# *Deviburs aphelenchus wangi* sp. n. (Nematoda: Ektaphelenchinae) feeding on *Aphelenchoides* sp.

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**Summary.** *Deviburs aphelenchus wangi* sp. n. is described and figured. The new species was isolated from pine packaging wood from the USA and inspected in Ningbo harbour, China in 2009. The new species is characterised by relatively slender body (a = 36.5 and 37.1 for males and females, respectively); three lines in the lateral field; stylet with relatively wide lumen but lacking basal knobs; vulval flap absent, very short post-uterine sac; non-functional and indistinct female rectum and anus; spicules relatively small (14.2-15.6 μm) with a flattened cucullus; two pairs of caudal papillae. The new species is morphologically close to *D. eproctatus* and *D. hunanensis* and can be distinguished by shape and size of spicules and smaller stylet. The separate species status is supported by ITS-RFLP patterns and molecular phylogenetic analysis based on ITS1/2 and partial LSU sequences, which revealed that the new species is close to *D. hunanensis*. The feeding habit of the new species is also observed and discussed.

**Key words:** Ektaphelenchidae, morphology, molecular taxonomy, new species.

In March 2009, during a routine inspection of imported packaging wood, a new species of *Deviburs aphelenchus* was isolated together with an *Aphelenchoides* species from pine packaging wood coming from USA. It is described and figured herein as *Deviburs aphelenchus wangi* sp. n.

## MATERIAL AND METHODS

Sawn samples taken from packaging wood were cut into small pieces no more than 1 cm wide. Nematodes were extracted by the modified Baermann funnel technique for 24 h. The feeding habit of the new species was observed on a slide in water and recorded. Multiplication on agar-fungi plates (*Botryotinia fuckeliana*) failed. Measurements were made on permanent slides fixed in TAF and processed to glycerol following the method of Seinhorst (1959). The light micrographs were made using a Zeiss Imager Z1 microscope equipped with a Zeiss AxioCam MRm CCD camera.

DNA samples of *Deviburs aphelenchus wangi* sp. n. were prepared according to Li *et al.* (2008). Two sets of primers (synthesised by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the ITS1/2 region and the D2D3 LSU region of rDNA, respectively. Primers for amplification of ITS1/2 were forward primer F194 (5'- CGT AAC AAG GTA GCT GTA G -3') (Ferris *et al.*, 1993) and reverse primer 5368r (5'- TTT CAC TCG CCG TTA CTA AGG -3') (Vrain, 1993). Primers for amplification of D2/D3 LSU were forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and reverse primer D3Br (5'-TCG GAA GGA ACC AGC TAC TA-3') (De Ley *et al*., 1999). PCR conditions were as described by Li *et al.* (2008). PCR products were separated on 1% agarose gels and visualised by staining with ethidium bromide. PCR products of sufficiently high quality were purified for cloning and sequencing by Invitrogen, Shanghai, China.

For ITS-RFLP profiles, suitable aliquots of the amplified ITS rDNA were digested for at least 3 h at 37° using 10 U of each of the five restriction endonucleases (*Rsa*I, *Hae*III, *Msp*I, *Hinf*I and *Alu*I) (Takara, Japan) following the manufacturer's instructions. Fragments were resolved by electrophoresis in a 2.5% agarose gel and stained with ethidium bromide.

The ITS1/2 and partial LSU sequences were analysed and aligned using the program ClustalW implemented in MEGA version 4.0 (Tamura *et al*., 2007). Phylogenetic trees were generated with the Neighbor Joining (NJ) method using the Tajima-Nei distance option. Bootstrapping analysis was performed with 1000 replicates.

