

RESEARCH

Open Access



# Morphological and molecular characterisation of *Helicotylenchus zengchengensis* n. sp. (Nematoda: Hoplolaimidae) from China, with a review on *Helicotylenchus* species in China

Yan Chen<sup>1</sup>, Yang Lu<sup>1</sup>, Hui Xie<sup>1\*</sup> and Chunling Xu<sup>1\*</sup>

## Abstract

*Helicotylenchus* comprises a significant group of plant-parasitic nematodes that primarily feed on plant roots. This study focuses on the description of a newly discovered species, *Helicotylenchus zengchengensis* n. sp., from China. The distinguishing characteristic of this species, as observed under a microscope, is the absence of head annulation. The stylet, measuring between 23.8 and 29.3  $\mu\text{m}$ , exhibits anteriorly flattened or indented stylet knobs. The elongated tail has a c value ranging from 15.8 to 22.6 and a c' value between 1.9 and 2.4. It bears 15 to 18 tail annuli, presenting a conical shape with projections. The phasmids are located at the 4th to 10th body annulations posterior to the anus, while no males were observed. Three phylogenetic trees were constructed to determine the evolutionary relationships using the 18S rRNA, ITS, and 28S D2-D3 expansion segments of the newly discovered species and related *Helicotylenchus* sequences obtained from the database. Based on the ITS sequences, a phylogenetic tree revealed that *H. zengchengensis* n. sp. clustered with *H. cuspicaudatus*. However, it was distinguished from *H. cuspicaudatus* when examining the 18S and 28S rRNA sequences and morphological characteristics. Additionally, comprehensive and diagnostic tables for all known species of *Helicotylenchus* in China are provided for reference.

**Keywords** Description, New species, Phylogenetic trees, Distribution in China

## Background

The genus *Helicotylenchus* Steiner, 1945 belongs to the family Hoplolaimidae Filipjev, 1934, the infraorder Tylenchomorpha De Ley and Blaxter, 2002, and the suborder

Tylenchina Thorne, 1949 (Decraemer and Hunt 2013). Nematodes in this genus are commonly known as spiral nematodes due to their spiral-shaped body in a relaxed and dead state, making them easily identifiable under a stereomicroscope (Crow 2013). There are over 230 valid species of *Helicotylenchus*, widely distributed worldwide and associated with many important crops (Uzma et al. 2015; Zameleh et al. 2020). Most species are ectoparasites, while a few are semi-endoparasites or occasional endoparasites. Some species, such as *H. pseudorobustus* (Steiner, 1914) Golden, 1956, *H. dihystra* (Cobb, 1896) Sher, 1961, and *H. multincinctus* (Cobb, 1893) Golden,

\*Correspondence:

Hui Xie

xiehui@scau.edu.cn

Chunling Xu

xuchunling@scau.edu.cn

<sup>1</sup> Research Center of Nematodes of Plant Quarantine/Guangdong Province Key Laboratory of Microbial Signals and Disease Control, Department of Plant Pathology, College of Plant Protection, South China Agricultural University, Guangzhou 510642, China



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

1956, can cause significant damage to crops (Subbotin et al. 2015; Rybarczyk-Mydlowska et al. 2019).

The morphological diagnostic characteristics of the genus *Helicotylenchus* include head shape, head annuli, stylet length, tail shape, and phasmid position relative to the anus. However, many species share similar diagnostic characteristics. Uzma et al. (2015) conducted a comprehensive study on the genus *Helicotylenchus*, providing diagnostic tables and line drawings for 224 species. Molecular identification and phylogenetic analyses based on conserved rRNA gene sequences have become powerful tools for species identification (Subbotin et al. 2011; Shokoohi et al. 2018; Rybarczyk-Mydlowska et al. 2019).

The first comprehensive work on the genus *Helicotylenchus* in China was conducted by Liu (2005), who provided important information on the morphology, localities, hosts, and references of 47 species in his book. Based on Liu's work, subsequent publications have revealed that 55 species have been recorded in China, with only *H. membranatus* Xie & Feng 1993 being the new species described from this country. In 2021, a new species of *Helicotylenchus* was discovered in soil samples collected from the bamboo rhizosphere in the Zengcheng District, Guangzhou, Guangdong Province, China. This study aimed to describe the new species morphologically and molecularly, analyze its phylogenetic relationship with other *Helicotylenchus* species based on rRNA gene sequences, and provide comprehensive and diagnostic tables for all *Helicotylenchus* species in China.

## Results

### *Helicotylenchus zengchengensis* n. sp.

Measurements of holotype female and 12 paratype females are listed in Table 1 and Figs. 1, 2, 3, 4.

#### Description:

Female. Body usually curved ventrally, forming spirals when killed by gentle heat, occasionally open 6-shaped. Body annuli 1.3–1.6  $\mu\text{m}$  wide at mid-body. The lateral field bearing four incisures with outer margins slightly crenate, with no areolation except on the cuticle in the oesophageal part and occasionally seen in the tail end; the two middle incisures were further apart than the two outer incisures and formed a U- or Y-shaped pattern in the tail end, about 1/5th–1/4th mid-body width in the middle body. Labial disc fused on ventral and dorsal sides without lateral lips, and sub-oval oral disc with oblong opening in *en face* view. The cephalic region was elevated and flattened anteriorly, continuous with the body. Head annulations were hardly seen under a light microscope (LM), but 1–2 annulations close to the first annulation of the body were seen under a scanning electron microscope (SEM). The cephalic framework is heavily

sclerotised. Stylet robust, 23.8–29.3  $\mu\text{m}$  long; stylet metenchium 12.2–14  $\mu\text{m}$  long, approximately half the length of stylet; stylet knobs rounded, anteriorly dented, or slightly flattened. Orifice of the dorsal oesophageal gland 9.5–13.4  $\mu\text{m}$  posterior to stylet base. Median bulb oval, 8.3–13.3  $\mu\text{m}$   $\times$  7–9.7  $\mu\text{m}$  with a prominent valvular apparatus. The oesophageal gland overlapped and wrapped over the anterior end of the intestine and the ventral side longest. Hemizonid located at 0–2 annuli anterior to the excretory pore. Excretory pore located above the level of the anterior part of oesophageal gland, 85.9–108  $\mu\text{m}$  apart from anterior end. Genital tract amphidelphic, outstretched, developing oocytes arranged in a single row, spermatheca subrounded. Vulva transverse, not protuberant, lateral vulval flaps absent, at 322.3–428.3  $\mu\text{m}$  apart from anterior end. Tail 27.8–33.5  $\mu\text{m}$  long, with 15–18 annuli, asymmetrical conical with variable ventral projections. Phasmids were rounded, located at 4–10th body striations or 5–14  $\mu\text{m}$  posterior to the anus.

Male. Not found.

### Type locality and habitat

The new species was recovered from the soil associated with bamboo in Zengcheng District, Guangzhou, Guangdong Province, China. (N23.449877°N, 113.847389°E).

### Etymology

The species name “zengchengensis” refers to the geological name where the new species was found.

### Type material

Holotype and 11 paratype females were deposited at the Laboratory of Plant Nematology, South China Agricultural University, Guangzhou, Guangdong Province, and two paratype females were deposited in National Parasitic Resources Center in Shanghai, China.

### Diagnose and relationships

*Helicotylenchus zengchengensis* n. sp. is characterized by the absence of head annulations under light microscopy (LM) and the presence of 1–2 annulations close to the first body annulation visible under scanning electron microscopy (SEM). The stylet length ranges from 23.8–29.3  $\mu\text{m}$  with anteriorly flattened or indented stylet knobs. The lateral field bears four incisures, with the inner two incisures positioned farther apart from the outer ones, and areolations present in the oesophageal region. The tail is long and asymmetrical conical with projections, consisting of 15–18 annuli. Phasmids are located at the 4–10th body annulations or at a distance of 5–14  $\mu\text{m}$  posterior to the anus. No males were found.

*Helicotylenchus zengchengensis* n. sp. can be readily distinguished from most species in the genus

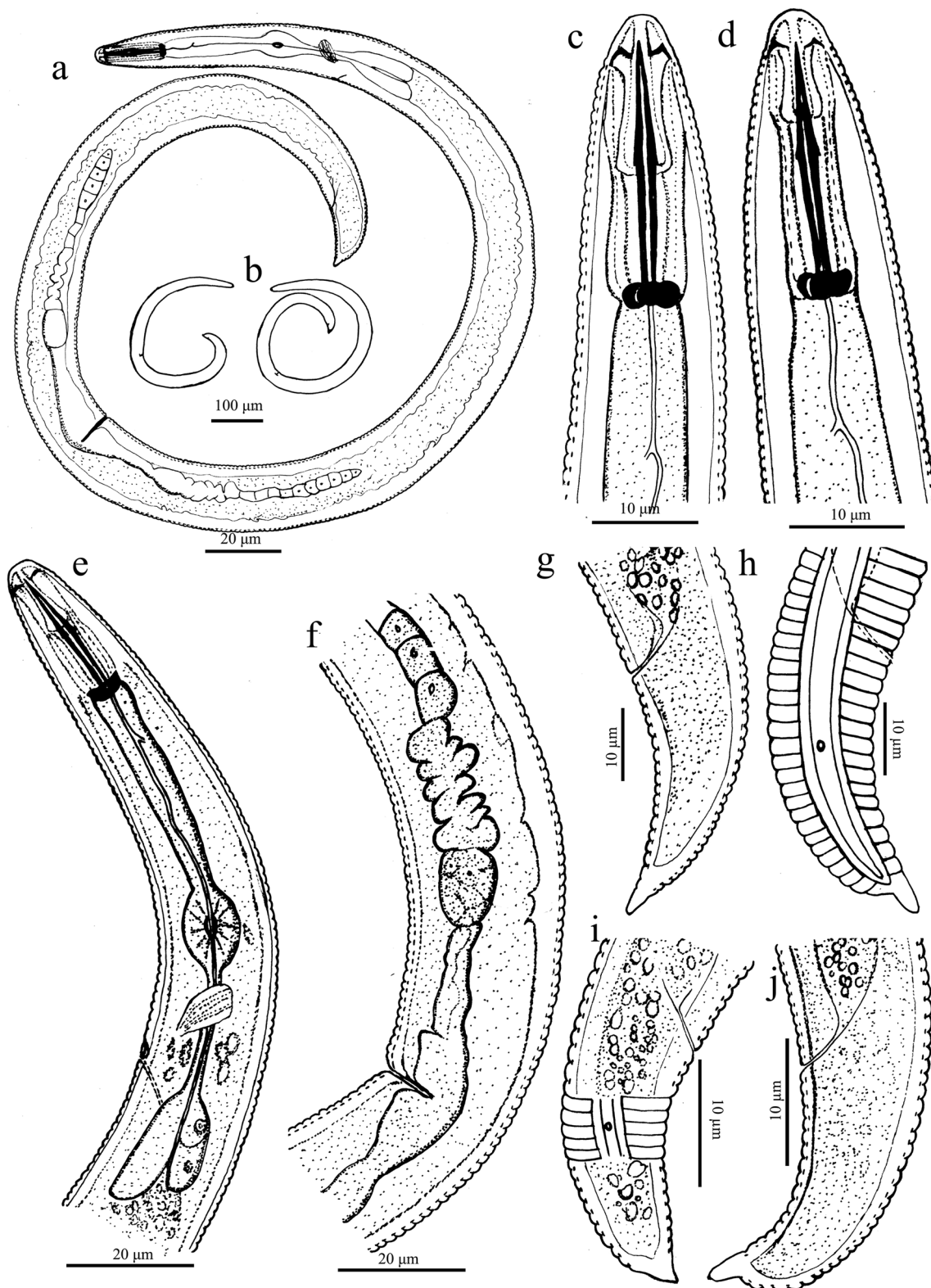
**Table 1** Morphometric data of the females of *Helicotylenchus zengchengensis* n. sp

