

A taxonomic appraisal of the original description, morphology and status of *Bursaphelenchus kolymensis* Korentchenko, 1980 (Aphelenchida: Aphelenchoididae)

Christer Magnusson^{1*} and Oleg A. Kulinich^{2**}

*Swedish University of Agricultural Sciences, Dep. of Plant and Forest Protection, Box 7044, S-75007, Uppsala, Sweden,

¹Present address: The Norwegian Crop Research Institute, Plant Protection Centre, Fellesbygget, N-1432 As, Norway, e-mail: christer.magnusson@planteforsk.nln.no

**Institute of Parasitology of Russian Academy of Sciences, Leninskii Prospect 33, 117071, Moscow, Russia.

²Present address: All-Russian Research Institute of Plant Quarantine, Pogranichnaya street 32, Bykovo, Moscow region, 140150, Russia.

Accepted for publication 1 June 1996

Summary. The taxonomic status of *Bursaphelenchus kolymensis*, a member of the pinewood nematode species complex (PWNSC) is unclear. Examination of the type specimens of *B. kolymensis* Korentchenko, 1980 revealed that the male has a broad beak- or spade-shaped bursa in lateral view and cucullus on the spicule. The female has a sub-cylindrical tail with a slender mucro. Comparison with related PWNSC species indicates that *B. kolymensis* is morphologically similar to *B. fraudulentus* Rühm, 1956 and *B. mucronatus* Mamiya & Enda, 1979, but distinct from *B. xylophilus* (Steiner & Buhner, 1934) Nickle, 1970. *Bursaphelenchus kolymensis* differs from *B. fraudulentus* by possessing a more slender mucro, a broad beak- or spade-shaped bursa and by having coniferous hosts. It is most similar to *B. mucronatus* populations from Russia and France, but differs from Japanese populations by its sub-cylindrical female tail and slender mucro, and the shape of the bursa. It is concluded that mating and genetic studies are required to resolve the specific status of *B. kolymensis*.

Key words: *Bursaphelenchus kolymensis*, morphology, *B. xylophilus*, *B. mucronatus*, *B. fraudulentus*.

The nematodes *Bursaphelenchus xylophilus* (Steiner & Buhner, 1934) Nickle 1970, *B. mucronatus* Mamiya & Enda, 1979, *B. kolymensis* Korentchenko, 1980, and *B. fraudulentus* Rühm, 1956 represent a group of morphologically similar species within the genus *Bursaphelenchus* Fuchs, 1937, with overlap in several morphological characters (Nickle, 1970). *Bursaphelenchus fraudulentus* is associated with deciduous tree hosts such as beech, birch, cherry, alder, oak and poplar (Rühm, 1956; Schauer-Blume & Sturhan, 1989) whereas the other three species are associated with conifers. Populations of *B. xylophilus* and *B. mucronatus* have been referred to as the "pinewood nematode species complex" (PWNSC) due to morphological variability between populations (Rutherford *et al.*, 1990; Webster *et al.*, 1990) and we consider *B. kolymensis* as a further member of the PWNSC.

Bursaphelenchus kolymensis was described by Korentchenko (1980) from specimens recovered

from the tracheae and the hemocoel of the Cerambycidae beetle *Monochamus sutor*, collected in the Upper Kolyma of the Magadan District, Russia. Juvenile *B. kolymensis* were present in 20% of dissected beetles (10 to 600 juveniles per beetle) and all these specimens were dauer stage juveniles (Korentchenko, 1980); nematodes were not found in the galleries of beetles. Adult nematodes used for describing the species were obtained by culturing dauer stage juveniles on fungus cultures (Korentchenko, 1980).

The taxonomic status of *B. kolymensis* is considered to be uncertain, therefore, we have examined the type material of this species to clarify the species status of *B. kolymensis* and to provide a comparison with morphologically similar species.