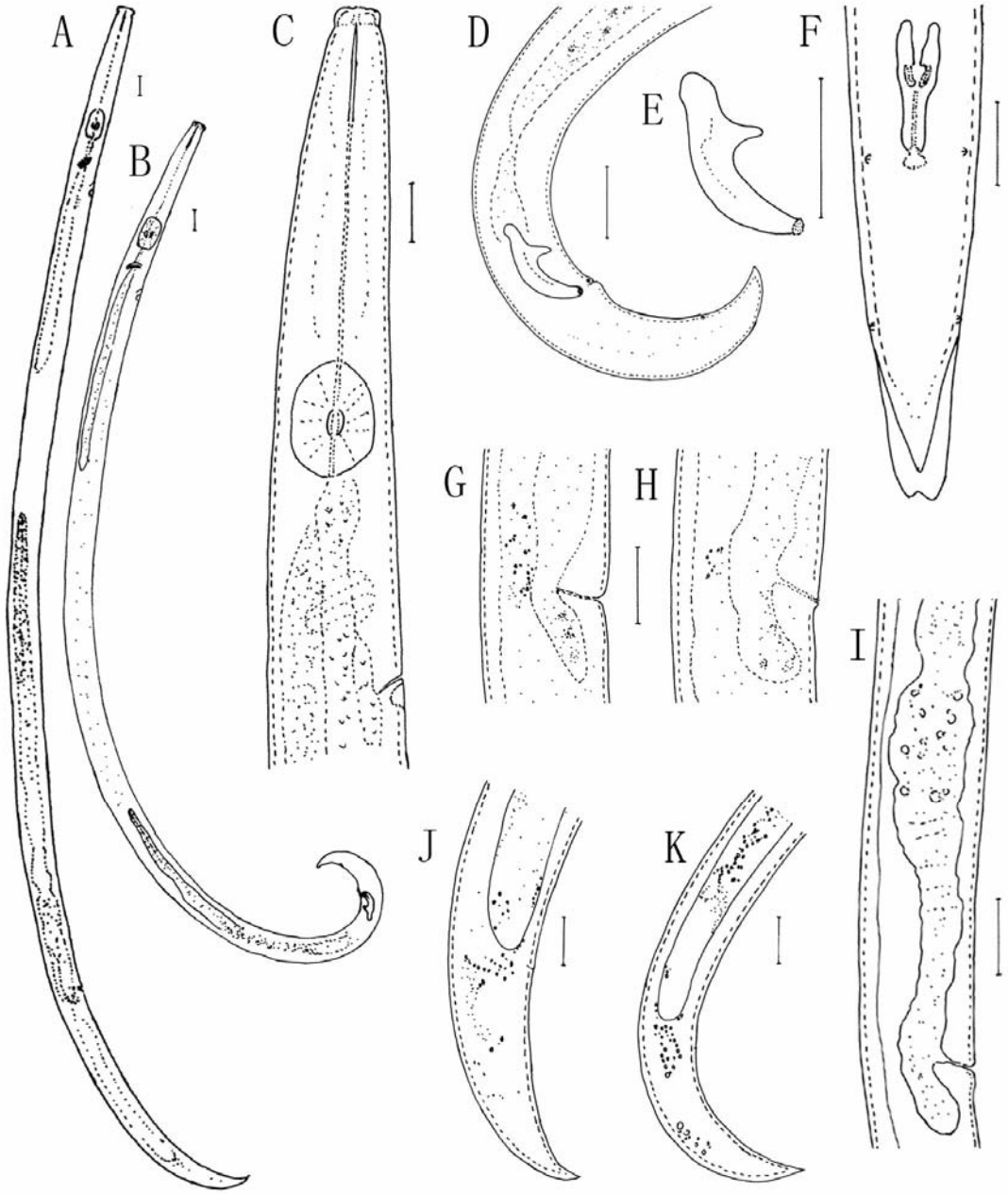


**Fig. 1.** *Devibursaphelenchus wangi* sp. n. A: Female; B: Male; C: Anterior body; D: Lateral view of male tail; E: Spicules; F: Ventral view of male tail; G-I: Vulva region; J, K: Variation of female tail. (Scale bars=10 μm).

## DESCRIPTIONS

### *Devibursaphelenchus wangi* sp. n. (Figs. 1-3)

**Measurements** (Table 1).

**Male.** Body slender, cylindrical, posterior region sharply curved ventrally when heat-killed. Cuticle weakly annulated, lateral field with three incisures (*i.e*., two ridges). Lip region offset, about 3.5 μm in height and 7.1 μm wide. Stylet short and relatively broad lumened, lacking basal swellings, conus forming *ca.* 38-40% of total length. Procorpus cylindrical. Median bulb strongly developed, elongate-oval, 15.7±0.8 μm long, 9.7±0.8 μm wide, with valve plates situated slightly posteriorly. Pharyngeal gland lobe slender and well developed, about six body diameters long, overlapping intestine dorsally. Nerve ring located at *ca.* 12-15 μm posterior to median bulb. Excretory pore located at *ca*

