

***Bursaphelenchus ogawanus* n. sp. isolated from bark material of *Fagus japonica* Maxim. from Japan**

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Summary - A *Bursaphelenchus* species belonging to the *abietinus* group was isolated from a bark sample of *Fagus japonica* infested by an unidentified *Dryocoetes* bark beetle. The species is typologically characterised by its two-lined lateral field, mitten-shaped spicule possessing a well-developed and dorsally truncate condylus, narrow triangular rostrum and a distinct cucullus, the presence of seven (P1, P2, P3 papilliform and P5 gland-opening like) male genital papillae, female vulva with side flap and the long, smoothly tapering and ventrally recurved female tail with strongly ventrally recurved distal part. The species is typologically close to *B. irokophilus* and *B. rainulfi* but can be distinguished from these two species by the shape of spicule rostrum, which is narrower in the new species. Phylogenetically, the new species form a well-supported clade with *B. rainulfi* but can be distinguished from them by the differences in the SSU and D2-D3 LSU sequences. The new species is described as *B. ogawanus* n. sp.

Keywords - *Bursaphelenchus abietinus* group, *Fagus japonica*, description, molecular phylogeny, morphology, morphometrics, new species, taxonomy.

The genus *Bursaphelenchus* Fuchs, 1937 is an important aphelenchoidid genus because of two lethal plant pathogens, *B. xylophilus* (Steiner & Buhner, 1934) Nickle, 1970, and *B. cocophilus* (Cobb, 1919) Baujard, 1989, and several biologically interesting species (e.g., Ryss & Subbotin, 2017; Kanzaki & Giblin-Davis, 2018). For example, *B. okinawaensis* Kanzaki, Maehara, Aikawa & Togashi, 2008, is a hermaphroditic species and an important model for sex determination (Kanzaki *et al.*, 2008; Shinya *et al.*, 2014, 2022); *B. sycophilus* Kanzaki, Tanaka, Giblin-Davis & Davies, 2014 and *B. suri* Kanzaki, Kruger, Greeff & Giblin-Davis, 2022, are highly derived obligate plant parasites (Kanzaki *et al.*, 2014, 2022); *B. sinensis* Marinari Palmisano, Ambrogioni, Tomiczek & Brandstetter, 2004, and its close relatives show a feeding dimorphism (phenotypic plasticity) (Kanzaki *et al.*, 2019a; Kanzaki & Giblin-Davis, 2020); *B. doui* Braasch, Gu, Burgermeister & Zhang, 2005 has two different dispersal stages, a dispersal fourth stage juvenile and phoretic adult form (Kanzaki *et al.*, 2013); *B. ulmophilus* Ryss, Polyanina, Popovichev & Subbotin, 2015, which has two dauer (dispersal) juvenile stages, and may be a weak parasite of the bark beetles, *Scolytus* spp. (Ryss *et al.*, 2015); and *B. tiliae* Tomalak & Malewski, 2014 dauers are found (possibly parasitize) the Malpighian tubules of adult *Ernoporus tiliae* (Tomalak & Malewski, 2014).

Intensive sampling of the genus may yield additional biological research materials (= satellite models). Most *Bursaphelenchus* species are entomophilic, *i.e.*, the nematodes are phoretically associated with various groups of insects, especially bark beetles (Rühm, 1956; Massey, 1974; Ryss *et al.*, 2005; Ryss & Subbotin, 2017; Kanzaki & Giblin-Davis, 2018). Thus, frequent field surveys of wood-boring insects and related

environments (substrates) are needed to collect *Bursaphelenchus* nematodes and related genera.

Here, a new *Bursaphelenchus* species isolated from a bark sample of a dead log of *Fagus japonica* Maxim. collected from Japan is described and illustrated based on its molecular profile and morphological characters.

Materials and methods

MATERIAL COLLECTION AND NEMATODE ISOLATION

An approximately 10 cm x 15 cm of bark sample was collected from a dead log of *F. japonica* fallen in Ogawa Forest Reserve, which is a natural broad-leaved forest located in a cool temperate zone, Kitaibaraki, Ibaraki, Japan (GPS: 36.936400 N, 140.587728 E, 615 m a.s.l.), on June 20, 2022. The bark sample was bored by an unidentified *Dryocoetes* sp. (Scolytinae).

The bark sample was placed in a plastic bag in the laboratory at room temperature (*ca* 25°C) and observed occasionally for 2 weeks. Nematodes captured in the water drops on the inner surface of the bag were collected using a micropipette and observed under a dissecting light microscope (S8 Apo; Leica). In addition to *Bursaphelenchus* sp., *Micoletzkyia* sp., *Parasitorhabditis* sp. and several unidentified panagrolaimids were recognised, but further analysis of the latter species was not conducted.

More than 100 adults (sex ratio approximately 1:1) of *Bursaphelenchus* sp. were collected using a stainless-steel insect pin (#00; Shiga Kontyu) and transferred to 1.0% malt-extract agar (MEA; 1.0% malt extract and 2.0% agar) previously inoculated with

the blue-stain fungus, *Ophiostoma minus* (Hedgc.). Propagated nematodes were examined under a light microscope (Eclipse 80i; Nikon) for identification at the intrageneric group level (Ryss & Subbotin, 2017; Kanzaki & Giblin-Davis, 2018). Thereafter, the nematode was subcultured using *O. minus* on MEA.

MORPHOLOGICAL OBSERVATION AND MICROGRAPHY

Adult nematodes were collected from a 2-week-old culture, heat-killed and fixed in TAF (triethanolamine:formalin:distilled water = 2:7:91) for 1 week. The fixed materials were processed with glycerine using a modified Seinhorst's method (Minagawa & Mizukubo, 1994) and mounted in glycerine according to de Maeseneer and d'Herde (as described in Hooper, 1986). The mounted materials were used for morphometric analyses and retained as type specimens. In addition, live adult nematodes from 2-week-old cultures were used for detailed morphological observations following Kanzaki (2013). Micrographs were obtained using a digital camera system (MC170 HD; Leica), and morphological drawings were generated using a drawing tube connected to the microscope. Drawings and micrographs were edited using Photoshop Elements 2019 software to generate figures.

MOLECULAR PROFILES AND PHYLOGENY

Bulk DNA samples were prepared from cultured nematodes (*ca* 20 individuals) following the methods described by Kikuchi *et al.* (2009) and Tanaka *et al.* (2012). In addition to bulk samples, five individuals were separately digested with the same methodology to confirm intraspecific variation of the sequences. The DNA sequence of

a *ca* 4.2-kb segment of the ribosomal DNA (rDNA) region, including the near-full length of the small subunit (SSU), ITS (ITS1, 5.8S rRNA and ITS2) and D1-D4 expansion segments of the large subunit (D1-D4 LSU), of bulk DNA sample and ITS of individual samples were determined following the methods of Ekino *et al.* (2017) and Kanzaki *et al.* (2019b). We also attempted to determine partial (*ca* 0.7 kb) segments of mitochondrial cytochrome oxidase subunit I (*mtCOI*) using the method of Kanzaki & Futai (2002), but the segment was not amplified by PCR probably because of mutations in the primer region sequences. Therefore, the phylogenetic analysis was conducted based on rDNA. The determined sequence was deposited in the GenBank database under accession number LC757019.

The sequences were compared with those of other parasitaphelenchid nematodes in the GenBank database. For this molecular phylogenetic analysis, the SSU and D2-D3 LSU were employed. The GenBank accession numbers of the compared sequences are listed in Supplementary Table S1.

The molecular phylogenetic status of the new species was determined by Bayesian analysis. First, the compared sequences were aligned using the MAFFT program (Katoh *et al.*, 2002; Kuraku *et al.*, 2013; available online at <https://mafft.cbrc.jp/alignment/server/index.html/>) with default settings. Base-substitution models for each gene were determined using the Akaike information criterion (AIC) in MEGA X (Kumar *et al.*, 2018). A combined Bayesian analysis was performed using MrBayes 3.2 (Huelsenbeck & Ronquist, 2000; Ronquist *et al.*, 20121); four chains were run for 4×10^6 generations, and Markov chains were sampled at intervals of 100 generations (Larget & Simon, 1999). Two independent runs were

performed, and after confirming the convergence of runs and discarding the first 2×10^6 generations as burn in, the remaining topologies were used to generate a 50% majority-rule consensus tree.

Results

MOLECULAR PROFILES AND PHYLOGENY

Molecular sequences are consistent between bulk and individual DNA samples. Based on the combined analysis, the new species clearly belonged to *B. abietinus* group *sensu* Kanzaki & Giblin-Davis, 2018 (Braasch *et al.*, 2009; Ryss & Subbotin, 2017; Kanzaki & Giblin-Davis, 2018; Torrini *et al.*, 2019), and was close to *B. rainulfi* Braasch & Burgermeister, 2002, with 100% posterior probability (Fig. 1, Suppl. Fig. S1).

Bursaphelenchus ogawanus n. sp.

(Figs 2-6)



MEASUREMENTS

See Table 1.

DESCRIPTION

Adults

Relatively small species in the genus, *i.e.*, body length ranging 468-502 μm and 482-625 μm for male and female, respectively. Body cylindrical, moderate to stout as the genus, *i.e.*, range of body length max. body diam. ratios (a values) of male and female are 27.7-30.8 and 24.3-33.8, respectively. Cuticle thin, annulated, lateral field with two lines (= relatively wide single band). Head distinctly offset from body, separated by a clear constriction, lip region in lateral view squarish rounded, *ca* twice as broad as high. Stylet with narrow lumen comprising a short cone *ca* one-third or a little more of total stylet length and a shaft with clear basal swelling. Procorpus cylindrical, 2.5-3 stylet lengths (\approx metacorpal lengths) long, ending in well-developed metacorpus (median bulb). Metacorpal valve clearly observed, present at middle of, or slightly posterior to, centre of metacorpus. Dorsal pharyngeal gland orifice opening into lumen of metacorpus mid-way between anterior end of metacorpal valve and anterior end of metacorpus. Pharyngo-intestinal junction *ca* 2 μm posterior to metacorpus. Dorsal pharyngeal glands overlapping intestine dorsally. Position of secretory-excretory pore varies among individuals, ranging from the level of posterior half of metacorpus to 0.6 metacorpal length posterior to metacorpus (57-157 and 75-156% metacorpal length posterior to the top of metacorpus in male and female, respectively). Nerve ring surrounding pharyngeal glands and intestine slightly posterior to pharyngo-intestinal junction. Hemizonid unclear in live material but distinctive in mounted one, located at *ca* 1.5 (1-2) metacorpal lengths posterior to secretory-excretory pore.

