BURSAPHELENCHUS SINENSIS SP. N. AND *B. THAILANDAE* BRAASCH *ET* BRAASCH - BIDASAK IN PACKAGING WOOD FROM CHINA

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Summary. *Bursaphelenchus sinensis* sp. n., found in coniferous wood (*Pinus* sp.) introduced from China as packaging material into Liefering (near Salzburg, Austria), is described and illustrated. The new species is characterized by relatively small body length, females and males averaging 540 and 594 µm respectively, a stylet length of 12-13 µm, lateral field about 1/8-1/13 of the maximum body width with two lines. The male spicules are unusual for the genus, separate, with a squared condylus, somewhat ventrally bent, rostrum not clearly differentiated, a ventral limb appearing anteriorly thickened and posteriorly weak and membranous. On the male, one single ventral and one pair of subventral preanal papillae, one pair of adanal sensilla without evident external structure, one pair of postanal papillae and gland openings at the beginning of the bursa, small oval bursa with central appendage. Female with a small vulval flap, tail conical, almost straight or slightly recurved ventrally, terminus almost pointed or rounded, sometimes with a small mucro. *Bursaphelenchus thailandae* Braasch *et* Braasch-Bidasak, was also recovered from the same wood sample containing *B. sinensis* sp. n. and some notes and morphometric data are given for the population.

A new species of *Bursaphelenchus* was recovered on September 2000 from coniferous wood (*Pinus* sp.) introduced from China as packaging material for granite stones into Liefering (near Salzburg, Austria). The wood showed blue staining by fungi, bore holes by *Monochamus* sp. and larvae of Scolytidae, probably *Orthotomicus* sp. The nematode is described herein as *Bursaphelenchus sinensis* sp. n.

Two more unidentified species of Aphelenchoididae, one of which later described as *Bursaphelenchus thailandae* Braasch *et* Braasch-Bidasak, 2002, were recovered from the same wood sample containing *B. sinensis* sp. n.

MATERIAL AND METHODS

The nematodes were extracted from wood fragments by the Baermann funnel technique and placed on Petri dishes with non-sporulating *Botrytis cinerea* Pers. ex Fr. for culturing on 5% (v/v) glycerol-supplemented potato dextrose agar. After three weeks, sub-cultures were started and maintained at 25 °C. Adults of *B. sinensis* sp. n. and *B. thailandae* were hand picked and collected in sterile water from 15-day-old cultures. All subsequent observations were made on nematodes alive in water or fixed in TAF or FP 4:1 hot solution and stained in cotton blue lactophenol or processed by the glycerol ethanol method (Seinhorst, 1959).

Type specimens were heat killed, fixed in FP 4:1 and processed by the glycerol ethanol method. Nematodes were drawn and measured with the aid of a camera lucida and a stage micrometer. For each character mean, standard deviation and range were calculated. For SEM observations, specimens of *B. sinensis* sp. n. reared on *B. cinerea* and fixed in FP 4:1 were studied with the aid of a scanning electron microscope Zeiss DSM 940 with cryopreservation Oxford CT 1500 B.

DESCRIPTIONS

BURSAPHELENCHUS SINENSIS sp. n.
(Table I; Figs 1 - 3)

Measurements of holotype male, allotype female, male and female paratypes are in Table I.

Body relatively short, cuticular annulations fine, inconspicuous, annules about 1 µm wide under light microscope. Lateral field narrow, about 1.2-2.0 µm wide (about 1/8-1/13 of the maximum body width) with two lines on midbody and near extremities.

Cephalic region hexalobate, offset by constriction, without any visible annulation, 2.4-4.8 µm high and 4.2-6.1 µm wide. Stylet thin, short, cone about 38-55% of the stylet length, shaft with distinct basal swellings.

Pharyngeal bulb round to oval, 12.1-18.2 µm long and 9.7-13.3 µm wide, with valve plates usually placed slightly posterior to centre of bulb, sometimes centrally. Dorsal oesophageal gland orifice opening into lumen of metacorpus 0.2-0.6 metacorpal valve lengths above metacorpal valve. Oesophago-intestinal junction 0.4-1.3 metacorpal valve lengths behind metacorpus. Oesophageal glands overlapping intestine mostly on dorsal side for 2.1-4.3 body widths at oesophago-intestinal junction.

Excretory pore usually posterior to the median bulb, 0.1-0.9 body diameters behind median bulb, sometimes at the same level of the base of the bulb or 4.8-8.5 µm

Table I. Measurements of *Bursaphelenchus sinensis* sp. n. (in µm).

