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Steinernema populi n. sp. (Panagrolaimomorpha, Steinernematidae), a new entomopathogenic nematode species from China

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Abstract

Steinernema populi n. sp. was recovered by baiting from beneath poplar trees in China. Morphological and molecular features provided evidence for placing the new species into the *Kushidai* clade. The new species is characterized by the following morphological features: third-stage infective juveniles (IJ) with a body length of 1095 (973–1172) µm, a distance from the anterior end to excretory pore of 77 (70–86) µm and a tail length of 64 (55–72) µm. The Body length/Tail length (*c*) ratio and Anterior end to Excretory pore/ Tail length × 100 (*E*%) of *S. populi* n. sp. are substantially greater than those of all other '*Feltiae–Kushidai–Monticolum*' group members. The first-generation males can be recognized by a spicule length of 66 (57–77) µm and a gubernaculum length of 46 (38–60) µm. The new species is further characterized by sequences of the internal transcribed spacer and partial 28S regions of the ribosomal DNA. Phylogenetic analyses show that *Steinernema akhursti* and *Steinernema kushidai* are the closest relatives to *S. populi* n. sp.

Introduction

Entomopathogenic nematodes (EPNs) of the family Steinernematidae Travassos, 1927 are lethal obligate pathogens of insects with a worldwide distribution (Poinar, 1979; Adams *et al*., 2007; Lis *et al*., 2021). Steinernematids have been successfully applied as commercial applications against many insect pests, such as scarab larvae in turf and lawns, insect pests in protected horticulture, mole crickets in lawns and turf, and black vine weevils in nursery plants (Lacey & Georgis, 2012; Shapiro-Ilan *et al*., 2020).

Steinernematidae comprises two genera: *Steinernema* and *Neosteinernema*. To date, more than 100 steinernematid species have been described from all continents except Antarctica, and this number is still growing (Cimen *et al*., 2016; Chaubey & Aasha, 2021). Based on phylogenetic analyses, family *Steinernematidae* can be divided into 12 multispecies clades: '*Affine*', '*Bicornutum*', '*Cameroonense*', '*Carpocapsae*', '*Costaricense*', '*Feltiae*', '*Glaseri*', '*Karii*', '*Khoisanae*', '*Kushidai*', '*Longicaudum*' and '*Monticolum*' (Spiridonov & Subbotin, 2016).

In recent years, a total of 17 steinernematids have been reported from China, of which two belong to the *Affine* clade: Steinernema bedding Qiu, Hu, Zhou, Pang and Nguyen 2005 and S. sichuanense Mráček, Nguyen, Tailliez, Boemare and Chen, 2006; one to Bicornutum-clade, namely, *S. ceratophorum* Jian, Peid and Hunt, 1997; five to Feltiae-clade, namely, *S. hebeiense* Chen, Li, Yan, Spiridonov and Moens, 2006; *S. xueshanense* Mráček, Liu and Nguyen, 2009; *S. xinbinense* Ma, Chen, Clercq, Waeyenberge, Han and Moens, 2012; *S. tielingense* Ma, Chen, Li, Han, Khatri-Chhetri, Clercq and Moens, 2012 and *S. cholashanense* Nguyen, Půža and Mráček, 2008; one to Glaseri-clade, *S. caudatum* Xu, Wang and Li, 1991; two to Karii-clade, namely, *S. aciari* Qiu, Zhou, Nguyen and Pang 2005 and *S. leizhouense* Nguyen, Qiu, Zhou and Pang, 2006a; one to Kushidai-clade, namely, *S. akhursti* Qiu, Hu, Zhou, Mei, Nguyen and Pang, 2005; four to Longicaudum-clade, namely, *S. longicaudum* Shen and Wang, 1992; *S. guangdongense* Qiu, Fang, Zhou, Pang and Nguyen 2004; *S. pui* Qiu, Zhao, Wu, Lv and Pang, 2011 and *S. taiwanensis* Tseng, Hou and Tang, 2018; and one to Monticolum-clade, namely, *S. changbaiense* Ma, Chen, Clercq, Han and Moens, 2012.

Table 1. Details on taxa used in the molecular analyses.

Nematode species			GenBank accession no.	
Species name	Isolate name	Geographic origin	ITS	28S
S. abbasi	S-01	India	AY248749	FJ935791
S. affine	B1	UK	AF331912	AF331899
S. akhursti	YNb112	China	DQ375757	AY177188
S. anatoliense	Al-Jubiha	Jordan	–	GU569043
S. apuliae	CS3	Italy	HQ416968	–
S. apuliae	Type	Apulia	–	GU569044
S. arasbaranense	IRAZ21	Iran	FJ860039	–
S. arenarium	Voronezh	Russia	DQ314288	AF331892
S. ashiunense	Type	Japan	DQ354694	FJ165550
S. beddingi	YNc174	China	AY603397	–
S. bicornutum	Type	Yugoslavia	AF121048	AF331904
S. boemarei	Grand Travers	France	FJ152414	GU569046
S. braziliense	Porto Murtinho	Brazil	–	FJ410326
S. carpocapsae	A24	China	GU395621	–
S. carpocapsae	NCR	Czech	–	KJ950292
S. ceratophorum	Type locality	China	AY230165	AF331888
S. changbaiense	JM-2011	China	JN865168	–
S. cholashanense	Tibet	China	EF431959	EF520284
S. cubanum	Pinar del Rio	Cuba	AY230166	–
S. diaprepesi	FL	USA	AF122021	GU569048
S. eapokense	Type locality	Vietnam	AY487921	–
S. everestense	104,185	Nepal	–	HM000104
S. feltiae	Malka	Jordan	EU200355	–
S. feltiae	Bodega Bay	California, USA	–	AF331906
S. glaseri	NC	USA	–	AF331908
S. glaseri	85,011	China	GU395635	–
S. guangdongense	GDc339	China	AY170341	AY169558
S. hebeiense	G6	China	DQ105794	–
S. hermaphroditum	T87	Indonesia	JQ687355	–
S. ichnusae	Sardinia	Italy	EU421129	–
S. innovationi	SGI-60	South Africa	KJ578793	KJ578794
S. intermedium	SC	USA	AF331916	AF331909
S. jeffreyense	J194	South Africa	KC897093	–
S. jollieti	–	Monsanto	–	GU569051
S. khoisanae	SF80	South Africa	DQ314287	GU569052
S. kraussei	Westphalia	Germany	AY230175	AF331896
S. kushidai	Hamakita	Shizuoka, Japan	AB243440	AF331897
S. longicaudum	CF1 VII	USA	AY230177	AF331901
S. loci	18	China	GQ497740	–
S. leizhouense	Type	China	AY170340	–
S. monticolum	Korea	South Korea	AF122017	–
S. monticolum	Type	USA	–	EF439651

(*Continued*)

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Table 1. (*Continued.*)

Nematode species			GenBank accession no.	
Species name	Isolate name	Geographic origin	ITS	28S
S. nguyeni	F2	South Africa	KP325084	–
S. oregonense	Oregon	USA	AF122019	–
S. oregonense	OS-10	USA	–	AF331891
S. pakistanense			AY230181	–
S. phyllophagae	Type strain	Florida, USA	FJ410327	FJ666054
S. puertoricense	Loiza	Puerto Rico	–	AF331903
S. puntauvense	Li 6	Costa Rica	–	EF187018
S. populi n. sp.	72-1	China	MZ367621	MZ367685.2
S. poinari	Tomsk	Czech Republic	KF241753	–
S. rarum	J1-USA	USA	DQ221116	–
S. riobrave	Type locality	USA	DQ835613	–
S. sangi	Type	Vietnam	AY355441	
S. siamkayai	T9	Thailand	AF331917	–
S. silvaticum	S16/056	Polish	MG543846	–
S. scapterisci	Type	China	–	GU395646
S. surkhetense	CS19	India	–	KU187262
S. tophus	ROOI-352	South Africa	KJ701241	KJ701240
S. texanum	Texas 28S	USA	–	EF152569
S. tielingense	Type locality	China	GU994201	–
S. thermophilum	SBIH1	Indian	MF919610	–
S. websteri	Peru strain	Peru	–	GU569058
S. weiseri	Turkey strain	Turkey	–	GU569059
S. xinbinense	LFS8	China	JN171593	GU994204
S. xueshanense	Yunnan	China	FJ666052	FJ666053
S. yirgalemense	Type	Ethiopia	AY748450	–
Caenorhabditis elegans	–	–	X03680	–
Panagrellus redivivus	JB-129	USA	–	AF331910

A recent survey was carried out from 2017 to 2020 in Jilin Province, China, to determine the occurrence and distribution of EPN. The survey resulted in the recovery of more than 40 isolates of EPN, with only one isolate of an unknown *Steinernema* being described as a new species in the current study. We utilized light microscopy (LM) and scanning electron microscopy (SEM) for morphological observation and morphometric analysis, as well as molecular observations, to fully describe and illustrate this new species.