Male

Gonad to right subventral of intestine, outstretched or with reflexed anterior end; three out of 10 examined individuals have reflexed anterior end. Spermatocytes arranged in two rows in anterior part of testis and single row in middle part of testis; pre-mature sperm (spermatozoa) formed in multiple rows in posterior end of testis. *Vas deferens* sometimes containing developed sperm, merging with distal part of intestine to form simple cloacal tube connected to cloacal opening (CO), occupying approximately 1/3 of total gonad length. Sperm amoeboid, *ca* 3-4 μm in diam. Spicules small, stout (mitten-shaped), paired. Capitulum of spicule distinctive, consisting of well-developed condylus and elongate triangular rostrum with bluntly pointed tip. Condylus distinct, slightly dorsally truncate with narrowly rounded to roundish-squared tip. Spicule blade (calomus-lamina complex) consisting of smoothly and clearly ventrally curved and well-cuticularised dorsal limb (lamina) and membranous calomus with smoothly weakly ventrally curved ventral contour, thus the blade forming somewhat half-circle shape. Distal tip of spicule forming a distinct rounded squarish cucullus. Bursal flap present, roundish-squared in shape, starting from level between second subventral paired papillae (P3) and distal ventral glandpapillae (P5), shape of distal end slightly varying among individuals, *i.e.*, with smoothly to irregularly rounded tip. Seven genital papillae (*i.e.*, one ventral papilla (P1) and three ventral-subventral paired papillae (P2, P3, P5)) present: precloacal P1 papilliform, ventral, slightly anterior to CO; adcloacal P2 papilliform, on subventral body; P3 located slightly posterior to mid-point between CO and tail tip; gland-opening-like P5 (glandpapillae) on ventral side, slightly posterior to P3 and tail tip, with internal connection (unclear secretory duct-like structure).

Female

Reproductive tract to right of intestine, monoprodelfic, comprising from anterior: ovary, oviduct, spermatheca, crustaformeria, uterus, vagina + vulva, and post-uterine sac. Ovary anteriorly outstretched. Oocytes in 2 rows in anterior parts of ovary, one well developed oocyte sometimes located at posterior end of ovary or in oviduct. Oviduct tube-like, connecting ovary and spermatheca, sometimes occupied by well-developed oocytes. Spermatheca formed by distinctive thick tissue as a branch or expansion of the gonad, *i.e.*, spermatheca with clearly closed anterior end and not a part of a simple tube, slightly irregular oval in shape, sometimes filled with well-developed sperm. Crustaformeria forming quadricalcolumella, not conspicuous, formed by relatively large, rounded cells. Uterus thick-walled, irregularly rounded shaped. Vagina slightly inclined anteriorly, a pair of three-celled structures located in uterus near vagina (uterus/post-uterine sac junction) where six (three pairs) of sclerotized pronged structures derived from each cell can be observed. Vulval opening forming horizontal dome-shaped slit in ventral view, slightly inclined anteriorly in lateral view. Vulval flap absent, but both sides of anterior vulval lip extending slightly, giving appearance of a short vulval flap in lateral view, although not a true flap (= side flap). Post-uterine sac conspicuous relatively short in the genus, *i.e.*, 2.0-2.8 vulval body diam. long, occupying *ca* 40% of vulva-anus distance, often containing sperm. Rectum and anus present, seemingly functional. Anus small, dome-shaped slit in ventral view. Tail long, elongate conoid with smoothly tapering distal end. Whole ventrally recurved, and distal part strongly ventrally recurved when killed by heat. Tail tip slightly varying in shape, narrowly rounded to digitate.

TYPE MATERIAL

The type material was obtained from a 2-week-old culture. The samples included a holotype male (United States Department of Agriculture Nematode Collection [USDANC] accession number T-795t), four paratype males (T-7862p-7865p) and five paratype females (T-7866p-7870p) deposited at the USDANC, Beltsville, MD, USA, and five paratype males (FPL-FFPRI accession number *Bursaphelenchus ogawanus* M-01-05) and five paratype females (*Bursaphelenchus ogawanus* F-01-05) deposited at the Forestry and Forest Products Research Center (FFPRI), Tsukuba, Japan. In addition to the type material, mass-fixed materials (fixed in TAF or processed in dehydrated glycerine) were deposited in the Kansai Research Center of the FFPRI.

TYPE HOST AND LOCALITY

The type material was collected from a 2-week-old culture of the laboratory population grown on the blue stain fungus, *O. minus* inoculated on 1.0% MEA. The culture was established from a bark sample obtained from a dead log of *Fagus japonica* infested by an unidentified *Dryocoetes* sp., which was found in the Ogawa Forest Reserve, Kitaibaraki, Ibaraki, Japan (GPS: 36.936400 N, 140.587728 E, 615 m a.s.l.) on June 20, 2022.

ETYMOLOGY

The specific epithet, ‘*ogawanus*’, is derived from the type locality of the nematode in the Ogawa Forest Reserve, Kitaibaraki, Ibaraki, Japan.

DIAGNOSIS AND RELATIONSHIPS

Besides its generic characters, *e.g.*, presence of a male bursal flap, *B. ogawanus* n. sp. is characterised by its two-lined lateral field, mitten-shaped spicule possessing a well-developed and dorsally truncate condylus, narrow triangular rostrum and a distinct cucullus, the number, arrangement and structure of male genital papillae (*i.e.*, P1, P2, P3 papilliform papillae and P5 glandpapillae), female vulva with a side flap and a long, smoothly tapering and ventrally recurved female tail with a strongly ventrally recurved distal part.

The combination of these characters suggests that the new species belongs to the *B. abietinus* group of the genus, which includes the following four species: *B. abietinus* Braasch & Schmutzenhofer, 2000, *B. hellenicus* Skarmoutsos, Braasch & Michalopoulou, 1998, *B. rainulfi* and *B. irokophilus* Torrini, Strangi, Mazza, Marianelli, Robersi & Kanzaki, 2019 (Ryss & Subbotin, 2017; Kanzaki & Giblin-Davis, 2018; Torrini *et al.*, 2019).

Bursaphelenchus ogawanus n. sp. shares a two-lined lateral field and a more-or-less elongate conical female tail with *B. irokophilus* and *B. rainulfi* (Braasch & Schmutzenhofer, 2000; Torrini *et al.*, 2019). These three species are typologically similar considering the intraspecific variation in each species in the characters typically used for speciation, *e.g.*, position of the secretory-excretory pore and the shapes of the spicule condylus and female tail tip (Braasch & Schmutzenhofer, 2000; Torrini *et al.*, 2019). Therefore, these three species can be distinguished based only on some minor characters. *Bursaphelenchus ogawanus* n. sp. is distinguished from *B. irokophilus* and *B.*

rainulfi by the rostrum shape of the male spicule, i.e., narrow triangular vs triangular in the latter two species (Torrini *et al.*, 2019). In addition, *B. ogawanus* n. sp. is distinguished from *B. irokophilus* by the body length of males, 490 (468-502) vs 606 (573-646) μm , and females, 559 (482-625) vs 759 (726-785) μm ; b value of males, 10.8 (10.3-11.3) vs 8.9 (8.4-9.4); maximum body diameter of males, 16.7 (15.6-18.1) vs 20.1 (18.4-21.9) μm and females, 21.1 (16.7-22.6) vs 28.9 (26.4-31.8) μm ; size of the median bulb in males, diameter x length = 7.3 (6.9-7.6) x 10.4 (10.1-10.8) μm vs 11.6 (10.4-12.4) x 16.6 (15.7-17.9) μm and females, 8.3 (7.6-9.0) x 11.2 (10.4-12.5) μm vs 14.0 (13.2-14.9) x 18.0 (17.4-18.9) μm ; secretory-excretory pore from the anterior end in males, 46 (40-50) μm vs 67 (61-70) μm and females, 48 (44-53) μm vs 73 (69-77) μm ; nerve ring from the anterior end in males, 51 (47-53) μm vs 47 (69-76) μm , and females, 52 (49-53) μm vs 76 (73-90) μm ; hemizonid from the anterior end in males, 58 (53-60) vs 83 (81-85) μm and females, 61 (57-64) μm vs 95 (90-100) μm ; anterior gonad length in females, 249 (200-274) μm vs 395 (365-441) μm ; anal body diameter in females, 8.9 (8.3-9.6) μm vs 10.5 (10.0-11.2) μm ; tail length in males, 29 (25-32) μm vs 37 (35-43) μm and females, 40 (37-45) μm vs 58 (50-62) μm ; *vas deferens* length in males, 98 (82-112) μm vs 131 (105-153) μm ; vulval body diameter in females, 18.0 (16.0-20.1) μm vs 23.3 (21.6-24.9) μm ; post-uterine sac length in females, 42 (36-47) μm vs 76 (55-91) μm , and vulva-anus distance in females, 107 (88-124) μm vs 159 (143-169) (Torrini *et al.*, 2019). In comparison, *B. ogawanus* n. sp. is distinguished from *B. rainulfi* only by the b values of males, 10.8 (10.3-11.3) vs 8.0 (6.8-9.2), and females, 12.1 (10.6-13.7) vs 9.1 (7.5-10.0). However, some values and measurements are slightly different but overlap slightly in range, including the c values of males, 17.1 (15.6-19.4) vs 25 (19-38),

and females, 14.1 (13.0-15.0) vs 17 (15-20), and male tail length, 29 (25-32) μm vs 23 (20-25) μm (Braasch & Schmutzenhofer, 2000).

Phylogenetically, *B. ogawanus* n. sp. is close to *B. rainulfi*, forming a well-supported clade with, but clearly distinguished from, that species (Fig. 1, Suppl. Fig. S1). Molecular sequences of *B. ogawanus* n. sp. showed 3-5 bp from three strains of *B. rainulfi* (AM397017, KF978103 and LC269965) in *ca* 1.5 kb of the SSU and *ca* 40-50 bp of differences from six strains of *B. rainulfi* (AM396575, EU295496, EU295498, KF978102, MT373702 and KU170776) in *ca* 0.7 kb of the D2-D3 LSU. Despite several uncertainly read parts in several sequences, the differences warrant an independent species status.