MATERIAL AND METHODS

For this study the original description of *B. kolymensis* was examined together with the holotype

and allotype specimens, on slides N 513 and N 514, respectively and paratype specimens (males, females and juveniles) kept on slides in the nematode collection of the Institute of Parasitology, Russian Academy of Sciences. Also, the morphology of *B. kolymensis* was compared with that of specimens obtained from populations of *B. fraudulentus* (B.f), *B. xylophilus* from USA (BxA) and Japan (BxJ, BxS-10), and *B. mucronatus* from France (BmF), Japan (BmJ, BmJ-M) and Russia (BmSU, BmR-921, BmR-931).

Populations of *B. xylophilus* and *B. mucronatus* were cultured in Scots pine (*Pinus sylvestris*) wood disks and a population of *B. fraudulentus* was cultured in birch wood disks (*Betula alba*). The wood disks (5-10 cm diameter) were inoculated with nematodes and maintained at 26° C for 1-2 months. Nematodes were extracted from chipped disks on a Baermann funnel, fixed in TAF, processed by the rapid glycerol-ethanol method (Seinhorst, 1959, 1962) and mounted in anhydrous glycerol on slides. Observations were also made of living specimens. Drawings were made using a compound microscope with interference contrast and photomicrographs were obtained using a Wild MPS 55 automatic photography system. Observations on spicule structure were made with processed whole nematode specimens and with spicules excised from fixed and living nematodes. The excised spicules were separated from each other by making a longitudinal division in the dorso-ventral plane. Spicule lengths were measured along the median line of the arc of the spicule.

RESULTS

Comments on the description of *B. kolymensis* Korentchenko, 1980

In her description of *B. kolymensis*, Korentchenko (1980) compares the species with *B. xylophilus* (syn. *B. lignicolus*) and *B. fraudulentus*, each of which have similar shaped spicules (Steiner & Buhner, 1934; Rühm, 1956). In the "Differential Diagnosis", *B. kolymensis* is reported to differ from *B. xylophilus* (syn. *B. lignicolus*) by (1) having the excretory pore at the level of, or anterior to, the medial bulb versus the level of the nerve ring; (2) the bursa being spade-shaped in lateral view versus sharply beak-shaped; and by (3) the female tail being sharply-conical with a prominent slender mucro versus cylindrical with a rounded tip, with or without a small peg or "mucron-like" projection. Also, *B. kolymensis* is reported to differ from *B. fraudulentus* by (1) having a separated spicule head, shorter rostrum and non-concave anterior facet of the capitulum; (2) the post-anal pair of caudal papillae being larger than the pre-anal pair in *B. kolymensis*, but being of equal size in

B. fraudulentus; and by (3) *B. kolymensis* being associated with a beetle of conifers (larch) whereas *B. fraudulentus* is associated with a beetle of deciduous trees.

Observations on the holotype and allotype of *B. kolymensis* Korentchenko, 1980

The holotype specimen of *B. kolymensis* is compressed, and the preservation of both the holotype and the allotype is relatively poor, with the internal organs being obscure.

The shapes of the spicules and bursa of the holotype male, and the tail and the mucro of the allotype female are presented in Fig. 1. The detailed morphology of the holotype spicule (Fig. 1A) can not be determined. The ventral aspect of the lamina is partly obscure and is indicated by a broken line conforming to the original description. The spicule length is 26 µm. The cucullus of the holotype spicule can not be resolved but Korentchenko (1980), whilst not commenting on the cucullus in the description, did provide an illustration of it in her Fig. 3. The bursa is clearly spade-shaped in lateral view (Figs. 1D & 2E), as originally reported by Korentchenko (1980). The excretory pore in the holotype and allotype are not visible.

The allotype females specimen is slightly compressed and the tail appears to be broadly sub-cylindrical with a distinct, slender mucro (Figs. 1I & 2C)

Observations on the paratypes of *B. kolymensis* Korentchenko, 1980

A single slide of paratypes is available which contains six females (Fig. 2A, B & D), one male (Fig. 2F) and three juvenile specimens. The shape of the bursa of the male is broadly beak-shaped in lateral view (Fig. 1E), thus differing slightly from that of the holotype (Fig. 1D). The distal end of the spicule has a distinct cucullus (Fig. 1B) and the length of the spicule is 22 µm. The position of the holotype and the paratype males on the slides only permit a lateral view, which limits observations on the structure of the caudal papillae, and consequently precludes comment being made of their relative size.