Materials and methods

Nematode isolation and rearing

The nematode isolate was recovered from soil samples collected from the rhizosphere of a poplar tree (*Populus* sp.) in Yushu city (44°59′30″N, 126°10′58″E), Jilin Province, China, during a survey in 2019. Collected soil samples (approximately 2 dm^3 vol.) were placed into plastic bags. Five last-instar *Galleria mellonella* (L.) larvae were placed into a small steel mesh pocket and put in these bags with soil, two pockets per plastic bag. These samples were transported to the laboratory.

For taxonomic studies, different life stages of *S. populi* were obtained from infected last-instar *G. mellonella* larvae exposed to 100 IJs/insect in a 15 cm-diameter Petri dish lined with moistened filter paper and kept in the dark at 25°C. The *G. mellonella* larvae died within 48 h after inoculation and insect cadavers turned reddish-brown after 2–5 days. After they died, the insect cadavers were transferred to a modified White trap (Kaya & Stock, 1997) and incubated at 25°C until IJ emerged.

First- and second-generation adults were obtained by dissecting infected insects approximately three days and five days, respectively, after the death of the host. The cadavers were dissected in Ringer's solution.

LM and SEM

For morphometric analysis, measurements were made on specimens fixed in triethanolamine formalin (TAF) (Courtney *et al.*,

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Table 2. Morphometrics of *Steinernema populi* n. sp. All measurements are in μm and take the form: mean ± standard deviation (range).

	First generation			Second generation		
	Male		Female	Male	Female	Infective juvenile
Character	Holotype	Paratypes (n = 25)	Paratypes (n = 25)	Paratypes (n = 25)	Paratypes (n = 25)	Paratypes (n = 25)
Body length (L)	1319.5	1378.4 ± 76.3 (1257.7–1514)	7025.9 ± 2586.9 (4038–13,762)	1184.5 ± 62.9 (1048–1287.2)	3271.5 ± 325.8 (2709.8–3930)	1094.6 ± 45.7 (973–1172)
Maximum body diameter (W)	81.1	82.3 ± 9.1 (66.3–95)	283.0 ± 66.5 (217–531)	69.9 ± 5.6 (60.1–80.9)	183.0 ± 21.1 (139–221)	36.3 ± 2.3 (32.6–41.4)
Anterior end to Excretory pore (EP)	106	107.8 ± 7.6 (94.9–121)	120.4 ± 22.0 (90.1–178)	94.7 ± 9.1 (75.3–114)	126.0 ± 12.5 (103–152)	76.7 ± 3.9 (70.0–86.1)
Anterior end to Nerve ring (NR)	127	126.9 ± 8.7 (107–143)	173.7 ± 14.1 (150–213)	117.6 ± 8.6 (97–133)	169.6 ± 15.3 (140–205)	106.2 ± 4.2 (97.7–113)
Oesophagus base (ES)	157	156.6 ± 12.5 (131–177)	241.3 ± 20.8 (213–278)	151.8 ± 12.2 (119–173)	224.2 ± 15.3 (202–251)	148.7 ± 6.1 (134–159)
Tail length (T)	49.2	50.9 ± 5.6 (39.2–68.)	62.2 ± 12.7 (41.3–87.5)	46.5 ± 4.8 (34.2–53.5)	64.3 ± 7.3 (46.8–78.6)	63.8 ± 5.0 (54.7–71.8)
Anal body width (ABW)	47.4	51.3 ± 5.1 (41.2–60.4)	93.4 ± 26.5 (60.1–157)	45.5 ± 1.8 (42.1–51.)	52.7 ± 7.3 (39.9–65.8)	22.6 ± 1.3 (21.1–26.5)
Spicule length (SpL)	57.4	66.0 ± 5.6 (57.4–76.6)	–	63.8 ± 6.4 (44.6–73.7)	–	–
Gubernaculum length (GuL)	44	46.2 ± 4.4 (38.1–60)	–	45.4 ± 3.9 (38.2–54.5)	–	–
GS% = (GuL/SpL) ×100	76.7	70.2 ± 7.3 (57.7–82.4)	–	71.5 ± 6.7 (61.5–85.9)	–	–
SW% = (SpL/ABW) ×100	121.1	129.3 ± 11.3 (107.4–159.5)	–	140.3 ± 14.7 (100.7–164.3)	–	–
Width at vulva	–	–	308.3 ± 79.4 (224–576)	–	193.3 ± 26.9 (144–249)	–
V% = vulva/L	–	–	51.5 ± 3.2 (44.7–60.1)	–	51.8 ± 2.3 (48.6–58.1)	–
a = L/W	16.3	16.9 ± 1.5 (14.8–20.2)	24.3 ± 5.0 (17.8–35.6)	17.0 ± 1.2 (14.6–19.4)	18.0 ± 2.1 (14–22.5)	30.3 ± 1.3 (28.0–32.8)
b = L/ES	8.4	8.9 ± 0.6 (7.7–10.1)	28.6 ± 8.5 (18.5–49.9)	7.8 ± 0.7 (6.8–9.9)	14.6 ± 1.4 (11.2–17.1)	7.4 ± 0.4 (6.8–8.5)
c = L/T	26.8	27.3 ± 2.8 (19.8–32.9)	111.7 ± 29.5 (74.9–181.5)	25.8 ± 3.3 (20.9–33.1)	51.2 ± 6.2 (41.2–65.1)	17.3 ± 1.4 (14.5–19.8)
c' = T/W	1.1	1.0 ± 0.1 (0.8–1.5)	0.7 ± 0.1 (0.5–0.9)	1.0 ± 0.1 (0.8–1.2)	1.2 ± 0.2 (0.9–1.5)	2.8 ± 0.3 (2.4–3.3)
D% = (EP/ES) × 100	67.5	69.1 ± 5.1 (59.3–78.4)	50.0 ± 8.7 (35.8–64.5)	62.6 ± 6.2 (47.4–73.1)	56.3 ± 4.7 (48.1–65.9)	51.6 ± 3.5 (46.8–60.7)
E% = (EP/T) × 100	215.4	213.6 ± 22.4 (170.6–273.6)	198.9 ± 43.4 (110.9–312.3)	206.1 ± 33.0 (145.9–288.6)	198.2 ± 30.7 (152.3–275.6)	120.7 ± 8.2 (105.2–139.5)
Hyaline tail (H)	–	–	–	–	–	21.9 ± 2.1 (17.5–24.9)
H% = (H/T) × 100	–	–		–	–	34.5 ± 4.0 (26.2–43.7)