REMARKS ON MORPHOMETRIC VALUES

Although *B. ogawanus* n. sp. is distinguished from *B. irokophilus* morphometrically, the values of its basic morphometrics, *e.g.*, *a*, *b*, *c* and *V*, are not clearly different between these two species, and most measurements showing clear differences reflect body size, which can be variable depending on the culture conditions. Thus, the morphometric variation between these two species (and *B. rainulfi*) could overlap with those of materials produced under variable culture conditions. Considering the intraspecific variation, morphometric values (and possibly some important typological characters, *e.g.*, position of secretory-excretory pore and female tail shape) overlap among closely related species in *Bursaphelenchus* (and often in other genera). Thus, the integrated approaches, *e.g.*, combination of morphological and morphometric characters and molecular markers (and hybridization experiments in case, those characters and

markers are too close), are necessary for the species identification in the present status, *i.e.*, many cryptic species complex had been described based on molecular markers and biological species concept. Therefore, we consider that morphometric values are useful to characterize the species, but not sufficient to distinguish closely related species.

Additional remarks

Detailed morphological characters of the *B. abietinus* group and systematics of the species groups have been discussed (*e.g.*, Ryss & Subbotin, 2017; Kanzaki & Giblin-Davis, 2018; Torrini *et al.*, 2019). Therefore, several typological and biological characters of the *B. abietinus* group are summarised here (Table 2) as additional remarks.

These five species, including *B. ogawanus* n. sp., could be typologically separated into two subgroups, two with a relatively short conical female tail (*B. abietinus* and *B. hellenicus*) and an elongate conical female tail (*B. irokophilus*, *B. rainulfi* and *B. ogawanus* n. sp.) (Table 2). This separation corresponds in part to their phylogenetic relationship, *i.e.*, a subclade (*B. abietinus* and *B. hellenicus*) has a short conical tail, and the basal (*B. irokophilus*) and the other two (*B. rainulfi* and *B. ogawanus* n. sp.) species have an elongated conical tail (Fig. 1, Suppl. Fig. S1). Also, the carrier insect species is in part in accordance with their phylogenetic status, *i.e.*, *B. rainulfi* is phoretically associated with *D. uniseriatus* Eggers (Shimizu *et al.*, 2013) and *B. ogawanus* n. sp. form a clade is possibly associated with *Dryocoetes* bark beetles, although the phoretic association of the species has not been proven and the carrier insect is necessary to be identified, and the other clade (*B. abietinus* and *B. hellenicus*) is associated with a

slightly larger bark beetle species (Fig. 1, Suppl. Fig. S1, Table 2). Further, the distribution is related in part to their phylogenetic relationship. Two close relatives forming well-supported clades, *i.e.*, *B. abietinus* + *B. hellenicus* and *B. rainulfi* + *B. ogawanus* n. sp., are mostly found in the European and Asian regions, respectively, but the natural distribution of *B. irokophilus* is uncertain because it has been isolated only from wood materials imported from Cameroon (Fig. 1, Suppl. Fig. S1, Table 2).

The host tree species are not linked to the phylogenetic relationships. Conifer associates (*B. abietinus*, *B. hellenicus* and *B. rainulfi*) and broad-leaved tree associates (*B. irokophilus* and *B. ogawanus* n. sp.) do not form a clade. Therefore, nematodes are hypothesised to co-speciate with carrier insects. In addition, *Dryocoetes* beetles are associated with several different groups of *Bursaphelenchus* spp.; namely, *B. leoni* Baujard, 1980, as “*B. borealis* Korentchenko, 1980” (*e.g.*, Korentchenko, 1980; Čermák *et al.*, 2013) (*B. sexdentati* group), *B. sachsi* Rühm, 1956 (*e.g.*, Rühm, 1956) (*B. hofmanni* group), *B. sinensis* Marinari Palmisano, Ambrogioni, Tomiczek & Brandstetter, 2004 (*e.g.*, Shimizu *et al.*, 2013) (*B. aberrans* group) and *B. tokyoensis* Kanzaki, Aikawa & Giblin-Davis, 2009 (*e.g.*, Kanzaki *et al.*, 2009) (*B. idius* group). Further isolation of *Bursaphelenchus* species, including *B. abietinus* and other species groups, is necessary to understand their diversity and evolutionary history in relation to their host trees and carrier insects.

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References

- Akbulut, S., Braasch, H. & Cebeci, H.H. (2013). First report of *Bursaphelenchus hellenicus* Skarmoutsos, Braasch, Michalopoulous (Nematoda: Aphelenchoididae) from Turkey. *Forest Pathology* 43, 402-406. DOI: 10.1111/efp.12045
- Ambrogioni, L., Irdani, T. & Caroppo, S. (2003). Records of *Bursaphelenchus* species on coniferous wood imported from Asian Russia and China to Italy. *Redia* 86, 139-146.
- Braasch, H. (2001). *Bursaphelenchus* species in conifers in Europe: distribution and morphological relationship. *EPPO Bulletin* 31, 127-142. DOI: 10.1111/j.1365-2338.2001.tb00982.x
- Braasch, H. & Burgermeister, W. (2002). *Bursaphelenchus rainulfi* sp. n. (Nematoda: Parasitaphelenchidae), first record of the genus *Bursaphelenchus* Fuchs, 1937 from Malaysia. *Nematology* 4, 971-978. DOI: 10.1163/156854102321122593
- Braasch, H. & Schmutzenhofer, H. (2000). *Bursaphelenchus abietinus* sp. n. (Nematoda: Parasitaphelenchidae) associated with fir bark beetles (*Pityokteines* spp.) from declining trees in Austria. *Russian Journal of Nematology* 8, 1-6.
- Braasch, H., Metge, K. & Burgermeister, W. (1999). *Bursaphelenchus*-Arten (Nematoda, Parasitaphelenchidae) in Nadelgehölzen in Deutschland und ihre ITS-RFLP-

- 382 Muster. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 51, 312-320.
- 383 Braasch, H., Tomiczek, C., Metge, K., Hoyer, U., Burgermeister, W., Wulfert, I &
384 Shönefeld, U. (2001). Records of *Bursaphelenchus* spp. (Nematoda,
385 Parasitaphelenchidae) in coniferous timber imported from the Asian part of Russia.
386 Forest Pathology 31, 129-140. DOI: 10.1046/j.1439-0329.2001.00233.x
- 387 Braasch, H., Burgermeister, W. & Gu, J. (2009). Revised intrageneric grouping of
388 *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchoididae). *Journal of*
389 *Nematode Morphology and Systematics* 12, 65-88.
- 390 Calin, M., Costache, C., Braasch, H., Zaulet, M., Buburuzan, L., Petrovan, V., Dumitru,
391 M., Mota M. & Vieira, P. (2015). New reports of *Bursaphelenchus* species
392 associated with conifer trees in Romania. *Forest Pathology* 45, 239-245. DOI:
393 10.1111/efp.12163
- 394 Carletti, B. (2008). *Bursaphelenchus* species with their natural vectors in Italy:
395 Distribution and essential diagnostic features. *Redia* 91, 111-117.
- 396 Carletti, B., Irdani, T., Ambrogioni, L., Pennacchio, F., & Francardi, V. (2008). New
397 occurrences of *Bursaphelenchus* species (Nematoda Parasitaphelenchidae)
398 phoretic associate of bark beetles in Italy. *Redia* 91, 67-71.
- 399 Čermák, V., Vieira, P., Gaar, V., Čudejková, M., Foit, J., Zouhar, M., Douda, O. & Mota,
400 M. (2013). On the genus *Bursaphelenchus* Fuchs, 1937 (Nematoda:
401 Parasitaphelenchinae) associated with wood and insects from declining forest
402 trees in the Czech Republic. *Forest Pathology* 43, 306-316. DOI:
403 10.1111/efp.12031
- 404 Chang, C.W. & Chen, P.C. (2020). [Identification of *Bursaphelenchus rainulfi*

- (Nematoda: Parasitaphelenchidae), a new record pine wood nematode species in Taiwan and its pathogenicity.] *Journal of Plant Medicine* 62, 31-39. DOI : 10.6716/JPM.202009_62(3).0004
- Dan, Y. & Yu, S.F. (2003). [Identification of *Bursaphelenchus* spp. on pine wood in Yunnan Province.] *Acta Phytopathologica Sinica* 33, 401-405. DOI :10.13926/j.cnki .apps .2003.05.004
- Ekino, T., Yoshiga, T., Takeuchi-Kaneko, Y. & Kanzaki, N. (2017). Transmission electron microscopic observation of body cuticle structures of phoretic and parasitic stages of Parasitaphelenchinae nematodes. *PLoS ONE* 12, e0179465. DOI: 10.1371/journal.pone.0179465
- Fuchs, A.G. (1937). Neue arasitische und halbparasitische Nematoden bei Borkenkäfern und einige andere Nematoden. 1 Teil. *Zoologische Jahrbücher (Systematik)* 70, 291-380.
- Gu, J., Braasch, H., Burgermeister, W. & Zhang, J. (2006). Records of *Bursaphelenchus* spp. intercepted in imported packing wood at Nigbo, China. *Forest Pathology* 36, 323-333. DOI: 10.1111/j.1439-0329.2006.00462.x
- Hooper, D.J. (1986). Handling, fixing, staining and mounting nematodes. In: Southey, J.F. (Ed.). *Laboratory methods for work with plant and soil nematodes*. London, UK, Her Majesty's Stationery Office, pp. 59-80.
- Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 1754-1755. DOI: 10.1093/bioinformatics/17.8.754
- Jiang, L., Li, X. & Zheng, J. (2007). First record of *Bursaphelenchus rainulfi* on pine

trees from eastern China and its phylogenetic relationship with intro-genus species.

Journal of Zhejiang University Science B. 8, 345-351. DOI:

10.1631/jzus.2007.B0345

Kanzaki, N. (2013). Simple methods for morphological observation of nematodes.

Nematological Research 43, 15-17. DOI: 10.3725/jjn.43.15

Kanzaki, N. & Futai, K. (2002). A PCR primer set for determination of phylogenetic

relationships of *Bursaphelenchus* species within *xylophilus* group. *Nematology* 4,

35-41. DOI: 10.1163/156854102760082186

Kanzaki, N. & Giblin-Davis, R.M. (2018). Diversity and plant pathogenicity of

Bursaphelenchus and related nematodes in relation to their vector bionomics.

Current Forestry Reports 4, 85-100. DOI: 10.1007/s40725-018-0074-7

Kanzaki, N. & Giblin-Davis, R.M. (2020). The genus *Berntsenus* Massey, 1974 is a

junior synonym of *Bursaphelenchus* Fuchs, 1937. *Nematology* 22, 677-695. DOI:

10.1163/15685411-00003332.

