DOI 10.2478/helm-2023-0029

HELMINTHOLOGIA, 60, 3: 227 – 239, 2023

# *Bursaphelenchus mucronatus* (Nematoda: Parasitaphelenchidae) associated with *Monochamus galloprovincialis* from Bosnia and Herzegovina and Georgia

V. ČERMÁK[1,3,*], B. NJEŽIĆ[4], N. NAZARASHVILI[5], E. GVRITISHVILI[5], K. TOMÁNKOVÁ[1], H. ORSÁGOVÁ[1], M. MAJESKÁ[2], J. FOIT[3], P. VIEIRA[6]

[1]Central Institute for Supervising and Testing in Agriculture, Division of Plant Pest Diagnostics, Šlechtitelů 773/23, 779 00 Olomouc, Czech Republic, *E-mail: *v.cermak@ukzuz.cz*; [2]Palacký University in Olomouc, Czech Advanced Technology Research Institute, Plant Genetics and Engineering, Šlechtitelů 27, 779 00 Olomouc, Czech Republic; [3]Mendel University in Brno, Faculty of Forestry and Wood Technology, Department of Forest Protection and Wildlife Management, Zemědělská 3, 613 00 Brno, Czech Republic; [4]University of Banja Luka, Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, 78 000 Banja Luka, Bosnia and Herzegovina; [5]State Laboratory of Agriculture, Plant Pest Diagnostic Department, 49 Godziashvili street, 0159 Tbilisi, Georgia; [6]United States Department of Agriculture, Agricultural Research Services, Mycology & Nematology Genetic Diversity & Biology Lab, Beltsville, MD 20705, USA

**Article info**

Received May 8, 2023
Accepted August 6, 2023

**Summary**

*Bursaphelenchus mucronatus* was detected in association with the pine sawyer beetle (*Monochamus galloprovincialis*) during the implementation and testing of cross traps with insect attractants as an efficient tool for detection survey for pine wood nematode (*Bursaphelenchus xylophilus*) in Bosnia and Herzegovina and Georgia in 2017 and 2018, respectively. This nematode was characterized by morphological, morphometric and molecular features. This is the first report of *B. mucronatus* in association with a *M. galloprovincialis* in Bosnia and Herzegovina and in Georgia.
**Keywords:** *Bursaphelenchus;* pine wood nematode; pine sawyer beetle; trap; detection survey

## Introduction

Detection surveys for quarantine pests are one of the most effective preventive measures given by the Regulation (EU) 2016/2031 of the European Parliament of the Council on protective measures against the introduction of non-native plant pathogens. The pine wood nematode *Bursaphelenchus xylophilus* and its non-european insect vectors (*Monochamus* spp.) are classified as quarantine pests according to Commission Implementing Regulation (EU) 2019/2072. Based on the established legislation long-term detection surveys are obligatory for all EU member countries. Following the Regulation (EU) 2016/2031, medium- and long-term monitoring programs of quarantine species are well established within the EU countries in order to protect the national forest resources, and to develop strategies against the potential introduction and spreading of this highly pathogenic nematode species to native pine forests. Within this scenario, national surveys for the pine wood nematode and its vectors have also been established in Bosnia and Herzegovina (BiH) and in Georgia.

Currently, the information regarding the distribution of species within the genus *Bursaphelenchus* in Bosnia and Herzegovina is to our knowledge not known. On the other hand, in case of Georgia, a total of 22 putative *Bursaphelenchus* species have been reported from the country so far (Mikaia *et al*., 2010). Nevertheless, from the morphologically closest species of the *xylophilus*-group only *B. fraudulentus* has been reported. In this study we report both morphological and molecular data of *Bursaphelenchus mucronatus* found during national surveys associated with the pine sawyer beetle *Monochamus galloprovincialis.* To our knowledge this represents the first report of this nematode species in both Bosnia and Herzegovina and Georgia.

* – corresponding author

## Materials and Methods

*Sampling, nematode extraction and cultivation*

Pheromone-baited black cross traps (Ecconex/Galloprotect Pack) were established as a part of the workshops focused on general surveys for forest quarantine species, including the pine wood nematode and its insect vectors. Two traps were hanged in the crowns of most abundant pine trees (approximately 20 m high), one in 2017 in the area of Banja Luka (BiH) on *Pinus nigra*, second in 2018 in Mamkoda park (Georgia) on *P. sylvestris*. Traps were checked for insects weekly (from June to September). Insects caught in the trap containers were transferred into plastic bottles, labelled, and transported to the laboratory. Although also other insects were caught in the traps, this study focused only on the identification and screening of known insect vectors of the pine wood nematode (i.e., *Monochamus* spp.). All collected vectors were identified morphologically to the species level before nematode analysis.

Nematodes were extracted by dissection and maceration of insects in sterile water followed by three hours incubation at room temperature. The presence or absence of nematodes was checked under the stereomicroscope (IPPC, 2016). The extracted nematode "dauer larvae" were fixed in 4 % formalin and transferred to pure glycerin according to De Grisse (1969) or prepared for molecular analysis. Where a large number of nematodes was available, a subset of living nematodes was transferred onto the plates containing the sporulating form of the fungus *Monilinia* sp., instead of *Botryotinia fuckeliana* (anamorph: *Botrytis cinerea*) in accordance with IPPC (2016), and cultured. Three weeks later, adults were fixed and transferred into glycerin as mentioned above.

*Morphological and morphometric analysis*

Morphological and morphometric analyses were based on the main diagnostic features for the genus *Bursaphelenchus* as described by Ryss *et al*. (2005) and Braasch *et al*. (2009). Specimens mounted in permanent slides were deposited in the nematological collection of the Division of Plant Pest Diagnostics in Olomouc, Czech Republic.

*Molecular analysis*

For molecular identification, DNA from single nematode specimens ("dauer larvae") was extracted and processed as follows: nematodes were homogenised in 50 µL of nematode lysis buffer (10mM Tris-HCl, pH 8.8; 1mM EDTA; 1 % Triton X-100 (v/v); 100 µg $ml^{-1}$ proteinase K) in a 1.5 mL Eppendorf tube using a micropestle. Samples were incubated at 55 °C for 1 h and subsequently at 95 °C for 10 min. The resulting DNA extracts were used as a template for each PCR reaction. The identification of individual nematodes was performed using two different *loci*, i.e., the ITS1-5.8S-ITS2 region of ribosomal DNA and the D2-D3 region of the 28S rDNA. The ITS1-5.8S-ITS2 region was amplified using the forward primer 5´-CGTAACAAGGTAGCTGTAG-3´ (Ferris *et al*., 1993) and the reverse primer 5´-TTTCACTCGCCGTTACTAAGG-3´ (26S, Vrain, 1993). For amplification of the D2-D3 region the forward primer D2A 5´-ACAAGTACCGTGAGGGAAAGTTG-3´ and the reverse primer D3B 5´-TCGGAAGGAACCAGCTACTA-3´ were used (De Ley *et al*., 1999).

The ITS1-5.8S-ITS2 PCR reaction was performed in a total volume of 50 µl containing 1x PCR buffer (75mM Tris-HCl pH 9.0; 50mM KCl; 20mM $(NH_4)_2SO_4$, 4mM $MgCl_2$, 400µM dNTPs, 600nM primers, 2 U of DNA polymerase (Biotools, Madrid, Spain) and 10 µl of undiluted template DNA, in a GenePro thermal cycler (Bioer Technology Co., LTD) using an initial denaturation step for 5 min at 94 °C, followed by 40 reaction cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 48 °C and extension for 2 min at 72 °C, with a final 10min extension at 72 °C. Six µl of the PCR product were digested for at least 2 hours at 37 ºC, using 1x restriction buffer and 5 U of enzyme (*Rsa*I, *Hae*III, *Msp*I, *Hinf*I and *Alu*I) in the total reaction volume of 10 µl. Restriction products were run on the Midori Green Advance (Nippon Genetics) stained 3 % TBE-buffered agarose gel (65 V, 60 min).

