

Description of *Bursaphelenchus ulmophilus* sp. n. (Nematoda: Parasitaphelenchinae) associated with Dutch elm disease of *Ulmus glabra* Huds. in the Russian North West

Alexander RYSS^{1,*}, Kristina S. POLYANINA²,
Boris G. POPOVICHEV² and Sergej A. SUBBOTIN^{3,4}

¹ Zoological Institute of the Russian Academy of Sciences, Universitetskaya Naberezhnaya 1,
St Petersburg 199034, Russia

² St Petersburg State Forest Technical University, Institutskiy Pereulok 5, St Petersburg 194021, Russia

³ Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road,
Sacramento, CA 95832, USA

⁴ Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences,
Moscow 117071, Russia

Received: 4 January 2015; revised: 13 April 2015

Accepted for publication: 13 April 2015; available online: 26 May 2015

Summary – A new species, *Bursaphelenchus ulmophilus* sp. n., from the *hofmanni* group is described morphologically and molecularly. This nematode species was found associated with Dutch elm disease of *Ulmus glabra* in parks of St Petersburg, Russia, and is vectored by adults and larvae of the bark beetles *Scolytus multistriatus* and *S. scolytus*. *Bursaphelenchus ulmophilus* sp. n. is characterised by the following features: body length 600-850 μm , stylet 12-14 μm long with base slightly and smoothly expanded, but lacking knobs, median bulb almost spherical in female and slightly ovoid in male, pharyngeal gland lobe dorsal, 4-5 body diam. long. This species has an oval spermatheca filled with spherical nucleic sperm 4-5 μm diam. Female post-uterine sac *ca* 0.5 of the vulva-anus distance and *ca* 3 vulval body diam. long, female tail reflexed, strongly hooked ventrally with a digitate or conically rounded tip. The male has seven caudal papillae arranged as 1 + 2 + 2 + 2, P1 is unpaired, anterior to cloacal opening, paired P2 at cloacal aperture, paired P3 and paired pore-like ‘gland papilla’ P4 at the lateral edges of the bursa which has the posterior border rounded to truncate. Phylogenetic analyses of the D2-D3 of 28S rRNA, partial 18S rRNA and ITS rRNA gene sequences revealed that *B. ulmophilus* sp. n. formed a clade with species of the *hofmanni* group and shared close relationships with *B. hofmanni* and *B. pinasteri*.

Keywords – bark beetle, Curculionidae, *hofmanni* group, molecular, morphology, morphometrics, new species, phylogeny, Scolytinae, *Scolytus multistriatus*, *Scolytus scolytus*, taxonomy.

Bursaphelenchus includes more than 100 nominal species, two of them being considered as economically important pests, namely *B. xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970, which causes the pine wilt disease of coniferous plant hosts with beetle vectors *Monochamus* spp. (Cerambycidae) in North America, East Asia and south Europe (Mota & Vieira, 2008), and *B. cocophilus* (Cobb, 1919) Baujard, 1989, responsible for the devastating red ring disease of coconut palm (*Cocos nucifera* L.), oil palm (*Elaeis guineensis* Jacquin) and other palms and transmitted by the palm weevil, *Rhynchophorus pal-*

marum L., in Central and South America (Dean, 1979; Griffith *et al.*, 2005).

After the anomalous summer of 2010 in the Russian Federation, when the temperature increased to seven degrees above the climatic norm, trees displayed wilt symptoms over large areas of forests and parks in the Russian northwest. In 2010 the July average temperature in the St Petersburg area was 24.4°C, in Moscow 26.1°C and in Elista 32.1°C, exceeding the climatic norm by 6.3°C, 7.7°C and 7.0°C, respectively (Wikipedia contributors, 2015). Among the woody plants that suffered were

* Corresponding author, e-mail: nema@zin.ru

Ulmus spp. (Ulmaceae), which were identified as being damaged by Dutch elm disease (DED). DED was first reported in the elms of south St Petersburg in 2002 (Dorofeeva & Tyushina, 2002; Dorofeeva, 2008) and is now widely distributed across parks of this city and other regions (Scherbakova & Mandelshtam, 2014). Morphological, cultural and molecular methods proved that the elm wilt in St Petersburg parks was caused by the European race of the fungus *Ophiostoma novo-ulmi* Brasier, 1991 (Kalko, 2008, 2009; Fedorova, 2009, 2010a, b).

During a survey of declining trees in St Petersburg parks during 2007–2014, we found a new species of *Bursaphelenchus* belonging to the *hofmanni* species group. This nematode was vectored by *Scolytus multistriatus* (Marsh., 1802) and *S. scolytus* (Fab., 1775) beetles and distinctly linked with DED symptoms. This is the third *Bursaphelenchus* species after *B. scolyti* Massey, 1974, which was recovered from the lesser European elm bark beetle, *S. multistriatus*, attacking *Ulmus americana* (L.) in the USA and *B. xerokarterus* Rühm, 1956. *Bursaphelenchus scolyti* is presently known to occur in the USA (Kanzaki *et al.*, 2009a) and has been found in association with *S. multistriatus* or other *Scolytus* species vectoring DED. *Bursaphelenchus xerokarterus* is known only from Germany and considered as *species inquirenda* (Braasch *et al.*, 2009). The entomoparasitic nematode, *Parasitaphelenchus oldhami* (Rühm, 1956), was found in the fat body of another vector of DED, the bark beetle *Hylurgopinus rufipes* (Tomalak *et al.*, 1988). But this nematode seems not to be the DED-associated pest because it may have contributed to a dramatic decline in the overwintering population of the vector beetle, whereas *Bursaphelenchus* spp. and their beetle vectors are usually considered as the synergists in wilt diseases (Mota & Vieira, 2008). Nevertheless, there are examples of the gradual change of the insect role in the Aphelenchoididae life cycle, at least from phoresy to the role of parasite. The dauer juveniles of *B. fagi* inhabiting Malpighian tubules demonstrate real pathogenicity to its vector *Taphrorychus bicolor* (Herbst, 1793) (see Tomalak & Filipiak, 2014); in the tracheal system and body cavity of the cerambycid vector beetle, *Acalolepta luxuriosa* (Bates, 1873), the endoparasitic adult form of *B. luxuriosae* was found with a degenerate digestive tract, thus indicating an endoparasitic feeding mode (Kanzaki *et al.*, 2009b). In the present study a new nematode species of the genus *Bursaphelenchus* from St Petersburg, Russia, is described.

Materials and methods

NEMATODE ISOLATION AND MORPHOLOGICAL OBSERVATION

Wood samples of *U. glabra* Huds., with distinct symptoms of DED, were collected in St Petersburg parks at the St Petersburg State Forest Technical University and the City Summer Garden. Simultaneously, adults and larvae of the bark beetles *S. multistriatus* and *S. scolytus* were collected from galleries in bark and the outer layer of wood and phloem. Nematodes were extracted from elm wood for 24 h at room temperature using a modified Baermann funnel technique.

The same technique was used to extract dauer juveniles from bark beetles and their larvae. Water was substituted with 0.9% NaCl and extractions started for 3 h at room temperature, and then continued at 24 h at 8°C in a refrigerator to prevent decay. Later we found that during extraction periods longer than 3 h the dauer juveniles (J3D) moulted to the next stage (J4D). Thus, we obtained the J3D by immediate dissection (within 1 h) of the adult beetle and their larvae. The morphological descriptions for the J3D and J4D stages are given separately.

For the morphological study, specimens were fixed in hot 4% formaldehyde, then processed to glycerin and mounted on permanent collection slides by a modification of Seinhorst's (1959) technique as described by Ryss (2003). Extracted nematodes were also multiplied on laboratory cultures of the fungus *Botryotinia fuckeliana* (de Bary) Whetzel, 1945 (= *Botrytis cinerea* Pers., 1794) growing on 2% potato dextrose agar at 22°C. Nematodes maintained in the culture were used for morphological and molecular studies.

DNA EXTRACTION, PCR, SEQUENCING AND PHYLOGENETIC ANALYSIS

DNA from nematode samples was extracted from several individuals using proteinase K. PCR and sequencing protocols were as described by Tanha Maafi *et al.* (2003). The primer set: D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (De Ley *et al.*, 1999) was used for amplification of the D2-D3 expansion segments of 28S rRNA gene, the primer set: F18STy12 (5'-CAG CCG CGG TAA TTC CAG C-3') and R18Ty12 (5'-CGG TGT GTA CAA AGG GCA GG-3') (Chizhov *et al.*, 2006) was used for amplification of the partial 18S rRNA gene and the primer set: F194 (5'-CGT AAC AAG GTA GCT GTA

G-3') (Ferris *et al.*, 1993) and 5368 (5'-TTT CAC TCG CCG TTA CTA AGG-3') (Vrain, 1993) was used for amplification of the ITS rRNA gene. The new sequences were submitted to GenBank under accession numbers KP331048, KP331049 and KR011752.

The D2-D3 expansion segments of 28S rRNA, 18S rRNA and ITS rRNA gene sequences of *Bursaphelenchus* from GenBank (Ye *et al.*, 2007; Pedram *et al.*, 2011; and others) were also used for phylogenetic reconstruction. Outgroup taxa for each dataset were chosen according to previously published data (Ryss *et al.*, 2013). The newly obtained and published sequences for each gene were aligned using ClustalX (Thompson *et al.*, 1997). The alignments were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) under the GTR model. BI analysis for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades.

Results

*Bursaphelenchus ulmophilus** sp. n. (Figs 1-7)

MEASUREMENTS

See Table 1.

DESCRIPTION

Adults

Body length 600-850 μm , curved ventrally. Stylet 12-14 μm long, its base slightly expanded, but without distinct knobs, cone forming 50% of its length. Four to five cephalic annuli, weakly visible under light microscopy. Excretory pore located mostly at posterior border of nerve ring. Lateral field with two bands in cross section, thereby appearing mostly as three lines in superficial view, but sometimes as four lines if distance between bands equals band width.

* The specific epithet is from the Latin and means 'elm-loving'.

Male

Similar to female in structure of anterior end. Testis situated on right subventral side of mid-intestine, long, anteriorly reflexed and with tightly packed polygonal spermatocytes, zone of spermatids distinct, consisting of two or three quartets of large separated cells, and a zone of large granulated polygonal immature sperm cells located posterior to spermatids. Sperm gradually decreasing in size to spherical mature sperm cells filling posterior 20-25% part of testis which has thick walls consisting of dark granular polygonal cells. These cells presumably have a secretory function as the spherical sperm cells lay amongst the secretion granules. Tail strongly hooked like an umbrella handle, terminating with mid-sized, membrane-like bursa with central chord, its length 5 μm along central line and 8-10 μm at borders, and 10 μm wide at base. Bursa varying from rounded to spade-like with truncate end with straight posterior edge. There are seven male tail papillae: mid-ventral unpaired P1 just anterior to cloacal opening, paired P2 at same level as P1 laterally, paired P3 shifted to 55-65% distance closer to bursa, and paired, small, pore-like P4 close to ventral mid-line at level of lateral edges of bursa. P4 pair can be considered as 'gland papillae' because of their small size and pore-like form, whereas other papillae are nipple-like. Spicule stout, rostrum and condylus well developed and separated (= *hofmanni* group). Angle between lines along capitulum (condylus-rostrum) and extending spicule end = 12-28°, point of intersection dorsal. Rostrum bluntly conical to rounded. Junction of rostrum and calomus rectangular. Condylus hemispherical to digitate, distinctly reflexed dorsally in most individuals. Spicular tip (lateral view) with small rounded cucullus, its width slightly more than its length, $1.2 \times 1.0 \mu\text{m}$, cucullus sometimes indistinct. Spicular lamina mid-point not excessively widened, bearing two lines, as typical for *hofmanni* group, comprising one curved line along dorsal lamina and second straight line along ventral lamina, additional third central straight line often distinct. Dorsal spicular lamina smoothly and symmetrically curved.