Kanzaki, N., Machara, N., Aikawa, T. & Togashi, K. (2008). First Report of

parthenogenesis in the genus *Bursaphelenchus* Fuchs, 1937: a description of

Bursaphelenchus okinawaensis sp. nov. isolated from *Monochamus maruokai*

(Coleoptera: Cerambycidae). *Zoological Science* 25, 861-873. DOI:

10.2108/zsj.25.861

Kanzaki, N., Aikawa, T. & Giblin-Davis, R.M. (2009). *Bursaphelenchus tokyoensis* n.

sp. (Nematoda: Parasitaphelenchinae) isolated from dead wood of the Japanese

red pine, *Pinus densiflora* Sieb. & Zucc., in Japan. *Nematology* 11, 171-180. DOI:

10.1163/156854109X429510

- 451 Kanzaki, N., Maehara, N., Aikawa, T. & Nakamura, K. (2013). An entomoparasitic
452 adult form in *Bursaphelenchus doui* (Nematoda: Tylenchomorpha) associated with
453 *Acalolepta fraudatrix*. *Journal of Parasitology* 99, 803-815. DOI: 10.1645/GE-
454 3253.1
- 455 Kanzaki, N., Tanaka, R., Giblin-Davis, R.M. & Davies, K. (2014). New plant-parasitic
456 nematode from the mostly mycophagous genus *Bursaphelenchus* discovered
457 inside figs in Japan. *PLoS ONE* 9, e99241. DOI: 10.1371/journal.pone.0099241
- 458 Kanzaki, N., Ekino, T. & Giblin-Davis, R.M. (2019a). Feeding dimorphism in a
459 mycophagous nematode, *Bursaphelenchus sinensis*. *Scientific Reports* 9, 13956.
460 DOI: 10.1038/s41598-019-50462-z
- 461 Kanzaki, N., Masuya, H., Ichihara, Y., Maehara, N., Aikawa, T., Ekino, T. & Ide, T.
462 (2019b). *Bursaphelenchus carpini* n. sp., *B. laciniatae* n. sp. and *B. cryphali*
463 *okhotskensis* n. subsp. (Nematoda: Aphelenchoididae) isolated from Japan.
464 *Nematology* 21, 361-388. DOI: 10.1163/15685411-00003220
- 465 Kanzaki, N., Kruger, M., Greeff, J.M. & Giblin-Davis, R.M. (2022). *Bursaphelenchus*
466 *suri* n. sp.: a second *Bursaphelenchus* syconial parasite of figs supports adaptive
467 radiation among section *Sycomorus* figs. *PLOS ONE* 17, e0265339. DOI:
468 10.1371/journal.pone.0265339
- 469 Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002). MAFFT: a novel method for
470 rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids*
471 *Research* 30, 3059-3066. DOI: 10.1093/nar/gkf436
- 472 Kikuchi, T., Aikawa, T., Oeda, Y., Karim, N. & Kanzaki, N. (2009). A rapid and precise
473 diagnostic method for detecting the pinewood nematode *Bursaphelenchus*

- 474 *xylophilus* by loop-mediated isothermal amplification (LAMP). *Phytopathology*
475 99, 1365-1369. DOI: 10.1094/PHYTO-99-12-1365
- 476 Korentchenko, E.A. (1980). [New species of nematodes from the family
477 Aphelenchoididae, parasites of trunk pests of *Larix dahurica*.] *Zoologicheskii*
478 *Zhurnal* 59, 1768-1780.
- 479 Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: molecular
480 evolutionary genetics analysis across computing platforms. *Molecular Biology*
481 *and Evolution* 35, 1547-1549. DOI: 10.1093/molbev/msy096
- 482 Kuraku, S., Zmasek, C.M., Nishimura, O. & Katoh, K. (2013). aLeaves facilitates on-
483 demand exploration of metazoan gene family trees on MAFFT sequence
484 alignment server with enhanced interactivity. *Nucleic Acids Research* 41, W22-
485 W28. DOI: 10.1093/nar/gkt389
- 486 Larget, B. & Simon, D.L. (1999). Markov chain Monte Carlo algorithms for the
487 Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16,
488 750-759. DOI: 10.1093/oxfordjournals.molbev.a026160
- 489 Massey, C.L. (1974). *Biology and taxonomy of nematode parasites and associates of*
490 *bark beetles in the United States*. Agricultural Handbook No. 446. Washington,
491 DC, USA, United States Department of Agriculture.
- 492 Michalopoulos-Skarmoutsos, H., Skarmoutsos, G., Kalapanida, M. & Karageorgos, A.
493 (2004). Surveying and recording of nematodes of the genus *Bursaphelenchus* in
494 conifer forests in Greece and pathogenicity of the most important species. In:
495 Mota, M. & Vieira, P. (Eds). *The pine wood nematode, Bursaphelenchus*
496 *xylophilus. Nematology: monographs and perspectives vol. 1*. Leiden, The

- 497 Netherlands, Brill, pp. 113-126. DOI: 10.1163/9789047413097_015
- 498 Minagawa, N. & Mizukubo, T. (1994). A simplified procedure of transferring
499 nematodes to glycerol for permanent mounts. *Japanese Journal of Nematology* 24,
500 75. DOI: 10.3725/jjn1993.24.2_75
- 501 Penas, A. C., Correia, P., Bravo, M. A., Mota, M. & Tenreiro, R. (2004). Species of
502 *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchidae) associated with
503 maritime pine in Portugal. *Nematology* 6, 437-453. DOI: 10.1163/15685
504 41042360573
- 505 Penas, A. C., Bravo, M. A., Naves, P., Bonirácio, L., Sousa, E. & Mota, M. (2006).
506 Species of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchidae) and
507 other nematode genera associated with insects from *Pinus pinaster* in Portugal.
508 *Annals of Applied Biology* 148, 121-131. DOI: 10.1111/j.1744-7348.2006.00042.x
- 509 Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget,
510 B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient
511 Bayesian phylogenetic inference and model choice across a large model space.
512 *Systematic Biology* 61, 539-542. DOI: 10.1093/sysbio/sys029
- 513 Rühm, W. (1956). *Die Nematoden der Ipiden. Parasitologische Schriftenreihe vol. 6.*
- 514 Ryss, A.Y. & Subbotin, S.A. (2017). [Coevolution of wood-inhabiting nematodes of the
515 genus *Bursaphelenchus* Fuchs, 1937 with their insect vectors and plant hosts.]
516 *Zhurnal Obshchei Biologii* 78, 32-61.
- 517 Ryss, A., Vieira, P., Mota, M. & Kulinich, O. (2005). A synopsis of the genus
518 *Bursaphelenchus* Fuchs, 1937 (Aphelenchida: Parasitaphelenchidae) with keys to
519 species. *Nematology* 7, 393-458. DOI: 10.1163/156854105774355581

- 520 Ryss, A., Polyanina, K., Popovichev, B.G. & Subbotin, S.A. (2015). Description of
521 *Bursaphelenchus ulmophilus* sp. n. (Nematoda: Parasitaphelenchinae) associated
522 with Dutch elm disease of *Ulmus glabra* Huds. in the Russian North West.
523 *Nematology* 17, 685-703. DOI: 10.1163/15685411-00002902
- 524 Shimizu, A., Tanaka, R., Akiba, M., Masuya, H., Iwata, R., Fukuda, K. & Kanzaki, N.
525 (2013). Nematodes associated with *Dryocoetes uniseriatus* Eggers (Coleoptera:
526 Scolytidae). *Environmental Entomology* 42, 79-88. DOI: 10.1603/EN12165
- 527 Shinya, R., Hasegawa, K., Chen, A., Kanzaki, N. & Sternburg, P.W. (2014). Evidence of
528 hermaphroditism and sex ratio distortion in the fungal feeding nematode
529 *Bursaphelenchus okinawaensis*. *G3: Genes, Genomics, Genetics* 4, 1907-1917.
530 DOI: 10.1534/g3.114.012385
- 531 Shinya, R., Sun, S., Dayi, M., Tsai, I., Miyama, A., Chen, A., Hasegawa, K.,
532 Antoshechkin, I., Kikuchi, T. & Sternberg, P.W. (2022). Possible stochastic sex
533 determination in *Bursaphelenchus* nematodes. *Nature Communications* 13, 2574.
534 DOI: 10.1038/s41467-022-30173-2
- 535 Skarmoutsos, G., Braasch, H. & Michalopoulou, H. (1998). *Bursaphelenchus hellenicus*
536 sp. n. (Nematoda, Aphelenchoididae) from Greek pine wood. *Nematologica* 44,
537 623-629. DOI: 10.1163/00572 5998X 00032
- 538 Tanaka, R., Kikuchi, T., Aikawa, T. & Kanzaki, N. (2012). Simple and quick methods
539 for nematode DNA preparation. *Applied Entomology and Zoology* 47, 291-294.
540 DOI: 10.1007/s13355-012-0115-9
- 541 Tomalak, M. & Malewski, T. (2014). *Bursaphelenchus tiliae* sp. n. (Nematoda:
542 Parasitaphelenchidae), a nematode associate of the bark beetle *Ernoporus tiliae*

(Panz.) (Coleoptera: Curculionidae, Scolytinae), in small-leaved lime, *Tilia cordata* Mill. *Nematology* 16, 1181-1196. DOI: 10.1163/15685411-00002845

Torrini, G., Strang, A., Mazza, G., Marianelli, L., Roversi, P.O. & Kanzaki, N. (2019). Description of *Bursaphelenchus irokophilus* n. sp. (Nematoda: Aphelenchoididae) isolated from *Milicia exelsa* (Welw.) C.C. Berg wood imported in Italy from Cameroon. *Nematology* 21, 957-969. DOI: 10.1163/15685411-00003268

Torrini, G., Paoli, F., Mazza, G., Simoncini, S., Strangi, A., Guidotti, A., Mori, E., Roversi, P.F. & Marianelli, L. (2020). First detection of *Bursaphelenchus abietinus* and *B. andrassyi* in Italy. *Forest Pathology* 50, e12627. DOI: 10.1111/efp.12627

Wang, H.-Y., Pan, C.-S. & Chen, Y. (2004). A new record species of genus *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchidae) in *Pinus massoniana* from China. *Journal of Xiamen University Natural Science* 43, 724-728.

