A new pin nematode, *Paratylenchus sinensis* n. sp. (Nematoda: Paratylenchinae) in the rhizosphere of White Mulberry from Zhejiang Province, China

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Short Title: *Paratylenchus sinensis* n. sp. from China

Abstract Pin nematode species are widely distributed and known to be associated with damage to a variety of shrubs, grasses, fruit and flowering trees. So far, no pin nematode species has been reported from Mulberry in China. During this study, a new *Paratylenchus* species recovered from the rhizosphere of mulberry tree, in Zhejiang Province is described and molecularly characterized. The species can be characterized by having swollen and slender females, four incisures in the lateral field, lip region conoid with anterior end flattened and without submedian lobes. The slender females have stylet 56.2 (50-62) μm long, excretory pore located at the level of nerve ring; vulval region with small advulval flap; tail conoid gradually tapers to form a finely pointed terminus. The swollen females comparatively larger than slender females, vulval lips enlarged, protruding and bulges out from the body contour, the tail is shorter than the slender female ending in a finely pointed terminus. The male is devoid of stylet, spicule 17.5 (16.8-18.2) μm long. Phylogenetic analysis based on partial 18S, ITS and D2-D3 expansion segments of the 28S rRNA gene sequences revealed that this new species grouped within paratylenchid species clade. Morphologically this new species belongs to the Group 10 of *Paratylenchus sensu lato* (stylet > 40 μm , 4 lateral lines and advulval flaps present).

Keywords *Gracilacus*, characterization, identification, molecular, morphology, SEM, phylogeny, taxonomy

Introduction

Mulberry is a common horticultural crop several areas of China. It adds value for the local farming communities such as sericulture (Liu and Wilson 2013), animal forage, paper and textile industry (Flora of China 2013), root bark for traditional medicine, and fruit production (Liu and Wilson 2016). In addition to providing economic returns, plantations of mulberry are considered important for stabilizing soils (He et al. 2007; Liu 2011; Qin et al. 2010). During the nematode inventory survey of Zhejiang Province, China, a population of unidentified long stylet pin nematode was recovered from the rhizosphere of Mulberry. Although no aboveground or root symptoms were detected in mulberry plants, soil infestation was high and prompted us to carry out an integrative taxonomic study to identify the species within this complex genus.

Pin nematodes of family Paratylenchidae are obligate ectoparasites of shrubs, grasses, fruit and flowering trees (Siddiqi 2000). These species are distributed across different climatic zones and environments (Raski 1976; Esser 1992; Ghaderi et al. 2014; Maria et al. 2018a; Li et al., 2019). Some of the species were reported to be pathogenic and causing reduced root system and poor growth, notable examples are *P. bukowinensis* Micoletzky 1922 on parsley and celery (Brzeski 1975; Brzeski & Radzikowska 1980); *G. epacris* (Allen & Jensen 1950) Goodey, 1963 on walnut, *G. capitatus* (Adams & Eichenmuller 1962) Siddiqi & Goodey 1964 on oak, *G. hamicaudatus* (Cid del Prado Vera & Maggenti 1988) Brzeski, 1998 on redwood, *P. projectus* Jenkins 1956 on alfalfa (Webster et al. 1967) and *P. shenzhenensis* Wang, Xie, Li, Xu, Yu & Wang 2013 on flamingo lily (Wang et al. 2013).

The taxonomy of pin nematodes has been intensively revised since the 1960s, the genus *Gracilacus* was erected by Raski (1962) for those *Paratylenchus* species having stylet length more than 48 µm. The validity of the genus *Gracilacus* has been questioned several times. The species were either synonymized to *Paratylenchus* (Brzeski 1963; Siddiqi and Goodey 1964; Geraert 1965; Brzeski 1998; Nguyen et al. 2004; Decraemer and Hunt 2006; Ghaderi et al. 2014), or treated as subgenus (Siddiqi 2000) or maintained (Raski 1976; Raski and Luc 1987; Maggenti et al. 1988; Raski 1991; Esser 1992; Andrassy 2007).

Previously, morphological identification alone rendered difficulties for this group due to their small sizes, overlapping diagnostic characters and uniformity in general appearances (Ghaderi et al. 2014), however, the DNA based strategies are sensitive and precise (i.e., any developmental

stage can be utilized for sequencing) so their use makes it possible to overcome limitations of morphological variation. Several nematologists successfully applied the 18S, 28S, and ITS sequences and provided useful taxonomic insights by studying the phylogenetic relationships among paratylenchid species (Van Den Berg et al. 2014; Yu et al. 2016; Maria et al. 2018a).