Characters	Holotype	Paratypes
n	1	12
L	608.9	608.4 ± 52.4 (496.5–688.9)
a	20.7	28.1 ± 3.5 (22.7–32.9)
b	5.5	4.9 ± 0.4 (3.8–5.5)
b′	4.9	4.6 ± 0.4 (3.6–5.2)
c	20.3	19.9 ± 1.9 (15.8–22.6)
c′	2.0	2.2 ± 0.2 (1.9–2.4)
V	57.7	60.4 ± 2.6 (54.8–64.9)
V′	60.7	63.7 ± 2.8 (57.7–69.3)
m	48.7	49.2 ± 1.2 (47.8–51.8)
G1	16.7	21.4 ± 5.4 (15.8–31.8)
G2	16.5	14.2 ± 1.6 (12.2–17.3)
Distance from anterior end to excretory pore %L	16.9	16.7 ± 1.8 (14.3–21.3)
Stylet length %L	4.2	4.3 ± 0.4 (3.7–5.2)
Head diameter	6.6	6.2 ± 0.3 (5.6–6.8)
Head height	3.3	3.4 ± 0.4 (3–4.1)
Stylet length	25.7	26 ± 1.5 (23.8–29.3)
Stylet cone length	12.5	12.9 ± 0.6 (12.2–14)
Stylet basal knob width	4.5	4.6 ± 0.3 (3.8–5.2)
Stylet basal knob height	2.1	2.2 ± 0.1 (2–2.3)
Distance from stylet base to the orifice of dorsal gland	11.6	10.9 ± 1.2 (9.5–13.4)
Median bulb length	12.1	11 ± 1.4 (8.3–13.3)
Median bulb width	8.8	8.4 ± 0.8 (7–9.7)
Distance from anterior end to median bulb valve	71.7	71.6 ± 4.6 (61.3–76.9)
Distance from anterior end to excretory pore	102.7	100.9 ± 7.1 (85.9–108)
Distance from anterior end to gland end	124.6	132.7 ± 10.5 (114.7–142.7)
Distance from anterior end to vulva	351.2	367.7 ± 35.4 (322.3–428.3)
Distance from vulva to anus	227.6	209.7 ± 25 (142.7–236.6)
Lateral field width	5.4	5 ± 0.8 (3.8–6.1)
Maximum body diameter	29.4	21.9 ± 2.8 (17.9–26.4)
Body diameter at anus	15.4	14.3 ± 0.7 (13.4–15.4)
Tail length	30.1	30.7 ± 1.7 (27.8–33.5)
Tail annuli	16	16.4 ± 1.1 (15–18)

All measurements are in  $\mu\text{m}$  and data of mean  $\pm$  SD are shown

*Helicotylenchus* based on three diagnostic characters: the absence of head annulations under LM, the posterior position of the phasmids relative to the anus, and a long tail. It shares similarities with *H. belli* (Sher, 1996), *H. ferus* (Eroshenko & Nguen Vu Thanh, 1981), *H. marethae* (Marais, Queneherve, Tiedt & Meyer, 2013), and *H. martini* (Sher, 1996) based on the former three diagnostic characters. However, it differs from *H. belli* mainly in tail shape (asymmetrical conical with a ventral projection vs. hemispherical without projection), shorter body length (496.5–688.9  $\mu\text{m}$  vs. 710–980  $\mu\text{m}$ ), and lower c value (15.8–22.6 vs. 27–48). It differs from *H. ferus* primarily in tail shape (asymmetrical conical with a ventral projection

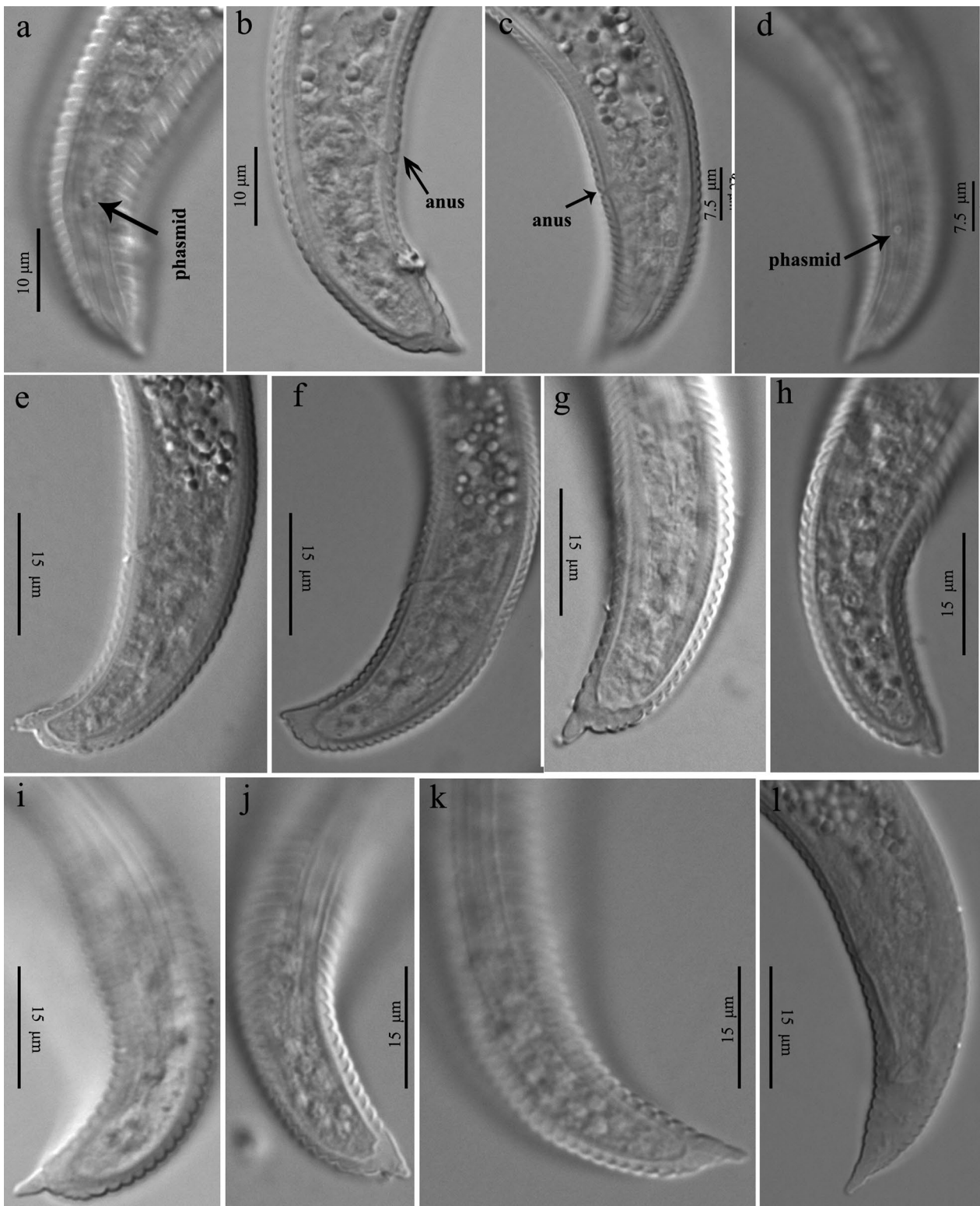
vs. hemispherical without projection), the number of body annuli from anus to phasmids (4–10 vs. 3–5), longer body length (496.5–688.9  $\mu\text{m}$  vs. 470–490  $\mu\text{m}$ ), higher a value (27.8–32.9 vs. 21–23), lower c value (15.8–22.6 vs. 43–45), and longer stylet length (23.8–29.3  $\mu\text{m}$  vs. 22–23  $\mu\text{m}$ ). It differs from *H. marethae* mainly in tail shape (asymmetrical conical and ventrally curved with a ventral projection vs. dorsally curved), lower c value (15.8–22.6 vs. 25.3–39.7), and higher “c” value (1.9–2.4 vs. 1.1–1.6). It differs from *H. martini* primarily in tail shape (asymmetrical conical with a ventral projection vs. smoothly rounded terminus) and a higher number of tail annuli (15–18 vs. 10).



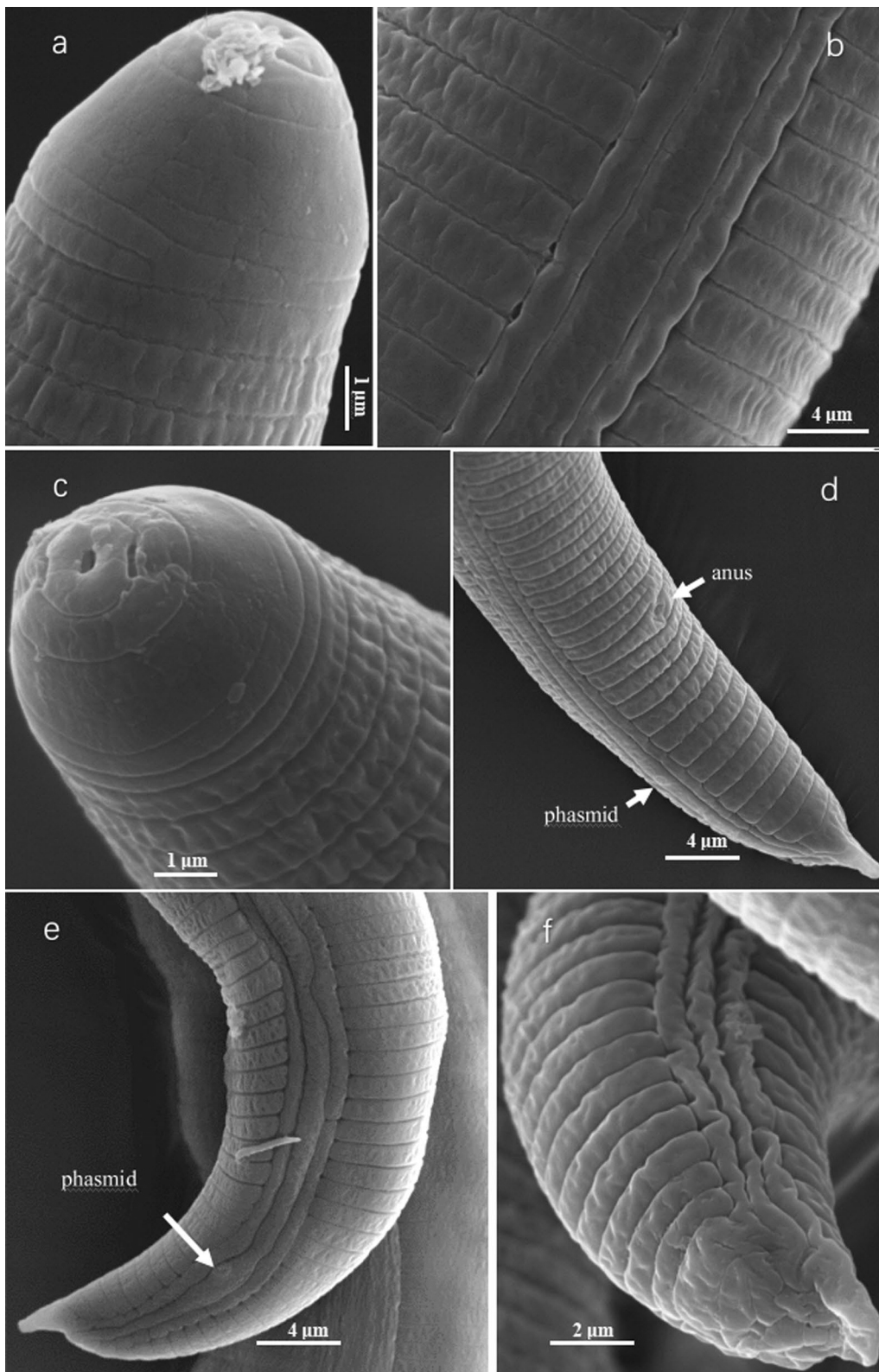
**Fig. 1** Line drawings of female *Helicotylenchus zengchengensis* n. sp. **a** and **b**. Entire body; **c** and **d** Head region; **e** Pharynx; **f** Vulva region; **g-j** Tails



**Fig. 2** Photomicroscopy of female *Helicotylenchus zengchengensis* n. sp. **a** and **b** Female entire body; **c** and **d** Anterior part; **e** Oesophageal part; **f** Genital part; **g** and **h** Incisures at middle body; **i** Vulva region



**Fig. 3** Photomicroscopy of tails of female *Helicotylenchus zengchengensis* n. sp



**Fig. 4** SEM photos of female *Helicotylenchus zengchengensis* n. sp. **a** and **c** Head; **b** Incisures in middle body; **d-f** Tails