Figure legends

Fig. 1. Phylogenetic relationship among *Bursaphelenchus abietinus* group species. The combined Bayesian tree was inferred from near full length of SSU and D2-D3 LSU. GTR + G + I model were applied to both SSU (AIC = 31525.931, lnL = -3248.5391) and D2-D3 LSU (AIC = 47026.21; lnL = -3132.4006). Analytical parameters were as follows: freqA = 0.26, freqC = 0.19, freqG = 0.26, freqT = 0.29; R(a) = 1.14, R(b) = 2.94, R(c) = 1.58, R(d) = 0.88, R(e) = 4.20, R(f) = 1.00; Pinva = 0.40; Shape = 0.38) for SSU and freqA = 0.21, freqC = 0.19, freqG = 0.32, freqT = 0.28; R(a) = 0.52, R(b) = 1.94, R(c) = 0.64, R(d) = 0.39, R(e) = 3.20, R(f) = 1.00; Pinva = 0.26; Shape = 0.79 for D2-D3 LSU. Posterior probability values exceeding 50% are given on appropriate clades. A subtree cropped from subfamily-wide tree (Suppl. Fig. S1) is provided here.

Fig. 2. *Bursaphelenchus ogawanus* n. sp. A: Whole body of female; B: Whole body of male. C: Lip and stylet region of a female; D: Anterior region of a male; E: Vulval region of female; F: Female tail; G: Male spicule; H, I: Male tail. All subfigures except for H (ventral view) are in right lateral view.

Fig. 3. Common adult characters of *Bursaphelenchus ogawanus* n. sp. A: Anterior region; B: Lip and stylet region in two different focal planes; C: Metacorporeal region in three different focal planes; D: Body surface of squashed individual where lateral field is indicated with small arrowheads. All subfigures are in left lateral view of an adult female. Labels are as follows: sc = stylet conus; ss = stylet shaft; bs = basal

swellings of stylet; go = dorsal pharyngeal gland orifice; p/I = pharyngo-intestinal junction (cardia); h = hemizonid; ep = secretory-excretory pore; mb = median bulb (metacarpus); nr = nerve ring.

Fig. 4. Variation of the position of secretory-excretory pore. All subfigures are right lateral view of different adult female individuals. The position of secretory-excretory pore is indicated with an arrow head in each subfigure.

Fig. 5. Male tail *Bursaphelenchus ogawanus* n. sp. A: Left lateral view in four different focal planes; B: Ventral view in two different focal planes. Labels are as follows: P + number = genital papillae labelled following Ryss *et al.* (2005); co = cloacal opening.

Fig. 6. Female characters of *Bursaphelenchus ogawanus* n. sp. in right lateral view. A: Posterior part of gonad; B: Oviduct to vulval region in two different focal planes; C: Tail region in two different focal planes. Labels are as follows: ov = ovary; od = oviduct; sp = spermatheca; cr = crustaformeria; ut = uterus; v = vulva; pus = post-uterine sac; int = intestine; rec = rectum; a = anus.

Fig. 7. Variation of female tail of *Bursaphelenchus ogawanus* n. sp. in right lateral view. Tail shape of 10 different individuals. A-H are in two different focal planes to show relative position of anus and tail tip. Anus is indicated with an arrow head in each subfigure.

Legend for Supplementary Figure

Supplementary Fig. S1. Phylogenetic relationship among Parasitaphelenchinae nematodes. Analytical model and parameters are described for the Fig. 1.

609 **Table 1.** Morphometric values of *Bursaphelenchus ogawanus* n. sp. All measurements
610 are in μm in a form: mean \pm s.d. (range).

	Male		Female
	Holotype	Paratypes	Paratypes
n	-	9	10
L	500	490 \pm 13 (468-502)	559 \pm 43 (482-625)
a	29.4	29.4 \pm 0.9 (27.7-30.8)	27.9 \pm 2.6 (24.3-33.8)
b	10.7	10.8 \pm 0.3 (10.3-11.3)	12.1 \pm 0.9 (10.6-13.7)
c	16.7	17.1 \pm 1.2 (15.6-19.4)	14.1 \pm 0.8 (13.0-15.0)
c'	2.5	2.3 \pm 0.2 (1.9-2.6)	4.5 \pm 0.2 (4.2-4.8)
T or V	65.0	61.6 \pm 3.4 (56.6-66.6)	73.7 \pm 0.5 (73.0-74.6)
M	38.5	39.1 \pm 3.8 (33.3-44.4)	36.1 \pm 1.8 (32.1-38.5)
Maximum body diam.	17.0	16.7 \pm 0.7 (15.6-18.1)	21.1 \pm 1.8 (16.7-22.6)
Lip diam.	5.9	6.0 \pm 0.2 (5.9-6.3)	6.3 \pm 0.3 (5.9-6.6)
Lip height	3.1	3.0 \pm 0.2 (2.8-3.5)	3.3 \pm 0.2 (3.0-3.5)
Lip diam./height ratio	1.9	2.0 \pm 0.1 (1.8-2.3)	1.9 \pm 0.2 (1.7-2.2)
Stylet conus	5.2	5.0 \pm 0.5 (4.2-5.6)	4.8 \pm 0.3 (4.3-5.2)
Stylet length	13.5	12.8 \pm 0.4	13.4 \pm 0.3

		(12.2-13.5)	(12.8-13.9)
Median bulb diam.	7.0	7.3 ± 0.3	8.3 ± 0.4
		(6.9-7.6)	(7.6-9.0)
Median bulb length	10.6	10.4 ± 0.3	11.2 ± 0.6
		(10.1-10.8)	(10.4-12.5)
Median bulb length/diam. ratio	1.5	1.4 ± 0.1	1.4 ± 0.0
		(1.3-1.6)	(1.3-1.4)
Secretory-excretory pore from anterior end	45	46 ± 2.9	48 ± 3.0
		(40-50)	(44-53)
Secretory excretory pore from posterior end of median bulb ¹	-1.7	0.0 ± 3.1	1.3 ± 2.9
		(-4.5-4.9)	(-3.1-6.3)
Relative position of secretory-excretory pore ²	84	100 ± 29	112 ± 25
		(57-157)	(75-156)
Nerve ring from anterior end	51	51 ± 2.1	52 ± 1.5
		(47-53)	(49-53)
Hemizonid from anterior end	57	58 ± 1.9	61 ± 2.5
		(53-60)	(57-64)
Hemizonid from posterior end of median bulb	10.8	11.9 ± 2.2	14.5 ± 2.0
		(8.7-15.3)	(11.5-17.0)
Hemizonid from secretory-excretory pore	12.5	11.9 ± 1.6	13.3 ± 2.9
		(9.7-15.3)	(7.6-18.1)
Gonad length ³	325	302 ± 21	249 ± 20
		(265-325)	(200-274)
Cloacal or anal body diam.	12.2	12.7 ± 0.4	8.9 ± 0.5
		(12.2-13.2)	(8.3-9.6)
Tail length	30	29 ± 1.9	40 ± 2.8
		(25-32)	(37-45)
Reflexed part of testis	0	12 ± 19	-
		(0-49)	
<i>Vas deferens</i> length	98	98 ± 10	-

		(82-112)	
Ratio of <i>vas deferens</i> to total gonad (%)	30.2	32.5 ± 3.6 (28.6-38.6)	-
Spicule length (chord) ⁴	12.6	12.3 ± 0.4 (11.8-12.8)	-
Spicule length (curve) ⁵	10.4	9.9 ± 0.7 (8.7-11.1)	-
Vulval body diam. (VBD)	-	-	18.0 ± 1.3 (16.0-20.1)
Post-uterine sac (PUS) length	-	-	42 ± 4.0 (36-47)
Vulva-anus distance	-	-	107 ± 10 (88-124)
PUS/VBD ratio	-	-	2.3 ± 0.2 (2.0-2.8)
Ratio of PUS to vulva-anus distance (%)	-	-	39.3 ± 1.9 (35.1-41.0)

611 ¹Calculated as secretory-excretory pore from anterior end – posterior end of median
612 bulb from anterior end. Thus, the negative value suggest that the pore is anterior to the
613 end of median bulb (overlapping with median bulb in the present species).

614 ²Relative position was calculated with a formula: (secretory-excretory pore from
615 anterior end – anterior end of median bulb from anterior end)/median bulb length. Thus,
616 the value 0 > = anterior to median bulb; 1-100 = overlapping with median bulb, 0 < =
617 posterior to median bulb.

618 ³Whole gonad length including *vas deferens* for male; anterior part of gonad (anterior
619 end of ovary to vulva) for female.

620 ⁴Condylus tip to distal end measured in a straight line.

621 ⁵Length curved along arc from capitulum depression to distal end.

622 **Table 2.** Some typological and biological characters of *Bursaphelenchus abietinus* group species.

Species	Female tail	S-E pore ¹	Host tree	Carrier insect	Distribution ²	References
<i>B. abietinus</i>	Conoid	0.5-1 body diam. posterior to MB	<i>Abies alba</i> Mill., <i>Picea abies</i> Link	<i>Pityokteines spinidens</i> (Reitter), <i>P. curvidens</i> (Germar), <i>P. voronzowi</i> (Jacobson)	Austria, Italy, Romania	Braasch & Schmutzenhofer (2000), Penas <i>et al.</i> (2006), Calin <i>et al.</i> (2015), Torrini <i>et al.</i> (2020)
<i>B. hellenicus</i>	Conoid	0.5-1 body diam. posterior to MB	<i>Larix</i> sp., <i>Pinus brutia</i> Ten., <i>P. halepensis</i> Mill., <i>P. sylvestris</i> L., <i>P. pinaster</i> Aiton, <i>P. yunnanensis</i> Franch.	<i>Tomicus piniperda</i> (L.), <i>Hylurgus ligniperda</i> (F.), <i>Ips sexdentatus</i> (Boerner & I.C.H.)	China, Germany, Greece, Italy, Portugal, Russia (Germany), Turkey	Skarmoutsos <i>et al.</i> (1998), Braasch <i>et al.</i> (1999, 2000), Braasch (2001), Michalopoulos-Skarmoutsos <i>et al.</i> (2004), Dan & Yu (2003), Penas <i>et al.</i> (2004), Jiang <i>et al.</i> (2007), Carletti (2008), Carletti <i>et al.</i> (2008), Akbulut <i>et al.</i> (2013), Torrini <i>et al.</i> (2020)
<i>B. rainulfi</i>	Elongate conoid	Overlapping with posterior half of MB	<i>Callitris columellaris</i> F.Muell., <i>Pinus caribaea</i> Morelet, <i>P. densiflora</i> Siebold & Zucc., <i>P. massoniana</i> Lambert, <i>Pinus</i> sp.	<i>Dryocoetes uniseriatus</i> Eggers	China, Japan, Germany (China), Malaysia, Taiwan, Thailand,	Braasch & Burgermeister (2002), Ambrogioni <i>et al.</i> (2003), Wang <i>et al.</i> (2004), Gu <i>et al.</i> (2006), Jiang <i>et al.</i> (2007), Shimizu <i>et al.</i> (2013), Chang & Chen (2020)

					USA (China)	
<i>B. irokophilus</i>	Elongate conoid	Bottom of MB to 0.5 MB length posterior to MB	<i>Milicia excelsa</i> (Welw.)	?	Cameroon (Italy)	Torrini <i>et al.</i> (2019)
<i>B. ogawanus</i> n. sp.	Elongate conoid	Mid MB to 0.6 MB length posterior to MB	<i>Fagus japonica</i>	<i>Dryocoetes</i> sp.	Japan	This study

623 ¹ Abbreviated as: S-E pore = secretory-excretory pore; MB = median bulb.

