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ANNEX 17

ENG

DIAGNOSTIC PROTOCOLS INTERNATIONAL STANDARD FOR PHYTOSANITARY MEASURES 27

DP 17: *Aphelenchoides besseyi*, *A. fragariae* and *A. ritzemabosi*

Produced by the Secretariat of the
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ISPM 27
Diagnostic protocols for regulated pests

DP 17: *Aphelenchoides besseyi, A. fragariae* and *A. ritzemabosi*

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2. Pest Information

Aphelenchoides species occur worldwide (Fortuner and Williams, 1975; CABI, n.d.). The majority of species within the genus *Aphelenchoides* Fischer, 1894 are mycetophagous, but a small group including *A. besseyi* (Christie, 1894), *A. fragariae* (Ritzema Bos, 1891) and *A. ritzemabosi* (Schwartz, 1911) also feed on higher plants. To date, 180 species of *Aphelenchoides* (and 19 of uncertain status) have been described (Sánchez-Monge *et al*., 2015). Plant-feeding *Aphelenchoides* species have the ability to survive unfavourable conditions in a quiescent stage. The members of this group are called foliar,leaf or bud nematodes because they are common and widespread parasites on these parts of plants. They are migratory ectoparasites and endoparasites of leaves, buds, stems and very occasionally corms, causing crinkling, blotching and reduced size of the leaves, resulting in a reduction of quality and yield of many ornamental and crop plants such as *Oryza sativa* (rice), *Fragaria* spp. (strawberry) and *Chrysanthemum* spp. (Hunt, 1993). It is important to identify the particular species in the infestation as the life cycle of each species is slightly different.

A. besseyi is known as the causal agent of white tip disease on its major host, *O. sativa*, wherever this host occurs worldwide. However, the nematode also infests *Fragaria* spp., where it is a cause of crimp disease recorded from Australia, the United States and more recently Europe. Other crops recorded as infested include grasses (*Panicum, Pennisetum* and *Setaria*), ornamentals (e.g. *Begonia* and *Chrysanthemum*) and vegetables (e.g. *Allium* and *Dioscorea*) (CABI, n.d.). The nematode was recently also identified as the causal agent of black spot disease on *Phaseolus vulgaris* (bean) (Chaves *et al*., 2013).

Plants of *O. sativa* susceptible to *A. besseyi* can be symptomless, but yield or quality loss occurs when symptoms are visible. Seed infested with *A. besseyi* has lowered viability and delayed germination (Tamura and Kegasawa, 1959), and diseased plants have reduced vigour and height (Todd and Atkins, 1958). The nematode is capable of withstanding desiccation and may be found in an anhydrobiotic state beneath the hulls of grains of *O. sativa*.

As with some other *Aphelenchoides* species, *A. besseyi* may be found between leaves and buds in *Fragaria* spp. and may cause distortion of the leaves, which is more noticeable on newly formed leaves after growth resumes in spring (Brown *et al.*, 1993). On *Fragaria* spp., *A. besseyi* appears in summer and is called the "summer crimp nematode" (Esser, 1966). It is a parasite of warm regions; according to EPPO/CABI (1997), *A. besseyi* is not found beyond 43° north latitude on rice or beyond 40° north on strawberries grown outdoors.

A. fragariae is an endoparasite and ectoparasite of the aerial parts of plants, and it has an extensive host range – more than 250 plant species in 47 families – and it is widely distributed in temperate and tropical regions throughout the world (EPPO, n.d.).

A. fragariae is a causal agent of crimp or spring dwarf disease on *Fragaria* spp. and can also cause serious damage to many other agricultural and ornamental crops, including ferns, foliage and flowering plants, and herbaceous and woody perennials (Kohl, 2011). *A. fragariae* is commonly found in the aerial parts of plants, corms and soil or growing media associated with host plants. It can be detected on leaves showing discoloured mosaic or angular spots. *A. fragariae* is responsible for an economic loss of millions of dollars each year in the ornamental nursery industry (Jagdale and Grewal, 2006). This nematode feeds on the epidermis, mesophyll and parenchyma tissues of leaves or fronds, resulting in chlorosis or vein-delimited lesions that turn necrotic, and in defoliation over time. In the absence of plant residues or wild host plants, *A. fragariae* can survive a few months in the soil (Ambrogioni and Greco, 2014). The nematode can be distributed over long distances in shipments of asymptomatic infested plants.

A. ritzemabosi is an obligate plant parasite, inhabiting leaves, buds and growing points. It may feed endoparasitically on mesophyll cells of leaves or ectoparasitically on buds and growing points (Siddiqi, 1974). Like *A. fragariae*, these nematodes do not enter the stem tissue but move within a water film on the surface to reach the leaves and buds. The leaves are invaded through the stomata. The nematodes

feed on and destroy the mesophyll cells, resulting in angular leaf spot in several hosts, and also causing dwarfing and leaf wilt. The cells in infested areas die and the leaves develop brown lesions delimited by the veins (Franklin, 1978). The nematodes exit the leaves through the stomata and once again migrate in a water film to infect flower buds (Southey, 1993).