Female

Ovary well developed, reaching pharyngeal gland lobe, situated on right subventral side of mid-intestine. Oviduct straight and wide, with wrinkled surface. Spermatheca small, oval, situated ventrally and to left side of proximal part of oviduct, with spherical non-cytoplasmic but nucleic sperm 4-5 μm diam. Spermatheca opening from left side to pre-crustaformerial chamber *via* a plicate sper-

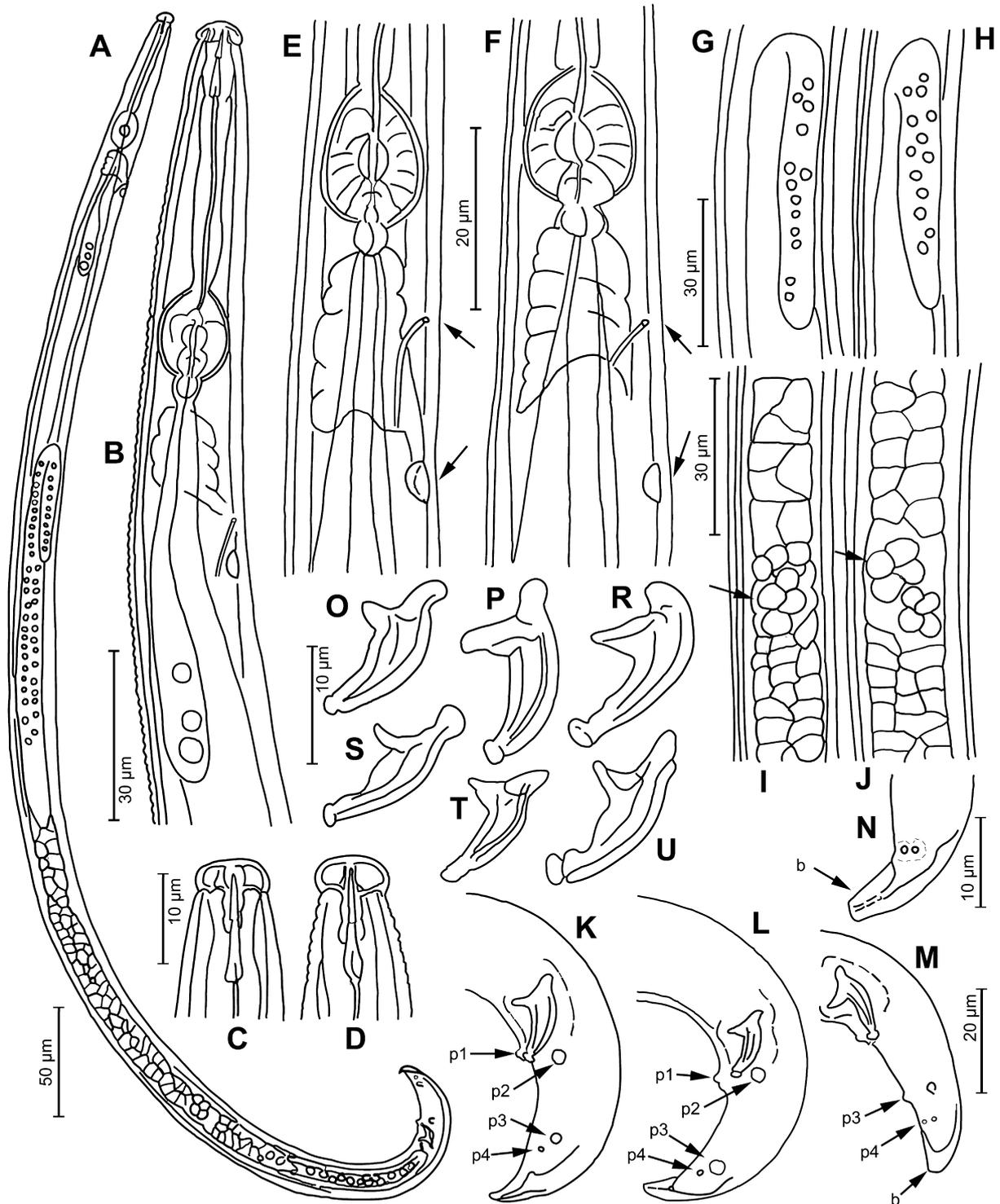


Fig. 1. *Bursaphelenchus ulmophilus* sp. n. Male. A: Body outline; B: Anterior region; C, D: Head and stylet; E, F: Excretory pore (upper arrow) and hemizonid (lower arrow); G, H: Reflexed testis tip with spermatogonia; I, J: Spermatids (arrows); K-N: Tail end (p1-p4 = male caudal papillae; b = bursa); O, P, R, S, T, U: Variations in spicule shape.

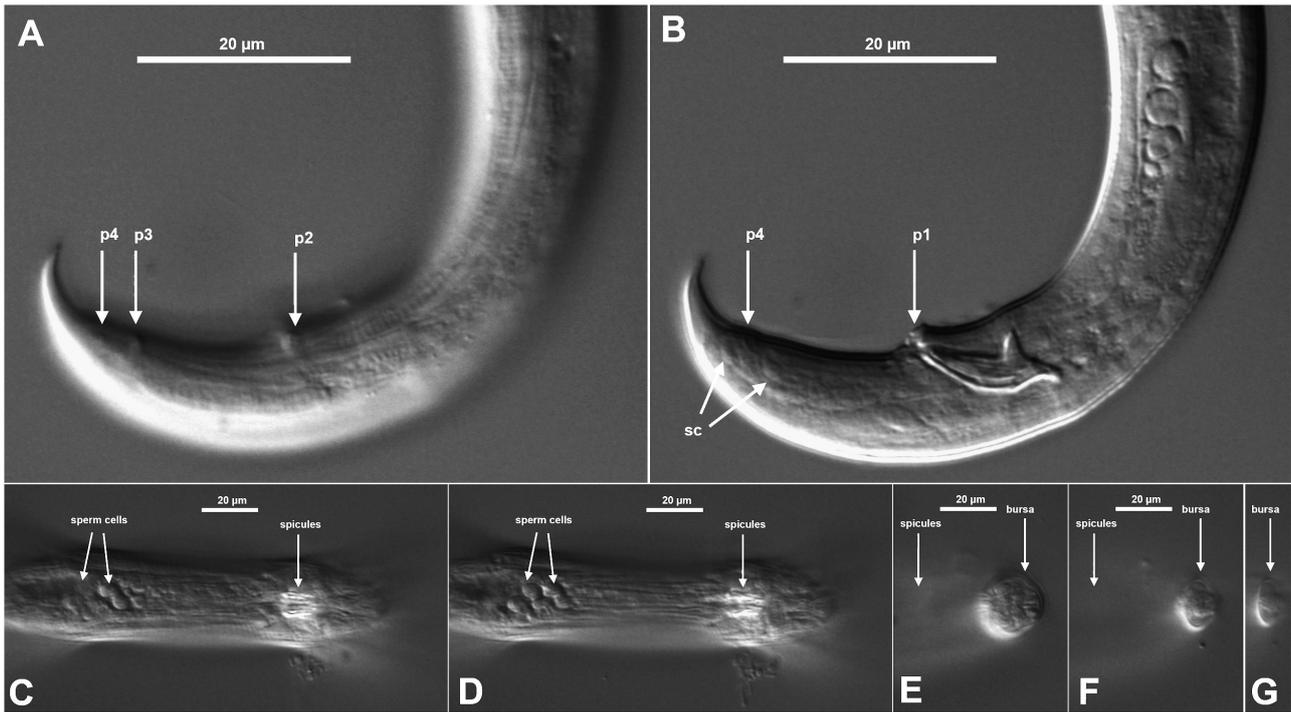


Fig. 2. *Bursaphelenchus ulmophilus* sp. n. A, B: Male tail at different optical levels, lateral view (p1-p4 = male caudal papillae; sc = sensory cells of papillae); C-G: Male tail focused at different levels, ventral view. Colour images available online at https://sites.google.com/site/ryssparasitology/files/2014_tail_sensory_100x_2s%20%281%29.gif and https://sites.google.com/site/ryssparasitology/files/2014_male_tail_anfas_40x.gif.

mathecal duct. Oviduct opening in pre-crustaformerial chamber from right side. Pre-crustaformerial chamber with small inner cavity, this chamber continuing proximally into crustaformerialia. Pre-crustaformerial chamber and crustaformerialia separated by sphincter with strong fibrillae. Crustaformerialia formed by large spherical cells containing cytoplasmic granules, joining with anterior uterus, walls of which consisting of large flattened cells. Vagina cuticular, rarely sloping anteriorly but mostly perpendicular to ventral body surface, vulval flap small, horseshoe-shaped with apex directed anteriorly and long sides (lateral ridges) directed laterally. No vulval papillae visible but, in lateral view on surface of vulval flap, a distinct small fold present in all studied specimens. Pair of three-celled structures situated laterally on both sides of vagina at uterus/post-uterine sac junction, bearing a well sclerotised, prong-like structure on inner surface of uterine wall. Posterior vulval lip massive, supported from inner side with semicircular fibrillary striped band. Post-uterine sac (PUS) very wide, empty or sometimes with round (possibly sperm) cells, its end hemispherical, not differentiated and devoid of rudimentary ovary. Ratio of PUS length to

vulval body diam. = 2.6-3.4. PUS forming 46-51% of vulva-anus distance. Tail reflexed, strongly hooked ventrally. Tail tip digitate or conically rounded.

Dauer juveniles from Scolytus multistriatus

J3D were obtained within 1 h from dissected beetle larva and adult beetles. Body thick, almost straight to slightly ventrally curved. Cephalic region hemispherical, low, with anterior thick hyaline cap-like cupola, continuous or weakly set off. Cephalic and body annulation indistinct. Stylet absent, but capillary stoma visible. Median bulb devoid of muscles and valve, elongate, spindle-shaped $11 \times 6 \mu\text{m}$, its posterior border $38\text{-}53 \mu\text{m}$ from anterior end. Excretory pore visible in two specimens, at the posterior border of nerve ring. Hemizonid just posterior to excretory pore. Pharyngeal gland lobe usually indistinct, in two specimens dorsal, $28\text{-}32 \mu\text{m}$. Genital primordium small, ovoid, $(10\text{-}12) \times (5\text{-}6) \mu\text{m}$, at (V) $53\text{-}73\%$ of body length. Tail narrowly conical, straight, its tip digitate, with hyaline zone $10\text{-}16 \mu\text{m}$ long. Based on the size of genital primordium, these dauer juveniles may be considered as belonging to the early stage J3D.