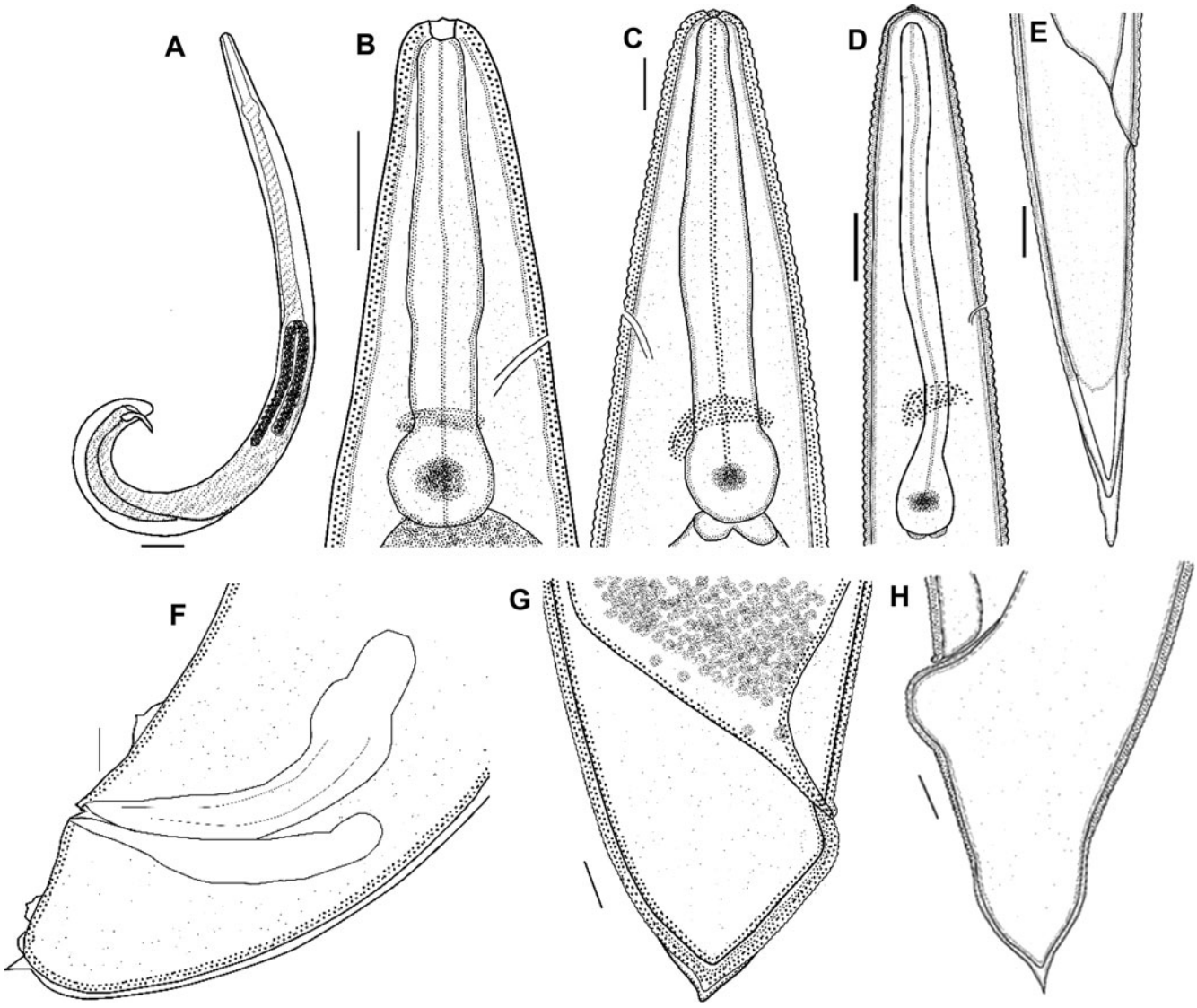

Fig. 1. *Steinernema populi* n. sp. line drawings. First-generation male: (a) total body, lateral view; (b) anterior end (lateral view), showing stoma region; (f) tail (lateral view), showing part of genital papillae, spicules and gubernaculum. First-generation female: (c) anterior end (lateral view), showing stoma region; (g) tail, lateral view. Second-generation female: (h) tail with mucron. Third-stage infective juvenile: (d) anterior end, lateral view, pharyngeal region, nerve ring and excretory pore; (e) tail, lateral view. Scale bars: (a) 75 μm; (b–d, g) 25 μm; (e, f, h) 10 μm.

1955) and mounted in anhydrous glycerine on slides (Seinhorst, 1959). Light microphotographs of the fresh individuals and mounted specimens were prepared using a Leica Microsystems TCS SP8 compound microscope (Mannheim, Germany). Micrographs were taken with an automatic camera system (Leica Application Suite X) mounted on a Leica microscope. Drawings and morphological analyses were performed using a drawing tube attached to an Olympus BHA light microscope (Tokyo, Japan) and Adobe Illustrator software version 2017 (Adobe, USA).

Adults of the first generation and IJ were fixed in 4% formalin buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 h at 8°C. They were post-fixed with 2% osmium tetroxide solution for 12 h at 25°C, dehydrated in a graded ethanol series, critical-point dried with liquid carbon dioxide, mounted on SEM stubs and coated with gold (Nguyen & Smart, 1995). Specimens were measured and photographed with the aid of a Regulus 8100 (Hitachi, Tokyo, Japan).

Molecular characterization

DNA was extracted from single females, using a modification of a method reported by Nguyen (2007a). The nematode was placed in 30 μl lysis buffer (50 mM magnesium chloride, 10 mM Dithiothreitol (DTT), 4.5% Tween 20, 0.1% gelatine and 1 μl proteinase K at 60 μg/ml) on the side of a 500 μl microcentrifuge tube, where it was cut into two or more pieces under a dissecting microscope. After being immediately stored at −80°C for at least 15 min, the tube was then incubated at 65°C for 1 h and then at 95°C for 10 min. After centrifugation for 2 min at 12,000 rpm, the supernatant (20 μl) was transferred to a clean microcentrifuge tube, where it was kept at −20°C.

A ribosomal DNA (rDNA) fragment containing the internal transcribed spacer (ITS) regions ITS1 and ITS2, and the 5.8S rRNA gene was amplified by polymerase chain reaction using the forward primer TW81: 5′-GTTTCCGTAGGTGAACCTGC-3′ and the reverse primer AB28: 5′-ATATGCTTAAGTTCAGCGGGT-3′ (Joyce *et al.*, 1994). The other fragment containing D2–D3 expansion segments of the 28S rDNA gene was amplified using the forward primer

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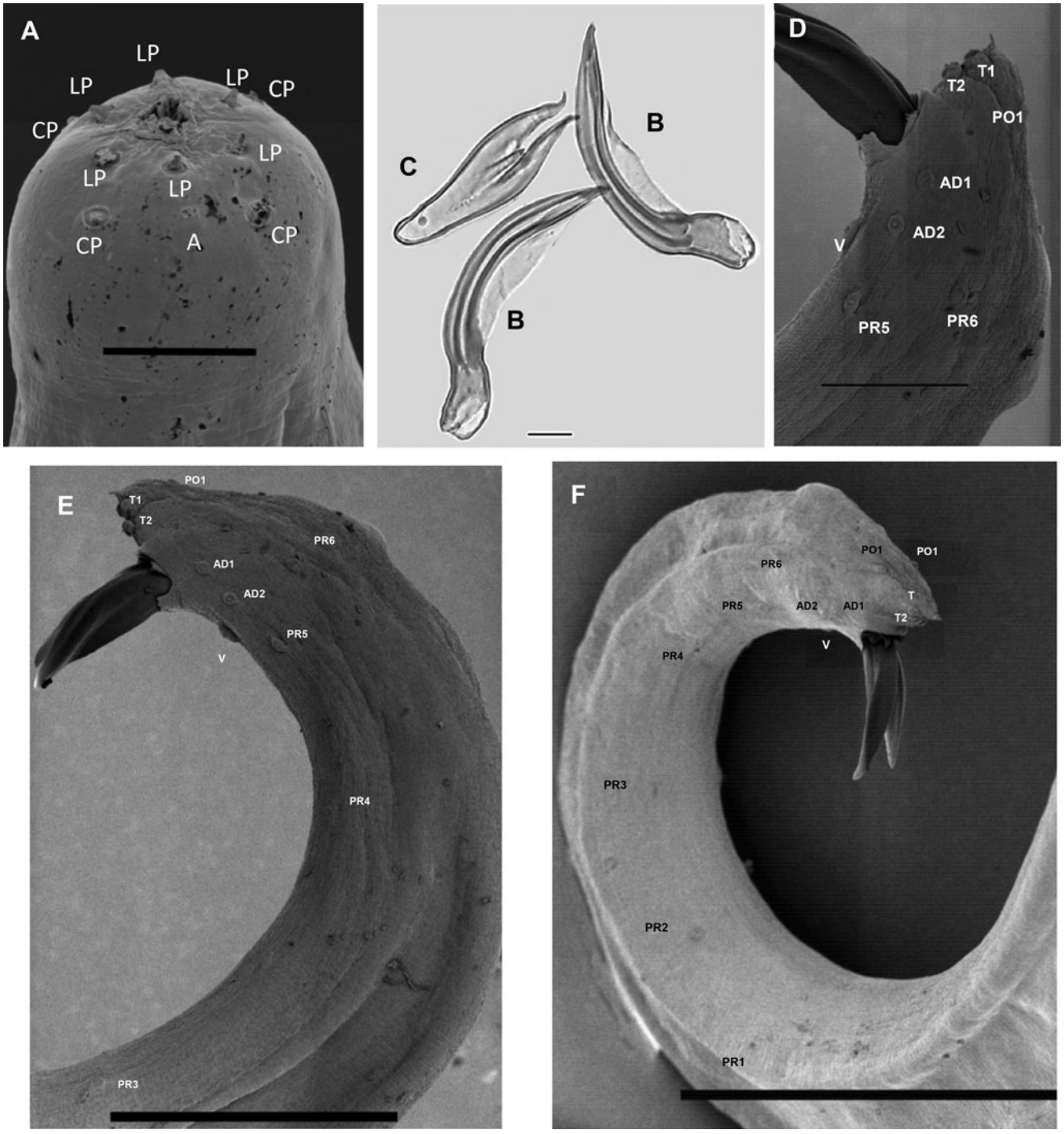

Fig. 2. *Steinernema populi* n. sp. LM and SEM photographs. First-generation male: (a) *en face* view, labial papillae (LP), cephalic papillae (CP), amphid (a); (b, c) spicule and gubernaculum; (d–f) tail region, showing 11 pairs of papillae and single ventral papilla, adanal papilla 1 (AD1); adanal papilla 2 (AD2); ventral papilla (V); terminal papillae1 (T1); terminal papillae 2 (T2); post-anal papillae 1 (PO1); post-anal papillae 2 (PO2); pre-anal papillae1–6 (Pr 1–6). Scale bars: (a) 5 μm; (b, c) 10 μm; (d) 20 μm; (e) 50 μm; (f) 100 μm.

D2F: 5′-CCTTAGTAACGGCGAGTGAAA-3′ and the reverse primer 536: 5′-CAGCTATCCTGAGGAAAC-3′ (Nguyen, 2007a).

Phylogenetic analysis

The newly obtained ribosomal DNA sequences of the ITS and D2–D3 regions of 28S were deposited in the GenBank (Altschul *et al.*, 1997) (table 1).

Multiple sequence alignments were produced by the default ClustalW configuration included in MEGA 7 and optimized manually in BioEdit (Hall, 1999). Pairwise distances were computed using MEGA 7 (Kumar *et al.*, 2016). *Caenorhabditis elegans* (X03680) and *Panagrellus redivivus* (AF331910) were used as the outgroup taxa for ITS and 28S phylogenetic analyses, respectively.