Paratylenchid nematodes are widely distributed in China, so far more than 30 species have been reported from different provinces (Maria et al. 2018a), 12 of them having long stylets (*P. aculentus* (Brown 1959) Raski 1962; *P. audriellus* Brown 1959; *P. bilineatus* (Brzeski 1995) Brzeski 1998; *P. epacris*; *P. guangzhouensis* Wang, Li, Xie, Xu & Wu 2016; *P. goodeyi* (Oostenbrink 1953) Raski 1962; *P. latescens* Raski 1976; *P. nanjingensis* Wang, Xie, Li, Wu and Xu 2014; *P. paralatescens* Maria, Cai, Powers, Ye, Zheng 2018; *P. raskii* Phukan & Sanwal 1979; *P. steineri* (Golden, 1961) Raski 1962; and *P. straeleni* (de Coninck, 1931) Raski 1976).

During this study, the examined nematode population was identified as a member of *Paratylenchus*. The population contains slender and swollen females and comparatively higher number of juveniles. The morphological characterization revealed that the species has aduvulval flap, four lateral lines, stylet longer than 40 µm and it belongs to the Group 10 of *Paratylenchus sensu* Ghaderi et al. (2014). The species was compared with all related species and described herein as a *Paratylenchus sinensis* n. sp.

Materials and methods

Nematode population sampling, extraction and morphological identification

Nematodes were extracted from soil samples using a modified Cobb sieving and flotation-centrifugation method (Jenkins 1964). For morphometric studies, nematodes were killed and fixed with hot formalin and processed to glycerine (Seinhorst 1959) as modified by De Gisse (1969). The drawings, measurements and light micrographs of nematodes were made with a Nikon Eclipse Ni-U 931845 compound microscope. For SEM examination, nematodes were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde, washed three times in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in a series of ethanol solutions and critical-point dried with CO₂. After mounting on stubs, the samples were coated

with gold at 6 to 10-nanometer thickness and the micrographs were made at 3 to 5 kV operating system (Maria et al. 2018b).

DNA extraction, PCR and sequencing

DNA samples were prepared according to Zheng et al. (2003). Four sets of primers (synthesized by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the near full-length 18S, expansion segments of 28S, and ITS region of rRNA. Nearly full length 18S region was amplified with two sets of primers 18s39F (5'-AAAGATTAAGCCATGCATG-3') and 18s977R (5'-TTTACGGTTAGAACTAGGGCGG-3'), the second set was 18s900F (5'-AAGACGGACTACAGCGAAAG-3') and 18s1713R (5'-TCACCTACAGCTACCTTGTTACG-3'), (Olson et al. 2017). Primers for amplification of ITS were TW81 (5'-GTTTCCGTAGGTGGTGAACCTGC3') and AB28 (5' ATATGCTTAAGTTCAGCGGGT3') (Joyce et al. 1994), the forward D2A (5' - ACAAGTACCGTGAGGGAAAGTTG-3') and the reverse D3B (5' - TCGGAAGGAACCAGCTACTA - 3') primers for amplification of D2-D3 28S (De Ley et al. 1999). PCR conditions were as described by Ye et al. (2007) and Powers et al. (2010). PCR products were evaluated on 1% agarose gels stained with ethidium bromide. PCR products of sufficiently high quality were sent for sequencing by Invitrogen, Shanghai, China. The newly obtained sequences were submitted to the GenBank database under accession numbers indicated on the phylogenetic trees.

Phylogenetic analyses

Newly obtained sequences of *P. sinensis* n. sp. (D2-D3 expansion segments of 28S, ITS and partial 18S rRNA) and the available sequences of other pin nematodes obtained from NCBI. These sequences were used for phylogenetic reconstructions of paratylenchid species. Outgroup taxa for the dataset were chosen according to previous published data (Van den Berg et al. 2014; Maria et al. 2018a). Multiple alignments of the different sequences were made using the FFT-NS-2 algorithm of MAFFT v. 7.205 (Kato & Standley 2013). Sequence alignments were manually visualized using BioEdit (Hall 1999) and edited by Gblocks ver. 0.91b (Castresana 2000) in Castresana Laboratory server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html)