A. ritzemabosi is rarely encountered in soil, where it cannot complete its life cycle or survive the winter. The nematode overwinters in dormant buds and growing points of *Chrysanthemum* spp. stools, which serve as a source of infestation (Hesling and Wallace, 1960). *A. ritzemabosi* survives unfavourable conditions through anhydrobiosis and can retain viability for some months in dried plant material. Like other *Aphelenchoides* species, *A. ritzemabosi* can reproduce on fungi, and soil fungi may therefore contribute to its survival in the absence of a host (Hooper and Cowland, 1986).

A. ritzemabosi was found in association with *Phytophthora cryptogea* on diseased *Gloxinia* plants (Stokes and Alfieri, 1969) and is linked with *Corynebacterium fascians* in the onset of "cauliflower disease" in strawberries (Crosse and Pitcher, 1952). Madej *et al.* (2000) found several plant-parasitic fungi in association with *A. ritzemabosi* on *Chrysanthemum* and *Zinnia* plants affected by this nematode, which increased the necrotic symptoms observed, i.e the number of necrosis.

A. ritzemabosi is a major pest of *Chrysanthemum* spp. in Australia, Europe, New Zealand and North America, and it has been reported on this host from several other countries (CABI/EPPO, 2000; EPPO, n.d.). Both *A. ritzemabosi* and *A. fragariae* cause damage to *Fragaria* spp. in several European countries as well as in Mexico (CABI/EPPO, 2000; EPPO, n.d.). The nematode has been recorded on a wide range of ornamental and other hosts from Asia, Europe, North America, Oceania and South America (CABI/EPPO, 2000; EPPO, n.d.) – about 200 host plant species according to Escuer and Bello (2000) and McCuiston (2007). *A. ritzemabosi* was reported as occurring in South Africa by Wager in 1972, but these records were made on the basis of symptoms only and the nematodes were not positively identified taxonomically. The first report of *A. ritzemabosi* in South Africa that was morphologically identified was on *Nerine* bulbs in nurseries (Swart *et al.*, 2007).

2. Taxonomic Information

Name:	*Aphelenchoides besseyi* Christie, 1942
Synonyms:	*Aphelenchoides oryzae* Yokoo, 1948; *Asteroaphelenchoides besseyi* (Christie, 1942) Drozdovsky, 1967
Taxonomic position:	Nematoda, Rhabditida, Tylenchina, Aphelenchoidea, Aphelenchoididae, Aphelenchoidinae, *Aphelenchoides*
Common names:	Preferred common name: rice leaf nematode (CABI, n.d.); other common names: summer crimp nematode, white tip, white tip nematode of rice (CABI, n.d.)
Name:	*Aphelenchoides fragariae* (Ritzema Bos, 1890) Christie, 1932
Synonyms:	*Aphelenchus fragariae* Ritzema Bos, 1891; *Aphelenchus olesistus* Ritzema Bos, 1893; *Aphelenchoides olesistus* (Ritzema Bos, 1893) Steiner, 1932; *Aphelenchus olesistus* var. *longicollis* Schwartz, 1911; *Aphelenchoides olesistus* var. *longicollis* (Schwartz, 1911) Goodey, 1933; *Aphelenchus pseudolesistus* Goodey, 1928; *Aphelenchoides pseudolesistus* (Goodey, 1928) Goodey, 1933; *Aphelenchus ormerodis* Jegen, 1920 (nec. Ritzema Bos, 1891)
Taxonomic position:	Nematoda, Rhabditida, Tylenchina, Aphelenchoidea, Aphelenchoididae, Aphelenchoidinae, *Aphelenchoides*
Common names:	Strawberry spring dwarf nematode, strawberry crimp nematode

Name: *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner and Buhrer, 1932

Synonyms: *Aphelenchoides ribes* (Taylor, 1917) Goodey, 1933; *Aphelenchus phyllophagus* Stewart, 1921; *Aphelenchus ribes* (Taylor, 1917) Goodey, 1923; *Aphelenchus ritzema-bosi* (Schwartz, 1911); *Pathoaphelenchus ritzemabosi* (Schwartz, 1911) Steiner, 1932; *Pseudaphelenchoides ritzemabosi* (Schwartz, 1911) Drozdovski, 1967; *Tylenchus ribes* Taylor, 1917

Taxonomic position: Nematoda, Rhabditida, Tylenchina, Aphelenchoidea, Aphelenchoididae, Aphelenchoidinae, *Aphelenchoides*

Common names: Chrysanthemum foliar nematode, leaf and bud nematode

3. Detection

3.1 Symptoms produced by the nematodes on host plants

A. besseyi, *A. fragariae* and *A. ritzemabosi* may occasionally be found in the growing media of infested hosts, but are most commonly found in infested plant foliage, including leaves, flowers, buds, and seed heads or pods. Symptoms of infestation by these nematodes vary according to the host.

