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Organisation Européenne et Méditerranéenne pour la Protection des Plantes
European and Mediterranean Plant Protection Organization

Normes OEPP
EPPO Standards

Diagnostic protocols for regulated pests
Protocoles de diagnostic pour les organismes réglementés

PM 7/39

Organization Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations

Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

Scope

EPPO Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are written according to a 'common format and content of a diagnostic protocol' agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use, etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all diagnostic protocols:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable
- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

References

EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.

FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).

IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).

IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).

OEPP/EPPO (2003) EPPO Standards PM 1/2 (12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris.

Definitions

Regulated pest: a quarantine pest or regulated non-quarantine pest.
Quarantine pest : a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

Outline of requirements

EPPO Diagnostic Protocols for Regulated Pests provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

Existing EPPO Standards in this series

Nineteen EPPO standards on diagnostic protocols have already been approved and published. Each standard is numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:

PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44

PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51

PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60

PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69
PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77
PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253
PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259
PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265
PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275
PM 7/10 (1) *Cacyreus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279
PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292
PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298
PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310
PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256
PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264
PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270
PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280
PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288
PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296

Several of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four 'contractor' diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 'intercomparison' laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostic protocols for regulated pests[1]
Protocoles de diagnostic pour les organismes réglementés

Aphelenchoides besseyi

Specific scope

This standard describes a diagnostic protocol for *Aphelenchoides besseyi*

Specific approval and amendment

Approved in 2003-09.

Introduction

Aphelenchoides besseyi (rice white-tip nematode) (Franklin & Siddiqi, 1972) infests rice and the consequent damage symptoms have given rise to its common name. It causes a characteristic whitening of the top 3–5 cm of the leaf tips, which later become necrotic, and a crinkling and distortion of the flag leaf enclosing the panicle; the latter is reduced in size, as are the grains. Symptoms may be confused with calcium and magnesium deficiency. *A. besseyi* also infests strawberries, where it is the causal agent of 'summer dwarf' or 'crimp' disease, a disease recorded from the USA, Australia and more latterly, Europe. Symptoms include leaf crinkling and distortion, and dwarfing of the plant, with an associated reduction in flowering. Symptoms may be confused with those caused by other *Aphelenchoides* species (leaf and bud nematodes).

In rice and strawberries, the nematode feeds ectoparasitically, but *A. besseyi* may also be endoparasitic, as in *Ficus elastica* and *Polianthes tuberosa*, in which it causes leaf drop and leaf lesions, respectively. On *Capsicum annuum* the infestation appears to result in rotting of the pods and premature pod drop, as in some fungal diseases. In the grass *Sporobolus poiretii*, the nematode stimulates growth, resulting in increased florescence. The nematode has also been found on *Boehmeria nivea*, numerous ornamental plants (including chrysanthemum, *Hibiscus*, and *Saintpaulia ionantha*) and grasses (*Panicum*, *Pennisetum*, and *Setaria*).

Specimens have been found in the soil of imported penjing (bonsai), but this may have arisen because of the practice of irrigating the plants in China with water from paddy fields where the nematode is a pest of rice. The nematode is capable of withstanding desiccation, and maybe found in a quiescent state beneath the hulls of rice grains. Thus there is also a risk of spread with the practice of using rice husks as packaging material, which has occurred on some imported consignments of plants.

Identity

Name: *Aphelenchoides besseyi* Christie 1942
Synonyms: *Aphelenchoides oryzae* Yokoo 1948
Asteroaphelenchoides besseyi (Christie 1942) Drozdovsky 1967
Taxonomic position: Nematoda: *Aphelenchida: Aphelenchina: Aphelenchoididae*
Bayer computer code: APLOBE
Phytosanitary categorization: EPPO A2 list no. 122; EU Annex designation: II/A1 on rice and II/A2 on strawberry.

Detection

The presence of the nematode on rice seed can be determined following EPPO Standard PM 3/38 (OEPP/EPPO, 1992). This method uses whole seeds, but recent work by Moretti *et al*. (1999) recommends the use of rice chaff or hull as an alternative testing material. Hoshino & Togashi (1999) have also described a simple extraction method said to be more efficient than the Baermann funnel technique. In foliage of other hosts, the nematode can be detected by inspecting cut leaves covered with water (the nematodes will swim into the water within 30 min if there is a heavy infestation), or by extracting nematodes from foliage using the Baermann funnel technique, or a mistifier apparatus. Both methods should be run for 48 h to detect low levels of infestation.

[1]The Figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

All developmental stages (females, males and juveniles) can be isolated from infested plant tissues or soil. Under the stereomicroscope, the round, relatively large median bulb is a distinctive feature of nematodes belonging to the Order *Aphelenchida*. These nematodes are vermiform, with most species appearing to be of stout structure and slow-moving. However, the few economically important species such as *A. besseyi* and leaf and bud nematodes tend to be of slim build, pale appearance, relatively long when compared to most other *Aphelenchoides* spp. and good swimmers (with a serpentine motion) in water (however, *A. blastophthorus*, a pest of ornamental plants and strawberries, rarely swims well and is of stouter structure).

Aphelenchoides spp. can be picked from the extract of any of these extraction methods and a temporary mount made on a glass slide. Suspect nematodes are collected into a drop of water on a slide, which is then slowly heated (to approximately 60 °C) until the nematodes become immobile. The body of *A. besseyi* killed by gentle heat is almost straight. The nematodes can be sealed on the slide with wax during this process, or they can be placed in a drop of fixative before sealing with wax. There is some difference in the appearance of water and fixed specimens (the former being preferable) but in fixed preparations some features such as stylets become more distinct. The examination then continues using a high power microscope (with objectives up to × 100 for examination under oil immersion).