	Holotype ♂	Allotype ♀	Paratypes ♂♂	Paratypes ♀♀
n			26	26
Body length	596.5	687.3	593.9 ± 68.9 (355.7 – 669.1)	539.6 ± 69.7 (450.1 – 722.4)
Body width (max)	19.4	21.8	18.4 ± 2.7 (10.9 – 21.8)	18.8 ± 2.1 (14.5 – 21.8)
a	30.8	31.6	32.6 ± 3.4 (28.6 – 46.8)	29.3 ± 2.6 (22.5 – 35.0)
Head height	3.6	3.6	3.4 ± 0.4 (2.4 – 3.6)	3.4 ± 0.4 (3.0 – 4.8)
Head width	5.4	6.1	5.0 ± 0.4 (4.2 – 6.1)	5.1 ± 0.5 (4.2 – 6.1)
Distance from anterior end to oesophago-intestinal junction	61.7	60.5	60.6 ± 6.4 (44.8 – 72.6)	54.8 ± 4.0 (48.4 – 62.9)
b	9.7	11.4	9.8 ± 1.0 (6.8 – 11.4)	9.8 ± 1.2 (7.8 – 11.9)
Distance from anterior end to posterior end of oesophageal glands	104.1	125.8	111.4 ± 9.6 (94.4 – 135.5)	101.5 ± 10.8 (78.7 – 125.8)
b'	5.7	5.5	5.3 ± 0.5 (3.6 – 6.2)	5.3 ± 0.5 (4.5 – 6.6)
Distance from anterior end to median bulb base	58.1	56.9	55.7 ± 5.8 (41.1 – 65.3)	51.0 ± 3.9 (46.0 – 59.3)
b1	10.3	12.1	10.7 ± 1.0 (7.5 – 12.4)	10.6 ± 1.3 (8.3 – 12.7)
Oesophageal glands overlapping intestine length	42.4	42.4	50.9 ± 5.6 (39.9 – 62.9)	47.5 ± 9.4 (27.8 – 65.3)
Oesophageal glands overlapping intestine length/body width oesophago-intestinal junction	2.7	3.2	3.4 ± 0.4 (2.7 – 4.2)	3.3 ± 0.6 (2.1 – 4.3)
Stylet length	12.7	12.7	12.5 ± 0.6 (11.5 – 13.3)	12.0 ± 0.4 (11.5 – 12.7)
m	42.9	42.9	43.7 ± 1.8 (40.0 – 45.5)	44.2 ± 4.1 (38.1 – 55.0)
Distance from base of median bulb to excretory pore	4.8	9.7	8.2 ± 4.4 (2.4 – 18.2)	6.5 ± 3.0 (1.2 – 13.3)
Distance from anterior end to excretory pore	62.9	66.6	62.2 ± 8.3 (42.4 –76.2)	57.3 ± 5.3 (49.6 – 66.6)
Distance from anterior end to hemizonid	72.6	75.0	77.9 ± 6.3 (60.5 – 87.1)	72.4 ± 4.8 (62.9 – 84.7)
Distance from hemizonid to excretory pore	9.7	8.5	15.6 ± 6.4 (3.6 – 32.7)	14.1 ± 5.2 (2.4 – 21.8)
V or T	79.1	72.4	62.7 ± 13.9 (34.4 – 80.7)	74.6 ± 1.0 (72.4 – 76.5)
Anterior genital branch length	-	211.8	-	161.3 ± 20.1 (121.0 – 211.8)
G1	-	30.8	-	30.0 ± 4.3 (21.4 – 37.9)
Postvulval branch length	-	99.2	-	79.8 ± 11.7 (60.5 – 101.6)
G2	-	14.4	-	15.0 ± 1.7 (12.0 – 18.5)
Vulva-anus distance	-	156.1	-	107.2 ± 15.7 (84.7 – 156.1)
Postvulval branch length % vulva-anus distance	-	63.6	-	74.9 ± 8.8 (62.5 – 91.5)
Tail length	26.6	32.7	27.6 ± 1.7 (21.8 – 31.2)	28.9 ± 3.0 (23.8 – 35.1)
c	22.4	21.0	21.6 ± 2.1 (16.3 – 25.0)	18.3 ± 1.6 (15.5 – 21.3)
c'	1.9	3.2	2.1 ± 0.2 (1.7 – 2.3)	3.3 ± 0.3 (2.6 – 4.2)
Spicule length	19.4	-	19.2 ± 1.7 (14.5 – 21.9)	-
Spicule length (chord)	16.9	-	16.3 ± 1.2 (12.1 – 18.2)	-
Spicule width	7.3	-	7.1 ± 0.7 (4.8 – 8.5)	-