**Table 1.** Measurements of *Deviбursaphelenchus wangi* sp. n., all measurements in μm; mean ± s.d. (range).

| | Female | | Male |
|---|---|---|---|
| | Holotype | Paratypes | Paratypes |
| n | - | 15 | 6 |
| L | 655.0 | 704.3±62.0 (606.0-803.3) | 614.3±22.9 (578.0-644.0) |
| a | 36.5 | 37.1±4.3 (26.4-45.1) | 36.5±2.4 (31.3-38.2) |
| b | 7.7 | 8.5±0.8 (7.6-10.6) | 7.2±0.4 (6.8-8.1) |
| b' | 3.2 | 3.5±0.3 (3.0-4.0) | 3.5±0.3 (3.3-4.0) |
| c | - | - | 17.5±0.9 (16.2-18.8) |
| c' | - | - | 2.8±0.1 (2.7-2.9) |
| V or T | 78.6 | 78.2±1.7 (73.7-80.0) | 29.1±0.6 (28.0-29.7) |
| Max body diam. | 17.9 | 19.1±3.3 (15.0-29.5) | 16.9±1.7 (15.2-20.6) |
| Lip diam. | 7.0 | 7.7±0.8 (6.9-9.0) | 7.1±0.7 (6.9-8.8) |
| Lip height | 3.1 | 3.7±0.5 (3.1-4.5) | 3.5±0.4 (3.1-4.0) |
| Stylet length | 16.8 | 17.1±0.3 (16.7-17.4) | 14.8±1.4 (12.4-16.6) |
| Median bulb length | 16.3 | 17.7±1.2 (16.3-19.8) | 15.7±0.8 (15.0-17.0) |
| Median bulb diam. | 10.3 | 11.0±1.8 (8.3-14.5) | 9.7±0.8 (8.3-11.1) |
| Median bulb length/diam. | 1.6 | 1.6±0.2 (1.3-2.0) | 1.6±0.2 (1.5-2.0) |
| Excretory pore position | 95 | 105.4±8.2 (94.0-119.1) | 98.2±3.2 (93.0-104.0) |
| Spicule (dorsal limb) | - | - | 18.3±0.6 (17.3-19.1) |
| Spicule (chord) | - | - | 15.0±0.5 (14.2-15.6) |
| Spicule (curved median line from the middle of condylus to the end) | - | - | 15.9±0.3 (15.4-16.4) |
| Ovary or testis length | 350 | 298.6±34.6 (256.0-355.0) | 180.7±4.7 (160.0-199.0) |
| Post-uterine sac length | 11.7 | 10.2±1.8 (7.6-13.2) | - |
| Blind sac | 85.0 | 107.0±14.8 (79.0-128.0) | - |
| Tail length | - | - | 35.2±2.2 (33.0-39.8) |

30-35 μm posterior to median bulb. Hemizonid located just posterior to excretory pore, but sometimes anterior to excretory pore. Testis single, about 180.7±4.7 μm long, spermatocytes arranged in two rows. Cloacal opening lips slightly protruding. Spicules arcuate, condylus rounded, elongated, lamina smoothly and symmetrically curved, rostrum conical with bluntly pointed tip. Distal ends of spicules forming a flattened cucullus. Tail strongly recurved, terminus finely pointed, spade-shaped terminal bursa clearly visible under light microscope. Two pairs of caudal papillae present: one pair located slightly precloacal and the second subventral pair located just anterior to the beginning of bursal flap.

**Females.** Body slightly ventrally arcuate when heat-relaxed. Cuticle and lip region similar to male. Ovary outstretched, developing oocytes in two rows. Vulva slightly inclined anteriorly, vulva lips not protruding, anterior vulva lip does not form a vulval flap. Vagina not sclerotized. Spermatheca elongate-

oval, sometimes containing round sperms. Post-uterine sac short, less than one body diameter long, rectum and anus indistinct. Intestine terminating as a blind sac. Tail conical, tapering to ventrally bent terminus, tail terminus finely rounded or sharply pointed.

**Diagnosis and relationships.** *Deviburs aphelenchus wangi* sp. n. is characterised by relatively slender body (a = 36.5 and 37.1 for males and females, respectively); three lines in the lateral field; stylet with relatively wide lumen and lacking basal knobs; relatively high vulva position (average 78%); vulval flap absent, very short postuterine sac; non-functional and indistinct female rectum and anus; spicules relatively small (14.2-15.6 µm) with a flattened cucullus; two pairs of caudal papillae; presence of a distinct bursal flap.

