and have shown a broad diversity with different countries and regions [5–11]. In Florida, USA, common genera of nematodes that damage turf include ectoparasitic *Belonolaimus*, *Trichodorus*, *Nanidorus*, *Helicotylenchus* and *Mesocriconema*, and endoparasitic *Hoplolaimus* and *Meloidogyne* [5]. A total of 29 species belonging to 22 genera in 15 families in North Carolina (NC) and South Carolina (SC), USA [10]; 28 species/taxa belonging to 16 genera and 12 families in Korea [6]; 52 different species/taxa belonging to 23 genera and 9 families in Belgium [8]; and 9 genera of plant-parasitic nematodes associated with turfgrass in southern Ontario, Canada [9] were reported. The nematodes associated with turfgrasses in NC and SC were molecularly characterized using 18S rDNA sequences [12]. *Meloidogyne graminis* (Sledge & Golden) Whitehead was reported to be occurring on golf greens of Yangjing and Zhuhai, Guangdong province [13]. However, an extensive survey of plant-parasitic nematodes associated with turfgrasses in Guangdong, China is needed. The objectives of this work were to: (i) identify the nematode species associated with turfgrasses in Guangdong province; (ii) characterize the most common species using morphological, morphometric and molecular methods; and (iii) analyze phylogenetic relationships among these nematode species using sequences of the 18S nuclear ribosomal RNA and the D2-D3 expansion segments of the 28S nuclear ribosomal RNA gene.

2. Material and Methods

2.1. Soil Sampling

During 2016–2020, samples for extracting nematodes were collected four times a year at a total of 33 locations of planted turfgrasses in Guangdong province (23.1317 °N, 113.2663 °E), China. Soil samples were collected from the root zone of bermudagrass (*Cynodon dactylon* (L.) Pers.) at 19 golf courses and 14 other locations in Guangdong province, China (sampling information shown in Table 1). Each sample consisted of 12 soil cores (1.5 cm diam. × 20 cm deep) sampled at roughly equal intervals in a zig-zag pattern across an area of 500 m^2 or less. Soil samples were combined for bulk sample and placed in sealed plastic bags. The sealed plastic bags were placed in sample boxes and stored at 4 °C before analysis to minimize changes in nematode populations.

2.2. Morphological Characterization

Nematodes were extracted from soil samples using the rapid centrifugal-flotation method [14]. Specimens were heat-killed, fixed in 3% formaldehyde and processed to glycerin using the formalin–glycerin method [15]. Measurements were performed with the aid of a camera lucida and a stage micrometer. The morphometric data were processed using Excel software [16]. Photomicrographs were taken with a Leica video camera (DFC490) attached via a C-mount Adapter fitted on a Leica microscope (DM4000B) and edited using Adobe Photoshop CS6.

The abbreviations and their definitions for the de Man's ratios and other indices used in tables are as follows:

n = number of specimens on which measurements are based
L = overall body length
V = % distance of vulva from anterior relative to body length

a = body length/greatest body diameter
b = body length/distance from anterior to esophago-intestinal valve
c = body length/tail length
c′ = tail length/tail diameter at anus or cloaca
VA = distance from vulva to anus
VBD = diameter of body at vulva
PUS = postuterine sac
MB = % distance of center of the middle esophageal bulb from anterior relative to esophageal length
T = % distance of testis relative to body length
R = ring number of body cuticle
Rs = ring number from anterior to base of stylet base
Rex = ring number from anterior to excretory pore
Roes = ring number from anterior to base of esophageal glands
Rv = ring number from vulva to tail tip
Ran = ring number from anus to tail tip
Rvan = ring number between vulva and anus

Table 1. Sampling locations and turf species.

Locations	Turf Species	Locations	Turf Species
Zhuhai Cuihu Golf	*Cynodon dactylon* (L.) Pers.	Sand River Golf	*Cynodon dactylon*
Zhuhai Jinwan Golf	*Cynodon dactylon*	Guangzhou Martyrs' Cemetery	*Zoysia tenuifolia* Willd. ex Trin.
Guangzhou Xiancun International Golf	*Cynodon dactylon*	Sun Yat-sen University	*Eleusine indica* (L.) Gaertn. *Sporobolus indicus* (L.) R. Br.
Guangzhou Lihu Golf	*Cynodon dactylon*	Guangzhou Baiyun Mountain	*Panicum repens* L. *Zoysia tenuifolia*
Guangzhou Fengshen Golf	*Cynodon dactylon*	Guangzhou Haizhuhu Park	*Zoysia tenuifolia*
Guangzhou Jiulonghu Golf	*Cynodon dactylon*	Guangzhou Liwanhu Park	*Panicum repens*
Guangzhou Nanhu Golf	*Cynodon dactylon*	South China Botanical Garden	*Cynodon dactylon*
Guangzhou Luhu Golf	*Cynodon dactylon*	Guangzhou Liuhuahu Park	*Eleusine indica*
Guangzhou Nansha Golf	*Cynodon dactylon*	Guangzhou People Park	*Panicum repens*
Guangzhou Lianhuashan Golf	*Cynodon dactylon*	Guangzhou Tianhe Park	*Sporobolus indicus*
Shunde Junan Golf	*Cynodon dactylon*	Guangzhou Shamian Park	*Panicum repens*
Nanhai Taoyuan Golf	*Cynodon dactylon*	Guangzhou Culture Park	*Eleusine indica* *Sporobolus indicus*
Dongguan Zhongxin Golf	*Cynodon dactylon*	Guangzhou Xiaogang Park	*Panicum repens* *Zoysia tenuifolia*
Dongguan Changan Golf	*Cynodon dactylon*	Guangzhou Yingzhou Ecological Park	*Cynodon dactylon*
Shenzhen Juhao Golf	*Cynodon dactylon*	Guangzhou Yuntai Park	*Zoysia tenuifolia*
Shenzhen Longgang Golf	*Cynodon dactylon*		
China Zhongshan Hot Spring Golf	*Cynodon dactylon*		
Huizhou Taojing Golf	*Cynodon dactylon*		

2.3. Molecular Characterization

One male or female was hand-picked and placed into 50 µL of worm lysis buffer (WLB) containing Proteinase K for DNA extraction [17]. DNA samples were stored at −20 °C until used as a PCR template.

The primers for small subunit (SSU) 18S amplification and DNA sequencing were forward primer 18S965 (5′ GGCGATCAGATACCGCCCTAGTT 3′) and reverse primer 18S1573R (5′ TACAAAGGGCAGGGACGTAAT 3′) [18]. Primers for large subunit (LSU) 28S amplification and DNA sequencing were forward primer D2A (5′ ACAAGTACCGT-GAGGGAAAGTTG 3′) and reverse primer D3B (5′ TGCGAAGGAACCAGCTACTA 3′) [19].

PCR reactions (25 µL) were performed using Dream Taq Green PCR Master Mix DNA polymerase (Thermo Fisher Scientific [China] Co. Ltd., Shanghai, China) according to the manufacturer's protocol. The thermal cycler program for PCR was as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 2 min. A final extension was performed at 72 °C for 10 min [20]. PCR products were cleaned using ExoSap-IT (Affymetrix Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. PCR products were sequenced by Guangzhou Tianyihuiyuan Gene Science & Technology Co., Ltd., Guangzhou, China, using an ABI PRISM 3730 sequencing system.

The rDNA SSU and LSU sequences from this project were deposited in GenBank under the accession numbers presented in Table 2 and compared with other nematode species in GenBank using the BLAST homology search program. The most similar sequences were downloaded for phylogenetic analysis. DNA sequences were aligned using Mega5.05 [21]. The model of base substitution in the SSU and LSU sets were evaluated using MODELTEST version 3.06 [22]. The Akaike-supported model, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 [23] running the chain for 1×10^6 generations and setting the 'burn-in' at 1000. The MCMC (Markov chain Monte Carlo) methods within a Bayesian framework were employed to estimate the posterior probabilities of the phylogenetic trees [24] using the 50% majority-rule.

Table 2. Nematode species and accession numbers in the GenBank database.

Nematode Species	Accession Numbers	
	18S	**28S**
Helicotylenchus dihystera (Cobb) Sher	OM670208	OM670211
Hemicriconemoides rosae Rathour, Sharma, Singh & Ganguly	OM339815	OM339816
Meloidogyne graminis	OM670242	OM687364
Mesocriconema xenoplax (Raski) Loof & De Grisse	OM671259	OM687363
Tylenchorhynchus leviterminalis Siddiqi, Mukherjee & Dasgupta	OM671280	OM671287

3. Results

3.1. Nematode Species

The prevalent plant-parasitic nematodes found among the 33 sampling locations were *Helicotylenchus dihystera* (Cobb), Sher, 1966; *Mesocriconema xenoplax* (Raski), Loof & De Grisse, 1989; *Meloidogyne graminis* (Sledge & Golden), Whitehead, 1968; *Hemicriconemoides* rosae, Rathour, Sharma, Singh & Ganguly, 2003; and *Tylenchorhynchus leviterminalis*, Siddiqi, Mukherjee & Dasgupta, 1982 (Table 3). *Mesocriconema xenoplax* was the most prevalent, with a detection rate (percentage of locations) of 60.6%, followed by *Meloidogyne graminis* with 27.3%, and other species with around 20% each.

3.2. Morphological Description

Because the SSU and LSU sequences aligned by ClustalW from the populations from different locations for each species in the present study were identical, they are considered as one species in the following description.

3.2.1. Description of *Helicotylenchus dihystera*

Female: Body spiral-shaped when heat-killed and distinctly annulated. Four incisures visible in lateral field with light microscopy. Stylet well-developed with rounded stylet knobs. Orifice of dorsal esophageal gland at about half stylet length behind stylet base. Lip hemispherical-shaped with lip rings. Median esophageal bulb oval with a well-developed valve. Excretory pore located at the anterior level of the esophageal gland. Hemizonion indistinct. The posterior esophageal glands overlapping intestines. Ovaries paired, outstretched, with oocytes in single row. Spermatheca without sperms. Vulva transverse

without vaginal membrane. Tail ventrally curved, dorsally convex-conoid to a narrow terminus which may form a slight projection (Figure 1).