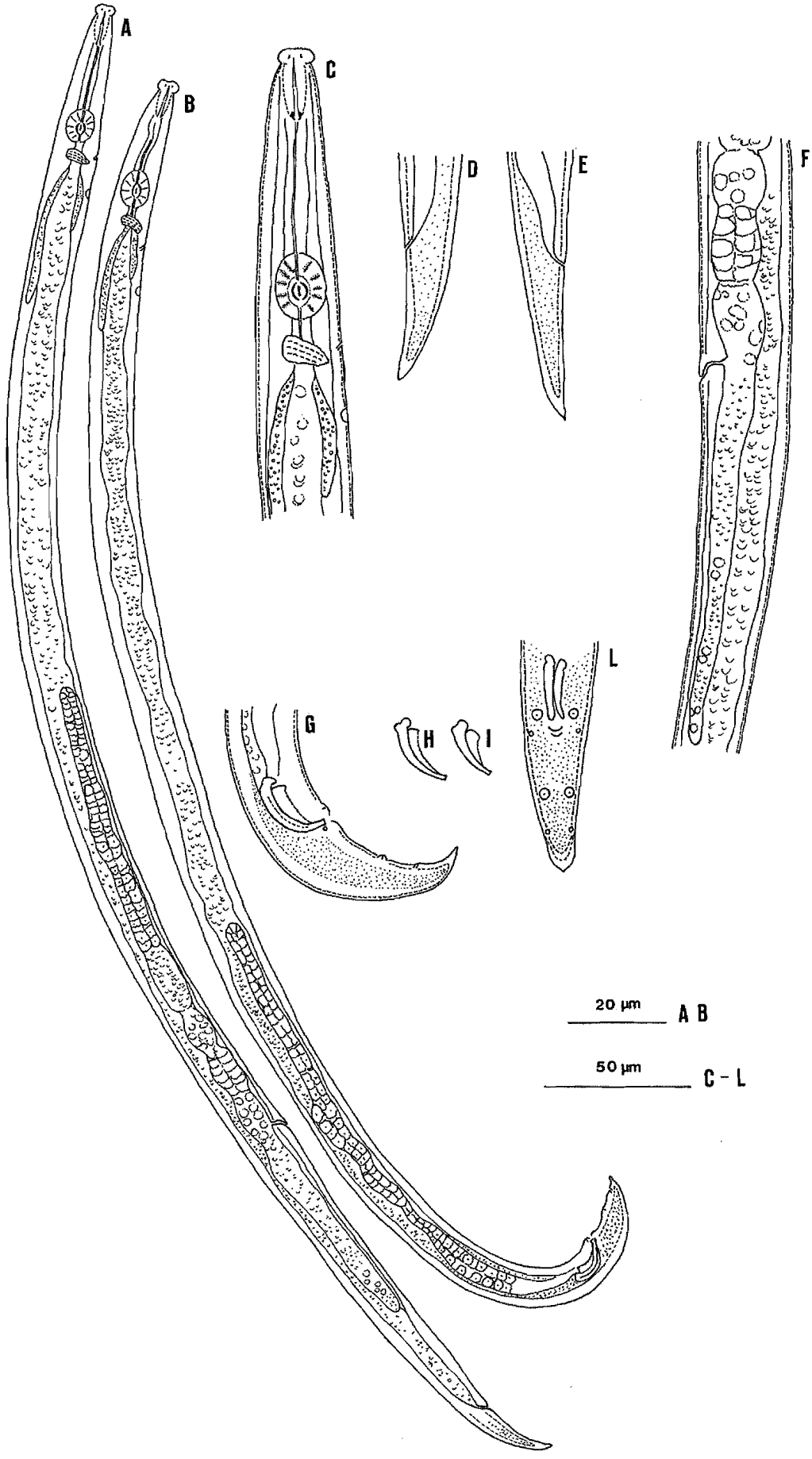

Fig. 1. *Bursaphelenchus sinensis* sp. n.: A, female; B, male; C, female oesophageal region; D, E, female tails; F, female vulval region; G, male tail; H, I, male spicules; L, male tail, ventral view.

anterior. Hemizonid 2.4-4.8 µm long, 2.4-32.7 µm posterior to the excretory pore. Gonads usually straight, sometimes recurved, reflexed part 1.0-4.2 body widths long, cells arranged in two or three rows in the growth zone.

Female. Relaxed nematodes slightly ventrally arcuate. Gonad with spermatheca differentiated, about 13.3-24.2 µm long and 7.3-15.7 µm wide, round-oval sperms. Preuteral gland developed, formed by two rows of 5-6 cells. Vulva posterior, the anterior lip modified into a small vulval flap, 1.8-2.4 µm long. Postvulval uterine branch long, equal to 74.9±8.8 (62.5-91.5)% of vulva-anus distance. Tail conical, almost straight or slightly recurved ventrally, terminus almost pointed or rounded, sometimes with a small mucro.

