

***Seinura lii* N. SP. AND *S. wuae* N. SP.
(NEMATODA: SEINURIDAE) FROM PINE WOOD IN
CHINA**

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*“Especialmente Convidada Pela Presidência da Comissão Editorial da Academia
Pernambucana de Ciência Agronômica”.*
(Specially invited by the Presidence of Editorial Commitee of the Pernambuco Academy of
Agricultural Science)

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Nota do Editor

Tenho encontrado espécimes do nematóide do gênero *Seinura* associados a tecidos lesionados de coqueiros, parasitados pelo fitonematóide *Bursaphelenchus cocophilus*, em diversas áreas do Nordeste do Brasil. Trata-se de um eficiente nematóide predador, sem grandes registros no Brasil. Esse fato motivou-me a solicitar a Dra. Ren E. Huang, Ph.D em Nematologia, com a qual mantenho correspondência científica, a republicação deste seu excelente artigo, na esperança de que nematologistas brasileiros possam se interessar por este fascinante organismo.

Editor Note

I have found specimens of the nematode belonging to the genus *Seinura*, associated to rotted tissue of coconut palms, parasitized by the plant parasitic nematode *Bursaphelenchus cocophilus* in several areas at Northeast, Brazil. This nematode is an efficient nematode predator, without many references in Brazil. This fact motivated me to ask authorization to republish this excellent article by Dr. Ren E. Huang, a PhD nematologist with which I maintain scientific correspondence. It's my intention that this article may motivate Brazilian nematologists to became interested to this fascinating organism.

ABSTRACT

***Seinura lii* N. SP. AND *S. wuae* N. SP.
(NEMATODA: SEINURIDAE) FROM PINE WOOD IN CHINA**

Seinura lii n. sp. and *S. wuae* n. sp. are described and illustrated from dying *Pinus massoniana* infested with *Bursaphelenchus xylophilus* in China. *Seinura lii* n. sp. is characterised by relatively long female and male bodies (763–1146 and

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831–1068 μm , respectively), stylet 16–20 μm long and lacking knobs, two rows of developing oocytes in the anterior gonad, welldeveloped postuterine sac (120–182 μm long), three lines or incisures in the lateral field, four pairs of subventral papillae on the male tail region, and an elongate–conoid female tail. *Seinura wuae* n. sp. is characterised by relatively long female and male bodies (819–1179 and 787–1062 μm , respectively), stylet 15–18 μm long and with small knobs, three lines or incisures in the lateral field, two or three rows of developing oocytes in the anterior gonad, long postuterine sac (103–172 μm long), two pairs of subventral papillae on the male tail region, and an elongate–conoid female tail.

Index terms: description, morphology, morphometrics, pine wood nematode, *Pinus massoniana*, SEM, taxonomy.

RESUMO

Seinura lii N. SP. E *S. wuae* N. SP. (NEMATODA: SEINURIDAE) EM PINHEIRO NA CHINA

Seinura lii n. sp. e *S. wuae* n. sp. foram descritos e ilustrados a partir de *Pinus massoniana*, infectado por *Bursaphelenchus xylophilus* na China. A espécie *Seinura lii* foi caracterizada por possuir machos e fêmeas com comprimento dos corpos relativamente longos (763 - 1.146 e 831 - 1.068 μm , respectivamente), estilete 16-20 μm , sem bulbos, duas linhas de oócitos em desenvolvimento na gônada anterior, saco pós-uterino bem desenvolvido, (120-182 μm de comprimento), campos laterais com três incisuras, quatro pares de papilas sub ventrais, na região da cauda dos machos e as fêmeas possuidoras de cauda longa e conoide. *Seinura wuae* foi caracterizada também por possuir fêmeas e machos com comprimento do corpo relativamente longo (819 - 1.179 e 787 - 1.062 μm , respectivamente), estilete com 15 - 18 μm , com pequenos bulbos, três incisuras no campo lateral, duas a três linhas de oócitos em desenvolvimento na região anterior da gônada, longo saco pós-uterino (103-172 μm de comprimento), dois pares de papilas sub ventrais na região da cauda dos machos e as fêmeas com cauda longa e conoide.

Termos para indexação: descrição morfológica, morfometria, nematóide do pinheiro, *Pinus massoniana*, SEM, taxonomia.

1. INTRODUCTION

In 2005, a thorough survey was conducted throughout China for the presence of pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhner, 1934) Nickle, 1970, in damaged coniferous forests. Three hundred samples infested with this nematode were obtained from dead or dying pine trees. We examined all worm

isolates from the samples and found three species of *Seinura* in samples from Jiangsu Province. Morphological characterisation of these three *Seinura* isolates indicated that two were new species and they are described herein as *Seinura lii* n. sp. and *Seinura wuae* n. sp.

Specimens were extracted from wood chips by a modified Baermann funnel technique (Dropkin, 1989), individuals picked out by means of a needle, and then multiplied on *Botrytis cinerea* grown on potato sucrose agar (PSA) at 25°C. Measurements (except for those mounted on glycerin) and descriptions were from nematodes that were heat-relaxed (65°C, 2 min) and fixed in glycerine/ formaldehyde, a mixture of formalin (40% formaldehyde), glycerin and distilled water in the ratio 10:1:89, respectively (Xie, 1999). Illustrations were done using a Leica DMLB2 light microscope fitted with a drawing tube. Measurements were taken using a ZEISS AXioPhot microscope with Axio Vision software. Permanent slides were made in glycerin after slowly processing the nematodes to glycerin using the method of Ryss (2003). Measurements were also made of nematodes mounted in fixative.