Amplification of the D2-D3 region was performed in a total volume of 25 µl using 1x PCRBIO Taq Mix (PCR Biosystems, London, UK), 400nM primers and 2 µl of undiluted template DNA, in a GenePro thermal cycler (Bioer Technology Co., LTD) using an initial denaturation step for 1 min at 95 °C, followed by 5 reaction cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 45 °C and extension for 30 s at 72 °C and 35 cycles of 15 s at 95 °C, 15 s at 54 °C and 30 s at 72 °C.

After ExoSAP purification, PCR products of both regions were submitted for direct Sanger sequencing to the Centre of Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany (Olomouc, Czech Republic) using the corresponding forward and reverse primers. Resulting sequence data were analysed using Geneious Bioinformatics software platform (Biomatters). The online bioinformatic tool RestrictionMapper (www.restrictionmapper.org) was used for in-silico calculations of ITS-restriction fragment sizes and virtual RFLP pattern, using five above mentioned restriction enzymes, which are known to generate species-specific ITS-RFLP profiles for the genus *Bursaphelenchus* (Burgermeister *et al*., 2009).

*Phylogenetic analysis*

The D2-D3 sequences obtained from nematodes collected in BiH and Georgia, as well as 10 sequences downloaded from the GenBank database (https://www.ncbi.nlm.nih.gov/), were used to reconstruct the phylogenetic relationships with other *Bursaphelenchus* species. *Aphelenchoides besseyi* (AY508109.1) was used as outgroup taxa. Phylogenetic analysis was conducted in MEGA X (Kumar *et al.,* 2018). DNA sequences were aligned using ClustalW with default options (Thompson *et al.*, 1994). The final alignment was visually checked. The optimal nucleotide substitution model for the Maximum Likelihood (ML) method was chosen according the Akaike Information Criterion (AIC). The evolutionary

Table 1. Morphometric comparison between the populations of *Bursaphelenchus mucronatus* from BiH and Georgia with the type population of *B. mucronatus* (Mamiya & Enda, 1979) from Japan. All measurements are in μm and in the form: mean ± s.d. (range).

| | Dauers Georgian population | ♀♀ Georgian population | ♂♂ Georgian population | Dauers BiH population | Dauers Mamiya & Enda 1979 | ♀♀ Mamiya & Enda 1979 | ♂♂ Mamiya & Enda 1979 |
|---|---|---|---|---|---|---|---|
| n | 5 | 25 | 12 | 5 | 30 | 40 | 35 |
| L | 597.5±19.8 (570-620) | 866.4± 77.0 (738-1018) | 675.3 ± 19.7 (652.5-723.0) | 589 ± 23.7 (558-620) | 590 (500-650) | 870 (700-980) | 790 (640-970) |
| a | 43.2±2.3 (39.5-45.6) | 40.9 ± 2.6 (35.0-45.7) | 42.6 ± 3.9 (35.8-50.4) | 41.7 ± 3.6 (35.8-45.0) | 39.8 (35.1-45.8) | 41.8 (36.5-45.9) | 44.0 (38.8-51.1) |
| b | 9.5* | 11.0 ± 1.2 (8.0-13.3) | 9.1 ± 0.4 (8.4-9.9) | 9.3* | 8.9 (7.9-9.6) | 12.6 (9.6-15.6) | 11.4 (9.0-14.7) |
| c | 20.7±1.40 (18.3-21.7) | 27.1 ± 2.8 (23.3-30.1) | 20.0 ± 1.1 (18.6-22.1) | 19.4 ± 1.3 (18.0-21.1) | 21.2 (19.7-24.4) | 26.2 (19.6-30.4) | 29.1 (25.7-35.6) |
| c´ | 3.6±0.3 (3.3-4.0) | 2.9 ± 0.2 (2.5-3.3) | 2.4 ± 0.1 (2.1-2.5) | 3.5 ± 0.2 (3.0-3.7) | | | |
| V [%] | - | 74.1 ± 2.0 (67.0-76.9) | - | - | | 75 (73-77) | |
| Stylet length | 8.5* | 13.3 ± 1.2 (10.2-15.1) | 13.6 ± 0.6 (12.8-14.6) | 8.8* | | 15.8 (14-16) | 15.0 (14-16) |
| Excretory pore position[1)] | 54.9±4.5 (49-58.1) | 83.7 ± 3.6 (73.6-89.5) | 76.7 ± 4.2 (70.5-84.9) | 63,3±7,2 (54.0-74.4) | | | |
| Pharynx length | 59.6* | 77.9 ± 4.2 (72.3-91.4) | 74.9 ± 3.0 (69.2-80.8) | 58.9* | | | |
| MB[2)] | 59.9±4.9 (54.7-67.9) | 66.6 ± 3.7 (62.6-78.3) | 64.9± 2.5 (61.5-69.5) | 58.0 ± 5.7 (50.7-67.0) | | | |
| Hemizonid position[1)] | - | 95.8± 5.2 (76.0-101.8) | 91.0 ± 2.5 (86.9-94.9) | - | | | |
| Anal/cloacal body diameter | 8.1±0.2 (7.9-8.4) | 11.1± 0.7 (10.1-12.2) | 14.0 ± 0.7 (12.7-14.8) | 8.2 ± 0.3 (7.8-8.5) | | | |
| Tail length | 29.1±2.8 (27.0-33.9) | 32.0± 2.2 (26.0-36.9) | 33.9 ± 1.9 (30.4-36.9) | 28.3 ± 2.55 (27.0-33.9) | | | |
| Post-uterine sac length | - | 156.5 ± 17.9 (125.0-195.6) | - | - | | | |
| Spicule length (curved median line) | - | - | 31.2 ± 1.5 (28.5-33.6) | - | | | 26.0 (23-29) |

[1)]Distance from anterior end; [2)]Distance from anterior end to base valves of median bulb, * - n=1 history was inferred by using the ML method based on the General Time Reversible (GTR) model with a discrete Gamma distribution of evolutionary rate differences among sites (five categories, + G) and invariant sites (+ I) (Nei & Kumar, 2000). The bootstrap consensus tree was inferred from 100 replicates (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates were collapsed.

## Results

*Insect Vector and Nematode morphological characterization*

As mentioned above, the main goal of our study was to detect potential insect vectors of pine wood nematode in BiH and Georgia, and therefore our analyses focused primarilly on the genus *Monochamus*. Forty specimens of *Monochamus galloprovincialis* were captured and processed in BiH. In Georgia, a total of 82 individuals of *M. galloprovincialis* were captured, of which 32 were analyzed.

The processed *M. galloprovincialis* specimens collected in BiH and Georgia contained only "dauer larvae" of nematodes settled in tracheae of the sawyer beetles. Morphometric analysis of dauer larvae from both countries is summarized in Table 1, while morphological diagnostic characters are shown in Figure 1. Morphological and morphometric analysis of adult stages of Georgian population recovered from the fungal cultures confirmed the species identification of these nematodes as *B. mucronatus* (Fig. 1 and Table 1).