3.1.1 Symptoms of *Aphelenchoides besseyi*

During early growth of *O. sativa*, the most conspicuous symptom caused by this nematode is the emergence of the chlorotic tips of new leaves from the leaf sheath (Figure 1(A)). These tips later dry and curl, while the rest of the leaf may appear normal. The young leaves of infested tillers can be speckled with a white splash pattern or have distinct chlorotic areas. Leaf margins may be distorted and wrinkled but leaf sheaths are symptomless. The flag leaf enclosing the panicle crinkles and distorts, and the panicle is reduced in size, as are the grains. Symptoms may be confused with calcium and magnesium deficiency. Infested panicles are shorter than normal panicles, with fewer spikelets and a smaller proportion of filled grain (Dastur, 1936; Yoshii and Yamamoto, 1951; Todd and Atkins, 1958). In severe infestations, the shortened flag leaf is twisted and can prevent the complete extrusion of the panicle from the boot (Yoshii and Yamamoto, 1950; Todd and Atkins, 1958). The panicles also often stay erect (Liu *et al.*, 2008) and discoloration can be observed on them (CABI, n.d.). The grain is small and distorted (Todd and Atkins, 1958) and the kernel may be discoloured and cracked (Uebayashi *et al.*, 1976) (Figure 1(B)). Infested plants mature late and have sterile panicles borne on tillers produced from high nodes.

On *Fragaria* spp., *A. besseyi* is the causal agent of "summer dwarf disease" (Perry and Moens, 2006). Symptoms include leaf crinkling and distortion, and dwarfing of the plant with an associated reduction in flowering (Figure 1(C)). Symptoms may be similar to and therefore confused with those caused by other *Aphelenchoides* species, emphasizing the importance of correct identification.

In *O. sativa* and *Fragaria* spp., *A. besseyi* feeds ectoparasitically, but the nematode may also be endoparasitic, as in *Ficus elastica* and *Polianthes tuberosa*, in which it causes leaf drop and leaf lesions, respectively. On *Capsicum annum* var. *longum* the infestation appears to result in rotting of the pods and premature pod drop, similar to some fungal diseases (Hockland and Eng, 1997). In the grass *Sporobolus poirettii*, this nematode stimulates growth, resulting in increased flowering.

3.1.2 Symptoms of *Aphelenchoides fragariae*

Common symptoms of plants damaged by *A. fragariae* are chlorosis; necrosis; distortion, deformation and dwarfing of the leaves, stems, flowers or bulbs; leaf tattering; and defoliation. The symptoms are often confused with symptoms caused by powdery mildew. Symptoms typically manifest as vein-delimited lesions or blotches that start as lightly chlorotic and then turn brown to black or necrotic and dry (Figure 2). Symptom expression, however, may be highly variable as a result of the different

characteristics of host plant species and the influence of environmental conditions. Infested plants sometimes do not exhibit symptoms until the plant is heavily infested with nematodes.

The shape and pattern of the blotches is closely related to the venation pattern of the leaf, the infested leaves appearing pale green to tan in colour or showing dark brown mosaic spots or angular necrotic lesions (Figure 3) (Knight *et al*., 2002; Khan *et al*., 2008; Kohl, 2011). On *Hosta* spp., leaf blotch symptoms appear as long, narrow necrotic patches bounded by longer veins, and in severe cases, the entire leaf dries and dies (Figure 4) (Zhen *et al.*, 2012). The leaf blotch symptoms on ferns appear as narrow, linear patches perpendicular to the midrib of the frond, corresponding to closely spaced lateral veins, as chevron-like stripes (Figure 5) (Cobon and O'Neill, 2011). On *Cyclamen* spp., *Begonia* spp. and *Andrographis paniculata*, infested leaves show water-soaked irregular patches that later turn brown (Figure 6) (Southey, 1993; dan Supriadi, 2008). In general, the blotches form more or less angular chlorotic areas in ternate or palmate leaves with reticulate venation or with main veins radiating from the petiole–lamina junction, while infected thicker and succulent leaves initially show water-soaked irregular patches that subsequently become necrotic without defined margins; ultimately, the entire leaf dies (Richardson and Grewal, 1993; Southey, 1993). On *Fragaria* spp., the initial symptoms of infestation are stunted plant growth and deformation of buds, leaves and flowers; infested plants show malformations including twisting and puckering of leaves, discoloured areas with hard and rough surfaces, undersized leaves with crinkled edges, tight aggregation or death of crowns, reddened and stunted petioles, and flower stalks with aborted or partly aborted flowers (Figure 7). Heavily infested plants do not produce fruit (Siddiqi, 1975).

For plants in general, *A. fragariae* infests buds or the crown, causing buds to decay, flowers to shrivel, and leaves, petioles and stems expanding from the infested buds to become misshapen, crinkled and stunted and to develop brown scars (Richardson and Grewal, 1993; Southey, 1993).

