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Morphological and molecular characterization of *Xiphinema* species from Shenzhen, China

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Summary

During a nematode biodiversity survey from 2012 to 2014 in Shenzhen, China, ten nematode populations (SZX1301–SZX1310) of *Xiphinema* were recovered from rhizosphere of different plants, namely *Acacia mangium* (SZX1306), *A. confuse* (SZX1309), *Blechnum orientale* (SZX1301, SZX1302, SZX1307, SZX1308), *Litchi chinensis* (SZX1304, SZX1310) in Tianxinshan and *Gleichenia linearis* (SZX1303, SZX1305) in Yangmeikeng environmental monitoring sites. Morphological and molecular profiles of these populations were determined. Three species of *Xiphinema*, i.e., *X. hunaniense* Wang & Wu, 1992, *X. brasiliense* Lordello, 1951 and *X. americanum* Cobb, 1913 *sensu lato* were identified using morphological characters and molecular data of partial 18S and 28S D2–D3 rDNA expansion segments. Four populations (SZX1301–SZX1304) were *X. hunaniense,* one population (SZX1305) *X. brasiliense,* and five populations (SZX1306–SZX1310) *X. americanum s.l.*. Phylogenetic analysis based on sequences of the 28S rDNA D2–D3 expansion segment revealed these three species are all distinct species and supported a close relationship with their corresponding species. This is the first report of *X. hunaniense, X. brasiliense* and *X. americanum s.l.* in their hosts except for *L. chinensis*.

Keywords: *Xiphinema* spp.; *Acacia mangium*; *Acacia confuse*; *Blechnum orientale*; *Gleichenia linearis*; *Litchi chinensis*; 28S rDNA; dagger nematode

Introduction

The genus *Xiphinema* Cobb, 1913 belonging to the family Longidoridae represents ectoparasitic root nematodes commonly known as the dagger nematode. There are approximately 260 nominal species in the genus to date (Gutiérrez-Gutiérrez *et al*., 2012). They are typically divided into two groups, namely *X. americanum*-group with about 50 species and non-*X. americanum*-group (Loof & Luc, 1990; Lamberti *et al*., 2000). The genus *Xiphinema* includes phytopathogenic species that damage a large number of wild and cultivated plants through direct feeding on root cells and transmission of several plant-pathogenic viruses (Taylor & Brown, 1997). It is of economic importance on grape, strawberry, hops, fruit trees and other crops. Nine species of *Xiphinema* have been shown to transmit nepoviruses (Decraemer & Robbins, 2007). Some species in the *X. americanum*-group can serve as vectors of several important plant viruses including *Tabacco ringspot virus*, *Tomato ringspot virus*, *Cherry rasp leaf virus* and *Peach rosette mosaic virus* that damage a wide range of crops (Taylor & Brown, 1997). Several species in the group are listed as quarantine organisms by some countries or regions such as the European and Mediterranean Plant Protection Organization. Therefore, accurate identification of the genus to the species level is crucial to implement appropriate control measures for these nematodes.

Currently, species identification of this genus is mainly based on morphological features and morphometrics. However, species belonging to *Xiphinema americanum*-group show conserved morphology and overlapping morphometrics (Coomans *et al*., 2001; Gutiérrez-Gutiérrez *et al*., 2012). Thus, DNA-based approaches including ribosomal DNA such as the 18S, D2-D3 expansion segments of 28S, ITS regions, and mitochondrial DNA have been employed for the molecular characterization and reconstruction of phylogenetic relationships within *Xiphinema* (Oliveira *et al*., 2004; Ye *et al*., 2004; He *et al*., 2005; Lazarova *et al*., 2006; Wu *et al*., 2007; Gutiérrez-Gutiérrez *et al*., 2012, 2013).

Some species of the genus *Xiphinema* are distributed worldwide, whereas others have limited distribution (Coomans, 1996; Coomans *et al*., 2001; Gutiérrez-Gutiérrez *et al*., 2012). So far, 14 species of *Xiphinema* (*X. americanum* Cobb, 1913*, X. brasiliense* Lordello, 1951*, X. brevicolle* Lordello & Costa, 1961*, X. diffusum* Lamberti & Blève-Zacheo, 1979*, X. elongatum* Schuurmans Stekhoven & Teunissen, 1938*, X. hunaniense* Wang & Wu, 1992*, X. imitator* Heyns, 1965*, X. incognitum* Lamberti & Blève-Zacheo, 1979*, X. insigne* Loos, 1949*, X. luci* Lamberti & Bleve-Zacheo, 1979*; X. oxycaudatum* Lamberti & Blève-Zacheo, 1979*, X. radicicola* Goodey, 1936*, X. taylori* Lamberti, Ciancio, Agostinelli & Coiro, 1992*, X. thornei* Lamberti & Golden, 1986) were reported in China (Luo *et al*., 2001; Pan *et al*., 2000; Teng *et al*., 2013; Wang *et al*., 1996; Wu, 2007; Xu *et al*., 1995; Zheng & Brown, 1999).

During a survey of nematode biodiversity in Yangmeikeng and Tianxinshan environmental monitoring sites in Shenzhen, China in 2012 – 2014, ten *Xiphinema* populations (designated as SZX1301 – SZX1310) were recovered from the rhizosphere collected from five plant species including Blechnoid (*Blechnum orientale* L.), Awn dichotoma (*Gleichenia linearis* Clarke.), Lychee (*Litchi chinensis* Sonn.), Acacia acacia (*Acacia mangium* Willd.) and Taiwan acacia (*A. confusa* Merr.).

The main objectives of this study were to: (i) identify the species of ten *Xiphinema* populations based on morphological and molecular approaches; and (ii) investigate their phylogenetic relationships with other species in the genus based upon sequence analysis of the 28S D2-D3 rDNA.

Materials and Methods

Sampling and Morphological Study

Soil samples were collected from the rhizosphere at a depth of 15 – 30 cm of different plants, namely Acacia acacia, Taiwan acacia*,* Blechnoid, Lychee in Tianxinshan and Awn dichotoma in Yangmeikeng environmental monitoring sites. Ten nematode populations from the rhizosphere of five plants in two environmental monitoring sites, Shenzhen, China, were presented in Table 1. Nematodes were extracted by a sieving and decanting method (Brown & Boag, 1988). Specimens were heat-killed, fixed in 3 % formaldehyde and processed to glycerin by the formalin-glycerin method (Hooper, 1970; Golden, 1990). Specimen preparation and measurements were as described in Golden & Birchfield (1972). Measurements of nematodes were performed with the aid of a camera lucida and a stage micrometer. The morphometric data were processed using Excel software (Ye, 1996). Photomicrographs were taken with a Leica video camera (DFC490) fitted on a Leica microscope (DM4000B), and edited using Adobe Photoshop CS5. Morphological identification of specimens for *Xiphinema* was done using the polytomous keys provided by Lamberti *et al*. (2000, 2004) and Loof & Luc (1990), with corresponding species descriptions.

Molecular Study

DNA extraction, amplification and sequencing: For each population, three females were hand-picked into distilled water for DNA extraction, amplification, and sequencing. They were placed into 50 µl of worm lysis buffer (WLB) containing Proteinase K for DNA extraction (Williams *et al*., 1992). DNA samples were stored at –20°C until used as a PCR template.

The primers for small subunit 18S amplification and DNA sequencing were forward primer 18S965 (5' GGCGATCAGATAC-

Table 1. Species and populations of *Xiphinema* in this study

Species	Population code	Locality	Host plant	18S Accession No.	28S D2-D3 Accession No.
Xiphinema hunaniense	SZX1301	Tianxinshan, Shenzhen	*Blechnum orientale*	KP793036	KP793046
Xiphinema hunaniense	SZX1302	Tianxinshan, Shenzhen	*Blechnum orientale*	KP793037	KP793047
Xiphinema hunaniense	SZX1303	Yangmeikeng, Shenzhen	*Gleichenia linearis*	KP793038	KP793048
Xiphinema hunaniense	SZX1304	Tianxinshan, Shenzhen	*Litchi chinensis*	KP793039	KP793049
Xiphinema brasiliense	SZX1305	Yangmeikeng, Shenzhen	*Gleichenia linearis*	KP793040	KP793050
Xiphinema americanum s.l.	SZX1306	Tianxinshan, Shenzhen	*Acacia mangium*	KP793041	KP793051
Xiphinema americanum s.l.	SZX1307	Tianxinshan, Shenzhen	*Blechnum orientale*	KP793042	KP793052
Xiphinema americanum s.l.	SZX1308	Tianxinshan, Shenzhen	*Blechnum orientale*	KP793043	KP793053
Xiphinema americanum s.l.	SZX1309	Tianxinshan, Shenzhen	*Acacia confuse*	KP793044	KP793054
Xiphinema americanum s.l.	SZX1310	Tianxinshan, Shenzhen	*Litchi chinensis*	KP793045	KP793055

CGCCCTAGTT 3') and reverse primer 18S1573R (5' TACAAAGGGCAGGGACGTAAT 3') (Mullin *et al*., 2005). Primers for large subunit 28S amplification and DNA sequencing were forward primer D2a (5' ACAAGTACCGTGAGGGAAAGTTG 3') and reverse primer D3b (5' TGCGAAGGAACCAGCTACTA 3') (Nunn, 1992).

The 25 µl PCR was performed using TaqMix DNA polymerase (Guangzhou Dongsheng Biotech Ltd., Guangzhou, 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 with 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 (Ye *et al*., 2007).