ModelFinder (Kalyaanamoorthy *et al.*, 2017) was used to select the best-fitting model using the Akaike information criterion.

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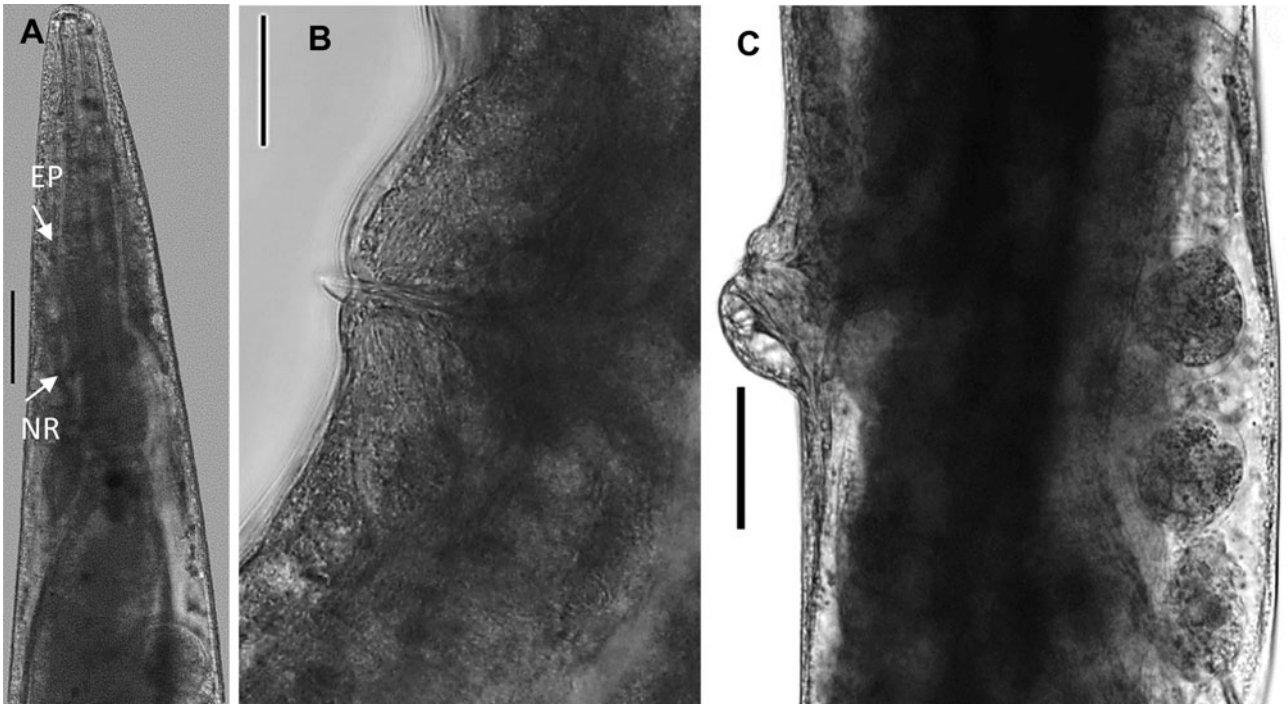

Fig. 3. *Steinernema populi* n. sp. LM and SEM photographs. First-generation female: (a) anterior region, showing pharynx, excretory pore (EP) and nerve-ring (NR) (arrow), lateral view; (b) vulva, lateral view. Second-generation female: (c) vulva, lateral view. Scale bars: (a, c) 50 µm; (b) 25 µm.

Bayesian inference (BI) analysis of the ITS and D2–D3 regions of 28S under the GTR + F + G4 and GTR + F + I + G4 model was employed to confirm the tree topology using MrBayes 3.2.6 (Ronquist *et al.*, 2012; Zhang *et al.*, 2020), running four chains for 1×10^7 generations. Burn-in sampled trees (25%) were discarded. The Markov chain Monte Carlo method was used within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) and generate a 50% majority-rule consensus tree. The software FigTree version 1.4.3 (Edinburgh, UK)was used to display and edit the trees.

Results

Description of Steinernema populi n. sp.

Measurements

The dimensions of the holotype and paratype specimens are provided in table 2.

Description

First-generation male. Body curved posteriorly, mostly J-shaped when heat-relaxed (fig. 1a). First-generation males are larger (average 1378 µm) than second-generation males (average 1185 µm). Cuticle smooth under LM, but striations visible with SEM. Head flattened, almost continuous with body contour, with six lips fused at base; each lip bearing a labial papilla. Outer circle of four cephalic papillae present. Amphidial apertures small, located posterior to lateral labial papillae (fig. 2a). Stoma shallow, narrow, usually with pronounced cheilorhabdions, posterior part funnel-shaped, well cuticularized. Pharynx with cylindrical procorpus and slightly swollen metacorpus. Nerve ring usually surrounding isthmus or anterior part of basal bulb. Excretory pore in centre of ventral side and located at ~2/3 of oesophagus distance from anterior end, rarely slightly posterior to nerve ring (fig. 1b). Testis monorchic, reflexed, consisting of germinal growth zone leading to seminal vesicle (fig. 1a). Spicules paired, symmetrical, slightly curved and yellow in colouration, distally bluntly pointed (fig. 2b, f). Manubrium of spicule, usually elongate (manubrium length/manubrium width of 1.4:1). Calomus distinct, but short. Lamina with two internal ribs, well curved. Velum extending from calomus almost to the end of lamina. Spicule terminus blunt (fig. 2b). Gubernaculum arcuate, *c.* 76% of spicule length, boat-shaped in lateral view, swollen at middle, with prominent narrow neck. Gubernaculum wings well divided; cuneus pointed (fig. 2c). Tail bluntly conoid, usually concave on ventral side; mucron sometimes absent, if developed, 1.5–2 µm long (figs 1f and 2e). Twenty-three genital papillae comprising 11 pairs and a single ventral papilla located just anterior to cloacal opening of paired papillae, five pairs subventral precloacal, one pair lateral precloacal, two pairs adcloacal subventral and three pairs postcloacal (one pair subdorsal, two pairs subventral terminal) (fig. 2d–f).

Second-generation male. General morphology similar to that of the first-generation male, but slightly smaller in body length and other morphometric values (table 2). Tail with same shape as that of the first-generation male and with or without mucron.

First-generation female. Body size varies significantly, rarely longer than 13,762 µm, usually C-shaped when heat-relaxed. Lip region and stoma region as in males (fig. 2a). Cuticle only faintly striated. First-generation females larger (average = 7026 µm) than second-generation females (average = 3272 µm). Pharynx with procorpus cylindrical and muscular, isthmus distinct, basal bulb enlarged. Nerve ring anterior to basal bulb, surrounding isthmus. Excretory pore located anterior to nerve ring (figs 1c and 3a). Reproductive system didelphic, amphidelphic, ovaries opposed, reflexed, glandular spermatheca, uterus in ventral position. Vulva in the form of transverse slit located slightly

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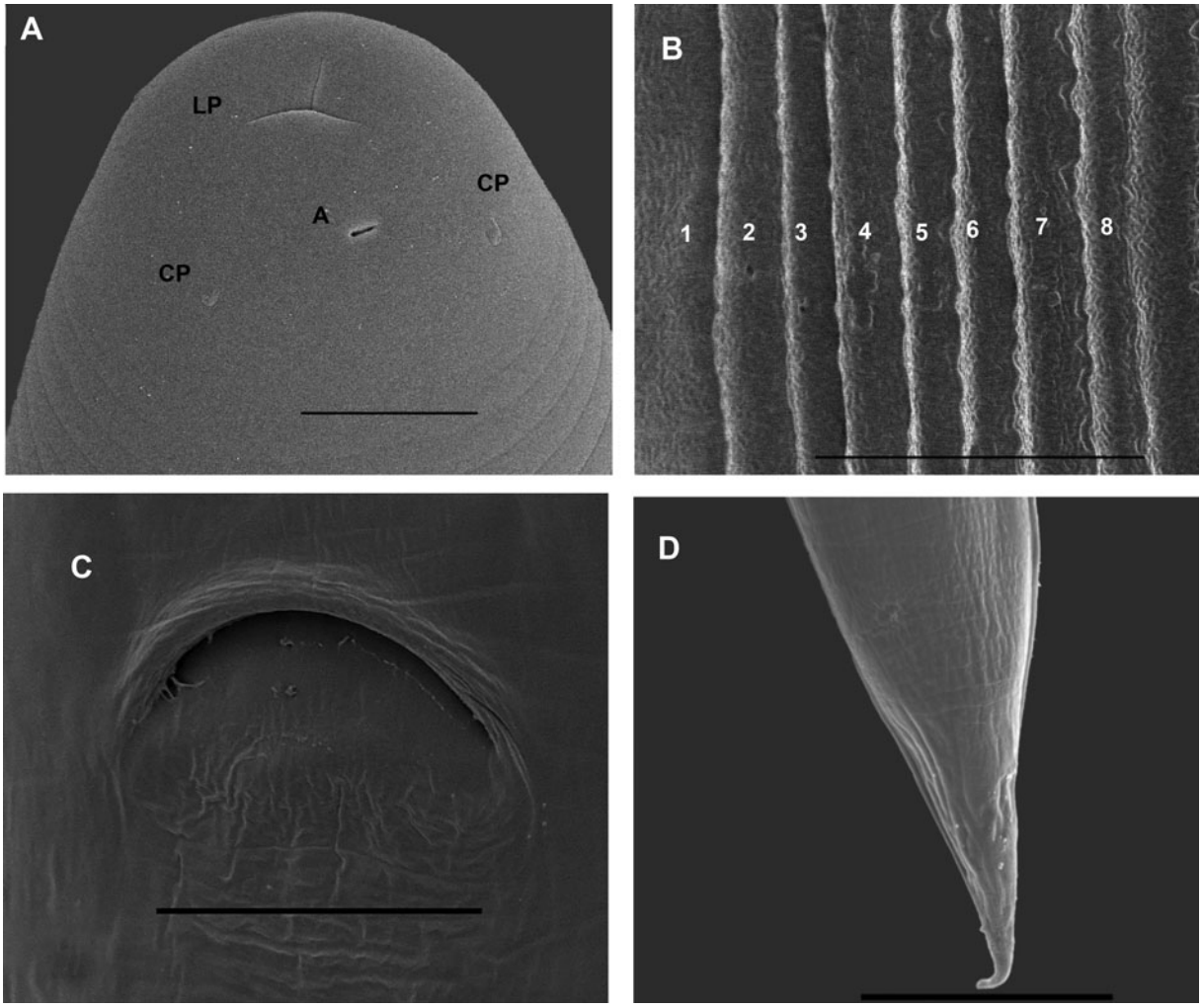