For scanning electron microscopy (SEM) examination, living adults were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated through a graded ethanol series, freeze-dried and sputter-coated with goldpalladium (see Wergin, 1981).

2. *SEINURA LII** N. SP. (FIGURES 1–3)

2.1. Measurements

See Table 1.

2.2. Description

– Female

Body slender, slightly ventrally curved when heatrelaxed. Cuticle with fine transverse striae. Three lines or incisures in lateral field. Lip region high, with six lips. Head set off by strong constriction. Stylet knobless or occasionally with small basal thickenings visible. Median bulb oblong (19–24 × 14–18 µm); valves posteriorly situated. Pharyngeal gland lobe extending 78–104 µm from base of median bulb.

* Patronym in honour of Li Chuan Dao for his 50 year career in forest pathology.

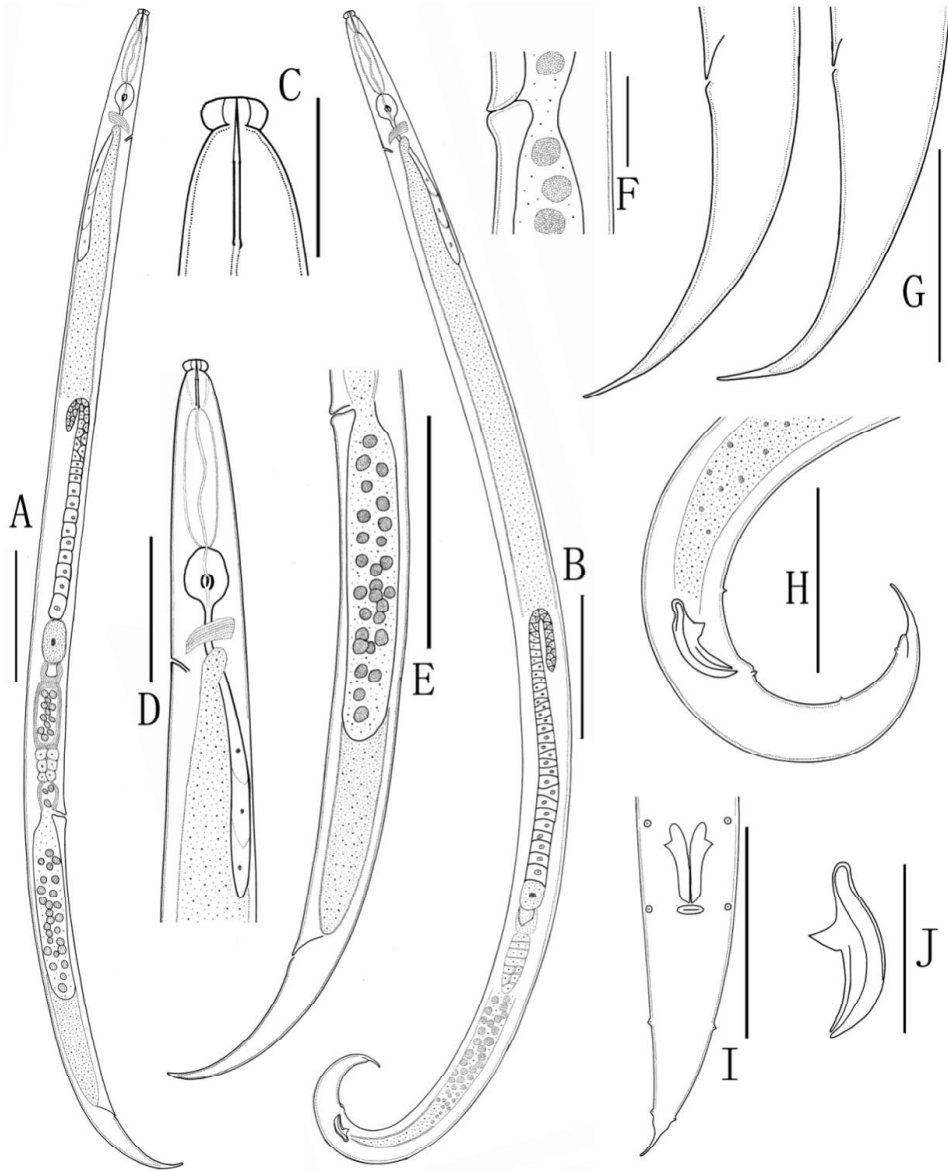


Figure 1. — *Seinura lii* n. sp. A: Entire view of female; B: Entire view of male; C: Lip region and stylet; D: Anterior region of female; E: Posterior region of female; F: Vulval region; G: Female tails; H: Lateral view of male tail; I: Ventral view of male tail; J: Spicules. (Scale bars: A, B, E = 100 μ m; C, F, J = 20 μ m; D, I = 50 μ m; G, H = 40 μ m.)