All paratype females have sub-cylindrical tails with long slender mucros (Figs. 1J-K & 2D), the mucros being similar in shape to that of the allotype (Figs. 1I & 2C).

Comment on the type material of *B. kolymensis* Korentchenko, 1980

Morphometrics obtained by re-measuring the type specimens differ slightly from those reported by

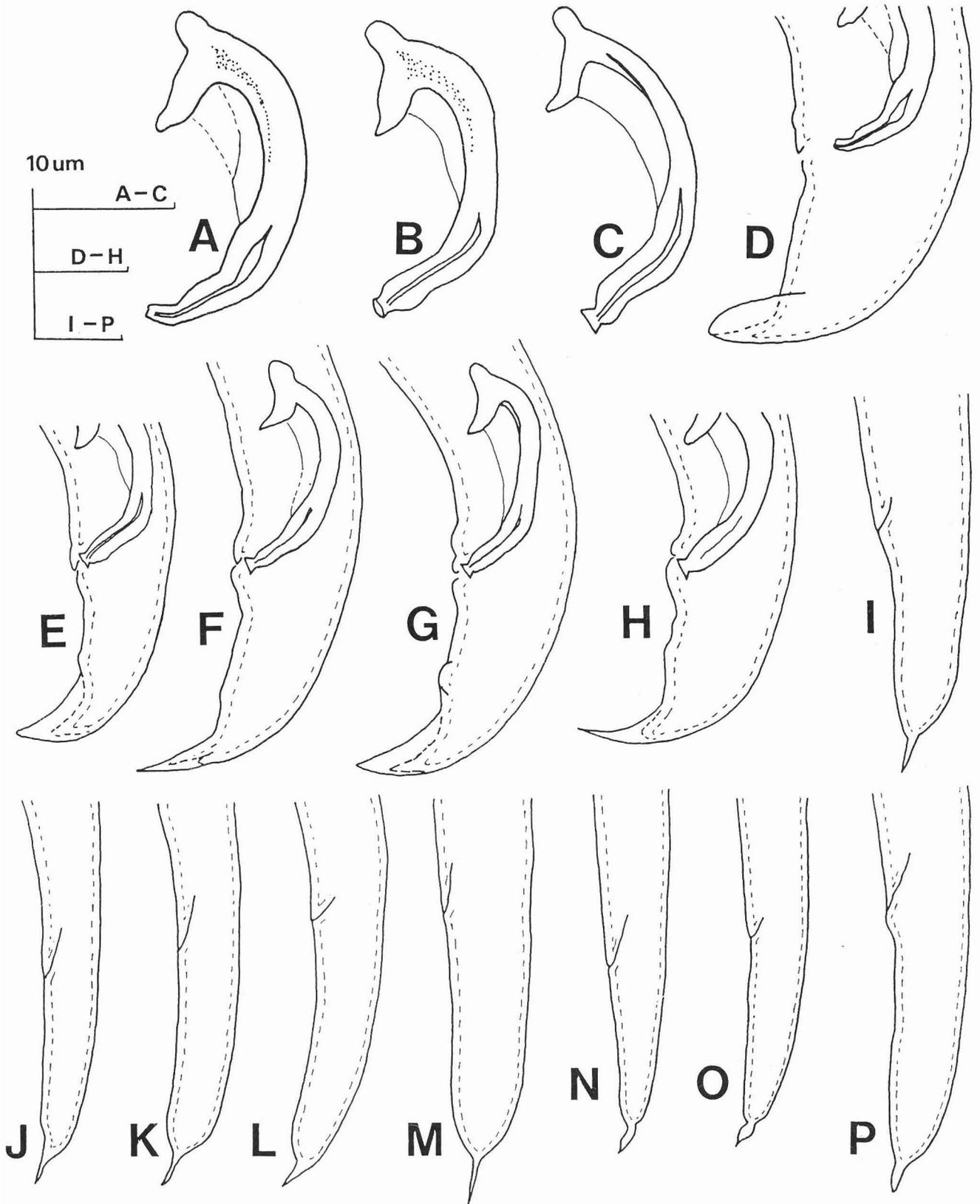


Fig. 1. Morphology of spicules, bursae and female tails in *Bursaphelenchus kolymensis* Korentchenko, 1980 and related species. A & D: *B. kolymensis* holotype; I: *B. kolymensis* allotype; B, E, J & K: *B. kolymensis* paratypes; C & L: *B. fraudulentus*; F: *B. xylophilus*, U.S.A. (BxA); G & M: *B. mucronatus*, Russia (BmSU); H, N & O: *B. mucronatus*, Japan (BmJ); P: *B. mucronatus*, Japan (BmJ-M).

Korentchenko (1980). The poor condition of several specimens (Fig. 2) precludes measurements being made of some characters, and thus some ratios can not be calculated.

Measurements obtained during this study from the type material:

Male holotype: L = 0.537 mm; a = 22.6; spicule = 26 μm .

Female allotype: L = 0.583 mm; a = 29.2; c = 24.9; c' = 2.3; V = 72%; stylet = 13.3 μm ; mucro = 4.4 μm .

Male paratype: L = 0.500 mm; a = 40.0; b = 9.1; spicule = 22 μm .

Female paratypes (n = 5): L = 0.518 (0.438-0.612) mm; a = 43.1 (41.3-44.1); b = 9.3-9.4; c = 22.0 (20.8-23.5); c' = 3.6 (3.1-3.9); V = 76 (72-79)%; stylet = 11.7 (10.0-11.3) μm ; mucro = 3.8 (3.5-4.0) μm .

Differences in the shape of the bursa and spicules between the holotype and the paratype are probably a result of the poor preservation of the specimens. Compression of the holotype specimen has probably contributed to the spade-like shape of the bursa in lateral view. It seems likely that the original drawing (Fig. 3O) of *B. kolymensis* (Korentchenko, 1980) was made from the holotype. Studies of the tail morphology in *B. xylophilus* and related species have revealed a considerable variability in the structure of the bursa of some populations (unpublished data). Differences between holotype and paratype specimens of *B. kolymensis* in the shape of their bursa is probably a result of natural variability or compression of the specimens. The observation of a cucullus on the spicule of the paratype specimen confirms the presence of this structure in *B. kolymensis*.