3.1.3 Symptoms of *Aphelenchoides ritzemabosi*

On *Chrysanthemum* spp., infestation from the soil, dead leaves or weed hosts progresses from the base of the plant upwards under moist conditions. Infested leaves show characteristic angular blotches delimited by the principal veins. The discoloration progresses from translucent yellowish and brownish green to dark brown. At a late stage, dead shrivelled leaves, hanging down, extend to the top of the plant (Figure 8). Although some stems of a given plant may bear dead leaves, other stems may be symptomless. The nematodes also invade and feed within the buds, sometimes killing the growing point and preventing flowering or producing malformed leaves with surface irregularities and rough brown scars.

On *Fragaria* spp., damage is most noticeable on newly formed leaves, which become puckered and distorted and may show rough, greyish feeding areas near the base of the main veins. The cauliflower disease of *Fragaria* spp., resulting in the continued production of axillary buds on affected crowns, was experimentally induced in *Fragaria* spp. runners through co-inoculation of *A. ritzemabosi* and *C. fascians* by Crosse and Pitcher (1952).

A. ritzemabosi causes polygonal blotches that are bound by veins on leaves of infested plants of *Nicotiana* spp., similar to symptoms on *Chrysanthemum* spp. (Shepherd and Barker, 1990; Johnson, 1998).

This nematode also infests many herbaceous plants; most show the typical interveinal leaf blotches and distortions of the upper leaves resulting from bud infestation. *A. ritzemabosi* is also associated with the death of lower leaves and buds and malformed growth of shoots in *Lavandula* spp. Woody plants such as *Buddleia* are also attacked, in which the nematode causes the death of buds and leaf distortions. Attacked *Viola* spp. are stunted, and affected leaves curl downwards, wither and die, while the undersides of leaves show typical water-soaked blotches (Thomas, 1968; Southey, 1993). Stunting and shoot blindness occurs on attacked *Crassula coccinia* (Atkinson, 1964). Combined infestation of *A. ritzemabosi* with *Ditylenchus dipsaci* in stem tissues shows as discoloration caused by feeding of the nematode after cell separation by *D. dipsaci*.

3.2 Extraction of the nematodes from plant material

3.2.1 Direct examination

In leaves infested with *A. besseyi, A. fragariae* or *A. ritzemabosi*, nematodes can be detected by inspecting cut leaves, especially small and young ones, immersed in tap water in a Petri dish under a stereomicroscope (the nematodes will swim into the water within 30 min if there is a heavy infestation).

3.2.2 Extraction methods

A. besseyi, A. fragariae and *A. ritzemabosi* can be extracted from plant material, soil or growing medium with suspected infestation using the Baermann funnel technique (Baermann, 1917), modified Baermann-tray method (Hooper and Evans, 1993), adapted sugar-flotation method (Coolen and D'Herde, 1972) or mistifier technique (Hooper *et al*., 2005). These extraction methods should be conducted for 48 h at room temperature to detect low levels of infestation. In heavily infested plant material, nematodes can be isolated by soaking plant material in water for 1 h. Any plant material to be tested should be cut into small pieces or sliced before extraction to increase the efficacy of extraction. Complementary information on extraction methods, including advantages and drawbacks, can be found in EPPO (2013a).

For the Baermann funnel technique (Hooper and Evans, 1993), a piece of rubber tubing is attached to a glass or plastic funnel stem and closed with a spring or screw clip. The funnel is placed in a suitable support and almost filled with water. Plant material containing nematodes is cut into small pieces, placed in a square of butter muslin, which is folded to enclose the material, and gently submerged in the water in the funnel. Nematodes emerge from the tissues and sink to the bottom of the funnel stem. After some hours, or preferably overnight, some of the water can be run off and examined for nematodes.

The modified Baermann-tray method for nematode extraction (Hooper and Evans, 1993) avoids the possibility that oxygen becomes limiting to the nematodes or that they lodge on the sloping funnel sides by using a shallow dish instead of a funnel and by supporting the material to be extracted on a sieve. The sieve is made from a plastic ring (cut from a polyethylene or Perspex[1] cylinder or a vinyl drainpipe) about 6–8 cm in diameter and 2 cm deep, with a piece of butter muslin stretched over one end and held by a rubber band or secured between two closely fitting rings; alternatively, nylon gauze can be stuck on or fused to the plastic ring. A milk filter or paper tissue is placed in the sieve and the chopped plant material is put on it. The sieve is then placed in water in a Petri dish or similar container. Small supports (e.g. glass rods or small feet attached to the sieve ring) are used to create a space of about 2 mm between the base of the sieve and the collecting dish. The material should be almost awash, and when it is not, more water should be added carefully between the outside of the sieve and the edge of the collecting dish. After a few hours, or overnight, the sieve is gently removed and the contents of the dish are examined for nematodes. The sieve can be re-immersed in fresh water for further extraction from the same sample.