Braasch (2009) re-established the genus *Deviburs aphelenchus* Kakuliya, 1967 belonging to Ektaphelenchinae, which contains five species: *D. typographi* Kakuliya, 1967; *D. eproctatus* (Sriwati, Kanzaki, Phan & Futai, 2008) Braasch, 2009; *D. hunanensis* (Yin, Fang & Tarjan, 1988) Braasch, 2009; *D. lini* (Braasch, 2004) Braasch, 2009 and *D. teratospicularis* (Kakuliya & Devdariani, 1965) Braasch, 2009.

*Deviburs aphelenchus wangi* sp. n. is particularly close to *D. eproctatus* and *D. hunanensis* in the shape of spicules and female tail. *Deviburs aphelenchus wangi* sp. n. is distinguished from *D. eproctatus* by the shape and size of spicules (15.4-16.4 µm *vs* 18.8-20.8 µm long measured along curved median line, condylus not recurved dorsally *vs* sometimes recurved dorsally ); different size of stylet (12.4-16.6 µm and 16.7-17.4 µm for males and females, respectively *vs* 15-20 µm and 19-22 µm); different ovary length (256-355 µm *vs* 152-243 µm) and testis length (160-199 µm *vs* 129-143 µm); different c ratio of males (c = 16.2-18.8 *vs* c = 13.3-15.0).

*Deviburs aphelenchus wangi* sp. n. is distinguished from *D. hunanensis* by the presence of three *vs* four lateral lines, absence *vs* presence of a functional rectum and anus, shorter stylet (12.4-16.6 µm and 16.7-17.4 µm for males and females, respectively, *vs* 19-21 µm and 20-26 µm); the shape of spicules (distal end of spicules with a distinct cucullus *vs* distal end of spicules obtuse, without cucullus).

*Deviburs aphelenchus wangi* sp. n. is distinguished from *D. typographi* by the body shape (a = 31.3-38.2 and 26.4-45.1 for males and females, respectively *vs* a = 21.2-22.5 and 20.2-20.9); shorter stylet (averaging 17 and 15 µm for females and males, respectively, *vs* 21-22 µm); the shape of female tail terminus (finely rounded or sharply pointed *vs* broadly rounded), the V value (V = 73.7-80.0 *vs* V = 85.3-85.8), and the size of spicules (14.2-15.6 µm *vs* 12 µm long measured in chord).

*Deviburs aphelenchus wangi* sp. n. is distinguished from *D. lini* by the shape and size of spicules (14.2-15.6 µm long *vs* 16-21 µm long measured in chord, rostrum bluntly pointed *vs* sharply pointed); different length of stylet (12.4-16.6 µm and 16.7-17.4 µm for males and females, respectively *vs* 17-21 µm and 18-23 µm); and the vulva structure (no sclerotization in the vulva region *vs* a strong half ring-like sclerotization in the anterior vulva part).

*Deviburs aphelenchus wangi* sp. n. is distinguished from *D. teratospicularis* by stylet length (averaging 17 and 15 µm for females and males, respectively, *vs* 18-22 µm); lack of basal swellings at the stylet *vs* presence of minute swellings in *D. teratospicularis*; finely rounded or pointed *vs* blunt tip of female tail; and by shape of spicules (rostrum position in the anterior part of spicules *vs* rostrum in the middle part of spicules due to very high condylus of *D. teratospicularis*, smoothly ventrally curved dorsal limb *vs* sunken distal part of dorsal limb).

**Molecular profiles and phylogenetic status.** The rDNA based sequences of ITS1/2 and D2D3 LSU are deposited in the GenBank database with the accession numbers GQ894739 and GQ903770, respectively. The molecular phylogenetic status of the new species is shown in Figures 4 and 5, and the ITS-RFLP profiles of rDNA are shown in Figure 6 and Table 2. The ITS-RFLP pattern of *D. wangi* sp. n. is different from the patterns of *D. hunanensis* and *D. lini* (Burgermeister *et al*., 2009).

**Table 2.** Sizes of PCR products and DNA restriction fragments obtained in ITS-RFLP analysis and calculated on sequencing results of the ITS1/2 regions

| Species | PCR product (bp) | Restriction fragments (bp)[1] | | | | |
|---|---|---|---|---|---|---|
| | | *Rsa* I | *Hae* III | *Msp* I | *Hinf* I | *Alu* I |
| *D. wangi* sp. n. | 966 | 403<br>335<br>155<br>73 | 717<br>249 | 769<br>197 | 488<br>433<br>45 | 798<br>73<br>65<br>15<br>15 |
| *D. hunanensis*[2] | 947 | 374<br>305<br>196<br>72 | 580<br>367 | 765<br>182 | 497<br>181<br>164<br>63<br>42 | 641<br>293<br>13 |

[1] Fragment sizes (bp) were calculated with a computer program DNASTAR MapDraw 5.01.