*Molecular characterization and phylogenetic analysis*

For both BiH and Georgian populations found during the survey, a molecular characterization was performed using the ITS1-5.8S-ITS2 and D2-D3 region of 28S of the ribosomal DNA generated from single dauer larvae specimens.

The amplification and sequencing of ITS1-5.8S-ITS2 region yielded an 884 bp sequence (corresponding to the entire PCR product sequence after primer trimming) for both BiH and Georgian populations (OK500292 and OK500293, respectively) sharing 99.7 % identity to each other: BiH sequence included one ambiguous base (A/G=R) at position 161 and G at 642 (which prevented amplicon cutting with *Rsa*I restriction enzyme at this position) while Georgian sequence had an A in position 642 (cutting motif for *RsaI*) and R at position 805. Both R bases come from an A+R double-peak presence in all raw data reads. From BiH population two specimens were analysed separately and sequenced twice in both directions resulting in 7 out of 8 raw data reads useful for consensus sequence generation. In all 7 of them a clear double-peak was observed. From Georgian population a single specimen was sequenced in both directions resulting in a double-peak presence in both raw data. Both BiH and Georgian sequences show high similarity (99 % for BiH due to R base at 161 position and 100 % for Georgian whose R is located out of section aligned with sequences deposited at NCBI (sequences AM396572.1 and MK584707.1, see Fig. 2).

The ITS-RFLP pattern was calculated virtually for the ITS sequences generated for both populations collected in BiH and Georgia. The virtual restriction profile generated for four enzymes were similar for both populations (Table 2) and showed the same reference ITS-RFLP pattern established for the *B. mucronatus* European type by Burgermeister *et al*. (2009). The only difference found between both populations was in the pattern generated by the *Rsa*I restriction enzyme (Table 2). The restriction profile of *Rsa*I of the population from Georgia corresponded to the profile of East-Asian type of *B. mucronatus* and differed from the reference profile for the European type of *B. mucronatus* (Burgermeister *et al*., 2009).

In case of D2-D3 region amplification and sequencing resulted in a sequence of 743 bp (corresponding to the entire PCR product sequence after primer trimming) for the population collected in BiH (OK523378) and partial 720 bp for the population collected in Georgia (OK523379). Both sequences shared 100 % identity to each other and also to the population of *B. mucronatus kolymensis* (MK584707) from Romania (Calin *et al*., 2020) and other sequence of *B. mucronatus* (AM396572.1) from Germany (data not shown). All four sequences clustered together in phylogenetic analysis (Fig. 2).

## Discussion

The genus *Bursaphelenchus* is widely distributed in the Northern Hemisphere, largely associated with conifer native forests. After the first detection of the pine wood nematode in Portugal (Mota *et al*., 1999), intense surveys have been conducted in all EU member state conifer forests. These intense surveys resulted in identification and characterization of a large list of *Bursaphlenchus* species in Europe, but also from Asia and the North America (Penas *et al*., 2004; Ryss *et al*., 2005; Kanzaki *et al*., 2012; Tomalak *et al*., 2013; Čermák *et al*., 2013; d'Errico *et al*., 2015 and Mitrea-Calin *et al*., 2020).

The diversity of the genus *Bursaphelenchus* in Bosnia and Herzegovina territories is still totally unknown, while to date a large number of species have been reported from Georgia (Kurashvili *et al*., 1980; Kakulia & Devdariani, 1965 and 1967; Kakulia, 1989; Mikaia *et al*., 2010). The first known report of the still valid representative of the genus *Bursaphelenchus* from Georgia was detection of *Bursaphelenchus piniperdae* in the frass of *Blastophagus minor* in Borjomi-Bakuriani coniferous forests in 1963 (Kakulia, 1967). After that, 22 putative species have been reported from Georgia in total (Mikaia *et al*., 2010) including nine species described originally from Georgia: *B. ernoporus*, Devdariani, 1975; *B. erosus* Kuraschvili, Kakulia & Devdariani, 1980; *B. georgicus* Devdariani *et al*., 1980; *B. hylesini* Devdariani, 1975; *B. populneus* Devdariani, 1973; *B. scalari* Kakulia, 1989; *B. tbilisyensis* Kakulia, 1989 and *B. sutoricus* Devdariani, 1974; *B. wecuae* Kurashvili *et al*., 1980. Hunt (1993) classified four of those species: *B. georgicus*, *B. populneus*, *B. tbilisyensis*, *B. wekuae* as *nomina nuda*. However, Ryss *et al*. (2005) included *B. georgicus* and *B. wekuae* into the list of valid *Bursaphelenchus* species and all together