Identification

Identification of *Aphelenchoides* nematodes is done using traditional microscopic techniques, measuring features characteristic for the species. While some investigation of electrophoresis and molecular diagnostic tools has been done (Ibrahim *et al*., 1994a,b), more testing needs to be done on a range of populations before a particular method can be recommended.

Order Aphelenchida

After Hunt (1993), nematodes of this Order are rarely obese except in some insect parasites. Cuticle thin, usually finely annulated and with lateral fields marked by 0 to more than 12 incisures. Cephalic region low, rounded, continuous or offset and with weak or moderate sclerotization. Four submedian cephalic papillae and six readily visible labial papillae (plus possibly six, more obscure, labial papillae) around the oral aperture. Amphidial apertures oval, pore-like, dorsosublateral on labial region. Stylet always present, length usually 10–20 µm, but exceptionally up to 185 µm; conus usually shorter than the shaft, but much longer in certain insect ectoparasitic forms. Basal swellings or knobs usually weakly developed or entirely absent. Oesophagus comprising a narrow, cylindrical procorpus, a strongly developed, offset, ovoid to rounded rectangular median bulb with crescentic valve plates and well-developed oesophageal glands forming a dorsally overlapping lobe in all genera apart from *Paraphelenchus* where the glands are small and enclosed in a nonoverlapping basal bulb. All three gland orifices (i.e. including the dorsal gland orifice) located within the median bulb. Isthmus usually short or absent. Nerve ring circumoesophageal or circumintestinal. Intestine cellular with distinct lumen. Rectum usually distinct except in some insect associates. Anus a broad transverse slit with an overhanging anterior lip, but absent or degenerate in some insect parasites or associates. Vulva posterior at 60–98%, usually in the form of a transverse slit or, exceptionally, an oval pore (*Aphelenchus*). Genital tract monoprodelphic, usually outstretched, but occasionally with a double flexure. Spermatheca axial, if present. Post-vulval sac usually present and may act as a spermatheca. Male genital system monorchic, outstretched. Sperm large, rounded, arranged in one or two rows in the gonoduct. Spicules typically rosethorn-shaped with prominent apex and rostrum, or derived therefrom, but elongate and cephalated in *Aphelenchus* and *Paraphelenchus*. Gubernaculum usually absent, but well-developed and elongate in *Aphelenchus* and *Paraphelenchus*. Bursa usually absent, but a true peloderan bursa with supporting ribs is present in *Aphelenchus* only, although some genera may have a terminal flap of cuticle (= bursa). Usually three pairs of caudal papillae present, but may number as few as two or as many as five pairs.

Characters of the genus Aphelenchoides

As with many other nematode genera, *Aphelenchoides* spp. are morphologically very similar. The following generic features help to distinguish the genus.

Length: generally from 0.2 to 1.3 mm, but most commonly 0.4–0.8 mm. *Heat-relaxed females*: die straight to ventrally arcuate. *Heat-relaxed males*: assume a 'walking-stick' shape with the tail region sharply curled ventrally. *Stylet*: very difficult to see under low power. Under high power it varies from clearly discernible to very faint. Generally about 10–12 µm long. Similarly basal knobs or swellings are sometimes clear but often indistinct. Unfortunately, measurements of these knobs have rarely been made. *Oesophagus*: procorpus cylindrical, leading to a characteristically prominent median bulb, which is usually spherical but sometimes oval, with central valve plates. *Pharyngeal gland*: overlaps the intestine, invariably on the dorsal side. *Vulva*: typically postmedian, usually between 60 and 75% of the body length. *Ovary*: monoprodelphic, typically outstretched, but may be flexed. *Post-vulval sac*: almost always present. *Tail shape*: variable. *Tail terminus*: can only be discerned at high power. The author considers that the presence or absence of mucros, and the shapes they portray, can be used to distinguish species, and is a key element in the identification of *Aphelenchoides besseyi*. A mucro is defined as a structure at the end of the tail terminus. *Spicules*: thorn-shaped, paired and separate. *Bursa*: absent.

Diagnosis of Aphelenchoides besseyi

The first step in diagnosis is to record and measure the critical morphological features on as many female specimens as are

available, ideally 20. In practice far fewer are usually available, and in such cases the nematologist should prepare the specimen(s) with great care to avoid spoiling the few features available. Males are not included in the keys presented here, but the shape and size of the spicules may assist in confirming the final identification. Sanwal (1961) produced a dichotomous key to 35 *Aphelenchoides* species that were recognized at the time, but to date over 140 species have been described. Fortuner (1970) devised a dichotomous key to *Aphelenchoides* species with star-shaped mucros, but the author considers this is no longer valid, because:

- *A. appendurus* has a leaf-like appendage, not a star-shaped mucro, and the original author did not consider it similar to any *Aphelenchoides* which had the latter character
- *A. ritzemabosi* has a box-like appendage with minute processes directed posteriorly (Web Fig. 1o,p,q), not a star-shaped mucro
- *A. silvester*, described in 1968, was not included, and neither are six other species with star-shaped mucros described since the key by Fortuner was published.

As *A. besseyi* can occur in both soil, foliage and rice husks, one has to consider all other *Aphelenchoides* nematodes that may be found. Unfortunately, many of these nematodes are difficult to identify because there is little to distinguish between them, a problem that is not facilitated by poor descriptions of the species themselves. Indeed, several authors have improved the original description for *A. besseyi*. In addition, studies on this species have shown the degree of variation in measurements made on populations from different hosts (Appendix 1).