The female tail shape, which is most clearly seen in the paratypes, is sub-cylindrical rather than sharply conical as stated by Korentchenko (1980), and the tail tip has a long slender mucro.

Korentchenko (1980) distinguished *B. kolymensis* from *B. xylophilus* (syn. *B. lignicolus*) partly on the position of the excretory pore and the tail shapes of males and females. We consider these characters as valid even though the excretory pore could not be observed in the type material. Some populations of *B. mucronatus* and *B. xylophilus* have a different position for the excretory pore (unpublished results).

When distinguishing *B. kolymensis* from *B. fraudulentus*, the characters of the latter species were obtained by Korentchenko (1980) from the original illustration by Rühm (1956) and not from type specimens. Korentchenko (1980) reported a difference in spicule structure and in the size of the male caudal papillae of these species. In our study of *B. fraudulentus* and of several populations of *B. mucro-*

natus the post-anal papillae may appear larger than the pre-anal papillae when specimens are in particular orientations. However, a definitive conclusion about size differences can not be made.

Light microscopy and SEM of male *B. xylophilus* and *B. mucronatus* (Nickle *et al.*, 1981; Giblin-Davis *et al.*, 1993) revealed that the two species each have seven papillae: one ventral preanal papilla, one pair of subventral preanal papillae, and two pairs of adjacent subventral postanal papillae at the anterior end of the bursal flap. Only one pair of preanal papillae and one pair of postanal papillae were reported for *B. kolymensis* by Korentchenko (1980), who used only light microscopy observations when preparing the species description. It is possible that 7 papillae are present but can only be observed with the aid of SEM.