Fig. 4. *Steinernema populi* n. sp. SEM photographs. Third-stage infective juvenile: (a) *en face* view, labial papillae (LP), cephalic papillae (CP), amphid (a); (b) lateral field in mid-body, ridges numbered 1–8; (c) anus, lateral view; (d) tail, lateral view. Scale bars: (a, c) 5 μm; (b) 50 μm; (d) 20 μm.

posterior to mid-body. Vulval lips slightly protruding, asymmetrical, with larger posterior lip (fig 3b). Tail length much shorter than anal body diameter, with slight postanal swelling. Tail blunt, conoid, lacking mucro. Postanal lips usually protruding, asymmetric with anterior lip smaller than posterior (fig. 1g).

Second-generation female. Similar to first-generation female but smaller and slenderer (table 2). Vulva shape and lips similar to those of first-generation female (fig. 3c). Vulva situated at mid-body (~55%). Pygmy forms observed. Excretory pore located more posteriorly than in first-generation female. Tail tip pointed but not mucronate (fig. 1h), tail longer than body width at anus. Postanal swelling slightly developed.

Third-stage infective juvenile. Body of heat-relaxed specimens almost straight or gently curved and slightly narrowed at the anterior and posterior ends. Head region continuous with body, rounded to slightly truncate, not annulated. Four conspicuous cephalic papillae and a pair of pore-like amphidial apertures laterally (fig. 4a). Lip region smooth, continuous; stoma closed. Cuticle transversely ribbed (fig. 1d). Lateral fields consisting of eight equally spaced and developed ridges in mid-body region (fig. 4b). Lateral field formula: 2, 4, 6, 8, 6, 2. Oesophagus with narrow corpus, slightly swollen metacorpus, isthmus surrounded by nerve ring. Excretory pore in the middle between anterior end and basal bulb (fig. 1d). Hemizonid distinct. Deirids not observed. Phasmid prominent in mid-tail just ventral to lateral field. Tail conoid with pointed terminus (fig. 4d). Hyaline portion occupying *c.* 35% of tail length.

Taxonomic summary

Type material. Holotype, first-generation male; paratype, third-stage juveniles, males and females of first and second generations mounted on slides and deposited in the Nematode Laboratory, Institute of Plant Protection, Jilin Academy of Agricultural Sciences, China.

Many males and females of the first generation and several third-stage infective juveniles were deposited in the US Department of Agriculture Nematode Collection (USDA NC), Beltsville, Maryland, USA (USDANC numbers are T-7612p to T-7628p).

Several males, females and infective juveniles were deposited in the Shenyang International Nematode Collection (SINC), National Parasitic Resource Center (SINC number is 20210555) (https://www.tdrc.org.cn/), China.

Type host. The natural host is unknown.

Type locality. Steinernema populi n. sp. was recovered by baiting with *G. mellonella* larvae from soil samples collected from the

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Table 3. Comparison of morphometrics of third-stage infective juveniles of *Steinernema populi* n. sp. with other members of clade III. Measurements are in μm and take the form: mean (range).

	Morphometric character[a]													
Species	*L*	*W*	EP	NR	ES	*T*	ABW	*a*	*b*	*c*	*D*%	*E*%	*n*	Reference
S. populi n. sp.	1095 (973–1172)	36 (33–41)	77 (70–86)	106 (98–113)	149 (134–159)	64 (55–72)	23 (21–27)	30 (24–33)	7.4 (6.8–8.5)	17 (15–20)	52 (47–61)	121 (105–140)	25	Present study
S. oregonense	980 (820–1110)	34 (28–38)	66 (60–72)	–	132 (116–148)	70 (64–78)	–	30 (24–37)	7.6 (6–8)	14 (12–16)	50 (40–60)	100 (90–110)	20	Liu & Berry (1996)
S. kraussei	951 (797–1102)	33 (30–36)	63 (50–66)	105 (99–111)	134 (119–145)	79 (63–86)	–	29 (–)	7.1 (–)	12.1 (–)	47 (–)	80 (–)	25	Nguyen (2007a)
S. tielingense	915 (824–979)	35 (32–38)	69 (64–73)	98 (90–105)	128 (120–135)	81 (74–85)	21 (19–23)	26 (23–28)	7 (6–8)	11 (9–13)	55 (47–61)	88 (85–94)	20	Ma *et al.* (2012b)
S. litorale	909 (834–988)	31 (28–33)	61 (54–69)	96 (89–104)	125 (114–133)	83 (72–91)	19 (16–22)	29.5 (27–31)	7.3 (6.7–7.9)	11 (9.7–11.9)	49 (44–56)	73 (68–84)	25	Yoshida (2004)
S. ichnusae	866 (767–969)	32 (27–35)	63 (59–68)	102 (94–108)	138 (119–148)	81 (76–89)	–	28 (24–32)	6.3 (5.6–6.9)	11 (8.8–12)	46 (42–49)	77 (68–83)	20	Tarasco *et al.* (2008)
S. silvaticum	860 (670–975)	30 (26–35)	62 (51–73)	96 (75–109)	121 (100–141)	75 (63–86)	17 (15–24)	31 (27–34)	7.1 (6.3–7.7)	11.4 (9.9–13.1)	50 (46–56)	–	21	Sturhan *et al.* (2005)
S. xueshanense	860 (768–929)	30 (29–33)	67 (60–72)	91 (81–96)	135 (130–143)	87 (80–92)	19 (17–21)	28 (26–32)	6.4 (5.8–7.0)	9.9 (9.0–11)	50 (46–52)	78 (70–90)	20	Mráček *et al.* (2009)
S. feltiae	849 (766–928)	29 (22–32)	63 (58–67)	113 (108–117)	136 (130–143)	86 (81–89)	–	30 (27–34)	6.4 (5.8–6.8)	10 (9.4–11)	46 (44–50)	74 (67–81)	25	Nguyen (2007b)
S. cholashanense	843 (727–909)	30 (26–35)	62 (59–65)	87 (72–97)	125 (110–138)	73 (60–80)	17 (16–19)	28 (24–34)	6.8 (6.1–7.2)	12 (10–14)	49 (46–53)	81 (76–91)	20	Nguyen *et al.* (2008)
S. sandneri	843 (708–965)	27 (23–32)	56 (44–64)	103 (83–118)	138 (123–151)	75 (64–86)	19 (15–24)	29 (23–33)	6.1 (5.5–6.9)	11.2 (11–13.2)	40 (36–45)	74 (63–86)	25	Lis *et al.* (2021)
S. akhursti	812 (770–835)	33 (33–35)	59 (55–60)	90 (83–95)	119 (115–123)	73 (68–75)	20 (19–20)	24 (23–26)	6.8 (6.6–7.2)	11 (10–12)	47 (45–50)	77 (73–86)	20	Qiu *et al.* (2005)
S. texanum	756 (732–796)	30 (29–34)	59 (52–62)	92 (84–102)	115 (111–120)	73 (60–79)	18 (17–20)	25 (22–27)	6.5 (6.2–7.0)	10 (9.6–12.5)	51 (46–53)	81 (76–88)	20	Nguyen *et al.* (2007)
S. citrae	754 (623–849)	26 (23–28)	56 (49–64)	98 (83–108)	125 (118–137)	71 (63–81)	14 (13–17)	30 (25–34)	6.0 (5.1–7.1)	15 (13–14)	44 (39–58)	110 (85–132)	25	Stokwe *et al.* (2011)
S. sangi	753 (704–784)	35 (30–40)	52 (46–54)	91 (78–97)	127 (120–138)	81 (76–89)	18 (17–19)	22 (19–25)	5.9 (5.6–6.3)	9.3 (8.7–10.2)	40 (36–44)	62 (56–70)	50	Phan *et al.* (2001)
S. weiseri	740 (586–828)	25 (24–29)	57 (43–65)	84 (72–92)	113 (95–119)	60 (49–68)	17 (14–19)	29 (25–33)	6.6 (5.7–7.2)	12 (10–14)	51 (44–55)	95 (–)	20	Mráček *et al.* (2003)
S. nguyeni	737 (673–796)	25 (22–28)	52 (47–58)	80 (74–86)	110 (101–121)	67 (61–73)	15 (13–17)	29 (27–33)	6.7 (6.2–7.4)	11 (10–12)	48 (43–57)	79 (70–86)	25	Malan *et al.* (2016)
S. jollieti	711 (625–820)	23 (20–28)	60 (53–65)	–	123 (115–135)	68 (60–73)	–	31 (25–34)	5.7 (4.9–6.4)	10.5 (9.0–11.7)	48 (46–50)	88 (–)	25	Spiridonov *et al.* (2004)
S. xinbinense	694 (635–744)	30 (28–31)	51 (46–53)	86 (75–90)	116 (109–125)	73 (65–78)	17 (16–19)	24 (21–25)	6.1 (5–7)	9.7 (8–11)	44 (40–47)	71 (65–78)	20	Ma *et al.* (2012a)
S. puntauvense	670 (631–728)	33 (31–38)	25 (20–30)	54 (46–69)	94 (81–103)	54 (51–59)	17 (15–18)	20 (17–23)	6.1 (7.1–7.9)	12 (11–13)	42 (25–50)	44 (35–56)	20	Uribe-Lorío *et al.* (2007)
S. hebeiense	658 (610–710)	26 (23–28)	48 (43–51)	78 (73–83)	107 (100–111)	66 (63–71)	–	26 (24–28)	6.2 (5.7–6.7)	10 (9.4–11)	45 (40–50)	72 (65–80)	20	Chen *et al.* (2006)
S. kushidai	589 (424–662)	26 (22–31)	46 (42–50)	76 (70–84)	111 (106–120)	50 (44–59)	–	22.5 (19–25)	5.3 (4.9–5.9)	11.7 (10–13)	41 (38–44)	92 (–)	50	Mamiya (1988)