Male: Not observed.

Morphometrics: See Table 4.

Remarks: Helicotylenchus dihystera was first described from soil around sugarcane roots (*Saccharum officinarum* L.) in Harwood, Australia. It is distributed worldwide, occurring in 3 countries in North America, 18 in Central America and Caribbean, 8 in South America, 8 in Oceania, 17 in Europe, 30 in Africa and 28 in Asia [25]. It is present in 7 provinces in China [25]. It has a broad host range including sugarcane, potato (*Solanum tuberosum* L.), banana (*Musa* spp. L.), rice (*Oryza sativa* L.), tea (*Camellia sinensis* (L.) Kuntze), avocado (*Persea americana* Mill.), coffee (*Coffea arabica* L.), maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.), sorghum (*Sorghum bicolor* (L.) Moench) and turfgrass [26–28]. Turfgrass hosts include Kentucky bluegrass (*Poa pratensis* L.) [29], bermudagrass (*Cynodon dactylon* (L.) Pers.), bentgrass (*Agrostis stolonifera* L.) and zoysiagrass (*Zoysia japonica* Steud.) [10,11,28]. In this study, *H. dihystera* was found in Zhuhai Cuihu Golf, Guangzhou Lihu Golf, South China Botanical Garden, Guangzhou Haizhuhu Park, Xiaogang Park and Yingzhou Ecological Park. The morphometric data and morphological characteristics of this present species match with American populations given by Zeng et al. [10].

3.2.2. Description of *Hemicriconemoides rosae*

Female: Body slightly ventrally curved when heat-killed. Cuticular sheath and rounded annuli distinct. Lateral field absent and without anastomoses. Lip region not set off, rounded with a prominent labial disc and two annuli. Cephalic framework well-developed. Stylet strong, long, with well-developed, anchor-shaped knobs. Procorpus and metacorpus amalgamated. Median esophageal bulb oblong, with well-developed valve. Isthmus narrow followed by distinct basal bulb, becoming vase-shaped. Excretory pore posterior to basal bulb end, 28–31 annuli from anterior end. Vulva with a membranous sheath, without vulval flap. Vagina distinct, sigmoid. Ovary monodelphic, prodelphic, outstretched with oocytes in a single row. Spermatheca well-developed, oblong to ovoid-shaped, without sperms. Anus visible. Tail dorsally convex-conoid, with a bluntly rounded or pointed tip (Figure 2).

Male: Not observed.

Morphometrics: See Table 5.

Remarks: *Hemicriconemoides rosae* was first collected from the rhizosphere of rose (*Rosa indica* L.) in the Bareilly district, Uttar Pradesh, India, and was originally described by Rathour et al. [30]. It was collected from sugarcane and redescribed by Khan et al. [31]. *Hemicriconemoides rosae* is known to occur only in rose and sugarcane in India and *Pilea cadierei* Gagnep. & Guill. in China. In this study, it was found in Guangzhou Lihu Golf, Culture Park, People Park, Sand River Golf, Shenzhen Juhao Golf and Dongguan Zhongxin Golf. Both morphology and morphometrics match the original description [30]. This is the first report of *H. rosae* on turfgrasses.

Table 3. Prevalent species of plant-parasitic nematodes in soil samples from rhizosphere of turf grasses in Guangdong, China.

Parasitic Nematodes	Locations	Detection Rates * (%)
Helicotylenchus dihystera	Zhuhai Cuihu Golf, Guangzhou Lihu Golf, South China Botanical Garden, Guangzhou Haizhuhu Park, Xiaogang Park, Yingzhou Ecological Park	18.2

Table 3. *Cont.*

Parasitic Nematodes	Locations	Detection Rates * (%)
Hemicriconemoides rosae	Guangzhou Lihu Golf, Culture Park, People Park, Sand River Golf, Shenzhen Juhao Golf, Dongguan Zhongxin Golf	18.2
Meloidogyne graminis	Zhuhai Cuihu Golf, Guangzhou Lihu Golf, Shunde Junan Golf, Shenzhen Juhao Golf, Dongguan Zhongxin Golf, Changan Golf, Huizhou Taojing Golf, Longgang Golf, China Zhongshan Hot Spring Golf	27.3
Mesocriconema xenoplax	Zhuhai Cuihu Golf, Zhuhai Jinwan Golf, Guangzhou Xiancun International Golf, Lihu Golf, Fengshen Golf, Jiulonghu Golf, Nanhu Golf, Luhu Golf, Nansha Golf, Lianhuashan Golf, Shunde Junan Golf, Nanhai Taoyuan Golf, Dongguan Zhongxin Golf, Dongguan Changan Golf, Shenzhen Juhao Golf, Longgang Golf, China Zhongshan Hot Spring Golf, Huizhou Taojing Golf, Tianhe Park, Guangzhou Baiyun Mountain Park	60.6
Tylenchorynchus leviterminalis	Liuhuahu Park, Liwanhu Park, Zhuhai Cuihu Golf, Guangzhou Jiulonghu Golf, Yuntai Park, Shamian Park, South China Botanical Garden	21.2

* refer to the proportion of the locations in which the nematode tests positive.

Table 4. Morphometrics of females of *Helicotylenchus dihystera* populations mounted in formalin–glycerin in this study compared to those reported by Zeng et al. [10]. All measurements in μm and in the format: mean ± s.d. (Range).

Character	Guangzhou Lihu Golf	Zhuhai Cuihu Golf	Guangzhou Haizhuhu Park	South China Botanical Garden	Guangzhou Xiaogang Park	Zeng et al. [10]
	H. dihystera	*H. dihystera*	*H. dihystera*	*H. dihystera*	*H. dihystera*	*H. dihystera*
n	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	15 ♀♀
L	650.0 ± 32.1 (610.0–699.0)	649.9 ± 28.9 (621.2–695.8)	682.4 ±89.7 (562.3–828.2)	648.6 ± 66.8 (527.2–762.8)	663.5 ± 36.6 (583.5–662.6)	649.8 ± 27.9 (620.2–693.3)
a	23.8 ± 2.9 (21.3–26.0)	22.9 ± 1.6 (19.2–24.3)	24.3 ± 2.4 (21.9–30.8)	25.7 ± 2.1 (21.5–28.8)	24.5 ± 3.5 (20.0–29.3)	21.9 ± 1.4 (19.8–23.6)
b	4.5 ± 0.6 (3.8–5.3)	4.5 ± 0.4 (4.0–5.0)	4.4 ± 0.8 (4.1–6.7)	5.1± 0.8 (4.8 -6.8)	5.0 ± 0.6 (4.3–6.9)	4.9 ± 0.2 (4.7–5.2)
c	38.3 ± 4.8 (35.3–46.5)	37.8 ± 1.9 (34.2–44.6)	36.7 ± 6.3 (29.9–46.9)	38.5 ± 4.3 (33.5–46.6)	31.9 ±3.8 (27.5–36.3)	36.1 ± 0.9 (35.2–37.2)

Table 4. *Cont.*

Character	Guangzhou Lihu Golf	Zhuhai Cuihu Golf	Guangzhou Haizhuhu Park	South China Botanical Garden	Guangzhou Xiaogang Park	Zeng et al. [10]
	H. dihystera	*H. dihystera*	*H. dihystera*	*H. dihystera*	*H. dihystera*	*H. dihystera*
C′	1.1 ± 0.1 (1.0–1.2)	1.1 ± 0.1 (1.0–1.2)	1.4 ± 0.2 (1.2–1.6)	1.2 ± 0.2 (1.0–1.4)	1.2 ±0.1 (1.0–1.3)	1.1 ± 0.1 (1.0–1.2)
V	62.6 ± 0.9 (61.0–63.5)	61.9 ± 1.5 (60.8–63.5)	64.5 ± 1.0 (63.0–65.5)	63.5 ± 1.2 (61.3–65.9)	63.1 ± 2.5 (59.6–67.2)	61.7 ± 1.7 (58.8–63.2)
Stylet length	24.2 ± 1.5 (23.0–26.0)	23.6 ± 0.5 (22.5–24.0)	24.5 ± 1.2 (23.2–26.4)	24.0 ± 1.0 (23.0 – 25.8)	24.6 ±2.8 (19.9–26.8)	23.7 ± 0.4 (23.0–24.0)
Excretory pore from anterior end	106.7 ± 3.1 (102.0–109.8)	108.0 ± 6.7 (101.5–116.5)	111.0 ± 1.0 (110.0–112.0)	105.3 ± 12.3 (93.8–136.2)	109.3 ± 6.3 (101.9–116.5)	109.0 ± 5.7 (101.0–116.6)