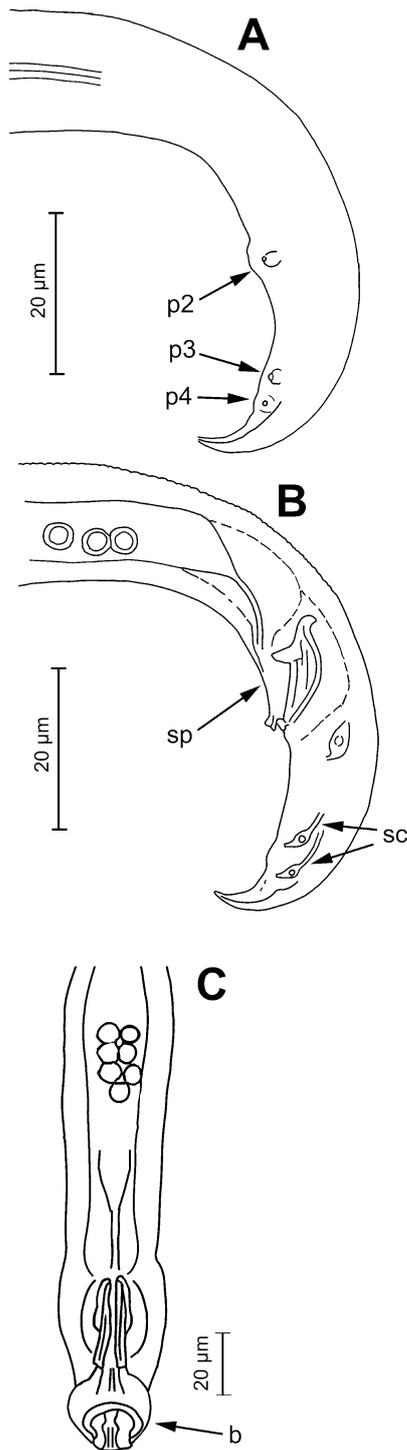


Fig. 3. *Bursaphelenchus ulmophilus* sp. n. A, B: Male tail at different optical levels, lateral view (p2-p4 = male caudal papillae; sc = sensory cells of papillae; sp = spicule); C: Male tail, ventral view (b = bursa).

Freshly moulted J4D obtained from J3D after 3-6 h in 0.9% NaCl water solution. Body slender, straight to ventrally curved. Cephalic region hemispherical, high, cephalic and body annulation indistinct, stylet indistinct, only capillary stoma is visible. Median bulb oval $10 \times 7 \mu\text{m}$, valve absent, its posterior border $52\text{--}55 \mu\text{m}$ from anterior end. Excretory pore indistinct. Pharyngeal gland lobe dorsal, $45\text{--}50 \mu\text{m}$. Genital primordium elongate, $97\text{--}134 \mu\text{m}$ long or 24-32% of body length. In female J4D vulva primordium at 66-76%. Tail sharply conical, straight to ventrally curved. Based on length of genital primordium, these dauer juveniles may be considered as belonging to pre-adult stage, *i.e.*, J4D. Consequently, juveniles of previous stage, from which these juveniles arose *via* a moult, belonging to J3D stage.

TYPE HABITAT AND LOCALITY

Cultures on *B. fuckeliana*-PDA medium were started from individuals isolated from the soft wood (1 cm deep) obtained from a dying elm, *Ulmus glabra* (Ulmaceae) showing symptoms of Dutch elm disease, *i.e.*, wilting, dark-coloured ring in cross-section of wilted branches, trunk with galleries of larvae and pupae of *Scolytus multistriatus* and *S. scolytus* (Curculionidae: Scolytinae). They were collected in the park of St Petersburg State Forest Technical University ($59.991923^{\circ}\text{N}$, $30.342697^{\circ}\text{E}$), St Petersburg, Russia, in all seasons during 2007-2015. Several cultures were started from dauer juveniles extracted from the haemocoel, fat body and tracheas of dissected female adults and larvae of *S. multistriatus* and *S. scolytus* collected simultaneously from galleries in the soft wood and bark from the same elm trees.

OTHER HABITAT AND LOCALITY

The species was also isolated from a dying elm, *U. glabra*, from Letniy Sad (St Petersburg, City Summer Garden, $59.945030^{\circ}\text{N}$, $30.336755^{\circ}\text{E}$) in August 2014.

TYPE MATERIAL

Type material obtained from 2-week-old cultures. Holotype male, 20 paratype females, 20 paratype males, ten paratype J3D dauers and nine paratype J4D dauers deposited in the Nematode Collection of the Zoological Institute RAS, St Petersburg, Russia. Four paratype males and four paratype females also deposited in the Nematode Collection of Wageningen Agricultural University, The Netherlands, and four paratype males and paratype

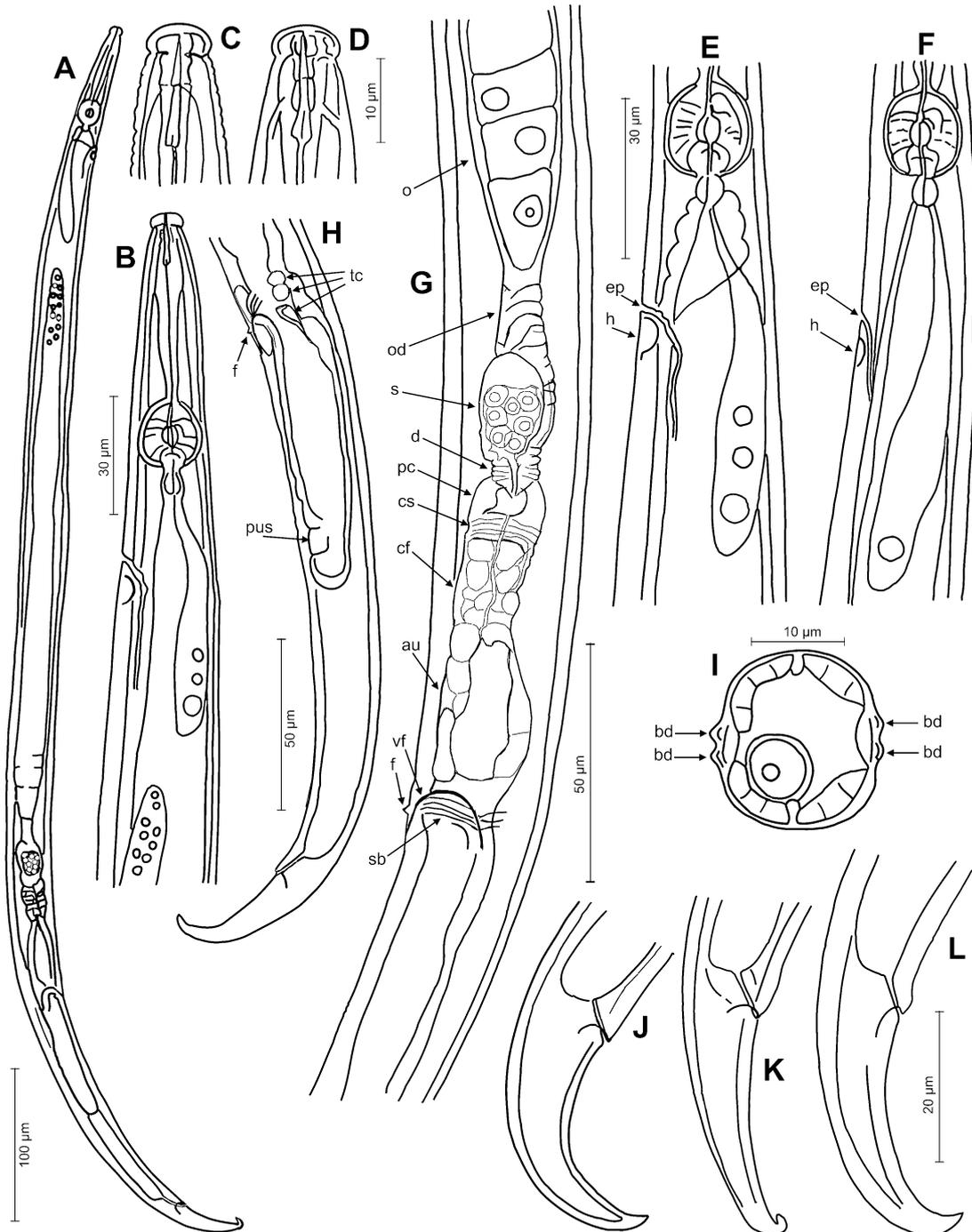


Fig. 4. *Bursaphelenchus ulmophilus* sp. n. Female. A: Entire body; B: Anterior region; C, D: Head and stylet; E, F: Gland lobe, median bulb, excretory pore (ep) and hemizonid (h); G: Anterior part of the female genital system (o = ovary; od = oviduct; s = spermatheca filled with sperm; d = spermathecal duct; pc = pre-crustaformerial chamber; cf = crustaformerial; cs = crustaformerial sphincter; au = anterior uterus; vf = vulval flap; f = vulval flap fold; sb = striped band of the posterior vulval lip); H: Posterior body part (tc = paired three-celled structure; f = vulval flap fold; pus = post-uterine sac); I: Body cross-section at ovary with two bands (= three lines) of lateral field (bd); J-L: Shape of tail.

