LAIMAPHELENCHUS PREISSII SP. NOV. (NEMATODA: APHELENCHINA) FROM NATIVE PINE CALLITRIS PREISSII IN SOUTH AUSTRALIA

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Summary
Laimaphelenchus preissii sp. nov. is described from bark of the Australian native conifer, Callitris preissii from Burdett, South Australia. This is the first record of Laimaphelenchus from Australia. The new species is characterised by the possession of a unique tail structure, with a single tubercle with many small projections, and the male has a bursa and two pairs of caudal papillae.

Key words: Laimaphelenchus preissii sp. nov., Nematoda, Aphelenchina, Callitris preissii, Australia

Introduction

Plant parasitic nematodes have been recognised as important pathogens of trees for more than 40 years (Sutherland & Webster 1993). The order Aphelenchida contains several genera of economic importance, namely Aphelenchoides Fischer, 1894 and Bursaphelenchus Fuchs 1937. The pine wood nematode, Bursaphelenchus xylophilus (Steiner & Buhrer 1934) Nickle 1970, is an important nematode causing death of pines in many countries (Suzuki 2003), and is internationally recognised as the most damaging pest of the coniferous forests and plantations. B. xylophilus has not been recorded in Australia. Nematode species belonging to these genera frequently co-habit with other genera such as Laimaphelenchus Fuchs, 1937 that are not pathogenic on conifers. Laimaphelenchus consists of nine valid species described from every continent except Australia (Hunt 1993; Swart 1997; Peneva & Chipev 1999). Since they are mostly found associated with moss, algae and lichen on trees, particularly conifers, and also in tunnels of wood borers (Hunt 1993), it is important to be able to recognise and distinguish them.

In 2000-2002, suspected symptoms of pine wilt disease were seen in pines trees at Heidelberg, Knoxfield and Williamstown, suburbs of Melbourne, Victoria (David Smith, pers. com.). A nematode tentatively identified as Bursaphelenchus hunanensis Yin, Fang & Tarjan, 1988 was extracted from an affected pine tree. Although the damage was limited to a relatively small number of trees, it was a strong warning of the risk of an incursion by B. xylophilus to the Australian forestry industries.

During a survey of nematodes associated with bark and wood in both indigenous and introduced pines in Australia, a new species of Laimaphelenchus was extracted from bark collected from Callitris preissii Miq. and is described in this paper.

Materials and Methods

Bark was collected from specimens of C. preissii growing in the road reserve at Burdett (34°59’ S, 139°22’ E), on the Murray Bridge to Mannum road, near Murray Bridge in South Australia, and from the Tailem Bend Forest Reserve (35°19’ S, 139°23’ E) near Tailem Bend. Bark was chipped with a hatchet, from each of 20 trees at each location, from a spot 1 m above the ground on the (shaded) south-eastern side of the tree. Cores of wood were also collected from the south-eastern side of each tree, using a borer (5 mm diameter). Samples were placed in an insulated container for transport back to Adelaide, and kept at 16°C until extraction. Using a mister, nematodes were extracted from sub-samples of 20–30 g material over 3 days. Nematodes collected from trees at Burdett were surface sterilised in 1% streptomycin for 10 min., washed 3 times with sterile water, and transferred to cultures of Botrytis cinerea Pers. on potato dextrose agar (PDA). Plates were

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incubated at 25°C, and were sub-cultured monthly. Nematodes were washed off the plates with water, and killed and fixed using hot 3% formalin, and left to harden for at least 2 weeks. Nematodes extracted from trees near Tailem Bend were also killed and fixed in 3% formalin. All nematodes were processed to glycerol, and mounted on glass slides, as described by Davies and Giblin-Davis (2004). Nematodes were examined using interference contrast microscopy.

Measurements are given in µm. Drawings and measurements were made from material mounted in glycerol, using a camera lucida. Body width and width of lateral fields was measured at mid-length. Body length was measured along the mid-line. Spicules were measured from the top of the condylus to the cucullus (Braasch & Schmutzenhofer 2000). De Man’s ratios were determined; respectively: a = length divided by greatest body width, b = length divided by distance from anterior end to start of oesophageal glands, b' = length divided by distance from anterior end to base of oesophageal glands c = length divided by tail length, c' = tail length divided by width at anus, m = conus as percentage of stylet length, MB = distance from anterior end of body to centre of median bulb x 100, divided by length of oesophagus; V = anterior end to vulva as percentage of body length, T = length of testis from cloaca to end or flexure as percentage of body length.