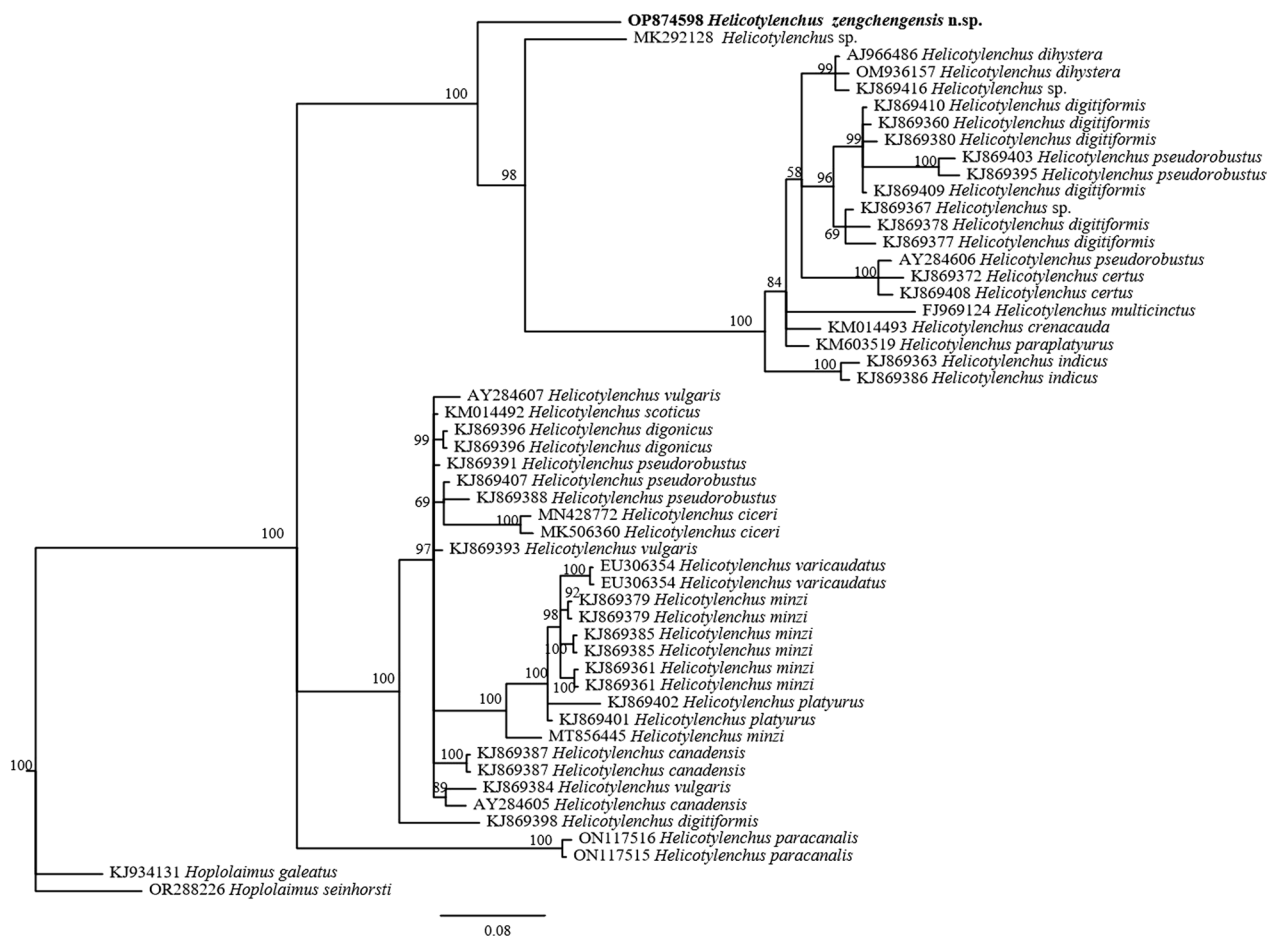
### Molecular characterization and phylogenetic analysis

In the study, molecular analysis was conducted on *Helicotylenchus zengchengensis* n. sp. Using conserved primers for the amplification of 18S partial rRNA segments, ITS, and the 28S D2-D3 expansion segments. The obtained sequences were deposited in GenBank with accession numbers OP874598 for 18S, OP828612–13 for ITS, OP828614, OQ912904, and OQ911481–82 for 28S D2-D3 expansion.

The assays using the Basic Local Alignment Search Tool (BLAST) revealed that the 18S rRNA gene sequence of *H. zengchengensis* n. sp. had the highest similarity (97.31%) to an unidentified *Helicotylenchus* species (Accession: MK292128) from rhizosphere soil of bamboo in America (Carta and Li 2019), followed by *H. dihystra* (Accession: AJ966486) with 96.57% identity, extracted from coastal soil in the United Kingdom (Meldal et al. 2007). A phylogenetic analysis of the 18S rRNA sequences (Fig. 5) showed that *H. zengchengensis* n. sp. is a unique species

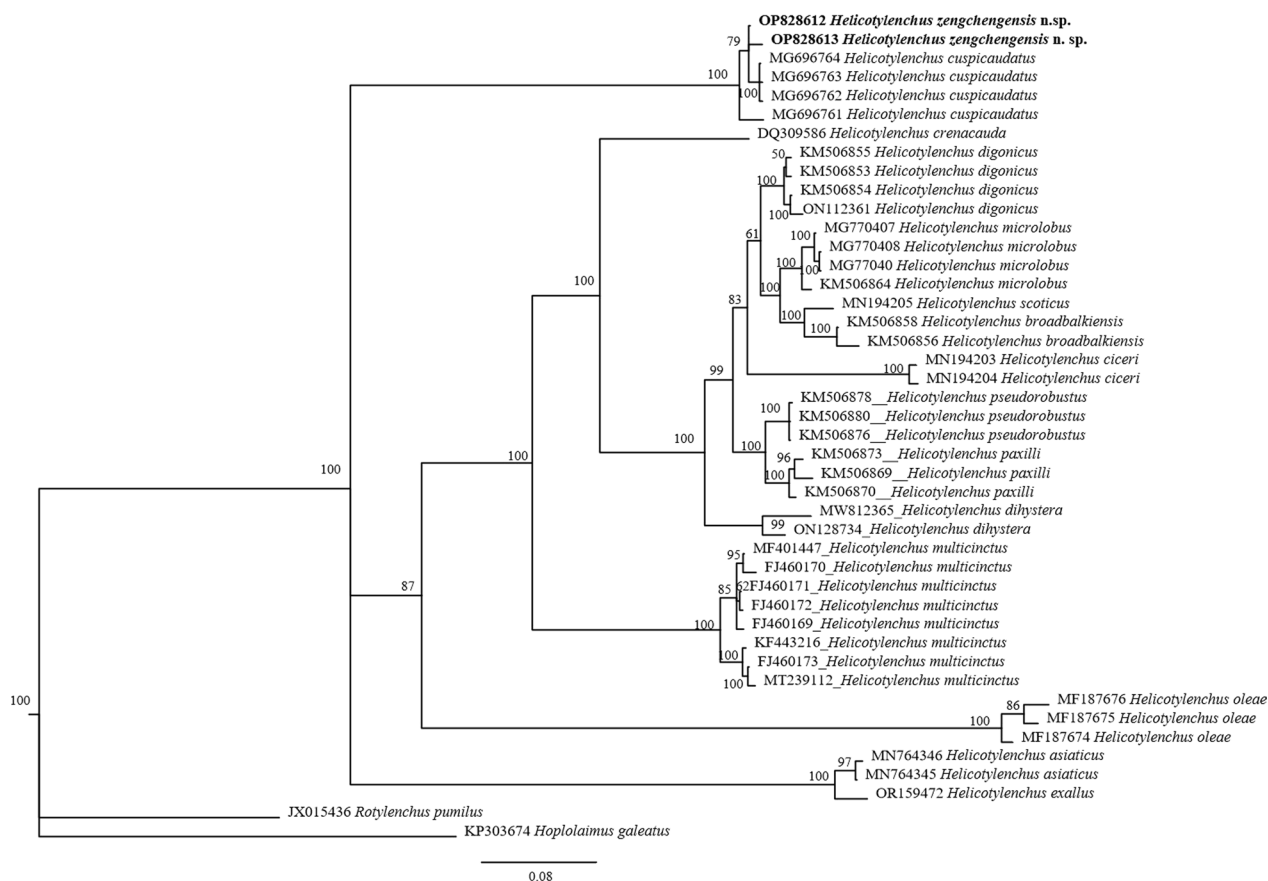
and formed a sister group with the unidentified species (Accession: MK292128), *H. dihystra*, *H. digitiformis* Ivanova, 1967, *H. pseudorobustus*, *H. certus* Eroshenko & Nguen Vu Thanh, 1981, *H. multicinctus*, *H. crenacuada* Sher, 1966, *H. paraplatyurus* Siddiqi, 1972, and *H. indicus*.

Regarding the two ITS sequences from two individuals, there were 27 base pair variations. BLAST search results showed that these sequences had 96.91% and 97% identity with a sequence from *H. cuspicaudatus* (Accession: MG696764) extracted from the rhizosphere soil of bamboo in China. Phylogenetic analysis of the ITS sequences (Fig. 6) confirmed that the two sequences of *H. zengchengensis* n. sp. clustered together and formed a well-supported clade with *H. cuspicaudatus* (PP=100%) and separated from other species. The four 28S rRNA gene sequences obtained from four individuals of *H. zengchengensis* n. sp. were all 791 bp long, exhibiting only three base pairs of variation. The BLAST results indicated that



**Fig. 5** Phylogenetic relationships of *Helicotylenchus zengchengensis* n. sp. with the species of the genus *Helicotylenchus*. The phylogenetic tree was constructed by Bayesian analysis using 18S rRNA partial sequences, with the optimal model GTR+I+G under AIC standard. Posterior probability values exceeding 50% are given on appropriate clades





**Fig. 6** Phylogenetic relationships of *Helicotylenchus zengchengensis* n. sp. with the species of the genus *Helicotylenchus*. The phylogenetic tree was constructed by Bayesian analysis using ITS-rRNA gene sequences, with the optimal model GTR+I+G under AIC standards. Posterior probability values exceeding 50% are given on appropriate clades

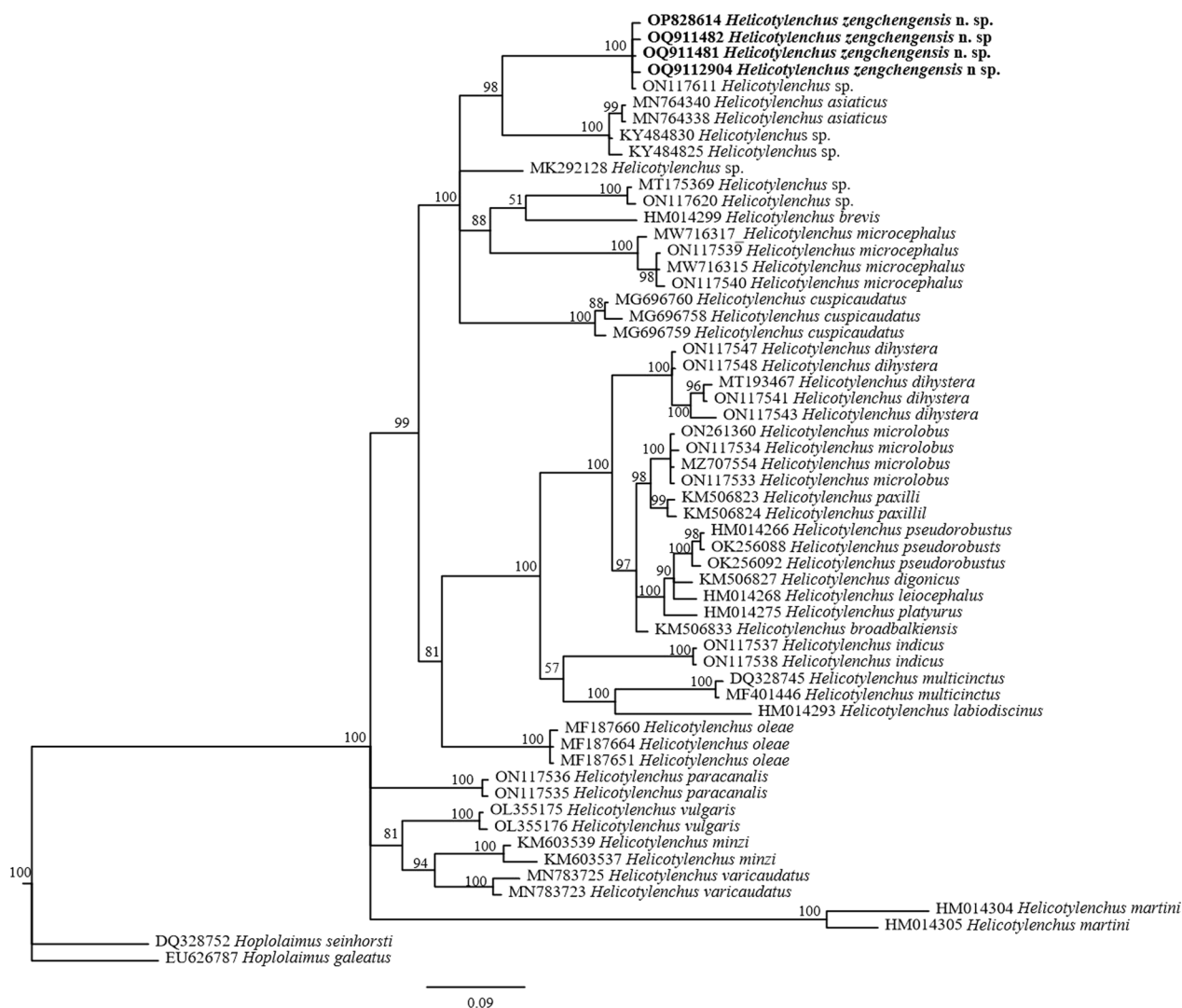
these sequences were most closely related to a sequence from an unidentified *Helicotylenchus* species (Accession: ON117611) with a 99.9% identity, and the species was extracted from rhizosphere soil of bamboo in Fujian Province, China. The Bayesian phylogenetic analysis of the 28S rRNA sequences (Fig. 7) revealed that the four sequences of *H. zengchengensis* n. sp. and the sequence of Accession ON117611 formed a distinct clade (PP = 100%) which is closely related to a sister group consisting of *H. asiaticus* Mwamular, Ma, Kim, Kim, Han & Lee, 2020 and the unidentified species (Accession: KY484830 and KY484825), but are less related with the three sequences from *H. cuspidatus* (Accession: MG696758–60).