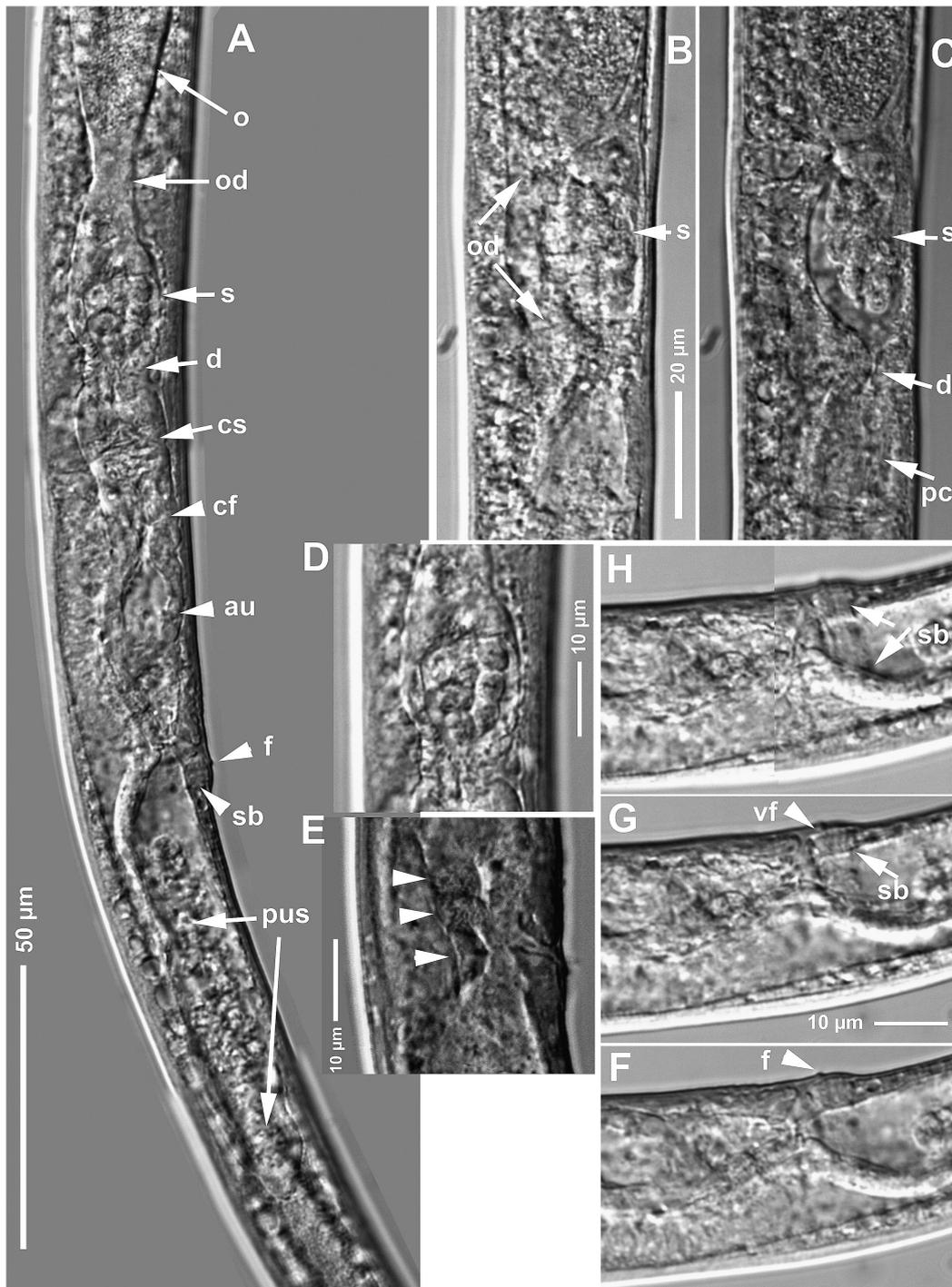


Fig. 5. *Bursaphelenchus ulmophilus* sp. n. Female genital system. A: Genital system sections; B, C: Arrangement of the spermatheca (ventral) and oviduct (dorsal); D: Sperm cells in spermatheca; E: Paired three-celled vaginal structure (arrows); F-H: Vulval region with flap (vf), vulval flap fold (f) and striped semicircular band of the posterior vulval lip (sb) in lateral view in different focal planes. Other abbreviations are the same as for Figure 4. Colour images available online at https://sites.google.com/site/ryssparasitology/files/vul_B_ulmf.gif.

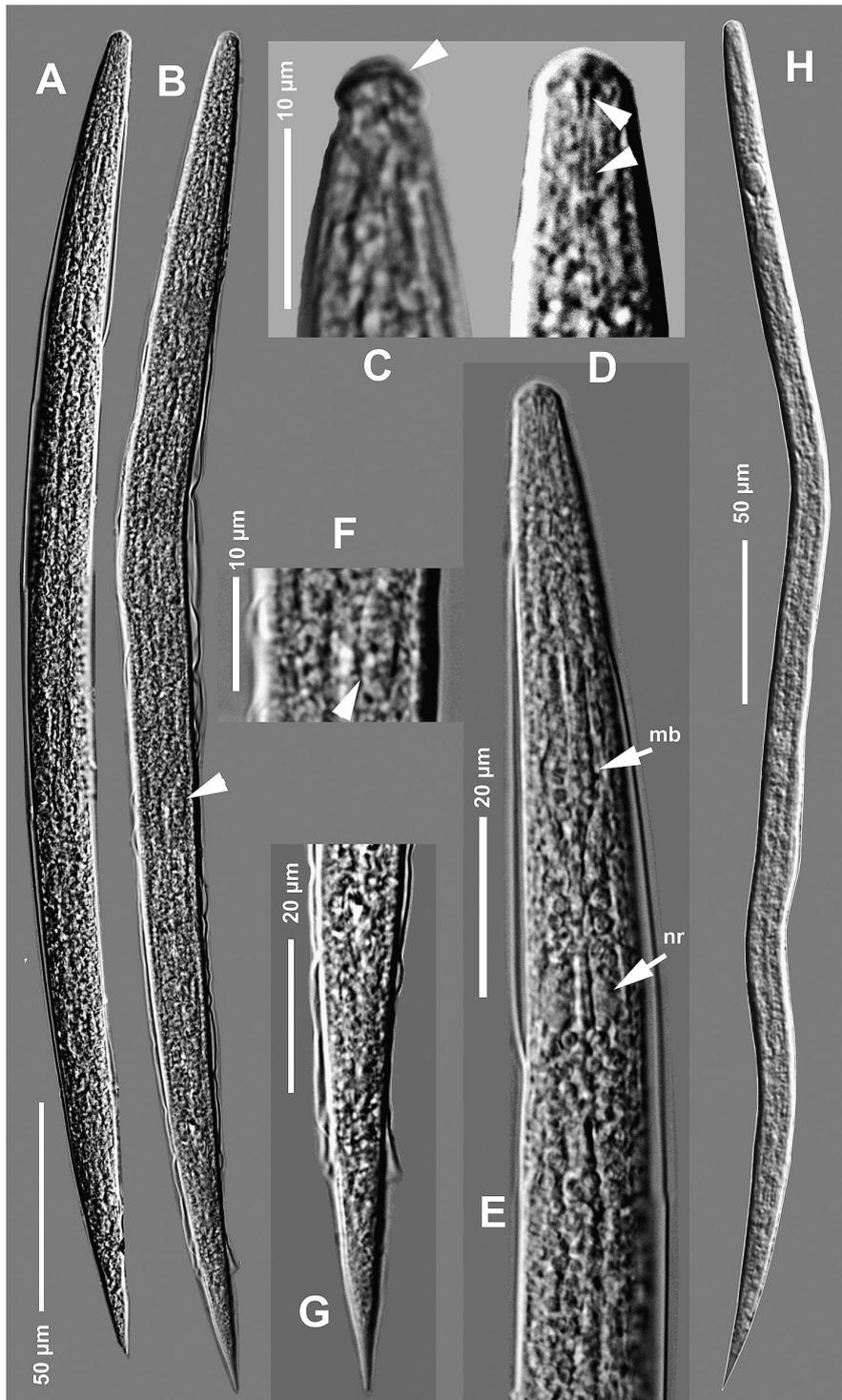


Fig. 6. *Bursaphelenchus ulmophilus* sp. n. Dauers. A, B: Dauer juvenile J3D (arrow = genital primordium); C, D: Head of dauer J3D with hyaline cap (arrow in C) and capillary-like stoma (arrow in D); E: Anterior region of dauer J3D (mb = median bulb; nr = nerve ring); F: Genital primordium in J3D; G: tail of J3D; H: Freshly moulted dauer J4D.

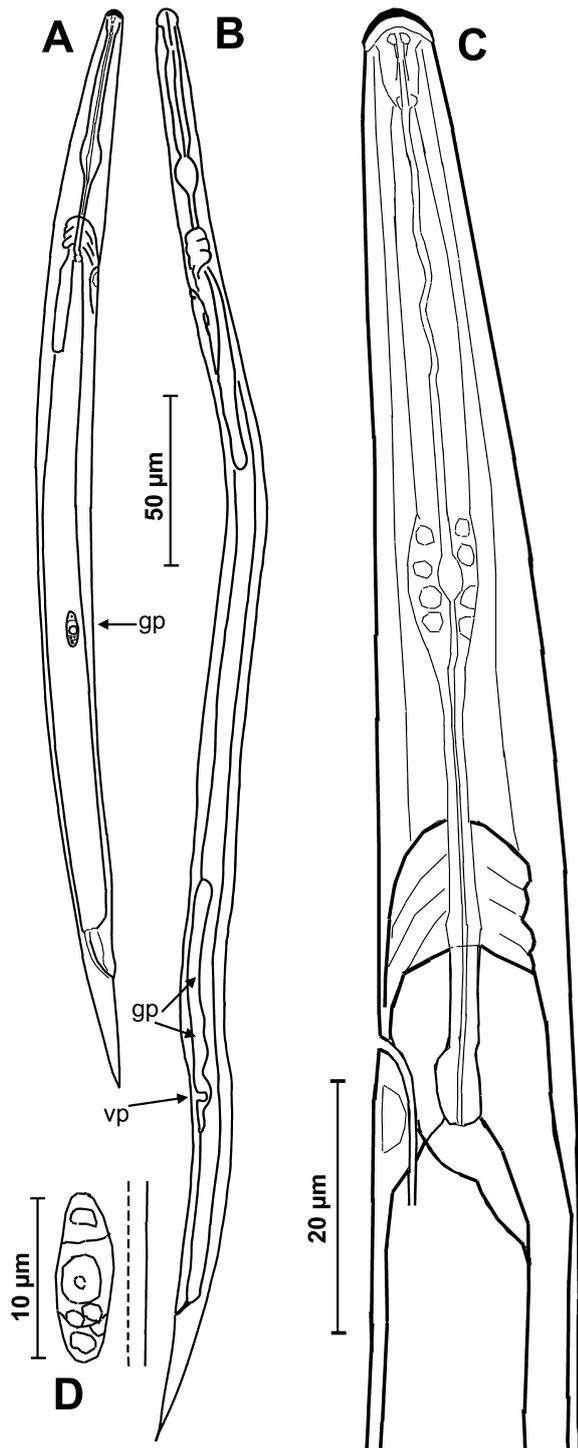


Fig. 7. *Bursaphelenchus ulmophilus* sp. n. Dauers. A: Daurer juvenile J3D (gp = genital primordium); B: Daurer juvenile J4D, freshly moulted (vp = vulval primordium); C: Anterior end of J3D; D: Genital primordium of J3D.

four females in the Nematode Collection of the University of California, Riverside, CA, USA.

DIAGNOSIS AND RELATIONSHIPS

Bursaphelenchus ulmophilus sp. n. is characterised by body length of 600-850 μm , stylet = 12-14 μm , stylet base slightly and smoothly expanded into three ridges, but without knobs, lateral field with two bands or ridges (*i.e.*, three lateral lines). Median bulb almost spherical in female and slightly ovoid in male, pharyngeal lobe dorsal 4-5 body diam. long. Spermatheca oval filled with spherical sperm 4-5 μm diam. Female PUS *ca* 0.5 of the vulva-anus distance and *ca* three vulval body diam. (VBD) long, female tail reflexed, strongly hooked ventrally, its tip digitate or conically rounded. Male with seven caudal papillae: 1 + 2 + 2 + 2, the mid-ventral unpaired P1 anterior to the cloacal opening, the paired P2 level with the cloacal aperture, the paired P3 and paired pore-like 'gland papilla' P4 at the lateral edges of the bursa which has the posterior border rounded to truncate.

Because of the two bands (three lines) that comprise the lateral field, broad spicule shape with small cucullus, bluntly conical rostrum and mostly prominent condylus, the new species belongs to the *hofmanni* group (Braasch *et al.*, 2009), as was also confirmed by molecular data. Among species of this group, the new species is close to *B. hofmanni* in body and spicule shape. It differs from the latter in the shape of condylus (not angular, slightly reflexed *vs* straight in *B. hofmanni*), shape of rostrum (digitate or blunt *vs* pointed in *B. hofmanni*, ratio of PUS to vulva-anus distance = 0.49 (0.46-0.51) *vs* 0.3-0.5 in *B. hofmanni* (data from Braasch, 1998).