PCR products were cleaned using an EZ Spin Column DNA Gel Extraction Kit (Bio Basic Inc., Markham, Ontario, Canada) according to the manufacturer's protocol before being sequenced by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, China) using an ABI PRISM 3730 sequencing system.

Phylogenetic analysis: The nematode sequences from this project were deposited in GenBank. We used DNA sequences with the highest matches with our populations from the GenBank database for phylogenetic analysis. DNA sequences were aligned using ClustalW (http://workbench.sdsc.edu, Bioinformatics and Computational Biology Group, Department of Bioengineering, UC San Diego, San Diego, CA, USA). The model of base substitution in the 28S rDNA sets was evaluated using MODELTEST version 3.06 (Posada & Crandall, 1998). The Akaike-supported model (GTR), the proportion of invariable sites (I), and the gamma distribution shape parameters and substitution rates (G) were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck & Ronquist, 2001) running the chain for 10^6 generations and setting the 'burn in' at 1000. We used MCMC (Markov Chain Monte Carlo) methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50 % majority-rule.

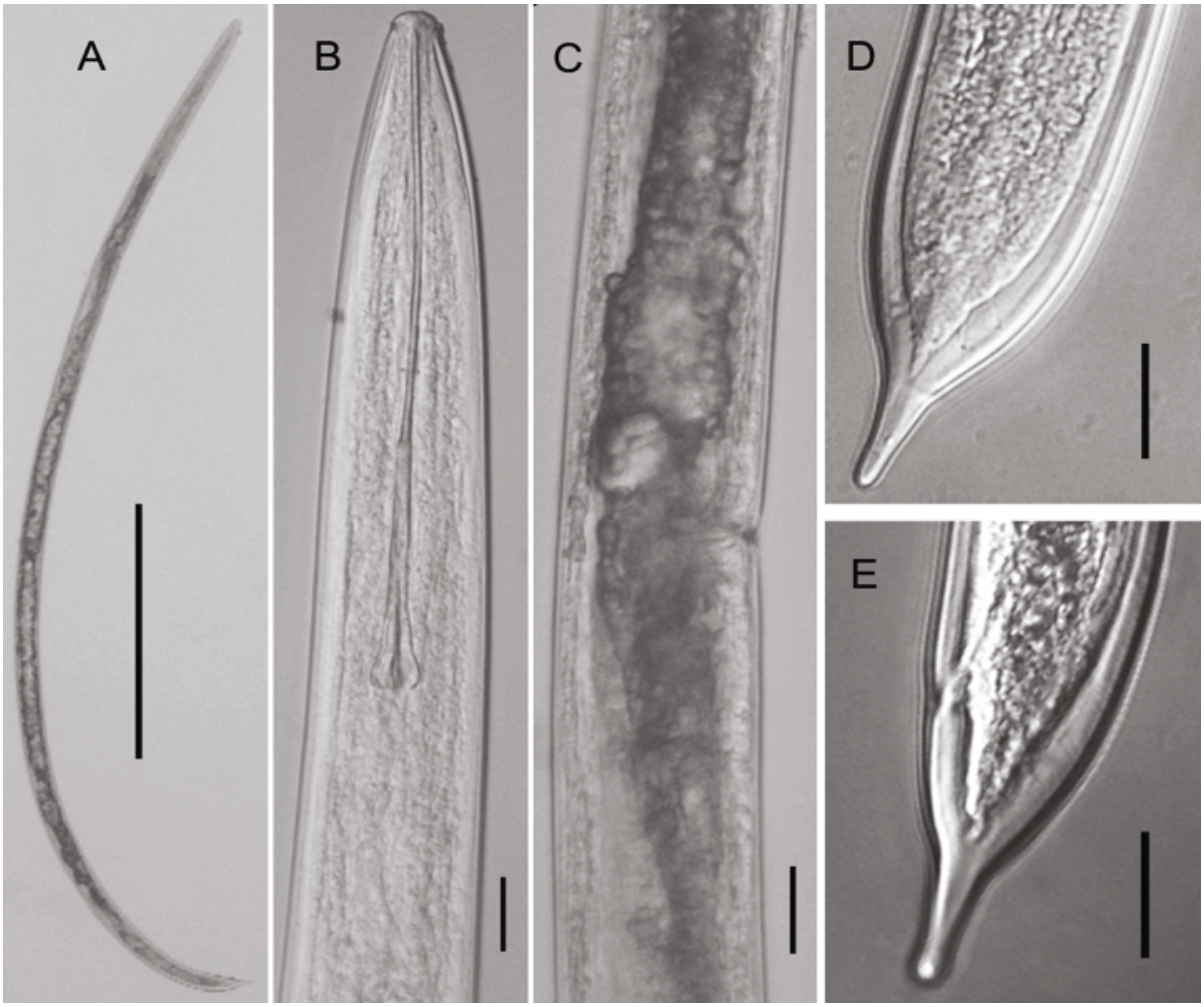

Fig. 1. Light micrographs of *Xiphinema hunaniense* from *Blechnum orientale*. A: Female entire body; B: Female anterior body; C: Reproductive system of female (in ventral view); D: Female tail (in ventral view); E: Female tail (in lateral view). Scale bars: A = 50 µm; B – E = 10 µm

Results

Through this study, four nematode populations (SZX1301 – SZX1304) were identified as *Xiphinema hunaniense*, one population (SZX1305) as *X. brasiliense*, and five populations (SZX1306 – SZX1310) as *X. americanum s.l.* (Table 1), representing the first report of these three species from the above-mentioned plant hosts except for lychee.

Morphological description

Morphometrics of females of ten populations of *Xiphinema* are presented in Table 2. Four populations (SZX1301 – SZX1304) of *X. hunaniense* are identical each other. All five populations of *X. americanum s.l.* (SZX1306 – SZX1310) showed little variation at morphometrics and molecular characteristics, thus considered different geographical populations belonging to the same species.

Xiphinema hunaniense Wang & Wu, 1992
(Fig. 1, Tables 1 & 2)

Female: Body 1810 – 2400 µm long, tapering slightly towards anterior and tail region, posterior end arcuate ventrally when heat-killed. Body cuticle smooth. Lip region 10 – 12 µm in diam., slightly offset from body profile. Amphids stirrup-shaped, with slit-like apertures. Stylet 173 – 193 µm long. Guide ring 86 – 108 µm from anterior end. Pharynx typical of genus. Vulva a transverse slit, anterior, occupies 23 % – 28 % of total body length, vagina thick-walled, occupying up to 50 % of body width. Reproductive system monodelphic, with a posterior reflexed gonad. Uterus short and undifferentiated, "Z"-organ absent. Tail dorsally conoid, slightly convex dorsally and concave ventrally, with a digitate, elongated peg (11 – 20 µm long), with three caudal pores on each side of tail (one on each at beginning of tail terminus, the other two on the dorsal side near anus).

