**LAIMAPHELENCHUS HEIDELBERGI SP. NOV. (NEMATODA: APHELENCHINA) FROM VICTORIA, AUSTRALIA, AND EMENDMENT OF THE DIAGNOSIS OF THE GENUS**

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**Summary**

*Laimaphelenchus heidelbergi* sp. nov. is described from wood of the exotic pine, *Pinus radiata*, from Heidelberg Park, Heidelberg, Victoria, Australia. This is the third record of *Laimaphelenchus* from Australia. The new species is characterised by having a unique tail structure, with a single tubercle with many tiny projections visible only with scanning electron microscopy, and the male has a spicule with two small protrusions on the ventral side about 2 µm from the distal end. The diagnosis of the genus is emended.

**Key words:** *Laimaphelenchus heidelbergi* sp. nov., Nematoda, Aphelenchina, *Pinus radiata*, Australia

**Introduction**

Records of nematodes from the order Aphelenchina from above-ground on conifers in Australia are few. McLeod *et al.* (1994) listed a number of plant nematodes in conifers in Australia, of which *Aphelenchoides bicaudatus* (Imamura) Filipjev & Schuurmans Stekhoven was the only species of aphelenchid. From a survey of pine plantations and native conifers, Zhao *et al.* (2006 a, b, c) collected and described two new species of *Laimaphelenchus* (Aphelenchoididae), the first records of the genus from Australia. Of these, *Laimaphelenchus preissii* Zhao, Davies, Riley and Nobbs, 2006 was collected from bark of the native pine *Callitris preissii*, and *Laimaphelenchus australis* Zhao, Davies, Riley and Nobbs, 2006 was collected from bark of the introduced commercial species *Pinus radiata*. In the present paper, a third Australian species of *Laimaphelenchus* is described, collected from wood samples from a dead *P. radiata* tree in Heidelberg, Victoria.

**Materials and Methods**

Wood was collected from a dead *P. radiata* tree standing in the Heidelberg Park, in Heidelberg, Victoria. Using an axe, wood was chipped from the trunk of the tree, from several spots 50 cm above the ground. The samples were placed in one plastic bag, which was placed in an insulated container for transport back to Adelaide, and then kept at 16°C for 6 months, until extraction. Using the Whitehead tray method, nematodes were extracted from sub-samples of 20 – 30 g wood chips over 3 days, at 25°C. Nematodes collected were surface sterilised in 1% streptomycin for 10 min., washed 3 times with sterilised water, and transferred to cultures of *Botrytis cinerea* Pers. on potato dextrose agar (PDA). Plates were incubated at 25°C, and were sub-cultured fortnightly. For morphological studies, nematodes were washed off the plates with water, killed and fixed using hot 3% formalin, and left to harden for at least 2 weeks. All nematodes were processed to glycerol, and mounted on glass slides, as described by Davies and Giblin-Davis (2004).

Measurements are in µm. Using interference contrast microscopy and a camera lucida, drawings and measurements were made from material mounted in glycerol. Body width and width of lateral fields was measured at mid-length. Body length was measured along the mid-line. Spicules were measured as a straight line from the top of the condylus to the tip of the blade (Braasch and Schmutzenhofer 2000). Morphometric ratios were determined; respectively: \( a = \) body length divided by greatest body width, \( b = \) body length divided by distance from anterior end to start of oesophageal glands, \( b' = \) body length divided by distance from anterior end to posterior extremity of oesophageal glands, \( c = \) body length divided by tail length, \( c' = \)
tail length divided by width at anus, \( m = \text{conus as percentage of stylet length} \); \( MB = \text{distance from anterior end of body to centre of median bulb x 100, divided by length of oesophagus} \); \( V = \text{anterior end to vulva as percentage of body length} \); \( T = \text{length of testis from cloaca to flexure as percentage of body length} \).

Nematodes were prepared for scanning electron microscopy (SEM) by modification of the method described by Heegaard et al. (1986). 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 dehydrated through an ethanol series, with 20 – 30 minutes 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, and left for 30 minutes. It 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 heidelbergi** sp. nov.  
(Figs 1-14)

**Measurements:** Table 1.

**Material examined**

**Holotype**

♂. Heidelberg Park, Heidelberg, Victoria, Australia, (37°45’ S, 145°04’ E), from culture on *B. cinerea* fungus. Australian National Insect Collection (ANIC) (slide no. xxx).