Other species of the *hofmanni* group (according to Braasch *et al.*, 2009) differ distinctly from the new species and differences are listed below based on characters taken from the species descriptions.

Bursaphelenchus ulmophilus sp. n. differs from *B. mazandaranense* Pedram, Pourjam, Ye, Atighi, Robbins & Ryss, 2011 in PUS length = 84 (76-89) *vs* 45 (38-57) μm , and the ratio of PUS to VBD = 3.1 (2.6-3.4) *vs* 2 (1.5-2.5); from *B. parvispicularis* Kanzaki & Futai, 2005 in c' = 3.4 (3.1-3.5) *vs* 4.4 (3.9-5.1), female tail tip reflexed *vs* slightly curved, tail tip conical to digitate *vs* cylindrically rounded, in spicule shape with line along the capitulum (condylus-rostrum) and line extending the spicule end crossing dorsally *vs* ventrally (this character was used in: Giblin-Davis *et al.*, 1993, 2006; Ryss *et al.*, 2005), the spicule is of moderate width with a prominent condylus flexed dorsally *vs* broad spicule and compact

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

Character	Male		Female	Dauer J3D	Dauer J4D
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes
n	–	20	20	10	9
L	694	690 \pm 37 (635-735)	827 \pm 12 (807-838)	337 \pm 26 (300-379)	409 \pm 18 (385-422)
a	47.9	46.2 \pm 4 (41.7-53.9)	30.7 \pm 1.6 (28.6-32.3)	20.9 \pm 2.5 (15.8-24.6)	28.7 \pm 5.5 (21.4-32.5)
b	10.8	11.1 \pm 1 (9.9-13.0)	12.7 \pm 0.1 (12.5-12.8)	4.4 \pm 0.6 (3.2-5.3)	6.1 \pm 0.4 (5.6-6.6)
b'	5.5	5.1 \pm 0.34 (4.7-5.5)	6.0 \pm 0.1 (5.9-6.1)	–	–
c	21.0	20.3 \pm 0.8 (19.2-21.8)	20.1 \pm 3.0 (15.6-23.2)	10.1 \pm 1.8 (6.7-12.1)	7.5 \pm 0.8 (6.6-8.4)
c'	2.3	2.1 \pm 0.4 (1.2-2.7)	3.4 \pm 0.2 (3.1-3.5)	4.0 \pm 0.5 (3.2-5.0)	5.8 \pm 1.1 (5.0-7.3)
V	–	–	74.0 \pm 1 (73.0-75.0)	–	–
Stylet	13.0	12.6 \pm 0.4 (12.0-13.0)	13.4 (12.5-14.0)	–	–
Cephalic region diam.	7.0	7.5 \pm 0.5 (7.0-8.0)	7.8 \pm 0.3 (7.5-8.0)	–	–
Cephalic region height	3.5	3.6 \pm 0.4 (3.0-4.0)	3.5 \pm 0.5 (3.0-4.0)	–	–
Median bulb length (L)	15.0	15.0 \pm 0.6 (14.0-16.0)	16.8 \pm 0.9 (16.0-18.0)	–	–
Median bulb diam. (D)	11.5	11.9 \pm 0.6 (11.0-13.0)	15.0 \pm 1 (13.0-16.0)	–	–
Median bulb L/D	1.3	1.3 \pm 0.1 (1.2-1.3)	1.2 \pm 0.04 (1.1-1.2)	2.0 \pm 0.2 (1.6-2.3)	1.4
Median bulb valve diam.	4	3.7 \pm 0.5 (3.0-4.5)	4.7 \pm 0.5 (4-5)	–	–
Excretory pore from anterior	89	79 \pm 10.8 (58-91)	91 \pm 1.5 (90-93)	–	–
Hemizonid from anterior	97	91 \pm 7.6 (76-99)	99 \pm 1.3 (98-101)	–	–
Nerve ring posterior border from anterior	89	82 \pm 6.5 (72-91)	88 \pm 3.4 (84-93)	78 \pm 7.4 (70-93)	68 \pm 5.5 (64-75)
Pharynx	64	63 \pm 4.4 (56-71)	65 \pm 0.9 (64-66)	87 \pm 6.9 (80-101)	53 \pm 1.5 (52-55)
Anterior to gland lobe end	127	135 \pm 7.9 (127-153)	138 \pm 3.1 (135-142)	–	–
Gland lobe	63	72 \pm 5.6 (63-82)	72 \pm 2.7 (70-76)	–	–
Gland lobe/body diam.	4.2	4.5 \pm 0.4 (4.1-5.1)	4.1 \pm 0.2 (3.9-4.2)	–	–
Max. body diam.	14.5	17 \pm 4.0 (13-26)	27 \pm 1.6 (25-29)	16 \pm 1.3 (14-19)	15 \pm 2.5 (13-18)
Posterior genital branch	–	–	84 \pm 5.0 (76-89)	–	–
Posterior genital branch/vulval diam.	–	–	3.1 \pm 0.3 (2.6-3.4)	–	–
Posterior genital branch/vulva-anus distance (%)	–	–	49 \pm 2.0 (46-51)	–	–
Tail	33	34 \pm 2.0 (30-36)	42 \pm 6.8 (36-53)	34 \pm 6.8 (27-50)	55 \pm 4 (50-58)
Tail diam.	14.5	15.3 \pm 1.0 (14.0-17.0)	12 \pm 2.0 (11.0-15.0)	8.6 \pm 1.2 (7.0-10.0)	9.7 \pm 1.3 (8.0-11.0)
Annuli (width of 10 at mid-body)	12	12 \pm 2.0 (10-15)	13 \pm 2.0 (11-15)	–	–
Spicule length (arc)	17.0	16.1 \pm 12.4 (13.0-18.0)	–	–	–
Spicule length (chord)	15.0	14.9 \pm 1.2 (13.0-17.0)	–	–	–
Spicule arc: width posterior to rostrum (lateral view)	3.4	3.9 \pm 0.4 (3.3-4.5)	–	–	–
Ratio distance from line between spicule rostrum and condylus ends to bottom of capitulum depression/rostrum-condylus length	0.2	0.2 \pm 0.04 (0.17-0.25)	–	–	–
Spicule length (along arc): capitulum width (distance between ends of rostrum and condylus)	2.0	2.1 \pm 0.1 (1.9-2.3)	–	–	–
Angle between lines: along capitulum (condylus-rostrum) and extending spicule end (dorsal intersection)	19.0°	18.6 \pm 5.3 (12.0-28.0)°	–	–	–

straight condylus; from *B. ratzeburgii* Rühm, 1956 in ratio of PUS to vulva-anus distance = 0.49 (0.46-0.51) vs 0.29 (0.28-0.3) (calculated from figures in original species description), female tail tip reflexed and digitate vs not reflexed and mucronate; from *B. gerberi* Giblin-Davis, Kanzaki, Ye, Center & Thomas, 2006 in spicule shape with the line along the capitulum (condylus-rostrum) and line extending the spicule end crossing dorsally vs ventrally (this character was used in: Giblin-Davis *et al.*, 1993, 2006; Ryss *et al.*, 2005), condylus and rostrum equally prominent vs condylus very long and truncate and rostrum short; from *B. anamurius* Akbulut, Braasch, Baysal, Brandstetter & Burgermeister, 2007 in spicule length = 16 (13-18) vs 10 (9-11) μm , condylus well developed vs completely reduced, vulval flap small but distinct vs absent, tail tip reflexed vs only slightly curved; from *B. corneolus* Massey, 1966 in spicule shape with condylus and rostrum of equal length vs condylus very long and truncate and rostrum very short; the ratio of PUS length to VBD = 3.1 (2.6-3.4) vs 4.5 (calculated from figures in original species description); ratio of PUS to vulva-anus distance = 0.49 (0.46-0.51) vs 0.70 (calculated from figures in original species description); from *B. curvicaudatus* Wang, Yu & Lin, 2005 in spicule length = 16 (13-18) vs 19 (17-22) μm , stylet length = 13 (12-14) vs 16 (14-17) μm , paired posterior male papillae ('gland papillae') vs unpaired single papilla, spicule condylus slightly reflexed dorsally vs dorsal line of condylus being a straight continuation of the spicule dorsal lamina line; from *B. osumiana* Kanzaki, Akiba, Kanetani, Tetsuka & Ikegame, 2014 in spicule shape with ratio of spicule length to its width posterior to rostrum = 3.9 (3.3-4.5) vs 2.0 (1.9-2.1) (calculated from figures in original species description), condylus prominent vs condylus small, not developed, cucullus well developed vs cucullus small and indistinct: PUS = 3.1 (2.6-3.4) vs 6.6 (5.4-7.2) VBD (calculated from measurements in original species description), female tail relatively shorter with $c' = 3.4$ (3.1-3.5) vs 4.3 (4.0-5.0); from *B. paracorneolus* Braasch, 2000 in spicule shape with line along capitulum (condylus-rostrum) and line extending the spicule end crossing dorsally vs ventrally; spicular lamina mid-point of *B. ulmophilus* sp. n. moderate in width and not mitten-shaped vs excessively widened to mitten-shaped, condylus slightly flexed dorsally vs condylus dorsal line appearing as a straight continuation of dorsal lamina, ratio of PUS to VBD = 3.1 (2.6-3.4) vs 2.0 (1.7-2.4) (calculated from measurements in original species description); from *B. paraparvispicularis* Gu, Wang, Duan, Braasch, Burgermeister & Zheng,

2010 in the presence of a small cucullus at the spicule tip vs lack of a distinct cucullus, presence vs absence of a vulval flap, female tail relatively long with $c' = 3.4$ (3.1-3.5) vs 2.8 (2.4-3.2), and tail tip reflexed vs only slightly ventrally curved; from *B. parapinasteri* Wang & Jang, 2007 in spicule shape which has the condylus long and slightly reflexed vs very short and perpendicular to the dorsal lamina of spicule (as in *B. xylophilus* group males), cucullus present vs absent, bursa bluntly rounded or truncated vs narrowly conical, female tail tip reflexed ventrally vs slightly curved ventrally and almost straight; from *B. pinasteri* Baujard, 1980 in body length of 830 (600-840) vs 600 (550-650) μm , cucullus on spicule tip present vs absent, and in spicule shape with line along capitulum (condylus-rostrum) and line extending the spicule end crossing dorsally vs ventrally, female tail tip reflexed vs straight, ratio of PUS to VBD = 3.1 (2.6-3.4) vs 4.6 (3.6-5.6); and from *B. sachsi* Rühm, 1956 in spicule shape with line along capitulum (condylus-rostrum) and line extending the spicule end crossing dorsally vs ventrally, cucullus present vs absent, ratio of spicule length to its width posterior to rostrum = 3.9 (3.3-4.5) vs 5.1 (calculated from figures in original species description), female tail tip reflexed vs slightly curved, and bursa spade-like to round vs narrowly conical.

Bursaphelenchus xerokarterus Rühm, 1956 is considered here as *species inquirenda* and is thus excluded from the comparison because its poor morphological description does not allow to be classified into any of the species groups (Braasch *et al.*, 2009). The species is an associate of Ulmaceae (*Ulmus foliacea* Gilib. and *Zelkova* sp.) and vectored by *S. scolytus* and *S. multistriatus* (Rühm, 1956). The species needs to be re-isolated and re-described (Kanzaki *et al.*, 2009a).