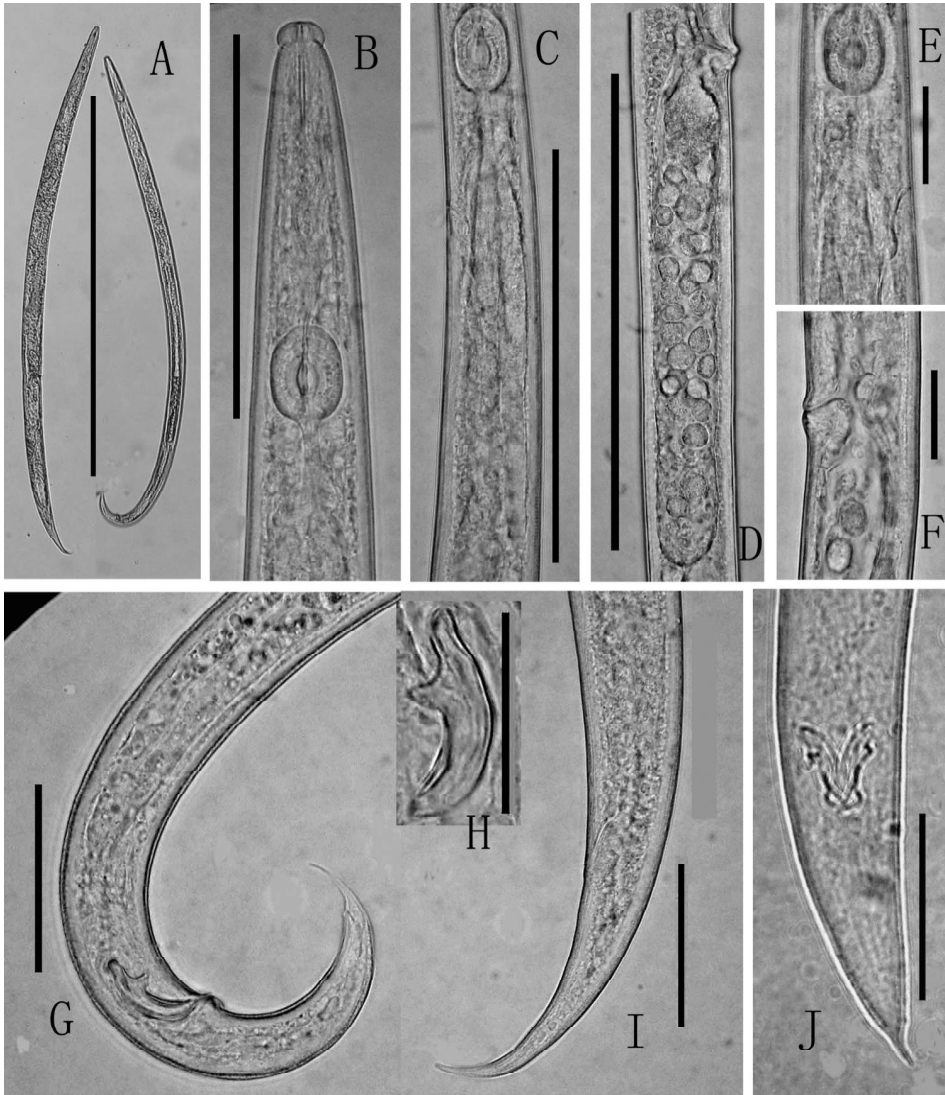


Figure 2. — Photomicrographs of *Seinura lii* n. sp. A: Entire view female (left) and male; B: Anterior region of female; C: Pharyngeal gland lobe; D: Postuterine sac with sperm; E: Excretory pore position; F: Vulval region; G: Lateral view of male tail; H: Spicules; I: Female tail; J: Ventral view of male tail. (Scale bars: A = 700 μ m; B = 80 μ m; C = 100 μ m; D = 140 μ m; E, F, H = 20 μ m; G, I, J = 40 μ m.)