The populations of *B. xylophilus* (Fig. 1F), *B. mucronatus* (Fig. 1G-H) and *B. fraudulentus* (Fig. 1C) used in our study have similar spicule morphology. The spicule of *B. fraudulentus* appears to have a more slender capitulum (Fig. 1C), but the projection of the spicules in the microscope is dependent on specimen orientation, the focal plane and the quality of the specimen. Based on spicule-shape, *B. kolymensis* is most similar to *B. xylophilus*, *B. mucronatus* and *B. fraudulentus*, and thus the morphology of the type material was compared with specimens from different populations of these three species. An emended differential diagnosis for *B. kolymensis* is presented here.

Emended differential diagnosis of *B. kolymensis* Korentchenko, 1980

Bursaphelenchus kolymensis can be distinguished from *B. xylophilus* by the sub-cylindrical shape of the female tail with a long slender mucro versus sub-cylindrical, usually with a broadly rounded terminus, occasionally with a small peg or projection; the excretory pore at level of or anterior to the median bulb versus between the pharyngeal intestinal junction and the nerve ring; a broad beak- or spade-shaped bursa versus sharply beak-shaped, in lateral view (Fig. 1F). From *B. fraudulentus* it can be distinguished by the shape of the mucro, the shape of the bursa and different host-plant, as *B. fraudulentus* is characterized by having a short mucro with a wide base (Fig. 1L), a sharp beak-shaped bursa in lateral view and is associated with deciduous trees. *Bursaphelenchus kolymensis* is similar to *B. mucronatus*, especially specimens from Russia (i.e. BmSU) and France (BmF), both of which have a similar tail-shape and mucro (Fig. 1M), but differs from Japanese specimens by the sub-cylindrical shape of the female tail, the slender mucro and the broadly