Male. Body J-shaped when killed by heath. Spicules paired, condylus squared, somewhat ventrally bent, sometimes slightly indented at the anterior margin, ventral limb weak, flexible with anterior thickened margin separated from and posterior to the tip of dorsal limb, remaining part of the limb membranous, rostrum not clearly differentiated; distal end of lamina truncate with a small thickening not expanded as a true cucullus. One single ventromedian papilla immediately above the cloa-

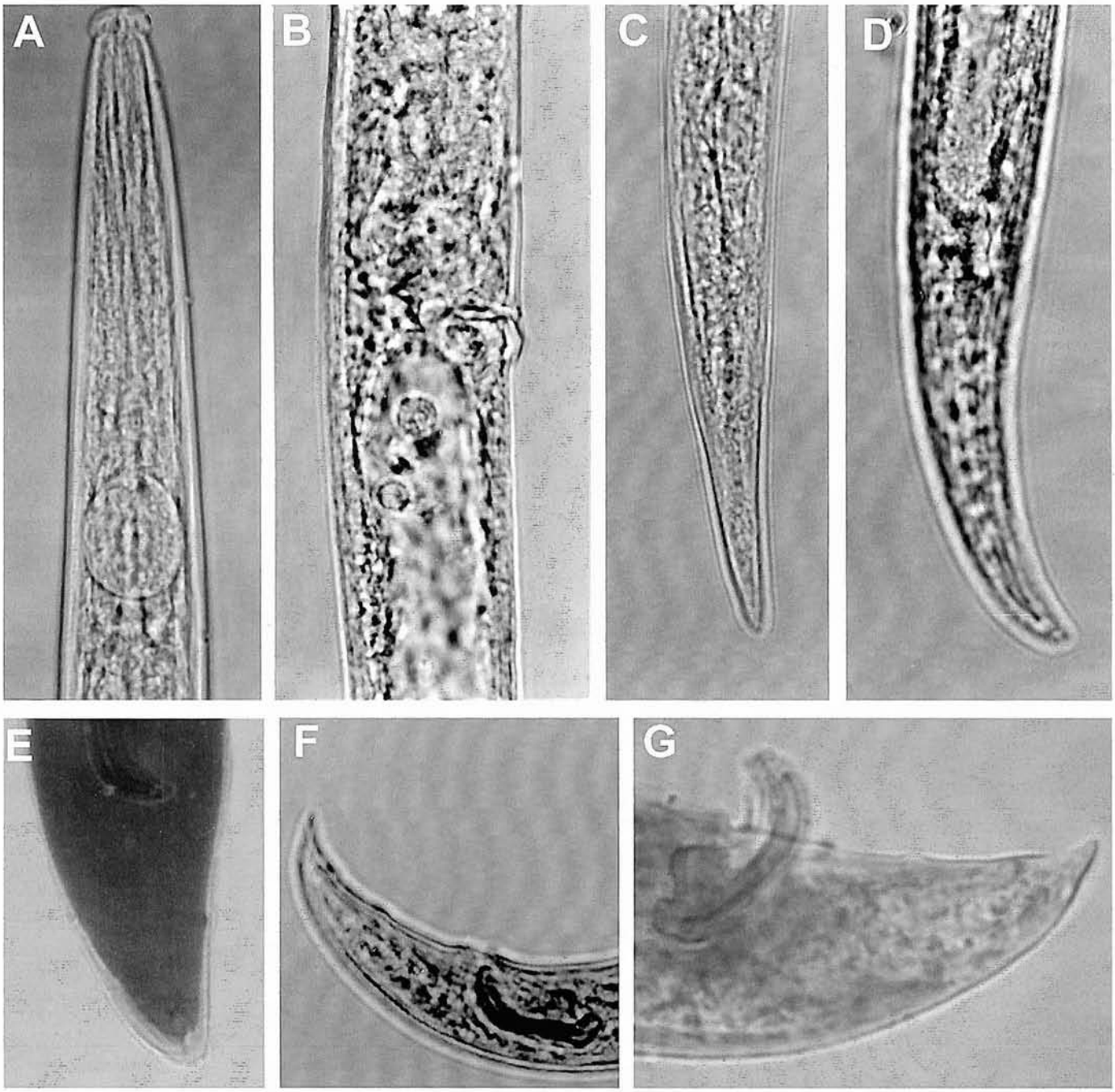

Fig. 2. LM photomicrographs of *B. sinensis* sp. n.: A, female anterior region; B, female vulval region; C, D, female tails; E, male tail, bursa; F, G, male tails.

ca, one pair of precloacal subventral papillae almost at the same level, one pair of ventrolateral sensilla without evident external structure at the cloaca level, one pair of postanal subventral papillae at 46.4±3.4 (37.5-54.2)% of tail length from cloaca; gland openings sometimes appearing as a pair of very small papillae, on the tail near the beginning of bursa at 73.1±2.6 (66.7-77.3)% of tail length from cloaca. Tail arcuate with obtuse or almost pointed talon-like terminus, bearing a small bursa oval with small central appendage. A few males were found with the same spicule and bursa shape, but different from the other males with the lip region a bit lower and squared, stylet 17.0-18.2 µm long with wider lumen and without basal knobs.