The adapted sugar-flotation method (Coolen and D'Herde, 1972) follows instructions for "mobile stages". Nematodes are released from plant material by means of a mixer that has two running speeds (e.g. Waring[1] blender). A container with a capacity of 0.5 litre is half filled with water. The sample is mixed with the water at low speed. The suspension is poured through a 1 000 µm sieve placed on a homogenization jar and rinsed with a fine, powerful, fan-shaped water jet produced by a low-volume fog spray nozzle until the jar contains 0.5 litre. After homogenization of the suspension by compressed air (about 1 min), a 100 ml aliquot is tapped off from the bubbling mixture into a centrifuge tube. Kaolin powder (1 ml) is added and the tube contents are thoroughly mixed by a mechanical stirrer (which is carefully cleaned after each operation). The mixture is centrifuged for 5 min at 1 800 *g*, after which the

[1] In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols 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.

supernatant is poured off. The residue is mixed with a sugar solution (∂ = 1.15) by mechanical stirring for at least 30 s. The suspension is centrifuged again for 4 min at 1 800 *g*. The sugar solution is poured into a sieve of 5–20 µm aperture sieve, which is placed in a small dish previously filled with the same liquid, until the meshes of the sieve are just covered. After about 1 min the dish is gently emptied sideways. The mobile stages on the sieve are washed with a spray atomizer into 100 ml water, ready for identification.

The mistifier technique, as described by Hooper *et al*. (2005), results in recovery of nematodes that are more active than the Baermann methods because oxygenation is better, and sap and decomposition products from the plant material, especially from bulbs such as *Narcissus* spp., which inactivate the nematodes, are washed away. A fine mist of water is sprayed over the plant material. A spray nozzle, passing about 4.5 litre water per hour, is used. Most systems use an intermittent spray of, for example, 1 min in every 10 min. Oil burner nozzles or gas jets can sometimes be adapted, and a water pressure of about 2.8 kg/cm^2 is usually required to produce a suitable mist. The plant material to be treated is cut into pieces 3–4 mm long and placed in a support in the funnel as described for the modified Baermann-tray method. Optimum sample size depends on the sieve diameter and water flow rate; increasing the sample size can decrease the efficacy of extraction. Nematodes collected in the tube attached to the funnel stem can be released in a beaker for further examination. Compared with the modified Baermann techniques, plant material will decompose much more slowly, thus allowing prolonged extraction times of up to two weeks. Several funnels can be set up on a rack and one or two nozzles can supply all of them. The whole apparatus can be set up on a bench if enclosed with a polyethylene cover and left to stand on a drainage tray.

More refined methods are required for detection of *A. besseyi* in *O. sativa* seeds. Moretti *et al.* (1999) recommend the use of *O. sativa* chaff or hull as an alternative testing material.

More recently, laboratories wishing to use universally tested methods have adopted the Seed Health Testing Method 7-025 for detection of *A. besseyi* on *O. sativa* produced by the International Seed Testing Association (see Remeeus and Pelazza, 2014). The seeds are dehulled using a mill with a 1 mm distance between the rolls (Critical Control Points) (e.g. TR120 rice husker[1] (Kett Electric Laboratory)). The kernels or hulls are transferred in a nylon sieve with a mesh of 0.25 mm to a beaker of 45 mm diameter. The beaker is filled with approximately 20 ml water and is left undisturbed for 24 h at 25 ± 2 °C. The sieve is removed from the beaker and squeezed gently. The contents of the beaker are examined for the presence of nematodes.

Under a stereomicroscope, stylet-bearing nematodes with a well-demarcated large metacorpus are separated from other nematodes present in the Petri dish and transferred with a pipette or a needle to a glass slide for microscopic examination.

4. Identification

The morphological terms used in this section are defined in EPPO (2013b).

Although *Aphelenchoides* can be identified to species level based on morphological examination, this method is possible only for adult specimens, as is the case for most other plant-parasitic nematode species. For precise species-level identification, the morphological characters of *Aphelenchoides* species need to be carefully examined under a high power microscope with at least ×1 000 magnification for use with immersion oil or by using a scanning electron microscope.

Because *Aphelenchoides* is very difficult to identify to species level using morphological characters alone, molecular diagnostic tools have been developed to support the morphological identification of *Aphelenchoides* species (Ibrahim *et al.*, 1994a, 1994b). Molecular methods can be applied to identification of all life stages, including the immature stages, and may be particularly helpful when there is a low level of infestation or when adult specimens are atypical or damaged. However, the specificity of currently available molecular tests may be limited as they have generally been developed and evaluated using a restricted number of species and populations from different geographic regions.

4.1 Morphological identification of aphelenchs

4.1.1 Preparation of aphelenchs for morphological identification

Individual nematodes of *Aphelenchoides* species can be picked from the extract produced by any of the extraction methods described in section 3.2.2 and collected in a drop of water on a slide. The nematodes are slowly heated (to approximately 60 °C) until they become immobile (Hooper *et al*., 2005). The habitus of nematodes killed by gentle heating is almost straight. The nematodes can be sealed on the slide with wax or they can be placed in a drop of fixative before sealing with wax. There are some differences in the appearance of water and fixed specimens, with the former being preferable, but in fixed preparations some features such as the stylet are more distinct.