Although the new species clustered with *H. cuspidatus* based on ITS-rRNA sequences and showed the highest identity with *H. cuspidatus*, it did not group with *H. cuspidatus* in the same clade based on the 28S rRNA gene sequences. In addition, it differed from *H. cuspidatus* in terms of morphological characteristics. These differences include a distinct lip annulus (smooth under LM vs. 4), a different location of phasmids (4–12

annuli posterior to anus vs. 5 annuli anterior to anus), a lower *c* value (15.8–22.6 vs. 36–33), and a higher *c'* value (1.9–2.4 vs. 1.1–1.8).

#### A diagnostic table of *Helicotylenchus* species in China

In China, 55 species have been described and redescribed. A table containing information on hosts and localities of 56 *Helicotylenchus* species in China (Table 2) is presented to facilitate the analysis of their geological and host distributions for further study. Among these species, *H. dihystra* stands out as the most frequently redescribed species with the broadest distribution and host range in China, followed by *H. digonicus* Perry in Perry, Darling and Thorne, 1959 and *H. pseudorobustus*. In addition, *H. californicus* Sher, 1966, *H. multicinctus*, *H. erythrinae* (Zimmermann, 1904) Golden, 1956, *H. exallus* Sher, 1966, *H. minzi* Sher, 1966, *H. abunaamai* Siddiqi, 1972, and *H. crenacauda* Sher, 1966 have widespread distributions, spanning five provinces (autonomous regions/cities) in China. For ease of species identification, a diagnostic table including information



**Fig. 7** Phylogenetic relationships of *Helicotylenchus zengchengensis* n. sp. with the species of the genus *Helicotylenchus*. The phylogenetic tree was constructed by Bayesian analysis using 28S rRNA D2-D3 gene sequences, with the optimal model GTR+I+G under AIC standards. Posterior probability values exceeding 50% are given on appropriate clades

on the 56 species is provided in Table 3, and the diagnostic characters used for identification are consistent with those given by Uzma et al. (2015).

## Discussion

The genus *Helicotylenchus* comprises a large family with 230 valid species. Species identification based on morphological studies is challenging due to shared diagnostic characteristics and significant intraspecific variation (Subbotin et al. 2011; Uzma et al. 2015). Molecular identification has proven to be a powerful tool, but most species have no sequencing data. Subbotin et al. (2011) conducted an extensive phylogenetic analysis of 54 isolates from 10 species, utilizing

89 sequences of the 28S rRNA D2-D3 expansion. They further expanded their research with another comprehensive study (Subbotin et al. 2015), focusing on six species within *Helicotylenchus*, and constructed phylogenetic trees using 154 sequences of 28S D2-D3 and 37 sequences of ITS. Their findings demonstrated that species could be distinguished from one another, although some species formed paraphyletic groups, and certain sequences submitted to GenBank were potentially misidentified. Subbotin et al.'s conclusions were further supported by Shokoohi et al. (2018) and Rybarczyk-Mydłowska et al. (2019). These researchers conducted comprehensive investigations involving numerous isolates from three different species.

**Table 2** Hosts and localities of *Helicotylenchus* species in China

Species	Hosts	Localities	References
<i>H. abunaamai</i> Siddiqi, 1972	tobacco ( <i>Nicotiana tabacum</i> ), strawberry ( <i>Fragaria ananassa</i> ), goosegrass ( <i>Eleusine indica</i> ), digitaria ( <i>Digitaria sanguinalis</i> ), bayberry ( <i>Myrica rubra</i> ), Grass	Anhui, Fujian, Guangdong, Hainan, Yunnan	Zhang and Chen (1994), Liu et al. (2012), Lin et al. (2017), Liu et al. (2017), Yu (2019)
<i>H. africanus</i> (Micoletzky, 1916) Andrásy, 1958	goosegrass, digitaria, dwarf lilyturf tuber ( <i>Ophiopogon japonicus</i> )	Anhui	Liu et al. (2017)
<i>H. agricola</i> Elmiligy, 1970	tobacco, peanut ( <i>Arachis hypogaea</i> ), apple ( <i>Malus domestica</i> ), grape ( <i>Vitis vinifera</i> ), peach ( <i>Prunus persica</i> ), plantain ( <i>Musa basjoo</i> ), fructus amomi ( <i>Amomum villosum</i> ), areca nut ( <i>Areca catechu</i> ), sandalwood ( <i>Santalum album</i> ), cinnamon ( <i>Cinnamomum cassia</i> )	Guangdong, Guizhou, Sichuan, Yunnan	Deng et al. (1992), Wu and Lu (1995), Xie et al. (1997a), Liu et al. (2012)
<i>H. anhelicus</i> Sher, 1966	apple	Guizhou	Feng et al. 1981, Liu (2005)
<i>H. australis</i> Siddiqi, 1972	honeysuckle ( <i>Lonicera japonica</i> ), Chinese rose ( <i>Rosa chinensis</i> ), lawn grass	Hainan, Sichuan	Li (1994a), Yuan et al. (2007), Yu (2019)
<i>H. apiculus</i> Roman, 1965	alocasia ( <i>Alocasia macrorrhiza</i> )	Fujian	Duan (2012)
<i>H. bambesae</i> Elmiligy, 1970	pea ( <i>Pisum sativum</i> ), green bean ( <i>Phaseolus vulgaris</i> ), cabbage ( <i>Brassica oleracea</i> ), Chinese cabbage ( <i>Brassica campestris</i> ), pear ( <i>Pyrus soratana</i> ), citrus ( <i>Citrus reticulata</i> ), sandalwood, <i>Trachelospermum jasminoides</i>	Anhui, Guangdong, Guizhou, Sichuan,	Lan et al. (1997a), Wu and Lu (1995), Xie et al. (1997a, b), Yang et al. (2018)
<i>H. belli</i> Sher, 1966	citrus, apple, pea	Guizhou, Fujian, Sichuan	Feng et al. 1981, Hu et al. (1991), Xie et al. (1997b), An (2009),
<i>H. caipora</i> Monteiro & de Mendonca, 1972	peach, citrus, grape	Sichuan	Deng et al. (1992), Lan et al. (1997b), Xie et al. (1997a)
<i>H. calliformicus</i> Sher, 1966	citrus, strawberry, rice ( <i>Oryza sativa</i> ), wheat ( <i>Triticum aestivum</i> ), sweet potato ( <i>Dioscorea esculenta</i> ), potato ( <i>Solanum tuberosum</i> ), sugarcane ( <i>Saccharum officinarum</i> ), horsebean ( <i>Vicia faba</i> ), rape ( <i>Brassica campestris</i> ), apple, olive ( <i>Olea europaea</i> ), lychee ( <i>Litchi chinensis</i> )	Beijing, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Shaanxi, Sichuan	Wang (1988), Fang and Yin (1993), Yin et al. (1994), Ye (1994), Li (1994a), Liu (2005), An (2009), Lin et al. (2017)
<i>H. carolinensis</i>	pineapple ( <i>Ananas comosus</i> )	Guangxi	Fang and Yin (1993)
<i>H. cavenessei</i> Sher, 1966	peach, spinach ( <i>Spinacia oleracea</i> ), lettuce ( <i>Lactuca sativa</i> ), choy sum ( <i>Brassica parachinensis</i> ), pelemo ( <i>Citrus grandis</i> ), citrus, areca nut, pomelo, banana ( <i>Musa paradisica</i> )	Guangdong, Guizhou, Hongkong, Xinjiang	Feng et al. (1981), Wu and Lu (1995), Xie and Feng (1996), Mijiti et al. (2007), Chen et al. (2008)
<i>H. concavus</i> Roman 1961	pea	Sichuan	Li (1994a)

**Table 2** (continued)

Species	Hosts	Localities	References
<i>H. cornus</i> Anderson, 1974	wheat, maize ( <i>Zea mays</i> ), sorghum ( <i>Sorghum bicolor</i> ), potato, sweet potato ( <i>Dioscorea esculenta</i> ), pea, sugarcane, flax ( <i>Linum usitatissimum</i> ), radish ( <i>Raphanus sativus</i> ), coriander ( <i>Coriandrum sativum</i> ), chinese cabbage, garlic ( <i>Bulbus Allii</i> ), spring onion ( <i>Allium fistulosum</i> ), pea, lychee	Guangdong, Guizhou, Sichuan, Tianjin	Feng et al. (1981), Yin et al. (1994), Li (1994a), Lan et al. (1997a)
<i>H. crassatus</i> Anderson, 1973	pea	Sichuan	Lan et al. (1997a)
<i>H. crenata</i> Sher, 1966	garlic, citrus, banana, longan ( <i>Dimocarpus longan</i> ), Chinese eaglewood ( <i>Aquilaria sinensis</i> ), tomato ( <i>Lycopersicon</i> )	Fujian, Guangdong, Hainan, Sichuan, Tianjin	Hu et al. (1991), Ye (1994), Wu and Lu (1995), Xie et al. (1997b), Liu and Zhang (1999), Yu (2019)
<i>H. cuspidatus</i>	esculentum)	Guangdong	Shen (2018), Wang et al. (2018)
<i>H. delhiensis</i> Khan & Nanjappa, 1972	bamboo ( <i>Bambusoideae</i> ), sugarcane	Sichuan	Xie et al. (1997a)
<i>H. digitiformis</i> Ivanova, 1967	citrus	Jiangsu, Shanxi, Sichuan, Tianjin	Deng et al. (1992), Li (1994b), Luo et al. (2002), Liu (2005), Huai et al. (2010), Pan et al. (2012), Fan (2019), Dong et al. (2022)
<i>H. digonicus</i> Perry in Perry, Darling & Thorne, 1959	poplar ( <i>Populus</i> sp.), apricot ( <i>Armeniaca vulgaris</i> ), wheat, maize, sorghum, peanut, garlic, creeping bentgrass ( <i>Agrostis stolonifera</i> )	Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hongkong, Hubei, Liaoning, Inner Mongolia, Shandong, Shanxi, Sichuan, Yunnan, Zhejiang	Wang (1988), Fang and Yin (1993), Li (1994a,b), Ye (1994), Wu and Lu (1995), Xie and Feng (1996), Lan et al. (1997a), Zheng et al. (1998a, b), Luo et al. (2002), Liu (2005), Yuan et al. (2007), Mei et al. (2008), An (2009), Qin (2009), Yang (2013), Fan (2019), Qi (2022)
<i>H. dihystra</i> (Cobb, 1893) Sher, 1961	44 host plants, including all kinds of trees, grass, crops, vegetables	Fujian, Guangdong, Guangxi, Hongkong, Hunan, Jiangsu, Jiangxi, Xinjiang, Yunnan, Zhejiang	Feng et al. (1981), Tang (1981), Wang (1988), Wang et al. (1991), Deng et al. (1992), Fang and Yin (1993), Li (1994a,b), Zhou (1996), Lan et al. (1997a), Xie et al. (1997b), Zheng et al. (1998a, b), Xie and Feng (1996), Liu and Zhang (1999), Li et al. (2000), Luo et al. (2002), Zhang et al. (2002), Liu (2005), Li et al. (2006), Xu et al. (2006), Zhou et al. (2007), Cheng (2008), Lin et al. (2008), Mei et al. (2008), An (2009), Li et al. (2009), Duan (2012), Fang et al. (2012), Teng et al. (2013), Yang (2013), Zhang et al. (2013), Lin et al. (2014), Xiao et al. (2014), Chen et al. (2015), Zheng et al. (2015), Lin et al. (2017), Zhang and Ding (2016), Qi (2022), Dong et al. (2022)
<i>H. egyptiensis</i> Tarjan, 1964	Over 50 host plants, including all kinds of trees, grass, crops, vegetables, herbal and ornamental plants	Guangdong, Guangxi, Guizhou, Hainan, Jiangxi	Feng et al. (1984), Fang and Yin (1993), Zhou et al. (2005), Shen (2018)
	tomato, ramie ( <i>Boerhaeria nivea</i> ), banyan ( <i>Ficus macrocarpa</i> ), philodendron ( <i>Philodendron</i> ), maize ( <i>Zea mays</i> )		