Type locality and habitat. Coniferous wood of *Pinus* sp. introduced from China as packaging material into Liefering (near Salzburg, Austria), on September 2000.

Type designations. Holotype male, allotype female and paratypes collected from a 15-day-old culture on *B. cinerea*.

Holotype male, allotype female, 21 male and 19 female paratypes kept in the nematode collection of the Istituto Sperimentale per la Zoologia Agraria, Florence, Italy; four slides (7 females, 5 males) deposited at the

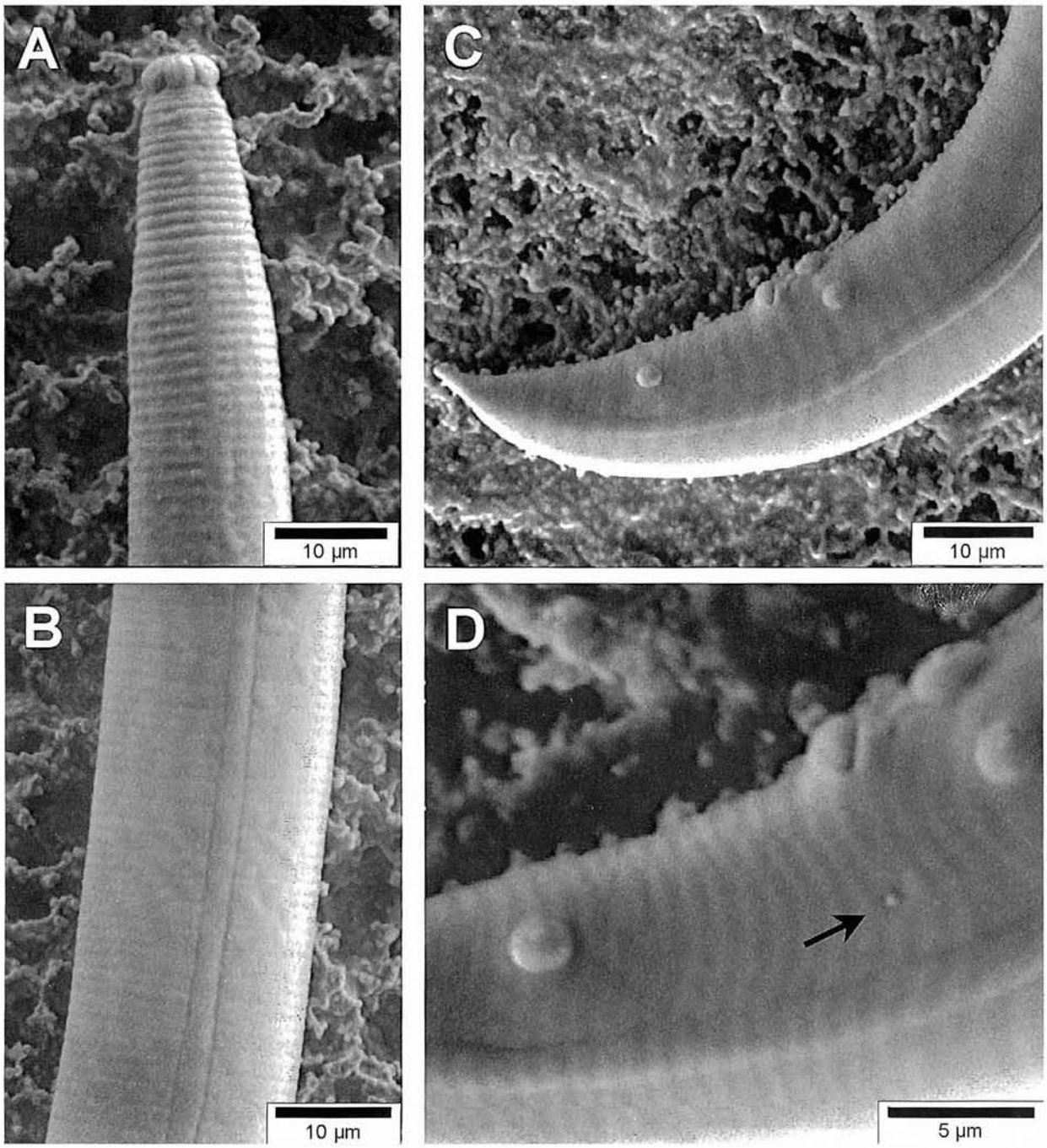

Fig. 3. SEM photomicrographs of adult male of *B. sinensis* sp. n.: A, anterior region; B, lateral field; C, male tail; D, detail of C, ventrolateral sensillum (arrow).

USDA Collection, Beltsville, Maryland, USA.