**Paratypes**

Ten males and 12 females on 14 slides, with various juveniles, from culture on *B. cinerea*, Waite Insect and Nematode Collection slide numbers WINC 063711 – 063724.

**Description**

**Female.** Long, slender (De Man’s a 28 – 40) nematodes (Fig. 2); habitus slightly ventrally arcuate, with curvature more pronounced in posterior region. When viewed with SEM, cuticle at anterior end, behind cephalic region, is tessellated in some specimens (Fig. 8). Body annules 0.8 µm wide at mid-body. Lateral fields with 3 incisures, occupying about 20% of body width, not areolate, extending to origin of tubercle (Figs 5, 9).

Cephalic region rounded, offset, not wider than body at base (Figs 3, 8). SEM shows a clear labial disc, not divided by ribs, with a clear demarcation between labial disc and area behind it. Cephalic region, behind labial disc, annulated, with 6 clear ribs perpendicular to the annules. Amphids pore-like, near anterior edge of post-disc area. Cephalic papillae present on ribs, also near anterior edge of post-disc area. Anterior cephalid a few micrometres anterior to level of conus base; posterior cephalid at level of stylet knobs.
Figure 1. *Laimaphelenchus heidelbergi* sp. nov. 1, male; 2, female; 3, male head; 4, spicules; 5, lateral field; 6, vulval region; 7, male tail. Scale bars = 50 µm, 1, 2, 7; 25 µm, 3-6.
Figure 2. Scanning Electron Microscopy observation of Laimaphelenchus heidelbergi sp. nov. 8, female head showing amphid and offset; 9, female anus and lateral lines; 10, vulva. Scale bars = 2 μm, 8; 10 μm, 9, 10.

Median bulb rounded to oval, 9 – 11 μm long by 12 – 14 μm wide. Valve plates usually in posterior half of bulb, occasionally central. Nerve ring located one twentieth to one third of a body width anterior to excretory pore, at point where lumen of intestinal tract widens.

Excretory pore conspicuous, 1 – 1.25 (n=5) body widths posterior to median bulb, 76 – 90 μm from anterior end. Hemizonid not seen.

Oesophageal glands overlap intestine on dorsal side, extending for 114 – 162 μm from anterior end of nematode.

Reproductive system outstretched, ovary with oocytes in a single column; conspicuous spermatheca filled with sperm cells; vagina sloping slightly towards anterior, not distally sclerotised, but having a relatively thick, refractile wall, and surrounded by a strong band of muscles (Fig. 6). Post vulval uterine sac 10 – 47 μm long, occupying 10 – 26% of distance from vulva to anus; containing many cells with small nuclei. Vulva without anterior flap; in some specimens appears slightly protruding (Figs 6, 10).

Tail conoid, ventrally curved, with a single offset tubercle covered by 20 – 30 knob-like appendages (seen only with SEM), including a prominent one at the tip.
Male. Morphology similar to that of female (Fig. 1). Testis outstretched, reflexed; reflexed part 40 – 80 µm long, about 15% of testis length; developing germ cells arranged in single column at anterior end of testis, usually in double column in the mid-part, and in single column at the distal end; area with double column occupying about 60% of the testis length. Spicules paired, 15 – 16 µm long; rosethorn-shaped, with prominent capitulum and broad rostrum with bluntly rounded tip (Fig. 4). Two small rounded protrusions on ventral side, separated by a notch 2 µm from distal end of spicule (Figs 4, 13). Gubernaculum absent. Caudal papillae 5, in three groups: one pair adanal, sub-ventral, at level of spicule rostrum; one pair sub-ventral at about 60% of distance between cloaca and tail tip, and one single mid-ventral on tail tip anterior to tubercle (Figs 7, 13).

Tail conoid, bearing single tubercle, with about 20 – 30 knob-like protrusions (seen only with SEM), including a prominent one at the tip (Fig. 14).

Type locality and habitat

Culture of nematodes extracted from wood chips collected from the trunk of *P. radiata* growing in Heidelberg, Victoria, Australia (37°45’ S, 145°04’ E). Chips collected by David Smith on 23 May 2003.
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Diagnosis

*Laimaphelenchus heidelbergi* sp. nov. differs from all other described species of *Laimaphelenchus* by the distinct tail shape with a single offset terminal tubercle covered by 20-30 tiny knob-like appendages in both sexes, and by having two small ventral protrusions near the distal end of the spicule. In addition, it is characterised by the following combination of morphological characters: three incisures in the lateral field; no vulval flap; two pairs of subventral caudal papillae, and a single ventral papilla near tail tip, including one pair at the level of the spicule rostrum, one at about 60% of distance between cloaca to tail tip, and a single papilla on the tail tip just before the tubercle.