For scanning electron microscopy (SEM), nematodes fixed in formalin were washed in three changes of water purified by reverse osmosis (RO). They were then immersed in a 0.05% solution of Tween 20, and sonicated for 60 seconds, using setting 4 on a GS UP 50 H sonication probe. The detergent was removed from the nematodes by three washes in filtered RO water, and they were then post-fixed and stained with 2% osmium tetroxide. After an hour, the nematodes were washed three times in filtered RO water. They were then dehydrated through an ethanol series, with 20-30 min. in each stage (30, 70, 80, 90, 95% ethanol, and then two changes of 100% ethanol). After this, a 1:1 solution of absolute ethanol and hexamethyldisilazane (HMDS) was added, left for 30 min. This was replaced with 100% HMDS, which was allowed to evaporate slowly overnight in a fume cupboard. For mounting of the dry nematodes on a stub, a piece of human hair was placed on a sticky disc on a stub. Nematodes were then placed along the hair, with some with the head and others the tail balanced on the hair. Stubs were coated with 3 nm of platinum, and viewed using a Philips XL30 Field Emission scanning electron microscope.

*Laimaphelenchus preissiae* sp. nov.

(FIGS 1 – 15)

*Measurements:* Table 1.

*Material examined*

**Holotype**

♂. Burdett, South Australia. Taken from nematode culture on *Botrytis cinerea* fungus. Australia National Insect Collection (ANIC) (slide no. 112).

**Paratypes**

Twenty males, 26 females and 54 juveniles have been examined. They were taken from nematode culture on *B. cinerea* fungus. Twenty four slides, numbered 004569-004597, are stored in the Waite Insect and Nematode Collection (WINC).

**Description**

*Female.* Long, slender (a 39 – 57) nematodes; habitus ventrally arcuate, with curvature more pronounced in posterior region. Body annules 1.5 µm wide at mid-body. Lateral fields with 4 incisures, occupying about 30% of body width, not areolate, extending to origin of tubercle.

Cephalic region rounded, offset, clearly wider than body at base. SEM shows a clear labial disc, not divided by ribs, no clear demarcation between labial disc and postlabial disc area. Anterior cephalid at level of conus base; posterior cephalid a few micrometres behind stylet knobs. Stylet 12 – 15 µm long, with small inconspicuous knobs.
Figure 1. Laimaphelenchus preissii sp. nov. 1, female; 2, male; 3, spicules; 4, vulval region; 5, lateral field; 6, male tail; 7, female anterior end; 8, female tail. Scale bars = 50 µm, 1, 2, 4 - 8; 25 µm, 3.
Figure 2. Scanning electron microscopy observation of *Laimaphelenchus preissii* sp. nov. 9, male head; 10, male tail; 11, male incisure; 12, male bursa and papillae. Scale bars = 1 µm, 9; 0.5 µm, 10; 5 µm, 11; 10 µm, 12.

Figure 3. Scanning electron microscopy observation of *Laimaphelenchus preissii* sp. nov. 13, female head; 14, female vulval flap; 15, female tail. Scale bars = 2 µm, 13; 10 µm, 14; 2 µm, 15.
Table 1. Morphometric data for Laimaphelenchus preissii sp. nov. (measurements µm; mean ± s.d. and (range) for paratypes)