**Table 2** (continued)

Species	Hosts	Localities	References
<i>H. erythrinae</i> (Zimmermann, 1904)	Golden, 1956	Fujian, Guangxi, Guizhou, Hubei, Jiangxi, Zhejiang, Shandong, Sichuan, Taiwan, Tianjin,	Feng et al. (1981), Wang (1988), Deng et al. (1992), Fang and Yin (1993), Li (1994a), Zhao et al. (1996), Xie et al. (1997a), Liu (2005), Yuan et al. (2007)
<i>H. exallus</i> Sher, 1966	wheat, barley ( <i>Hordeum vulgare</i> ), maize, sweet potato, sesame ( <i>Sesamum indicum</i> ), coffee ( <i>Coffea arabica</i> ), tea ( <i>Camellia sinensis</i> ), pea, mung bean ( <i>Vigna radiata</i> ), string bean ( <i>Phaseolus vulgaris</i> ), coriander, apple, peach, grape, garlic, persimmon ( <i>Diospyros kaki</i> ), banana, bayberry, sunflower ( <i>Helianthus annuus</i> ), barnyard grass ( <i>Echinochloa crusgalli</i> ), bullbena ( <i>Hemarthria compressa</i> ), sage ( <i>Salvia japonica</i> ), sugarcane ( <i>Saccharum sinensis</i> )	Fujian, Guangdong, Guangxi, Guizhou, Jiangxi, Heilongjiang, Hongkong, Hubei, Jilin, Liaoning, Sichuan, Tianjin,	Wang (1988); Hu et al. (1991); Wang et al. (1991); Fang and Yin (1993); Ye (1994); Li (1994a, b); Wu and Lu (1995); Xie and Feng (1996); Qiao et al. (2011)
<i>H. graminophilus</i> Fotedar & Mahajan, 1974	citrus, onion, grape, Chinese mustard ( <i>Brassica albuglaba</i> ), mooli ( <i>Raphanus sativus</i> ), cryptomeria ( <i>Cryptomeria japonica</i> ), tomato, mulberry ( <i>Morus alba</i> L.)	Yunnan	Zhao et al. (1996), Lan et al. (1997a), Xie et al. (1997b), Luo et al. (2002), Wang and Cai (2005), An (2009)
<i>H. hydrophilus</i> Sher, 1966	maize	Guizhou, Liaoning, Sichuan	Feng et al. (1981), Li (1994a, b)
<i>H. imperialis</i> Rashid & Khan, 1972	chaoitan pepper ( <i>Capsicum annuum</i> ), loquat ( <i>Eriobotrya japonica</i> ), citrus, olive ( <i>Canarium aleosum</i> )	Henan	Li et al. (1986)
<i>H. indicus</i> Siddiqi, 1963	tomato, citrus	Guizhou, Sichuan	Feng et al. (1981), Li (1994b), Zhang et al. (1998)
<i>H. labiatus</i> Roman, 1965	bluegrass ( <i>Poa annua</i> ), olive, tea, grass	Guizhou, Guangdong, Jiangxi, Shanxi,	Feng et al. (1984), Xu et al. (2013), Dong et al. (2022)
<i>H. labiodiscinus</i> Sher, 1966	peach	Sichuan	Lan et al. (1997b),
<i>H. leioccephalus</i> Sher, 1966	peach	Sichuan	Feng et al. (1981), Wang (1988), Lan (1993), Li (1994a), Lan et al. (1997b),
<i>H. lobus</i> Sher, 1966	plum ( <i>Prunus cerasifera</i> ), maize, soybean ( <i>Glycine max</i> ), mustard ( <i>Brassica juncea</i> ), leek ( <i>Allium tuberosum</i> ), citrus, apple, pea, sugarcane	Guizhou, Hubei, Sichuan,	Feng et al. (1981), Wang (1988), Fang and Yin (1993), Lan (1993), Li (1994b), Lan et al. (1997a)
<i>H. martinii</i> Sher, 1966	apple	Guizhou	Feng et al. (1981)
<i>H. membranatus</i> Xie & Feng 1993	peanut, pea, citrus	Guizhou, Sichuan	Feng et al. (1981), Lan et al. (1997a)
<i>H. microlobus</i> Perry in Perry, Darling & Thorne, 1959	green onion ( <i>Allium fistulosum</i> )	Hongkong	Xie and Feng (1993)
<i>H. microcephalus</i> Sher, 1966	tomato, strawberry, creeping bentgrass, paspalum	Guangdong, Hainan	Lin et al. (2017), Xia et al. (2022)
<i>H. minzi</i> Sher, 1966	cryptomeria, arrowroot ( <i>Maranta arundinacea</i> ), peacock arrowroot ( <i>Calathea koyana</i> )	Hebei, Shanxi, Zhejiang	Zhou et al. (2005), Mei et al. (2008)
	potato, pea, citrus, fuchsia ( <i>Fuchsia hybrida</i> ), platycystis ( <i>Platycladus orientalis</i> ), elm ( <i>Ulmus pumila</i> )	Guizhou, Hebei, Hubei, Inner Mongolia, Sichuan	Feng et al. (1981), Wang (1988), Zhang et al. (1998), Xu et al. (2006)

**Table 2** (continued)

Species	Hosts	Localities	References
<i>H. multinctus</i> (Cobb, 1893)	banana, rice, maize, sesame ( <i>Sesamum indicum</i> ), grape, sugarcane	Anhui, Guangdong, Guizhou, Hubei, Sichuan, Yunnan	Wang (1988), Ye (1994), Li (1994a, b), Wu and Lu (1995), Wang et al. (1996), Luo et al. (2002), Xiao et al. (2014)
<i>H. mucronatus</i> Siddiqi, 1964	<i>T. jasmminoides</i> , fevervine ( <i>Paederia scandens</i> )	Anhui	Liu et al. (2017)
<i>H. oleae</i> Insera, Vovlas & Golden, 1956	quercus ( <i>Quercus mongolica</i> ), setaria ( <i>Setaria viridis</i> ), graveolens, chestnut ( <i>Castanea mollissima</i> )	Liaoning	Zhao et al. (1996), Liu (2005), Yuan et al. (2007)
<i>H. paraconcaus</i> Rashid & Khan, 1972	strawberry, citrus, orange ( <i>Citrus sinensis</i> ), onion ( <i>Allium fistulosum</i> )	Guangdong, Hongkong	Xie and Feng (1996), Lin et al. (2017)
<i>H. pisi</i> Swarup & Sethi, 1968	peach, grape	Anhui, Sichuan	Wang et al. (1996), Lan et al. (1997b),
<i>H. platyurus</i> Perry in Perry, Darling & Thorne, 1959	tea, grape, cucumber ( <i>Cucumis sativus</i> ), ginger ( <i>Zingiber officinale</i> ), tobacco ( <i>Nicotiana tabacum</i> ), celery ( <i>Apium</i> )	Guangdong, Hubei, Shaanxi, Sichuan,	Wang (1988), Ye (1994), Li (1994a, b a, b), Luo et al. (2002), Li et al. (2012)
<i>H. pseudorobustus</i> (Steiner, 1914)	Over 40 host plants, including fruit trees, grass, crops, vegetables, herbal and ornamental plants	Anhui, Beijing, Fujian, Guangdong, Guangxi, Hebei, Henan, Hubei, Liaoning, Shandong, Shanghai, Shanxi, Tianjin, Xingjiang, Yunnan	Wang (1988), Wang et al. (1991), Fang and Yin (1993), Ye (1994), Li (1994a), Xie et al. (1997a), Zhao et al. (1996), Li et al. (2006), Xu et al. (2006), Wang et al. (2008), An (2009), Duan (2012), Teng et al. (2013), Zheng et al. (2015), Liu et al. (2017), Dong et al. (2022)
<i>H. pteraceris</i> Singh, 1971	strawberry, elm	Zhejiang	Mei et al. (2008), Lin et al. (2017)
<i>H. reynosus</i> Razjivin, O'Reilly & Millan, 1973	wheat, sorghum, sugarcane	Jiangxi, Tianjin	Feng et al. (1984), Liu (2005)
<i>H. rotundicauda</i> Sher, 1966	strawberry, creeping bentgrass	Fujian	Xie et al. (2004), Dong et al. (2022)
<i>H. sachari</i> Razjivin, O'Reilly & Millan, 1973	peach	Sichuan	Lan et al. (1997b)
<i>H. sieversii</i> Razjivin, 1971	citrus	Guizhou	Feng et al. (1981)
<i>H. spitsbergensis</i> Loof, 1971	apple, pea	Sichuan	Li (1994a), Lan et al. (1997a)
<i>H. truncates</i> Roman, 1965	citrus	Sichuan	Xie et al. (1997b)
<i>H. tumidicaudatus</i> Phillips, 1971	cactus ( <i>Opuntia dillenii</i> )	Inner Mongolia	Zhang et al. (1998)
<i>H. tunisiensis</i> Siddiqi, 1963	grape	Sichuan	Li (1994a, b)
<i>H. variabilis</i> Phillips, 1971	potted landscapes, green bean, sugarcane, pea, citrus, sand kernel, privet ( <i>Ligustrum lucidum</i> ), elm	Guangdong, Guizhou, Jiangxi, Zhejiang, Sichuan	Ye (1994), Wu and Lu (1995), Xie et al. (1997b), Zheng et al. (1998a, b)
<i>H. varicaudatus</i> Yuen, 1964	peach, banana, quercus, jujube ( <i>Ziziphus jujuba</i> )	Liaoning, Sichuan	Lan et al. (1997a), Liu (2005), Yuan et al. (2007),
<i>H. willmottae</i> Siddiqi, 1972	sandalwood ( <i>Santalum album</i> )	Guangdong	Wu and Lu (1995)
<i>H. zengchengensis</i> n. sp.	bamboo	Guangdong	This study

**Table 3** Diagnostic data on species of the genus *Helicotylenchus* females in China (data all from original description)

Species	Male	L (mm)	a	c	c'	V	Head annu	Stylet length (µm)	Tail shape*	Tail annu	Phasmid anter./poster. annu
<i>H. abunaamai</i>	absent	0.56 (0.52–0.63)	26.5 (25–29)	38 (33–44)	1.3 (1.1–1.4)	61.5 (59–65)	4	21.6 (21–22)	CC, SH–VP	9 (7–11)	1–5/
<i>H. africanus</i>	present	0.62–0.98	33–41	25–36	1.6–2.3	58–64	4–5	27–31	TAP, BT–VP	11–18	3–8/
<i>H. agricola</i>	absent	0.48–0.61	21.5–27	27–39	1–1.3	62–65	4–5	22–25	CON, SH–VP	7–10	4–7/
<i>H. anhelicus</i>	present	0.57–0.78	26–32	33–46	1–1.9	57–64	5	29–32	H	11	4–5/
<i>H. apiculus</i>	present	0.5–0.6	21–29	30–41	–	61–66	5–7	24	SP–VP	–	5/
<i>H. australis</i>	absent	0.67 (0.6–0.8)	32 (27–37)	53 (41–69)	1.2 (0.9–1.6)	60 (58–61)	–	21.5 (20–24)	H	13 (8–10)	1–11/
<i>H. bambesae</i>	absent	0.55–0.59	21–22	34–42	1–1.3	62–63	5–6	22–24	VP	7–9	2–7/
<i>H. belli</i>	absent	0.71–0.98	27–33	27–48	1.2–1.6	57–62	0	27–29	H	16	7/
<i>H. caipora</i>	present	0.61–0.73	27.9–35.4	32.6–51.3	1.3–2.0	57.3–63.3	4–5	24.3–27.1	CC–SRT	6–11	7/2–3
<i>H. californicus</i>	present	0.58–0.78	27–32	32–50	0.8–1.3	59–64	4	24–27	L–VP	8	2/
<i>H. carolinensis</i>	absent	0.68–0.82	27–32	35–49	0.9–1.3	61–64	4	25–28	IR–H	12	6/
<i>H. cavenessei</i>	absent	0.56–0.7	24–32	33–64	1–1.3	56–62	4	24–27	IR–H	13	6/
<i>H. concavus</i>	absent	0.64–0.86	26–33	42–51	0.8–1	61–65	0	29–32	H	10–12	2/3
<i>H. cornutus</i>	present	0.67–0.84	25–32	37–59	0.6–1.2	61–65	4–5	24–27	IR–VP	5–11	2–10/
<i>H. crassatus</i>	absent	0.72 (0.65–0.78)	31 (27–34)	59 (45–71)	0.79 (0.63–1)	61–64	4	28 (26–30)	IR–VP	3–9	6/2
<i>H. crenicauda</i>	absent	0.57–0.77	24–29	31–52	1–1.3	59–64	4	24–28	IND–VP	8	4–6/
<i>H. cuspicaudatus</i>	absent	0.58–0.72	23–30	26–33	1.6–2.5	60–67	4	25–28	CON–VA	19–23	5/
<i>H. delhiensis</i>	absent	0.54–0.7	21–30	43–54	–	59–65	2–3	21–22	CON–R	7	7/5
<i>H. digitiformis</i>	absent	0.7–0.8	26–31	30–39	1.1–1.4	60–62	5	27–30	DIGI	14–16	1–4/
<i>H. digonicus</i>	absent	0.5–0.79	23–33	41–63	0.7–0.9	58–64	4–5	24–28	H, SH–VP	4–10	1–5/
<i>H. dihystra</i>	present	0.59–0.79	27–35	35–49	0.8–1.2	60–65	4–5	24–29	CON–SH–VP	6–12	5–11/
<i>H. egyptiensis</i>	absent	0.78 (0.69–0.85)	29 (26–32)	29 (25–33)	1.7 (1.4–1.9)	60 (59–62)	4–6	26 (24–28)	MUC	8–15	5/2
<i>H. erythrinae</i>	present	0.48–0.61	23–26	27–34	1–1.6	60–65	4–5	23–26	MUC	6–12	4/2
<i>H. exallus</i>	present	0.61–0.78	26–32	30–52	0.7–1.2	59–63	4	25–28	CON, SH–VP	12	3–5/
<i>H. graminophilus</i>	absent	0.7–0.84	25–32	32–40	–	64–68	Indist	25–29	BLC	8–12	7/2–4
<i>H. hydrophilus</i>	present	0.7–0.92	26–31	35–55	0.8–1.2	59–65	4	28–32	L–VP	7	4–5/
<i>H. imperialis</i>	present	0.48–0.59	25–33	36–43	–	65–71	4–5	27–29	R	8–12	4–10/
<i>H. indicus</i>	absent	0.45–0.63	23–32	33–47	–	60–65	4	21–23	CC, BL–T	11	6/6
<i>H. labiatus</i>	absent	0.5–0.6	21–24	35–48	–	61–64	3–4	25	CC	–	8/
<i>H. labiodiscinus</i>	absent	0.48–0.66	22–29	27–42	1–1.4	58–63	0	2327	IR–H	15	7–8/
<i>H. leiocephalus</i>	absent	0.68–0.86	25–31	31–61	0.9–1.5	55–63	0	24–29	H	12	2/
<i>H. lobus</i>	absent	0.74–0.95	30–34	34–46	1–1.6	57–65	5	28–31	SH–VP	9	3/2