Hockland (2002) has therefore attempted to reduce the number of comparisons that need to be made by selecting only those *Aphelenchoides* species that also have a star-shaped mucro, together with those pest species that might also be encountered in foliage. A polytomous key and a dichotomous key are included for ring testing. It should be noted that the following procedure relies heavily on the original descriptions and drawings of species which at times can appear contradictory. For example, the tail shape for *A. aligarhiensis* is described as elongate-conoid, but the accompanying drawing does not infer this. There is also no accompanying value for 'c', which is an indicator of tail shape, being the value of tail length divided by body width at anus. Similarly, the excretory pore for *A. jonesi* is said to be opposite the nerve ring, but the accompanying drawing infers that it is posterior to the nerve ring. In such cases the written description has been the one included in this protocol. Where possible, original data has been supplemented by additional published information for the most commonly encountered species.

Polytomous key

Several key features have been selected to produce a small polytomous key (Table 1). The selected features have been given codes: the shape of the mucro (A), pvs. length (B), tail shape (C), stylet length (D), the number of lateral lines (E) and the relative positions of the excretory pore and nerve ring (F). Supplementary drawings of the types of mucros and tail shapes are provided in Web Figs 1 and 2 to aid categorization.

Table 1 Polytomous key for some of the key characters distinguishing *Aphelenchoides besseyi*

Species	Code					
	A	B	C	D	E	F
A. besseyi	1	1	1	1	1	1
A. hylurgi	1	1	1	1	4	3
A. unisexus	1	1	1/3	1	3	3
A. asteromucronatus	1	1	1/3	2	1	3
A. siddiqii	1	1	3/4	1	1	1/2/3
A. asterocaudatus	1	1/2	1	1	3	3
A. andrassyi	1	1/2	2/3	1/2	2	4
A. wallacei	1	1/2	3	3	1	1
A. goodeyi	1	2	1	1	1	3
A. lichenicola	1	2	1	1/2	1	2/3
A. silvester	1	2	1	1/2	1	4
A. jonesi	1	2	1	1/3	1	2
A. brevistylus	1	2	1/2	2	3	1
A. aligarhiensis	1	2	2/3	1	1	1
A. blastophthorus	2	2	1/2	3	1	2
A. ritzemabosi	4	2	2	1	1	3
A. fragariae	2/4	2	2	1	3	2/3

Note. *A. nonveilleri* and *A. saprophilus*, mentioned in previous editions of this key, are now considered by the author to be *species indeterminatae*.

Each suspect specimen should therefore be examined and assigned a set of codes according to the following categorization:

- A mucro shape: 1 = star (Web Fig. 1 a–f); 2 = single terminal mucro (Web Fig. 1g–m); 3 = bifurcate (Web Fig. 1n); 4 = other (Web Fig. 1o–t); 5 = no mucro (Web Fig. 1 μ–v)
- B length of the postvulval sac (pvs): 1 = pvs length 33% or less the distance between the vulva and the anus; 2 = pvs length more than 33% the distance between the vulva and the anus; 3 = no pvs
- C tail shape: 1 = conoid: shape of a cone, with both sides of the tail surface tapering at an equal angle to the tail tip. Total length not exceeding × 5 anal body width (Web Fig. 2a); 2 = elongate-conoid: an elongated cone, with a length × 5 or more anal body widths long (Web Fig. 2b,c); 3 = dorsally convex-conoid: tail shape that at its first appearance is curved ventrally. The dorsal side of the tail is curved in a convex manner before it joins with the ventral surface. The ventral surface is usually concave, but from some viewpoints may appear straight. It may be any length (Web Fig. 2d,e); 4 = subcylindroid: both sides of the tail appear to run parallel for most of their length, and end with a hemispherical or subhemispherical tail tip (Web Fig. 2f).
- D stylet length (μm): 1 = 10–13; 2 = less than 10; 3 = more than 13
- E lateral lines (number of): 1 = 4 lines; 2 = 3 lines; 3 = 2 lines; 4 = unknown
- F relative position of the excretory pore and nerve ring: 1 = excretory pore anterior to, or level with, the anterior level of the nerve ring (Web Fig. 3 a,b); 2 = excretory pore level with the nerve ring (Web Fig. 3c); 3 = excretory pore posterior to, or opposite the posterior level of the nerve ring (Web Fig. 3d,e).

Table 2 Dichotomous key to female *Aphelenchoides* spp.

1.	Star-shaped mucro	2
	No star-shaped mucro	*A. besseyi*
2.	Pvs up to 33% of distance from vulva to anus	3
	Pvs more than 33% of distance from vulva to anus	*A. aligarhiensis*, *A. brevistylus*, *A. lichenicola*
3.	Tail shape conoid or elongate conoid	4
	Tail shape subcylindrical	*A. siddiqii*
4.	Stylet length 10–12.5 µm	5
	Stylet length outside this range	*A. asteromucronatus*, *A. hylurgi*, *A. wallacei*
5.	Four lateral lines	6
	Less or more than four lateral lines	*A. andrassyi*, *A. asterocaudatus*, *A. unisexus*
6.	Excretory pore anterior to, or level with, anterior level of nerve ring	*A. besseyi*
	Excretory pore level with nerve ring	*A. goodeyi*, *A. jonesi*, *A. silvester*

The set of codes obtained should then be compared with those set out in Table 1, which should allow a provisional diagnosis to be made.

As with all identifications involving the use of morphological characters, the combination of several key features is the key to a positive diagnosis. In the polykey there is some overlap of codes, and users are advised to refer to original descriptions if in doubt about a diagnosis, or refer to the database (Table 3) for further guidance. Alternatively, a dichotomous key, using the same set of characters, may also help to determine the identity of the nematode.