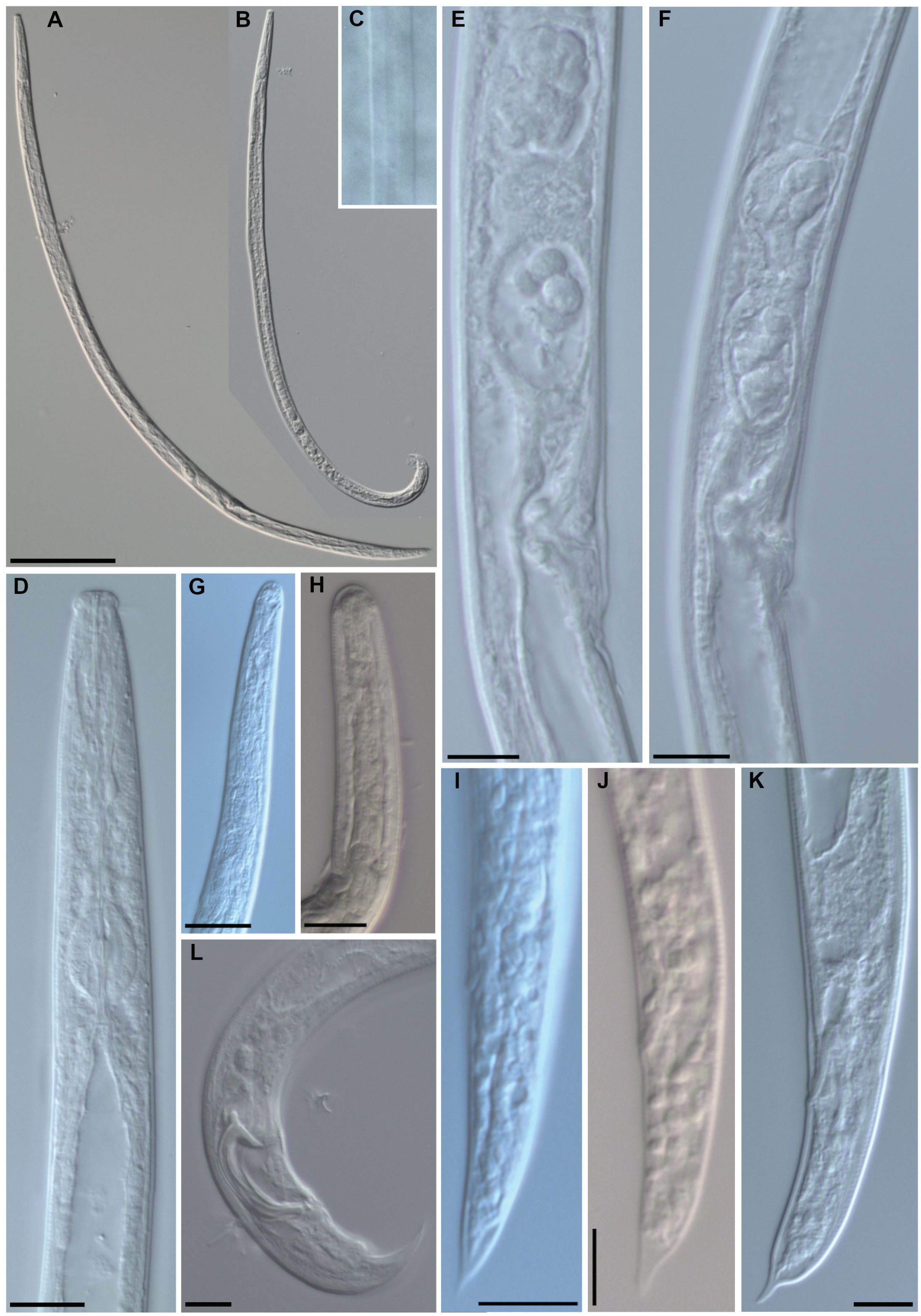


Fig. 1. Light micrograph of *Bursaphelenchus mucronatus*. A: Female entire body; B: Male entire body; C: Lateral field with four incisures; D: Female anterior region; E, F: Vulva; G, H: Dauer larvae anterior part; I, J: Dauer larvae tail tip; K: Female tail tip; L: Mail tail; A, B,C, D, E, F, G, I, K, L: Georgian population; H, J: BiH population; A, B – 100 μm; C-L – 10 μm.

Table 2. Virtual ITS-RFLP patterns of *Bursaphelenchus mucronatus* from BiH and Georgia.

| | | BiH population, OK523378 | | | | | Georgian population, OK523379 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ITS-PCR product | *Rsa*I | *Hae*III | *Msp*I | *Hinf*I | *Alu*I | *Rsa*I | *Hae*III | *Msp*I | *Hinf*I | *Alu*I |
| Fragment sizes (~bp) | 884* | **490** | 623 | 355 | 411 | 678 | **412** | 623 | 355 | 411 | 678 |
| | | **412** | 196 | 304 | 232 | 246 | **264** | 196 | 304 | 232 | 246 |
| | | **22** | 105 | 265 | 120 | | **226** | 105 | 265 | 120 | |
| | | | | | 87 | | **22** | | | 87 | |
| | | | | | 49 | | | | | 49 | |
| | | | | | 25 | | | | | 25 | |

* The amplification and sequencing of ITS1-5.8S-ITS2 region yielded an 884 bp sequence (corresponding to the entire PCR product sequence after primer trimming) for both BiH and Georgia populations (OK523378 and OK523379, respectively)

listed 18 species from Georgia, but at the same time considered *B. populneus* and *B. tbilisyensis* as *nomina nuda.* Based on the drawings of *B. populneus* and *B. ernoporus* re-published in Mikaia *et al*. (2010) and description of *B. tbilisyensis* in Kakulia (1989) we consider them as valid species. We consider only *B. hylesini* as *nomen nudum.* In total, we include 23 *Bursaphelenchus* species in the list of species described from Georgia (see Table 3). However, the majority of them has been insufficiently described and molecularly characterized to allow their classification into the groups (Braasch *et al*., 2009).

Six species were associated with longhorn beetle vectors (Cerambycidae): *B. fraudulentus* associated with *Cerambys cerdo acuminatus* Motsch (Kakulia *et al*., 1980); *B. georgicus* associated with *Rhopalopus macropus* Pens (Devdariani *et al*., 1980); *B. populneus* associated with *Saperda populnea* L. (Kakulia *et al*., 1980); *B. scalari* associated with *Saperda scalaris* L. (Kakulia, 1989); *B. sutoricus* associated with *Monochamus sutor* L. (Devdariani, 1974) and *B. tbilisyensis* associated with *Saperda carcharias* L. (Kakulia, 1989).

Due to the association of *B. mucronatus* with Europe- and

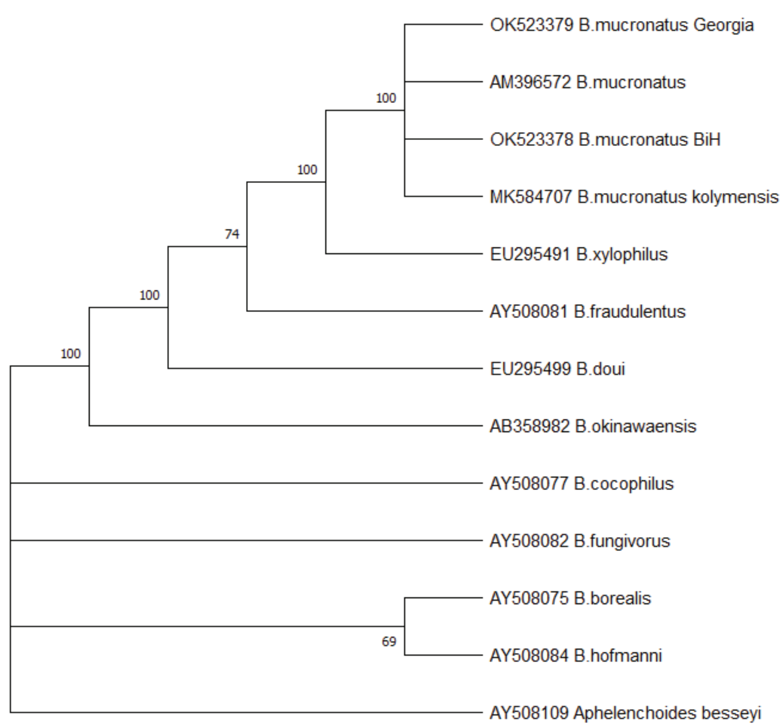


Fig. 2. Phylogenetic tree resulting from alignment of 28S rRNA gene sequences.
The tree was inferred by Maximum Likelihood method under the GTR + G + I model. Bootstrap values were obtained from 100 replicate. Values higher than 50% are presented. OK523378 and OK523379 are the original sequences.

Table 3. List of *Bursaphelenchus* species described from Georgia.