624 ² Isolation from imported wood or packing material are described as “origin (inspected country)”.

625 **Supplementary Table S1.** Nematode species compared in the phylogenetic analysis
626 and their GenBank accession numbers.

Species	Accession number	
	SSU	D2-D3 LSU
<i>Potensaphelenchus stammeri</i> (outgroup 1)	AB368535	MN017236
<i>Bursaphelenchus abruptus</i> (outgroup 2)	AB067756	AY508073
<i>Bursaphelenchus ogawanus</i> n. sp.	LC757019¹	
<i>Bursaphelenchus abietinus</i>	AY508011	AY508074
<i>Bursaphelenchus acaloleptae</i>	LC269963	AB650013
<i>Bursaphelenchus africanus</i>	JF317266	HM623784
<i>Bursaphelenchus anamurius</i>	FJ768949	FJ768949
<i>Bursaphelenchus andrassyi</i>	KF164829	MN879888
<i>Bursaphelenchus antoniae</i>	AM279709	KY498773
<i>Bursaphelenchus arthuroides</i>	HQ599188	HQ599190
<i>Bursaphelenchus borealis</i>	AY508012	AY508075
<i>Bursaphelenchus braaschae</i>	GQ845409	GQ845408
<i>Bursaphelenchus burgermeisteri</i>	JF317267	JF317269
<i>Bursaphelenchus carpini</i>	LC425133 ¹	
<i>Bursaphelenchus</i> cf. <i>poligraphi</i>	LC425135 ¹	
<i>Bursaphelenchus chengi</i>	KT599479	EU107359
<i>Bursaphelenchus clavicauda</i>	AB299221	AB299222
<i>Bursaphelenchus cocophilus</i>	AY509153	AY508077
<i>Bursaphelenchus conicaudatus</i>	AB067757	AB299227
<i>Bursaphelenchus corneolus</i>	HQ407406	HQ407405
<i>Bursaphelenchus crenati</i>	KU683736	KU683737
<i>Bursaphelenchus cryphali okhotskensis</i>	LC425131 ¹	
<i>Bursaphelenchus doui</i>	AB299223	DQ899733
<i>Bursaphelenchus eggersi</i>	AY508013	AY508078
<i>Bursaphelenchus eremus</i>	MK301111	AM396568
<i>Bursaphelenchus eucarpus</i>	MG840410	MG840392

<i>Bursaphelenchus fagi</i>	-	JX683686
<i>Bursaphelenchus firmae</i>	AB650015	AB650014
<i>Bursaphelenchus fraudulentus</i>	AB067758	AY508079
<i>Bursaphelenchus fungivorus</i>	AY508016	AY508082
<i>Bursaphelenchus geraerti</i>	MG264562	MG264565
<i>Bursaphelenchus gerberae</i>	AY508024	AY508092
<i>Bursaphelenchus gillanii</i>	KJ653442	KJ653443
<i>Bursaphelenchus hellenicus</i>	AY508017	AY508083
<i>Bursaphelenchus hibisci</i>	LC627322 ¹	
<i>Bursaphelenchus hildegardae</i>	AM397013	AM396569
<i>Bursaphelenchus hirsutae</i>	LC269966 ¹	
<i>Bursaphelenchus hofmanni</i>	AY508018	KF496910
<i>Bursaphelenchus hylobianum</i>	AY508019	KT806477
<i>Bursaphelenchus irokophilus</i>	MK544843	MK544842
<i>Bursaphelenchus kesiyae</i>	LC087116	LC087117
<i>Bursaphelenchus kiyoharai</i> NK215	AB597255	AB597256
<i>Bursaphelenchus koreanus</i>	JX154585	JX154584
<i>Bursaphelenchus laciniatae</i>	LC425132	LC425132
<i>Bursaphelenchus leoni</i>	MN907406	MN907407
<i>Bursaphelenchus luxuriosae</i>	AB097864	AB299228
<i>Bursaphelenchus macromucronatus</i>	LC579929 ¹	
<i>Bursaphelenchus masseyi</i>	-	JQ287495
<i>Bursaphelenchus michalskii</i>	MH815102	MH457128
<i>Bursaphelenchus microcarpae</i>	LC596447 ¹	
<i>Bursaphelenchus mucronatus kolymensis</i>	AB932856	DQ364688
<i>Bursaphelenchus niphades</i>	AB849465	AB849475
<i>Bursaphelenchus okinawaensis</i>	AB358983	AB358982
<i>Bursaphelenchus paraburgeri</i>	HQ727727	HQ727726
<i>Bursaphelenchus paracorneolus</i>	AY508027	AY508095
<i>Bursaphelenchus paraleoni</i>	MN419334	MN419326

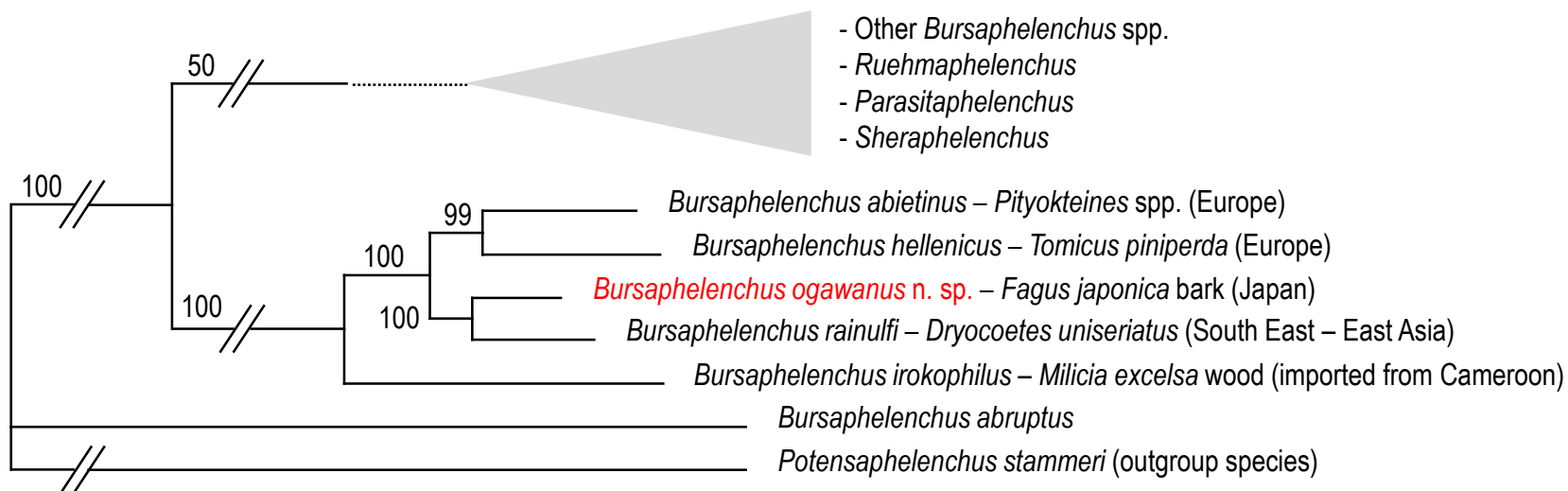
<i>Bursaphelenchus paraluxuriosae</i>	JF966206	JF966204
<i>Bursaphelenchus parantoniae</i>	KT223041	KT223042
<i>Bursaphelenchus paraparvispicularis</i>	GQ421483	GQ429010
<i>Bursaphelenchus parapinasteri</i>	KT878515	KT878516
<i>Bursaphelenchus parathailandae</i>	JN377724	JN377722
<i>Bursaphelenchus parayongensis</i>	MW367550	MW367554
<i>Bursaphelenchus parvispicularis</i>	AB218829	AB368537
<i>Bursaphelenchus piceae</i>	KT315781	KF772174
<i>Bursaphelenchus pinasteri</i>	AM397016	AM396574
<i>Bursaphelenchus platzeri</i>	AY508026	AY508094
<i>Bursaphelenchus poligraphi</i>	LC425134 ¹	
<i>Bursaphelenchus populi</i>	HQ699855	FJ998281
<i>Bursaphelenchus posterovulvus</i>	KF314804	KF314807
<i>Bursaphelenchus pterocarpi</i>	MH820122	MH827564
<i>Bursaphelenchus rainulfi</i>	LC269965	AM396575
<i>Bursaphelenchus ratzeburgii</i>	MG264564	MG264569
<i>Bursaphelenchus rockyi</i>	MH393459	MH396441
<i>Bursaphelenchus rufipennis</i>	AB368529	AB368530
<i>Bursaphelenchus sakishimanus</i>	LC027461	LC027462
<i>Bursaphelenchus saudi</i>	KT806480	KT806482
<i>Bursaphelenchus seani</i>	AY508029	AY508099
<i>Bursaphelenchus sexdentati</i>	AY508031	AY508101
<i>Bursaphelenchus sinensis</i>	AB232162	AB368538
<i>Bursaphelenchus singaporensis</i>	AM397018	AM396576
<i>Bursaphelenchus tadamiensis</i>	AB635399 ¹	
<i>Bursaphelenchus taedae</i>	OM976850	OM976872
<i>Bursaphelenchus taphrorychi</i>	-	MF422699
<i>Bursaphelenchus thailandae</i>	MK301110	DQ497184
<i>Bursaphelenchus tokyoensis</i>	AB430445	AB430446
<i>Bursaphelenchus tryphloeii</i>	-	FJ998283