Dichotomous key

The author offers a short dichotomous key (Table 2) especially for this protocol. Only characters from female nematodes have been considered. This is complemented by Web Figs 1–3, showing critical features, and Table 3, giving more details of those *Aphelenchoides* spp. that also have a star-shaped mucro, together with those pest species that might also be encountered in foliage.

Description of Aphelenchoides besseyi

This description is modified from Hunt (1993). Web Fig. 4 illustrates the main diagnostic features of the species.

Female: body slender, straight to slightly arcuate ventrally when relaxed. Cephalic region rounded, unstriated, slightly offset and wider than body at lip base. Lateral fields about one-fourth as wide as body, with four incisures. Median oesophageal bulb oval, with a distinct valvular apparatus slightly behind its centre. Excretory pore usually near anterior edge of nerve ring. Post-vulval sac narrow, inconspicuous, not containing sperms, 2.5–3.5 times anal body width but less than one-third the distance from the vulva to the anus. Tail conoid 3.5–5 anal body widths long. Terminus bearing a mucro of diverse shape with three to four pointed processes.

Male: about as numerous as females. Posterior end of body curved by about 180° in relaxed specimens. Tail conoid, with terminal mucro with two to four pointed processes. Spicules typical of the genus except that the proximal end lacks an apex and has only a moderately developed rostrum.

Possible confusion with similar species

The star-shaped mucro is a very distinctive feature possessed by only 16 other species described in the genus. These species can be distinguished from *A. besseyi* by following the guidance given in the keys. Those species that remain indistinguishable apart from the position of the excretory pore, a feature often difficult to see, can be further distinguished by additional features: *A. goodeyi* has a convex-conoid tail and is generally shorter in its body length (0.46–0.61 mm compared with 0.57–0.88 mm for *A. besseyi*); *A. jonesi* generally has a lower value for 'a' (20–28, compared with 26–58) and a slightly higher range of values for its stylet length (11–14 µm, compared with 10–12.5 µm); *A. silvester* has a convex-conoid tail shape, a slightly lower value for 'b' (8–9.7, compared with 9–13.1) and is generally shorter in body length (0.48–0.56 mm, compared with 0.57–0.88 mm). Males are commonly found when *A. besseyi* is numerous, but they have not been described for *A. goodeyi*, *A. jonesi* or *A. silvester*.

Apart from the shape of the mucro, *A. besseyi* differs from other plant-parasitic species of this genus occurring in strawberries (*A. blastophthorus*, *A. fragariae* and *A. ritzemabosi*) as follows: *A. besseyi* has a postvulval sac that is always less than a third of the distance from the vulva to the anus, whereas those of the other species are longer than this; the tail of *A. besseyi* has a conoid shape, similar to *A. blastophthorus,* but shorter than that of *A. fragariae* and *A. ritzemabosi*, which tend to be elongate-conoid; the excretory pore is usually positioned near the anterior edge of the nerve ring in *A. besseyi*, whereas in the other species it is either level with or posterior to the nerve ring; the spicules of *A. besseyi* are distinctive in that the proximal end lacks a dorsal process (or apex) and only a moderately developed ventral one (rostrum), whereas those of *A. blastophthorus* are comparatively large for the genus, have a rather stout dorsal limb which is characteristically flattened about mid-way along its arch, its distal end is curved ventrally to give it a hooked or knobbed appearance, and the apex and rostrum are pronounced structures, those of *A. fragariae* have a moderately developed apex and rostrum, whilst the smoothly curved spicules of *A. ritzemabosi* seem to lack a dorsal or ventral process at all.

Table 3 Database of morphological characters for *Aphelenchoides besseyi* and selected species

Species	mucro shape	length of pvs	tail shape	position of excretory pore and nerve ring	a	b	length(mm)	stylet	L.L.	c
A. aligarhiensis	star	× 5.5 body width, extending to more than 50% of v-a distance	elongate-conoid	excretory pore slightly anterior to nerve ring	25–35	7.0–9.0	0.50–0.70	10	4	13–22
A. andrassyi	star	× 3 vulval body width	elongate conoid	not possible to advise	23–28	3.2–3.9	0.39–0.44	9.0–10.0	3	6.0–12.0
A. asterocaudatus	star	× 2 vulval body width	conoid	excretory pore posterior to nerve ring	24.6	9.6	0.62	12	2	16
A. asteromucronatus	star	× 1 vulval body width or less	conoid	excretory pore at level of lower edge of nerve ring	32–39	5.5–9.5	0.39–0.54	9	4	10.9–14.5
A. besseyi – (author)	star	2.5–3.5 anal body width	conoid	excretory pore usually	32–42	10.2–11.4	0.66–0.75			17–21
(all descriptions)		but less than 33%	conoid	near the anterior edge	26.6–58	9.0–13.1	0.57–0.88	10–12.5	4	13.8–21
		v-a distance		of the nerve ring						
A. blastophthorus	single terminal mucro	approx. 50% v-a distance	conoid	excretory pore approx. opposite nerve ring	32–47	9.3–11	0.68–0.9	15–19.5	4	16–21
A. brevistylus	star	33–66% v-a distance	conoid	excretory pore anterior to nerve ring	29.1–35.0	8.1–10.5	0.39–0.63	6.0–8.0	2	11.1–15.7
*A. fragariae**	no mucro behind the nerve ring	more than 50% v-a distance	elongate conoid	excretory pore level with or close behind nerve ring	45–63	8–15	0.45–0.80	10–11	2	12–20
A. goodeyi	star	× 3 vulval body width	convex-conoid	excretory pore close behind nerve ring, level with its hind edge	29–39	n/a	0.46–0.61	11.5–12.5	4	14–18
A. hylurgi	star	× 1.5 body width	conoid	excretory pore slightly posterior to nerve ring	26.6	10.2	0.57	13	obscure	14.7
A. jonesi	star	nearly × 2 body widths long. Nearly 50% v-a distance	conoid	excretory pore opposite nerve ring	20–28	9.3–11.0	0.72–0.99	11.0–14.0	4	16–22
A. lichenicola	star	50% or more v-a distance	elongate-conoid	excretory pore opposite or close to posterior margins of the nerve ring	32–43	7.0–10.5	0.53–0.69	9.5–10	4	15–17
A. nonveilleri	star	× 3 body width	conoid	not known	31	n/a	0.597	12.8	3	17
A ritzemabosi	tail terminus invariably ending in a box-like structure which has two to four minute processes pointing posteriorly	50% or more the v-a distance	elongate conoid	excretory pore 0.5–2 body widths posterior to nerve ring	40–45	10–13	0.77–1.2	12	4	18–24
A. siddiqii	star	× 0.5–1.0 body width	subcylindrical	excretory pore usually opposite the posterior margins of the nerve ring, sometimes reaching near its anterior margins	26.66–38.88	7.14–9.72	0.37–0.70	11–12.5	4	14.11–19.64
A. silvester	star	no information	convex-conoid	no information	37–38	8.0–9.7	0.48–0.56	9.5–10	4	15–16
A. unisexus	star	20%–33% v-a distance	conoid	excretory pore at the level of the posterior margin of the nerve ring, or just posterior to it.	30–36.9	7.9–10.2	0.48–0.76	10.0–11.0	2	13.0–17.5
A. wallacei	star	× 2 body widths long	convex-conoid	excretory pore anterior to the nerve ring	22–23	7.8–8.5	0.69–0.73	13.5–14	4	15–17