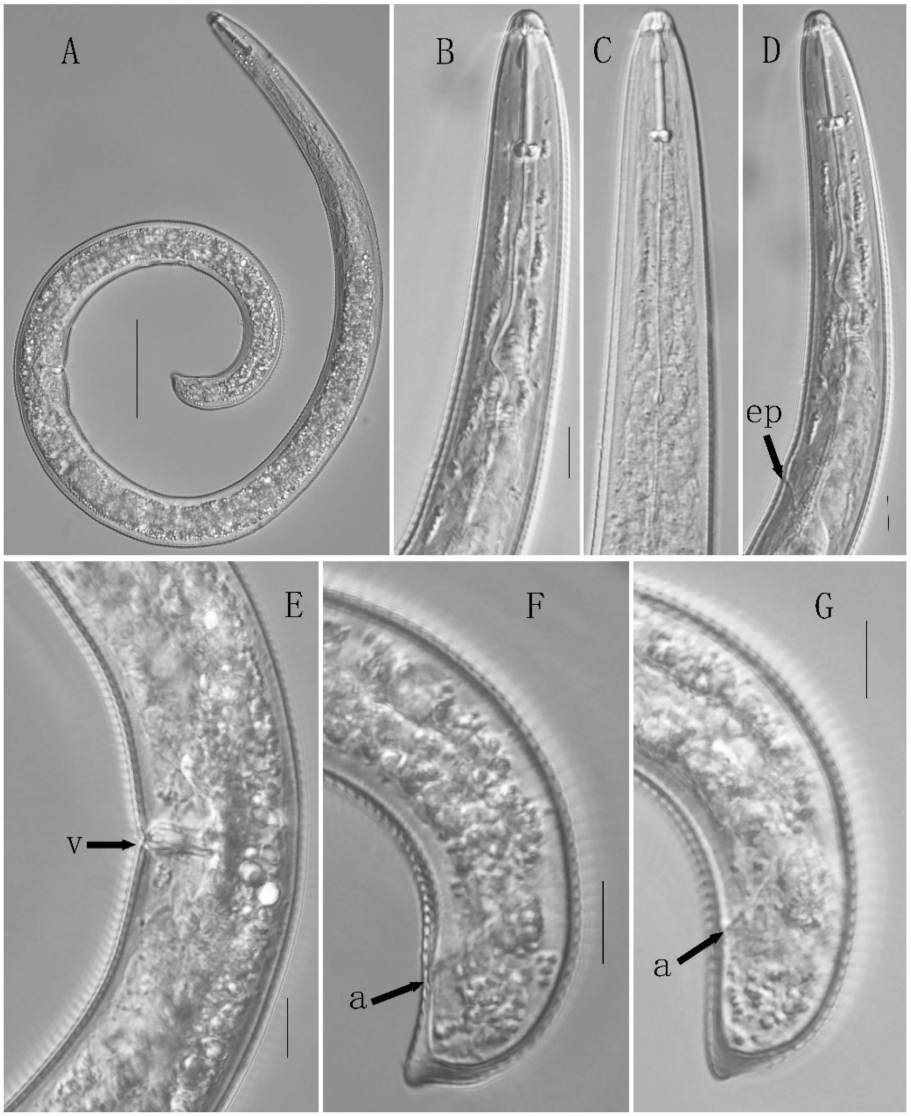

Figure 1. Adult female of *Helicotylenchus dihystera* in lateral view. (**A**): Entire body; (**B**,**C**): Anterior body; (**D**): Anterior body (ep arrow refers to excretory pore); (**E**): Vulva (v arrow); (**F**,**G**): Tails (a arrow refers to anus). (Scale bars: (**A**) = 50 µm; (**B**–**G**) = 10 µm).

3.2.3. Description of *Meloidogyne graminis*

Second-stage juvenile: Body cylindrical, tapering to the posterior end. Head without distinct cephalic framework and annuli. No obvious contraction between the head and the body. Four incisures visible in lateral field. Stylet small with rounded knobs. Median esophageal bulb elongated with well-developed valves. Tail with a distinct, relatively long hyaline terminus and rounded tip (Figure 3).

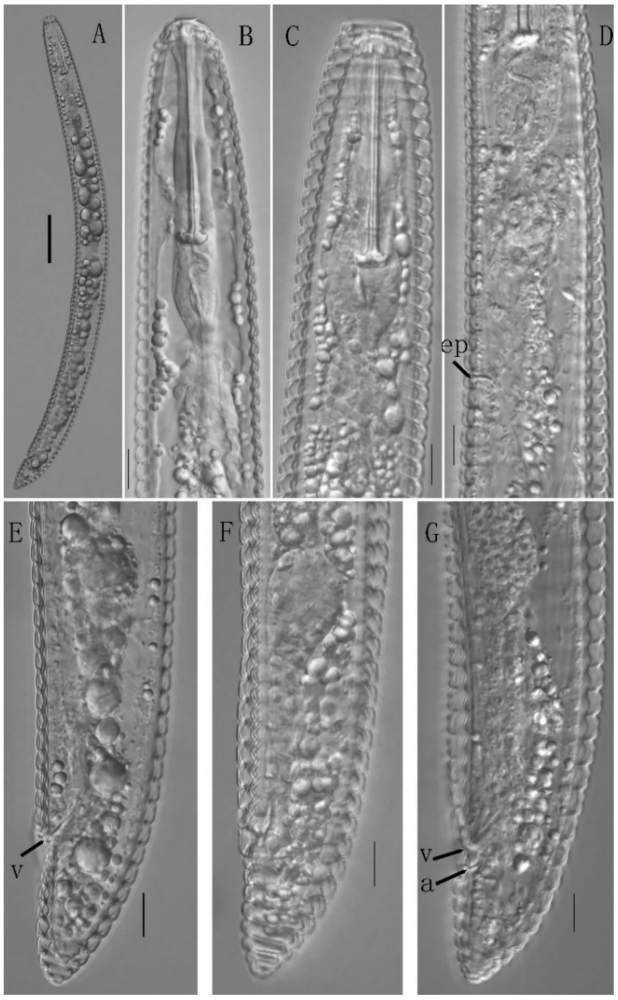

Figure 2. Adult female of *Hemicriconemoides rosae*. (**A**): Entire body; (**B**,**C**): Anterior body; (**D**): Excretory pore (ep arrow); (**E**–**G**): Tails (a refers to anus, v refers to vulva) (Scale bars: (**A**) = 50 µm; (**B**–**G**) = 10 µm).

Table 5. Morphometrics of females of *Hemicriconemoides rosae* populations mounted in formalin–glycerin. All measurements in µm and in the format: mean ± s.d. (Range).

Character	Bermuda Grass Population
n	10 ♀♀
L	477.7 ± 22.6 (434.3–509.1)
a	14.8 ± 1.4 (11.2–16.2)
b	4.8 ± 0.3 (4.5–5.4)

Table 5. *Cont.*

Character	Bermuda Grass Population
c	25.0 ± 7.8 (19.9–38.7)
c′	1.0 ± 0.2 (0.8–1.2)
V	93.5 ± 0.4 (92.8–94.3)
Body diameter	32.6 ± 4.1 (28.5–43.5)
Stylet length	52.2 ± 1.4 (49.9–54.8)
Pharynx length	100.5 ± 4.9 (91.5–107.7)
Anal body diameter	19.9 ± 2.5 (16.2–22.7)
Tail length	20.5 ± 5.3 (12.6–25.5)
Excretory pore from anterior end	135.0 ± 9.0 (125.4–144.6)
R	103.8 ± 3.9 (97.0–110.0)
Rs	13.6 ± 1.0 (12.0–15.0)
Roes	23.8 ± 1.6 (21.0–26.0)
Rex	30.0 ± 1.1 (28.0–31.0)
Rv	8.8 ± 0.8 (8.0–10.0)
Ran	5.6 ± 1.1 (4.0–7.0)
Rvan	3.2 ± 1.3 (2.0–5.0)

Male: Body long and vermiform-shaped. Cuticle striated. Four incisures visible in lateral field. Head with well-developed cephalic framework, without annuli. No obvious contraction exists between the head and the body. Stylet robust with distinct rounded knobs. Median esophageal bulb oval with a well-developed valve. Spicules paired, separated, slightly curved ventrally, with a slender gubernaculum, near tail terminus. Tail short, with rounded tip (Figure 3).

Female: Not observed.

Morphometrics: See Table 6.

Remarks: *Meloidogyne graminis* was first described from grass in Florida by Sledge [32] and has been reported in other states in USA [10,11,33–35]. It has also been recorded in Venezuela [36], Brazil [37], Germany [38], the Netherlands [39], China [13] and India [40]. Turfgrass hosts of *M. graminis* include bermudagrass (*Cynodon* spp.), St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), zoysiagrass (*Zoysia* spp.), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.), seashore paspalum (*Paspalum vaginatum* Swartz), bentgrass (*Agrostis* spp.), and bluegrass (*Poa* spp.) [10,11,34,41,42]. In this study, *M. graminis* was found in turfgrass in Zhuhai Cuihu Golf, Guangzhou Lihu Golf, Shunde Junan Golf, Shenzhen Juhao Golf, Dongguan Zhongxin Golf, Changan Golf, Huizhou Taojing Golf, Longgang Golf and China Zhongshan Hot Spring Golf, Guangdong province, China. Both morphology and morphometrics match the description of other population [10].

3.2.4. Description of *Microcinema xenoplax*

Female: Body slightly curved ventrally when heat-killed. Body rings distinct with smooth margins. Anastomoses rare. Head broad, first annule entire or emarginated laterally.

Submedian lobes well-developed. Lip region conspicuous, elevated. Four labial plates distinct, well-separated. Stylet strong and long with anchor-shaped knobs. Esophagus typical Criconematid esophagus. Excretory pore located near the esophageal basal bulb. Vulva distinctly open with lips separated, two protruberances bearing anterior lip visible in ventral view. Vagina always sigmoid in lateral view. Ovary monodelphic, prodelphic. Tail broadly rounded to conoid, terminus button-shaped (Figure 4).