Diagnosis and relationships. Relatively short nematodes. Stylet thin, short, with distinct basal swellings. Lateral field with two lines. In the female anterior vulva lip forming a small flap covering vulva. Postvulval uterine branch occupying about 62.5-91.5% of vulva-anus distance, tail conical, almost straight or slightly ventrally recurved, terminus almost pointed or rounded.

Bursaphelenchus sinensis sp. n. can be distinguished from all other *Bursaphelenchus* species by the spicules, which are separate and characterized by a squared condylus, ventrally bent, ventral limb weak, with anterior part thickened separate and posterior to the anterior margin of the dorsal limb, remaining part membranous, rostrum not clearly differentiated.

Because of the stylet with distinct basal swellings, small vulval flap, spicule shape, the new species appears similar to *B. aberrans* Yusheng, Kan *et* Jun.

Nevertheless *B. aberrans* differs from the new species by the presence of four lateral lines (two in *B. sinensis* sp. n.), shape of the lip region, anteriorly flattened and somewhat squared in *B. aberrans* (Yusheng *et al.*, 2002), rounded in *B. sinensis* sp. n., excretory pore always posterior to the median bulb (usually posterior, but sometimes at the same level or anterior to the base of the bulb in the new species), shorter post-uterine branch (about 50-60% *vs* 62.5-91.5% of vulva-anus distance in the new species), oocytes in single row *vs* two-three rows in the new species, female and male gonads straight, sometimes reflexed in the new species, shape of female tail, more ventrally curved in *B. aberrans*. Moreover, the spicules in *B. aberrans* appear lacking the membranous ventral limb and the small thickening on the distal end of lamina described for *B. sinensis* sp. n.; the bursa-like flap of cuticle on the tail in *B. aberrans* is conical and narrow, in the new species oval with a small appendage; *B. aberrans* has an adanal pair and two post-cloacal pairs of papillae, the new species three adanal papillae (one single ventral median and one pair subventral), one pair of sensilla, one pair of post-cloacal papillae and gland outlets.

Bursaphelenchus gonzalezi Loof resembles *B. sinensis* sp. n. in the aberrant shape of the spicules, which have a weakly sclerotized ventral protuberance and lack a distinct rostrum, but differs from the new species by the shorter spicules, 13.0 µm (Yin *et al.*, 1988) *vs* 14.5-21.9 µm in the new species, a ventral rod-like sclerotization and the wide lamina of the spicules. Moreover, *B. gonzalezi* can be distinguished by the longer body (690.0-1100.0 µm for females *vs* 450.1-722.4 µm in *B. sinensis* sp. n.; 580.0-970.0 µm for males *vs* 355.7-669.1 µm in *B. sinensis* sp. n., by the number of lateral lines (which are four in *B. gonzalezi*, two in the new species), the stylet bearing inconspicuous basal knobs, which appear distinct in the new species, female without vulval flap, which is present in the new species, female tail thinner with a subacute terminus, conical with terminus almost pointed or rounded in *B. sinensis* sp. n., male tail with two subventral pairs and one subdorsal pair of postanal papillae, while *B. sinensis* sp. n. has only one postanal pair and a pair of gland openings sometimes appearing as very small papillae.

Biological characteristics: nematodes of the new species are not easily reared on *B. cinerea* and multiply slowly on this host.

Etymology: the species name is derived from the latin name of China.

BURSAPHELENCHUS THAILANDAE
Braasch *et* Braasch-Bidasak, 2002
(Table II, Fig. 4)

REMARKS

The species, recently described by Braasch and Braasch-Bidasak (2002) from *P. merkusi* Jungh *et* de Vriese in Thailand, was found together with *B. sinensis* sp. n. in pine wood fragments introduced from China to Austria and easily reared on *B. cinerea*.

The nematodes from *B. cinerea* cultures differ from those of the original description by the longer body (747.8-1194.3 µm *vs* 640.0-820.0 µm for females; 706.6-981.3 µm *vs* 435.0-720.0 µm for males) and the position of the vulva (73.6-77.9 *vs* 72.0-73.0). Median bulb elongate-ovoid 14.5-23.0 µm long, 9.7-18.2 µm wide with conspicuous metacorpal valve 5.4-7.3 µm long, 3.6-4.8 µm wide. Dorsal oesophageal gland opening into lumen of metacorpus 1.8-3.6 µm above valve plates. Excretory pore level or posteriorly adjacent to hemizonid, 0.9-2.2 body diameters behind median bulb. Hemizonid conspicuous, 6.1-7.3 µm long, at 94.4-133.1 µm from the anterior end.

Male tail curled, the extent of curl variable, bursa usually very small and inconspicuous, often indistinguishable with light microscopy, more developed in a few specimens. The variations of the bursa are similar to those described by Braasch and Braasch-Bidasak (2002). The spicule shape appears to correspond closely to those of the original description but with condylus longer (6.1-7.3 µm *vs* 5.0 µm). Three pairs of caudal papillae are present, one preanal, one subventral postanal at 47.3±5.4 (40.7-64.3)% of tail length from cloaca, another smaller more median at 69.0±6.1 (48.0-82.1)% of tail length, as described by Braasch and Braasch-Bidasak (2002).