141 using options for a less stringent selection (minimum number of sequences for a conserved or a
142 flanking position: 50% of the number of sequences +1; maximum number of contiguous non-
143 conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half).
144 Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using
145 MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The best-fit model of DNA evolution was
146 obtained using JModelTest V.2.1.7 (Darriba et al. 2012) with the Akaike Information Criterion
147 (AIC). The best-fit model, the base frequency, the proportion of invariable sites, and the gamma
148 distribution shape parameters and substitution rates in the AIC were then given to MrBayes for
149 the phylogenetic analyses. Unlinked general time-reversible model with invariable sites and a
150 gamma-shaped distribution (GTR + I + G) was used for the D2-D3 expansion segments of 28S
151 rRNA, partial 18S, and GTR + G model for ITS. These BI analyses were run separately per
152 dataset using four chains for 2×10^6 generations for all of the molecular markers. A combined
153 analysis of the three genes was not undertaken due to some sequences not being available for all
154 species. The Markov Chains were sampled at intervals of 100 generations. Two runs were
155 conducted for each analysis. After discarding burn-in samples and evaluating convergence, the
156 remaining samples were retained for further analyses. The topologies were used to generate a
157 50% majority-rule consensus tree. Bayesian posterior probabilities (BPP) are given on
158 appropriate clades. Trees from all analyses were visualized using FigTree software V.1.42
159 (<http://tree.bio.ed.ac.uk/software/figtree/>).

160

161 **Results and description**

162

163 *Paratylenchus sinensis* * n. sp. (Figs 1-5, Table 1)

164

165 Young or slender female: Body slender, ventrally arcuate when heat relaxed; cuticle finely
166 annulated; lateral field with four incisures; SEM en face view showed smooth lip region without
167 submedian lobes, oral aperture slit-like with two prominent ridges laterally; amphidial apertures
168 narrow; lip region conoid with anterior end flattened, continuous with the rest of the body; labial
169 framework sclerotization weak; stylet flexible, ventrally curved, cone *ca.* 90% of the total stylet
170 length; stylet knobs rounded; dorsal pharyngeal gland opening 4.0-5.0 μm behind stylet knobs;

* The specific epithet is formed from the Latinised name of the country of origin.

171 median pharyngeal bulb elongate, bearing distinct large valve; isthmus short slender, surrounded
172 by nerve ring; basal bulb pyriform, pharyngeal-intestinal valve inconspicuous; excretory pore
173 situated at the level of nerve ring; hemizonid immediately posterior to excretory pore; gonad
174 short, prodelphic, spermatheca rounded filled with sperm; vulva a transverse slit occupying half
175 of the corresponding body width, vulval lips not protruding with advulval flaps (only discernible
176 under SEM), post uterine sac absent; anus indistinct; tail slender, conoid, finely annulated,
177 gradually tapers to form a finely pointed terminus.

178

179 Mature or swollen female: Body variously swollen in size, often with coiled or spiral posterior
180 region; lip region similar to slender female; stylet of majority of mature females protruding out;
181 median bulb enlarged 20.0-25.0 μm ; isthmus slender; excretory pore located at the level of nerve
182 ring; body enlarges till vulva and narrows down preceding vulva; vulval lips enlarged, protruding
183 bulges out from the body contour; advulval flaps present (discernible under SEM); anus
184 indistinct; tail smaller than in slender female, conoid, finely annulated, gradually tapers to form a
185 finely pointed or rounded terminus.

186

187 Male: Body more slender than female, tapering towards both ends, posterior region ventrally
188 arcuate when heat relaxed; cuticle smooth with fine annulations; labial region similar to that of
189 female but narrower, continuous with body, sclerotization in labial region weak; stylet lacking;
190 pharynx rudimentary, procorpus, metacarpus, and basal bulb inconspicuous; excretory pore
191 located 64.6 (54.7-77.6) μm away from anterior end; testis outstretched, with small spermatozoa;
192 spicule slender, slightly curved; gubernaculum curved; bursa absent; tail cylindrical, tapering
193 gradually to a finely pointed tip.

194

195 Juveniles: Only two forms were detected, the early life-stage juveniles lack stylet representing
196 under-develop pharynx components; anus indistinct; posterior body ends with a finely rounded
197 terminus; the other life-stage was observed after moulting of early stage juvenile; this stage of
198 individuals have stylet and all components of pharynx but only shorter than that of the adult
199 females; genital primordial under develop; anus indistinct; posterior body ends with a finely
200 rounded or pointed terminus.

201

Type host and locality

The type specimens were extracted from the rhizosphere of white mulberry (*Morus alba* L.) at Haining County, Jiaxing City, Zhejiang Province, P. R. China on May 2019. The geographical position of the sampling site is (120°50'0" E; 30°28'43" N).