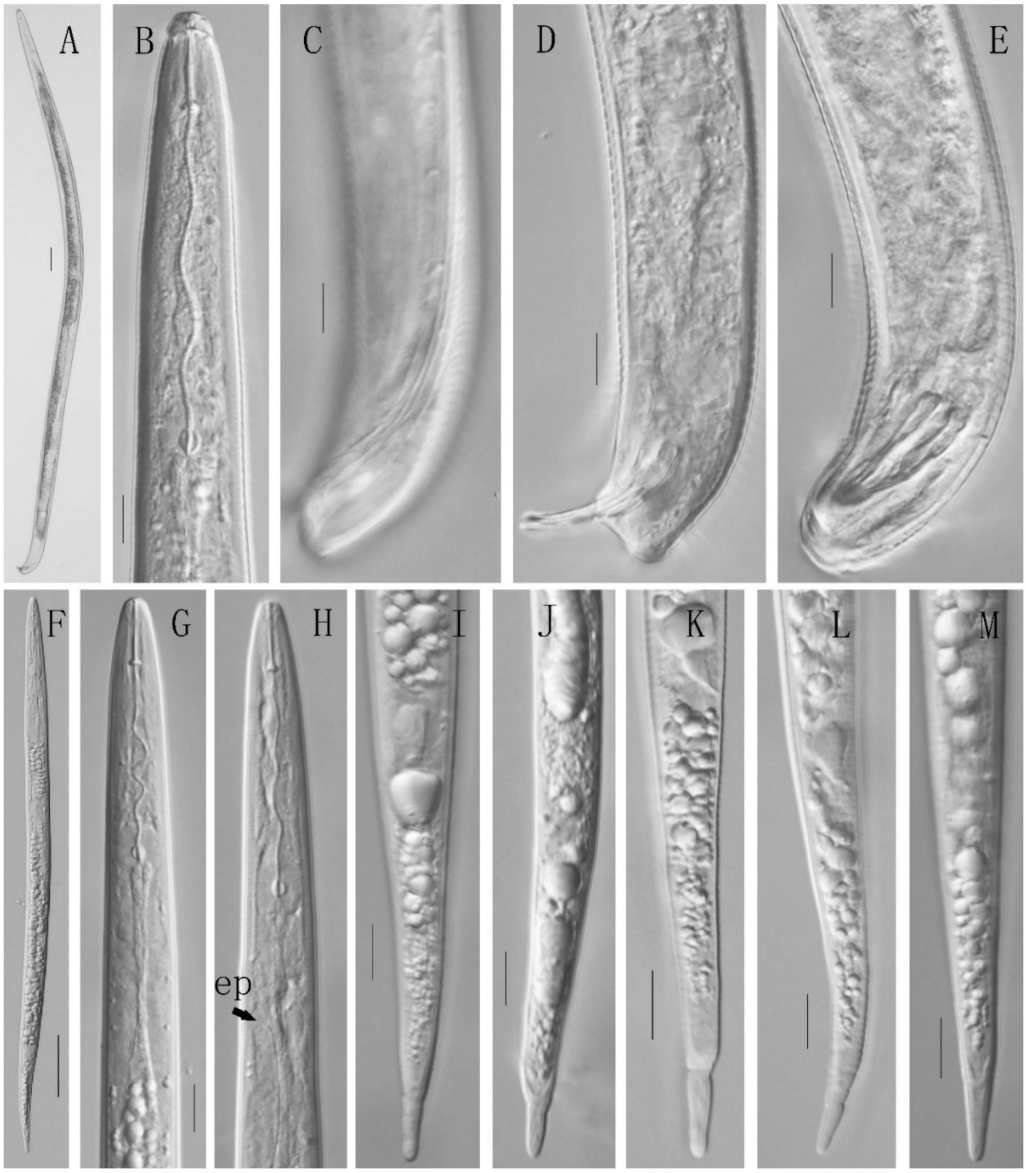

Figure 3. Adult male (**A**–**E**) and juvenile (**F**–**M**) of *Meloidogyne graminis*. (**A**): Entire male body; (**B**): Anterior male body; (**C**): Lateral field; (**D**,**E**): Male tails; (**F**): Entire juvenile body; (**G**): Anterior juvenile body; (**H**): Excretory pore (ep arrow); (**I**–**M**): Tails (Scale bars: (**A**,**F**) = 50 µm; (**B**–**E**,**G**–**M**) = 10 µm).

Male: Not observed.

Morphometrics: See Table 7.

Remarks: *Mesocriconema xenoplax* was first documented from grapevines (*Vitis vinifera* L. var. *sultanina*) in California [43]. It has been recorded in North America [44], South America [45], Europe [46,47], South Africa [48], Australia [49], New Zealand [50], China [51], India [52], Japan [53] and Iran [54]. Turfgrass hosts of *M. xenoplax* include tall fescue (*Festuca arundinacea* J. C. D. von Schreber) [55] and bermudagrass, creeping bentgrass and zoysiagrass [10,11]. In this study, *M. xenoplax* was detected in 18 golf courses and 2 parks

(Table 3). Both morphology and morphometrics fit the description of other population [10]. This was the most widely distributed nematode species on turfgrasses in Guangdong province, China.

Table 6. Morphometrics of second-stage juveniles of *Meloidogyne graminis* populations mounted in formalin–glycerin in this study compared to those reported by Zeng et al. [10]. All measurements in μm and in the format: mean ± s.d. (Range).

Character	Zhuhai Cuihu Golf	Guangzhou Lihu Golf	Shunde Junan Golf	Zeng et al. [10]
	M. graminis	*M. graminis*	*M. graminis*	*M. graminis*
n	15	15	15	15
L	410.6 ±19.8 (393.4–440.0)	392.5 ±16.9 (378.0–422.0)	401.9 ± 13.4 (374.6–430.6)	392.4 ± 16.8 (368.2–420.2)
a	28.0 ± 1.3 (26.5–30.0)	26.9 ± 1.7 (22.0 -29.5)	27.0 ± 1.1 (26.5–30.0)	24.9 ± 1.8 (22.7–27.9)
b	4.2 ± 0.3 (3.8–4.5)	4.3 ± 0.2 (4.0–4.7)	4.2 ± 0.4 (4.0 – 4.6)	4.3 ± 0.2 (4.1–4.7)
c	6.7 ± 0.3 (6.3–7.8)	6.6 ± 0.5 (6.0–7.0)	6.7 ± 0.4 (6.0–7.5)	6.5 ± 0.4 (6.0–6.9)
c′	5.7 ± 0.3 (5.5–6.4)	5.5 ± 0.5 (4.3–6.5)	5.6 ± 0.3 (4.5 -6.5)	5.5 ± 0.6 (4.8–6.4)
Body width	14.5 ± 0.6 (13.0–16.2)	15.0 ± 1.2 (13.5–17.0)	15.0 ± 0.4 (14.5–16.0)	15.9 ± 1.3 (13.9–17.7)
Stylet length	11.8 ± 0.4 (11.0–12.5)	11.5 ± 0.2 (11.0–12.0)	11.0 ± 0.5 (10.0–11.8)	11.7 ± 0.4 (11.0–12.0)
Body diam. at stylet basal knob	9.5 ± 0.5 (9.0–10.0)	9.4 ± 0.3 (9.0–10.0)	9.6 ± 0.5 (9.0–11.0)	9.7 ± 0.3 (9.3–10.0)
Pharynx length (Head to metacarpus base)	96.0 ± 7.0 (90.0–108.5)	90.5 ± 5.0 (81.5–96.0)	91.8 ± 4.5 (84.0–96.0)	90.7 ± 5.2 (81.3–96.2)
Anal body width	10.8± 0.5 (9.5–11.5)	11.0± 0.8 (10.0–12.5)	10.6 ± 0.4 (8.5–11.8)	11.2 ± 0.9 (10.0–12.6)
Tail length	62.0 ± 5.0 (56.0–69.0)	61.0 ± 3.5 (54.0–64.5)	61.5 ± 1.6 (58.0–67.0)	61.0 ± 3.8 (53.9–64.5)
Hyaline tail part	11.5 ± 1.0 (10.5–13.5)	11.2 ± 0.6 (10.0–12.5)	11.5 ± 1.9 (10.0–12.5)	11.5 ± 0.8 (10.2–12.6)
Lip width	5.6 ± 0.5 (5.0–6.5)	5.5 ± 0.2 (5.0–5.7)	5.5 ± 0.6 (5.0–5.8)	5.4 ± 0.2 (5.1–5.6)
Lip height	2.5 ± 0.2 (2.3–2.8)	2.2 ± 0.1 (2.2–2.3)	2.4 ± 0.2 (2.2–2.6)	2.2 ± 0.1 (2.2–2.3)
Metacorpus length	10.0 ± 0.3 (9.5–10.5)	10.0 ± 0.5 (9.0–10.7)	10.0 ± 0.2 (9.5–10.5)	10.0 ± 0.7 (9.1–10.8)
Metacorpus width	7.6 ± 0.5 (7.0–8.5)	7.7 ± 0.3 (7.0–8.5)	7.5 ± 0.2 (7.0–8.0)	7.8 ± 0.5 (7.1–8.3)
LipL/W	2.2 ± 0.2 (2.0- 2.5)	2.4 ± 0.1 (2.2–2.5)	2.3 ± 0.3 (2.1–2.5)	2.4 ± 0.1 (2.3–2.5)
Metacorpus L/W	1.3 ± 0.1 (1.3–1.4)	1.3 ± 0.0 (1.2–1.3)	1.3 ± 0.1 (1.3–1.4)	1.3 ± 0.0 (1.2–1.3
H% tail	23.0 ± 3.5 (18.0–29.0)	20.5 ± 2.0 (17.5–22.9)	21.0 ± 2.0 (18.5–25.5)	18.9 ± 2.0 (16.7–21.8)

3.2.5. Description of Tylenchorhynchus leviterminalis

Female: Body ventrally curved to open C-shaped when heat-killed. Cuticle annuli distinct, 1.8–2.0 μm wide at mid-body, 2.0–2.3 pm at the tail region. Lateral field with four incisures. Lip region not offset, hemispherical. Cephalic framework slightly sclerotized. Stylet with rounded knobs. Median bulb well-developed, rounded to slightly ovate. Secretory-excretory pore (ep) near anterior end of saccate basal bulb, hemizonid two annuli anterior to ep. Ovaries outstretched. Vagina straight. Spermatheca rounded

with sperms. Tail distinctly clavate with 18–22 annuli and with large hemispherical smooth hyaline portion (Figure 5).