Female gonad outstretched with oval spermatheca filled with sperms 21.8-84.7 µm long, 18.2-37.5 µm wide. Vulval lips strongly protruding. Tail slim, conoid, whiplike tapering sometimes with a mucronate structure.

A molecular check of the population using ITS-RFLP confirms the species (Hoyer U., pers. comm.).

Table II. Measurements of *Bursaphelenchus thailandae* (in µm).

	♂♂	♀♀
n	20	20
Body length	829.7 ± 71.4 (706.6 – 981.3)	910.6 ± 148.2 (747.8 – 1194.3)
Body width (max)	27.0 ± 2.8 (20.6 – 33.9)	27.8 ± 4.8 (21.8 – 36.3)
a	30.7 ± 3.1 (26.9 – 33.9)	32.8 ± 2.3 (28.2 – 37.9)
Head height	3.7 ± 0.4 (3.0 – 4.8)	4.0 ± 0.5 (3.6 – 4.8)
Head width	7.1 ± 0.8 (5.4 – 7.9)	8.0 ± 0.6 (7.3 – 8.5)
Distance from anterior end to oesophago-intestinal junction	76.3 ± 2.4 (72.6 – 79.9)	77.6 ± 5.0 (69.0 – 88.3)
b	10.9 ± 1.0 (9.3 – 13.1)	11.7 ± 1.5 (10.0 – 14.4)
Distance from anterior end to posterior end of oesophageal glands	153.0 ± 11.2 (131.9 – 170.6)	156.5 ± 11.8 (139.2 – 179.1)
b'	5.5 ± 0.7 (4.3 – 7.1)	5.9 ± 1.1 (4.9 – 8.6)
Distance from anterior end to median bulb base	72.5 ± 2.2 (69.0 – 77.4)	73.6 ± 5.0 (65.3 – 84.7)
b1	11.5 ± 1.2 (9.9 – 13.8)	12.3 ± 1.5 (10.6 – 15.3)
Oesophageal glands overlapping intestine length	76.6 ± 10.9 (52.0 – 93.2)	78.9 ± 11.0 (55.7 – 95.6)
Oesophageal glands overlapping intestine length/body width oesophago-intestinal junction	4.2 ± 0.7 (2.9 – 5.1)	4.2 ± 0.8 (2.6 – 5.6)
Stylet length	13.9 ± 0.5 (13.3 – 14.5)	14.0 ± 0.6 (13.3 – 15.1)
m	42.4 ± 3.1 (33.3 – 46.2)	42.9 ± 2.4 (36.4 – 45.5)
Distance from anterior end to excretory pore	116.9 ± 8.4 (99.2 – 133.1)	116.1 ± 10.1 (101.6 – 140.4)
V or T	70.1 ± 7.7 (59.9 – 80.3)	76.0 ± 1.1 (73.6 – 77.9)
Anterior genital branch length	583.6 ± 99.3 (423.5 – 774.4)	452.6 ± 153.2 (290.4 – 776.8)
G1	-	48.7 ± 9.1 (38.5 – 65.8)
Postvulval branch length	-	84.8 ± 17.8 (60.5 – 121.0)
G2	-	9.3 ± 1.0 (7.8 – 11.7)
Vulva-anus distance	-	169.6 ± 31.3 (139.2 – 242.0)
Postvulval branch length % vulva-anus distance	-	50.1 ± 6.0 (41.0 – 63.8)
Tail length	31.0 ± 1.6 (29.0 – 33.9)	50.8 ± 6.0 (42.4 – 72.6)
c	26.8 ± 2.6 (20.9 – 31.2)	18.0 ± 2.3 (15.1 – 23.8)
c'	2.0 ± 0.2 (1.6 – 2.3)	3.8 ± 0.4 (3.0 – 4.7)
Spicule length (chord)	17.7 ± 0.5 (16.9 – 18.2)	-
Spicule width	6.7 ± 0.6 (5.4 – 7.3)	-