**Table 3** (continued)

Species	Male	L (mm)	a	c	c'	V	Head annu	Stylet length (µm)	Tail shape*	Tail annu	Phasmid anter./poster. annu
<i>H. martini</i>	present	0.45–0.58	25–31	21–31	1.5–2.5	54–60	0	22–25	H–SRT	10	/3–12
<i>H. membranatus</i>	absent	0.56 (0.53–0.61)	24.5 (23–28)	43 (38–55)	1.0 (0.8–1.2)	65 (62–68)	4	26	SCY/BLC	7–10	2/3
<i>H. microlobus</i>	absent	0.65–0.72		30–40		59–63		28–31	H	10	4/2
<i>H. microcephalus</i>	absent	0.55–0.71	27–32	30–42	1.2–1.5	61–66	Indist	23–27	TAP–VP	10	5–3/
<i>H. minzi</i>	present	0.68–0.8	27–34	46–71	0.5–1	59–64	5	26–29	IR–H	11	2–4/
<i>H. mucronatus</i>	present	0.54 (0.49–0.59)	25.6 (25–28)	29 (26–33)	0.55 <sup>#</sup>	60 (58–63)	4	22 (21–23)	MUC	9 <sup>#</sup>	3/
<i>H. multinctus</i>	present	0.47–0.53	24–30	35–46	0.8–1	65–69	3–4	22–24	H	6–12	2–6/
<i>H. oleae</i>	absent	0.67–0.93	33–36	37–45	1.1–1.7	58–65	4–5	29–31	BLC	16–21	4–7/
<i>H. paraconcaus</i>	absent	0.62–0.71	24–42	39–52	?	64–68	0	26–29	CON–R	12	10/8
<i>H. pisi</i>	absent	0.7–0.87	17–29	28–41	–	60–65	3–4	26–29	VP	12	/3
<i>H. platyurus</i>	absent	0.8–0.93	27–32	32–46	1–1.6	57–60	4–5	28–32	H	9–17	6/2
<i>H. pseudorobustus</i>	absent	0.6–0.82	27–34	32–52	0.9–1.4	59–64	4–5	26–30	L–VP	7–12	2–7/
<i>H. pteracercus</i>	absent	0.62–0.73	25–29	41–68	0.6–1.08	57.1–65	5	23.5–25.2	VP	10–15	16/3
<i>H. reynosus</i>	absent	0.58–0.7	24.7–26.4	41.2–42	–	62.1–65.3	4–5	25.2–26.2	CONI–DIGI	7–9	6–5/
<i>H. sacchari</i>	present	0.57–0.69	20.5–26.7	26–30.7	–	65–67.3	5–6	22–24	CYL–SRT	9–11	2–0/
<i>H. sieversii</i>	present	0.74 (0.72–0.82)	24 (22–26)	40 (38–42)	–	62 (62–65)	5	32.4 (2–34)	BL–R	9–10	2–1/
<i>H. spitsbergensis</i>	absent	0.79–1.08	28–38	36–42	–	56–68	4–5	27–31	TRAP–CON	13	2/3
<i>H. truncates</i>	absent	0.43–0.5	20–32	34–50	–	60–65	3–4	20–22	T	–	7/1
<i>H. tumidicaudatus</i>	present	0.81 (0.72–0.89)	27 (24–31)	58 (38–36)	0.7(0.5–1.0)	63 (60–67)	Indist	27 (25–28)	H	–	12–5/
<i>H. tunisiensis</i>	absent	0.98 (0.88–1.1)	30.3 (28–33)	49 (45–55)	1 <sup>#</sup>	57.3 (56–58)	4	34 (32–36)	OBT–R	9 <sup>#</sup>	7/
<i>H. variabilis</i>	present	0.62 (0.51–0.72)	25 (23–29)	46 (35–53)	1.0(0.8–1.4)	63 (56–66)	0	25 (23–26)	BR	12	5–10/
<i>H. varicaudatus</i>	present	0.62 (0.58–0.67)	18–26	39–50	–	62 (60–63)	5	31 (29–33)	IR–R	6–11	3/2
<i>H. willmottae</i>	absent	0.68 (0.6–0.79)	31 (28–33)	47 (40–60)	1.1 (0.9–1.2)	63 (59–67)	5–6	26 (24.5–28)	SCY	12–17	1–8/
<i>H. zengchengensis</i>	absent	0.61 (0.5–0.69)	28 (23–33)	20 (16–23)	2.2 (1.9–2.4)	60 (55–65)	Indist	26 (24–29)	DC, VP	19 (14–25)	/4–10

Ann. annulations, anter. anterior, poster. posterior, Indist. Indistinct, \*Column: all the abbreviations were cited from Uzma et al. (2015), BL Bluntly, BLC Bluntly conoid, BR Broadly rounded, CC Convex-conoid, CON Conoid, CONI Conical, CON–VA Conoid ventrally arcuate, CYL Cylindrical, DC Dorsally curved, DIGI Digitate, H Hemispherical, IND Indented, IR Irregular, L Large, MUC Mucronate, OBT Obtusely, R Rounded, SCY Sub-cylindrical, SH Short, SRT Smooth rounded terminus, T Truncate, TAP Tapering, TRAP Trapezoid, VP Ventral projection. <sup>#</sup>The data were measured or counted based on the line drawings in the original references



They combined the 28S D2-D3 expansion segments with morphological characteristics in their studies. It is noteworthy that, although over 20 species within *Helicotylenchus* were sequenced, the majority of DNA sequences available in GenBank pertain to only a few species, such as *H. dihystrera*, *H. pseudorobustus*, and *H. microlobus* Perry in Perry, Darling and Thorne, 1959. Consequently, achieving reliable molecular identification for *Helicotylenchus* species remains a substantial undertaking.

Of the 55 known species in China, two species—*H. microlobus* and *H. membranatus*—were not included in the study by Uzma et al. (2015). Firoza and Maqbool (1993) synonymized *H. microlobus* with *H. pseudorobustus*, and Uzma et al. (2015) accepted their decision. However, Subbotin et al. (2015) and Mwamula et al. (2020) concluded that these two species could be distinguished from each other based on 28S rRNA D2-D3 expansion segments, and these two species were in two separate clades in this study (Fig. 6). Therefore, we also consider *H. microlobus* a valid species. Shen (1992), Xie et al. (2004), and Dong et al. (2022) reported *H. rotundicauda* Sher, 1966 in China. Since *H. rotundicauda* has been synonymized as *H. dihystrera* (Fortuner 1981; Liu 2005), we transferred their information to *H. dihystrera*. Similar actions were taken on other five species identified in China, i.e., *H. bradys* Thorne and Malek, 1968, *H. glissus* Thorne and Malek, 1968, *H. nannus* Steriner, 1945, *H. plumariae* Khan & Basir, 1964, and *H. talonus* Siddiqi, 1972 which were synonymized as *H. pseudorobustus*, *H. dihystrera*, *H. dihystrera*, *H. indicus* Siddiqi, 1963, and *H. bambesae* Elmiligy, 1970, respectively, based on morphological characteristics (Fortuner 1987, 1991; Firoza and Maqbool 1993, 1994). Two other species, *H. caribensis* Roman, 1965 and *H. boringuensis* Roman, 1965, were considered species inquirendae by Fortuner (1981). Liu (2005) followed this action and listed them as unconfirmed or species inquirendae, Uzma et al. (2015) also accepted mostly taxonomical status of these species, and we did not include these eight species in this study.

*H. dihystrera*, *H. pseudorobustus*, *H. digonicus*, and *H. multicinctus* are among the most frequently documented species worldwide. Notably, *H. pseudorobustus* and *H. dihystrera* can parasitize a wide range of crops, impeding their growth, as Subbotin et al. (2015) reported. On the other hand, *H. multicinctus* is known to induce necrotic lesions on banana roots, ultimately resulting in stunted plant growth, as outlined by Gowen et al. (2005). Although there have been no reported crop losses attributed to these nematodes in China, it is imperative to conduct further examinations to understand their potential threats fully.

## Conclusions

This study describes a new species within the genus *Helicotylenchus*, named *H. zengchengensis* n. sp., based on its morphological and molecular characteristics. This new species represents the second new species of *Helicotylenchus* described in China. The identification of this new species contributes to the global diversity of *Helicotylenchus*, and the DNA sequences obtained will serve as a valuable resource for future molecular diagnostics within the genus. Furthermore, the diagnostic and comprehensive tables included in this report provide information on the geographical distribution and host range of all *Helicotylenchus* species in China and will significantly facilitate the identification of new record species and research on nematode diversity.

## Methods

### Nematode population and morphological identification

Soil associated with bamboo was collected from the Zengcheng District, Guangzhou City, Guangdong Province, China (N23.449877° N, 113.847389° E). The nematode was isolated from soil using a modified Barman funnel method, then killed by heating at 65°C for 3 min and fixed with FG fixation (formalin: glycerol: distilled water = 10:1:89) for more than a week, dehydrated using the glycerol-ethanol method and mounted on permanent slides (Xie and Feng 1993). The observation, measurement, and photography of nematodes were performed using a ZEISS Scope A1 light microscope (LM) equipped with a microscopic imaging system and a digital photography camera. For scanning electron microscopy (SEM) studies, the nematodes were observed and photographed under 10 kV using an FEI XL-30-ESEM scanning electron microscope at the Testing Center of South China Agricultural University.

### DNA extraction, PCR sequencing, and phylogenetic analyses

DNA was extracted from a single nematode using a proteinase K assay (Mundo-Ocampo et al. 2008). The primers used for amplifying rRNA 28S D2-D3, ITS, and 18S partial sequences and the annealing temperature for PCR reaction were described by Dey ley et al. (1999), Vrain et al. (1992), and Holterman et al. (2006), respectively. PCR amplification was performed in a 40 µL volume of the mixture, including 2 µL DNA template, 2 µL of each primer, 20 µL 2×Super Taq PCR StarMix (with Dye) (Genestar, Beijing, China), and 14 µL sterile water. The procedure was as follows: pre-denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 28S and 55°C for ITS for 30 s, and 54°C for 18S rRNA for

1 min, extension at 72°C for 1 min, and a final extension at 72°C for 8 min. PCR products were purified using a Gel Extraction Kit (CWBI, Jiangsu, China). The targeted fragments were cloned into the pMD18-T vector (Takara, Japan), and positive colonies were sequenced by Sangon Biotech Co., Ltd. (Shanghai, China). All obtained sequences were submitted to the GenBank database. *Helicotylenchus zengchengensis* n. sp. sequences were aligned with other species in the genus *Helicotylenchus* using BLAST in GenBank under default parameters.