| No. | status | Species | Insect vector | Associated plant/note | Group* | Reference |
|---|---|---|---|---|---|---|
| *1* | *valid* | *B. chitwoodi* Rühm, 1956 | *Hylastes ater* (Fabricius) (Coleoptera: Scolytidae) | *Pinus* sp. (Pinales: Pinaceae), exact pine species is not mentioned in the text<br>*Pinus sosnowskyi* Nakai, *Pinus pityosa* Steven (Pinales: Pinaceae) | Hofmanni group | Kakulia & Shalibashvili, 1976a<br>Kakulia, Shalibashvili, Gorgadze, 1983 |
| *2* | *valid* | *B. crenati* Rühm, 1956 | *Hylesinus crenatus* (Fabricius) (Coleoptera: Scolytidae) | *Fraxinus excelsior* L. (Oleales: Oleaceae) | Xylophilus group | Kurashvili *et al.*, 1980 |
| *3* | *valid* | *B. eggersi* Rühm, 1956 | *Hylurgops palliatus* (Gyllenhal) (Coleoptera: Scolytidae) | *Picea orientalis* (L.) (Pinales:Pinaceae); *Abies* sp., *Larix* sp.; *Picea orientalis* (L.), *Pinus cedrus* L. (Pinales: Pinaceae) | Eggersi group | Kakulia & Maglakelidze, 1973<br>Kurashvili *et al.*, 1980 |
| *4* | *valid* | *B. eidmanni* Rühm, 1956 | *Ips typographus* L. (Coleoptera: Scolytidae)<br>Georgia *Ips typographus* L. (Coleoptera: Scolytidae) | *Abies* sp., *Larix* sp., *Picea orientalis* (L.), *Pinus cedrus* L., *P. sosnowskyi* Nakai (Pinales: Pinaceae) | Eidemani group | Kakulia, 1971<br>Kakulia & Devdariani, 1975**<br>Kurashvili *et al.*, 1980 |
| *5* | *valid* | *B. eremus* Rühm, 1956 | *Scolytus intricatus* (Ratzeburg) (Coleoptera: Scolytidae) | *Populus gracilis* Grossh., *Salix* sp. (Salicales: Salicaceae), *Castanea vulgaris* Hance, *Quercus iberica* Steven ex Bieb., *Q. pedunculata* Ehrh., *Q. sessiliflora* Salisb. (Fagales: Fagaceae), *Ulmus foliacea* Gilib. (Urticales: Ulmaceae) | Eremus group | Kurashvili *et al.*, 1980 |
| *6* | *valid* | *B. ernoporus* Devdariani, 1975 | *Ernoporicus fagi (Fabricius)* (Coleoptera: Scolitidae) | *Fagus* sp. (Fagales: Fagaceae), *Carpinus* sp. (Fagales: Betulaceae) | Insufficient description: not considered for Grouping | Kakulia & Devdariani, 1975**<br>Mikaia *et al.*, 2010<br>Hunt, 1993 (*nomen nudum*)<br>Ryss *et al.*, 2005 (*nomen nudum*) |
| *7* | *valid* | *B. erosus* Kurashvili et al., 1980 | *Orthotomicus erosus* (Woll.) (Coleoptera: Scolytidae) | *Abies* sp., *Picea orientalis* (L.), *Pinus sosnowskyi* Nakai (Pinales: Pinaceae)<br>*Pinus pityusa* Steven | Eidemani group | Kurashvili *et al.*, 1980<br>Kakulia, Shalibashvili, Gorgadze, 1983 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *8* | *valid* | *B. eucarpus* Rühm, 1956 | *Scolytus mali* (Bechstein & Scharfenberg) (Coleoptera: Scolytidae) | *Malus domestica* Borkh., *Prunus* sp., *Sorbus* sp. (Rosales: Rosaceae), Ulmaceae gen. sp. (Urticales) | Sexdentati group | Kakulia & Devdariani, 1975**<br>Kurashvili *et al.*, 1980 |
| *9* | *valid* | *B. fraudulentus* Rühm, 1956 | *Saperda carcharias* (L.) (Coleoptera: Cerambycidae) | - | Xylophilus group | Kakulia & Devdariani, 1975**<br>Kurashvili *et al.*, 1980 |
| *10* | *valid* | *B. georgicus* Devdariani et al., 1980 | *Rhopalopus macropus* Germar (Coleoptera: Cerambycidae) | *Quercus iberica* Schur. (Fagales: Fagaceae) | Insufficient description: not considered for grouping | Devdariani *et al.*, 1980<br>Kakulia, 1989<br>Hunt, 1993 (*nomen nudum*)<br>Ryss *et al.*, 2005 |
| *11* | *valid* | *B. idius* Rühm, 1956 | *Pityogenes chalcographus* L. (Coleoptera: Scolytidae) | *Pinus* sp. (Pinales: Pinaceae), *Carpinus caucasica* Grossh. (Betulales: Betulaceae), *Juglans* sp. (Juglandales: Juglandaceae), *Populus tremula* L. (Salicales: Salicaceae), *Quercus iberica* Steven ex Bieb. (Fagales: Fagaceae) | Six-incisure ungrouped species | Kakulia & Devdariani, 1975**<br>Kurashvili *et al.*, 1980 |
| *12* | *valid* | *B. incurvus* Rühm, 1956 | *Dendroctonus micans* (Kugel.) (Coleoptera: Scolytidae) | *Abies* sp., *Picea orientalis* (L.), *Pinus sylvestris* L. (Pinales: Pinaceae) | Sexdentati group | Kurashvili *et al.*, 1980 |
| *13* | *valid* | *B. mucronatus* Mamiya & Enda, 1979 | *Monochamus galloprovincialis* (Coleoptera: Cerambycidae) | *Pinus sylvestris* (L.) (Pinales: Pinaceae) | Xylophilus group | This article |
| *14* | *valid* | *B. nuesslini* Rühm, 1956 | *Pityokeines curvidens* (Germar) (Coleoptera: Scolytidae) | Not mentioned in the text. | Piniperdae group | Kakulia & Shalibashvili, 1976b |
| *15* | *valid* | *B. piniperdae* Fuchs, 1937 | *Blastophagus* piniperda (Coleoptera: Scolytidae) | *Pinus sosnowskyi* Nakai, *Pinus pityosa* Steven (Pinales: Pinaceae) | Piniperdae group | Kakulia, Devdariani, 1965<br>Kakulia, 1967<br>Kurashvili *et al.*, 1980<br>Kakulia, Shalibashvili, Gorgadze, 1983 |
| *16* | *valid* | *B. populneus* Devdariani, 1937 | *Saperda populnea* L. (Coleoptera: Cerambycidae) | *Populus* sp. | Insufficient description: not considered for grouping | Devdariani, 1973*<br>Mikaia *et al.*, 2010<br>Hunt, 1993 (*nomen nudum*)<br>Ryss *et al.*, 2005 (*nomen nudum*) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *17* | *valid* | *B. ratzerburgii* Rühm, 1956 | *Scolytus ratzeburgii* Janson (Coleoptera: Scolytidae) | *Betula* sp. (Betulales: Betulaceae) | Hofmanni group | Kurashvili *et al.*, 1980 |
| *18* | *valid* | *B. scalari* Kakulia, 1989 | *Saperda scalaris* L. (Coleoptera: Cerambycidae) | *Alnus glutinosa* L. | Insufficient description: not considered for grouping | Kakulia, 1989 |
| *19* | *valid* | *B. sexdentati* Rühm, 1960 | *Ips sexdentatus* (Boerner) (Coleoptera: Scolytidae) | *Picea orientalis* (L.), *Pinus sosnowskyi* Nakai, Pinus pityosa Steven (Pinales: Pinaceae) | Sexdentati gropu | Kakulia & Devdariani, 1975**<br>Kurashvili *et al.*, 1980<br>Kakulia, Shalibashvili, Gorgadze, 1983 |
| *20* | *valid* | *B. sutoricus* Devdariani, 1974 | *Monochamus sutor* (L.) (Coleoptera: Cerambycidae) | *Pinus* sp. (Pinales: Pinaceae) | Sexdentati group | Devdariani, 1974 |
| *21* | *valid* | *B. tbilisiensis* Kakulia, 1989 | *Saperda carcharias* L. (Coleoptera: Cerambycidae) | Not mentioned in the text. | Insufficient description: not considered for grouping | Kakulia, 1989<br>Hunt, 1993 (*nomen nudum*)<br>Ryss *et al.*, 2005 (*nomen nudum*) |
| *22* | *valid* | *B. wekuae* Kakulia et al., 1978 | *Trypophloeus* sp. (erroneously named as *Trypodendron sygnatum*) (Coleoptera: Scolytidae) | *Carpinus caucasica* Grossh. (Betulales: Betulaceae), *Fagus orientalis* Lipsky. (Fagales: Fagaceae) | Insufficient description: not considered for Grouping | Kakulia *et al.*, 1978**<br>Kurashvili *et al.*, 1980<br>Kakulia, 1989<br>Hunt, 1993 (*nomen nudum*)<br>Ryss *et al.*, 2005 |
| *23* | *valid* | *B. xerocarterus* Rühm, 1956 | *Scolytus scolytus* (Fabricius) (Coleoptera: Scolytidae) *Scolytus scolytus* (Fabricius), *S. multistriatus* (Marsh.) (Coleoptera: Scolytidae) | *Ulmus foliacea* Gilib., *Zelkova* sp. (Urticales: Ulmaceae), *Carpinus caucasica* Grossh. (Betulales: Betulaceae), *Juglans* sp. (Juglandales: Juglandaceae), *Populus nigra* L. (Salicales: Salicaceae) | Hofmanni/Abietinus group | Kakulia & Devdariani, 1967<br>Kakulia & Devdariani, 1975**<br>Kurashvili *et al.*, 1980<br>Ryss *et al.*, 2005 |
| *24* | *species inquirendae vel incertae sedis* | *B. conurus* (Steiner, 1932) | - | - | - | Kakulia, 1989<br>Hunt, 1993<br>Ryss *et al.*, 2005 |
| *25* | *species inquirendae vel incertae sedis* | *B. conjuctus* | - | - | - | Kakulia, 1989<br>Hunt, 1993<br>Ryss *et al.*, 2005 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *26* | *species inquirendae vel incertae sedis* | *B. rüehmi* | - | - | - | Kakulia, 1989<br>Hunt, 1993<br>Ryss *et al.*, 2005 |
| *27* | *nomen nudum* | *B. hylesini* | - | - | - | Devdariani, 1975**<br>Kurashvili *et al.*, 1980<br>This article (Nomina Nuda) |
| *28* | Transfer to other genus | *B. lignophilus* (Körner, 1954) Meyl, 1961 | - | *Laimaphelenchus lignophilus* (Körner, 1954) Goodey, 1960 | - | Kakulia, 1989<br>Ryss *et al.*, 2005 |
| *29* | Transfer to other genus | *B. teratospicularis* Kakulia & Devdariani, 1965 | - | *Devibursaphelenchus teratospicularis* | - | Kakulia & Devdariani, 1965<br>Kakulia, 1989<br>Ryss *et al.*, 2005<br>Braasch, 2009 |
| *30* | Transfer to other genus | *B. typographi* Kakulia, 1967 | - | *Devibursaphelenchus typographi* | - | Kakulia, 1967<br>Kakulia, 1989<br>Ryss *et al.*, 2005<br>Braasch, 2009 |