4.1.2 Identification of the family Aphelenchoididae

The family Aphelenchoididae is characterized by a large metacorpus and pharyngeal glands usually not enclosed in a bulb (overlapping). The dorsal pharyngeal gland opens into the metacorpus. Males have caudal papillae.

A. besseyi, *A. fragariae* and *A. ritzemabosi* belong to the family Aphelenchoididae, members of which share the morphological characteristics outlined in Table 1.

Table 1. Main morphological characteristics shared by the family Aphelenchoididae

Body part	Characteristic
Body form	Vermiform, not swollen
Lateral field	Usually with four or fewer incisures (two to four, rarely six)
Stylet	Slender, with narrow lumen and usually with small basal knobs or swellings
Pharynx	Isthmus rudimentary or absent, nerve ring circumpharyngeal to circumintestinal, pharyngeal glands lobe-like and long, dorsally overlapping intestine
Post-uterine sac	Usually present
Spicule	Rose thorn-shaped or derived therefrom
Adanal bursa	Rarely present (reported to date only from *Pseudoaphelenchus*)
Gubernaculum	Absent
Tail shape	Both sexes similar, conoid, with pointed or rounded, often mucronate, terminus

4.1.3 Identification of the genus *Aphelenchoides*

As with many other nematode genera, *Aphelenchoides* species are morphologically very similar; these nematodes are vermiform, with most species appearing stout and slow-moving. However, the few economically important species such as *A. besseyi*, *A. fragariae* and *A. ritzemabosi* tend to be slim, pale and relatively long when compared with most other *Aphelenchoides* species. They are also good swimmers (with a serpentine motion).

The members of the genus can be diagnosed by the following morphological characteristics (Figure 9):

- body length from 0.2 to 1.3 mm, but most commonly from 0.4 to 0.8 mm
- heat relaxed females become straight to ventrally arcuate (Figure 9(A))

- heat relaxed males assume a walking-stick shape with the tail region curled ventrally (Figure 9(B))
- cuticle finely annulated, lateral field with two to four (rarely six) incisures (Figure 9(D))
- stylet very difficult to see under low power microscopy; under high power, the stylet varies from clearly discernible to very faint. Generally about 10–12 µm long. Similarly, basal knobs or swellings are sometimes clear but often indistinct.
- pharynx: pharyngeal procorpus long and slender; metacorpus well developed, spherical to rounded-rectangular, with central valve plates; oesophageal gland lobe long, with dorsal overlap of the intestine (Figure 9(C))
- vulva typically post-median, usually between 60 and 75 percent of the body length
- ovary monoprodelphic, typically outstretched, but may be flexed
- post-vulval sac almost always present
- tail shape conoid to variable (Figure 9(E, F)), in male more strongly curved ventrally and papillae variable
- tail terminus without or with one or more mucros (Figure 9(G, H)) (a mucro is defined as a structure at the end of the tail terminus). Mucros can be definitively discerned only at ×1 000 with oil immersion. The presence or absence of mucros, and the shapes they assume, can be used to distinguish species, and are a key element in the identification of *A. besseyi*, *A. fragariae* and *A. ritzemabosi*.
- spicules well-developed, thorn-shaped, paired and separate
- bursa absent.

Aphelenchoides species can be distinguished from species of other genera encountered in soil and plant material by using the key in Table 2.

Table 2. Key to distinguish *Aphelenchoides* species from species of other genera in soil and plant material

1	Stylet present	**2**
	– Stylet absent	**NAS**
2	Four-part pharynx with a cylindrical procorpus followed by a valvulated metacorpus, slender isthmus and glandular basal bulb	**3**
	– Two-part pahrynx, anterior part slender, posterior part expanded, glandular and muscular (Note that the cylindrical procorpus and the valvulated metacorpus are considered separate parts)	**NAS**
3	Dorsal pharyngeal gland outlet in metacorpus; metacorpus very large, often nearly as wide as the diameter of the body	**4**
	– Dorsal pharyngeal gland outlet in procorpus behind stylet knobs; metacorpus moderate to reduced in size (less than three-fourths body width)	**NAS**
4	Pharyngeal glands lobe-like, long dorsal overlap of intestine	**5**
	–Pharyngeal glands pyriform, no overlap of intestine; or pharyngeal glands lobe-like, ventral overlap of intestine	**NAS**
5	Lateral fields with four or fewer incisures; stylet with basal knobs or swellings; female tail conoid, elongate conoid, convex conoid or subcylindroid to a pointed or narrowly rounded terminus; male spicules robust, thorn-shaped; adanal bursa absent	**6**

	– Lateral fields with six or more incisures; stylet without basal knobs; female tail short, subcylindroid and with broadly rounded terminus; male spicules slender, tylenchoid; adanal bursa present	**NAS**
6	Tail of both sexes short, usually less than four times anal body width	**7**
	– Tail of both sexes elongate to filiform, usually more than four times anal body width	**NAS**
7	Stylet slender, often about 10–12 µm and usually less than 20 µm; vulval flap absent; male without small bursa-like flap at tail tip	***Aphelenchoides***
	– Not with the above combination of characteristics	**NAS**

NAS, not *Aphelenchoides* spp.