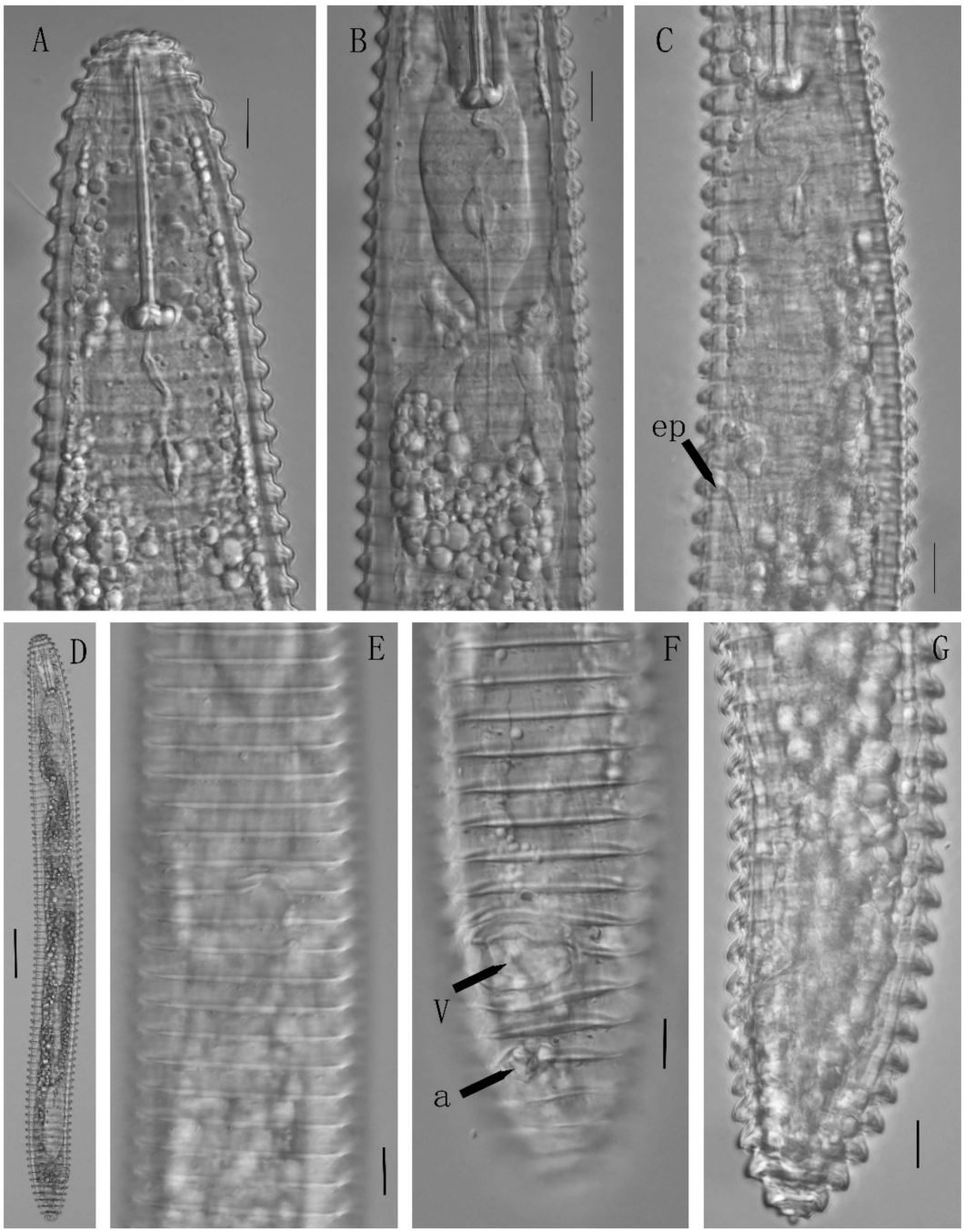

Figure 4. Adult female of *Mesocriconema xenoplax*. (**A**): Anterior body; (**B**): Esophagus; (**C**): Excretory pore (ep arrow); (**D**): Entire body; (**E**): Rings (annules); (**F**): Vulva (v arrow) and anus (a arrow); (**G**): Tail (Scale bars: (**D**) = 50 µm; (**A**–**C**,**E**–**G**) = 10 µm).

Male: Similar to female in general. Testis single, outstretched. Spicules slightly curved ventrally, paired, separate. Gubernaculum well developed. Tail conoid and pointed. Bursa enveloping tail (Figure 5).

Morphometrics: See Table 8.

Table 7. Morphometrics of females of *Mesocriconema xenoplax* populations mounted in formalin–glycerin in this study compared to those reported by Zeng et al. [10]. All measurements in μm and in the format: mean ± s.d. (Range).

Character	Guangzhou Lihu Golf	Zhuhai Cuihu Golf	Guangzhou Baiyun Mountain Park	Guangzhou Tianhe Park	Zeng et al. [10]
	M. xenoplax	*M. xenoplax*	*M. xenoplax*	*M. xenoplax*	*M. xenoplax*
n	10 ♀♀	10 ♀♀	10 ♀♀	10 ♀♀	15 ♀♀
L	553.9 ± 28.0 (502.9–603.5)	542.6 ± 49.5 (455.8–622.0)	444.4 ±51.2 (371.3–578.2)	386.6 ± 53.8 (304.2–503.8)	532.8 ± 44.7 (472.7–632.2)
a	13.0 ± 0.6 (12.4–13.6)	13.2 ± 0.7 (12.5–13.5)	9.8 ± 1.4 (8.9–12.8)	9.7 ± 1.1 (8.5–12.6)	12.6 ± 0.9 (11.3–14.1)
b	4.5 ± 0.2 (4.0–5.2)	4.6 ± 0.2 (4.3–5.0)	4.0 ± 0.4 (3.3–4.9)	4.2± 0.5 (3.3–4.8)	4.7 ± 0.3 (4.2–5.2)
c	31.0 ± 4.8 (25.5–41.0)	30.5 ± 3.5 (24.5–36.3)	23.2 ± 2.6 (20.8–25.3)	21.5 ± 2.1 (18.0–23.9)	29.1 ± 3.4 (23.9–35.3)
c′	0.8 ± 0.1 (0.7–0.9)	0.7 ± 0.1 (0.6–0.8)	0.7 ± 0.2 (0.6–0.9)	0.7 ± 0.1 (0.6–0.8)	0.8 ± 0.1 (0.7–0.9)
V	92.5 ± 0.5 (91.5–93.7)	92.3 ± 0.4 (91.5–93.5)	93.7 ± 0.6 (91.9–94.6)	92.5 ± 0.7 (91.5–94.6)	92.3 ± 0.4 (91.7–92.9)
Stylet length	56.5 ± 1.9 (56.5–61.5)	55.3 ± 1.5 (52.5–58.8)	54.0 ± 2.3 (52.8–57.6)	52.0 ± 2.1 (60.0–80.0)	55.3 ± 1.6 (52.3–58.2)
Pharynx length (Head to metacarpus base)	119.3 ± 7.4 (105.4–132.0)	118.0 ± 6.0 (108.5–128.9)	110.5 ± 9.5 (98.8–132.6)	95.0 ± 5.0 (87.8–180.6)	114.2 ± 5.1 (109.6–122.9)
Excretory pore from anterior end	136.7 ± 7.5 (130.6–150.0)	133.8 ± 9.2 (128.5–141.6)	130.5 ± 4.7 (126.3–141.4)	132.8 ± 5.5 (127.9–140.0)	127.7 ± 5.0 (120.6–133.8)
R	100.8 ± 3.3 (87.0–108.0)	101.9 ± 3.1 (97.0–109.0)	87.0 ± 3.0 (84.0–92.0)	96.0 ± 13.0 (82.0–110.0)	102.8 ± 4.2 (96.0–112.0)
Rs	13.0 ± 0.5 (12.0–14.0)	13.5 ± 0.4 (13.0–14.5)	13.0 ± 1.0 (12.0–14.0)	15.0 ± 1.0 (14.0–16.0)	13.4 ± 0.5 (13.0–14.0)
Roes	25.0 ± 1.5 (21.0–27.0)	24.5 ± 0.9 (21.0–25.0)	23.0 ± 2.0 (21.0–26.0)	26.0 ± 3.0 (22.0–29.0)	25.5 ± 0.9 (23.0–26.0)
Rex	29.0 ± 0.7 (26.0–33.0)	29.0 ± 1.5 (25.0–32.0)	24.0 ± 2.0 (22.0–26.0)	27.0 ± 2.0 (25.0–30.0)	28.2 ± 1.3 (25.0–30.0)
Rv	8.0 ± 0.9 (6.0–10.0)	8.5 ± 0.7 (7.0–11.0)	7.0 ± 1.0 (6.0–8.0)	7.0 ± 1.0 (6.0–8.0)	8.6 ± 0.7 (8.0–10.0)
Ran	4.5 ± 1.0 (4.0–7.0)	4.8 ± 0.5 (4.0–8.0)	4.0 ± 1.0 (3.0–5.0)	6.0 ± 1.0 (5.0–7.0)	4.3 ± 0.5 (4.0–5.0)
Rvan	4.0 ± 0.8 (2.0–5.0)	4.3 ± 0.5 (3.0–5.0)	2.5 ± 0.2 (2.0–3.0)	2.3 ± 0.5 (2.0–3.0)	4.3 ± 0.6 (3.0–5.0)

Remarks: *Tylenchorhynchus leviterminalis* was first described from banana, mango (*Mangifera indica* L.) and jackfruit (Artocarpus heterophyllus Lam.) in India [56]. It was reported from banana (*Musa* spp.) in Sistan and Baluchestan province; from grasses at the College of Agriculture (Badjgah Region), Shiraz University, Fars province, Iran [57,58]; from banana, sugarcane and bamboo (*Bambusa* spp.) in Taiwan [59]; from strawberry (*Fragaria ananassa* Duch.) in China mainland [60]; and from sugarcane in Japan [61]. In this study, *T. leviterminalis* was found in turfgrass samples from Liuhuahu Park, Liwanhu Park, Zhuhai Cuihu Golf, Guangzhou Jiulonghu Golf, Yuntai Park, Shamian Park and South China Botanical Garden, Guangdong province, China. Both morphology and morphometrics fit the description of other populations [56]. This is the first record of *T. leviterminalis* on turfgrasses *C. dactylon*, *E. indica*, *P. repens* and *Z. tenuifolia*.