Legend: * = grouping is based on Braasch *et al.* (2009); ** original publication not available to the authors

Asia-widely distributed *Monochamus* species (Evans *et al*., 1996; Akbulut *et al*., 2017; Löbl & Smetana, 2010), the nematode occurrence should copy the habitat of its insect vectors. Up to now *B. mucronatus* has been already reported from 22 European and Mediterranean countries: Austria and Bulgaria (Braasch, 2001), Finland (Tomminen, 1989), Croatia (Đođ *et al*., 2016), France (Baujard *et al*., 1979), Germany (Braasch *et al*., 1999), Greece (Skarmoutsos & Skarmoutsos, 1992), Hungary (Tóth & Elekes, 2013), Italy (Marinari-Palmisano *et al*., 1992), Israel (Gu *et al*., 2006), Lithuania (Stanelis, 2005), Norway (Magnusson *et al*., 2004), Poland (Brzeski & Baujard, 1997), Portugal (Penas *et al*., 2004), Romania (Mitrea-Calin *et al*., 2020), Russia (Braasch, 1991), Serbia (Bašic *et al*., 2014), Slovenia (Urek & Širca, 2005), Spain (Escuer *et al*., 2004), Sweden (Schroeder & Magnusson, 1992), Turkey (Akbulut *et al*., 2006) and Ukraine (Kozlovsky, 2013). *Bursaphelenchus mucronatus* is mainly associated with *M. galloprovincialis* in Europe (Tomminen *et al*., 1989; Vincent *et al*., 2008, Penas *et al*., 2004; Braasch *et al*., 1999). Association with *M. sutor* is rarer in Europe as reported only from Romania (Mitrea-Calin *et al*., 2020), Spain (Abelleira *et al*., 2015) and Sweden (Schroeder & Magnusson, 1992). Anyway, it is probable that *B. mucronatus* might be vectored by other *Monochamus* species present in the region as well (Togashi *et al*., 2008). Besides *M. galloprovincialis*, also *M. saltuarius*, *M. sartor* and *M. sutor* occur in Bosnia and Herzegovina. Although only *M. galloprovincialis* and *M. sutor* are reported from Georgia, it is likely that some other species known from neighbouring countries (i.e. *M. saltuarius*, *M. sartor* and *M. urussovii*) might occur in Georgia (Löbl & Smetana, 2010). As authors know the association of *B. mucronatus* with other *Monochamus* species (*M. saltuarius*, *M. sartor* and *M. urussovi*) has not been reported in Europe.

*B. mucronatus* as a native European species (Ryss *et al*., 2005; Pereira *et al*., 2013) is not pathogenic to coniferous trees and is not considered as a pest in European conditions. However, as data from Portugal and Spain have shown (Penas *et. al*., 2004; Vincent *et al*., 2008) it can be very quickly displaced by its harmful relative *B. xylophilus* after first occurrence in new area. And reciprocally, a certain risk is involved in planting of non-European coniferous tree species (Pötzelsberger *et al*., 2020). Therefore, the permanent and detailed detection survey including laboratory analysis of wood and insect samples and survey of wood packaging material is highly recommended for monitoring of distribution of insect vectors and relative species of *Bursaphelenchus* as an integral part of the discrimination of high-risk areas for the potential establishment of *B. xylophilus*, early findings of new outbreaks and creation of an emergency plan.

**Conflict of Interest, Ethical Approval and/or Informed Consent**

Authors state no conflict of interest and this article does not contain any studies with human participants or animals.

**Acknowledgements**

This research was supported by the Czech Development Agency (CZDA) via the projects "Food Safety Improvement in Bosnia and Herzegovina" and "Food Safety Improvement in Georgia". The authors kindly thank to Petr Vaculík for foreign project management and support, to the Administration of the Tbilisi National Park for the insect trapping permission and to Oleg Gorgadze for kind providing of the original book of Kakulia (1989).

**References**

Abelleira, A., Ibarra, N., Aguín, O, Mosquera, P., Abelleira-Sanmartín, A., Sorolla, A., Ares, A., Mansilla, P. (2014): First report of *Bursaphelenchus mucronatus kolymensis* (Nematoda: Aphelenchoididae) on Monochamus sutor (Coleoptera: Cerambycidae) in Spain. *For Pathol*, 45(1): 82 – 85. DOI: 10.1111/efp.12139

Akbulut, S., Togashi, K. Linit, M.J. (2017): Cerambycids as plant disease vectors with special reference to Pine Wilt. In: Wang, Q. (Ed) *Cerambycidae of the World: Biology and Pest Management*. CRC Press, Florida, USA, pp. 223 – 266.