*Data from more than one source.

Requirements for a positive diagnosis

The procedures for detection described in this protocol should have been followed. Measurements should be made of the diagnostic features listed as characteristic. As many individuals as possible (up to 20) should be examined to increase confidence in the data, but in phytosanitary work it is recognized that few specimens may be available. Comparison should be made with codes listed in the polytomous key, or the procedure for the dichotomous key should have been followed. Confirmation of the identification of *A. besseyi* should be by comparison with the features listed in the database. Any confusion with other species should be resolved by referring to this species comparison chart or the original descriptions.

Report on the diagnosis

A report on the execution of the protocol should include:

- information and documentation on the origin of the infested material
- description of the damage symptoms
- measurements and drawings or photographs of the nematode
- an indication of the magnitude of the infestation
- comments as appropriate on the certainty or uncertainty of the identification.

Permanent slides and/or culture of the nematodes may also be required.

Further information

Further information on this organism can be obtained from: Dr D. Hunt, CABI-Bioscience, Egham TW20 9TY, UK; Prof. Dr G. Karssen, Plant Protection Service, Ministry of Agriculture, Nature Management and Fisheries, 15 Geertjesweg, P.O. Box 9102, 6700 HC Wageningen, Netherlands.

Acknowledgements

This protocol was originally drafted by: S. Hockland, Central Science Laboratory, York (GB).

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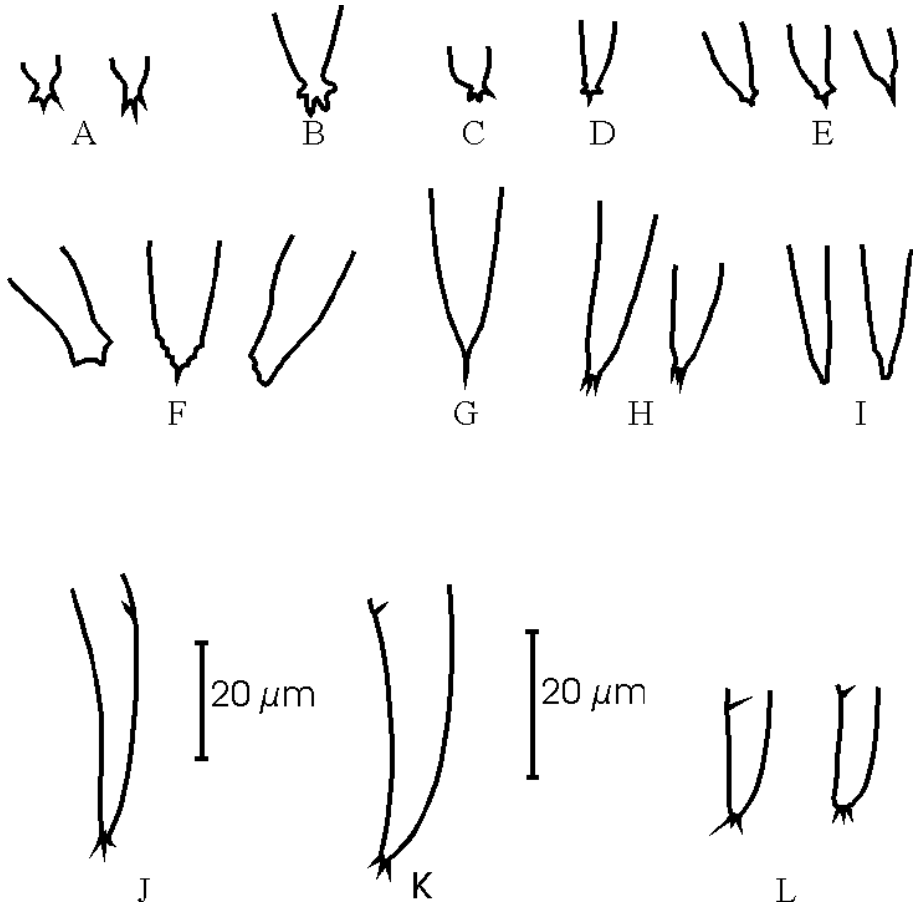
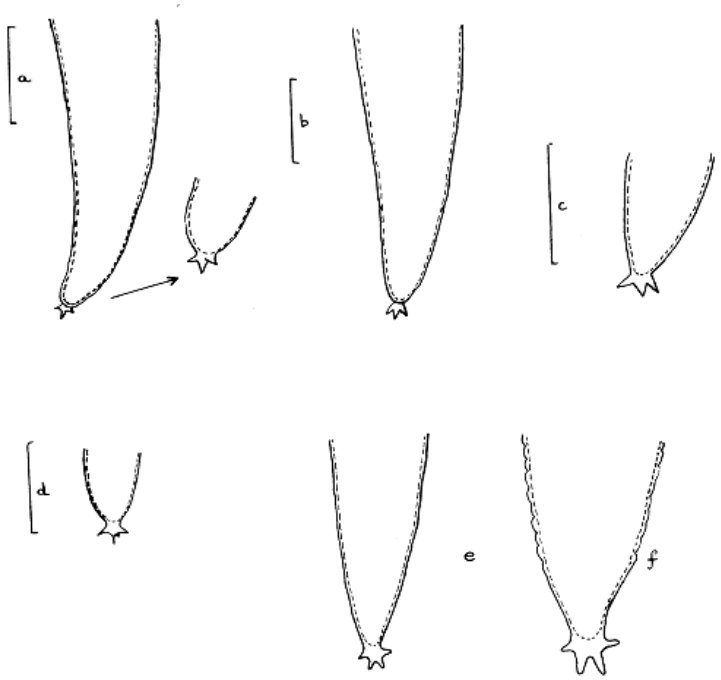
Appendix 1 Supplementary drawings to aid categorisation of mucro type and tail shape (scale measurements not provided on many descriptions)