|                | Holotype male | Allotype female | Paratype males | Paratype females |
|----------------|---------------|-----------------|----------------|------------------|
|                | n 1           | 1               | 20             | 26               |
| L              | 971           | 1071            | 1088 ± 61      | (1000 - 1218)    |
|                |               |                 | 1185 ± 74      | (1007 - 1386)    |
| a              | 51.0          | 56.3            | 45.3 ± 5.9     | (36.7 - 51)      |
|                |               |                 | 48.9 ± 4.9     | (39.3 - 57.1)    |
| b              | 12.8          | 12.5            | 13.5 ± 1.4     | (10.9 - 15.6)    |
|                |               |                 | 14.5 ± 1.5     | (11.8 - 17.8)    |
| b'             | 6.2           | 5.1             | 5.8 ± 1.0      | (5.0 - 7.7)      |
|                |               |                 | 6.1 ± 0.5      | (5.5 - 6.9)      |
| c              | 22.7          | 20.5            | 25.4 ± 4.3     | (17.6 - 34.7)    |
|                |               |                 | 28.3 ± 5.8     | (19.1 - 39.3)    |
| c'             | 2.7           | 4.7             | 2.3 ± 0.4      | (1.6 - 3.2)      |
|                |               |                 | 2.9 ± 0.6      | (1.8 - 4.5)      |
| V or T         | 59.8          | 68.9            | 63.2 ± 6.1     | (55.5 - 71.7)    |
|                |               |                 | 69.8 ± 1.5     | (66.5 - 71.3)    |
| MB             | 42.4          | 36.4            | 41.9 ± 4.5     | (37.0 - 47.8)    |
|                |               |                 | 37.8 ± 3.4     | (33.3 - 43.5)    |
| m              | 47.1          | 41.2            | 45 ± 1.3       | (43.3 - 47.1)    |
|                |               |                 | 43.6 ± 3.2     | (38.9 - 46.9)    |
| Anterior end   | 67            | 76              | 83 ± 14        | (71 - 105)       |
| to valves of   |               |                 | 71 ± 5         | (64 - 79)        |
| median bulb    |               |                 |                |                  |
| Oesophagus     | 157           | 210             | 200 ± 30.6     | (152 - 233)      |
| length         |               |                 | 188 ± 14.0     | (164 - 200)      |
| Stylet length  | 13            | 13              | 13 ± 0.9       | (11 - 17)        |
| Head width     | 7             | 7.5             | 7 ± 0.4        | (7 - 7.5)        |
|                |               |                 | 7 ± 0.2        | (6.5 - 7)        |
| Head height    | 3             | 3               | 3 ± 0.4        | (2 - 3)          |
| Tail length    | 43            | 52              | 44 ± 7.1       | (32 - 57)        |
|                |               |                 | 44 ± 9.1       | (32 - 64)        |
| Anus to vulva  | -             | 281             | -              | 314 ± 24.1       |
|                |               |                 |                | (271 - 364)      |
| Anterior end   | -             | 738             | -              | 828 ± 57.1       |
| to vulva       |               |                 |                | (707 - 1000)     |

Median bulb rounded to oval, 14.7 – 16 µm long, 12.5 – 14 µm wide. Nerve ring located near excretory pore at point where lumen of the intestinal tract widens.

Excretory pore conspicuous, about 1.5 body widths posterior to nerve ring, 100 – 114 µm from anterior end. Hemizonid not seen.

Oesophageal glands variable, usually one dorsal but may be two lobes (one small ventral and one large dorsal), overlap of intestine on dorsal side extending for 164 – 200 µm.

Reproductive system with outstretched ovary with oocytes in a single row; conspicuous spermatheca filled with sperm cells; vagina sloping anteriorly, not sclerotised distally. Post-vulval uterine sac 86 – 157 µm long, occupying one third to one half of distance from vulva to anus; containing many cells with prominent nuclei. Vulva with well-developed anterior vulval flap, posterior lip about twice width of anterior.

Tail conoid, ventrally curved, with one broad tubercle with about 10 projections (seen only with SEM), including a prominent projection at the tip.

**Male.** Morphology similar to that of female. Testis outstretched; developing germ cells in single file. Spicules paired, 22 – 28 µm long; rosethorn-shaped, with prominent capitulum and rostrum broad with bluntly rounded tip. No gubernaculum. Two pairs of caudal papillae present, one pair analan, subventral; second pair subventral at about 60% of distance to tail tip.

Tail conoid, with a small bursa formed by extension of cuticle in region of the lateral fields, running from level of second pair of papillae to beginning of tubercle. Tail tip with single dorso-ventrally flattened tubercle, with about 20 projections (seen only with SEM), including a prominent terminal projection.

**Juveniles.** Range of lengths for juvenile stages: J2: 286 – 429 µm (n = 18); J3: 500 – 714 µm (n = 18); J4: 786 – 1071 µm (n = 18).

**Type locality and habitat**
Bark on trunk of *C. preissii* growing on roadside at Burdett, South Australia (34°59’ S, 139°22’ E). Collected by Z. Zhao on 6 November 2003.

**Diagnosis**

*Laimaphelenchus preissii* sp. nov. is a member of the group of species characterised by the anterior vulval lip being strongly developed, overlapping the vulval slit and the posterior vulval lip. The unique morphology of the male bursa, the body length and the morphology of the tail tip of both sexes distinguish this species from all other described species of *Laimaphelenchus*.