<i>Bursaphelenchus tusciae</i>	AY508033	AY508104
<i>Bursaphelenchus ulmophilus</i>	-	KP331049
<i>Bursaphelenchus vallesianus</i>	AM397020	AM396578
<i>Bursaphelenchus wuae</i>	AB918706	AB918707
<i>Bursaphelenchus xylophilus</i>	AB067760	AY508107
<i>Bursaphelenchus yongensis</i>	AM397023	AM396581
<i>Bursaphelenchus</i> sp. 104J7	MK292121	MK292121
<i>Bursaphelenchus</i> sp. 5286	MF800855	MF807217
<i>Bursaphelenchus</i> sp. “FukauraB”	LC425136 ¹	
<i>Bursaphelenchus</i> sp. isolate Hyl5	MT556023	MT556020
<i>Bursaphelenchus</i> sp. isolate O1	MT556021	MT556018
<i>Bursaphelenchus</i> sp. isolate O117	MG865772	MG865773
<i>Bursaphelenchus</i> sp. isolate O2	MT556022	MT556019
<i>Bursaphelenchus</i> sp. isolate O217	MG865769	MG865770
<i>Bursaphelenchus</i> sp. isolate T217	MG865767	MG865768
<i>Bursaphelenchus</i> sp. isolate TV17	MG865792	MG865793
<i>Bursaphelenchus</i> sp. isolate Z17	MG865787	MG865788
<i>Bursaphelenchus</i> sp. JG-2011d	JN377728	JN377726
<i>Bursaphelenchus</i> sp. LC-2016	KT884896	KT884895
<i>Bursaphelenchus</i> sp. Ulm-04	-	MH548901
<i>Parasitaphelenchus costati</i>	LC269967 ¹	
<i>Parasitaphelenchus frontalis</i>	LC383740	LC383742
<i>Ruehmaphelenchus ameicanus</i>	OK668309	OK668310
<i>Ruehmaphelenchus asiaticus</i>	-	AM269475
<i>Ruehmaphelenchus digitulus</i>	JN377732	JN377730
<i>Ruehmaphelenchus formosanus</i>	AB808718	MH590283
<i>Ruehmaphelenchus fujiensis</i>	LC593613 ¹	
<i>Ruehmaphelenchus juliae</i>	LC031813 ¹	
<i>Ruehmaphelenchus quercophilus</i>	KY689022	KY689023
<i>Ruehmaphelenchus</i> sp. “FukauraR”	LC425137 ¹	

<i>Ruehmaphelenchus</i> sp. NK202	AB368534	AB597984
<i>Ruehmaphelenchus</i> sp. “Wakayama”	LC593614	LC593615
<i>Sheraphelenchus entomophagus</i>	KC875226	KC875232
<i>Sheraphelenchus sucus</i>	AB808720	AB808721

627 ¹ Long (*ca* 4.2 kb) sequence including SSU, ITS and D1-D4 LSU.