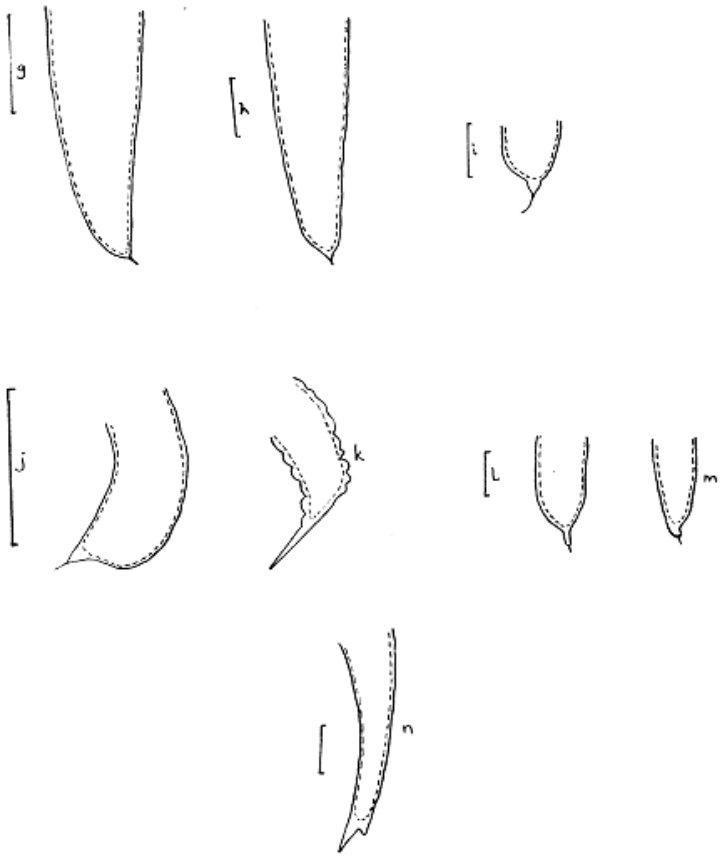


'star-shape' mucros:
a *besseyi* **b** *nonveilleri* **c** *siddiqii* **d** *unisexus* **e** *lichenicola* (described as star-shape, though variants may have an axial ray more prominent than others)
'leaf-like' mucros: f *appendurus* **'single terminal' mucro: g** *blastophthorus*
'box-like' mucros: h *ritzemabosi* **no mucro: i** *fragariae*
tail shapes: conoid: j *besseyi* **elongate-conoid: k** *aligarhiensis* (described as such by the original description, but could be considered conoid from associated drawing (no 'c' values available) **sub-cylindrical: l** *siddiqii*

Fig. 1 Tail terminus types of *Aphelenchoides* species (After Hockland, 2002).
(a) - (f): star shape. a. *A. aligarhiensis* b. *A. asterocaudatus* c. *A. besseyi* d. *A. goodeyi* (all scale bars 10µm) e. and f. *A. nonveilleri* (x 1100 and x2200 respectively)



(g) - (m): single terminal mucro: g. *A. richardsoni* h. *A. nechaleos* i. *A. vaughani* j. *A. tsalolikhini* l. and m. *A. submersus*
(n): bifurcate: *A. bicaudatus* (all scale bars 10µm)



(o) - (t): other: o.p.q. *A. ritzemabosi* r. *A. sphaerocephalus* s. *A. gynotylurus* t. *A. helicosoma* (scale bars all 10µm)

(u) – (v): no mucro: u. *A. microstylus* (scale bar 10µm) v. *A. obtusus* (x1,250)