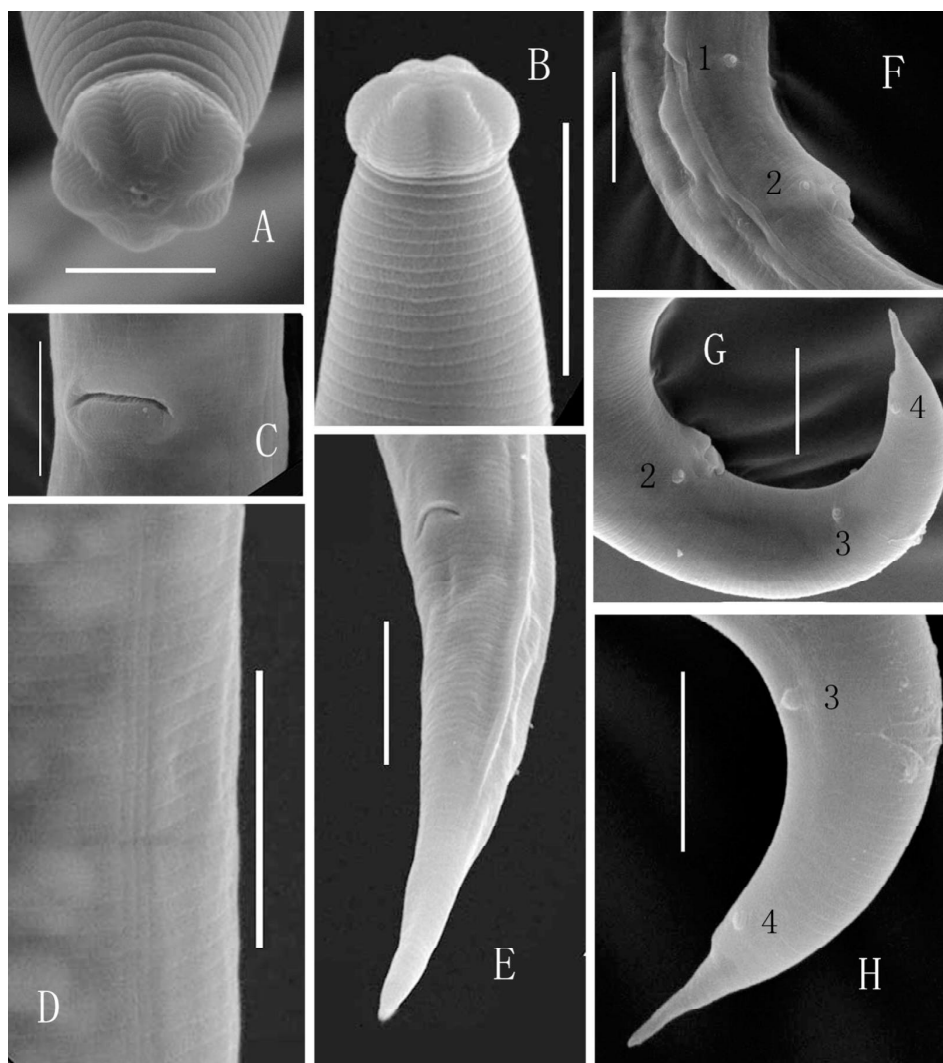


Figure 3. — Scanning electron micrographs of *Seimura lili* n. sp. A: Frontal view of lip region; B: Lateral view of head; C: Vulval region; D: Lateral field with three lines; E: Female tail; F, G, H: Three subventral papillae on male tail (1 = precloacal pair; 2 = adcloacal pair; 3, 4 = postcloacal pairs). (Scale bars: A = 5 μ m; B, C, D, E, F, G, H = 10 μ m.)

Table 1. - Morphometrics of *Seinura lili* n. sp.

Character	Female			Male	
	Holotype (glycerin)	Paratypes (glycerin)	Paratypes (fixed*)	Paratypes (glycerin)	Paratypes (fixed*)
n	-	14	30	8	30
L	910	885 ± 67.0 (763-1038)	1000 ± 80.5 (810-1146)	857 ± 26.1 (831-908)	958 ± 53.3 (865-1068)
a	41	40 ± 1.8 (36.4-42.2)	34.4 ± 3.92 (28-40)	42.0 ± 1.39 (40.4-44)	36.8 ± 2.70 (32.0-43)
b	9.84	9.8 ± 0.52 (8.9-10.7)	9.7 ± 0.65 (8.9-11.1)	9.8 ± 0.56 (9.2-10.7)	9.7 ± 0.47 (9.0-11)
c	16.0	15.5 ± 2.3 (12.6-18.5)	13.6 ± 0.85 (11.3-15.7)	20 ± 1.2 (18.4-21.5)	17.6 ± 2.18 (14.6-22.3)
c'	4.18	4.3 ± 0.39 (3.8-5.1)	4.2 ± 0.33 (3.4-5.0)	3.2 ± 0.27 (2.8-3.6)	3.1 ± 0.27 (2.7-3.6)
V	68.8	68.7 ± 0.86 (66.7-69.8)	67.5 ± 1.08 (64.1-69.5)	-	-
Stylet	18.5	18.0 ± 0.76 (16.8-19.2)	18.1 ± 0.82 (16.3-20.1)	17.1 ± 0.57 (16.4-18.0)	17.7 ± 0.47 (16.8-18.7)
Postuterine sac length	130	134 ± 17.7 (120-161)	153 ± 13.6 (126-182)	-	-
Spicule length	-	-	-	20.7 ± 0.50 (20.0-21.5)	20.6 ± 0.76 (19.2-22)
Tail length	57	57 ± 5.9 (52-69)	73 ± 3.9 (68-84)	43 ± 3.4 (42-50)	56 ± 4.6 (48-64)

Values in μm are in the form: mean \pm SD (range).

* Nematodes measured in fixative.

Excretory pore located posterior to nerve ring, ca 17–25 μm from base of median bulb. Ovary monodelphic, outstretched or reflexed, not reaching pharyngeal gland lobe. Developing oocytes arranged in two rows anteriorly, single row posteriorly. Spermatheca often invisible; when visible sperms present. Vulva posteriorly located, flap absent. Postuterine sac with sperms, highly developed, ca 2/5–2/3 of vulva–anus distance long, most sperms 6–8 μm in diam. Anus and rectum visible. Tail slightly recurved, elongate–conoid, terminus pointed.

– Male

Body slender, ventrally curved when heat–relaxed. Cuticle, lip region, excretory pore, median bulb, stylet and pharyngeal gland lobe similar to female. Testis reflexed, not reaching pharyngeal gland lobe. Developing spermatocytes in arranged in two rows anteriorly, single row posteriorly. Spicules paired; apex elongate, rostrum prominent. Tail arcuate, terminus pointed, talon–like in lateral view. Tail tip 8.3–14 μm long. Four pairs of subventral papillae present: first pair precloacal, second pair adcloacal, third and fourth pairs postcloacal. Bursa and gubernaculum absent.

2.3. Type host and locality

The type specimens were collected from a culture on *Botrytis cinerea*. The culture was started from specimens collected from dying *Pinus massoniana* Lamb infested with *B. xylophilus* in Liyang county, Jiangsu Province, China.

2.4. Type material

Slides of holotype female (S.L.f–No.1); paratype females (S.L.f–No.2–15) and paratype males (S.L.m–No.1–8) deposited in the Institute of Forest Protection, Nanjing Forestry University, Nanjing, China. Other paratype material deposited in the USDA Nematode Collection, Beltsville, MD, USA, slides T–5451 to T–5456 (females) and T–5457 to T–5461 (males).