Type material

Holotype female, 15 slender females, 3 male paratypes, 7 swollen females and juveniles (slide numbers ZJU-24-01-ZJU-24-13) were deposited in the nematode collection of Zhejiang University, Hangzhou, China. 5 slender females, 2 male paratypes and 3 swollen females were deposited in Nematode collection in the Institute for Sustainable Agriculture, CSIC, Córdoba, Spain.

Diagnosis and relationships

The new species can be characterized by having swollen and slender females, four incisures in the lateral field, advulval flap and lip region conoid with anterior end flattened, submedian lobes absent. The slender females have stylet 56.2 (50-62) μ m long, excretory pore located at the level of nerve ring; tail conoid gradually tapering to a finely pointed terminus. The swollen females comparatively larger than slender ones, with enlarged median bulb; vulval lips enlarged, protruding and bulges out from the body contour, tail shorter than in the slender female ending in a finely pointed terminus. The male devoid of stylet, spicules 17.5 (17.0-18.0) μ m long. Based on the 4 lateral lines, presence of advulval flap and stylet longer than 40 μ m the new species belong to the Group 10 of *Paratylenchus sensu* Ghaderi et al. (2014). The group contains 22 nominal species however, with the proven validity of *P. audriellus* by Esmaeili et al. (2016) now this group consists of 23 species.

The new species was compared with all species of Group 10, however, it can easily be differentiated from *P. anceps* Cobb 1923; *P. capitatus*; *P. epacris*; *P. intermedius* Raski 1962; *P. mirus* Raski 1962; *P. paraperaticus* Kashi, Karegar & Kheiri 2009; *P. peraticus* Raski 1962 and *P. verus* Brzeski 1995 by having shorter stylet viz. less than 70 μ m.

It can further be differentiated from *P. aonli* Misra & Edward 1971; *P. brasiliensis* Huang &

233 Raski 1986; *P. hamicaudatus* and *P. longilabiatu*s (Huang & Raski 1986) Brzeski, 1998 by the
234 position of excretory pore. For the new species, it is located at the level of nerve ring however, in
235 the aforementioned species it is at the level of median bulb valve or anterior. In addition to this *P.*
236 *crenatus* Corbett 1966 is the only species of the Group 10 where excretory pore is located
237 opposite the end of basal pharyngeal bulb.

238 Based on the stylet length and position of excretory pore the species comes close to *P.*
239 *audriellus*; *P. corbetti* (Ganguly & Khan 1990) Siddiqi 2000; *P. goodeyi*; *P. ivorensis* Luc & de
240 Guiran 1962; *P. marylandicus* Jenkins 1960; *P. pandatus* (Raski 1976) Siddiqi 1986; *P. parvulus*
241 (Raski 1976) Siddiqi 1986; *P. straeleni*; *P. robustus* Wu 1974.

242 The new species can be differentiated from: *P. audriellus* by swollen female life stage (present
243 vs absent), lip morphology (conoid with anterior end flattened vs truncated), vulval flap
244 (indistinct vs distinct), female tail terminus morphology (conoid with finely pointed tip vs claw-
245 like terminus with sharply pointed tip), lower V = 76.8 (74.8-79.6) vs 81.4 (80-84) and shorter
246 spicules 17.5 (17.0-18.0) vs 19.7 (19.0-20.0) μ m; *P. corbetti* by having smooth cuticle of mature
247 female vs punctuations and tubercles, submedian lobes (absent vs present), the wider body of
248 slender females a = 26.2 (21.6-31.2) vs 10-14, a shorter tail in males c = 12.4 (11.3-13.4) vs 12-19
249 and shorter spicules length 17.5 (17.0-18.0) vs 25.0-28.0 μ m; *P. goodeyi* by the lip morphology
250 (conoid with anterior end flattened vs rounded), spermatheca morphology (rounded vs elongated
251 three times longer than the corresponding body diameter), male stylet (absent vs present), shorter
252 spicules length 17.5 (17.0-18.0) vs 21.0 μ m and the length of mature female (same or slightly
253 longer vs stouter); *P. ivorensis* by swollen female life stage (present vs absent), lip morphology
254 (conoid with anterior end flattened vs rounded), vulval flap (indistinct vs distinct), slender female
255 tail terminus morphology (conoid with finely pointed tip vs rounded, conoid or hook shape
256 terminus) and shorter spicules length 17.5 (17.0-18.0) vs 20.0 μ m; *P. marylandicus* by swollen
257 female life stage (present vs absent), vulval flap (indistinct vs distinct), cloacal extension of
258 cuticle (absent vs cuticle bulges out at spicules level), male tail morphology (cylindrical tapering
259 to a pointed terminus vs stubby conoid tail with rounded terminus), shorter body length of female
260 313 (272.5-351.5) vs 390 (340-420) μ m, shorter stylet length 56.2 (50-62) vs 66.7 (63-71.4), and
261 shorter spicules length 17.5 (17.0-18.0) vs 19.5 μ m; *P. pandatus* by swollen female life stage
262 (present vs absent), lip morphology (conoid with anterior end flattened vs rounded), submedian
263 lobes (absent vs present), vulval flap (indistinct vs distinct), spermatheca morphology (rounded