SEQUENCE AND PHYLOGENETIC ANALYSIS

The D2-D3 of 28S rRNA gene alignment included 61 sequences of *Bursaphelenchus* and two sequences of *Panagrolaimus* and *Panagrellus*, which were selected as outgroup taxa, and was 813 bp in length. Phylogenetic analysis resulted in the majority consensus BI tree presented in Figure 8. The new species clustered with *B. hofmanni* and its sequence differs from that of *B. hofmanni* by 4.9% (35 bp).

The partial 18S rRNA gene alignment included 51 sequences of *Bursaphelenchus* and two sequences of *Pseudaphelenchus vindai* and *Tylaphelenchus jiaae* (selected as outgroup taxa) and was 762 bp in length. The majority consensus BI tree is given in Figure 9. The new species

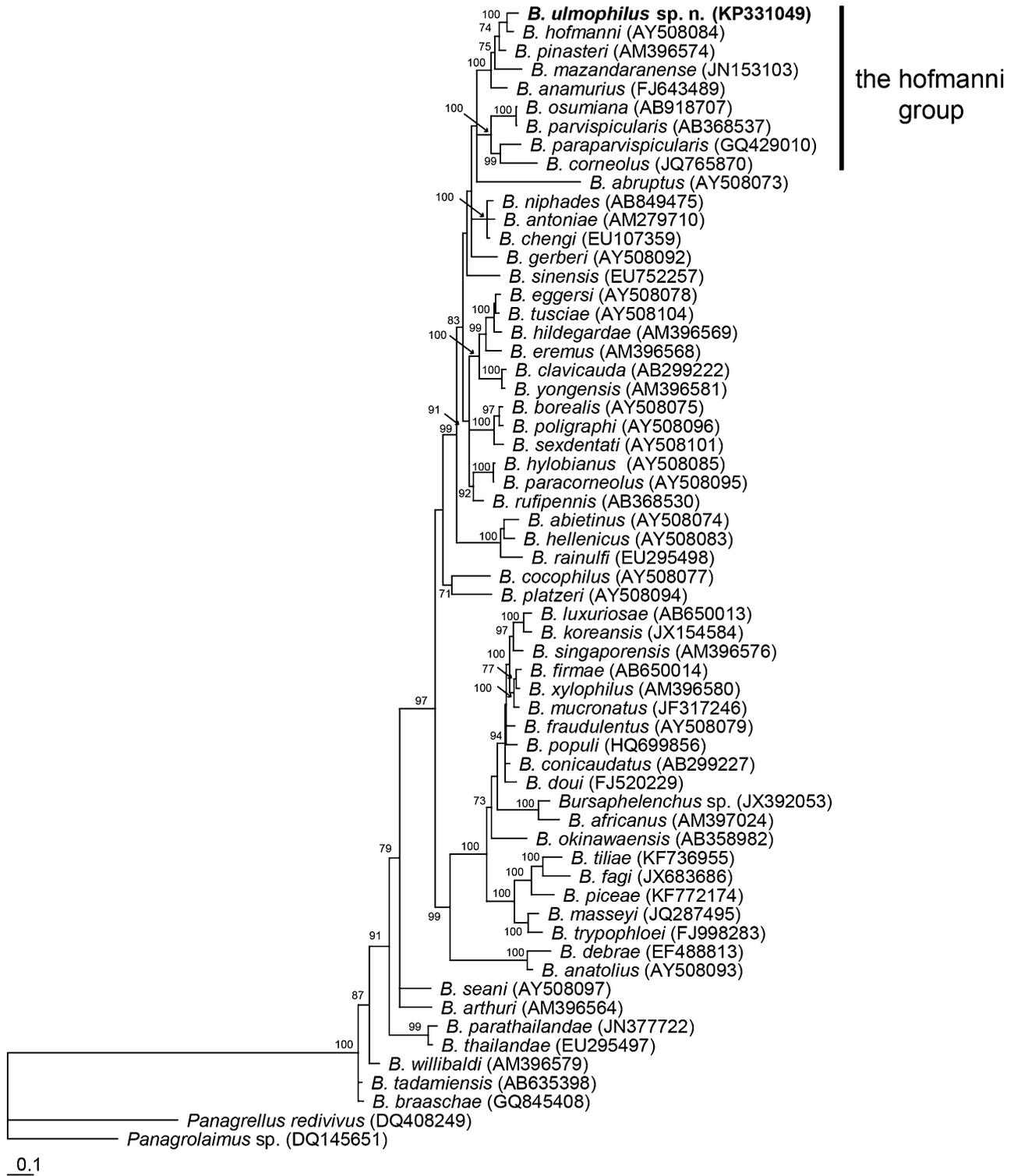


Fig. 8. Phylogenetic relationships within *Bursaphelenchus* spp. as inferred from the Bayesian analysis of D2-D3 28S rRNA gene sequences. Posterior probability values more than 70% are given on appropriate clades. New sequence indicated in bold.

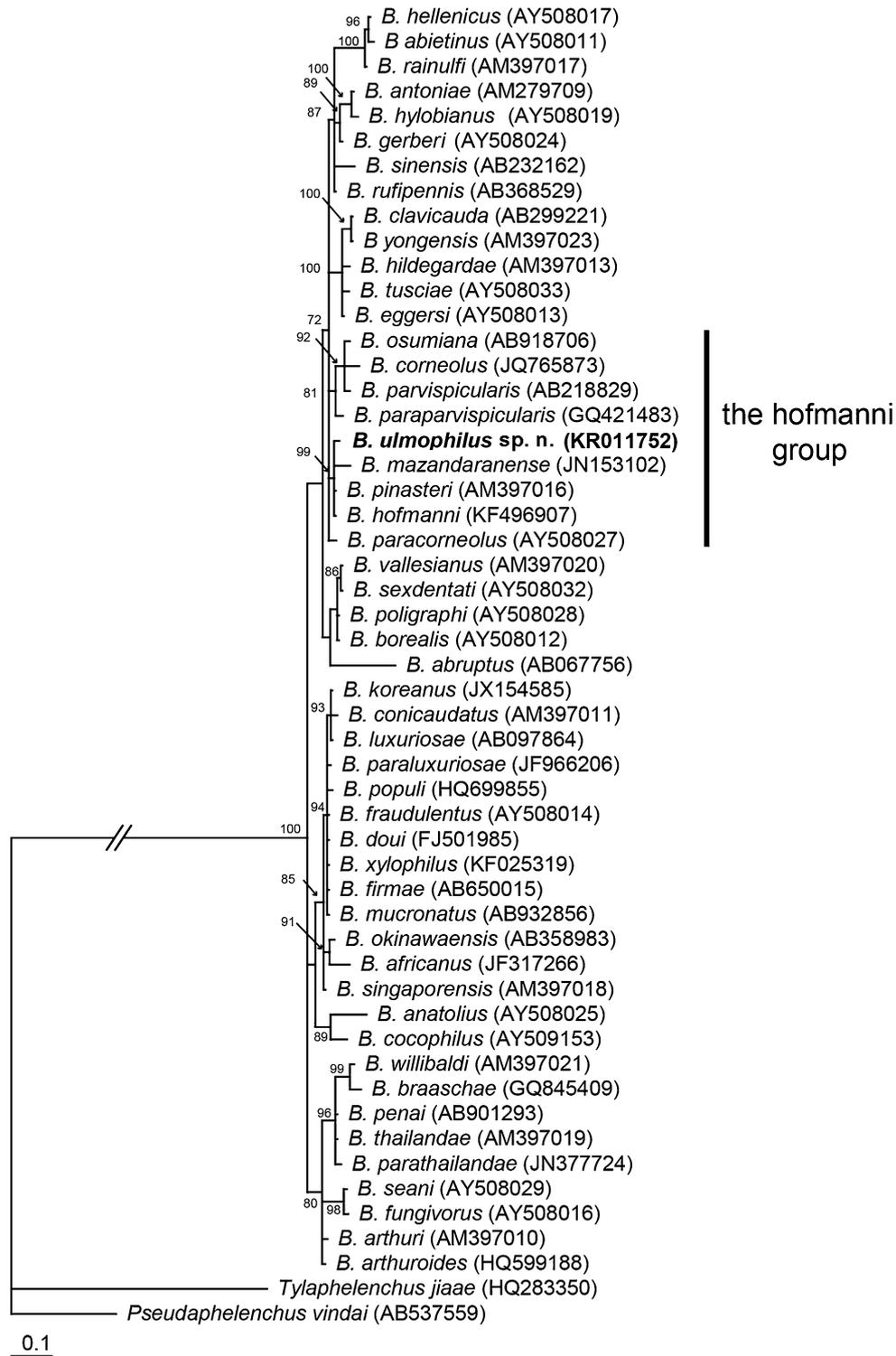


Fig. 9. Phylogenetic relationships within *Bursaphelenchus* spp. as inferred from the Bayesian analysis of partial 18S rRNA gene sequences. Posterior probability values more than 70% are given on appropriate clades. New sequence indicated in bold.