2.5. Diagnosis and relationships

Seinura lii n. sp. is characterised by relatively long female and male bodies (763–1146 and 831–1068 μm , respectively), presence of three lines or incisures in the lateral field, a knobless stylet, two rows of developing oocytes in the anterior gonad,

well-developed postuterine sac (120–182 μm long), four pairs of subventral papillae on the male tail, and an elongate-conoid female tail.

Seinura lii n. sp. belongs to the Group I category of *Seinura* species (sensu Shahina & Hunt, 1995), which is characterised by the presence of a postuterine sac. It can be distinguished from all but six species in this group by the shape of the female tail (elongate-conoid vs filiform or spicate). The new species differs from *S. wuae* n. sp. by having the anterior developing oocytes arranged in two vs two to three rows; smaller V (64–69.5 vs 69.6–75.2); four vs two pairs of caudal papillae; and stylet knobs lacking vs present.

Of the remaining five similar species, *S. clavata* Bajaj & Bhatti, 1982 differs by female tail terminus clavate vs pointed; shorter female body (590–620 vs 763–1038 μm); higher V (74–76 vs 66.7–69.8); and smaller a value (26–34 vs 36.4–42.2). *Seinura hechlerae* Chaturvedi & Khera, 1979 has a shorter female body (320–470 vs 763–1038 μm) and smaller a value (20–28 vs 36.4–42.2). *Seinura informis* Gagarin, 2001 has the female tail tip blunt vs pointed; shorter female body (430–536 vs 763–1038 μm); and smaller a value (19–25 vs 36.4–42.2). *Seinura nodosa* Andrassy, 1966 has the female tail terminus knobbed vs pointed; shorter female body (390–400 μm); and smaller a value (23.2 vs 36.4–42.2). *Seinura sutura* Massey, 1971 has vulval flap present vs absent; shorter female body (580–650 vs 763–1038 μm); and smaller a value (29–32 vs 36.4–42.2) whilst *S. tritica* Bajaj & Bhatti, 1982 has developing oocytes arranged in two to five rows vs two and one rows; shorter female body (570–700 vs 763–1038 μm); and higher V (79–81 vs 66.7–69.8).

3. *SEINURA WUAE*** N. SP. (FIGURES 4–6)

3.1. Measurements

See Table 2.

3.2. Description

– Female

Body slender, slightly ventrally curved when heatrelaxed. Cuticle with fine transverse striae. Three lines or incisures in lateral field. Head set off by constriction; lip region high, with six lips. Stylet with small basal knobs, although at times difficult