264 vs elongated double longer than the corresponding body diameter), shorter body length of female
 265 313 (272.5-351.5) vs 380 (330-420) μm , shorter stylet length 56.2 (50-62) vs 66 (63-70) μm ,
 266 distance of excretory pore from anterior end 80.6 (72-88.6) vs 103 (94-119) μm , and shorter
 267 spicule length 17.5 (16.8-18.2) vs 20 (19-22) μm ; *P. parvulus* by swollen female life stage
 268 (present vs absent), lip morphology (conoid with anterior end flattened vs disc shape), submedian
 269 lobes (absent vs present), vulval flap (indistinct vs distinct), tail terminus morphology (conoid
 270 with finely pointed tip vs bluntly rounded) and males (present vs absent); *P. straeleni* by swollen
 271 female life stage (present vs absent), lip morphology (conoid with anterior end flattened vs
 272 rounded), posterior uterine branch (absent vs present), higher V value = 76.8 (74.8-79.6) vs 85
 273 (80-82), vulval flap (indistinct vs distinct), shorter spicule length 17.5 (17.0-18.0) vs 19.0-21.0
 274 μm and the early juveniles of new species are without stylet vs stylet present in all juvenile
 275 stages; *P. robusta* by lip morphology (conoid with anterior end flattened vs rounded),
 276 rudimentary posterior uterine branch (absent vs present), lower V value = 76.8 (74.8-79.6) vs 81-
 277 85, longer tail 30.2 (25.2-34.2) vs 19-26 μm and the male (present vs absent) and tail
 278 morphology (conoid with finely pointed tip vs rounded or notched with depression near tip); *P.*
 279 *yokooi* (Toida, Ohshima & Hirata 1983) Brzeski 1998 by lip morphology (conoid with anterior
 280 end flattened vs truncated), submedian lobes (absent vs present), the position of excretory pore
 281 (at the level of nerve ring vs more anterior), vulval flap (indistinct vs distinct), tail terminus
 282 morphology (finely pointed tip vs subacute). Excretory pore distance of swollen female 75.7
 283 (73.077.5) vs 37 (32-40) μm , wider body of swollen female $a = 26.2$ (21.6-31.2) vs 11 (8-13).
 284

285 *Molecular characterization and phylogenetic relationships of P. sinensis n. sp. with other*
 286 *Paratylenchus species*

288 The new species was molecularly characterized using partial 18S, D2-D3 segments of 28S, and
 289 ITS region of the rDNA gene and deposited in the GenBank. Based on the phylogenetic analysis
 290 of the 18S gene (Fig.6), *Paratylenchus* species formed two highly and moderately supported clades
 291 with 1.00 and 0.91 BPP, respectively. *Paratylenchus sinensis* n. sp. (MN129018-MN129019) is
 292 related with *P. colinus* (Huang & Raski 1986) Brzeski 1988 (KP966494) and both species are in a
 293 clade with *P. shenzhenensis* (KJ636431, KF6684494-KF668504) and *P. variatus* Jairajpuri 1982
 294 (AY919230) (possible syn. of *P. aquaticus* for van den Berg et al. 2014). The pairwise sequence

identity of *P. sinensis* n. sp. with aforementioned species are 95-98% with 29-89 nucleotide differences.

The 28S gene (Fig. 7) analysis indicated that *P. sinensis* n. sp. (MN129026-MN129029) was separated from all other *Paratylenchus* spp. and showed a certain relationship with *Paratylenchus* species having long stylet i.e. *P. audriellus* (KU291238), *P. colinus* (KP966492), *P. paralatescens* (MH200616), *P. wuae* Yu, Ye & Powers 2016 (KM061782) and an unidentified *Paratylenchus* sp. from Nanjing (KR232932). The pairwise sequence identities of *P. sinensis* n. sp. with the aforementioned species are 86-90 % with 72-82 nucleotide differences.