[2] According to Burgermeister *et al*., 2009.

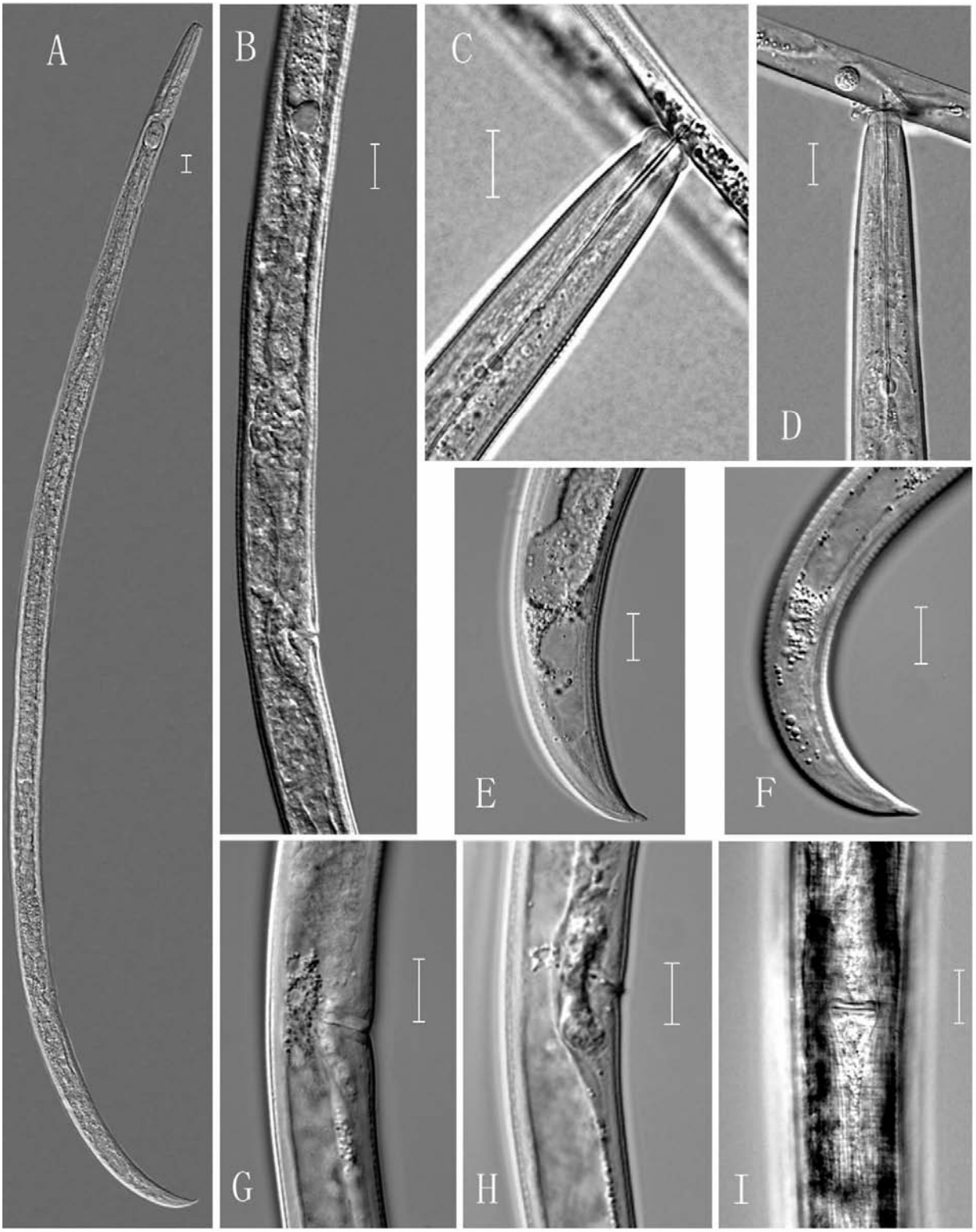


**Fig. 2.** Light photomicrographs of *Devibursaphelenchus wangi* sp. n. (female) A: Whole body; B: Vulva region; C, D: Feeding on *Aphelenchoides* sp.; E, F: Tail; G, H: Vulva region (lateral view); I: Vulva region (ventral view). (Scale bars=10 μm).