Phylogenetic analysis was used to evaluate the correlations between the new species and other *Helicotylenchus* species. The phylogenetic trees were constructed by Bayesian inference (BI) using MrBayer 3.2.1 as described by Ronquist et al. (2012). Clustal X was used for sequence alignment with default parameters. Conserved regions were selected with Gblocks 0.91b. The NEX format of aligned sequences was calculated and transformed by PAUP 4.0b4a. The best-fit model was determined by MrModeltest 2.3 based on the Akaike Information Criterion (AIC) with default parameters. Bayesian analysis was initiated with a random starting tree. Four independent Markov Chain Monte Carlo (MCMC) models were used and run for  $2 \times 10^6$  generations and sampled once every 100 generations. After discarding the first 25% aged samples, the remaining samples were retained to generate a 50% majority rule consensus tree. Posterior probabilities (PP) were given on the clades. For 18S rRNA sequence analysis, 49 sequences from 17 identified and two unidentified species from *Helicotylenchus* were chosen, with *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935 (Accession: KJ934131) and *Hop. seinhorsti* Luc, 1958 (Accession: OR288226) as the outgroup. For the ITS sequence analysis, 40 sequences from 13 species from *Helicotylenchus* were selected, with *Rotylenchus pumilus* (Perry in Perry, Darling & Thorne, 1959) Sher, 1961 (Accession: JX015436) and *Hop. galeatus* (Accession: KP303674) as the outgroup. For the 28S rRNA sequences analysis, 46 from 21 identified species and six from unidentified species from *Helicotylenchus* were selected, with *Hop. galeatus* (Accession: EU626787) and *Hop. seinhorsti* (Accession: DQ328752) as the outgroup.

#### Abbreviations

LM	Light microscope
SEM	Scanning electron microscope
ITS	Internal transcribed spacer
BLAST	Basic Local Alignment Search Tool
PP	Posterior probabilities
MCMC	Markov Chain Monte Carlo
AIC	Akaike Information Criterion
BI	Bayesian inference

#### Acknowledgements

We thank Huizhong Wang and Sihua Yang in our lab for their help in the phylogenetic trees and SEM assay.

#### Author contributions

YC identified this species and performed all related research. YL performed part of the molecular diagnosis assay. CX drew the new species, prepared the figures, and wrote the manuscript. HX revised the manuscript. All authors read and approved the final manuscript.

#### Funding

This work was financially supported by the Science & Technology Basic Resources Investigation Program of China (2018FY100304).

#### Availability of data and materials

The holotype and most of the paratypes were conserved in our laboratory in the department of Plant Pathology, South China Agricultural University in Guangzhou, and one paratype were deposited in the National Parasitic Resources Center in Shanghai.

#### Declarations

##### Ethics approval and consent to participate

Not applicable.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare that they have no competing interests.

Received: 4 July 2023 Accepted: 3 January 2024

Published online: 25 January 2024

#### References

- An C. Description of 15 nematode species of citrus root in Yongchun and studies on new technologies for nematodes separation. Xiamen: Xiamen University; 2009. p. 20–42.
- Carta LK, Li S. PCR amplification of a long rDNA segment with one primer pair in agriculturally important nematodes. *J Nematol*. 2019;51:1–8. <https://doi.org/10.21307/jofnem-2019-026>.
- Chen XG, Liu J, Hong ZZ, Huang JH, Zeng YS. Morphological and molecular characterization of two species of plant-parasitic nematodes associated with golf turfgrasses in Guangdong, China. *J Zhongkai Univ Agric Eng*. 2015;28(4):6–11. <https://doi.org/10.3969/j.issn.1674-5663.2015.04.002>.
- Chen Y, Zhuo K, Li XD, L JL. Identification of four species of the family Hoplolaimidae in Guangzhou. *Nematol Res China*. 2008;2:1–7.
- Cheng Y. Identification and harmfulness of parasitic nematode species on peanut. Fuzhou: Fujian Agriculture and Forestry University; 2008. p. 16–7.
- Crow WT. Spiral nematode *Helicotylenchus* spp. (Nematoda: Tylenchida: Hoplolaimidae). 2013. <http://edis.ifas.ufl.edu/>
- De Ley P, Felix M, Frisse LM, Nadler SA, Sternberg PW, Thomas WK. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology*. 1999;1:591–612.
- Decraemer W, Hunt DJ. 2013. Structure and Classification. In: Perry RN & Moens M. *Plant Nematology*. CAB International; 2013. p. 3–37.
- Deng D, Lan Q, Xie C, He W. Plant parasitic nematode study on fruit trees in Sichuan (II)—Survey and identification of helical nematodes damaging peach trees. *Sichuan Fruits Technol*. 1992;20(1):13–9.
- Dong Y, Jin P, Zhang H, et al. Distribution and prevalence of plant-parasitic nematodes of turfgrass at golf courses in China. *Biology*. 2022;11:1322. <https://doi.org/10.3390/biology11091322>.
- Duan Y. Investigation and species identification on the plant-parasitic nematodes of ornamental plants in Fujian. Fuzhou: Fujian Agriculture and Forestry University; 2012. p. 35–9.

- Fan JL. Plant parasitic nematode species and community structure under the rhizosphere of fruit trees in Shanxi province. Taiyuan: Shanxi Agricultural University; 2019. p. 21–7.
- Fang Y, Yin G. Study on spiral nematodes (*Helicotylenchus*: Hoplolaiminae) from the roots of the crips in Guangxi Zhuang Autonomous region. *J South China Normal Univ.* 1993;34:65–70.
- Fang AQ, Tan JJ, Ye JR, Hao DJ. Species identification of rhizosphere parasitic nematodes of pine tree in Jiangsu province. *J Nanjing Forestry Univ.* 2012;36(5):109–14. <https://doi.org/10.1007/s11783-011-0280-z>.
- Feng Z, Li S, Li W, Fang Y, Wu Y. Studies on the parasitic nematodes of agricultural crops in China. 1. Studies on the parasitic nematodes of agricultural crops in Guizhou. Technical Bulletin of Plant Quarantine Research. Beijing, Institute of Plant Quarantine Ministry of Agriculture, People's Republic of China. 1981.
- Feng Z, Li S, Li W, Wu Y. Identification on parasitic nematodes of agricultural crops in China. Technical Bulletin of Plant Quarantine Research. Beijing, Institute of Plant Quarantine Ministry of Agriculture, People's Republic of China. 1984.
- Firoza K, Maqbool MA. Three new species of the subfamily Hoplolaiminae (Nematoda:Hoplolaimida) from Pakistan. *Pak J Nematol.* 1993;11:69–78.
- Firoza K, Maqbool MA. (1994) Description of *Helicotylenchus meloni* n. sp. and *H. striatus* n. sp. (Nematoda: Hoplolaimidae) from Pakistan. *Pak J Nematol.* 1994;12(2):109–16.
- Fortuner R. A reappraisal of Tylenchina (Nema). 8. The family Hoplolaimidae Filipev, 1934. *Revue Nématol.* 1987;10(2):219–32.
- Fortuner R. The Hoplolaiminae. In: William RN, editor. Manual of agriculture nematology. New York: Marcel Dekker; 1991. p. 669–719.
- Gowen SR, Queneherve P, Fogain R. Nematodes parasites of bananas and plantains. In: Luc M, Sikora RA, Bridge J (eds) Plant Parasitic Nematodes In Subtropical And Tropical Agriculture (2nd edition). CABI publishing; 2005. p. 611–644
- Holterman M, Wurff AVD, Elsen SVD, Megen HV, Bongers T, Holobachov O, et al. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Pakistan J Zool.* 2006;23:1792–800.
- Hu K, Yang B, Wang Q, Zhu W. An investigation of plant nematodes around the roots of citrus trees in Sichuan province. *J Southwest Agric Univ.* 1991;13(3):258–64.
- Huai J, Tan J, Ye J, Qu H. Preliminary Survey and identification of rhizosphereparasitic nematodes of poplar Tree in Jiangsu province. *J Huazhong Agric Univ.* 2010;29(3):277–81.
- Lan Q. Identification of parasitic nematodes collected from the root soil of apple trees. *Southwest Horticulture.* 1993;1:4–7.
- Lan Q, Deng D, Xie C, Zhao X, He W. Identification of Hoplolaimidae species collected from the root soil of pear trees. *Southwest China J Agric Sci.* 1997a;10:40–5. <https://doi.org/10.16213/j.cnki.scjas.1997.s1.009>.
- Lan Q, Zhao X, He W, Deng D, Xie C. Description of helical nematodes damaging peach trees in Sichuan province. *Southwest China J Agric Sci.* 1997b;10:76–80.
- Li D. List of parasitic nematodes on plants and edible fungi in Sichuan Province, China. *J Southwest Agric Univ.* 1994a;16(1):1–10.
- Li D. A description of some species of parasitizing nematodes of *Helicotylenchus* on plant roots in Sichuan province. *J Southwest Agric Univ.* 1994b;16(3):273–7.
- Li S, Feng Z, Xu K, Li W. Description on the nematodes of crops in Henan province. *Acta Agriculural Universitatis Henanensis.* 1986;20(3):349–57. <https://doi.org/10.16445/j.cnki.1000-2340.1986.03.012>.
- Li H, Shen P, Xu J. Investigations on parasitic nematodes of import and export garden plants in Jiangsu province. *J Nanjing Agric Univ.* 2000;23(1):34–8.
- Li W, Yang Y, Li S, Hu X. Preliminary investigation on parasitic nematode species of flowers and plants in Rosaceae. *Southwest China J Agric Sci.* 2006;19(5):906–11. <https://doi.org/10.16213/j.cnki.scjas.2006.05.030>.
- Li H, Gao J, Zhang Y. Types of vegetable garden plant parasitic nematodes in Taigu. *J Shanxi Agric Sci.* 2009;37(8):54–9.
- Li JL, Zhao HH, Li XH, Kong FY. Identification of plant nematode species on tobacco in Hubei province. *Hubei Agric Sci.* 2012;51(21):4795–8. <https://doi.org/10.14088/j.cnki.issn0439-8114.2012.21.043>.
- Lin LF, Hu XQ, Liu CG, Li WF, Zhou YL. Identification of plant parasitic nematodes from rhizospheric soil of *Erigeron breviscapus* in Yunnan. *Plant Prot.* 2008;34(3):69–73. <https://doi.org/10.3969/j.issn.0529-1542.2008.03.018>.
- Lin LF, Zhou YL, Liu CG, Zhou YL, Yang L, Hu XQ, et al. Record of plant parasitic nematodes from rhizosphere soil of *Eriobotrya japonica* in Yunnan. *Acta Agric Univ Jiangxiensis.* 2014;36(3):562–4. <https://doi.org/10.13836/j.jjau.2014091>.
- Lin LF, Liu CG, Li Y, Long YY, Hu XQ. Preliminary species identification of parasitic nematodes from rhizosphere soil of grapes in Mengzi city. *Plant Diseases Pests.* 2017;8(2):20–5. <https://doi.org/10.19579/j.cnki.plant-d.p.2017.02.006>.
- Liu WZ. Description of the species of plant parasitic nematodes. Beijing: China Agriculture Press; 2005. p. 179–245.
- Liu G, Zhang S. Identification of parasitic nematodes on longan in Fujian China. *J Fujian Agric Univ.* 1999;28(1):59–65.
- Liu YT, Liu YJ, Wang WP, Xie Y, Du PP, Wang Y. Identification of ectoparasitic nematodes on rhizosphere of tobacco in Wenshan, Yunnan Province. *J Yunnan Agric Univ.* 2012;27(4):490–6. [https://doi.org/10.3969/j.issn.1004-390X\(n\).2012.04.005](https://doi.org/10.3969/j.issn.1004-390X(n).2012.04.005).
- Liu TY, Wu HP, Hu P, Wang H, Ye C, Ma HQ, et al. Description of the spiral nematodes from plant rhizosphere in Dashushan scenic spot, Hefei City Anhui Province. *Plant Protect.* 2017;43(4):97–103. <https://doi.org/10.3969/j.issn.0529-1542.2017.04.018>.
- Luo SL, Zhangsun DT, He PC. Description of some species in genus *Helicotylenchus* around the grape roots from four provinces in China. *Plant Prot.* 2002;28(6):5–8.
- Mei YY, Zheng W, Zheng JW. Description of four species in the genus *Helicotylenchus*. *Plant Prot.* 2008;34(5):116–8.
- Meldal BH, Debenham NJ, De Ley P, De Ley IT, Vanfleteren JR, Vierstraete AR, et al. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Mol Phylogenet Evol.* 2007;42(3):622–36. <https://doi.org/10.1016/j.ympev.2006.08.025>.
- Mijiti G, Supi R, Xie H. Investigation on new record category of parasitic nematode around fruit tree roots in the south of Xinjiang. *J Xinjiang Agric Univ.* 2007;30(3):40–3.
- Mundo-Ocampo M, Troccoli A, Subbotin SA. Synonymy of a fenestrata supported by phylogenetics with molecular and morphological characterisation of *H. koreana* comb. n. and *H. orientalis* comb. n. (Tylenchida: Heteroderidae). *Nematology.* 2008;10(5):611–32. <https://doi.org/10.1163/156854108785787190>.
- Mwamula AO, Na H, Kim YH, Kim YH, Han G, Lee DW. Characterization of a new spiral nematode, *Helicotylenchus asiaticus* n. sp., and three known species from Korea; with comments on the validity of *Helicotylenchus microlabus* Perry in Perry, Darling & Thorne, 1959. *Eur J Plant Pathol.* 2020;157:565–81. <https://doi.org/10.1007/s10658-020-02022-9>.
- Pan YW, Tan JJ, Ye JR. Description of several nematode species of Tylenchida and Dorylaimida from Polar rhizosphere in Jiangsu province. *Scientia Silvae Sinicae.* 2012;48(8):161–5.
- Qi JX. Species identification and community diversity analysis of plant parasitic nematodes in rhizospheres of *Pyrus sinkiangensis* and *Ficus carica* in Xinjiang. Urumqi: Xinjiang Agricultural University; 2022. pp. 24–33.
- Qiao LY, Huang JL, Lu XH, Liu ZM. Investigations on mulberry parasitic nematodes in Guangxi. *J South Agric.* 2011;42(11):1344–9.
- Qin YT. Studied on nematodes of onion soil and it's population changing in Jiayuguan Gansu. Lanzhou: Gansu Agricultural University; 2009. p. 21–2.
- Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Hohn S, et al. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 2012;61:539–42.
- Rybarczyk-Mydlowska K, Dmowska E, Kowalewska K. Phylogenetic studies on three *Helicotylenchus* species based on 28S rDNA and mtCOI sequence data. *J Nematol.* 2019. <https://doi.org/10.21307/jofnem-2019-033>.
- Shen C. Preliminary results of investigation of nematode disease of garden in Beijing. *ACTA Pytopathologica Sinica.* 1992;22(3):263–4. <https://doi.org/10.13926/j.cnki.apps.1992.03.022>.
- Shen D. Investigation and identification of plant-parasitic nematodes on landscape and ornamental plants in Guangzhou. Guangzhou: South China Agricultural University; 2018. p. 21–22,31–39.
- Shokoohi E, Marais M, Fourie H, Panahi H. Morphological and molecular characterization of three *Helicotylenchus* species from Iran. *Zootaxa.* 2018;4388(2):207–20. <https://doi.org/10.11646/zootaxa.4388.2.4>.
- Subbotin SA, Inerra RN, Marais M, Mullin P, Powers TO, Roberts PA, et al. Diversity and phylogenetic relationships within the spiral nematodes of *Helicotylenchus* Steiner, 1945 (Tylenchida:Hoplolaimidae) as inferred from