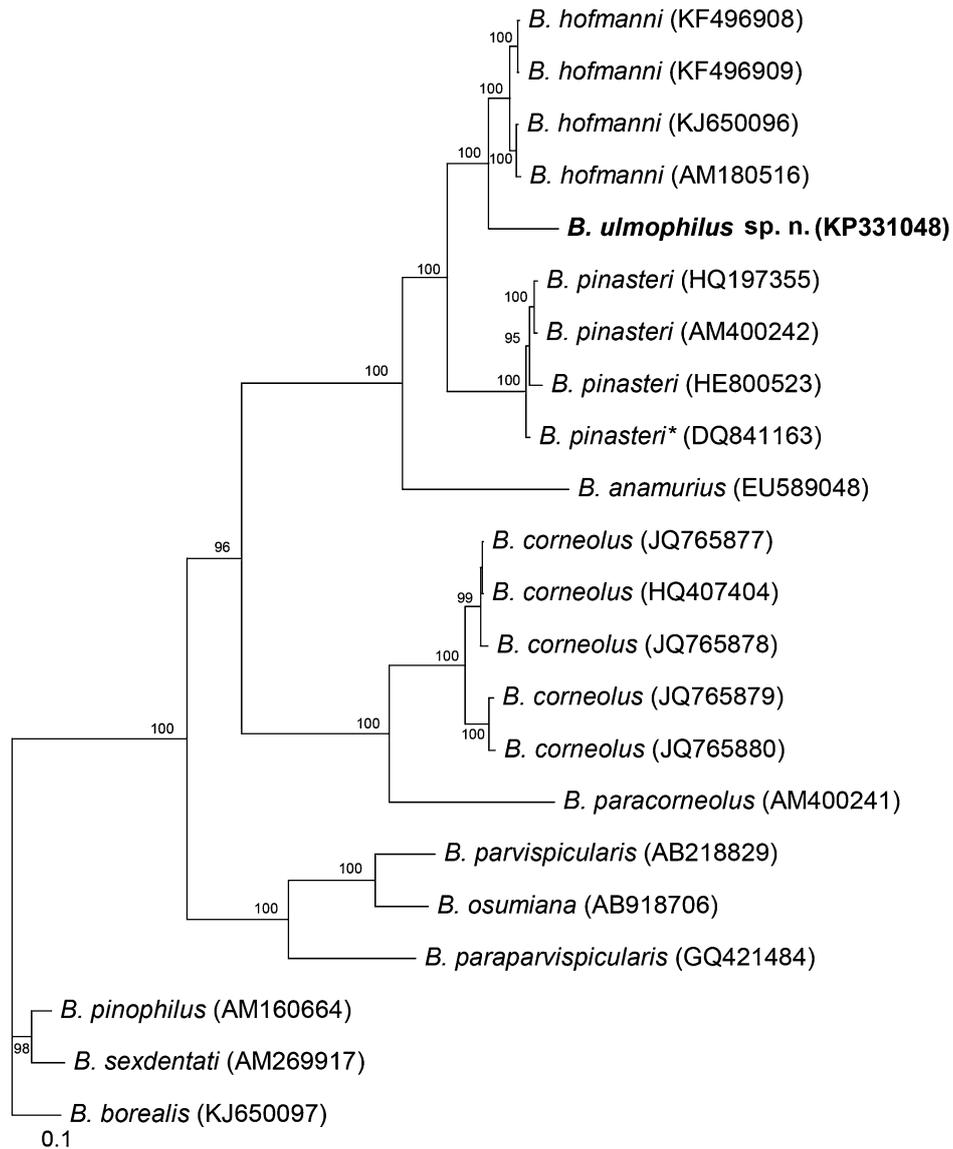


Fig. 10. Phylogenetic relationships within *Bursaphelenchus* from the *hofmanni* group as inferred from the Bayesian analysis of ITS rRNA gene sequences. Posterior probability values more than 70% are given on appropriate clades. New sequence indicated in bold. * Originally identified in GenBank as *B. hofmanni* by Urek *et al.* (2007).

formed a clade with *B. hofmanni*, *B. mazandaranense* and *B. pinasteri* and its sequence differs from those of first two species in three deletions/insertions, 7 bp (0.9%) and three deletions/insertions, respectively, and was identical with that of *B. pinasteri*.

The ITS rRNA gene alignment included 22 sequences of *Bursaphelenchus* and was 1296 bp in length. The majority consensus BI phylogenetic tree is presented in Figure 10. The new species formed a highly supported

clade (PP = 100) with *B. hofmanni*. The ITS rRNA gene sequence of the new species differs from those of *B. hofmanni* by 12.7-13.0% (112-117 bp).

Discussion

The new species is attributed to the *hofmanni* group, a group which includes species associated with conifer-

ous and deciduous woody plants and vectored by representatives of the family Curculionidae, subfamily Scolytinae (Table 2). In the D2-D3 of 28S and 18S rRNA gene trees (Ryss *et al.*, 2013 and the present study) the *hofmanni* group species from coniferous hosts are mixed with

species from deciduous hosts. Such evolutionary patterns might indicate the existence of two trends running in opposite directions, *i.e.*, a transition from Pinaceae hosts to deciduous hosts and *vice versa*. In the clade containing *B. parvispicularis*, *B. osumiana*, *B. corneolus* and *B. para-*

Table 2. Records of species from the *hofmanni* group with list of vectors (Curculionidae) and plant hosts.

Species	Country and region	Vector	Associated plant	Reference
Section 1: Deciduous plant host				
<i>B. ulmophilus</i> sp. n.	Russia, St Petersburg	<i>Scolytus multistriatus</i> Marsh., <i>S. scolytus</i> (Fab., 1775)	<i>Ulmus glabra</i> Buds.	This paper
<i>B. mazandaranense</i>	Iran	?	<i>Fagus orientalis</i> Lipsky	Pedram <i>et al.</i> (2011)
<i>B. parvispicularis</i>	Japan, Kyoto Prefecture	Scolytinae	<i>Quercus mongolica</i> Fisch. ex Ledeb. var. <i>grosseserrata</i>	Kanzaki & Futai (2005)
<i>B. ratzeburgii</i>	Germany Georgia	<i>S. ratzeburgii</i> Jans., 1856 <i>S. ratzeburgii</i>	<i>Betula verrucosa</i> Ehrh. <i>B. verrucosa</i>	Rühm (1956) Kurashvili <i>et al.</i> (1980)
Section 2: Pinaceae plant host				
<i>B. hofmanni</i>	Germany Austria Czech Republic Portugal Slovenia China, Yunnan Province	? <i>Pityokteines curvidens</i> Germ. ? ? ? ?	<i>Picea abies</i> (L.) <i>Abies alba</i> Mill. Imported coniferous wood <i>Pinus pinaster</i> Aiton <i>Pinus</i> sp. <i>Pinus armandii</i> Franchet	Braasch (1998) Braasch (2000); Tomiczek (2000) Braasch (2001) Penas <i>et al.</i> (2002) Urek & Sirca (2003) Dan & Yu (2003)
<i>B. anamurius</i>	Turkey, Mersin	?	<i>Pinus brutia</i> Ten	Akbulut <i>et al.</i> (2007)
<i>B. corneolus</i>	USA, New Mexico	<i>Dendroctonus adjunctus</i> Blandford, 1897	<i>Pinus ponderosa</i> P. & C. Lawson	Massey (1966)
<i>B. curvicaudatus</i>	China, Jiangsu, intercepted from Mexico ship	?	Coniferous wood packaging	Wang <i>et al.</i> (2005)
<i>B. osumiana</i>	Japan, Kagoshima Prefecture, islands Yakushima and Tanegashima	?	<i>Pinus armandii</i> Franch var. <i>amamiana</i> (Koidz.) Hatusima	Kanzaki <i>et al.</i> (2014)
<i>B. paracorneolus</i>	Germany Russia (intercepted wood)	? ?	<i>Picea abies</i> , <i>Pinus sylvestris</i> L. <i>Larix sibirica</i> Ledeb.	Braasch (2000) Braasch (2001)
<i>B. paraparvispicularis</i>	China, Hong Kong, inspected in Ningbo harbour	?	Coniferous wood packaging	Gu <i>et al.</i> (2010)
<i>B. parapinasteri</i>	China, Fujian?	?	<i>Pinus massoniana</i> Lamb.	Wang & Zhang (2007)
<i>B. pinasteri</i>	France Germany Spain	? ? ?	<i>Pinus pinaster</i> Aiton <i>Pinus sylvestris</i> L. <i>Pinus pinaster</i> , <i>P. pinea</i> L.	Baujard (1980) Schönfeld <i>et al.</i> (2001) Escuer <i>et al.</i> (2004)
<i>B. sachsi</i>	Germany Slovakia	<i>Dryocoetes autographus</i> (Ratz., 1837) <i>D. autographus</i>	<i>Picea excelsa</i> (Lamb.) <i>Picea abies</i>	Rühm (1956) Tenkácová & Mítuch (1987)

parvispicularis, only the first named species has a deciduous plant host, the other three species only being known from coniferous trees (Figs 8, 9, and the 28S rRNA and 18S rRNA gene trees in Ryss *et al.*, 2013). In a neighbouring clade of the *hofmanni* group, *B. mazandaranense* and *B. ulmophilus* sp. n. have deciduous host species, whereas the other three species (*B. pinasteri*, *B. hofmanni*, *B. anamurius*) all have coniferous hosts (Figs 8-10 and the 28S rRNA and 18S rRNA gene trees in Ryss *et al.*, 2013). Similar conclusions on the evolution of relationships between *Bursaphelenchus* spp. and their woody Pinaceae and deciduous hosts were drawn by Kanzaki (Kanzaki, 2006; Kanzaki *et al.*, 2014). The absence of strong co-evolution pattern of species in the *hofmanni* group with their plant hosts may be explained by their type of feeding: the nematodes feed and multiply in the wood of dying and dead trees and thus they do not depend on deep physiological relations with their plant hosts.

It is remarkable that the J3D stage is vectored by adult bark-beetles and their larvae, these dauers quickly moulting to the J4D stage. In the *B. xylophilus* group the dauers belong to the JD4 stage which is found on the pupae and vectored by adult cerambycid beetles (Ryss, 2008). The new results indicate a possible difference in the transmission stages between the species groups of *Bursaphelenchus*.

In the results of this study we found and described a new nematode species *B. ulmophilus* sp. n. associated with the DED symptoms. Thus, the DED association might include not only fungi of the genus *Ophiostoma* and beetle vectors of the genus *Scolytus*, as it has been known earlier, but also a nematode species. This association is similar to that for the pinewood disease, which includes *Ophiostoma* fungi, *Monochamus* (Cerambycidae) beetle vectors and the nematode *B. xylophilus* (Ryss *et al.*, 2005; Mota & Vieira, 2008). *Ophiostoma* fungi and *Bursaphelenchus* nematodes may have synergistic effects and might play a major role in inducing wilt diseases in woody plants. We cannot exclude the possibility that the fungi may also play important roles in the nematode life cycle, although such hypotheses need to be proved by experimental studies. It is possible that other nematode-fungus associations will be discovered for other well-known 'fungi-caused' plant diseases as, recently, in Colorado (USA) the role of *B. masseyi* in sudden aspen decline (SAD) of *Populus tremuloides* Michx. caused by the fungus *Cytospora chrysosperma* (Pers.) Fr., using the bark beetle *Trypophloeus populi* Hopkins, 1915 as a vector, was demonstrated (Tomalak *et al.*, 2013).

Acknowledgements

The first two authors, who prepared the morphological part of the paper, acknowledge support from grant 14-14-00621 of the Russian Science Foundation; the molecular part was done by the fourth author; insect and wood sampling by the third author. The authors would like to express appreciation to Prof. S.G. Medvedev and Prof. A.O. Frolov (ZIN RAS) for their help with microscopic equipment and technical advices on microscopy.