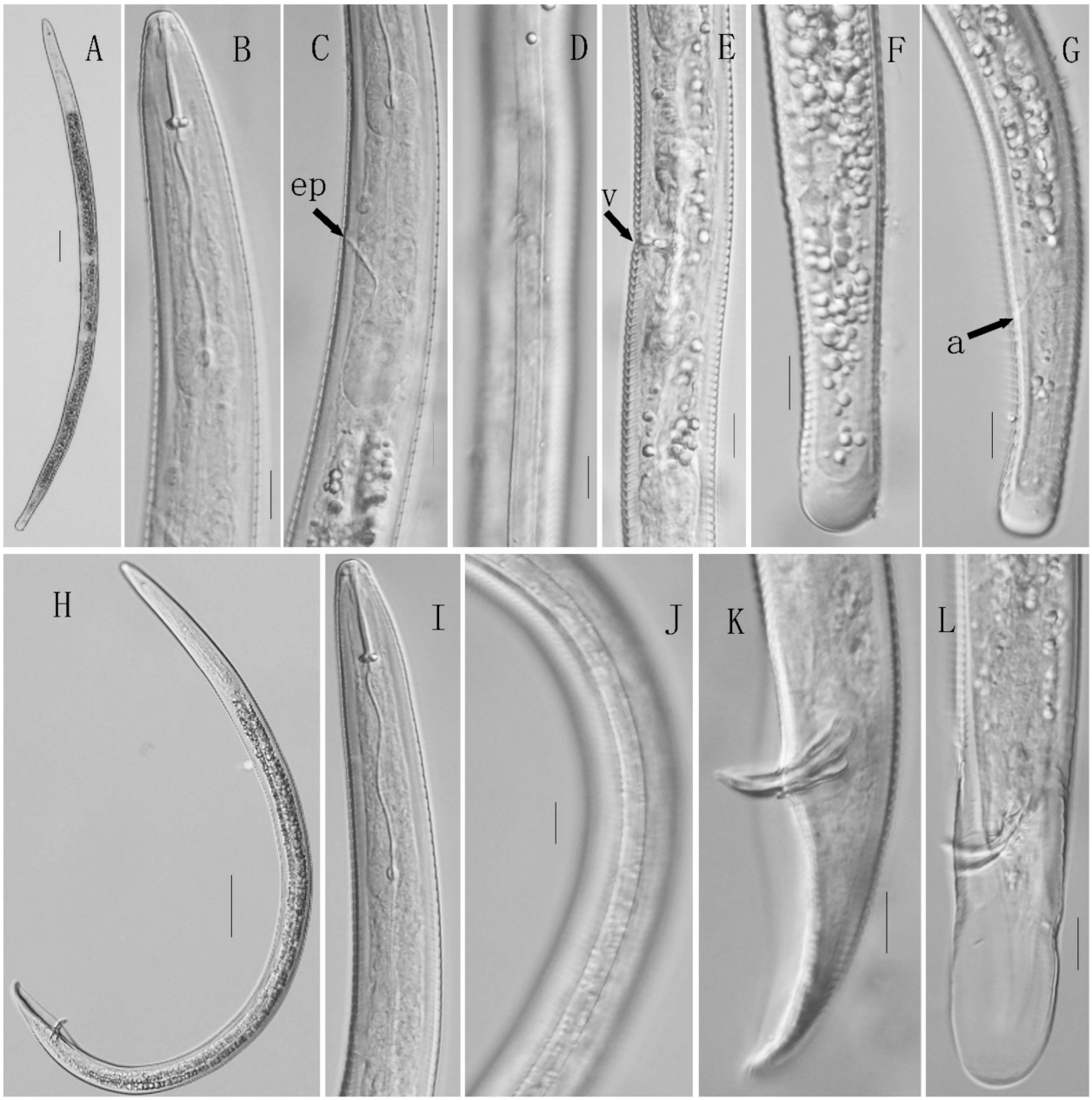

Figure 5. Adult male and female of *Tylenchorhynchus leviterminalis*. (**A**): Entire female body; (**B**): Anterior female body; (**C**): Excretory pore (ep arrow); (**D**): Female lateral field; (**E**): Reproductive system (arrow v refers to vulva); (**F**,**G**): female tails (arrow a refers to anus); (**H**): Entire male body; (**I**): Anterior male body; (**J**): Male lateral field; (**K**): Male tail (lateral view); (**L**): Male tail (subventral view) (Scale bars: (**A**,**H**) = 50 µm; (**B**–**G**,**I**–**L**) = 10 µm).

3.3. Molecular Characterization and Phylogenetic Relationships

A 602-bp 18S rDNA and a 574-bp 28S D2-D3 expansion segment of *H. dihystera* in this study were amplified and sequenced. A BLASTN search of this species matches well with its corresponding species. From 18S sequence, the study *H. dihystera* and a population of *H. dihystera* (JX069950) in GenBank yielded 602 total characters with 99.83% identity; intraspecific sequence variation for *H. dihystera* was 0.17% (1 nucleotide, nt). The study *H. dihystera* shared 581 (581/582 = 99.83%), 579 (579/582 = 99.48%) and 579 (579/582 = 99.48%) identical nucleotides with *H. pseudorobustus* (KJ869397), *H. digitiformis* (KJ869410) and *H. crenacauda* (KM014493), respectively. From the 28S sequence, alignment of the study *H. dihystera* with another population of *H. dihystera* (HM014261) in GenBank revealed 99.83% identity; in-

traspecific sequence variation for *H. dihystera* was 0.17% (1 nt). The study *H. dihystera* shared 556 (556/575 = 96.70%) identical nucleotides with *H. microlobus* (MN764324), *H. pseudorobustus* (MF996708).

Table 8. Morphometrics of *Tylenchorynchus leviterminalis* populations mounted in formalin–glycerin. All measurements in μm and in the format: mean ± s.d. (Range).

Character	Liuhuahu Park		Liwanhu Park	
	T. leviterminalis		*T. leviterminalis*	
n	10 ♀♀	10 ♂♂	10 ♀♀	10 ♂♂
L	642.4 ± 17.7 (620.3–661.2)	628.6 ± 32.2 (560.2–662.2)	667.5 ± 36.6 (606.5–722.0)	687.5 ± 36.0 (620.5–791.0)
a	29.3 ± 1.4 (27.9–30.0)	29.7 ± 2.8 (27.5–34.8)	27.5 ± 2.5 (25.0–31.0)	29.5 ± 2.6 (26.5–34.0)
b	4.5 ± 0.3 (4.1–5.0)	4.0 ± 0.8 (3.0–5.1)	4.8 ± 0.2 (4.4–5.3)	5.5 ± 0.6 (4.9–6.5)
c	14.0 ± 1.1 (12.9–16.0)	13.5 ± 0.9 (12.5–14.6)	15.0 ± 0.8 (13.5–16.3)	17.0 ± 2.2 (13.1–21.3)
c′	3.5 ± 0.5 (2.8–4.3)	2.8 ± 0.2 (2.3–3.2)	2.6 ±0.3 (2.1–3.2)	2.2 ± 0.3 (1.6–3.1)
V	55.5 ± 0.5 (54.0–56.5)	- -	54.5 ± 1.5 (51.0–56.2)	- -
Stylet length	22.5 ± 0.5 (21.0–23.0)	21.0 ± 6.0 (20.2–21.8)	20.6 ± 0.8 (19.0–21.8)	19.6 ± 0.8 (18.8–21.6)
MB	48.3 ± 1.8 (46.4–51.3)	47.5 ± 2.2 (45.1–50.1)	48.6 ± 1.6 (46.8–50.4)	50.6 ± 3.2 (47.1–53.8)
Excretory pore from anterior end	101.0 ± 6.0 (95.0–110.5)	- -	109.4 ± 6.6 (102.0–125.5)	- -
Tail annules	16.0 ± 3.0 (12.0–20.0)	- -	17.0 ± 2.0 (15.0–21.0)	- -
T	- -	56.0 ± 3.5 (52.0–60.0)	- -	50.5 ± 5.5 (44.0–57.5)
Spicule length	- -	23.3 ± 0.8 (21.5–24.6)	- -	22.3 ± 1.8 (20.5–25.6)

A 606-bp 18S rDNA and a 696-bp 28S D2-D3 expansion segment of *H. rosae* in this study were amplified and sequenced. A BLASTN search of this species matches well with its corresponding species. From 18S sequence, the study *H. rosae* and a population of *H. rosae* (MW938290) in GenBank yielded 606 total characters with 99.83% identity; intraspecific sequence variation for *H. rosae* was 0.17% (1 nt). It shared 601 (601/606 = 99.17%), 595 (595/598 = 99.50%), 604 (604/606 = 99.67%) and 518 (518/524 = 98.85%) identical nucleotides with *H. fujianensis* (MH444628), *H. wessoni* (HM116035), *H. wessoni* (KJ934163) and *H. chitwoodi* (JQ708170), respectively. From the 28S sequence, alignment of the study *H. rosae* with two other populations of *H. rosae* (MW938526 and MK371813) in GenBank revealed 99.57% identity; intraspecific sequence variation for *H. rosae* was 0.43% (3 nt). It shared 600 (600/641 = 93.60%), 637 (637/697 = 91.39%) and 634 (634/696 = 91.09%) identical nucleotides with *H. wessoni* (KF856520), *H. wessoni* (HM116035), *H. ortonwilliamsi* (MN888469) and *H. brachyurus* (MN720101), respectively.

A 606-bp 18S rDNA and a 682-bp 28S D2-D3 expansion segment of *M. graminis* in this study were amplified and sequenced. A BLASTN search of this species matches well with its corresponding species. From 18S sequence, the study *M. graminis* and three populations of *M. graminis* (KP901050, KP901044 and JN241854) in GenBank yielded 606 total characters with 99.51–99.83% identities; intraspecific sequence variations for *M. graminis* were 0.33–0.49% (2–3 nt). It shared 598 (598/606 = 98.68%), 596 (596/607 = 98.19%) and 596 (596/607 = 98.19%) identical nucleotides with *M. ardenensis* (EU669946), *M. incognita* (MT102326) and *M. hapla* (MK102780), respectively. From the 28S sequence, alignment of the study *M. graminis* with other two populations of *M. graminis* (JN019329 and KP901075) in

GenBank revealed 99.71% identity; intraspecific sequence variation for *M. graminis* was 0.29% (2 nt). It shared 662 (662/683 = 96.93%), 620 (620/692 = 89.60%), 618 (618/684 = 90.35%), 620 (620/684 = 90.64%), 609 (609/667 = 91.30%) and 623 (623/684 = 91.08%) identical nucleotides with *M. marylandi* (JN019350), *M. minor* (KC241977), *M. luci* (LN626951) *M. haplanaria* (MK102786), *M. javanica* (JQ317915) and *M. enterolobii* (MZ602648), respectively.