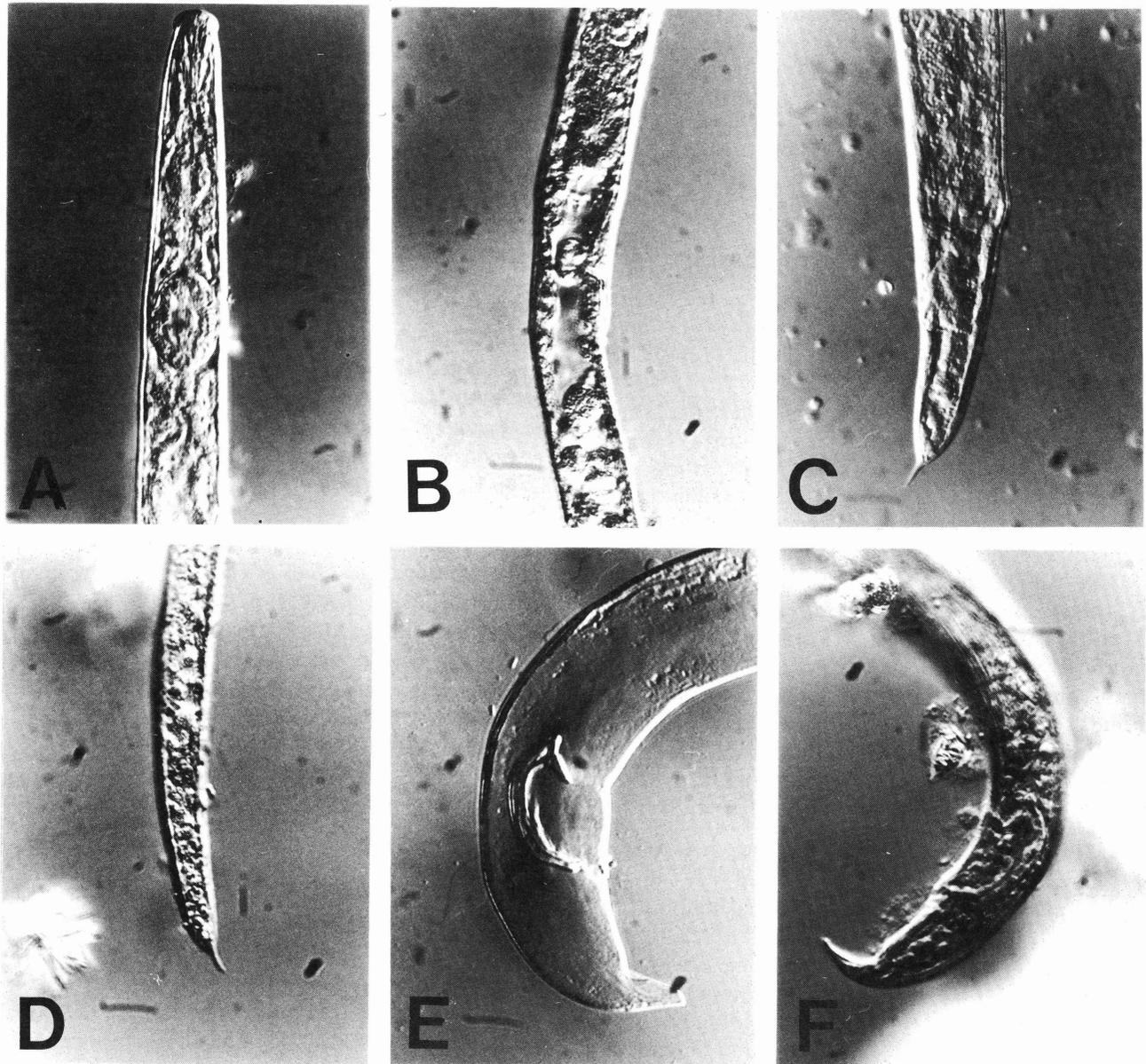


Fig. 2. Type material of *Bursaphelenchus kolymensis* Korentchenko, 1980. A: Female paratype anterior end; B: Female paratype at level of vulva; C: Female allotype tail; D: Female paratype tail; E: Male holotype tail; F: Male paratype tail.

beak or spade-shaped bursa. The female tail of *B. mucronatus* from Japan is variable (Fig. 1N-P), but has a more conical shape and a thicker mucro and males (Fig. 1H) have a sharply beak-shaped bursa in lateral view.

DISCUSSION

Bursaphelenchus kolymensis is morphologically distinct from *B. xylophilus*, but very similar in morphology to *B. fraudulentus* and *B. mucronatus*. As a

result of differences in the morphology of the bursa in lateral view (males) and the mucro (females) between *B. kolymensis* and *B. fraudulentus*, together with the latter species association with deciduous trees, *B. kolymensis* may be considered most similar to *B. mucronatus*.

Bursaphelenchus mucronatus is widespread in Asia (Mamiya & Enda, 1979; Mamiya, 1986; Baojun & Wang, 1989; Kulinich & Kolossova, 1995) and is considered common in Europe, from where it has been frequently reported (De Guiran & Boulbria,

1986; McNamara & Stoen, 1988; Schroeder & Magnusson, 1989; Tomminen *et al.*, 1989; Braasch, 1991). Although relationships between different geographical populations of the nematode from its extensive distribution area remain to be clarified, studies on the taxonomic relationships of the PWNSC based on crossings, DNA and enzyme analysis have revealed differences in affinity between *B. mucronatus* populations from Japan and Europe (De Guiran *et al.*, 1985; Webster *et al.*, 1990; Abad *et al.*, 1991; Beckenbach *et al.*, 1992; Riga *et al.*, 1992; Bolla & Boschert, 1993). Our study indicates a closer affinity of *B. kolymensis* to populations of *B. mucronatus* from Russia and France than to populations from Japan. However, it can be concluded that the complicated and intricate taxonomic situation of the PWNSC precludes synonymization of *B. kolymensis* Korentchenko, 1980 with *B. mucronatus* Mamiya & Enda, 1979. As a result of the large variability in morphological characters of PWNSC it will probably require mating and genetic studies to unequivocally resolve the specific status of *B. kolymensis*.