**Fig. 3**. Light photomicrographs of *Deviburs aphelenchus wangi* sp. n. (male) A: Whole body; B, C: Anterior body; D: Lateral field; E, F: Spicules; G & I: Tail (ventral view, showing papillae and bursa); H: Tail. (Scale bars=10 μm).

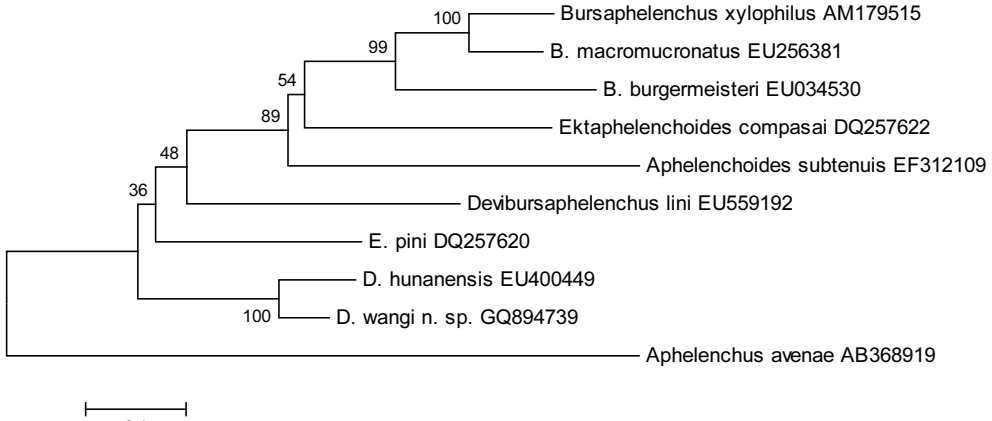


**Fig. 4.** Molecular phylogenetic status of *Deviburs aphelenchus wangi* sp. n. based on ITS1/2 sequences. *Aphelenchus avenae* served as the outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