A 633-bp 18S rDNA and a 718-bp 28S D2-D3 expansion segment of *M. xenoplax* in this study were amplified and sequenced. A BLASTN search of this species matches well with its corresponding species. From 18S sequence, the study *M. xenoplax* and a population of *M. xenoplax* (MF095022) in GenBank yielded 633 total characters with 99.84% identity; intraspecific sequence variation for *M. xenoplax* was 0.16% (1nt). It shared 628 (628/634 = 99.05%), 628 (628/634 = 99.05%) and 629 (629/634 = 99.21%) identical nucleotides with *M. curvatum* (AY919186), *M. nebraskense* (KY574845) and *M. discus* (MF094892), respectively. From the 28S sequence, alignment of the study *M. xenoplax* with one other population of *M. xenoplax* (MN888463) in GenBank revealed 99.72% identity; intraspecific sequence variation for *H. rosae* was 0.28% (2 nt). It shared 639 (639/663 = 96.38%), 669 (669/709 = 94.36%), 658 (658/703 = 93.60%) and 650 (650/697 = 93.26%) identical nucleotides with *M. curvatum* (MN720094), *M. antipolitanum* (MN888461), *M. onoense* (MZ220549) and *M. ornatum* (MW938536), respectively.

A 602-bp 18S rDNA and a 704-bp 28S D2-D3 expansion segment of *T. leviterminalis* in this study were amplified and sequenced. A BLASTN search of this species matches well with its corresponding species. From 18S sequence, the study *T. leviterminalis* and a population of *T. leviterminalis* (EU368585) in GenBank yielded 602 total characters with 99.83% identity; intraspecific sequence variation for *T. leviterminalis* was 0.17% (1 nt). It shared 582 (582/591 = 98.48%), 577 (577/591 = 97.63%), 500 (500/506 = 98.81%) and 577 (577/592 = 97.47%) identical nucleotides with *T. microconus* (KX789741), *T. clarus* (KX789740), *T. annulatus* (LC540653) and *T. claytoni* (KJ934130), respectively. From the 28S sequence, alignment of the study *T. leviterminalis* with three other populations of *T. leviterminalis* (KJ475547, KJ461550 and KJ475546) in GenBank revealed 99.57–99.86% identities; intraspecific sequence variations for *T. leviterminalis* were 0.14–0.43% (1–3 nt). It shared 682 (682/706 = 96.60%) and 682 (682/705 = 96.74%) identical nucleotides with *T. agri* (MG491667) and *T. annulatus* (MT193442), respectively.

Phylogenetic analyses of the partial 18S and 28S D2-D3 were performed to examine the relationships among the most common species from this study and related species from Genbank. The dendrogram inferred from 18S rDNA sequences (Figure 6) using *Aphelenchoides besseyi* Christie, 1942 as an outgroup demonstrates: (i) four monophyletic clades with 100% posterior probability (pp) corresponding to the Meloidogynidae, Telotylenchidae, Criconematidae and Hoplolaimidae families; (ii) with 100% pp, *M. graminis* from this study is grouped into the Meloidogynidae-clade with six other species/populations of *Meloidogyne*, *T. leviterminalis* into the Telotylenchidae-clade with five other species/populations of *Tylenchorynchus*, both *M. xenoplax* and *H. rosae* into the Criconematidae-clade with five other species/populations of their respective genera, and *H. dihystera* into the Hoplolaimidae-clade with four other species/populations of *Helicotylenchus* from GenBank; (iii) species belonging to the Meloidogynidae and Telotylenchidae are grouped in a well-supported (pp = 99%) monophyletic clade. The Meloidogynidae, Telotylenchidae and Criconematidae also form a monophyletic clade with 99% pp.

The tree inferred from D2-D3 expansion segments of 28S rDNA (Figure 7) using *Aphelenchoides besseyi* Christie, 1942 as an outgroup demonstrates: (i) four distinct clades are the same as those identified by 18S rDNA sequences; (ii) the families Meloidogynidae and Telotylenchidae are in a highly-supported (pp = 100%) monophyletic clade, which together with the Hoplolaimidae forms an additional clade (pp = 100%); species/populations belonging to the genera *Mesocriconema* and *Hemicriconemoides* are in a well-supported (pp = 100%) monophyletic clade; (iii) *Meloidogyne graminis* from this study is clustered in a monophyletic clade with two other populations of *M. graminis* (JN019329 and KP901075) with 100% pp, *T. leviterminalis* is in a well-supported (pp = 100%) monophyletic clade with

three other populations of *T. leviterminalis* (KJ475547, KJ461550 and KJ475546), *H. dihystera* is in a monophyletic clade with one other population of *H. dihystera* (HM014261) with 100% pp, *M. xenoplax* is in a monophyletic clade with one other population of *M. xenoplax* (MN888463) with 100% pp, and *H. rosae* is in a highly-supported (pp = 99%) monophyletic clade with two other populations of *H. rosae* (MW938526 and MK371813).

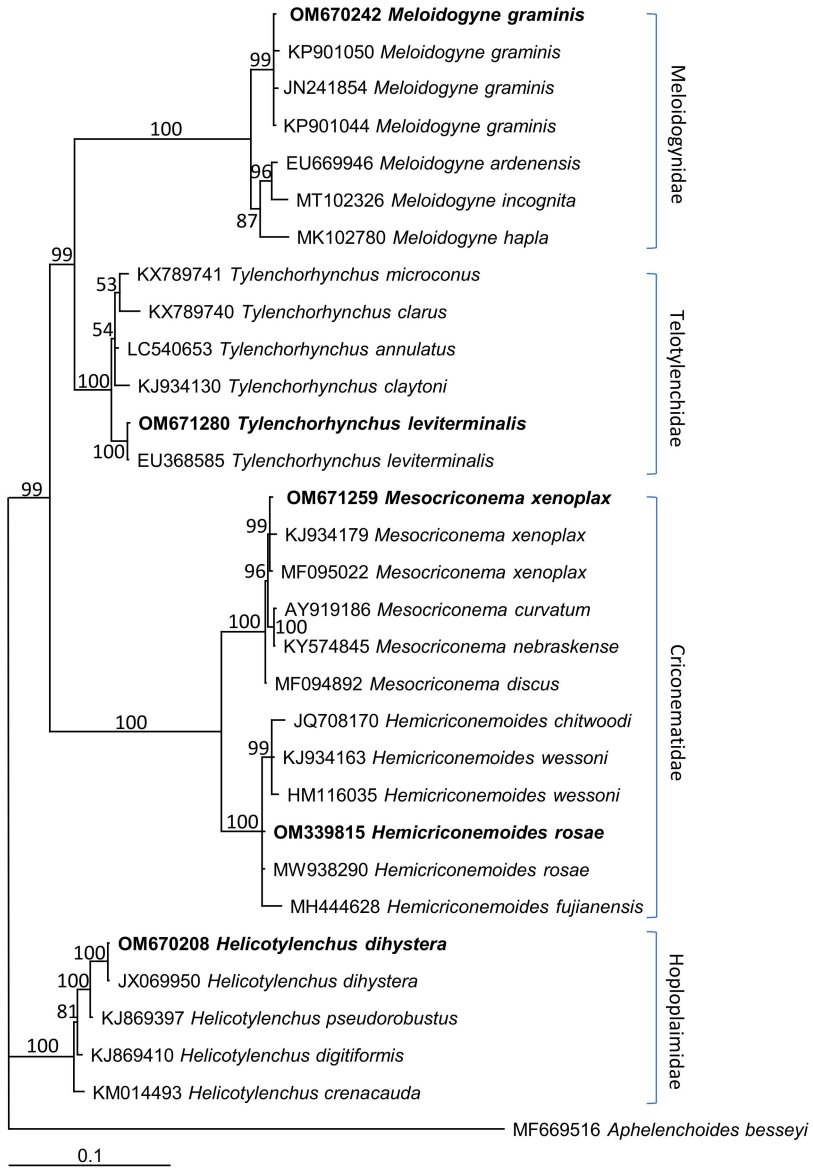

Figure 6. The 10,001st Bayesian tree inferred from 18S under the GTR + I + G model (lnL = 6925.1914; freqA = 0.2438; freqC = 0.2192; freqG = 0.2803; freqT = 0.2568; R(a) = 1.2950; R(b) = 2.2321; R(c) = 1.0279; R(d) = 0.7318; R(e) = 4.2831; R(f) = 1; Pinvar = 0.3455; Shape = 0.6090). Posterior probability values exceeding 50% are given on appropriate clades. The new species is in bold font.