Similar to 18S tree, the ITS gene tree indicated that the *Paratylenchus* species formed two clades (Fig.8). *Paratylenchus sinensis* n. sp. (MN129021-MN129024) clustered within second clade, in a subclade that represents *Paratylenchus* species having long stylet i.e. *P. nanjingensis*, *P. aculentus*, *P. paralatescens*, *P. guangzhouensis*, *P. bilineatus*, and *P. wuae*. The pairwise sequence identities of *P. sinensis* n. sp. with the aforementioned species are 82-85 % with 84-110 nucleotide differences.

Our phylogenetic analysis revealed that the long stylet *Paratylenchus* species are not monophyletic. The new species only grouped with long stylet *Paratylenchus* species in the 28S gene analysis indicating the conserved nature of this gene, while in the ITS gene analysis the new species arranged separately and within short stylet *Paratylenchus* species clade. The genus *Paratylenchus* constitutes more than 100 species but only a dozen of species have been provided with molecular sequences. To this point, we postulate that due to the limited number of available sequences the relationship between long and short paratylenchids is not well resolved.

Discussion

Several diagnostic compendium and keys have been proposed for *Paratylenchus* species identification (Raski 1991; Esser 1992; Brzeski 1998), recently Ghaderi et al. (2014) proposed a grouping scheme based on stylet length, number of lateral lines and presence/absence of vulval flap. This scheme works conveniently in terms of morphological identification but this grouping does not correspond with phylogenetic positioning of the species. The *P. sinensis* n. sp. has a longer stylet (56.2 (50-62), 4 lateral lines and a small advulval flap. In our phylogenetic analyses some of the species having long stylet (>40 μ m) showed paraphyly in all three ribosomal gene trees (Figs

6-8). It is also noted that several other long stylet species (i.e. *P. aculentus*, *P. audriellus*, *P. bilineatus*, *P. colinus*, *P. guangzhouensis*, *P. nanjingensis*, *P. paralatescens* and *P. wuae*) grouped together and form a highly supported clade regardless with the number of lateral lines, or presence of vulval flap. To this point, we postulate that phylogenetic grouping of *Paratylenchus* species might not only correspond with the stylet length.

Several *Paratylenchus* species were described as having swollen females of considerable sizes (Raski 1962, 1976; Toida et al. 1983; Huang & Raski 1986; Cid del Prado Vera & Maggenti 1988). It is also observed that the body swelling of *Paratylenchus* species is a relative character and is most likely to detect swollen females in species described with only non swollen females (Esser 1992). The swollen females have been detected for *P. sinensis* n. sp. however, none of the long stylet species that have been molecularly characterized in recent years were described with swollen females.

Paratylenchids represent a significant variation in stylet length, ranges from 10-120 μ m. The stylet length is related to the feeding habit, the short stylet species are epidermal feeder while long stylet species are cortical feeder (Siddiqi 2000). The difference in stylet length may originate from the different food source or species plasticity.

In China, the long stylet *Paratylenchus* species were reported from Asian elm, bamboo, camellia, grapevine, Japanese white birch, *Pinus* spp. and medicinal herb (*Arabis pendula* L.). The detection of several life-stages of *P. sinensis* n. sp. in the mulberry rhizosphere suggesting this new species is associated with the host. However, detailed research is needed to explore the extent of damage that this species could pose on the mulberry growth.

In conclusion, the current study presents the integrative identification of *P. sinensis* n. sp. recovered from the rhizosphere of mulberry. Based on the morphology the species belong to the group 10 of *Paratylenchus* species *sensu* Ghaderi et al. (2014). The phylogenetic analysis of *P. sinensis* n. sp. is not well resolved therefore it is presumed that the extension of molecular analysis with inclusion of more species will be helpful for recovering phylogenetic relationships and evolutionary phylogenies. In addition to this, special concern should be directed toward pin nematodes identification as occurrence of cryptic species and divergences between species have been listed in this group of nematodes (Akyazi et al. 2015; Esmaeili et al. 2016).

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Compliance with Ethical Standards

Conflict of interest All the authors certify that 1) do not have any actual or potential conflict of interest, 2) the study described is original and has not been published previously, and is not under consideration for publication elsewhere, 3) all prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected. We also certify that all authors have reviewed the manuscript and approved the final version of the manuscript before submission.