4.1.4 Identification of *Aphelenchoides* to species level

The identification of species in the *Aphelenchoides* genus is complex and requires a systematic approach. It is generally agreed that a combination of morphological and molecular methods is required for the most reliable identification. The first step in diagnosis is to record and measure the critical morphological features of as many female specimens as are available, ideally 20. In practice, far fewer adult specimens are usually available, and in such cases, the nematologist should prepare the specimen(s) with great care to avoid damaging the few features available, leaving sufficient juveniles or females for analysis by molecular tools. Males are not included in the keys presented in this diagnostic protocol, but the shape and size of their spicules may assist in confirming the final identification.

The plant-infesting *Aphelenchoides* species include *A. besseyi*, *A. blastophthorus*, *A. fragariae*, *A. ritzemabosi* and *A. subtenuis*, which live in the above-ground parts of plants. They can be distinguished from other species of the genus by their slender body and the more posterior position of the hemizonid: six to ten annules behind the excretory pore (versus one to three annules) (Thorne, 1961).

Aphelenchoides is a large genus. Allen (1952) provided a key to the four species of bud and leaf nematodes (*A. besseyi*, *A. fragariae*, *A. ritzemabosi* and *A. subtenuis*). Sanwal (1961) produced a dichotomous key to the 35 *Aphelenchoides* species that were recognized at the time. Fortuner (1970) devised a dichotomous key to 11 *Aphelenchoides* species with star-shaped mucros. Baranovskaya (1981) provided a dichotomus key to 97 species with descriptions of 105 species. Shahina (1996) provided a compendium to 141 *Aphelenchoides* species and used tail terminus to divide these species into four groups: (1) tail simple without any outgrowth or mucronate structure; (2) tail terminus with one or sometimes two mucronate structures; (3) tail with tetramucronate spine or star-shaped; and (4) tail outgrowth other than with a spine or star-shaped. EPPO (2004) devised a polytomous key to 17 *Aphelenchoides* species including 14 species with star-shaped mucros and 3 species of bud and leaf nematodes without star-shaped mucros (*A. blastophthorus*, *A. fragariae* and *A. ritzemabosi*), and divided the tail terminus of *Aphelenchoides* species into five groups: (1) with star-shaped mucro; (2) with a single mucro; (3) bifurcate; (4) mucro shape belonging to other type at tail tip; and (5) without mucro.

A. besseyi differs from other plant-parasitic species of the genus by having a star-shaped mucro, although non-pathogenic species of *Aphelenchoides* also have star-shaped mucros. *A. besseyi* is the most common plant-parasitic species with a star-shaped mucro although plant-parasitic species can be found in strawberries (*A. blastophthorus*, *A. fragariae* and *A. ritzemabosi*) as follows: *A. besseyi* has a post-vulval sac that is always less than one-third of the distance from the vulva to the anus, whereas sacs 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; and the spicules of *A. besseyi* are distinctive in that the proximal ends have an indistinct dorsal process (or apex) and have only a moderately developed ventral one (rostrum), while spicules of *A. blastophthorus* are

comparatively large for the genus, have a rather stout dorsal limb that is characteristically flattened about midway along its arch, with its distal end curved ventrally to give it a hooked or knobbed appearance, and the apex and rostrum are pronounced structures, spicules of *A. fragariae* have a moderately developed apex and rostrum, and the smoothly curved spicules of *A. ritzemabosi* seem to lack a dorsal or ventral process.

A. besseyi, *A. blastophthorus*, *A. fragariae*, *A. ritzemabosi* and *A. subtenuis* live as parasites in buds and leaves of plants. *A. saprophilus*, a fungivorous species, is also often found in damaged or diseased plant material, including bulbs and corms. Andrássy (2007) provided a key to 47 *Aphelenchoides* species found in Europe, including the six species encountered in buds and leaves. A short dichotomous key to *Aphelenchoides besseyi*, *A. fragariae* and *A. ritzemabosi* is given in Table 3.

For this diagnostic protocol, which concentrates on the three leaf and bud nematode species *A. besseyi*, *A. fragariae* and *A. ritzemabosi*, a dichotomous polytomous key is offered that should allow the reader to proceed reliably to the relevant specialist section. It is frequently not possible to determine a singular character in a short key that is required for identification, while a polytomous key allows the combination of a range of characters for a provisional identification.

As *A. besseyi*, *A. fragariae* and *A. ritzemabosi* can all occur in a wide range of habitats, including occasionally in planting media, all *Aphelenchoides* nematodes that may be found in these habitats need to be considered in a diagnosis. Unfortunately, many of these nematodes are difficult to identify because there is little to distinguish them, a problem not alleviated by the poor descriptions of the species themselves. However, several authors have improved the original descriptions for the three targeted species. In addition, studies on *Aphelenchoides* species have shown the degree of variation in measurements made on populations from different hosts.