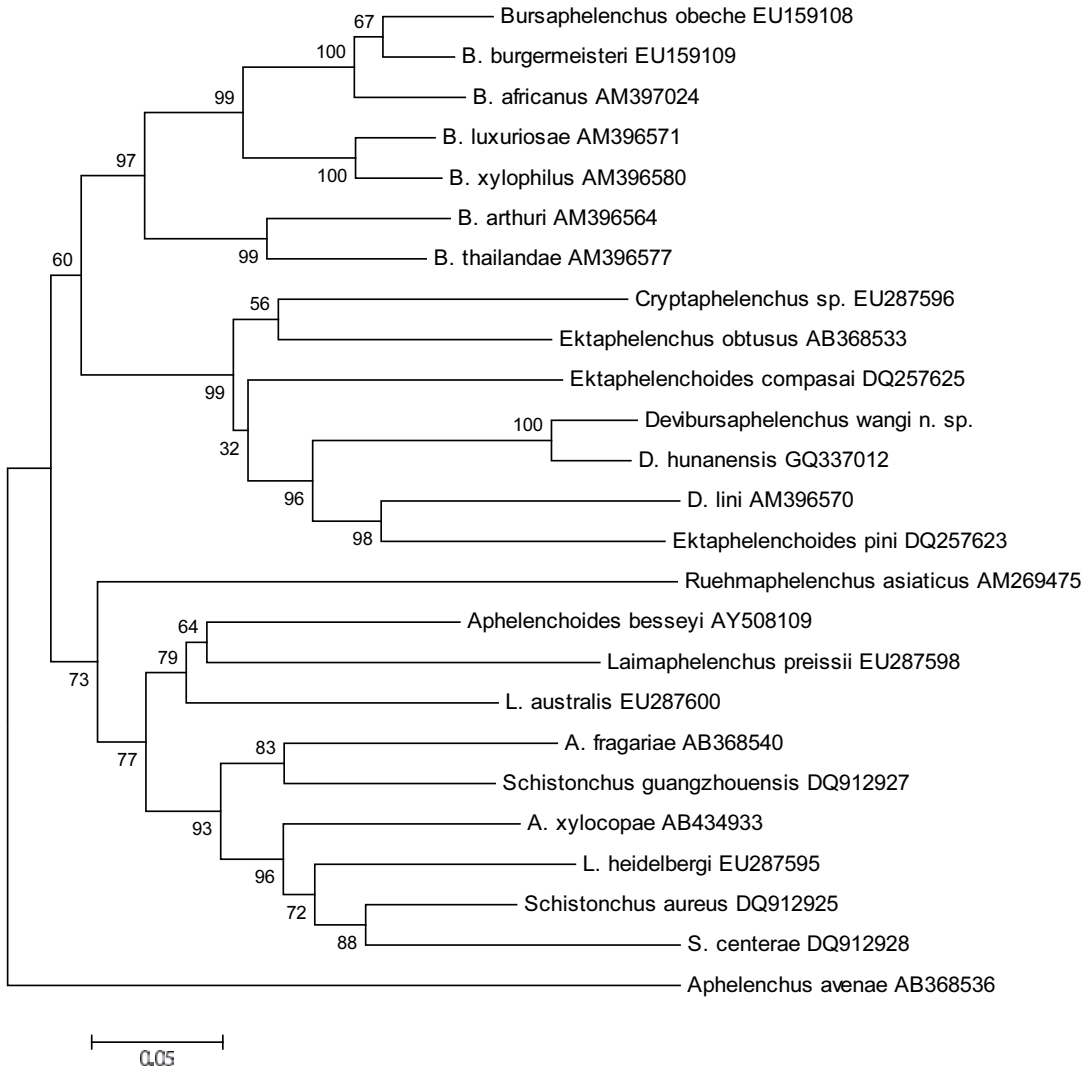


**Fig. 5.** Molecular phylogenetic status of *Devibursaphelenchus wangi* sp. n. based on partial LSU sequences. *Aphelenchus avenae* served as the outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

The two molecular trees show that the new species is close to *D. hunanensis* (Figs 4 and 5), and that the genus *Devibursaphelenchus* is close to other genera of Ektaphelenchinae such as *Ektaphelenchoides*, *Ektaphelenchus* and *Cryptaphelenchus* (Fig. 5).

**Type habitat and locality.** Packaging wood of *Pinus* sp. from USA, inspected in Ningbo Entry-exit Inspection and Quarantine Bureau, China, in 2009.

**Feeding habitat.** Seven specimens of *Devibursaphelenchus wangi* sp. n., including males, females and juveniles were found feeding on *Aphelenchoides* sp. The bodies of *Aphelenchoides* sp. were penetrated by the stylets of *D. wangi* sp. n., which could not easily be separated from their food (Fig. 2).

**Types.** Holotype male, ten female and five male paratypes (slide numbers 848-1 to 848-11) deposited in the nematode collection of Ningbo Entry-exit Inspection and Quarantine Bureau, China. One paratype male and four paratype females (slide numbers 9319 and 9320) deposited in the Canadian National Collection of Nematodes, Ottawa, Ontario, Canada. One paratype male and four paratype females (slide numbers 848-12 and 848-13) deposited in the Institute of Biotechnology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China.

**Etymology.** *D. wangi* sp. n. is named after Wang Songqing, the deputy director of Ningbo Entry-exit Inspection and Quarantine Bureau, who had supported the authors' research.

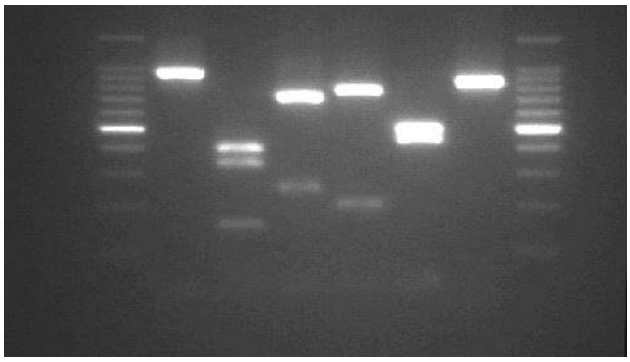

**Fig. 6.** ITS-RFLP pattern of *Deviбursaphelenchus wangi* sp. n. M = Molecular size marker (100 bp ladder); Lane 1: rDNA amplification product; Lanes 2-6: Digestion products obtained with *Rsa*I, *Hae*III, *Msp*I, *Hinf*I and *Alu*I. Sizes of PCR product and its restriction fragments are shown in Table 2.