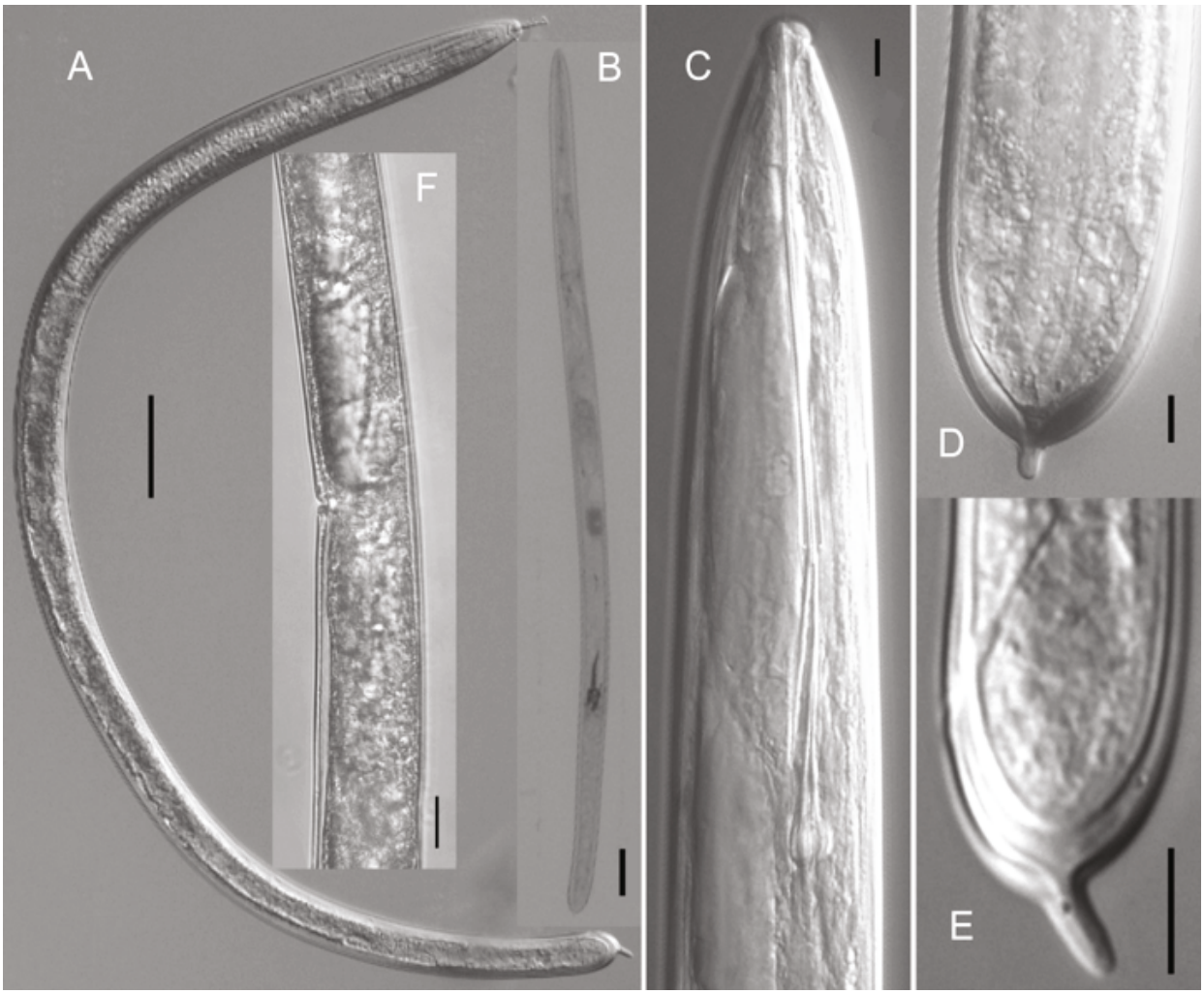

Fig. 2. Light micrographs of female *Xiphinema brasiliense* from *Gleichenia linearis*. A, B: Entire body; C: Anterior body; D: Tail in ventral view; E: Tail in lateral view; F: Reproductive system. Scale bars: A = 500 µm; B, C, E = 20 µm; D = 10 µm

Table 2. Morphometrics of females of *Xiphinema* spp. populations mounted in formalin–glycerin in this study. All measurements in µm and in the format: mean ± s.d. (Range)

	X. hunaniense	*X. hunaniense*	*X. hunaniense*	*X. hunaniense*	*X. brasiliense*	*X. americanum s.l.*	*X. americanum s.l.*	*X. americanum s.l.*	*X. americanum s.l.*	*X. americanum s.l.*
	SZX1301	SZX1302	SZX1303	SZX1304	SZX1305	SZX1306	SZX1307	SZX1308	SZX1309	SZX1310
Host	*Blechnum orientale*	*Blechnum orientale*	*Gleichenia linearis*	*Litchi chinensis*	*Gleichenia linearis*	*Acacia mangium*	*Blechnum orientale*	*Blechnum orientale*	*Acacia confusa*	*Litchi chinensis*
n	10	10	10	10	5	10	10	10	10	10
L	2098.8 ± 96.1 (1989.6 – 2200.0)	2236.4 ± 176.9 (2001.8 – 2400.0)	2110.5 ± 100.0 (1998.7 – 2202.7)	2018.6 ± 141.1 (1810.0 – 2179.3)	1643.1 ± 346.8 (1108.0 – 2100.0)	1792.6 ± 80.7 (1669.0 – 1888.0)	1808.6 ± 86.7 (1691.0 – 1904.0)	1876.1 ± 144.7 (1696.0 – 2105.6)	1785.6 ± 116.2 (1643.8 – 1932.0)	1807.5 ± 94.8 (1683.0 – 1909.0)
a	42.1 ± 3.4 (39.7 – 48.0)	46.9 ± 4.3 (42.5 – 52.7)	46.8 ± 3.1 (43.0 – 50.1)	46.2 ± 2.5 (43.0 – 49.1)	38.4 ± 5.5 (30.0 – 47.9)	45.1 ± 1.0 (43.8 – 46.2)	45.6 ± 1.0 (44.5 – 47.1)	46.8 ± 4.1 (38.1 – 50.1)	46.6 ± 2.2 (44.7 – 50.4)	44.7 ± 2.1 (41.0 – 46.0)
b	5.9 ± 0.3 (5.6 – 6.4)	6.1 ± 0.2 (5.9 – 6.5)	6.0 ± 0.2 (5.9 – 6.3)	5.6 ± 0.5 (5.0 – 6.1)	5.5 ± 0.3 (5.2 – 6.0)	6.8 ± 0.4 (6.1 – 7.2)	6.7 ± 0.6 (5.9 – 7.3)	6.7 ± 0.6 (5.9 – 7.7)	6.6 ± 0.4 (6.1 – 7.0)	6.4 ± 0.7 (5.3 – 7.0)
c	45.9 ± 5.7 (38.0 – 51.6)	48.0 ± 3.8 (41.7 – 51.0)	40.4 ± 3.8 (37.1 – 46.8)	39.9 ± 2.2 (37.6 – 43.0)	41.6 ± 2.8 (39.0 – 48.1)	63.0 ± 5.8 (57.4 – 69.4)	64.1 ± 9.8 (56.2 – 78.7)	68.3 ± 10.3 (56.3 – 84.9)	64.5 ± 9.1 (56.1 – 76.7)	64.3 ± 4.0 (58.1 – 69.2)
c’	1.6 ± 0.1 (1.5 – 1.7)	1.6 ± 0.1 (1.6 – 1.7)	1.9 ± 0.3 (1.7 – 2.3)	2.1 ± 0.4 (1.7 – 2.6)	1.2 ± 0.1 (1.0 – 1.3)	1.1 ± 0.1 (1.0 – 1.4)	1.1 ± 0.1 (0.9 – 1.2)	1.1 ± 0.2 (0.9 – 1.3)	1.1 ± 0.1 (1.0 – 1.2)	1.1 ± 0.1 (1.0 – 1.3)
V	27.6 ± 0.4 (27.0 – 28.0)	26.9 ± 0.8 (26.0 – 27.8)	26.0 ± 1.6 (23.3 – 27.2)	26.8 ± 0.8 (26.0 – 28.0)	27.3 ± 1.0 (26.0 – 29.0)	51.8 ± 1.7 (50.3 – 54.5)	52.4 ± 1.5 (50.9 – 54.7)	51.9 ± 1.1 (50.6 – 53.2)	52.5 ± 1.6 (50.8 – 54.5)	52.7 ± 0.9 (51.6 – 53.9)
Odontostyle	121.1 ± 4.6 (116.8 – 128.6)	120.6 ± 4.2 (116.9 – 127.7)	114.0 ± 3.1 (109.0 – 117.2)	115.2 ± 3.7 (110.3 – 119.0)	123.8 ± 4.9 (116.0 – 132.0)	89.0 ± 1.0 (87.9 – 90.2)	93.1 ± 3.0 (89.8 – 95.9)	94.8 ± 7.5 (82.7 – 107.6)	89.0 ± 2.4 (85.9 – 92.3)	92.5 ± 4.0 (88.5 – 97.2)

Odontophore	66.9 ± 3.5 (63.8 – 71.3)	65.1 ± 0.7 (64.2 – 66.2)	70.3 ± 1.0 (68.9 – 71.4)	64.3 ± 4.9 (60.0 – 70.0)	69.5 ± 4.8 (65.0 – 80.0)	50.4 ± 1.5 (48.9 – 52.3)	52.0 ± 1.7 (49.7 – 53.8)	53.2 ± 2.4 (49.6 – 58.0)	51.0 ± 1.6 (48.9 – 53.2)	51.1 ± 2.7 (48.7 – 55.6)
Onchiotyle	187.9 ± 4.6 (181.8 – 192.9)	185.7 ± 4.2 (182.2 – 192.9)	184.3 ± 3.9 (177.9 – 188.2)	179.5 ± 5.9 (172.7 – 189.0)	193.3 ± 9.3 (182.0 – 212.0)	139.5 ± 2.3 (136.9 – 142.5)	145.0 ± 4.6 (139.8 – 149.6)	142.4 ± 7.0 (132.3 – 150.3)	140.0 ± 3.4 (136.8 – 145.5)	143.7 ± 6.2 (137.2 – 152.8)
Tail	46.5 ± 7.2 (40.2 – 55.9)	46.6 ± 2.1 (44.0 – 49.0)	52.7 ± 6.8 (42.9 – 59.3)	50.8 ± 5.9 (44.2 – 57.9)	39.7 ± 8.9 (26.9 – 51.0)	28.6 ± 2.2 (26.0 – 31.9)	28.7 ± 3.8 (24.2 – 32.6)	27.8 ± 3.2 (23.9 – 33.2)	28.0 ± 2.8 (25.1 – 31.5)	28.2 ± 2.5 (26.3 – 32.6)
Width at lip	11.0 ± 0.8 (10.0 – 12.0)	10.9 ± 0.6 (10.0 – 11.5)	10.5 ± 1.0 (9.7 – 12.0)	10.5 ± 0.5 (10.0 – 11.0)	11.2 ± 0.9 (10.0 – 12.6)	10.6 ± 0.4 (10.0 – 11.0)	10.6 ± 0.4 (10.0 – 11.0)	10.6 ± 0.4 (10.0 – 11.2)	10.6 ± 0.4 (10.0 – 11.0)	10.6 ± 0.4 (10.0 – 11.0)
Width at mid. body	50.1 ± 4.6 (44.3 – 55.0)	47.9 ± 4.8 (43.8 – 56.1)	45.2 ± 1.3 (44.0 – 46.7)	43.6 ± 0.9 (42.1 – 44.4)	43.0 ± 8.7 (32.2 – 60.0)	39.7 ± 2.4 (36.2 – 42.0)	39.7 ± 1.3 (38.0 – 41.5)	40.5 ± 6.0 (34.9 – 55.2)	38.4 ± 3.7 (33.7 – 43.2)	40.5 ± 1.5 (38.9 – 42.9)
Width at anus	28.6 ± 3.2 (25.3 – 32.9)	28.8 ± 1.1 (27.4 – 30.0)	27.3 ± 3.4 (23.8 – 32.4)	24.8 ± 3.0 (22.1 – 29.6)	33.8 ± 8.3 (23.4 – 46.7)	25.2 ± 0.5 (24.5 – 25.7)	25.4 ± 1.2 (23.5 – 26.4)	25.4 ± 1.7 (23.2 – 27.9)	25.6 ± 0.8 (24.6 – 26.5)	25.3 ± 3.1 (22.3 – 29.5)
Pharynx	355.9 ± 25.2 (332.0 – 386.7)	367.3 ± 21.0 (339.3 – 388.8)	350.1 ± 16.8 (329.3 – 372.4)	360.3 ± 33.8 (312.1 – 401.6)	301.5 ± 65.2 (205.2 – 375.0)	264.1 ± 22.6 (238.3 – 300.5)	273.5 ± 28.6 (239.7 – 304.0)	280.6 ± 24.1 (242.4 – 322.8)	271.3 ± 29.7 (241.0 – 304.0)	283.8 ± 31.9 (247.5 – 333.3)
Oral aperture to ring guide	101.5 ± 3.4 (98.0 – 106.7)	102.5 ± 4.8 (97.3 – 108.2)	94.1 ± 5.7 (85.5 – 100.0)	97.5 ± 1.3 (95.9 – 99.3)	115.1 ± 3.7 (109.2 – 120.0)	74.6 ± 2.7 (72.7 – 79.4)	78.3 ± 4.8 (72.0 – 84.5)	79.7 ± 4.2 (71.6 – 86.9)	73.8 ± 2.6 (70.9 – 76.7)	74.7 ± 8.0 (69.3 – 88.9)