[a]Abbreviations as in Table 2; –, Measurements not available.

Table 4. Comparison of morphometrics of first-generation males of *Steinernema populi* n. sp. with other members of clade III. Measurements are in μm and take the form: mean (range). Data for new species in bold.

	Morphometric character[a]							
Species	SpL	GuL	W	D%	SW%	GS%	MUC	*n*
S. akhursti	90 (85–100)	64 (58–68)	131 (115–150)	56 (52–61)	180 (140–200)	71 (65–77)	P	25
S. tielingense	88 (79–98)	62 (49–70)	129 (111–159)	71 (64–78)	191 (176–212)	73 (59–82)	A	20
S. puntauvense	77 (71–81)	34 (30–40)	119 (101–139)	67 (45–85)	170 (140–200)	65 (55–75)	P	19
S. xueshanense	76 (66–91)	49 (41–60)	144 (97–159)	80 (73–87)	152 (93–172)	64 (58–95)	A	20
S. litorale	75 (67–89)	53 (44–64)	96 (82–111)	40 (34–56)	174 (154–200)	71 (62–81)	P	25
S. oregonense	71 (65–73)	56 (52–59)	138 (105–161)	73 (64–75)	151 (–)	79 (–)	A	20
S. feltiae	70 (65–77)	41 (34–47)	75 (60–90)	60 (51–64)	113 (99–130)	59 (52–61)	p	25
S. weiseri	68 (62–72)	53 (46–57)	112 (84–138)	49 (39–60)	180 (150–240)	80 (70–85)	A	20
S. cholashanense	66 (60–71)	39 (32–45)	137 (73–204)	64 (50–85)	115 (92–144)	71 (61–85)	P	20
S. ichnusae	66 (64–67)	44 (43–46)	137 (73–204)	62 (59–65)	139 (120–162)	67 (64–69)	A	20
S. nguyeni	66 (58–75)	43 (30–55)	82 (58–106)	48 (38–57)	215 (185–279)	66 (46–81)	P	20
***S. populi* n. sp.**	**66 (57–77)**	**46 (38–60)**	**82 (66–95)**	**69 (59–78)**	**129 (107–160)**	**70 (58–82)**	**P/A**	**25**
S. citrae	65 (57–80)	44 (32–59)	103 (87–113)	58 (47–67)	198 (156–233)	68 (48–89)	P	20
S. jollieti	64 (55–70)	54 (45–60)	115 (98–135)	64 (53–83)	145 (–)	84 (–)	A	12
S. kushidai	63 (48–72)	44 (39–60)	97 (75–156)	51 (42–59)	150 (–)	70 (–)	A	20
S. sangi	63 (58–80)	40 (34–46)	159 (120–225)	49 (42–63)	150 (120–160)	60 (50–70)	P	20
S. sandneri	60 (53–65)	44 (39–50)	155 (124–178)	51 (42–59)	111 (97–127)	79 (61–83)	P	25
S. texanum	60 (55–66)	45 (39–53)	99 (81–116)	67 (58–73)	157 (127–203)	75 (62–84)	A	20
S. hebeiense	57 (51–63)	46 (38–50)	86 (74–98)	51 (48–59)	140 (120–170)	80 (60–90)	A	20
S. xinbinense	56 (49–62)	35 (30–41)	103 (90–126)	45 (41–50)	137 (114–156)	63 (54–72)	P	20
S. silvaticum	51 (42–64)	37 (30–43)	65 (52–78)	60 (45–63)	155 (–)	73 (–)	P	26
S. kraussei	49 (42–53)	33 (29–37)	128 (110–144)	53 (–)	110 (–)	67 (–)	P	–

[a]Abbreviations as in table 1.
MUC, mucron; P, present; A, absent; NA, measurements not available.

rhizosphere of a poplar tree (*Populus* sp.) in Yushu city (44° 59′30″N, 126°10′58″E), Jilin Province, China.

Etymology. The specific epithet refers to the *Populus*.

Diagnoses and relationships

Steinernema populi n. sp. was characterized by the morphology and morphometrics of IJ and males (table 2). The IJ of *S. populi* n. sp. can be recognized by the largest body length of 1095 μm, oesophagus length of 149 μm, a tail length of 64 μm, ratios b = 7.4 and c = 17, E% = 121 and H% = 35 (table 2). An excretory pore is located in approximately the mid-pharynx region, and lateral field pattern is 2, 4, 6, 8, 6, 2. The male of the first generation is characterized by a curved spicule length of 66 μm, a gubernaculum length of 44 μm, maximum body diameter of 82 μm, D% = 69 and the presence of a short tail mucron in the second generation (sometimes also present in first-generation males) (table 2).

Steinernema populi n. sp. belongs to species of the '*Feltiae–Kushidai–Monticolum*' superclade, which comprises over 20 species. Phylogenetic analyses also placed *S. populi* n. sp. in the *Kushidai* clade (as proposed by Spiridonov and Subbotin, 2016). Molecular data show that within this clade, *S. populi* n. sp. is sister to the pair of *S. akhursti* and *S. kushidai*.

The IJ of *S. populi* n. sp. can be distinguished from *S. akhursti* (Qiu et al, 2005) by body length (1095 vs. 812 μm), the distance from head to excretory pore and nerve ring, which is higher, and by excretory pore, which is more posterior (D% 55 vs. 47). The oesophagus length is much longer (149 vs. 119 μm), while the tail is much shorter (64 vs. 73 μm), and it has a higher E% (121 vs. 77) (table 3). The first-generation males of *S. populi* n. sp. differ from that of *S. akhursti* by much shorter spicule and gubernaculum (66 vs. 90 μm and 46 vs. 64 μm, respectively), higher D% (69 vs. 56) and lower SW% (129 vs. 180) (table 4).

Steinernema populi n. sp. differs from *S. kushidai* (Mamiya, 1988) by its longer body length of IJ (1095 vs. 589 μm), bigger maximum body diameter (36 vs. 26 μm), longer distance from anterior end to excretory pore (77 vs. 46 μm), longer tail length (64 vs. 50 μm) and higher ratio c and higher E% (17 vs. 11.7 and 121 vs. 92, respectively) (table 3). The first-generation males of the new species differ from that of *S. kushidai* in higher D% (69 vs. 51) and lower SW% (129 vs. 150) (table 4).

The IJ of *S. populi* n. sp. differs from *S. cholashanense*, *S. hebeiense*, *S. tielingense*, *S. xinbinense* and *S. xueshanense* by the longest body length and oesophagus length, the highest ratios b, c and E%, and the shortest tail. The position of the IJ nerve ring at 106 μm is more anterior than that in *S. feltiae* at 113 μm, and

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Table 5. Pairwise analysis of the differences in base pairs of the ITS regions between closely related *Steinernema* species and *S. populi* n. sp. Data for new species in bold.