** Patronym in honour of Wu Xiao Qin, one of the discoverers of this species.

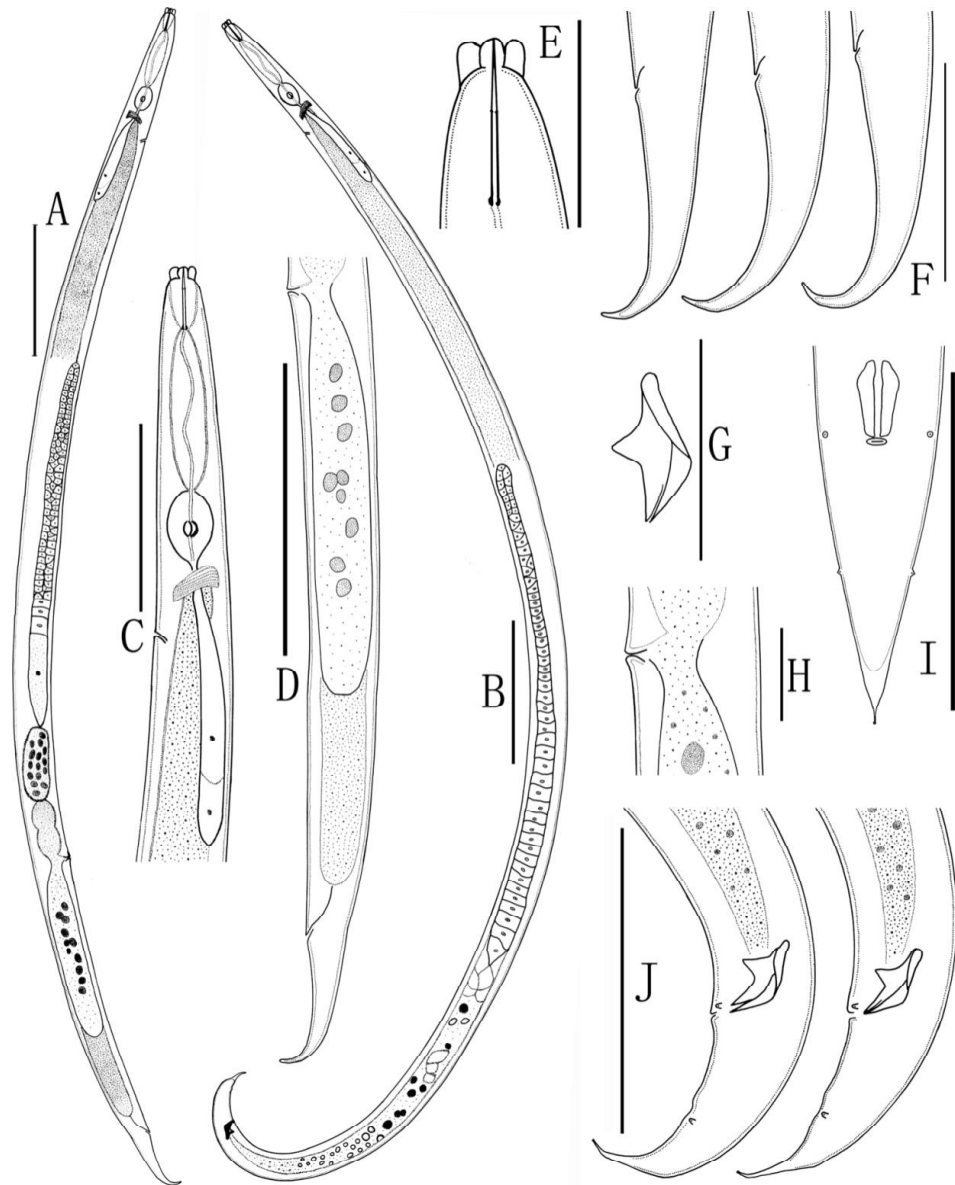


Figure 4. — *Seimura wuae* n. sp. A: Entire view of female; B: Entire view of male; C: Anterior region of female; D: Posterior region of female; E: Lip region and stylet; F: Variation in female tail shape; G: Spicules; H: Vulval region; I: Ventral view of male tail; J: Lateral view of male tails. (Scale bars: A, B, D = 100 μ m; E, G, H = 20 μ m; C, I, J = 50 μ m; F = 40 μ m.)

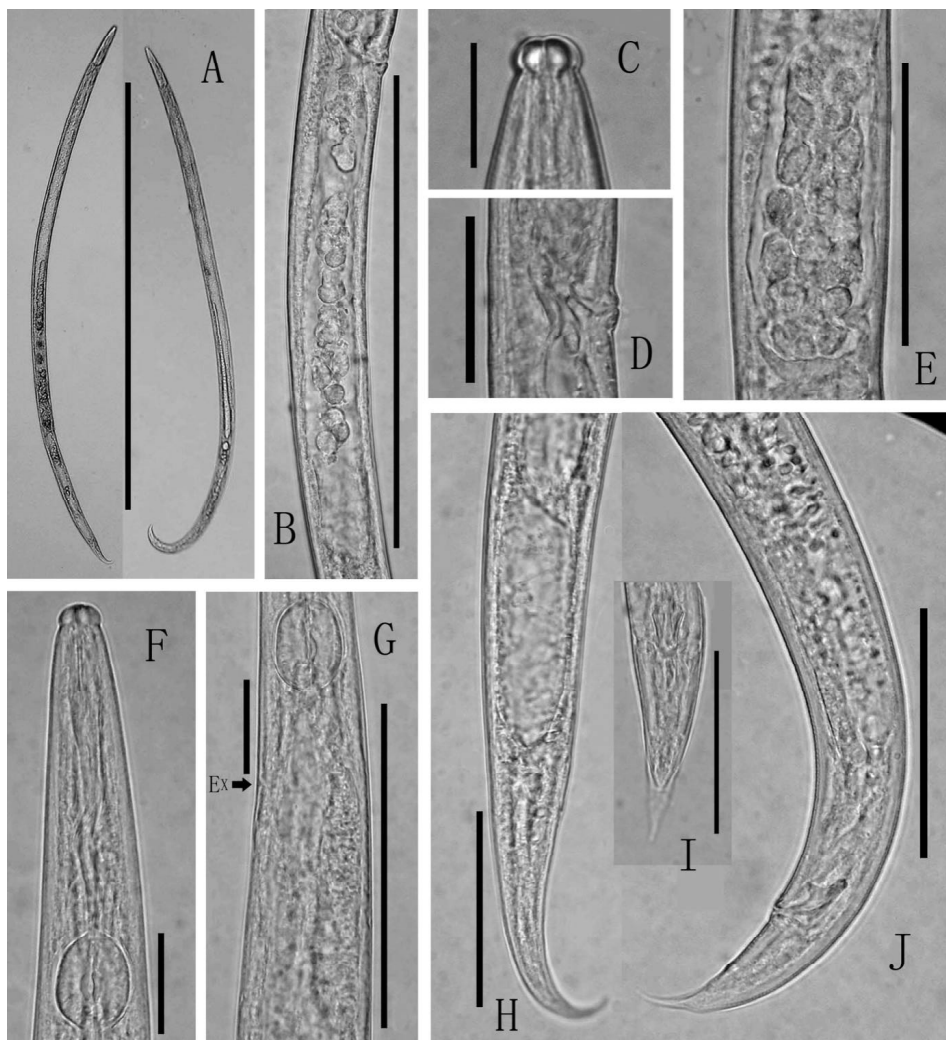


Figure 5. — Photomicrographs of *Seinura wuae* n. sp. A: Entire view of female (left) and male; B: Postuterine sac; C: Lip region; D: Vulval region; E: Spermatheca with sperm; F: Anterior region of female; G: Excretory pore position and pharyngeal gland lobe (Ex: excretory pore); H: Female tail; I: Ventral view of male tail; J: Lateral view of male tail. (Scale bars: A = 800 μ m; B = 140 μ m; C = 15 μ m; D = 30 μ m; E, J = 50 μ m; F = 20 μ m; G = 20, 70 μ m; H, I = 40 μ m.)