Male: Not found.
The morphology and morphometrics of four studied populations (SZX1301 – SZX1304) of *X. hunaniense* agreed with the description of type populations except for a lower a (39.7 – 52.7 *vs* 51.0 – 57.0) and c (37.1 – 51.6 *vs* 53.0 – 63.0) values (Wang & Wu, 1992). A revised polytomous key code sensu Loof & Luc (1990) for *X. hunaniense* identification is: A1-B4-C4-D4-E1-F2-G2-H2-I3-J4-K2-L1.

Xiphinema brasiliense Lordello, 1951
(Fig. 2, Tables 1 & 2)
Female: Body 1108-2100 µm long, cylindrical, straight or ventrally arcuate to form an open "C" shape when heat-killed. Lip region continuous with the rest of the body or offset from body profile by a depression. Stylet 182 – 212 µm long. Guide ring 109 – 120 µm from anterior end. Reproductive system monodelphic, with an anterior reflexed gonad. Vulva anteriorly located at 26 % – 29 % of total body length, vagina 1/3 to 1/2 body diam. long, posteriorly obliquely bent. Tail broadly conoid ending with a well-developed axial peg, 8 – 12 µm long. Four caudal pores present on each side of tail.
Male: Not found.
Xiphinema brasiliense population (SZX1305) agreed with type (Lordello, 1951) and other populations (Cordero, 2003), except for a higher b and c value (5.50 *vs* 5.05 and 41.60 *vs* 24.55) (Lordello, 1951), but lie within the ranges of those reported by Cordero (2003). A revised polytomous key code sensu Loof and Luc (1990) for *X. brasiliense* identification is: A1-B4-C5-D5-E1-F2-G2(3)-H1(2)-I3-J5-K?-L1.

Xiphinema americanum s.l. Cobb, 1913
(Fig. 3, Tables 1 & 2)
Female: Body 1644 – 2110 µm in length, cylindrical, tapering gradually towards the anterior extremity, and more abruptly posteriorly

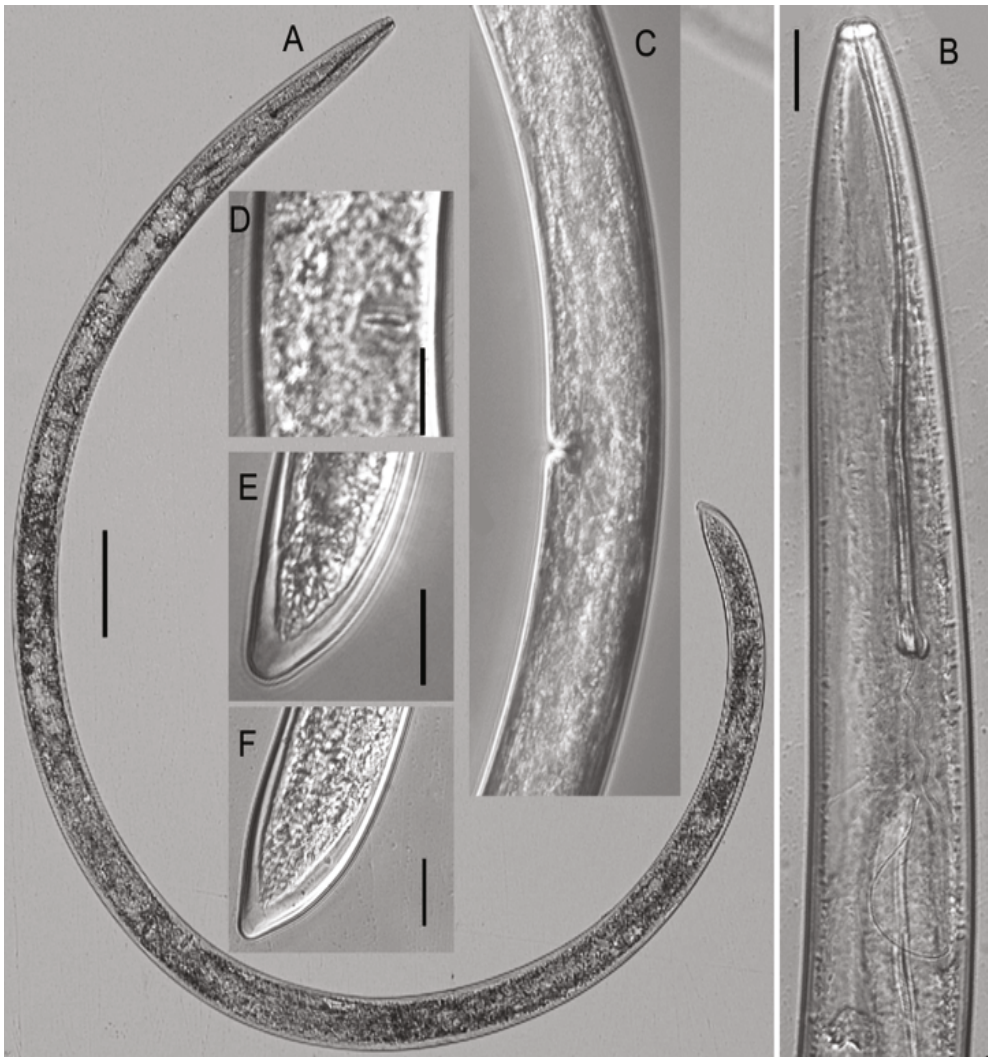

Fig. 3. Light micrographs of female *Xiphinema americanum s.l.* from *Acacia mangium*. A: Entire body; B: Anterior body; C: Reproductive system; D: Vulva (in ventral view); E: Tail in lateral view; F: Tail in ventral view. Scale bars: A = 100 µm; B – F = 20 µm

in the tail region, ventrally arcuate to form a closed "C" shape when heat-killed. Cuticle finely transversely striated. Lip region broadly rounded, set off from the rest of body by a depression, 10 – 11 µm in diam. Amphids large, stirrup-shaped, with wide aperture, as a straight transverse slit. Stylet 132 – 153 µm long, basal flanges 7.5 – 9.5 µm wide. Guiding ring 69 – 89 µm from anterior end. Pharynx dorylaimoid with the anterior part tubular, bearing a strongly refringent mucro at 48 – 54 µm from the base of the odontophore, pharyngeal basal bulb containing three nuclei, nucleus of dorsal pharyngeal gland located at 25 % of the length from the beginning of basal bulb, and two ventro-sublateral nuclei situated at 50 % of the bulb. Oesophago-intestinal valve heart-shaped. Reproductive system amphidelphic with reflexed branches about equally developed. Vulva slit-like, situated in mid-body region. Vagina 11 – 15 µm in length, occupying about 1/3 of the corresponding body diam., pars proximalis vaginae 5 – 6 µm long, pars distalis vaginae 7 – 10 µm long. Uteri long, 35 – 45 µm, not clearly separated from the oviduct, without spermatheca. Rectum length 1/2 of the body diam. at anus. Tail short conoid, with rounded terminus and four lateral pores.

five studied populations were classified as *X. americanum s.l.* due to limited diagnostic morphological characters and DNA data.

The codes of the Chinese *Xiphinema americanum s.l.* populations and close species using both *X. americanum*-group polytomous identification keys (Lamberti *et al*., 2000; Lamberti *et al*., 2004) were presented in Tables 3,4. The codes of the Chinese *X. americanum s.l.* populations are: A2, B2, C2, D1, E1(2), F1, G2, H2(3), I2, J2 sensu Lamberti *et al*. (2000) and A3(4), B2, C2, D2, E2, F1, G2, H2, I2 sensu Lamberti *et al*. (2004). Further, after sorting the codes with other data in the keys the Chinese populations showed identical numbers to some other species in *X. americanum*-group.