Research Involving Human Participants and/or Animals No specific permits were required for the described fieldwork studies. The samples from wild plants were obtained in public areas, forests, and other natural areas studied and do not involve any species endangered or protected in China. The sites are not protected in any way.

Informed Consent All the authors certify that the work carried out in this research followed the principles of ethical and professional conduct have been followed. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Figure legends

Fig. 1. Line drawings of *Paratylenchus sinensis* n. sp. A-C: Anterior region of slender female, male and early juvenile, respectively; D, E: Slender female vulval region (SEM); F: Slender female vulval region (LM); G: Posterior region of male showing gonad; H: Posterior region of slender female showing gonad; I: Male tail; J, K: Slender female tails; L: Tail region of swollen female; M: posterior region of swollen female showing gonad; N-Q: Variation in body shapes of swollen females (Scale bars = A-K=10 μ m; L-O =40 μ m).

Fig. 2. Light photomicrographs of *Paratylenchus sinensis* n. sp. A: Entire male body; B: Entire slender female body; C-D: Pharyngeal region of slender female, arrows showing position of excretory pore (exp); E, F: Pharyngeal region of male; G, H: Vulval regions; I: Male tail; J, K: Tail regions of slender female; L: Posterior region of slender female showing gonad; M: Posterior region of male showing gonad (Scale bars = A, B=40 μ m, B-I =40 μ m; L-O =10 μ m). Abbreviations (exp= excretory pore; sp= spermatheca; v= vulva; a=anus).

Fig. 3. Light photomicrographs of swollen female of *Paratylenchus sinensis* n. sp. A: Anterior region; B-C: Variation in body shapes; D-E: Anterior region with protracted stylets; F: Posterior region showing gonad; G-I: Vulval regions (Scale bars = B, C=40 μ m, A, D-I=10 μ m). Abbreviations (exp= excretory pore; sp= spermatheca; v= vulva; a=anus).

Fig. 4. Light photomicrographs of *Paratylenchus sinensis* n. sp. A, B: Juveniles; C: Entire slender female body; D: Entire male body; E-F: Swollen females (Scale bars = A-F =40 μ m).

Fig. 5. Scanning Electron Micrographs of *Paratylenchus sinensis* n. sp. female. A-B: *En face* view of slender females; C: *En face* view of swollen female; D-F: Vulval regions of slender females; G: Posterior region of slender female; H: Anus of slender female; I: Lateral lines; J, K: Posterior region of swollen female; (Scale bars = A-D, F, I=5 μ m; E, H= 10 μ m; G, J=20 μ m; K=30 μ m). Abbreviations (v= vulva; a=anus).

Fig. 6. Phylogenetic relationships within populations and species of the genus *Paratylenchus* as

inferred from Bayesian analysis using the partial 18S rRNA gene sequence dataset with the GTR + I + G model. Bayesian posterior probability more than 0.70 is given for appropriate clades. Newly obtained sequences are indicated in bold.

Fig. 7. Phylogenetic relationships within populations and species of the genus *Paratylenchus* as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequence dataset with the GTR + I + G model. Bayesian posterior probability more than 0.70 is given for appropriate clades. Newly obtained sequences are indicated in bold.

Fig. 8. Phylogenetic relationships within populations and species of the genus *Paratylenchus* as inferred from Bayesian analysis using the ITS rRNA gene sequence dataset with the GTR + G model. Bayesian posterior probability more than 0.70 is given for appropriate clades. Newly obtained sequences are indicated in bold.

Table 1. Morphometrics of *Paratylenchus sinensis* n. sp. female. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