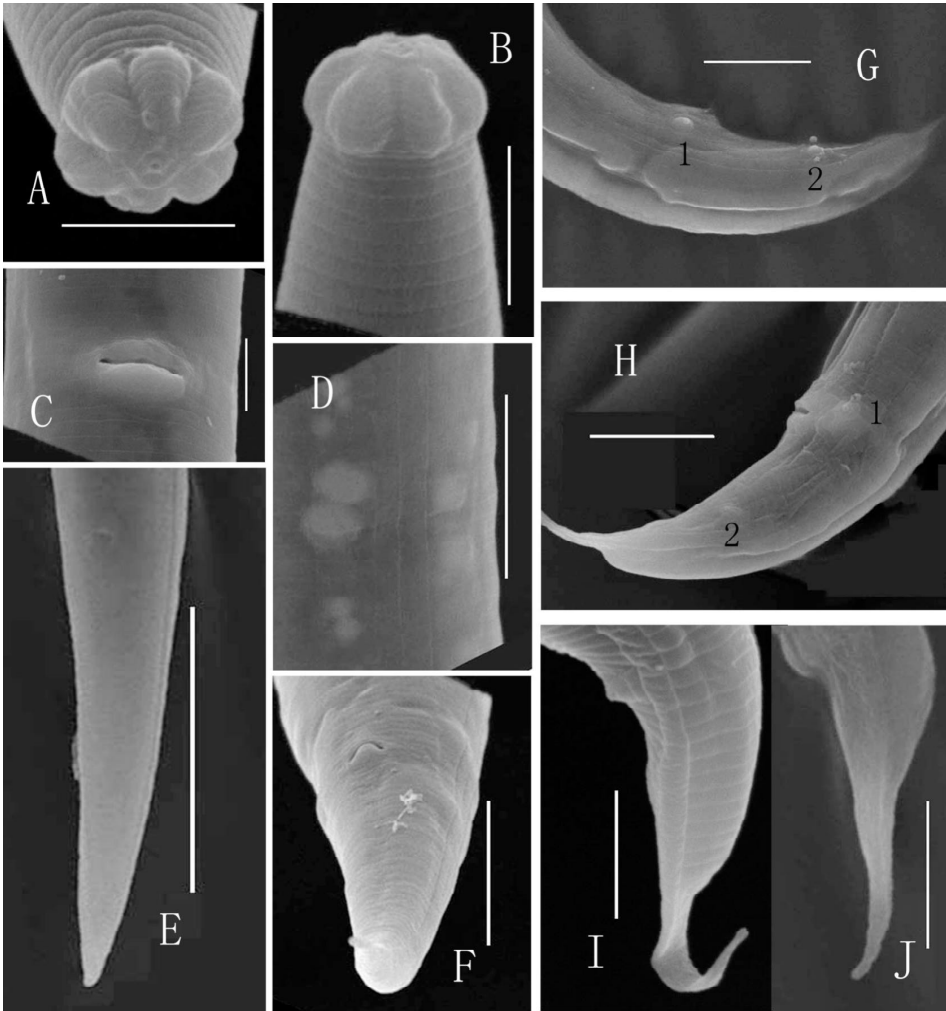


Figure 6. — Scanning electron micrographs of *Seimura wuae* n. sp. A: Frontal view of lip region; B: Lateral view of head; C: Vulval region; D: Lateral field with three lines; E, F: Female tails; G, H: Subventral papillae on male tail (1 = adcloacal pair; 2 = postcloacal pair); I, J: Male tail tip. (Scale bars: A, B, C, I, J = 5 μ m; D, F, G, H = 10 μ m; E = 20 μ m.)

Table 2. - Morphometrics of *Seinura wuae* n. sp.

Character	Female			Male	
	Holotype (glycerin)	Paratypes (glycerin)	Paratypes (fixed*)	Paratypes (glycerin)	Paratypes (fixed*)
n	-	10	30	7	30
L	855	902 ± 67.2 (831-1014)	972 ± 82.7 (819-1179)	861 ± 67.0 (787-953)	955 ± 60.5 (838-1062)
a	43.4	45.9 ± 1.8 (42.9-48.3)	34 ± 3.62 (29-40)	45.8 ± 1.1 (44-47.5)	34 ± 3.39 (28-40)
b	9.7	10.1 ± 0.78 (9.2-11.5)	11.3 ± 0.66 (10.0-12.5)	9.0 ± 0.58 (8.2-9.8)	11.8 ± 0.55 (10.9-12.7)
c	18.29	19.7 ± 1.05 (17.8-21.5)	20 ± 1.03 (18.8-22.4)	27.2 ± 2.4 (24.4-29.8)	25.1 ± 1.82 (22.0-30.7)
c'	4.0	4.1 ± 0.21 (3.7-4.4)	3.5 ± 0.32 (2.7-4.1)	2.5 ± 0.25 (2.2-2.8)	2.6 ± 0.31 (2.0-3.2)
V	73.5	73.3 ± 0.4 (72.6-73.8)	72.9 ± 1.15 (69.6-75.2)	-	-
Stylet	15.8	16.1 ± 0.69 (15.3-17.5)	16.4 ± 0.67 (15.2-18.1)	15.8 ± 0.60 (15.0-16.8)	16.4 ± 0.55 (15.2-17.3)
Postuterine sac length	110	119 ± 23 (103-135)	144 ± 15.8 (123-172)	-	-
Spicule length	-	-	-	15.8 ± 0.63 (14.7-16.6)	13.8 ± 1.05 (12.9-16.2)
Tail length	47	45 ± 4.3 (39-52)	50 ± 3.0 (43-58)	32 ± 1.7 (28-33)	38 ± 2.3 (33-42)

Values in μm are in the form: mean \pm SD (range).