- analysis of the D2–D3 expansion segments of 28S rRNA gene sequences. *Nematology*. 2011;13(3):333–45.
- Subbotin SA, Vovlas N, Yeates GW, Hallmann J, Kiewnick S, Chizhov VN, et al. Morphological and molecular characterisation of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 and related species (Tylenchida: Hoplolaimidae) with a phylogeny of the genus. *Nematology*. 2015;17(1):27–52. <https://doi.org/10.1163/15685411-00002850>.
- Tang C. Studies on the plant-nematodes in south Fujian I. The species of Tylenchida. *ACTA Zool Sinica*. 1981;27(4):345–51.
- Teng WF, Tan JJ, Ye JR. Species survey of Hoplolaimidae parasites in rhizosphere soil of garden trees in Nanjing city. *J Nanjing Forestry Univ*. 2013;37(3):82–6.
- Uzma I, Nasira K, Firoza K, Shahina F. Review of the genus *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae) with updated diagnostic compendium. *Pak J Nematol*. 2015;33(2):115–60. <https://doi.org/10.18681/2015.v33.i02.p01201507310001>.
- Vrain TC, Wakarchuk DA, Levesque AC, Hamilton RI. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundam Appl Nematol*. 1992;15:563–73.
- Wang M. Identifying of cultivated plant nematodes in Hubei province (continued). *J Huazhong Agric Univ*. 1988;7(4):337–48.
- Wang SJ, Cai QJ. Investigation and identification of nematodes in the rhizosphere of *Arytomeria fortunei* in Fujian. *Acta Agric Univ Jiangxiensis*. 2005;27(1):92–5. <https://doi.org/10.13836/j.jjau.2005021>.
- Wang S, Wu X, Qiu W. Identification of six Hoplolaimidae (Nematoda: Tylenchida) species collected from the root soil of grapes. *Luoye Guoshu*. 1991;2:5–14. <https://doi.org/10.13855/j.cnki.lygs.1991.02.002>.
- Wang J, Zhang C, Wu W, Leng C, Li S, Zhang T, et al. Investigations on parasitic nematodes of vitis in Anhui. *J Anhui Agric Univ*. 1996;23(1):18–21. <https://doi.org/10.13610/j.cnki.1672-352x.1996.01.005>.
- Wang XM, Zhao HH, Liu WZ, Gao HX, Zou ZF. The identification of nematode species from rhizosphere soil of *Allium sativum* in Ji'nan and Pingdu in Shandong province. *J Qingdao Agric Univ*. 2008;25(4):285–8. <https://doi.org/10.3969/j.issn.1674-148X.2008.04.010>.
- Wang HH, Shen D, Huang SB, Zhuo K. First record of *Helicotylenchus cuspidatus* on *Bambusa chungii* in China. *Plant Prot*. 2018;44(2):75–80. <https://doi.org/10.16688/j.zwbh.2017492>.
- Wu Y, Lu A. An investigation of southern medicinal plant nematoids. *J Chinese Med Mater*. 1995;18(6):275–8. <https://doi.org/10.13863/j.issn1001-4454.1995.06.002>.
- Xia YH, Li J, Xu FF, Lei B, Li HL, Wang K, et al. Identification and a culture method for a *Helicotylenchus microlobus* from tomato in China. *BMC Zool*. 2022;7:42. <https://doi.org/10.1186/s40850-022-00144-7>.
- Xiao Y, Zhou X, Zhang S. Identification of *Helicotylenchus* and *Hoplolaimis* species parasitized banana in Fujian, China. *J Fujian Agric Forestry Univ*. 2014;43(6):573–8. [https://doi.org/10.13323/j.cnki.j.fafu\(nat.sci\).2014.06.003](https://doi.org/10.13323/j.cnki.j.fafu(nat.sci).2014.06.003).
- Xie H, Feng Z. A new species of genus *Helicotylenchus* Steiner, 1945: *Helicotylenchus membranatus* sp. nov. *Acta Phytopathologica Sinica*. 1993;23(4):337–9. <https://doi.org/10.13926/j.cnki.apps.1993.04.014>.
- Xie H, Feng Z. A new species of genus *Helicotylenchus* Steiner, 1945: *Helicotylenchus membranatus* sp. Nov. (Tylenchida: Hoplolaimidae). *ACTA Pytopathologica Sinica*. 1993;23(4):337–9. <https://doi.org/10.13926/j.cnki.apps.1993.04.014>.
- Xie H, Feng Z. Description of the five species of the genus *Helicotylenchus* from Hongkong. *J Huazhong Agric Univ*. 1996;15(1):30–3.
- Xie C, He W, Lan Q, Deng D. Nematode species parasitizing grape and their distribution in Sicuan province. *Southwest China J Agric Sci*. 1997a;10:85–9.
- Xie C, Zhao X, He W, Deng D, Lan Q. Genera and species of citrus rhizosphere nematodes in Sichuan, China. *Southwest China J Agric Sci*. 1997b;10:65–75.
- Xie ZC, Chen LF, Zhang SS. Identification of plant nematodes species on strawberry in Fujian China. *J Laiyang Agric College*. 2004;21(2):99–103.
- Xu CL, Zhou CN, Chen C, Xie H. Description of new record species of the subfamily Hoplolaiminae Filipjev of potato in China (II). *J Huazhong Agric Univ*. 2006;25(4):364–70. <https://doi.org/10.13300/j.cnki.hnlkxb.2006.04.006>.
- Xu YM, Zhao ZQ, Wang JM, Huo SY, Jiang C. Description of a new record *Helicotylenchus indicus* in Shanxi province China. *J Shanxi Agric Univ*. 2013;33(6):517–9. <https://doi.org/10.13842/j.cnki.issn1671-8151.2013.06.013>.
- Yang YB. Identification of parasitic nematode species and investigation on nematode diseases of longan, litchee and guava in Fujian, China. *Fuzhou: Fujian Agriculture and Forestry University*; 2013. p. 142–6.
- Yang Q, Wu H, Wu X, Lin F, Yang Z. *Helicotylenchus bambesae* was firstly recorded in Anhui province. *Nematol Res China*. 2018;7:24–27.
- Ye W. Plant parasitic nematodes in Guangdong province. *Animal Plant Quarantine*. 1994;1:48–52.
- Yin Y, Gao X, Feng Z. Investigation of parasitic nematodes on lychee in Guangdong province. *J South China Agric Univ*. 1994;15(3):22–7.
- Yu J. Investigation and identification of soil nematodes species in Hainan province. *Guangzhou: South China Agricultural University*; 2019. p. 31–42.
- Yuan H, Zhao CK, Zhao HH. Identification of the species in the Hoplolaiminae (Nematoda) from Shandong province. *J Qingdao Agric Univ*. 2007;24(2):120–2. <https://doi.org/10.3969/j.issn.1674-148X.2007.02.011>.
- Zameleh FM, Karegar A, Ghaderi R, Hesar AM. Morphological and molecular characterisation of *Helicotylenchus ciceri* n. sp. and *H. scoticus* Boag & Jairajpuri, 1985 (Nematoda: Hoplolaimidae) from Iran. *Nematology*. 2020;22(6):611–26. <https://doi.org/10.1163/15685411-00003326>.
- Zhang S, Chen Y. Studies on nematodes of Bayberry (*Myrica rubra*). *J Fujian Agric Univ*. 1994;23(2):172–4.
- Zhang SL, Ding L. Identification of *Helicotylenchus* species parasitizing in Taiwan *Morus laevigata*. *Fujian Agric Sci Technol*. 2016;7:9–11. <https://doi.org/10.13651/j.cnki.fjnykj.2016.07.004>.
- Zhang X, Wang D, Shi A, Li Q. The investigation and identification of plant-parasitic nematodes on ornamental plants in Baotou Area. *For Res*. 1998;11(2):175–8. <https://doi.org/10.13275/j.cnki.lykxyj.1998.02.011>.
- Zhang S, Xiao R, Lin N, Ai H. Identification of parasitic nematode species from Chinese olive in Fujian, China. *J Fujian Agric Forestry Univ*. 2002;31(4):445–51.
- Zhang S, Lin G, Chen T, Zhang S. Identification of parasitic nematode in roots of *Chamaedorea elegans*. *J Fujian Agric Forestry Univ*. 2013;42(2):143–8. [https://doi.org/10.13323/j.cnki.j.fafu\(nat.sci\).2013.02.011](https://doi.org/10.13323/j.cnki.j.fafu(nat.sci).2013.02.011).
- Zhao H, Liu W, Duan Y, Liu Y. Identification of the species in the Hoplolaiminae from northern area in China. *J Shenyang Agric Univ*. 1996;27(3):190–5.
- Zheng JW, He SS, Oian RT, Li DB. Description of three species in genus *Helicotylenchus* (Nematoda: Hoplopamidae) on potted landscapes from Zhejiang China. *J Zhejiang Agric Univ*. 1998a;24:563–6.
- Zheng J, He S, Qian R, Li D. Description of three species in Genus *Helicotylenchus* (Nematoda: Hoplopamidae) on potted landscapes from Zhejiang, China. *J Zhejiang Agric Univ*. 1998b;24(5):563–6.
- Zheng XL, Wu WJ, Ruan ZY, Jin XX, Li DP, Xie H. Description of two species of the genus *Helicotylenchus* from *Chamaedorea elegans* and *Calathea koyana*. *J Shanghai Jiaotong Univ (chin Ed)*. 2015;33(3):9–13. <https://doi.org/10.3969/j.issn.1671-9964.2015.03.002>.
- Zhou G. Identification of two Hoplolaimidae species. *ACTA Agriculturae Shanghai*. 1996;12(2):77–80.
- Zhou CN, Li Y, Chen C, Xu CL, Xie H. Two new geological record species of the genus *Helicotylenchus* in China. *J Zhongkai Univ Agric Technol*. 2005;18(4):29–31. <https://doi.org/10.3969/j.issn.1674-5663.2005.04.007>.
- Zhou YL, Guo YL, Zhang W, Hu XQ. Preliminary description on meggranate parasitical nematodes in Yunnan. *Acta Agric Univ Jiangxiensis*. 2007;29(6):937–9. <https://doi.org/10.13836/j.jjau.2007195>.