628



10% difference within ca 2.6 kb of aligned SSU and D2-D3 LSU

Fig. 1

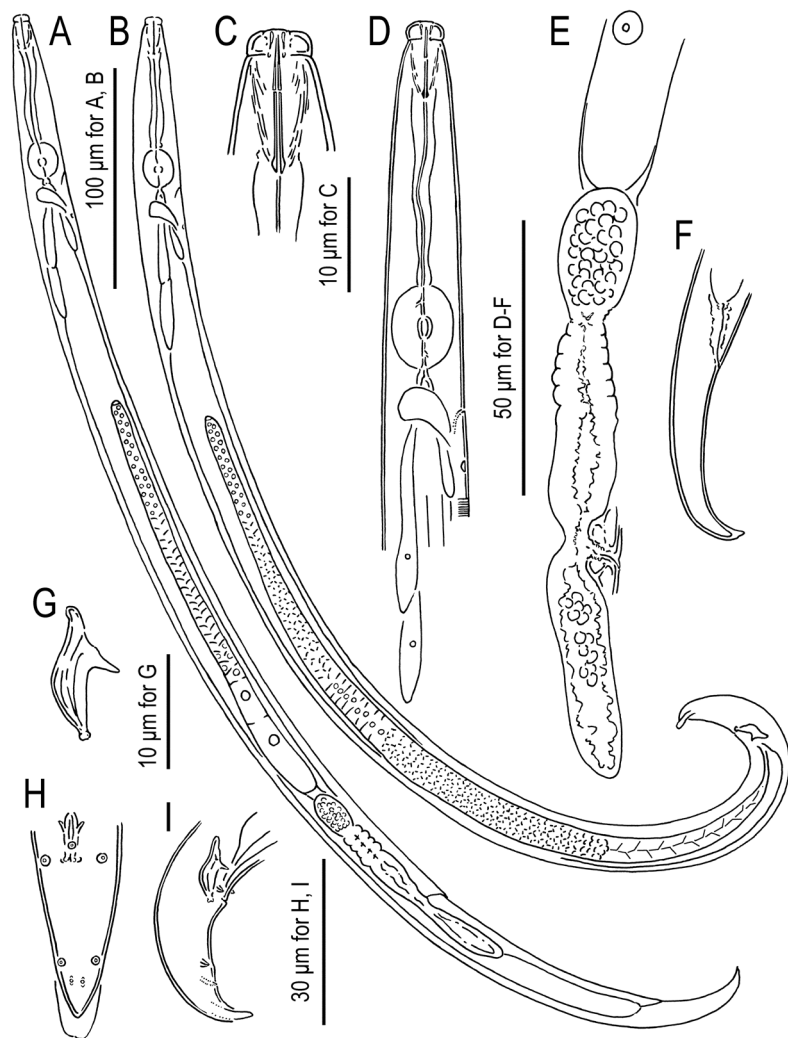


Fig. 2

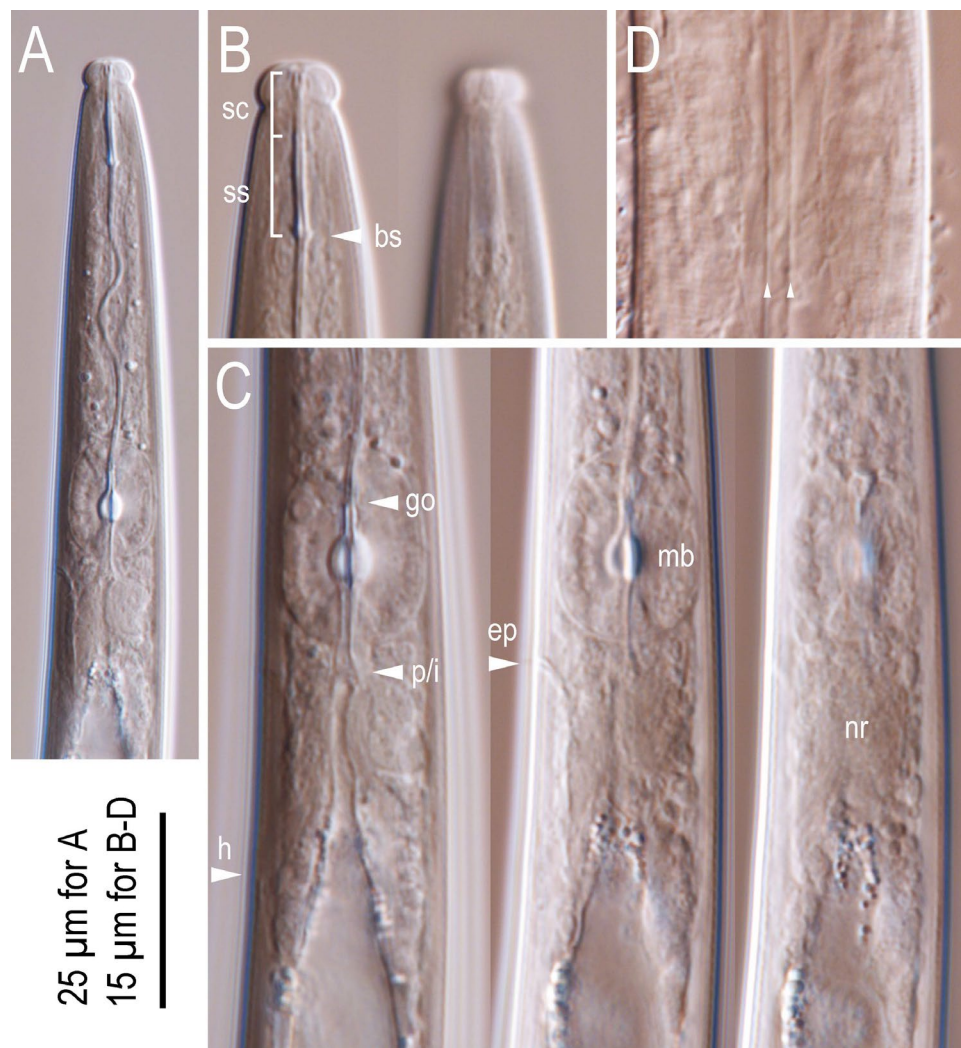


Fig. 3

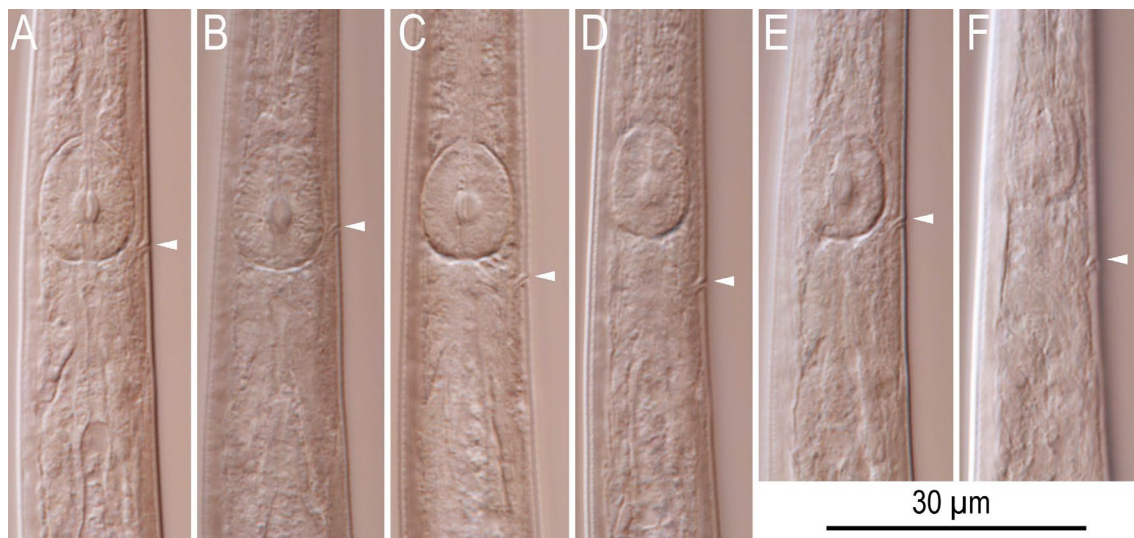


Fig. 4

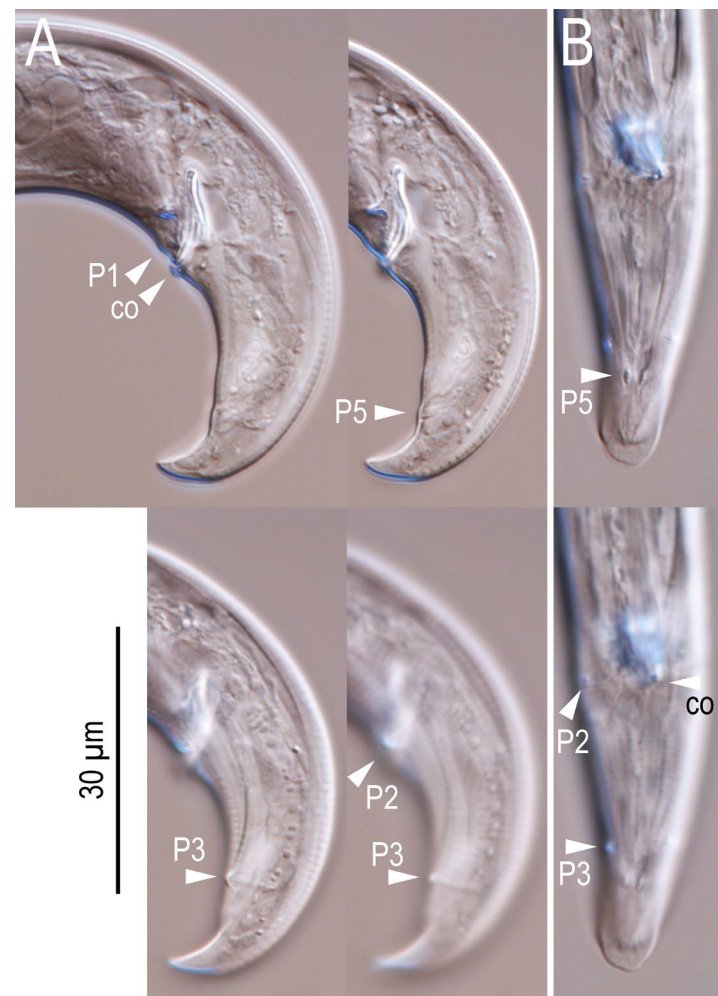


Fig. 5

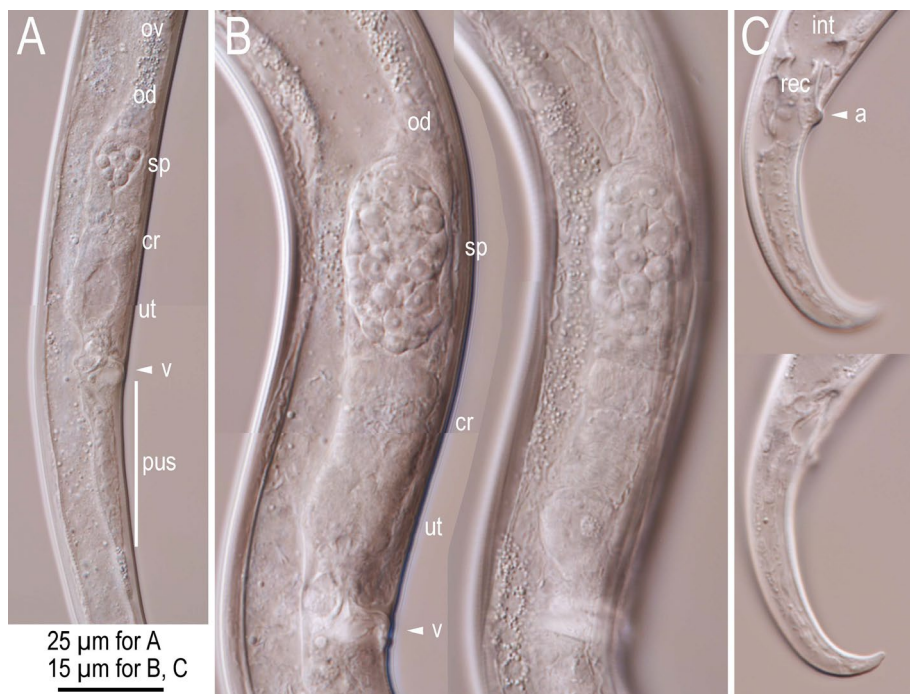


Fig. 6

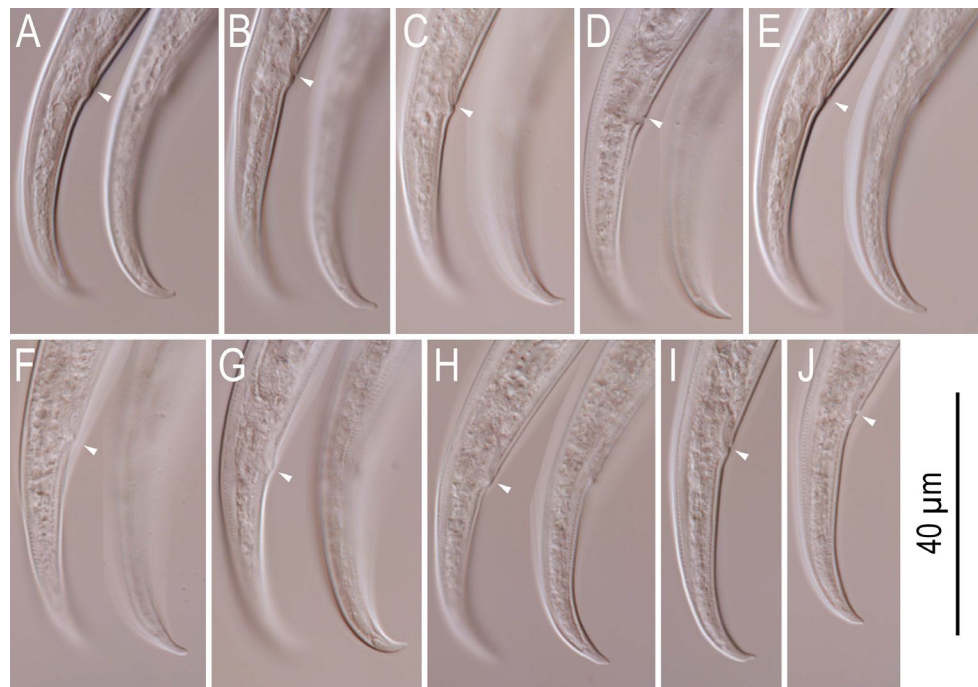
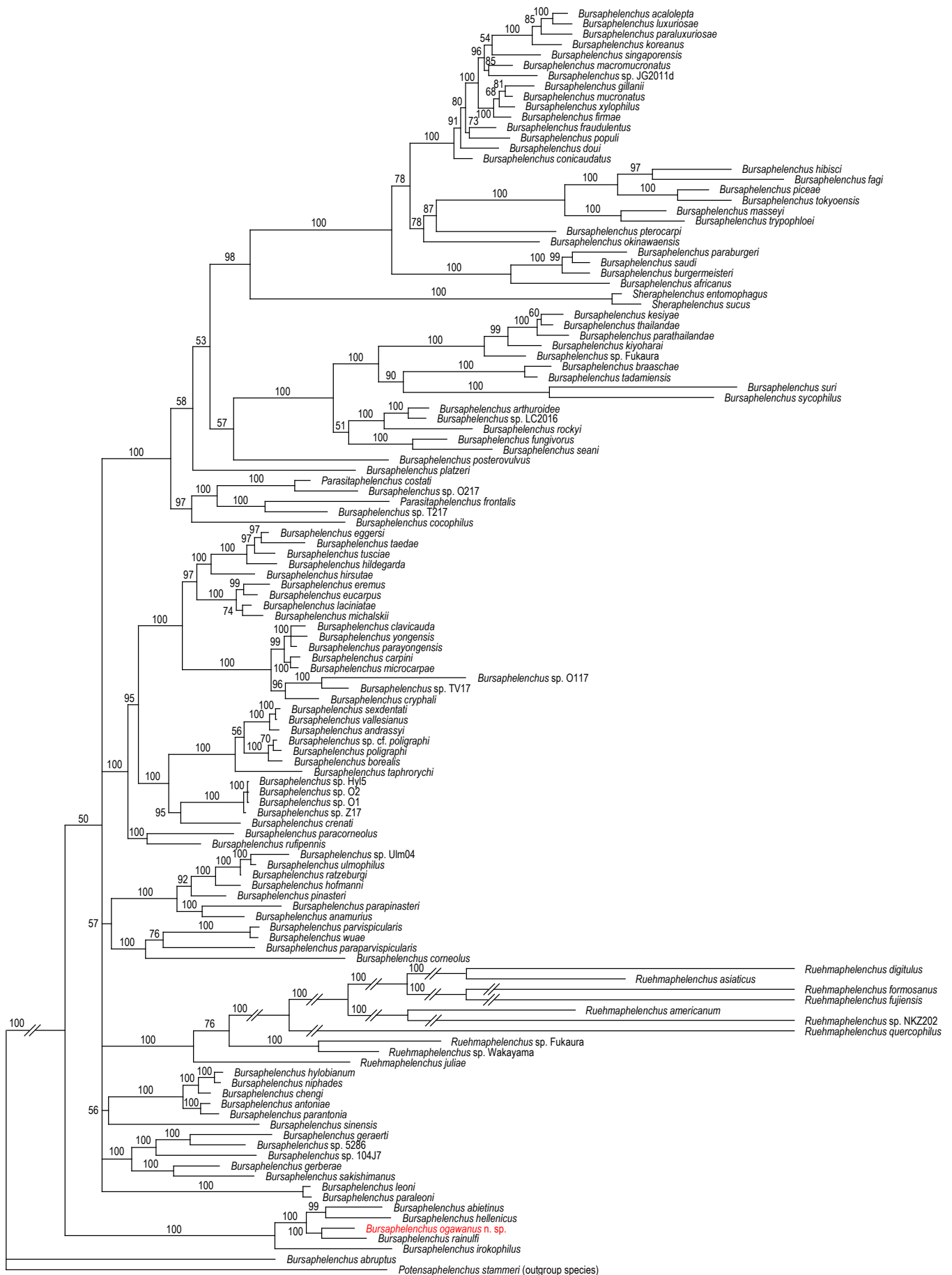


Fig. 7



10% difference within ca 2.6 kb of aligned SSU and D2-D3 LSU