Bačić, J., Stare, B.G., Urek, G., Širca, S. (2014): First Report of *Bursaphelenchus mucronatus kolymensis* Associated with Pinus sylvestris in Serbia. *Plant Dis*, 98(12): 1745 – 1745. DOI: 10.1094/PDIS-06-14-0594-PDN

Baujard, P., Boulbria, A., Ham, R., Laumond, C., Scotta La Masse`se, C. (1979): Premieres Donnees Sur La Nematofaune Associee Aux Deperissements Du Pin Maritime Dans L'ouest De La France [First Data on Nematofauna Associated with Maritime Pine Dieback in Western France]. *Ann. Sci. For.*, 36: 331 – 339 (In French)

Braasch, H. (1991): First detection of *Bursaphelenchus mucronatus* Mamiya & Enda, 1979 in Germany and its presence in wood imported from USSR, with further details on the description of the species. *Arch Phytopathol Pfl*, 27: 209 – 218

Braasch, H., Metge, K., Burgermeister, W. (1999): *Bursaphelenchus*-Arten (Nematoda, Parasitaphelenchidae) in Nadelgehölzen in Deutschland und ihre ITS-RFLP-Muster. *Nachr. bl. Dtsch. Pflanzenschutzd.*, 51: 312 – 320

Braasch, H. (2001): *Bursaphelenchus* species in conifers in Europe: distribution and morphological relationships. *Bull OEPP,* 31: 127 – 142. DOI: 10.1111/j.1365-2338.2001.tb00982.x

Braasch, H., Burgermeister, W. Gu, J. (2009): Revised intrageneric grouping of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchoididae). *J Nematode Morphol System*, 12(1): 65 – 88

Braasch, H. (2009): Re-establishment of *Devibursaphelenchus* Kakuliya, 1967 (Nematoda, Aphelenchoididae) and proposal for a new combination of several Bursaphelenchus species. *J Nematode Morphol System*, 12(1): 1 – 5

Brzeski, M.W., Baujard, P. (1997): Morphology and morphometrics of *Bursaphelenchus* (Nematoda: Aphelenchoididae) species from pine wood of Poland. *Ann Zool*, 47: 305 – 319

Burgermeister, W., Braasch, H., Metge, K., Gu, J., Schröder, T., Woldt, E. (2009): ITS-RFLP analysis, an efficient tool for differentiation of *Bursaphelenchus* species. *Nematology*, 11(5): 649 – 668. DOI: 10.1163/156854108/399182

Calin, M., Cean, M., Costache, C., Rusinque, L., Paraschiv, M., Camacho, M., Vieira, P., Cristea, S., Nóbrega, F., Inacio, M. L. (2020): First detection of *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoididae) on *Monochamus sutor* (Coleoptera: Cerambycidae) in Romania. *For Pathol*, 50: DOI: 10.1111/efp.12578

Čermák, V., Vieira, P., Gaar, V., Čudejková, M., Foit, J., Široká, K., Mota, M. (2012): *Bursaphelenchus pinophilus* Brzeski & Baujard, 1997 (Nematoda: Parasitaphelenchinae) associated with nematangia on *Pityogenes bidentatus* (Herbst, 1783) (Coleoptera: Scolytinae), from the Czech Republic. *Nematology*, 14(3): 385 – 387. DOI: 10.1163/156854111X614502

De Grisse, A.T. (1969): Redescription ou modification de quelques techniques utilisees dans l'etude des nematodes phytoparasitaires [Redescription or modification of some techniques used in the study of plant parasitic nematodes]. *Meded. Rijksfakulteit Landbowwetenschappen Gent*, 34: 351 – 369 (In French)

De Ley, P., Félix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W., Thomas, K.W. (1999): Molecular and morphological characterization of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology*, 1(6): 591 – 612. DOI: 10.1163/156854199508559

Devdariani, T.G. (1973): Two new nematode species from beech bark beetle (*Taphrophynchus bicolor* Herbst.) from the East Georgia. *Soobshch Akad Nauk Gruz SSR*, 50(1): 213 – 216 (In Georgian)

Devdariani, T.G. (1974): A new nematode species from *Monochamus sutor*. *Soobshch Akad Nauk Gruz SSR*, 76: 709 – 712 (In Russian)

Devdariani, T.G., Kakulia, G.A., Khavatashili, D.D. (1980): New species of nematode of small maple Capricorn beetle (*Rhopalopus macropus*). *Soobshch Akad Nauk Gruz SSR*, 98: 457 – 459 (In Georgian)

Đođ, N., Lukić, I., Çota, E., Pernek, M. (2016): Wood nematode species spectrum in the Mediterranean pine forests of Croatia. *Period Biol*, 117: 505 – 512. DOI: 10.18054/pb.2015117.4.3442

d'Errico, G., Carletti, B.E., Schröder, T., Mota, M., Vieira, P., Roversi, P.F. (2015): An update on the occurrence of nematodes belonging to the genus *Bursaphelenchus* in the Mediterranean area. *Forestry*, 88(5): 509 – 520. DOI: 10.1093/forestry/cpv028

Escuer, M., Arias, M., Bello, A. (2004): Occurrence of the genus *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchida) in Spanish conifer forests. *Nematology*, 6(1): 155 – 156. DOI: 10.1163/156854104323073035

Evans, H.F., McNamara, D.G., Braasch, H., Chadoeuf, J. Magnusson, C. (1996): Pest Risk Analysis (PRA) for the territories of the European Union (as PRA area) on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. *Bull OEPP*, 26: 199 – 249.

Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783 – 791. DOI: 10.1111/j.1558-5646.1985.tb00420.x

Ferris V.R, Ferris J.M., Faghihi, J. (1993): Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. *Fundam Appl Nematol*, 16: 177 – 184

Gu, J., Braasch, H., Burgermeister, W., Zhang, J. (2006): Records of *Bursaphelenchus* spp. intercepted in imported packaging wood at Ningbo, China. *For Pathol*, 36: 323 – 333. DOI: 10.1111/j.1439-0329.2006.00462.x

Hunt, D.J. (1993): *Aphelenchida, Longidoridae and Trichodoridae, Their systematics and bionomics*. CABI, London, UK: 356 pp

IPPC (International Plant Protection Convention) (2016): *ISPM 27. Annex 10. Bursaphelenchus xylophilus* (2016). Rome, IPPC, FAO: 36 pp.

Kakulia, G.A., Devdariani, T.G. (1965): A new species of nematode *Bursaphelenchus teratospicularis* Kakulia & Devdariani, sp. nov. (Nematoda: Aphelenchoidea). *Soobshch Akad Nauk Gruz SSR*, 38: 187 – 191 (In Georgian)

Kakulia, G. A. (1967): Nematofauna of Pine shoot beetles (*Blastophagus piniperda* & *Blastophagus minor*) in Borjomi-Bakuriani coniferous forests. Helminthofauna of animals and plants in Georgia: *Collection*, *Institute of Zoology*, *Tbilisi*: 55 – 54 (In Georgian)

Kakulia, G.A. & Devdariani, T.G. (1967). Nematode fauna of *Scolytus scolytus* F. in East Georgia. *Soobshch Akad Nauk Gruz SSR*, 46: 469 – 474 (In Georgian)

Kakulia, G.A. (1971): Nematode fauna of *Ips typographus* in the Georgian SSR. *Parazit. Sbor.* 2, *Tbilisi*: 53 – 56 (In Russian)

Kakulia, G.A., Maglakelidze, L. (1973): Nematode fauna of *Hylurgops palliatus* in the Georgian SSR. *Parazit. Sbor.* 3, *Tbilisi*: 76 – 78 (In Russian)

Kakulia, G.A., Shalibashvili K. (1976a): The nematode fauna of *Hylaster ater* in the pine forests of the Pitsundskii and Ritsinskii reserves. *Zapovedniki Gruzii*, *Sbornik Trudov*, 4: 259 – 262. (In Russian)

Kakulia, G.A., Shalibashvili K. (1976b): The nematode fauna of *Pityokeines curvidens* Germ. in the conifer forests of Abkhaziya. *Zapovedniki Gruzii, Sbornik Trudov*, 4: 317 – 320 (In Russian)