The Russian population BmSU is very similar to *B. kolymensis* both in female and male characters. This population was isolated from larch wood imported to Finland from Siberia (A. Lahtinen personal communication) and sent to the senior author by the Finnish Plant Inspection Service. The taxonomic identity of these specimens, and their relationship to *B. kolymensis*, will remain uncertain until the latter species has been re-isolated and further studied.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support provided by the Royal Swedish Academy of Sciences for this collaborative study and the Russian Foundation of Fundamental Researches. We sincerely thank Dr. E.A. Korentchenko (Russia) for providing paratypes of *B. kolymensis* and Professor K. Futai (Japan) for populations BxS-10 and BmJ-M and Dr D. Sturhan (Münster, Germany) for populations Bf, BmF and BmJ of *B. mucronatus*. We thank Dr. R.M. Giblin-Davis (USA) for constructive criticism and Mrs. Kajsa Goransson (Uppsala, Sweden) for technical assistance with photography.

REFERENCES

- Abad, P., Tares, S., Brugier, N. & De Guiran, G. 1991. Characterization of the relationships in the pinewood nematode species complex (PWNSC) (*Bursaphelenchus* spp.) using a heterologous *unc-22* DNA probe from *Caenorhabditis elegans*. *Parasitology* 102: 303-308.
- Baojun, Y. & Wang, Q. 1989. Distribution of the pinewood nematode in China and susceptibility of some Chinese and exotic pines to the nematode. *Canadian Journal of Forest Research* 19: 1527-1530.
- Beckenbach, K., Smith, M.J. & Webster, J.M. 1992. Taxonomic affinities and intra- and interspecific variation in *Bursaphelenchus* spp. as determined by polymerase chain reaction. *Journal of Nematology* 24: 140-147.
- Bolla, R.I. & Boschert, M. 1993. Pinewood nematode species complex: interbreeding potential and chromosome number. *Journal of Nematology* 25: 227-238.
- Braasch, H. 1991. Erster Nachweis von *Bursaphelenchus mucronatus* Mamiya und Enda, 1979 in Deutschland und sein Vorkommen in Holzimporten aus der UdSSR nebst Ergänzungen zur Beschreibung dieser Art. *Archiv für Phytopathologie und Pflanzenschutz* 27: 209-218.
- De Guiran, G. & Boulbria, A. 1986. Le nematode des pins. Caracteristiques de la souche française et risque d'introduction et d'extention de *Bursaphelenchus xylophilus* en Europe. *EPPO Bulletin* 16: 445-452.
- De Guiran, G., Lee, M.J., Dalmasso, A. & Bongiovanni, M. 1985. Preliminary attempt to differentiate pinewood nematodes (*Bursaphelenchus* spp.) by enzyme electrophoresis. *Revue de Nématologie* 8: 88-90.
- Giblin-Davis, R.M., Mundo-Ocampo, M., Baldwin, J.G., Norden, B.B. & Batra, S.W.T. 1993. Description of *Bursaphelenchus abruptus* n. sp. (Nemata: Aphelenchoididae), an associate of a digger bee. *Journal of Nematology* 2: 161-172.
- Korentchenko, E.A. 1980. [New species of nematodes from the family Aphelenchoididae, parasites of stem pests of the Dahurian Larch]. *Zoologicheskoy Zhurnal* 59: 1768-1780.
- Kulinich, O.A. & Kolossova, N.V. 1995. The potential of the pinewood nematode *Bursaphelenchus xylophilus* to become established in countries of the former USSR. *Russian Journal of Nematology* 3: 35-48.
- Mamiya, Y. 1986. Interspecific hybridization between *Bursaphelenchus xylophilus* and *B. mucronatus* (Aphelenchida: Aphelenchoididae). *Applied Entomology and Zoology* 21: 159-163.
- Mamiya, Y. & Enda, N. 1979. *Bursaphelenchus mucronatus* n. sp. (Nematoda: Aphelenchoididae) from pine wood and its biology and pathogenicity to pine trees. *Nematologica* 25: 353-361.
- McNamara, D.G. & Stoen, M. 1988. A survey for *Bursaphelenchus* spp. in pine forests in Norway. *EPPO Bulletin* 18: 353-363.
- Nickle, W.R. 1970. A taxonomic review of the genera of the Aphelenchoidea (Fuchs, 1937) Thorne, 1949 (Nematoda: Tylenchida). *Journal of Nematology* 2: 375-392.
- Nickle, W.R., Golden A.M., Mamiya, Y., & Wergin, W.P. 1981. On the taxonomy and morphology of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner &

- Buhrer 1934) Nickle 1970. *Journal of Nematology* 13: 385-392.
- Riga, E., Beckenbach, K. & Webster, J.M. 1992. Taxonomic relationships of *Bursaphelenchus xylophilus* and *B. mucronatus* based on interspecific and intraspecific cross-hybridization and DNA analysis. *Revue de Nématologie* 15: 391-395.
- Rühm, W. 1956. Die Nematoden der Ipiden. *Parasitologische Schriftenreihe*, Jena 6: 1-435.
- Rutherford, T.A., Mamiya, Y. & Webster, J.M. 1990. Nematode-induced pine wilt disease: Factors influencing its occurrence and distribution. *Forest Science* 36: 145-155.
- Schauer-Blume, M. & Sturhan, D. 1989. Vorkommen von Kiefernholz nematoden (*Bursaphelenchus* spp.) in der Bundesrepublik Deutschland? *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes. (Braunschweig)* 41: 133-136.
- Schroeder, L.M. & Magnusson, C. 1989. Tallvednematoden - ett hot mot svensk skog? *Skogsfakta* 64: 1-4.
- Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69.
- Seinhorst, J.W. 1962. On the killing, fixation and transferring to glycerine of nematodes. *Nematologica* 8: 29-32.
- Steiner, G. & Buhrer, E.M. 1934. *Aphelenchoides xylophilus* n.sp., a nematode associated with blue-stain and other fungi in timber. *Journal of Agricultural Research* 48: 949-951.
- Tomminen, J., Nuorteva, M., Pulkkinen, M. & Väkevää, J. 1989. Occurrence of the nematode *Bursaphelenchus mucronatus* Mamiya & Enda 1979 (Nematoda: Aphelenchoididae) in Finland. *Silva Fennica* 23: 271-277.
- Webster, J.M., Anderson, R.V., Baillie, D.L., Beckenbach, K., Curran, J. & Rutherford, T.A. 1990. DNA probes for differentiating isolates of the pinewood nematode species complex. *Revue de Nématologie* 13: 255-263.

Магнуссон К., Кулинич О. А. Таксономическая оценка первоописания, морфология и статус *Bursaphelenchus kolymensis* Korentchenko, 1980 (Aphelenchida: Aphelenchoididae).

Резюме. Группа видов древесных нематод включает виды: *Bursaphelenchus xylophilus*, *B. mucronatus* и *B. kolymensis*, причем таксономический статус последнего вида до сих пор не уточнен. Изучение типовых препаратов *B. kolymensis* Korentchenko, 1980 показало, что самцы имеют широкую клювовидную или лопатовидную бурсу при латеральном положении тела, и кукулюс на конце спикул. У самок субцилиндрический хвостовой отдел с тонким мукро. Сравнение *B. kolymensis* с другими представителями этого комплекса видов показывает его близость к *B. fraudulentus*, от которого отличается формами мукро и бурсы, а также растением-хозяином, и к изолятам *B. mucronatus* из Франции и России. От изолята *B. mucronatus* из Японии *Bursaphelenchus kolymensis* отличается хвостом самки, мукро и формой бурсы самца. Делается вывод о необходимости дальнейших генетических исследований для определения таксономического статуса *B. kolymensis*.