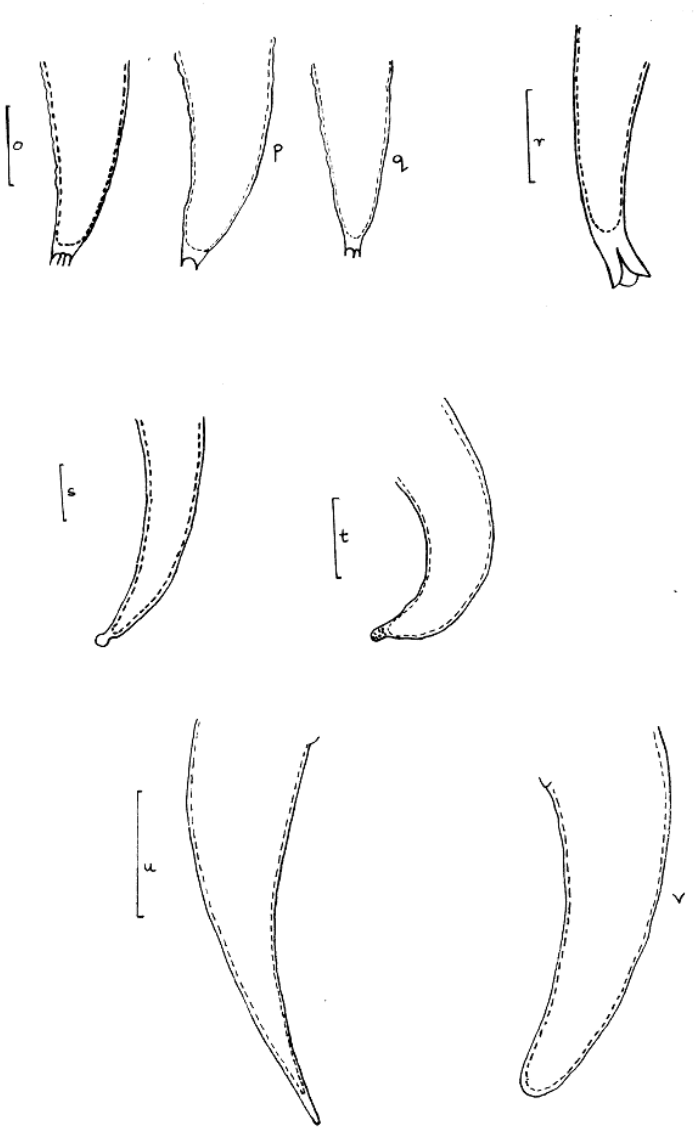


Fig. 2 Drawings of tail shapes in *Aphelenchoides* species.
1. conoid: a. *A. blastophthorus* **2. elongate-conoid:** b. *A. andrassyi* (no scale bar) c. *A. chalonus* **3. dorsally convex-conoid:** d. *A. fluviatilis* (x 1100) e. *A. franklini* **4. sub-cylindroid:** f. *A. subtenuis* (scale bars = 10μm)

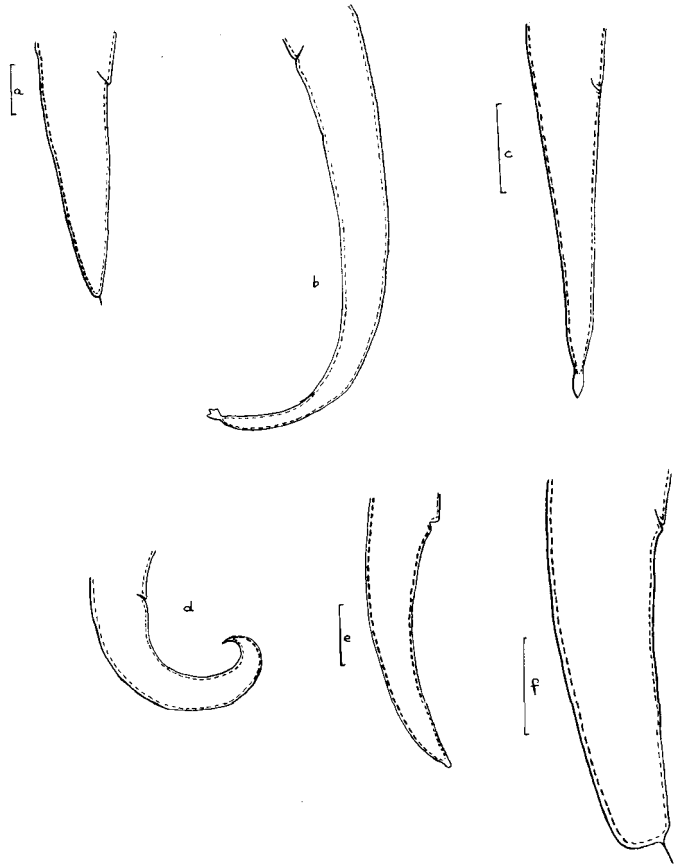


Fig. 3 Positions of the excretory pore relative to the nerve ring in *Aphelenchoides* species.
1. Excretory pore is anterior to, or level with the anterior edge of the nerve ring: a. *A. longiurus* b. *A. blastophthorus*
2. Excretory pore is level with the nerve ring (from behind the anterior point to in front of the posterior point): c. *A. cibolensis*
3. Excretory pore is level with the posterior edge of the nerve ring (d), or posterior to it (e): d. *A. arcticus* e. *A. ritzemabosi* (scale bar = 10μm)