Relationships

The genus *Laimaphelenchus* contains two groups of species (Baujard 1981; Hunt 1993), one with females having a vulval flap and one without (Table 2). *Laimaphelenchus heidelbergi* sp. nov. belongs to the group without a flap. The other species in this group are *L. pannocaudus* Massey, 1966, *L. phloesini* Massey, 1974, *L. pini* Baujard, 1981, *L. patulus* Swart 1997 and *L. australis*. *Laimaphelenchus heidelbergi* sp. nov. is separated from *L. penardi* (Steiner, 1914) Filipjev and Schuurmans Stekhoven 1941, *L. deconincki* Elmily and Geraert, 1972, *L. pensobrinus* Massey, 1966, *L. cocuccii* Doucet, *L. unituberculus* Bajaj and Walia, 2000, *L. preissii* and *L. helicosoma* Peneva and Chipev, 1999, which have a flap.

In body length (see Table 2 for measurements), *L. heidelbergi* sp. nov. females are larger than *L. patulus*, *L. phloesini*, *L. pini*, and *L. australis*; and shorter than *L. preissii*. *Laimaphelenchus heidelbergi* sp. nov. females are similar in size to those of *L. pannocaudus*, *L. penardi*, *L. deconincki*, *L. pensobrinus*, *L. cocuccii*, *L. unituberculus*, and *L. helicosoma* (see Table 2).

The lateral fields of *L. heidelbergi* sp. nov. have three incisures, similar to those of *L. patulus*, *L. cocuccii*, and *L. deconincki*, and differing from those of *L. helicosoma*, *L. pensobrinus* and *L. penardi* which have two incisures, and from *L. preissii*, *L. australis*, *L. pannocaudus*, *L. unituberculus*, *L. pini*, and *L. phloesini*, which have four.

The post vulval uterine sac of *L. heidelbergi* sp. nov. is similar in length to that of most species of *Laimaphelenchus*, but shorter than that of *L. pannocaudus*, *L. penardi* and *L. preissii*. In having a relatively thick refractile wall, the vagina of *L. heidelbergi* sp. nov. is similar to that in *L. deconincki*, *L. cocuccii* and *L. unituberculus*. The tail tip differs from that of all described species except *L. preissii*, in that it has one offset tubercle with about 20 – 30 knob-like appendages that can only be seen with SEM, but it differs from *L. preissii* by the structure of the projections, which are smaller and in having more of them.

The labial disc of *L. heidelbergi* sp. nov. is clear, with a demarcation between labial disc and post-disc area. This structure differs from that of *L. patulus*, where there is with no clear demarcation between labial disc and post-disc area (Swart 1997). *Laimaphelenchus heidelbergi* sp. nov. also differs from *L. cocuccii*, which lacks a labial disc.

The spicule shape of *L. heidelbergi* sp. nov. is similar to that of *L. patulus*, but the presence of the two small ventral protrusions near the distal end of the blade is unique in *Laimaphelenchus*.

According to the general description of *Laimaphelenchus* (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. A pair of caudal papillae near the tail tip occurs commonly in the genus (Baujard 1981; Swart 1997), appearing in *L. patulus*, *L. pannocaudus*, *L. phloesini*, *L. pini*, *L. penardi* and *L. pensobrinus*. *Laimaphelenchus heidelbergi* sp. nov. has two pairs of subventral caudal papillae and a single ventral papilla, including one pair at the level of the spicule rostrum, one at about 60% of distance between cloaca and tail tip, and a single papilla on the tail tip just before the tubercle. The latter has not been described for other species of *Laimaphelenchus*. 
**Table 1.** Morphometric data for *Laimaphelenchus heidelbergi* sp. nov. (measurements µm± s.d.)