Molecular Phylogenetic Relationships

The 18S rDNA (633 – 796 bp) and 28S D2–D3 expansion segment (773 – 860 bp) were amplified and sequenced. Sequences of the rDNA were compared using blastN search from a diverse collection of *Xiphinema* species from GenBank and were used to construct phylogenetic trees with highest match sequences.

The alignment for the partial 18S rDNA included 67 sequences. Four studied populations (KP793036 – KP793039) of *X.*

Table 3. The codes of the Chinese *Xiphinema americanum s.l.* populations and the close species in *X. americanum*-group sensu Lamberti *et al*. (2000)

Species/Codes	A	J	C	H	B	D	E	F	G	I
X. americanum s.l.	2	2	2	2(3)	2	1	1(2)	1	2	2
X. taylori	2	2	2	3(2)	3(2)	1	1	1(2)	2	2(1)
X. diffusum	2	2	2(1)	2(1)	2	1	1	1	2	1(2)
X. incognitum	2	2	2(1)	2(3)	2(3)	1(2)	1	1	2(1)	2
X. brevicolle	2	2	3(2)	3	3(2)	1	2(3) (1)	1	2	2(1)
X. parabrevicolle	2	2	3(2)	3	3(2)	1	2(1)	1	2	1(2)

Male: Not found.

A morphometric analysis of five studied populations of *X. americanum s.l.* (SZX1306 – SZX1310) revealed that specimens from these populations differed from Cobb's paratypes of the *X. americanum* by having a longer body (1644 – 2106 *vs* 1400 – 1500 µm), a lower a value (38 – 50 *vs* 50 – 57), a higher c value (56 – 85 *vs* 45 – 54), a lower c' value (0.9 – 1.4 *vs* 1.7 – 2.0), a longer odontostyle (83 – 108 *vs* 65 – 73 µm), a longer odontophore (49 – 58 *vs* 41 – 46 µm) and a more posterior guide ring (71 – 89 *vs* 51 – 55 µm) (Lamberti & Golden, 1984), from *X. incognitum* by having a smaller lip (10.0 – 11.2 *vs* 11.0 – 13.0 µm), from *X. diffusum* by having a smaller lip (10.0 – 11.2 *vs* 11.0 – 13.0 µm), a more posterior guide ring (69.3 – 88.9 *vs* 60.0 – 64.0 µm), from *X. taylori* by having a shorter (1644 – 2106 *vs* 2100 – 2500 µm), a higher c' value (0.9 – 1.4 *vs* 0.7 – 0.8) and a longer tail (23.9 – 33.2 *vs* 19.5 – 22.0 µm), from *X. parabrevicolle* by having a higher c' value (0.9 – 1.4 *vs* 0.7 – 0.8), a smaller lip (10.0 – 11.2 *vs* 12.5 – 14.0 µm) (Gutiérrez-Gutiérrez *et al*. 2012), from *X. brevicolle* by having a shorter stylet (137 – 143 *vs* 144 – 173 µm) (Lordello & Costa 1961; Lamberti *et al*. 1991; Luc *et al*. 1998), a lower b value (5.3 – 7.7 *vs* 7.0 – 10.5) (Lordello & Costa 1961). Nevertheless, these *hunaniense* from China had 100 % identities (647/647=100 %) based on alignments of the sequences of 18S rDNA. No 18S sequence of *X. hunaniense* was available for comparison in GenBank. Alignment of the 18S sequences from the studied population (KP793040) of *X. brasiliense* from China with one Brazil population of *X. brasiliense* (AY297836) from GenBank revealed 99 % identity (688/694=99 %). The sequences of 18S rDNA from five studied populations (KP793041 – KP793045) of *X. americanum s.l.* from China shared 100 % identities (633/633=100 %). These sequences are also identical to four other populations from Brazil (AY297822), Czech Republic (HM163212), Belgium (AY580057) and Japan (AB604340). However, the sequenced 18S fragments are only 633 – 796 bp without sufficient divergent sites to examine the phyloge-

Table 4. The codes of the Chinese *Xiphinema americanum s.l.* populations and *X. incognitum* and *X. brevicolle* sensu Lamberti *et al*. (2004)

Species/Codes	G	H	A	C	B	D	E	F	I
X. americanum s.l.	2	2	3(4)	2	2	2	2	1	2
X. incognitum	2	2	3	2	2	2	2	1	2
X. brevicolle	2	2	5	1	2	23	23	1	12

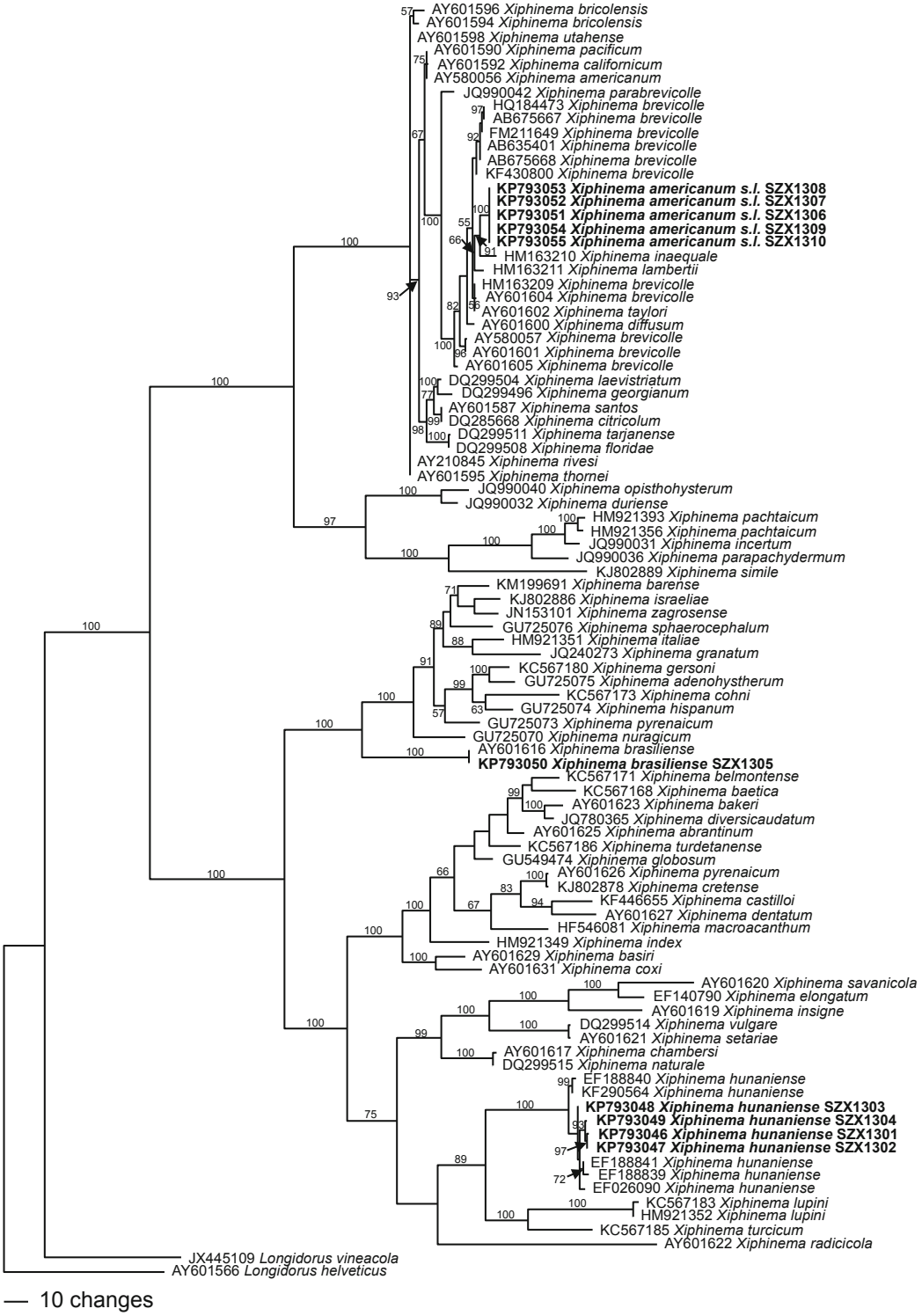

Fig. 4. The 10001st Bayesian tree inferred from *Xiphinema* spp. 28S D2-D3 under GTR+I+G model (-lnL=10448.2939; AIC=20916.5879; freqA=0.2519; freqC=0.2207; freqG=0.3013; freqT=0.2261; R(a)=0.9498; R(b)=2.5514; R(c)=2.4833; R(d)=0.5243; R(e)=4.4187; R(f)=1; Pinva=0.3354; Shape=0.8037). Posterior probability values exceeding 50% are given on appropriate clades

netic relationships among dagger species, no significant clades were generated with strong support, thus the 18S tree is presented. The alignment for the D2–D3 of 28S rDNA included 93 sequences. The sequences of 28S rDNA from four studied populations (KP793046 – KP793049) of *X. hunaniense* shared 99 % identities with 2 nucleotide differences. The alignment of the 28S sequences from these four studied populations with other five populations of *X. hunaniense* (EF026090, EF188839, EF188840, EF188841, KF290564) from GenBank revealed 98 % – 99 % identities with 6 – 14 nucleotide differences. The blastn search of the 28S sequence of the Chinese population (KP793050) of *X. brasiliense* revealed a 99 % match (763/766=99 %, 3 nucleotide differences) with one Brazilian population of *X. brasiliense* (AY601616) from GenBank. Five studied populations (KP793051 – KP793055) of *X. americanum s.l.* shared 99 % – 100 % identities with 0 – 9 nucleotide differences. The alignment of the 28S sequences from these five studied populations with 11 populations of *X. brevicolle* (AB635401, AB675667, AB675668, AY580057, AY601601, AY601604, AY601605, FM211649, HM163209, HQ184473, KF430800) and some other species such as *X. lambertii* (HM163211)*, X. diffusum* (AY601600)*, X. taylori* (AY601602), etc. from GenBank revealed 98 % – 99 % identities with 10 – 20 nucleotide differences.