	Species	Acc. no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	***S. populi* n. sp.**	**MZ367621**	–																					
2	*S. akhursti*	DQ375757	84	–																				
3	*S. kushidai*	AB243440	103	63	–																			
4	*S. cholashanense*	EF431959	127	97	124	–																		
5	*S. xinbinense*	JN171593	131	107	130	36	–																	
6	*S. kraussei*	AY171264	133	112	133	44	38	–																
7	*S. sangi*	AY355441	133	105	131	82	90	88	–															
8	*S. jollieti*	AY171265	133	110	138	62	70	76	100	–														
9	*S. silvaticum*	MG543846	135	114	137	48	42	37	98	78	–													
10	*S. tielingense*	GU994201	136	108	135	43	41	43	89	68	54	–												
11	*S. sandneri*	MW078536	137	125	145	56	49	26	96	86	47	54	–											
12	*S. xueshanense*	FJ666052	139	106	135	33	62	69	95	85	72	67	82	–										
13	*S. texanum*	EF152568	140	107	147	68	78	77	99	85	84	71	92	86	–									
14	*S. oregonense*	AF122019	144	114	141	56	67	70	98	87	72	68	83	53	88	–								
15	*S. citrae*	EU740970	144	115	149	58	66	70	109	72	72	71	79	76	77	81	–							
16	*S. weiseri*	AY171268	147	111	145	55	60	72	103	68	75	70	84	70	78	74	37	–						
17	*S. ichnusae*	EU421129	148	113	145	56	67	75	100	73	76	77	84	69	75	75	45	31	–					
18	*S. nguyeni*	KP325084	151	123	155	66	73	79	118	84	80	78	87	82	79	88	23	45	51	–				
19	*S. litorale*	AB243441	154	115	145	64	71	83	107	67	85	80	90	75	86	85	45	28	35	50	–			
20	*S. feltiae*	AF121050	154	120	156	73	72	84	116	83	87	81	93	86	92	89	59	53	46	64	56	–		
21	*S. hebeiense*	DQ105794	172	140	167	101	105	109	139	106	108	104	117	111	108	111	84	82	86	85	80	100	–	
22	*S. monticolum*	AF122017	231	210	227	192	193	196	220	182	198	195	204	191	196	202	190	191	195	197	182	199	202	–

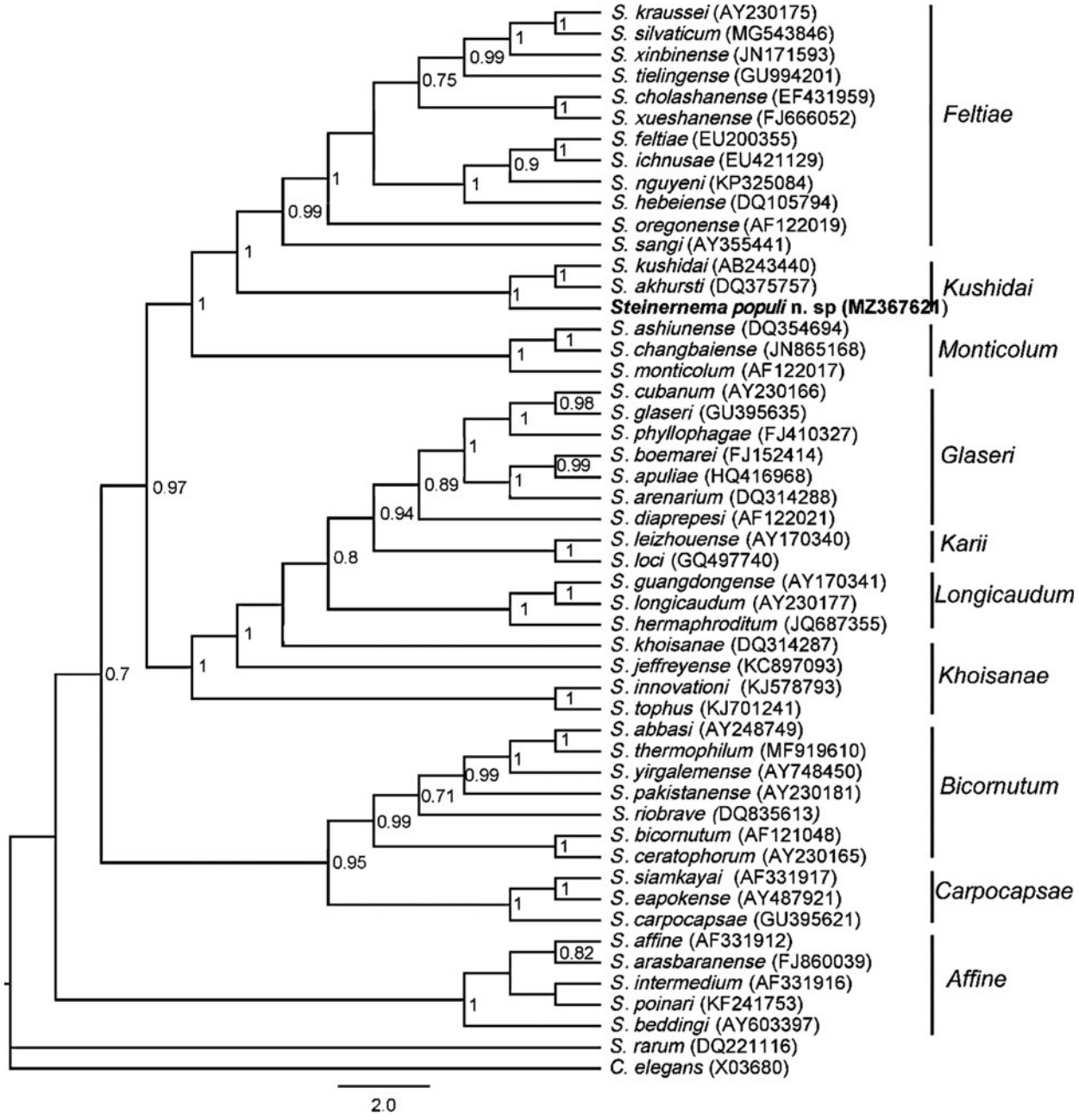

Fig. 5. Bayesian 70% majority-rule consensus tree inferred from ITS rDNA of *Steinernema populi* n. sp. under the GTR + F+G4 model. BPPs of more than 70% are given for appropriate clades. The new sequence is in bold font. The scale bar indicates the number of nucleotide substitutions per site.

posterior than that in *S. puntauvense* at 54 µm, *S. nguyeni* at 80 µm, the tail (64 µm) is longer than *S. weiseri* (60 µm) and shorter than *S. kraussei* (79 µm) (table 3). The males of *S. populi* n. sp. differ from *S. cholashanense*, *S. hebeiense*, *S. tielingense*, *S. xinbinense* and *S. xueshanense* by the slenderest maximum body diameter. The SW% is higher at 129 than that of *S. cholashanense* at 115 and lower than *S. tielingense* at 191 (table 4).

Molecular characterization and phylogenetic analysis

Steinernema populi n. sp. was characterized genetically by the sequences of the ITS (MZ367621) and D2–D3 (MZ367685.2) regions of 28S rDNA. The BLAST search performed using the ITS-rDNA sequence revealed that the highest similarity with all currently available ITS sequences of *Steinernema* is less than 90.94% with *S. akhursti* (accession number DQ375757) and 88.41% with *S. kushidai* (AB243440). Based on the 28S rDNA sequence, the new species showed 97.61% similarity to *S. weiseri* (FJ165549) and 96.26% similarity to *S. akhursti* (KF289902).

Steinernema populi n. sp. is characterized genetically by sequences of ITS and D2–D3 regions of rDNA. The sequence of ITS regions of *S. populi* n. sp. is 739 bp. Pairwise distances (table 5) showed that the new species differs from *S. akhursti*, its closest taxon, by 84 bp. Distances from the new species and others are presented in table 5.

For ITS regions, BI analysis shows that the alignment resulted in 1493 characters, of which 265 are constant, 493 variable characters are parsimony-uninformative and 735 characters

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Table 6. Pairwise analysis of the differences in base pairs of the D2–D3 regions between closely related *Steinernema* species and *S. populi* n. sp. Data for new species in bold.