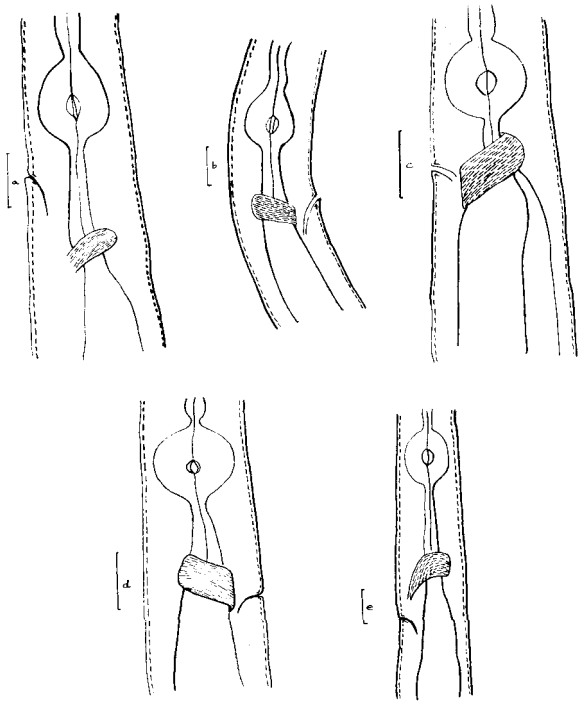
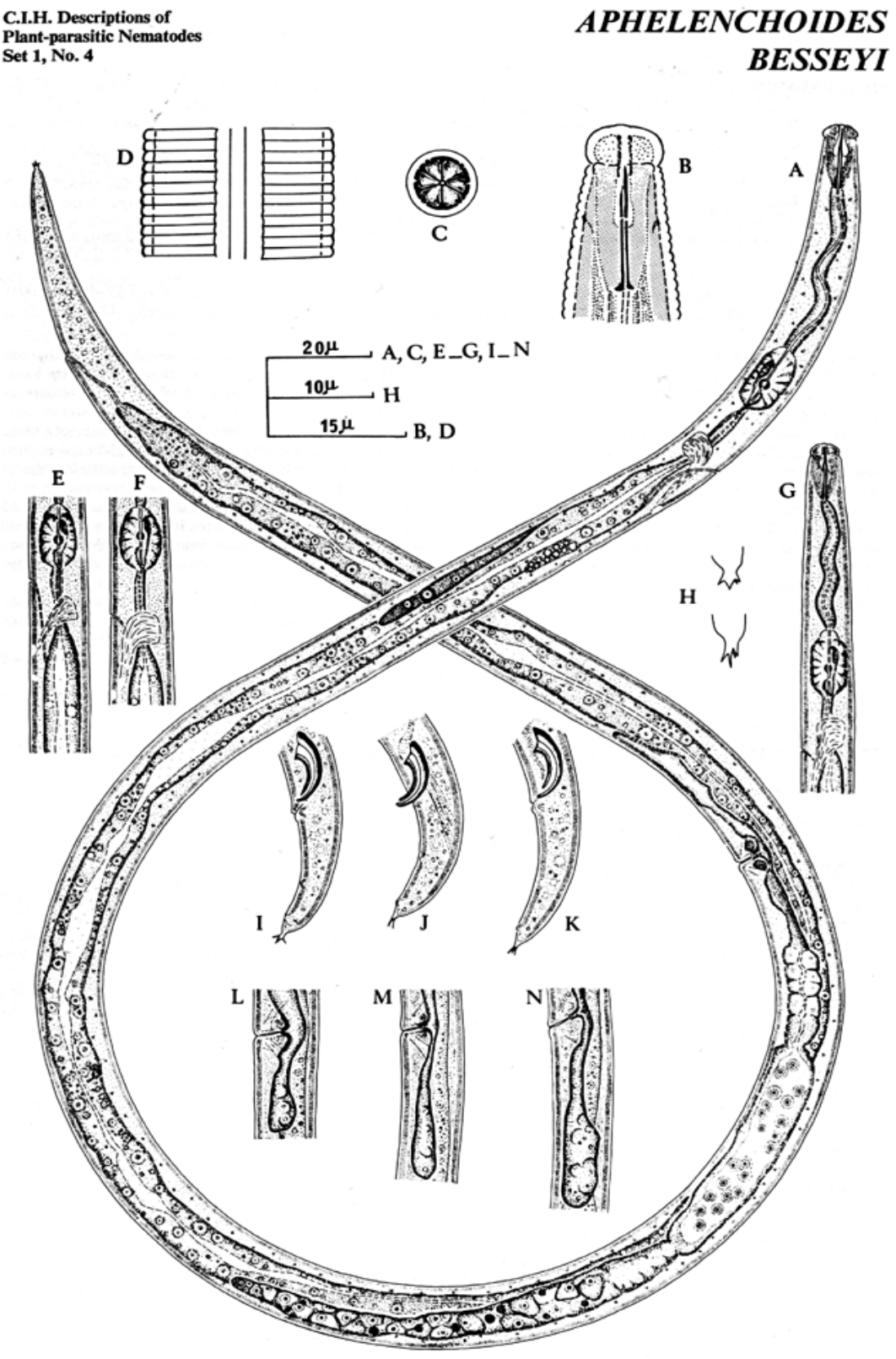


Fig. 4 Description of *Aphelenchoides besseyi*



Aphelenchoides besseyi Christie. A. Female. B. Female head end. C. Female *en face* view. D. Lateral field. E & F. Variation in female median oesophageal bulb and position of excretory pore with respect to nerve ring. G. Male anterior end. H. Female tail termini showing variation in shape of mucro. I–K. Male tail ends. L–N. Variation in post-vulval uterine sac. (B and D original: the rest after Fortuner, 1970.)