The phylogenetic tree inferred from D2–D3 of the 28S rDNA (Fig. 4) using *Longidorus vineacola* Ye & Robbins, 2003 and *L. helveticus* Lamberti, Kunz, Grunder, Molinari, De Luca, Agrostinelli & Radicci, 2001 as outgroups suggested that: *i*) all the selected xiphinematids are in a monophyletic clade in relation to *L. vineacola* with 100 % pp; *ii*) two distinct clades of *Xiphinema* species are highly supported (pp=100 %)*,* representing *X. americanum*-group and non-*X. americanum*-group. In *X. americanum*-group, seven populations (HM921393, HM921356, KJ802889, JQ990031, JQ990032, JQ990036, JQ990040) are in a clade and 35 other populations including five studied ones of *X. americanum s.l.* (KP793051 – KP793055) are in another clade with 100 % support; *iii*) five studied populations of *X. americanum s.l.* are in a monophyletic clade with 100 % support, and are in a highly-supported (pp=100 %) monophyletic clade with 16 other populations of *Xiphinema*, and they are sister to *X. parabrevicolle* (JQ990042); *iv*) four studied populations of *X. hunaniense* (KP793046 – KP793049) and the population of *X. brasiliense* (KP793050) are clustered with other members of non-*X. americanum*-group, and these two species are in two separate clades; *v*) four studied populations of *X. hunaniense* are in a highly-supported (pp=100 %) monophyletic clade with five other populations of *X. hunaniense* (EF026090, EF188839, EF188840, EF188841, KF290564) from GenBank, and they are sister to *X. lupini* (KC567183, HM921352) and *X. turcicum* (KC567185); *v*) the studied population *X. brasiliense* (KP793050) is in a highly-supported (pp=100 %) clade with the Brazilian population of *X. brasiliense* (AY297836), and they are in a highly-supported (pp=100 %) clade with 12 other species (*X. nuragicum, X. pyrenaicum, X. hispanum, X. adenohystherum, X. sphaerocephalum, X. italiae, X. zagrosense, X. granatum, X. cohni, X. gersoni, X. israeliae, X. barense*) from GenBank.

Discussion

Species identification on *Xiphinema americanum*-group is difficult or even impossible due to conservative and overlapping morphological and morphometric characters. This group may contain many cryptic species that are morphologically indistinguishable but may be phylogenetically distant to one another (Gutiérrez-Gutiérrez *et al*. 2010, 2012; Barsi & De Luca 2008; Oliveira *et al*. 2005, 2006; Wu *et al*. 2007; Ye *et al*. 2004). In the present study, analysis of morphology and morphometrics indicated that five studied populations (SZX1306 – SZX1310) were very similar to some members belonging to *X. americanum*-group. The codes of these five populations using polytomous identification keys revealed identical numbers to some other species in this group such as *X. incognitum* (Tables 3,4). Molecular analysis based on D2–D3 of 28S rDNA sequences revealed that these five studied populations and other members within *X. americanum*-group such as *X. brevicolle* show high similarity. Thus, these five studied populations were classified as *X. americanum s.l.*. Compared with *X. incognitum* from Fujian (Wu 2007) and Japan (Shishida 1983)*,* a closest species to the Chinese *X. americanum s.l.*, no obvious morphometrics difference was found. The identification codes of these two species revealed identical numbers. However, 28S rDNA sequence alignment of five populations of the Chinese *X. americanum s.l.* with an American population (AY601597) of *X. incognitum* in GenBank revealed 96 % identity with 27 nucleotide differences. In addition to D2–D3 of 28S rDNA, other molecular markers, such as ITS-rRNA and the protein-coding mitochondrial gene, cytochrome oxidase c subunit I (COI) were successfully used for diagnosis and reconstruction of phylogenetic relationships within some species of *X. americanum*-group (Gutiérrez-Gutiérrez *et al*. 2012; Lazarova *et al*., 2006). In the future, sequencing these markers on five studied populations of *X. americanum s. l.* will help to characterize this species and investigate its phylogenetic relationship with other sequenced dagger species.

Morphological intraspecific variation in dagger nematodes from different geographic locations is common (Tarjan, 1969; Brown & Topham, 1985; Cho & Robbins, 1991). In the present study, no obvious differences were found in morphological and morphometric characters amongst four populations of *X. hunaniense* (SZX1301 – SZX1304) and amongst five studied populations of *X. americanum s.l.* from two sites and five plants. This is largely due to the sampling being from the same sites, the same city or the same hosts; for example, two populations (SZX1301 & SXZ1302) of *X. hunaniense* were from the same host (*B. orientale*) in different spots of the same site (Tianxinshan), so were *X. americanum s.l.* populations, SZX1307 and SXZ1308 (Table 1). Compared with other populations, most of the morphological characteristics from populations of *X. hunaniense* fit within the ranges of previous reports (Wang & Wu, 1992; Robbins & Wang, 1998; Zheng & Brown, 1999). The minor differences were only present in a few characters such as a

and c values. However, the molecular data confirmed identity of this species. Morphometrics of the studied *X. brasiliense* population agreed with those of type population except for b and c values, but the analysis of the 28S D2–D3 rDNA sequences revealed their identity. Therefore, all these morphological differences amongst populations were considered as intraspecific variation.

Xiphinema hunaniense and *X. brasiliense* belong to *X. radicicola*-group. They are difficult to separate in morphology both possessing a relatively short body size, an anteriorly situated vulva and a simple posterior uterus lacking a "Z" organ or other ornamentation, but can be differentiated by sequence of 28S D2–D3 expansion region (Wu *et al*., 2007). In this study, a comparison of the ranges of the morphometrics of females from *X. hunaniense* and *X. brasiliense* showed that most characters are partialy overlapping except for a lower c' value (1.0 – 1.3 *vs* 1.5 – 2.6) and a more posterior guide ring (109 – 120 *vs* 86 – 108 µm) of *X. brasiliense* (Table 1). However, analysis of molecular data (28S D2–D3 rDNA) of these two species indicated their identify being only 89 % – 90 % (110 – 112 nucleotide differences). Molecular phylogenetic analysis based on sequences of the 28S D2–D3 expansion region revealed that they are in separate clades (Fig. 4). *Xiphinema hunaniense* was once considered as a junior synonym of *X. radicicola* (Loof *et al*., 1996), but it was re-established as a valid species by Robbins & Wang (1998) and Zheng & Brown (1999). Alignment of sequences of the 28S D2–D3 expansion region from *X. hunaniense* and a Vietnam population of *X. radicicola* indicated that they have 83 % identity with 153 nucleotide differences. Molecular phylogenetic analysis based on sequences of the 28S D2–D3 revealed that they are clearly different species (Fig. 4). Thus these three species are all valid species and can be differentiated by molecular data of 28S D2–D3 rDNA.

Xiphinema brasiliense has been found in Brazil (Lordello, 1951), Guatemala, Ceylon and Nigeria (Cohn & Sher, 1972), the Ivory Coast and Australia (Luc, 1981), Peru (Alkemade & Loof, 1990), India (Loof *et al*., 2001), Venezuela (Cordero, 2003), Taiwan (Ni *et al*., 2003; Chen *et al*., 2004), China (Wu, 2007). Liu *et al*. (1995) identified a population from *Sageretia theezans* Brongn in Guangdong as *X. brasiliense* only based on morphology, but Song *et al*. (1998) considered this population as *X. hunaniense* by morphological observation compared with a population of *X. hunaniense* from bonsai in Shanghai. In this study, a comparison of the ranges of the morphometrics of females from *S. theezans* in Guangdong (Liu *et al*., 1995) and our population of *X. brasiliense* from *Gleichenia linearis* in Shenzhen, Guangdong revealed that almost all characters of both populations are very similar except for a more anteriorly-located guide ring from the *S. theezans* population (94 – 108 *vs* 109 – 120 µm) (Table 2). Sequence analysis of the 28S D2–D3 supported species identity of our population. Therefore, it is necessary to identify species of nematodes by combination of morphological and molecular data. So far, *X. brasiliense* has been reported from various hosts including *Solanum tuberosum* L., *Litchi chinensis, Citrus* sp. L., *Mangifera indica* L., *Persea americana* Mill., *Musa* sp. L., *Saccharum officinarum* L., *Sorghum bicolor* L., *Andropogum bicornis* L., *Clidermia hirta* L. D. Don., *Prunus persica* L., *Euterpes edulis* Mart., and *Butia capitata* (Mart.) Becc. (Alkemade & Loof, 1990; Cordero, 2003; Diaz-Silveira & Herrera, 1998; Lordello, 1951; Oliveira *et al*., 2003; Wu, 2007). *Gleichenia linearis* is a new host record for *X. brasiliense.*