References

- Akbulut, S., Braasch, H., Baysal, I., Brandstetter, M. & Burgermeister, W. (2007). Description of *Bursaphelenchus anamurius* sp. n. (Nematoda: Parasitaphelenchidae) from *Pinus brutia* in Turkey. *Nematology* 9, 859-867.
- Baujard, P. (1980). Trois nouvelles espèces de *Bursaphelenchus* (Nematoda: Tylenchida) et remarques sur le genre. *Revue de Nématologie* 3, 167-177.
- Braasch, H. (1998). *Bursaphelenchus hofmanni* sp. n. (Nematoda, Aphelenchoididae) from spruce wood in Germany. *Nematologica* 44, 615-621.
- Braasch, H. (2000). *Bursaphelenchus paracorneolus* sp. nov. (Nematoda: Parasitaphelenchidae) aus Koniferenholz in Deutschland und Bemerkungen zu seiner Biologie und Verbreitung. *Annales Zoologici* 50, 177-182.
- Braasch, H. (2001). *Bursaphelenchus* species in conifers in Europe: distribution and morphological relationships. *EPPO Bulletin* 31, 127-142.
- Braasch, H., Burgermeister, W. & Gu, J. (2009). Revised intra-generic grouping of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchoididae). *Journal of Nematode Morphology and Systematics* 12, 65-88.
- Chizhov, V.N., Chumakova, O.A., Subbotin, S.A. & Baldwin, J.G. (2006). Morphological and molecular characterization of foliar nematodes of the genus *Aphelenchoides*: *A. fragariae* and *A. ritzemabosi* (Nematoda: Aphelenchoididae) from the Main Botanical Garden of the Russian Academy of Sciences, Moscow. *Russian Journal of Nematology* 14, 179-184.
- Dan, Y. & Yu, S. (2003). [Identification of *Bursaphelenchus* spp. on pine wood in Yunnan Province.] *Acta Phytopathologica Sinica* 33, 401-405.
- De Ley, P., Felix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W. & Thomas, W.K. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1, 591-612.
- Dean, C.G. (1979). *Red ring disease of Cocos nucifera L. caused by Rhadinaphelenchus cocophilus (Cobb, 1919) Goodey, 1960. An annotated bibliography and review.* Technical Com-

- munication, No. 47. Farnham Royal, UK, Commonwealth Agricultural Bureaux.
- Dorofeeva, T.B. (2008). [Epiphytotic disease of elm in Saint Petersburg.] *Zashchita i Karantin Rasteniy* 3, 59.
- Dorofeeva, T.B. & Tyushina, G.N. (2002). [Graphiosis of Ulmaceae in Saint-Petersburg and the measures to control it.] *Ekologia Bolshogo Goroda* 6, 57-61.
- Escuer, M., Árias, M. & Bello, A. (2004). Occurrence of the genus *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchida) in Spanish conifer forests. *Nematology* 6, 155-156.
- Fedorova, S.M. (2009). [Phytosanitary situation of the suburb parks of Saint Petersburg.] *Zashchita i Karantin Rasteiniy* 8, 15-17.
- Fedorova, S.M. (2010a). [Diseases of the woody and bush plants in park plantations of the museums Pavlovsk and Gatchina and the efficiency of the biological preparations in the control of Dutch elm disease.] Candidate of Science Dissertation Thesis, St Petersburg – Pushkin, All-Russian Institute of Plant Protection, 130 pp.
- Fedorova, S.M. (2010b). [Epiphytoty of the Dutch elm disease in historical parks of Saint-Petersburg.] In: *Problemy ozele-neniya krupnykh gorodov. Conference Proceedings 9-11 February 2010, Moscow*, pp. 72-77.
- Ferris, V.R., Ferris, J.M. & Faghihi, J. (1993). Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. *Fundamental and Applied Nematology* 16, 177-184.
- Giblin-Davis, R.M., Mundo-Ocampo, M., Baldwin, J.G., Norden, B.B. & Batra, S.W.T. (1993). Description of *Bursaphelenchus abruptus* n. sp. (Nemata: Aphelenchoididae), an associate of a digger bee. *Journal of Nematology* 25, 161-172.
- Giblin-Davis, R.M., Kanzaki, N., Ye, W., Center, B.J. & Thomas, W.K. (2006). Morphology and systematics of *Bursaphelenchus gerberae* n. sp. (Nematoda: Parasitaphelenchidae), a rare associate of the palm weevil, *Rhynchophorus palm-arum* in Trinidad. *Zootaxa* 1189, 39-53.
- Griffith, R., Giblin-Davis, R.M., Koshy, P.K. & Sosamma, V.K. (2005). Nematode parasites of coconut and other palms. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*, 2nd edition. Wallingford, UK, CABI Publishing, pp. 493-527.
- Gu, J., Wang, J., Duan, W., Braasch, H., Burgermeister, W. & Zheng, J. (2010). Description of *Bursaphelenchus parvispicularis* n. sp. (Nematoda: Aphelenchoididae) found in packaging wood from Hong Kong, China. *Nematology* 12, 557-566.
- Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754-755.
- Kalko, G.V. (2008). [Dutch elm disease in Saint Petersburg.] *Mikologia i Fitopatologia* 6, 564-571.
- Kalko, G.V. (2009). [Ophiostoma-caused wilt of elms in Saint Petersburg.] *Zashchita i Karantin Rasteniy* 3, 48-49.
- Kanzaki, N. (2006). [Taxonomy and systematics of *Bursaphelenchus* nematodes.] *Journal of the Japanese Forest Society* 88, 392-406.
- Kanzaki, N. & Futai, K. (2005). Description of *Bursaphelenchus parvispicularis* n. sp. (Nematoda: Parasitaphelenchidae) isolated from a dead oak tree, *Quercus mongolica* var. *grosseserrata*. *Nematology* 7, 751-759.
- Kanzaki, N., Giblin-Davis, R.M. & Center, B.J. (2009a). Redescription of four species of North American *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchinae) from Massey's type material. *Nematology* 11, 129-150.
- Kanzaki, N., Maehara, N., Aikawa, T., Giblin-Davis, R.M. & Center, B.J. (2009b). The first report of a putative "entomoparasitic adult form" in *Bursaphelenchus*. *Journal of Parasitology* 95, 113-119.
- Kanzaki, N., Akiba, M., Kanetani, S., Tetsuka, K. & Ikegame, H. (2014). *Bursaphelenchus osumiana* n. sp. (Tylenchomorpha: Aphelenchoididae) isolated from dead *Pinus armandii* var. *amamiana* in Osumi Islands in Japan. *Nematology* 16, 903-916.
- Kurashvili, B.E., Kakulia, G.A. & Devdariani, T.G. (1980). [Parasitic nematodes of the bark-beetles in Georgia.] Tbilisi, Georgia, Metsniereba.
- Massey, C.L. (1966). The nematode parasites and associates of *Dendroctonus adjunctus* (Coleoptera: Scolytidae) in New Mexico. *Annals of the Entomological Society of America* 59, 425-440.
- Mota, M. & Vieira, P. (Eds) (2008). *Pine wilt disease: a worldwide threat to forest ecosystems*. Dordrecht, The Netherlands, Springer.
- Pedram, M., Pourjam, E., Ye, W., Robbins, R.T., Atighi, M.R. & Ryss, A. (2011). Description of *Bursaphelenchus mazandaranense* sp. n. (Nematoda: Parasitaphelenchidae) from Iran. *Russian Journal of Nematology* 19, 121-129.
- Penas, A.C., Bravo, M.A., Pires, J. & Mota, M. (2002). *Bursaphelenchus* species found in maritime pine in Portugal. *Nematology* 4, 473.
- Rühm, W. (1956). Die Nematoden der Ipiden. *Parasitologische Schriftenreihe* 6, 1-435.
- Ryss, A., Vieira, P., Mota, M. & Kulinich, O. (2005). A synopsis of the genus *Bursaphelenchus* Fuchs, 1937 (Aphelenchida: Parasitaphelenchidae) with keys to species. *Nematology* 7, 393-458.
- Ryss, A.Y. (2003). Express technique to prepare collection slides of nematodes. *Zoosystematica Rossica* 11, 257-260.
- Ryss, A.Y. (2008). Entomophilic juveniles of the nematode *Bursaphelenchus mucronatus* (Nematoda, Aphelenchida, Parasitaphelenchidae) obtained from the cerambycid vector *Monochamus urussovi* (Coleoptera, Cerambycidae) under experimental conditions. *Entomological Review* 88, 108-114.
- Ryss, A.Y., McClure, M.A., Nischwitz, C., Dhiman, C. & Subbotin, S.A. (2013). Redescription of *Robustodorus megadorus* with molecular characterization and analysis of

- its phylogenetic position within the family Aphelenchoididae. *Journal of Nematology* 45, 237-252.
- Scherbakova, L.N. & Mandelshtam, M.Y. (2014). Elms of Saint Petersburg: after the third ring. In: Musolin, D.L. & Selikhovkin, A.V. (Eds). *The Kataev memorial readings – VIII. Pests and diseases of woody plants in Russia. Proceedings of the international conference, Saint Petersburg (Russia), November 18-20, 2014*. Saint-Petersburg, State Forest Technical University, p. 97.
- Schönfeld, U., Apel, K.H. & Braasch, H. (2001). Nematoden der Gattung *Bursaphelenchus* (Nematoda, Parasitaphelenchidae) in den Kiefernwäldern des Landes Brandenburg – Ergebnisse eines Monitorings. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 53, 180-184.
- Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4, 67-69.
- Tanha Maafi, T., Subbotin, S.A. & Moens, M. (2003). Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology* 5, 99-111.
- Tenkáčová, I. & Mituch, J. (1987). Nematodes new for the fauna of the Czechoslovak Socialist Republic with the affinity to scolytids (Coleoptera, Scolytidae). *Helminthologia* 24, 281-291.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876-4882.
- Tomalak, M. & Filipiak, A. (2014). *Bursaphelenchus fagi* sp. n. (Nematoda: Parasitaphelenchidae), an insect-pathogenic nematode in the Malpighian tubules of the bark beetle, *Taphrotrychus bicolor* (Herbst.) (Coleoptera: Curculionidae, Scolytinae), in European beech, *Fagus sylvatica* L. *Nematology* 16, 591-606.
- Tomalak, M., Welch, H.E. & Galloway, T.D. (1988). Interaction of parasitic nematode *Parasitaphelenchus oldhami* (Nematoda: Aphelenchoididae) and a bacterium in Dutch elm disease vector *Hylurgopinus rufipes* (Coleoptera: Scolytidae). *Journal of Invertebrate Pathology* 52, 301-308.
- Tomalak, M., Worrall, J. & Filipiak, A. (2013). *Bursaphelenchus masseyi* sp. n. (Nematoda: Parasitaphelenchinae) – a nematode associate of the bark beetle, *Trypophloeus populi* Hopkins (Coleoptera: Curculionidae: Scolytinae) in aspen, *Populus tremuloides* Michx., affected by sudden aspen decline in Colorado. *Nematology* 15, 907-921.
- Tomiczek, C. (2000). A survey for *Bursaphelenchus* spp. in conifers in Austria and implications to Austria forests. In: *XXI IUFRO World Congress 2000*. Kuala Lumpur, Malaysia, p. 399. [Abstr.]
- Urek, G. & Sirca, S. (2003). [Plant parasitic nematodes affecting the above ground plant parts in Slovenia.] In: *Slovenskega Posvetovanje o Varstvu Rastlin, Slovenije, 4-6 March 2003*, pp. 486-488.
- Urek, G., Sirca, S. & Geric, B. (2007). Morphometrical and molecular characterization of *Bursaphelenchus* species from Slovenia. *Helminthologia* 44, 37-42.
- Vrain, T.C. (1993). Restriction fragment length polymorphism separates species of the *Xiphinema americanum* group. *Journal of Nematology* 25, 361-364.
- Wang, H.Y. & Zhang, S.S. (2007). Description of *Bursaphelenchus parapinasteri* sp. nov. (Nematoda, Parasitaphelenchidae) in wood of *Pinus massoniana* from Fujian Province, China. *Acta Zootaxonomica Sinica* 32, 906-912.
- Wang, J.C., Yu, B.Y. & Lin, M.S. (2005). Description of a new species (Nematoda, Aphelenchoididae) isolated from wood packaging material. *Acta Zootaxonomica Sinica* 30, 314-319.
- Wikipedia contributors (2015). [Anomalous heat in Russia (2010).] Available online at <https://ru.wikipedia.org/?oldid=68912367> (accessed 18 March 2015).
- Ye, W.M., Giblin-Davis, R.M., Braasch, H., Morris, K. & Thomas, W.K. (2007). Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43, 1185-1197.