|                | Holotype male | Allotype female | Paratype males | Paratype females |
|----------------|---------------|-----------------|----------------|-----------------|
|                | Mean±s.d.     | Range           | Mean±s.d.      | Range           |
| *n*            | 681±738       | 567-752         | 750±77.3       | 533-895         |
| *L*            | 39.0±2.7      | 34.3-44.7       | 39.9±2.4       | 28.6-40.5       |
| *a*            | 10.2±1.3      | 8.9-13.18       | 12.0±1.3       | 8.6-14.2        |
| *b*            | 10.2±1.3      | 8.9-13.18       | 12.0±1.3       | 8.6-14.2        |
| *c*            | 19.1±2.7      | 15.2-24.2       | 18.6±2.7       | 10.7-22.3       |
| Head width (µm) | 5.5±0.4       | 5.0-6.0         | 5.8±0.3        | 5.0-6.0         |
| Head height (µm) | 2.1±0.3       | 1.5-2.0         | 2.3±0.4        | 2.0-3.0         |
| Tail length (µm) | 36.2±4.4      | 29.0-43.0       | 41.1±6.5       | 33.0-48.0       |
| Anus to vulva (µm) | -             | -               | 184.7±30.2     | 114.0-229.0     |
| Anterior end to vulva (µm) | 522.6±48.3 | 433.0-609.0 |
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Table 2. Comparative morphology of *Laimaphelenchus* species.

| Species   | L of female (µm) | Incisures in lateral field | L uterine sac (µm) | Tail structure | Spicule L (µm) | Spicule form | Caudal papillae (pairs) | Reference |
|-----------|------------------|---------------------------|-------------------|----------------|----------------|---------------|------------------------|-----------|
| **With vulval flap** |                  |                           |                   |                |                |               |                        |           |
| *cocucci* | 570-740          | 3                         | 40-63             | 4 tubercles with protrusions | -              | -             | -                      | Doucet 1992 |
| *deconincki* | 690-770         | 3                         | 32-46             | 4 tubercles with protrusions | -              | -             | -                      | Elmiligy & Geraert 1971 |
| *helicosoma* | 619             | 2                         | 30                | poorly developed tubercle | 22-25          | 3             |                        | Peneva & Chipev 1999 |
| *penardi*   | 640-810          | 2                         | 110-126           | 4 fringed tubercles | 16-23          | 3             |                        | Baujard 1981 |
| *pensobrinus* | 460-610        | 2                          | c. 40             | 4 tubercles, no fringes | c. 33          | 2             |                        | Massey 1966 |
| *preissii*  | 1007-1386        | 4                         | 86-157            | 1 tubercle with 10 protrusions | 22-28          | 2             |                        | Zhao *et al.* 2006 b |
| *unituberculus* | 690-800        | 4                          | c. 40             | 1 tubercle with protrusions | 14-15          | 3             |                        | Bajaj & Walia 2000 |
| **Without vulval flap** |                  |                           |                   |                |                |               |                        |           |
| *australis* | 371-459          | 4                         | 21-45             | 3 or 4 tubercles, each with 4-6 protrusions | 17-21          | 3             |                        | Zhao *et al.* 2006 a |
| *heidelbergi* | 533-895         | 3                         | 10-47             | 1 tubercle with 20-30 knob-like protrusions | 15-16          | 2 and 1 single | this paper             |           |
| *pannocaudus* | 690-960         | 4                          | c. 100            | 1 tubercle with 3 ragged protrusions | c. 25          | 3             |                        | Massey 1966 |
| *patulus*   | 460-530          | 3                         | 25-40             | 4 fringed tubercles | 20-22          | 2 or 3        |                        | Swart 1997 |
| *phloesini* | 430-510          | 4                         | 28-50             | 3 fringed tubercles | 17-20          | 3             |                        | Massey 1974 |
| *pini*      | 350-470          | 4                         | c. 18-30          | 4 fringed tubercles | 12-17          | 3             |                        | Baujard 1981 |

L = length. Spicules drawn from original descriptions; not to scale.

**Etymology:**

Named for the place, Heidelberg, from which it was collected.