**Relationships**

The species of *Laimaphelenchus* are divided into those with and those without a vulval flap (Baujard 1981; Hunt 1993). *Laimaphelenchus preissii* sp. nov. belongs to the first group. It has lateral fields with four incisures and a long post-uterine sac. Females of *L. preissii* sp. nov. (1007 – 1385 µm) are closest to *L. pannocaudus* Massey, 1966 (700 – 1060 µm) in body length. They are larger than all other described species (*L. pannocaudus* (Steiner, 1914) Filipjev & Schuurmans Stekhoven, 1941, 573 – 800 µm; *L. deconincki* Elmiligy and Geraert, 1972, 690 – 770 µm; *L. pensobrinus* Massey, 1966, 610 µm; *L. cocuccii* Doucet, 1992, 570 – 740 µm; *L. unituberculus* Bajaj and Walia, 2000, 690 – 800 µm; *L. helicosoma* Peneva and Chipev, 1999, 619 µm; *L. patulus* Swart, 1997, 450 – 530 µm; *L. phloeosini* Massey, 1974, 430 – 510 and *L. pini* Baujard, 1981, 350 – 470 µm). *Laimaphelenchus preissii* sp. nov. is distinguished from *L. pannocaudus* by the absence of a vulval flap in the latter, and is similar to *L. penardi, L. deconincki, L. pensobrinus, L. cocuccii, L. unituberculus, and L. helicosoma* in having vulval flap. However, in *L. preissii* sp. nov. the vagina is not surrounded by a thick cuticularised tube and is not sclerotized, which differs from *L. deconincki, L. cocuccii* and *L. unituberculus*. *Laimaphelenchus preissii* sp. nov. has a long vulval sac, as does *L. penardi* and *L. pannocaudus*, but all other species have shorter sacs. The tail tip differs from all described species in that the one broad tubercle has about 10 projections that can only be seen with SEM. These are smaller than in other species. The labial disc is similar to that of *L. patulus*, in not being divided by ribs and with no clear demarcation between labial disc and post-labial disc area (Swart 1997). It differs from *L. cocuccii*, which lacks a labial disc.

Males of *L. preissii* sp. nov. are longer than all described species, and the presence of a bursa distinguishes it from all recognised species. The spicule shape is similar to that of *L. penardi*. Two pairs of caudal papillae are present; one pair adanal and subventral, and a second pair subventral at about 60% of the distance from the cloaca to the tail tip. The projections on the tail tip are smaller than found in any other described species. The labial plate is the same as in the female.

**Etymology**

Named for the species of conifer, *Callitris preissii*, from which it was first collected.

**Discussion**

The genus *Laimaphelenchus* contains nine valid species recorded from Europe, India, South Africa, North America, Argentina and Antarctica (Hunt 1993; Swart 1997; Peneva & Chipev 1999). This is the first record of the genus from Australia.

*Laimaphelenchus preissii* sp. nov. was found in the bark of native pine *C. preissii*, but not in the wood. It appears to be feeding on fungi or lichens growing on the bark of the tree. It was cultured successfully on *Botrytis cinerea*, and also on *Monilinia fructicola* and *Rhizoctonia solani*. The best growth occurred on *B. cinerea*. No insects appeared to be associated with it on the host pine trees.

According to the general description of *Laimaphelenchus* in Hunt (1993), three pairs of caudal papillae are present in the male: a preanal pair, an adanal pair and a pair midway to the tail tip. In *L. preissii* sp. nov. there is an adanal, subventral pair, and a second pair subventral at about 60% of distance to tail tip. A pair of caudal papillae near the tail tip seems to be a common feature in the genus (Baujard 1981; Swart, 1997), including *L. patulus, L. pannocaudus, L. phloeosini, L. pini, L. penardi* and *L. pensobrinus*, but does not occur in this new species.
In having a bursa on the male tail and a broad tubercle with many tiny projections on the tail tip of both sexes, *L. preissii* sp. nov. is unique in the genus. *Laimaphelenchus lignophilus* (Körner, 1954) was described as having a bursa on the male tail, but according to Hunt (1993) it is now considered as a species incertae sedis. Morphological assignment of *preissii* sp. nov. to the genus *Laimaphelenchus* has been confirmed by comparison of the sequence of the rDNA 18S gene with those of related genera of nematodes, including *Aphelenchoidea* and *Bursaphelenchus* (Weimin Ye & Giblin-Davis, pers. com.).

*Laimaphelenchus preissii* sp. nov. provides a good example of the difficulties associated with taxonomy of the aphelenchids based on morphology alone. Presence of the vulval flap and bursa are indicative of *Bursaphelenchus*, so it requires examination with SEM to detect the projections on the tail that define it as *Laimaphelenchus*. There is a strong need for development of molecular markers to allow confirmation of the taxonomy of the economically important aphelenchids associated with trees.

Acknowledgements

We would like to thank the following people for their contribution to this paper: Weimin Ye and Robin Giblin-Davis, The University of Florida, for comparison of DNA sequences; Mike Hodda, CSIRO Entomology, for providing references and advice; Ian Smith, The University of Melbourne, and Charlma Phillips, Forestry SA, for organising sample collection. This work was funded by the Forest and Wood Products Research and Development Corporation.

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