## DISCUSSION

The genus *Deviбursaphelenchus* clearly belongs to Ektaphelenchinae with the following typical characters: stylet with relatively wide lumen, rectum and anus obscure in females, intestine ending in a blind sac. The molecular phylogenetic analysis also showed that *Deviбursaphelenchus* species cluster together with those of the genera of Ektaphelenchinae (Fig. 5). Additionally, the predatory behaviour of *Deviбursaphelenchus* species supports their placement within Ektaphelenchinae. Among the genera of Ektaphelenchinae, only *Deviбursaphelenchus* has a bursa, which is otherwise typical for Parasitaphelenchinae.

The genus *Deviбursaphelenchus* differs from *Ektaphelenchus* by having a male tail with a terminal bursa, but if the male is absent, the females of these two genera are similar especially for those *Ektaphelenchus* species without distinct basal swellings (all the stylets of *Deviбursaphelenchus* species are without basal swellings).

According to Braasch (2009), the genus *Deviбursaphelenchus* is characterised by a slender body; strong stylet (20-26 μm long) with relatively wide lumen and usually without basal thickenings; metacorpus elongate-oval, with valve plates located posterior to the middle of bulb; excretory pore posterior to metacorpus; vulva relatively posterior (V = 76-80), not protruding and without flap; vagina sclerotized; post-uterine branch short (up to 1.5 times the corresponding body diameter long); rectum and anus obscure in females; male tail strongly recurved, with distinct terminal bursa seen in dorso-ventral position; two pairs of male caudal papillae; spicules strong, almost straight and arcuate in their distal part, with prominent rostrum, relatively high condylus, without cucullus, and in some species with a hook-like appendix at the distal end, with appearance of a cucullus.

The re-establishment of *Deviбursaphelenchus* is supported by this new species description. However, the new species lacks a highly sclerotized vagina shown in other species of the genus (Braasch, 2009) and has a cucullus. Therefore, these features may not be used for genus characterisation.

*Deviбursaphelenchus hunanensis* has several features in common with the other *Deviбursaphelenchus* species (Braasch, 2009), but it was described as having four lateral lines (two refractive inner lines), and distinct rectum and anus of females (Yin *et al*., 1988). Gu *et al*. (2006) reported that *D. hunanensis* was detected in Ningbo, China, but the rectum and anus of the reported species were not clearly seen. The identity of this species with the originally described *D. (B.) hunanensis* remains questionable.

Braasch (2004) observed that a specimen of *D. lini* was feeding on a *Bursaphelenchus xylophilus* juvenile. *D. hunanensis* has been observed feeding on *B. mucronatus* and *B. rainulfi* (unpubl. observations). *Deviбursaphelenchus wangi* sp. n. has been observed feeding on *Aphelenchoides* sp. Therefore, we assume that *Deviбursaphelenchus* species are predatory on other nematodes and cannot be multiplied on fungi. It will be very interesting and of practical interest to investigate the potential efficiency of *Deviбursaphelenchus* species for a possible future control strategy of *B. xylophilus*.

Because packaging wood is a circulating product and there is no phytosanitary treatment mark, the exact geographic origin of the new species remains unclear. The vector of the new species is also unknown.

## ACKNOWLEDGEMENT

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**Jianfeng Gu, Jiangling Wang, Jingwu Zheng.** *Devibursaphelenchus wangi* sp. n. (Nematoda: Ektaphelenchinae), питающийся на *Aphelenchoides* sp.

**Резюме.** Приводится описание и иллюстрации для *Devibursaphelenchus wangi* sp. n. Новый вид выделен из прибывшей из США упаковки, изготовленной из древесины сосны. Древесина упаковки была обследована в гавани Нингбо в Китае в 2009 году. Новые вид характеризуется сравнительно тонким телом (a = 36.5 и 37.1 для самцов и самок, соответственно); тремя линиями латерального поля, стилетом со сравнительно широким просветом и без базальных утолщений, отсутствием вульварной складки, не функционирующим и не различимым ректумом и анальным отверстием у самок; сравнительно небольшими (14.2-15.6 μm) спикулами с уплощенным кукулюсом, двумя парами хвостовых папилл. Новый вид морфологически близок к *D. eproctatus* и *D. hunanensis,* но может быть дифференцирован от них по размеру и форме спикул и меньшей длине стилета. Независимый статус этого нового вида подтвержден спектрами ITS-RFLP, а также результатами молекулярно-филогенетического анализа, основанного на сравнении полной ITS последовательности и частичной LSU последовательности. Показано, что новый вид близок к *D. hunanensis*. Исследован характер питания нематод нового вида.