**Genus: Laimaphelenchus** Fuchs, 1937 syn. *Ruidosaphelenchus* Laumond and Carle, 1971

**Diagnosis,** emended from Hunt (1993)

(The following diagnosis includes information, particularly from SEM studies, not available to Hunt in 1993. He had 7 species to consider; there are now 13. Differences from Hunt’s diagnosis are indicated in bold.)
Aphelenchoidinae. Medium sized to large nematodes about 530 – 1400 µm long (Table 2). Arcuate to strongly ventrally arcuate to C-shaped or spiral when heat relaxed. Body fairly stout to slender (a ratio range 21 – 57 in females, 23 – 67 in males) and weakly or distinctly annulated. Lateral fields with rarely two, three or four incisures, extending to origin of tail terminus. Cephalic region low to moderately low (head length 33-50% diameter), rounded, offset. Labial disc present or absent. Amphid openings pore-like, where seen opening just behind labial disc. Stylet reasonably well-developed, 9 – 15 µm long, with small basal knobs that vary from inconspicuous to distinct. Conus 30 – 50 % of stylet length. Lumen of stylet narrow, distinct. Procorpus cylindrical, leading to a well-developed rounded to rounded-rectangular median bulb with strong crescentic valve plates situated in anterior or posterior half of bulb, rarely centrally. Oesophageal gland usually dorsal, 3 – 5 body widths long; rarely with a longer dorsal and a shorter ventral lobe. Nerve ring one to two body widths posterior to median bulb. Excretory pore at level of nerve ring or up to two body widths behind it. Vulva posteriorly situated, usually at about 70 %, but ranging from 50 – 85 % of body length. Anterior vulval lip may or may not form a flap over the genital opening; with slight protrusion of lips (except in L. australis, lips flat). Vagina anteriorly directed or perpendiccular to body, with a cuticular annulus at point where it joins the uterus in some, surrounded by a relatively thick refractile tube or strong musculature in other species. Genital tract monoprodelphic, outstretched, with developing oocytes in a single column. Post vulval uterine sac less than one to four body widths long. Rectum and anus present. Tail conoid, tapering to a distinctive, offset terminus, bearing tubercles that vary in degree of development: some species with one simple tubercle with finger or knob-like protrusions; others with three to four pedunculate tubercles with or without fringed margins. However, the single tubercle of L. preissii bears many small projections, rather than four pedunculate tubercules with fringes. Thus, the structure of the terminus varies between species of Laimaphelenchus. Although the single tubercle of L. heidelbergi sp. nov. and L. preissii is obvious with the light microscope, it cannot be seen in detail without SEM. Sequencing of LSU, SSU and mtCOI genes (Zhao 2006c; Ye and Giblin-Davis, unpublished data) confirmed that L. heidelbergi sp. nov. and L. preissii belong to the genus Laimaphelenchus, and the genus has therefore been emended here. These nematodes provide a good example of the difficulties associated with the taxonomy of the aphanelenchids based on light microscopy alone, as examination with SEM is needed to detect the knob-like protrusions on the terminal tubercle that define them as Laimaphelenchus.

Discussion

This is the third record of the genus Laimaphelenchus from Australia, and the second from the common commercial forest tree, P. radiata. Laimaphelenchus heidelbergi sp. nov. was found in a rotted wood sample of P. radiata. It was not collected from non-rotting samples from the same tree that were extracted soon after collection. Part of the sample was stored in a sealed plastic bag in a constant temperature room at 16˚C. After 6 months, when a second extraction of the sample was made, the wood chips looked wet and appeared more rotten than did those of the earlier sample. We suspect that L. heidelbergi sp. nov. does not normally occur in the wood of the tree. Lichens were present on bark in the sample, and it could have fed on them. It came from the bark of the tree, like L. preissii and L. australis, the nematode could have moved into the rotted chips to feed on fungi growing in them. Laimaphelenchus heidelbergi sp. nov. was cultured successfully on Botrytis cinerea, indicating that it can feed and develop on fungi. No insects appeared to be associated with it in the samples examined.

Hunt’s (1993) diagnosis of Laimaphelenchus describes the tail as conoid, tapering to a distinctive, offset terminus, bearing four pedunculate tubercles with fringed margins. However, L. heidelbergi sp. nov., L. preissii and L. unituberculatus have a single terminus (tubercle) on the tail, bearing many small projections, rather than four pedunculate tubercules with fringes. Thus, the structure of the terminus varies between species of Laimaphelenchus. Although the single tubercle of L. heidelbergi sp. nov. and L. preissii is obvious with the light microscope, it cannot be seen in detail without SEM. Sequencing of LSU, SSU and mtCOI genes (Zhao 2006c; Ye and Giblin-Davis, unpublished data) confirmed that L. heidelbergi sp. nov. and L. preissii belong to the genus Laimaphelenchus, and the genus has therefore been emended here. These nematodes provide a good example of the difficulties associated with the taxonomy of the aphanelenchids based on light microscopy alone, as examination with SEM is needed to detect the knob-like protrusions on the terminal tubercle that define them as Laimaphelenchus.
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Acknowledgements

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