Kakulia, G.A., Devdariani, T.G., Maglakelidze, L.M. (1980): Nematodes of Cerambycidae, parasites of trees in Eastern Georgia. *Konferentsiya Ukrainskogo Parazitlogicheskogo Obshchestva. Tezisy dokladov. Chast* II, 109 – 110. (In Georgian)

Kakulia, G.A., Shalibashvili, G., Goargadze, O. (1983): Nematodes of bark beetles of Pitsunda Nature Reserve. *Nature Protection of Georgia 9*, *Academy of Sciences of the Georgian SSR*: 155 – 163. (In Georgian)

Kakulia, G.A. (1989): *Parasites of insects and biological methods of their control*. *Academii Nauk Gruzinskoi SSR*, Tbilisi, 212 pp. (In Russian)

Kanzaki, N., Maehara, N., Aikawa, T., Matsumoto, K. (2012): *Bursaphelenchus firmae* n.sp. (Nematoda: Aphelenchoididae), isolated from *Monochamus grandis* Waterhouse that emerged from dead firs, Abies firma Sieb. Et Zucc. *Nematology*, 14(4): 395 – 404. DOI: 10.1163/156854111X602974

Kozlovsky, M. (2013): *Bursaphelenchus mucronatus* as a cause of dying a secondary fir forests in Ukrainian Carpathians. In: Schröder, T. (Ed) *Pine Wilt Disease Conference 2013*, pp. 100, Braunschweig, ISSN: 1866-590X.

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol*, 35: 1547 – 1549. DOI: 10.1093/molbev/msy096

Kurashvili, B.E., Kakulia, G.A.,Devdariani, T.G. (1980): *Parasitic nematodes of the bark-beetles in Georgia*. Tbilisi, Georgia, Metsniereba, 172 pp. (In Russian)

Löbl, I., Smetana, A. (eds.) (2010): *Cataloque of Palearctic Coleoptera*, Vol. 6, Chrysomeloidea. Stenstrup, Apollo Books, 924 pp.

Magnusson, C., Overgaard, H., Nyeggen, H., Thunes, K., Salinas, S.H., Hammeraas, B. (2004): Survey of the pine wood nematode (PWN) B*ursaphelenchus xylophilus* in Norway 2002. *Grønn kunnskap*, 8: 1 – 13

Mamiya, Y., Enda, N. (1979): *Bursaphelenchus mucronatus* n. sp. (Nematoda: Aphelenchoididae) from pine wood and its biology and pathogenicity to pine trees. *Nematologica*, 25(3): 353 – 361

Marinari Palmisano, A., Ambrogioni, L., Caroppo, S. (1992): *Bursaphelenchus mucronatus* on *Pinus pinaster* in Italy. *Redia*, 75: 517 – 527

Mikaia, N., Devdariani, T., Gninenko, Y. (2010): Representatives of Nematodes of the Genus *Bursaphelenchus* (Aphelenchida, Parasitophelenchidae) in Georgia. *Bull. Georgian Natl. Acad. Sci.*, 4(1): 146 – 151

Mitrea-Călin, M., Inácio, M.L., Costache, C., Nóbrega, F., Mota, M., Cristea, S., Braasch, H. (2020): New observation on occurrence and distribution of *Bursaphelenchus* spp. (Nematoda: Aphelenchoididae) in conifers in Romania. *For Pathol*, 50: e12629. DOI: 10.1111/efp.12629

Mota, M.M., Braasch, H., Bravo, M.A., Penas, A.C., Burgermeister, W., Metge, K., Sousa, E. (1999): First report of *Bursaphelenchus xylophilus* in Portugal and in Europe, *Nematology*, 1(7): 727 – 734. DOI: 10.1163/156854199508757

Nei, M.,Kumar, S. (2000): *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

Penas, A.C., Correia, P., Bravo, M.A., Mota, M., Tenreiro, R. (2004): Species of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchidae) associated with maritime pine in Portugal. *Nematology*, 6(3): 437 – 453. DOI: 10.1163/1568541042360573

Pereira, F., Moreira, C., Fonseca ,L., van Asch, B., Mota, M., Abrantes, I., Amorim, A. (2013): New insights into the phylogeny and worldwide dispersion of two closely related nematode species, *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*. *PLoS One*, 8: e56288. DOI: 10.1371/journal.pone.0056288

Pötzelsberger, E., Spiecker, H., Neophytou, C., Mohren, F., Gazda, A., Hasenauer, H. (2020): Growing non-native trees in European forests brings benefits and opportunities but also has its risks and limits. *Curr For Rep*, 6: 339 – 353. DOI: DOI: 10.1007/s40725-020-00129-0

Ryss, A., Vieira, P., Mota, M., Kulinich, O. (2005): A synopsis of the genus *Bursaphelenchus* Fuchs (Nematoda: Parasitaphelenchidae) with a key to species. *Nematology*, 7(3): 393 – 458. DOI: 10.1163/156854105774355581

Schroeder, L. M., Magnusson, C. (1992): Transmission of *Bursaphelenchus mucronatus* (Nematoda) to branches and bolts of *Pinus sylvestris* and *Picea abies* by the cerambycid beetle *Monochamus sutor*. *Scand J For Res*, 7: 107 – 112

Skarmoutsos, G, Skarmoutsou, H. (1999): First Record of *Bursaphelenchus* Nematodes from Pine Forests in Greece. *Plant Dis*, 83(9): 879. DOI: 10.1094/PDIS.1999.83.9.879D

Stanelis, A. (2005): The Occurence of *Bursaphelenchus mucronatus* Mamya & Enda, 1979 in Lithuania. *Acta Zool Litu,* 15: 1, 62 – 63, DOI: 10.1080/13921657.2005.10512611

Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22: 4673 – 4680

Togashi, K., Taga, Y., Iguchi, K., Aikawa, T. (2008): *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoididae) vectored by *Monochamus urussovi* (Coleoptera: Cerambycidae) in Hokkaido, Japan. *J For Res*, 13(2): 127 – 131. DOI: 10.1007/s10310-007-0057-1

Tomalak, M., Worrall, J., Filipiak, A. (2013): *Bursaphelenchus masseyi* sp.n. (Nematoda: Parasitaphlenchinae) – 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(8): 907 – 921. DOI: 10.1163/15685411-00002729

Tomminen, J., Nuorteva, M., Pulkkinen, M., Väkevä, J. (1989): Occurrence of the nematode *Bursaphelenchus mucronatus* Mamiya & Enda 1979 (Nematoda: Aphelenchoididae) in Finland. *Silva Fenn (Hels)*, 23(4): 271 – 277. DOI: 10.14214/sf.a15547

Tóth, Á and Elekes, M. (2013): Report on the survey for *Bursaphelenchus xylophilus* and the occurrence of other Bursaphelenchus species in Hungarian coniferous forests. *Bull OEPP*, 14(1): 152 – 154. DOI: 10.1111/epp.12012

Urek G., Širca S. (2005): First Report of the East-Asian Type of *Bursaphelenchus mucronatus* in *Pinus sylvestris* in Slovenia. *Plant Dis*, 89(9): 1015. DOI: 10.1094/PD-89-1015B

Vincent, B., Altemayer, V., Roux-Morabito, G., Naves, P., Sousa, E, Lieutier, F. (2008): Competitive interaction between *Bursaphelenchus xylophilus* and the closely related species *Bursaphelenchus mucronatus*. *Nematology*, 10(2): 219 – 230. DOI: 10.1163/156854108783476403

Vrain T.C. (1993): Restriction fragment length polymorphism separates species of the *Xiphinema americanum* group. *J Nematol*, 25: 361 – 364