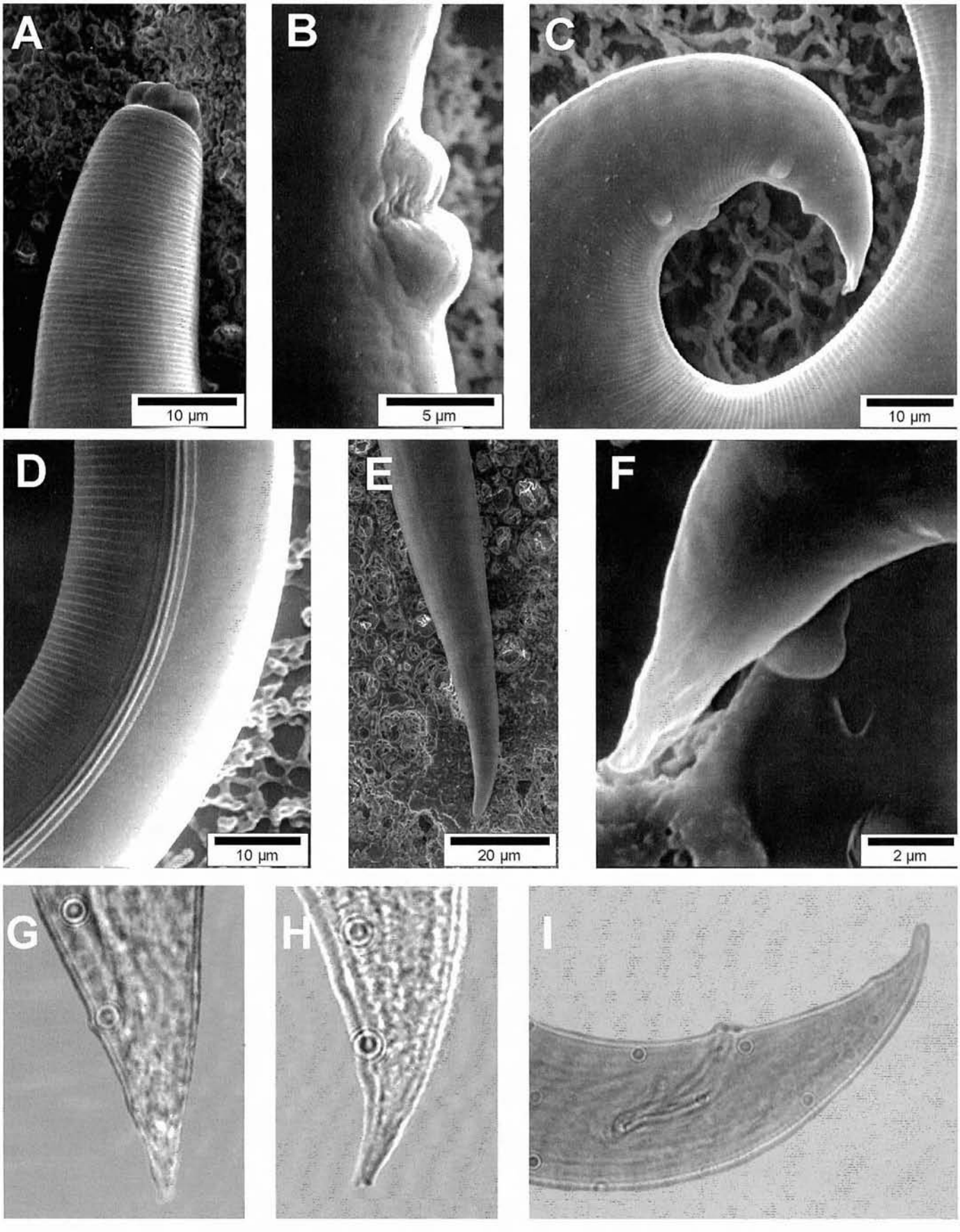

Fig. 4. SEM and LM photomicrographs of *Bursaphelenchus thailandae*: A, male anterior region; B, female vulval region; C, male posterior region; D, lateral field; E, female tail; F-H, male tails, bursa; I male posterior region.

DISCUSSION

Bursaphelenchus sinensis sp. n. cannot easily be attributed to any of the groups in the genus *Bursaphelenchus* (Braasch, 2001). The finding of a few males with cephalic region flattened and stylet longer with wider lumen and without basal knobs, but showing the same spicule shape as the other males, suggests the possible presence of a morphologically different insect parasitc generation. In fact the nematodes are not easily reared on *B. cinerea* and multiply very slowly on this host. A parasitic generation with morphological differences between free-living and parasitic adults has been observed in other *Bursaphelenchus* and *Parasitaphelenchus* species (Massey, 1966; Vosilite, 1990). Males which differed from the other males only by head shape and shape and size of the stylet were found in a population attributed to *B. aberrans* (Braasch and Braasch-Bidasak, 2002). A more careful comparison of *B. sinensis* sp. n. with *B. aberrans* was impossible as the authors were unable to obtain the necessary material from China. *Bursaphelenchus thailandae* and *B. aberrans* were found in coniferous wood of *Pinus* spp. in Thailand (Braasch and Braasch-Bidasak, 2002) and also in packaging wood from China (Tomiczek *et al.*, 2003).

The finding of *B. sinensis* sp. n. and the apparently frequent occurrence of other *Bursaphelenchus* species in packaging wood (Braasch and Braasch-Bidasak, 2002) shows the great risk of spreading these nematodes through this material.

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