	Species	Acc.no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	***S. populi* n. sp.**	**MZ367685.2**	–																					
2	*S. akhursti*	AY177188	11	–																				
3	*S. weiseri*	GU569059	11	12	–																			
4	*S. texanum*	EF152569	14	17	13	–																		
5	*S. oregonense*	AF331891	16	12	8	12	–																	
6	*S. feltiae*	GU994202	16	13	5	12	7	–																
7	*S. litorale*	JQ795723	16	15	5	14	9	4	–															
8	*S. tielingense*	AF331906	16	15	7	14	7	4	6	–														
9	*S. xueshanense*	FJ666053	16	17	11	14	12	12	14	14	–													
10	*S. ichnusae*	EU421130	17	14	6	11	8	3	5	7	12	–												
11	*S. jollieti*	GU569051	17	16	6	15	10	7	7	9	15	8	–											
12	*S. kraussei*	AF331896	18	17	11	16	10	10	12	10	12	11	15	–										
13	*S. cholashanense*	EF431959	18	21	13	16	12	10	12	8	14	13	15	11	–									
14	*S. sandneri*	MW078535	19	21	13	20	14	14	14	12	14	15	17	8	12	–								
15	*S. kushidai*	AF331897	20	17	17	26	19	18	18	18	20	19	21	19	22	20	–							
16	*S. xinbinense*	GU994204	20	21	13	18	12	10	12	8	14	13	13	10	7	9	20	–						
17	*S. hebeiense*	DQ399664	22	22	14	19	15	13	15	16	18	14	18	15	19	19	25	19	–					
18	*S. citrae*	GU004534	22	23	15	22	19	14	14	14	24	15	17	22	20	24	22	22	25	–				
19	*S. nguyeni*	KR815816	25	27	17	23	21	16	16	16	25	17	19	24	21	26	24	24	27	12	–			
20	*S. silvaticum*	MG547577	26	29	19	28	24	20	22	20	24	23	25	20	19	22	30	20	25	24	30	–		
21	*S. monticolum*	EF439651	26	28	26	28	27	29	29	29	31	30	30	31	31	33	29	33	36	37	37	43	–	
22	*S. sangi*	GU569057	42	44	46	51	48	47	48	47	47	50	50	50	47	50	37	49	53	52	52	54	48	–

Note: GS, gubernaculum length divided by spicule length; SW, spicule length divided by anal body width; V%, distance from anterior end to vulva/Body length; a, Body length/Maximum body diameter; b, Body length/Oesophagus base; c, Body length/Tail length; c′, Tail length/Maximum body diameter; D%, Anterior end to excretory pore in % of pharynx length; E%, (Anterior end to Excretory pore/ Tail length) × 100; H, Hyaline tail.

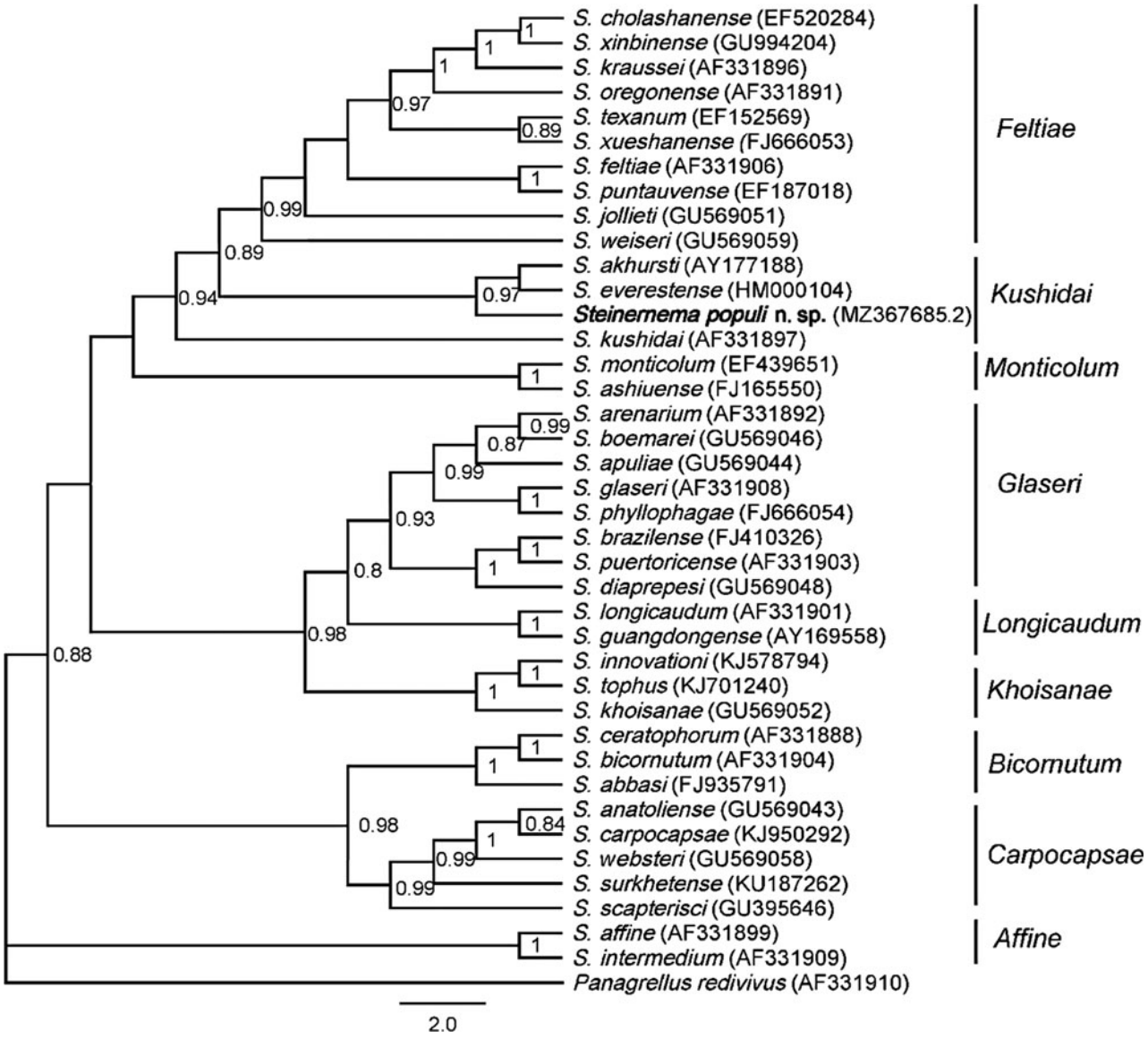

Fig. 6. Bayesian 70% majority-rule consensus tree inferred from D2–D3 regions of 28S rDNA of *Steinernema populi* n. sp. under the GTR + F+I + G4 model. BPPs of more than 70% are given for appropriate clades. The new sequence is in bold font. The scale bar indicates the number of nucleotide substitutions per site.

(included) are parsimony-informative. The phylogenetic relationships between 51 species of *Steinernema* are presented in fig. 5. In this consensus tree, 18 species of the '*Feltiae–Kushidai–Monticolum*' superclade form a monophyletic assemblage in which the new species, *S. kraussei*, *S. silvaticum*, *S. xinbinense*, *S. tielingense*, *S. xueshanense*, *S. oregonense*, *S. sangi*, *S. cholashanense*, *S. feltiae*, *S. ichnusae*, *S. nguyeni*, *S. hebeiense*, *S. kushidai*, *S. akhursti*, *S. monticolum, S. changbaiense* and *S. ashiunense* form a monophyletic group, *S. populi* n. sp. as a member of the *Kushidai* clade, and sister to a clade (Bayesian posterior probability (BPP): 1.0) that encompasses *S. akhursti* and *S. kushidai* (fig. 5).

The sequence of D2–D3 regions of *S. populi* n. sp. is 890 bp. Pairwise distances (table 6) show that the new species differs from its closest taxa, *S. akhursti* and *S. kushidai*, by 11 bp and 20 bp, respectively. These data indicate that the new nematode is a new species when comparing these distances with those of other species in table 6.

For D2–D3 regions, BI analysis shows that the alignment resulted in 1044 characters, of which 444 are constant, 343 variable characters are parsimony-uninformative and 257 characters (included) are parsimony-informative. The phylogenetic relationships between 40 *Steinernema* species are presented in fig. 6. The 16 species, *S. populi* n. sp., *S. cholashanense*, *S. xinbinense*, *S. kraussei*, *S. oregonense*, *S. texanum*, *S. xueshanense*, *S. feltiae*, *S. puntauvense*, *S. jollieti*, *S. weiseri*, *S. akhursti*, *S. everestense*, *S. kushidai*, *S. ashiuense* and *S. monticolum*, form a monophyletic group. In this clade, the new species, *S. akhursti* and *S. everestense* (BPP: 0.97), form a monophyletic group; *S. monticolum* and *S. ashiuense* form another monophyletic group.

Morphological studies and molecular analyses show that the studied nematode is a new species evolving independently from its sister taxa. In general, morphological and molecular data confirm the status of *S. populi* n. sp. as a new species according to the phylogenetic and evolutionary species concepts (Adams, 1998).

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

Author contributions. C.L. Tian# and F. Zhu# contributed equally to this work. Conceptualization, Methodology, Software, Validation, Investigation, Writing-original draft, Writing-review & editing, Visualization. X.Y. Li: Writing-review and editing, Supervision. J.H. Zhang : Soil sample collection, Formal analysis. V. Půža: Morphological identification. D. Shapiro-Ilan: Writing-review and editing. D. Zhao: Observation SEM. J.W. Liu: Soil sample collection. J.J. Zhou: Raise Galleria mellonella. Y. Ding: Raise Galleria mellonella. J.C. Wang: Data curation, Visualization, Supervision. J. Ma: Visualization, Supervision. X.F. Zhu: Nematodes drawing. M.H. Li* and J.P. Li* were co-correspondence. Conceptualization, Resources, Writing-review and editing, Supervision, Project administration, Funding acquisition.

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