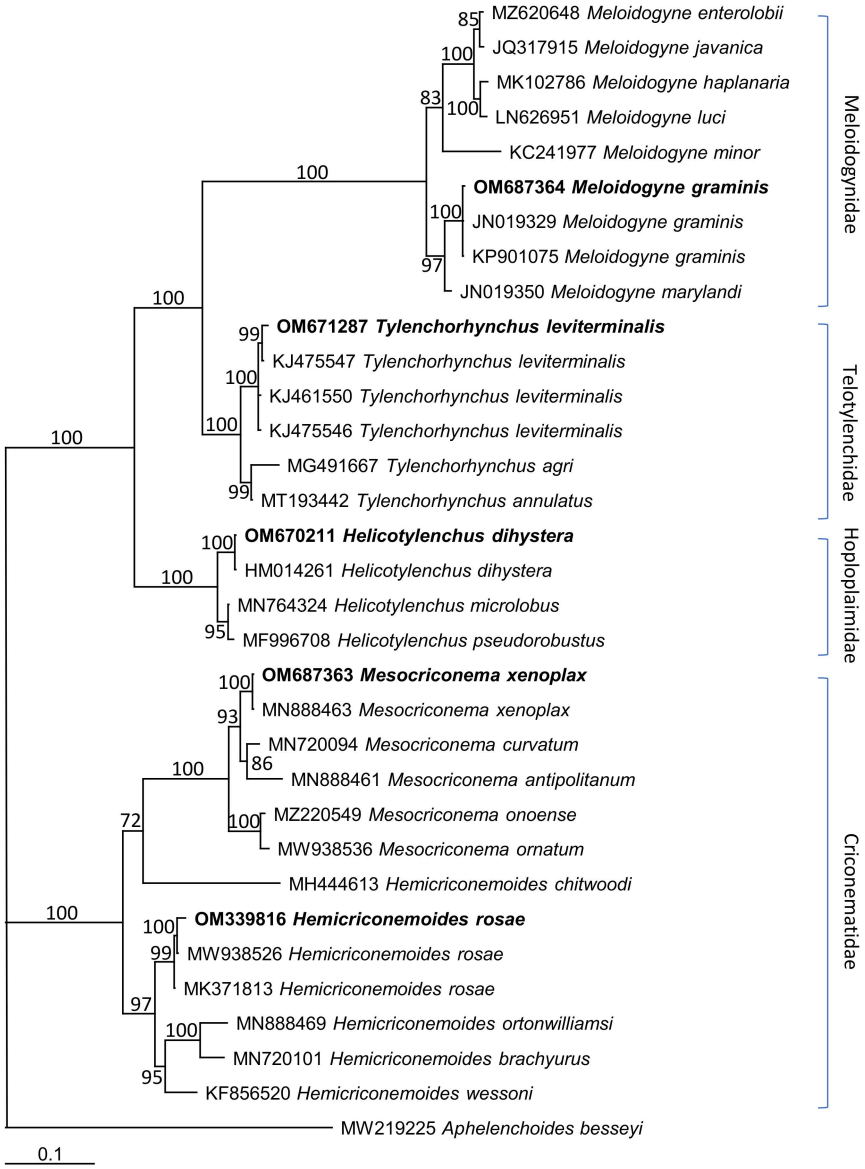

Figure 7. The 10,001st Bayesian tree inferred from D2-D3 under the GTR+I+G model (lnL = 5789.4619; freqA = 0.1932; freqC = 0.2222; freqG = 0.3323; freqT = 0.2523; R(a) = 0.6368; R(b) = 2.4422; R(c) = 1.4785; R(d) = 0.3450; R(e) = 3.8018; R(f) = 1; Pinvar = 0.2939; Shape = 1.3309). Posterior probability values exceeding 50% are given on appropriate clades. The new species is in bold font.

4. Discussion

Prior to this work, there have been several research papers illustrating a broad diversity of nematodes associated with turfgrasses in the United States [5,10,11,28,62], Canada [7,63,64], Argentina [65], Germany, Sweden, Norway [66,67], Belgium [8], Israel [68] and Korea [6]. Previous research showed that the species of *Hemicriconemoides* and *Tylenchorynchus* associated with turfgrasses include *H. chitwoodi* Esser, 1960 and *H. wessoni* Chitwood & Birchfield, 1957 in *Cynodon dactylon* [10,11]; *H. brachyurus* (Loos, 1949) Chitwood & Birchfield, 1957 in *Poa pratensis* and *Zoysia japonica* [6]; *T. claytoni* Steiner, 1937 in *P. pratensis* and *Z. japonica* [6,10,11]; *T. dubius* in *Agrostis stolonifera* and *P. pratensis* [69]; *T. annulatus* in *Z. japonica*; and *T. thermophilus* in *Z. japonica* and *P. pratensis* [6]. The work presented here provides new records of *H. rosae* and *T. leviterminalis* associated with turfgrasses.

The genus *Hemicriconemoides* Chitwood & Birchfield, 1957 morphologically differ from other genera within Criconematidae by possessing a loosened outer cuticular sheath cov-

ering the cuticle of their body; in females, the sheath is attached to the main body at the head and vulva. This genus is represented by 52 species [70]. The species *H. rosae* in this study morphologically agreed with the populations described by Rathour et al. [30] and Khan et al. [31]. Its status as a distinct species is corroborated by molecular sequences of 18S and 28S D2-D3 (Figures 6 and 7). *Hemicriconemoides rosae* was first found around the rhizosphere of rose (*Rosa indica*) [30]. It was also documented as occurring in sugarcane [31] and *Pilea cadierei* [71]. In the present study, it was extracted from rhizosphere soils of multiple turfgrasses including *Cynodon dactylon*, *Eleusine indica*, *Panicum repens* and *Sporobolus indicus*, thus extending associated plant range of *H. rosae*.

The genus *Tylenchorhynchus* was established by Cobb (1913) for *T. cylindricus* found in southern California [72]. This genus contains 111 species that parasitize a wide variety of plants [73]. Handoo [73] defined the valid and most significant differentiating characters and prepared a key and a compendium containing morphometric and related details of these valid species. Siddiqi et al. [56] first described *T. leviterminalis* from banana, mango and jackfruit in India. The morphological and morphometric characters of the studied *T. leviterminalis* fit the original description [56]. Its presence as a distinct species is supported by molecular sequences of 18S and 28S D2-D3 (Figures 6 and 7). Some hosts of *T. leviterminalis* were reported, including banana (*Musa* spp.) [55], sugarcane [57,59], bamboo (*Bambusa* spp.) [57] and strawberry (*Fragaria ananassa*) [60]. In the present study, *T. leviterminalis* was first reported on turfgrasses *C. dactylon*, *E. indica*, *P. repens* and *Z. tenuifolia*.

The genus *Helicotylenchus* Steiner, 1945 belongs to the family Hoplolaimidae. There are over 200 species within the genus [74]. Morphological identification of *Helicotylenchus* species is often difficult because of high intra- and interspecific variability and a lot of poorly described species [75,76]. Application of non-morphological characters such as DNA sequences can help to confirm classical morphology-based identifications and resolve some of the problems experienced in the identification of *Helicotylenchus* species [76]. *Helicotylenchus dihystera* is the type species of the genus. The morphometric data and morphological characteristics of *H. dihystera* in this study match with other populations given by Zeng et al. [10] and Siddiqi [27]. Phylogenetic analysis inferred from sequences of 18S and 28S D2-D3 segment support the presence of *H. dihystera* (Figures 6 and 7).

The genus *Mesocriconema* Andrássy, 1965, belonging to the family Criconematidae, is characterized by having thick, rounded, protruding, retrorse cuticular annulations. This genus contains 90 species [77], some of which are morphologically very close to each other. Molecular and morphological analyses of species within *Mesocriconem*, from North America, were used to differentiate formally described members of the genus as well as lineages lacking a formal description [78,79]. Powers et al. [79] distinguished 24 haplotype groups by using COI sequences. In this study, molecular and morphological analyses were employed to determine the species *M. xenoplax*; both morphology and morphometrics of *M. xenopax* matched the description by Zeng et al. [10], and its presence as a distinct species is supported by molecular sequences of 18S and 28S D2-D3 (Figures 6 and 7). *Mesocriconema xenoplax* has a wide host range. Rootstocks of most species in the genus *Prunus* L. support populations of *M. xenoplax*. It also infects various other fruit trees such as grapes [80]. The turfgrass hosts of *M. xenoplax* include tall fescue (*Festuca arundinacea* J. C. D. von Schreber) [55] and bermudagrass, creeping bentgrass and zoysiagrass [10,11]. In this study, *M. xenoplax* was detected in the rhizosphere soils from *C. dactylon*, *P. repens*, *S. indicus* and *Z. tenuifolia.* Pathogenicity examinations are needed for these turfgrasses in the future.

Root-knot nematodes (*Meloidogyne* spp.) have been one of the most prevalent species on turfgrasses worldwide [6,8,10,11,40,63,64]. So far, reported *Meloidogyne* species associated with turfgrasses include *M. incognita* (Kofoid & White) Chitwood, 1949 [65], *M. graminis*, *M. graminicola* Golden & Birchfield, 1965 [6], *M. marylandi* Jepson & Golden, 1987 [6,62], *M. minor* Karssen et al., 2004 [63] and *M. naasi* Franklin, 1965 [8,10]. Zeng et al. [10,11] and Sánchez-Arce et al. [7] showed that *M. graminis* was one of the most prevalent species in golf course turfgrasses. Sánchez-Arce et al. [7] and Zhuo et al. [13]

reported that *M. graminis* was associated with bermudagrass. Zeng et al. [10,11] showed that *M. graminis* was found in two grass species (bermudagrass and zoysiagrass). In the present study, *M. graminis* was also detected in bermudagrass from 9 golf courses. Our report, along with previous publications, shows a strong association of *M. graminis* and bermudagrass, but more extensive sampling is needed. *Meloidogyne minor* was previously associated with yellow patch disease on *Agrostis stolonifera* var. *stolonifera* L. on golf greens [63]. *Meloidogyne* spp. have variations in pathogenicity, but a recent survey in SC indicated severe damage caused by *M. graminis* on golf course turfgrasses, particularly bermudagrass [81]. Although the pathogenicity of some *Meloidogyne* species to turfgrasses still needs to be further examined, root-knot nematodes are undoubtedly worthy of attention in the future.

5. Conclusions

Morphologically, this study clarified the identity of the most common plant-parasitic nematodes on golf course turf in Guangdong. Molecularly, it characterized these species using sequences of the 18S nuclear ribosomal RNA (rRNA) and the D2-D3 expansion segments of the 28S rRNA gene, showing little intraspecific variation of the sequences with respective corresponding species from GenBank. Phylogenetically, it investigated the phylogenetic relationships among these plant-parasitic nematode species based on sequences of the 18S rRNA and the D2-D3 expansion segments of the 28S rRNA gene, revealing correct phylogenetic placements of the five species in this study. Both sequences of the 18S rRNA and the D2-D3 expansion segments of the 28S rRNA gene verified morphological-based identification of these species in the present study. Five nematode species are described, including *H. dihystera*, *H. rosae*, *M. graminis*, *M. xenoplax* and *T. leviterminalis*, with new records of *H. rosae*, and *T. leviterminalis* associated with turfgrass. This work was a first step for future study including pathogenicity assay, relationship examination with other pathogens and development of control measures of these turf nematodes to provide more precise and effective management options to turf superintendents. This is the first report on molecular characterization and phylogenetic relationships of plant-parasitic nematodes associated with turfgrasses in Guangdong, China.

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