Xiphinema americanum s.l. and *X. hunaniense* have been reported from China. *Xiphinema_americanum s.l.* is reported from Jiangsu, Zhejiang, Hunan, Guangxi, Shandong, Hebei and Inner Mongolia, Sichuan, Yunnan (CABI, 2011; Wang & Wu, 1992; Xu *et al*., 1995). *Xiphinema hunaniense* is reported from Hunan, Zhejiang, Guangxi, Fujian, and Shanghai (Pan *et al*., 2000; Wang *et al*., 1996; Wang & Wu, 1992; Xu *et al*., 1995; Wu *et al*., 2007; Song *et al*., 1998). Our study added new records to the list. These two species are associated with various plants. *Xiphinema hunaniense* has been reported from *Camellia japonica* L., *Citrus grandis* (L.) Osbeck, *Citrus sinensis* (L.) Osbeck, *Cycas revolute* L., *Euphoria longana* Lam., *Eriobotrya japonica* Lindl., *Vitis vinifera* L., *Hibiscus rosasinensis* L., *Litchi chinensis, Mangifera indica, Pyrus pyrifolia* var. *yokoyama, Pinus* sp. L., *Prunus* sp. L., *Buxus sinica* L., *Camellia sasanpua* L., *Ligustrum quihoui* L., *L. lucidum* L. and *Juniperus chinensis* L. (Chen *et al*., 2004; Long *et al*., 2014; Wu *et al*., 2007; Zheng *et al*., 1999) and *X. americanum s.l*. from agricultural, horticultural and forest soils, including *Agropyron cristatum* Gaertn., *Amygdalus persica* L., *Castanea mollissima* Bl., *Citrus aurantium* L., *Coffea arabica* L., *Crataegus* sp. L., *Cynodon dactylon* Pers., *Daucus carota* L., *Diospyros kaki* Thunb., *Eucalyptus tereticornis* Sm., *Fragaria* sp. L., *Ilex crenata* Thunb., *Juglans regia* L., *Ligustrum* sp. L., *Litchi chinensis, Malus* sp. Mill., *Persea americana*, *Podocarpus macrophyllus* L., *Pyrus* sp. L., *Rosa indica* L., *Urtica urens* L., *Vitis* sp. L., *Zea mays* L. (Brodie *et al*., 1969; Cohn, 1969; Cohn & Mordechai, 1969; Cohn & Orion, 1970; Cho *et al*., 1991; Goodey *et al*., 1965; Griffin *et al*., 1996; Morton, 1987; Norton & Varon De Agudelo, 1984; Sakai *et al*., 2011; Siddiqui *et al*., 1973; Wang *et al*., 1992; Zhao *et al*., 2012). To our knowledge, this is the first report of *X. hunaniense* on *B. orientale* and *G. linearis* and *X. americanum s.l.* on *A. mangium, A. confuse* and *B. orientale.*

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References

Alkemade, J. R. M., Loof, P. A. A. (1990): The genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) in Peru. *Rev. Nématol.,* 13: 339 – 348

Barsi, L., De Luca, F. (2008): Morphological and molecular characterization of two putative *Xiphinema americanum*-group species, *X. parasimile* and *X. simile* (Nematoda: Dorylaimida) from Serbia. *Nematology*, 10: 15 – 25

Brodie, B. B., Good, J. M., Adams, W. E. (1969): Population dynamics of plant nematodes in cultivated soil: effect of sod-based rotations in cecil sandy loam. *J. Nematol.*, 1: 309 – 312

Brown, D. J. F., Boag, B. (1988): An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. *Nematol. Mediterr.,* 16: 93 – 99

Brown, D. J. F., Topham, P. B. (1985): Morphometric variability between populations of *Xiphinema diversicaudatum* (Nematoda: Dorylaimoidea). *Rev. Nematol.,* 8: 15 – 26

CABI. (2011): *Xiphinema brevicollum* Lordello & Da Costa. [Distribution map]. Distribution Maps of Plant Diseases (October) (1st Edition), Map 1119

Chen, D., Ni, H., Yen, J., Cheng, Y., Tsay, T. (2004): Identification and variation of *Xiphinema hunaniense* populations from Taiwan. *Plant Pathol. Bull.*, 13: 155 – 166

Cho, M. R., Robbins, R. T. (1991): Morphological variation among 23 *Xiphmema americanum* populations. *J. Nematol.,* 23: 134 – 144

Cohn, E. (1969): The occurrence and distribution of species of *Xiphinema* and *Longidorus* in Israel. *Nematologica,* 15: 179 – 192

Cohn, E., Orion, D. (1970): The pathological effect of representative *Xiphinema* and *Longidorus* species on selected host plants. *Nematologica,* 16: 423 – 428

Cohn, E., Mordechai, M. (1969): Investigations on the life cycles and host preference of some species of *Xiphinema* and *Longidorus* under controlled conditions. *Nematologica,* 15: 295 – 302

Cohn, E., Sher, S. A. (1970): A contribution to the taxonomy of the genus *Xiphinema* COBB, 1913. *J. Nematol.*, 4: 36 – 65

Coomans, A. (1996): Phylogeny of the Longidoridae. *Russ. J. Nematol.,* 4: 51 – 60

Coomans, A., Huys, R., Heyns, J., Luc, M. (2001): Character analysis, phylogeny, and biogeography of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae). *Annales du Musée Royal de l'Afrique Centrale (Zoologie), Tervuren, Belgique,* 287: 1 – 239

Cordero, M. (2003): Morphometrics of a population of *Xiphinema brasiliense* (Nematoda, Dorylaimida) from Tachira, Venezuela. *Nematol. Mediterr.,*31: 39 – 41

Decraemer, W., Robbins, R. T. (2007): The who, what and where of Longidoridae and Trichodoridae. *J. Nematol.,* 39: 295 – 297

Diaz-Silveira, M. F., Herrera, J. O. (1998): An overview of nematological problems in Cuba. *Nematropica,* 28: 151 – 163

Golden, A. M. (1990): Preparation and mounting nematodes for microscopic observation. In: Zuckerman, B. M., Mai, W. F., Krusberg, L. R. (Eds) *Plant Nematology Laboratory Manual*. Revised Edition, Amherst, MA, USA: University of Massachusetts Agricultural Experiment Station, pp. 197 – 205

Golden, A. M., Birchfield, W. (1972): *Heterodera graminophila* n. sp. (Nematoda: Heteroderidae) from grass with key to closely related species. *J. Nematol.,* 4: 147 – 154

Goodey, J. B., Franklin, M. T., Hooper, D. J. (1965): *The Nematode Parasites of Plants Catalogued Under Their Hosts*. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England. 3th Edition, 214pp.

Griffin, G. D., Asay, K. H., Horton, W. H. (1996): Factors affecting population trends of nematodes on rangeland grasses. *J. Nematol.*, 28: 107 – 114

Gutiérrez-Gutiérrez, C., Palomares-Rius, J., Cantalapiedra-Navarrete, C., Landa, B. B., Esmenjaud, D., Castillo, P. (2010): Molecular analysis and comparative morphology to resolve a complex of cryptic *Xiphinema* species. *Zool. Scr.*, 39: 483 – 498. DOI: 10.1111/j.1463-6409.2010.00437.x

Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Decraemer, W., Vovlas, N., Prior, T., Palomares-Rius, J., Castillo, P. (2012): Phylogeny, diversity, and species delimitation in some species of the *Xiphinema americanum*-group complex (Nematoda: Longidoridae), as inferred from nuclear and mitochondrial DNA sequences and morphology. *Eur. J. Plant Pathol.,* 134: 561 – 597

Gutiérrez-GutiéRrez, C., Cantalapiedra-Navarrete, C., Remesal, E., Palomares-Rius, J. E., Navas-CortéS, J. A., Castillo, P. (2013): New insight into the identification and molecular phylogeny of dagger nematodes of the genus *Xiphinema* (Nematoda: Longidoridae) with description of two new species. *Zool. J. Linn. Soc.,* 169: 548 – 579

He, Y., Subbotin, S. A., Rubtsova, T. V., Lamberti, F., Brown, D. J. F., Moens, M. (2005): A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology,* 7: 111 – 124

Hooper, D. J. (1970): Handling, fixing, staining and mounting nematodes. In: Southey, J. F. (Ed) *Laboratory Methods For Work With Plant And Soil Nematodes.* 5th Edition, London: Her Majesty's Stationery Office, pp. 39 – 54

Huelsenbeck, J. P., Ronquist, F. (2001): Mr Bayes: Bayesian inference of phylogenetic trees. *Bioinformatics,* 17: 1754 – 1755

Lamberti, F., Golden, A. M. (1984): Redescription of *Xiphinema americanum* Cobb, 1913 with comments on its morphometric variations. *J. Nematol.,*14: 204 – 206

Lamberti, F., Hockland, S., Agostinelli, A., Moens, M., Brown, D. J. F. (2004): The *Xiphinema americanum* group. 3. Keys to species identification. *Nematol. Mediterr.*, 32: 53 – 56

Lamberti, F., Molinari, S., Moens, M., Brown, D. J. F. (2000): The *Xiphinema americanum*-group. I. Putative species, their geographical occurrence and distribution, and regional polytomous identification keys for the group. *Russ. J. Nematol.,* 8: 65 – 84

Lamberti, F., Ciancio, A., Agostinelli, A., Coiro, M. I. (1991): Relationship between *Xiphinema brevicolle* and *X. diffusum* with a redescription of *X. brevicolle* and descriptions of three new species of *Xiphinema* (Nematoda: Dorylaimida). *Nematol. Mediterr.,* 19: 311 – 326