* Nematodes measured in fixative.

to discern. Median bulb oblong (16–19 × 10–12 µm); valves posteriorly situated. Pharyngeal gland lobe extending 75–86 µm from base of median bulb. Excretory pore located posterior to nerve ring, 18–25 µm from base of median bulb. Ovary monodelphic, outstretched, not reaching pharyngeal gland lobe. Developing oocytes arranged in two or three rows. Spermatheca visible, sperms present. Vulva posteriorly located, flap absent. Postuterine sac well developed, ca 2/5–2/3 of vulva–anus long. Anus and rectum visible. Tail, median to elongated conoid, recurved.

– Male

Body slender, ventrally curved when heat-relaxed. Cuticle, lip region, excretory pore, median bulb, stylet and pharyngeal gland lobe similar to female. Testis outstretched or reflexed, not reaching pharyngeal gland lobe. Developing spermatocytes arranged in two rows anteriorly, single row posteriorly. Spicules paired; apex and rostrum prominent. Tail curved to spicate terminus. Two pairs of subventral papillae present: first pair adcloacal, second pair postcloacal. Bursa and gubernaculum absent.

3.3. Type host and locality

The type specimens were collected from a culture on *Botrytis cinerea*. The culture was started from specimens collected from dying *Pinus massoniana* Lamb infested with *B. xylophilus* in Yizheng county, Jiangsu Province, China.

3.4. Type material

Slides of holotype female (S.W.f–No.1); paratype females (S.W.f–No.2–11) and paratype males (S.W.m–No.1–7) deposited in the Institute of Forest Protection, Nanjing Forestry University, Nanjing, China. Other paratypes deposited in the USDA Nematode Collection, Beltsville, MD, USA, slides T–5462 to T–5469 (females) and T–5470 to T–5477 (males).

3.5. Diagnosis and relationships

Seinura wuae n. sp. is characterised by relatively long female and male bodies (819–1179 and 787–1062 µm, respectively), a stylet bearing small basal knobs, two or three rows of developing oocytes, well-developed postuterine sac (103–172 µm

long), three lines or incisures in the lateral field, two pairs of subventral papillae on the male tail, and an elongate-conoid female tail.

Seinura wuae n. sp. belongs to the Group I category of *Seinura* species (sensu Shahina & Hunt, 1995). It can be distinguished from all but six species in this group by the elongate-conoid vs filiform or spicate female tail.

The new species differs from *S. lii* n. sp. by having the developing oocytes arranged in two to three vs two rows; higher V (69.6–75.2 vs 64–69.5); two vs four pairs of caudal papillae; and stylet knobs present vs absent.

Of the remaining five similar species, *S. clavata* differs by female tail terminus clavate vs pointed; shorter female body (590–620 vs 831–1014 μm); and smaller a value (26–34 vs 42.9–48.3). *Seinura hechlerae* has a shorter female body (320–470 vs 831–1014 μm); and smaller a value (20–28 vs 42.9–48.3). *Seinura informis* has the female tail tip blunt vs pointed; shorter female body (430–536 vs 831–1014 μm); and smaller a value (19–25 vs 42.9–48.3). *Seinura nodosa* has the female tail terminus knobbed vs pointed; shorter female body (390–400 vs 831–1014 μm); smaller a value (23.2 vs 42.9–48.3). *Seinura sutura* has vulval flap present vs absent; shorter female body (580–650 vs 831–1014 μm); and smaller a value (29–32 vs 42.9–48.3); whilst *S. tritica* has the developing oocytes arranged in two to five rows vs two to three rows; shorter female body (570–700 vs 831–1014 μm); and higher V (79–81 vs 72.6–73.8).

4. DISCUSSION

Only three *Seinura* isolates in total were found in 300 samples taken from dead or dying pine trees infested with the pine wood nematode, *B. xylophilus*, during a nationwide survey for this nematode in China. This suggests that *Seinura* species do not commonly occur with pine wood nematodes in China although *Seinura* spp. have been frequently found in trees infested with pine wood nematodes in Florida, USA (Esser, 1987). Esser speculated that *Seinura* spp. may have promise as a biological control agent of pine wood nematode as they are predatory on other nematodes (Linford, 1937; Linford & Oliveira, 1937; Hechler, 1963; Wood, 1975). As pine wilt disease is epidemic in China, further studies on the predatory lifestyle and biological control potential of *S. lii* n. sp. and *S. wuae* n. sp. on pine wood nematode are needed.

5. ACKNOWLEDGEMENTS

The authors thank Dr Alexander Ryss (Zoological Institute, Russian Academy of Sciences, Russia) for technical assistance on morphological taxonomy of plant-parasitic nematodes and for providing related literature. The authors are grateful to Dr Fayyaz Shahina (National Nematological Research Centre, University of Karachi, Pakistan), Dr David Hunt (CABI Bioscience, UK Centre, Egham, UK) and Dr Vladimir Gagarin (Institute of Inland Waters Biology, Russian Academy of Sciences, Russia), for providing key *Seinura* data. Sincere thanks also go to Dr Xiao Liu (Department of Developmental Biology, Stanford University Medical Center, CA, USA) for critically reviewing the manuscript and for providing useful suggestions. We acknowledge the China Inspection and Quarantine Nanjing Bureau for providing a ZEISS AXioPhot microscope equipped with AXio Vision measurement software. The project was sponsored by the State Forestry Administration and was supported by the Natural Science Foundation of China.

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