	holotype	Paratype				
	Female	slim Females	males	obese Females	Juveniles	J4 or pre adult
n	1	20	5	10	6	5
Body length	304.8	313.2 $\pm$ 24.5 (272.5-351.5)	297.8 $\pm$ 26.2 (277.0-334.8)	338.5 $\pm$ 30.5 (300.4-378.1)	261.8 $\pm$ 4.9 (254.6-268.6)	263.6 $\pm$ 6.7 (256.2-269.1)
a	25.4	26.2 $\pm$ 2.7 (21.6-31.2)	30.5 $\pm$ 3.3 (26.6-34.5)	19.3 $\pm$ 2.3 (15.8-22.4)	-	23.3 $\pm$ 1.3 (22.1-25.1)
b	2.9	2.9 $\pm$ 0.2 (2.6-3.2)	-	3.6 $\pm$ 0.4 (3.2-4.4)	-	2.4 $\pm$ 0.1 (2.2-2.5)
c	9.9	10.4 $\pm$ 1.2 (8.2-12.6)	12.4 $\pm$ 0.8 (11.3-13.4)	12.9 $\pm$ 1.2 (10.6-15.1)	-	9.0 $\pm$ 0.4 (8.7-9.5)
c'	0.3	0.2 $\pm$ 0.0 (0.2-0.3)	3.0 $\pm$ 0.3 (2.5-3.4)	0.4 $\pm$ 0.1 (0.4-0.5)	-	0.3 $\pm$ 0.0 (0.2-0.3)
V/T	76.0	76.8 $\pm$ 1.4 (74.8-79.6)	34.1 $\pm$ 4.1 (28.0-37.2)	78.2 $\pm$ 1.2 (76.6-80.0)	-	77.2 $\pm$ 1.3 (75.5-78.4)
Lip height	2.1	2.1 $\pm$ 0.2 (1.7-2.7)	1.7 $\pm$ 0.2 (1.5-2.0)	2.7 $\pm$ 0.3 (2.3-3.0)	-	2.5 $\pm$ 1.1 (1.7-4.3)
Lip width	4.9	5.0 $\pm$ 0.4 (4.3-5.7)	3.8 $\pm$ 0.2 (3.6-4.1)	6.5 $\pm$ 0.6 (5.8-7.3)	-	4.3 $\pm$ 1.2 (2.1-5.1)
Stylet	55.7	56.2 $\pm$ 4.0 (50-62)	-	54.0 $\pm$ 4.7 (46.5-61.0)	-	49.6 $\pm$ 3.3 (44.7-53.2)
Stylet %	18.3	18.0 $\pm$ 0.9 (15.2-19.1)	-	16.0 $\pm$ 1.6 (13.4-19.0)	-	19.4 $\pm$ 0.4 (18.8-19.8)
Median bulb Length	16.9	19.1 $\pm$ 2.1 (14.2-22.3)	-	21.5 $\pm$ 1.8 (20.3-24.6)	-	18.0 $\pm$ 1.3 (16.3-19.1)
Median bulb width	7.1	8.3 $\pm$ 1.3 (6.5-10.6)	-	11.0 $\pm$ 0.7 (9.7-11.5)	-	6.4 $\pm$ 0.7 (5.8-7.4)
Excretory pore	78.2	80.6 $\pm$ 4.8 (72.0-88.6)	64.6 $\pm$ 8.5 (54.7-77.6)	75.7 $\pm$ 2.7 (72.6-77.5)	64.7 $\pm$ 5.0 (55.5-69.5)	70.9 $\pm$ 2.0 (68.9-72.8)
Pharynx	104.2	107.6 $\pm$ 6.0 (96.1-119.0)	-	93.5 $\pm$ 8.0 (80.2-107.8)	69.5 $\pm$ 6.7 (57.1-75.4)	109.6 $\pm$ 3.6 (105.6-115.5)
Body diam.	12	12.1 $\pm$ 1.3 (9.5-14.2)	9.7 $\pm$ 1.0 (8.2-10.6)	17.7 $\pm$ 1.6 (15.5-20.0)	13.2 $\pm$ 1.5 (10.7-14.7)	11.5 $\pm$ 0.8 (10.2-12.4)
Vulval body diam.	10.6	10.6 $\pm$ 1.2 (8.8-13.1)	-	15.1 $\pm$ 1.4 (14.3-18.0)	-	10.0 $\pm$ 1.0 (9.2-11.4)
Anal/Cloacal body diam.	8.3	7.4 $\pm$ 0.8 (6.0-8.3)	8.0 $\pm$ 0.5 (7.3-8.7)	7.3 $\pm$ 0.8(6.5-8.6)	-	7.6 $\pm$ 0.8 (7.0-8.5)
From vulva to tail term.	73.1	72.4 $\pm$ 5.2 (60.7-83.2)	-	73.4 $\pm$ 5.3 (63.7-79.7)	-	60.3 $\pm$ 3.8 (56.6-65.8)
Tail	30.9	30.2 $\pm$ 2.6 (25.2-34.2)	24.2 $\pm$ 3.2 (21.2-29.5)	26.3 $\pm$ 1.9 (23.5-29.6)	-	28.5 $\pm$ 1.3 (26.3-29.3)
Spicule length	-	-	17.5 $\pm$ 0.6 (16.8-18.2)	-	-	-
Gubernaculum	-	-	4.3 $\pm$ 0.6 (3.4-5.1)	-	-	-