Larget, B., Simon, D. L. (1999): Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Mol. Biol. Evol.,* 16: 750 – 759

Lazaroa, S. S., Mallock, G., Oliveira, C. M. G., Hubschen, J., Neilson, R. (2006): Ribosomal and mitochondrial DNA analyses of

Xiphinema americanum-group populations. *J. Nematol.,* 38: 404 – 410

Liu, J., Wang, D., Chen, J., Ye, B., Huang, G. (1995): The new record of *Xiphinema brasiliense* for China and the observation of this nematode larvae. *Plant Quarant. (Shanghai),* 9: 327 – 330 (In Chinese)

Long, H., Ling, X., Li, Y., Zheng, Y. (2014): Molecular and morphological identification of *Xiphinema hunaniense* on the *Juniperus chinensis* imported from Thailand. *Plant Pathol. Quarant.,* 4: 92 – 98

Loof, P. A. A., Coomans, A., Baujard, P., Luc, M. (2001): On five species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) recently described from India. *Nematology,* 3: 277 – 283

Loof, P. A. A., Luc, M, Baujard, P. (1996): A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the *X. americanum*-group: Supplement 2. *Syst. Parasitol.,* 33: 23 – 29

Loof, P. A. A., Luc, M. (1990): A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the *X. americanum*-group. *Syst. Parasitol.,* 16: 35 – 66

Lordello, L. G. E., Costa C. P. (1961): A new nematode parasite of coffee roots in Brazil. *Rev. Bras. Biol.,* 21: 363 – 366

Lordello, L. G. E. (1951): *Xiphinema brasiliense*, nova espécie de nematóide do Brasil, parasita de *Solanum tuberosum* L. *Bragantia,* 11: 87 – 90

Luc, M. (1981): Observations on some *Xiphinema* species with the female anterior genital branch reduced or absent (Nematoda: Longidoridae). *Rev. Nématol.,* 4: 157– 167

Luc, M., Coomans, A., Loof, P. A. A., Baujard, P. (1998): The *Xiphinema americanum*-group (Nematoda: Longidoridae). 2. Observations on *Xiphinema brevicollum* Lordello & da Costa, 1961 and comments on the group. *Fund. Appl. Nematol.,* 21: 475 – 490

Luo, S., Zhangsun, D., He, P. (2001): Investigation of *Xiphinema* species around grape roots from four provinces of China. *Acta Horticu. Sin.,* 28: 451 – 452 (In Chinese)

Morton, J. (1987): Lychee. In: *Fruits of warm climates*. USA: Miami, FL, pp. 249 – 259

Mullin, P. G., Harris, T. S., Powers, T. O. (2005): Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. *Nematology, 7*: 59 – 79. DOI: 10.1163/1568541054192199

Ni, H. F., Cheng, Y. H., Chen, R. S., Tsay, T. T., Chen, D. Y. (2003): Discrimination of *Xiphinema* species from Taiwan by rDNA-RFLP analysis. *Plant Pathol. Bull.,* 12: 235 – 241

Norton, D. C., Varon De Agudelo, F. (1984): Plant-parasitic nematodes associated with maize in Cauca and Valle del Cauca, Colombia. *Plant Dis*., 68: 950 – 952

Nunn, G. B. (1992): *Nematode Molecular Evolution*. PhD dissertation. UK: University of Nottingham

Oliveira, C. M. G., Brown, D. J. F., Neilson, R., Monteiro, A. R., Ferraz, L. C. C. B., Lamberti, F. (2003): The occurrence and geographic distribution of *Xiphinema* and *Xiphidorus* species (Nematoda: Longidoridae) in Brazil. *Helminthologia,* 40: 41 – 54

Oliveira, C. M. G., Hübschen, J., Brown, D. J. F., Ferraz, L. C. C. B., Wright, F., Neilson, R. (2004): Phylogenetic relationships among *Xiphinema* and *Xiphidorus* nematode species from Brazil inferred from 18S rDNA sequences. *J. Nematol.,* 36: 153 – 159

Oliveira, C. G., Fenton, B., Malloch, G., Brown, D. J. F., Neilson, R. (2005): Development of species-specific primers for the ectoparasitic nematode species *Xiphinema brevicolle*, *X. diffusum*, *X. elongatum*, *X. ifacolum* and *X. longicaudatum* (Nematoda: Longidoridae) based on ribosomal DNA sequences. *Ann. Appl. Biol.,* 146: 281– 288

Oliveira, C. M. G., Ferraz, L. C. C. B., Neilson, R. (2006): *Xiphinema krugi*, species complex or complex of cryptic species? *J. Nematol.,* 38: 418 – 428

Pan, C. S., Zheng, J. W., Zhou, X. P., Neilson, R., Brown, D. J. F. (2000): Preliminary assessment of the occurrence of longidorid and trichodorid nematodes (Nematoda: Longidoridae and Trichodoridae) in Xiamen, Fujian Province, China. *Russ. J. Nematol.,* 8: 153 – 159

Posada, D., Criandall, K. A. (1998): Modeltest: testing the model of DNA substitution. *Bioinformatics,* 14: 817 – 818

Robbins, R. T., Wang, S. (1998): A comparison between *Xiphinema radicicola* and *X. hunaniense*. In *Program and abstracts of the 24th international symposium of the European Society of Nematologists*, 4 – 9th, August 1998, Dudee, Scotland, 100.

Sakai, H., Takeda, A., Mizukubo, T. (2011): First report of *Xiphinema brevicolle* Lordello et Costa,1961 (Nematoda: Longidoridae) in Japan. *ZooKeys*, 135: 21 – 40
doi: 10.3897/zookeys.135.1716

Shishida, Y. (1983): Studies on nematodes parasitic on woody plants. 2. Genus *Xiphinema* Cobb, 1913. *Japanese J. Nematol.*, 12: 1 – 14

Siddiqui, I. A., Sher, S. A., French, A. M. (1973): *Distribution of Plant Parasitic Nematodes in California*. State of California Department of Food and Agriculture, Division of Plant Industry, 324pp.

Song, S., Gao, H., Zhou, G. (1998): Plant parasitic nematodes of bonsai in Shanghai. I. *Xiphinema hunaniense* – new record in Shanghai. *Acta Agric. Shanghai,* 14: 73 – 75 (In Chinese)

Tarjan, A. C. (1969): Variation within *Xiphinema americanum* group (Nematoda: Longidoridae). *Nematologica,* 15: 241 – 252

Taylor, C. E., Brown, D. J. F. (1997): *Nematode Vectors of Plant Viruses*. Wallingford, UK: CAB International, 296pp.

Teng, W., Tan, J., Ye, J., Fang A. (2013): Aphelenchida and *Xiphinema* spp. from rhizosphere of ornamental trees in Nanjing, eastern China. *J. Beijing For. Univ.,* 35: 88 – 94 (In Chinese)

Wang, S., Wu, X., Zhang, C. (1992): Occurrence and distribution of *Xiphinema americanum* in orchard in Jiaodong peninsula, China. *Decidous Fruits*, 2: 23 – 24 (In Chinese)

Wang, S., Wu, X. (1992): Two species of *Xiphinema* Cobb, 1913 (Dorylaimida: Longidoridae) from around grape roots in China. *Acta Phytopathol. Sin.,* 22: 117 – 123 (In Chinese)

Wang, S., Chiu, W. F., Yu, C., Li, C., Robbins, R. T. (1996): The occurrence and geographical distribution of longidorid and trichodorid nematodes associated with vineyards and orchards in China. *Russ. J. Nematol.,* 4: 145 – 153

Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R., Waterston, R. H. (1992): A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics,* 131: 609 – 624

Wu, Y., Zheng, J., Robbins, R. T. (2007): Molecular Characterization of a *Xiphinema hunaniense* population with morphometric data of all four juvenile stages. *J. Nematol.,* 39: 37 – 42

Wu, Y. (2007): *Studies on morphology and molecular classification of major populations of the genus Xiphinema in China*. PhD dissertation, China: Zhejiang University (In Chinese)

Xu, J., Fu, P., Cheng, H. (1995): A taxonomic study on species of *Xiphinema* from seven provinces of China (Nematoda: Longidoridae). *J. Nanjing Agric. Univ.,* 18: 37 – 42

Ye, W. (1996): Applying Microsoft Works spread sheet in statistics for morphometric data of nematode identification. *Afro-Asian J. Nematol.,* 6: 203 – 211

Ye, W., Giblin-Davis, R. M., Braasch, H., Morris, K., Thomas, W. K. (2007): Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.,* 43: 1185 – 1197. DOI: 10.1016/j.ympev.2007.02006

Ye, W., Szalanski, A. L., Robbins, R. T. (2004): Phylogenetic relationships and genetic variation in *Longidorus* and *Xiphinema* species (Nematoda: Longidoridae) using ITS1 sequences of nuclear ribosomal DNA. *J. Nematol.,* 36: 14 – 19

Zheng, J., Brown, D. J. F. (1999): *Xiphinema insigne* LOOS, 1949 and *X. hunaniense* WANG & WU, 1992 from Hangzhou, China, and synonymization of *X. savaryi* Lamberti, Troccoli & Agostinelli, 1997 with *X. insigne*, and *X. siamense* Lamberti, Troccoli & Agostinelli, 1997 with *X. radicicola* Goodey, 1936 (Nematoda: Longidoridae). *Russ. J. Nematol.,* 7: 127 – 137