As with all identifications involving the use of morphological characters, the combination of several key features is crucial to a positive diagnosis. In the polytomous key there is some overlap of codes, and users are advised to refer to original descriptions if in doubt about a diagnosis or to refer to Table 6 for further guidance and proceed to molecular testing to confirm.

A flow diagram of the identification process is provided in Figure 10.

Reference material can be found through various resources; for example, Q-bank (see http://www.q-bank.eu/Nematodes) or Nematode Collection Europe (NCE) (see http://www.nce.nu).

Table 3. Simplified key to distinguish *Aphelenchoides besseyi*, *A. fragariae* and *A. ritzemabosi* from other species

1	Post-vulval sac length more than one-third the distance between the vulva and the anus	**2**
	– Post-vulval sac length less than one-third the distance between the vulva and the anus; star-shaped mucro present	***A. besseyi***
2	Lateral field with three or four incisures	**3**
	– Lateral field with two incisures, body slender (a = 45–63), cephalic region almost continuous with body contour	***A. fragariae***
3	Tail terminus with a single mucro	**Other species**
	– Tail terminus with two to four processes pointing posteriorly giving it a paintbrush-like appearance, usually four incisures, stylet about 12 µm long, post-vulval sac usually more than half the distance between the vulva and the anus	***A. ritzemabosi***

4.2 Morphological identification of *Aphelenchoides besseyi*

4.2.1 Morphological characteristics

Details and views are provided in Figure 11.

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. Metacorpus 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 sperm, 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. Often as numerous as females. Posterior end of body curved by about 180 degrees in relaxed specimens. Tail conoid, with terminal mucro with two to four pointed processes. Spicules typical of the genus except that the proximal ends lack a distinct apex and have only a moderately developed rostrum. The dorsal limb spicules measure 18–21 µm (mean 19.2 µm).

4.2.2 Identification using morphological keys

In this diagnostic protocol an attempt has been made to reduce the number of comparisons required by selecting only those *Aphelenchoides* species that have a star-shaped mucro, together with those pest species that might also be encountered in foliage. It should be noted that the following procedure relies heavily on the original descriptions and drawings of species, which are sometimes contradictory. For example, the tail shape for *A. aligarhiensis* is described as elongate conoid, but the accompanying drawing does not show this. There is also no accompanying value for c´, which is an indicator of tail shape (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 shows it to be posterior to the nerve ring. In such cases the written description is the one included in this diagnostic protocol. Where possible, original data have been supplemented by additional published information for the most commonly encountered species.

4.2.2.1 Dichotomous key for *Aphelenchoides besseyi*

A short dichotomous key is provided in this diagnostic protocol as an added value for identification. Only characters from female nematodes have been considered. The key is complemented by Figure 11, showing critical features, and by Table 2 and Table 6, which provide more details of those *Aphelenchoides* species that have a star-shaped mucro together with those pest species that might also be encountered in foliage. After the key has been consulted, a check on the probable identity of the nematode should be made with reference to Table 3 and the relevant species description.

Dichotomous key to distinguish *A. besseyi* from other related species of *Aphelenchoides*

1	Star-shaped mucro present	**2**
	– Star-shaped mucro absent	**not *A. besseyi***
2	Post-vulval sac up to one-third of the length of the distance from the vulva to the anus	**3**
	– Post-vulval sac more than one-third of the length of the distance from the vulva to the anus	***A. aligarhiensis*, *A. brevistylus*, *A. fujianensis*, *A. lichenicola***
3	Tail shape is conoid or elongate conoid	**4**
	– Tail shape is subcylindroid	***A. siddiqii***

4	Stylet length in the range 10–12.5 µm – Stylet length outside the range 10–12.5 µm	**5** ***A. asteromucronatus, A. hylurgi, A. wallacei***
5	Four lateral lines – Fewer or more than four lateral lines	**6** ***A. andrassyi, A. asterocaudatus, A. unisexus***
6	Excretory pore anterior to, or level with, the anterior level of the nerve ring – Excretory pore level with the nerve ring	***A. besseyi*** ***A. goodeyi, A. jonesi, A. silvester***

4.2.2.2 Polytomous key for *Aphelenchoides* species

Polytomous keys allow the easy addition of new species into an identification procedure. For this diagnostic protocol several important features of species related to *A. besseyi* have been selected to produce a small polytomous key (Table 4). The selected features have been given codes. It has been feasible to convert only six morphological characters into codes. Each specimen for identification should be examined and assigned a set of these codes (A–F) according to the following categorization (Figure 12).

A. Tail terminus or mucro shape

1 = star (Figure 12(A) (a–f))

2 = single terminal mucro (Figure 12(A) (g–m))

3 = bifurcate (Figure 12(A) (n))

4 = other (Figure 12(A) (o–t))

5 = no mucro (Figure 12(A) (u–v))

B. Length of the post-vulval sac

1 = one-third or less the distance between the vulva and the anus

2 = more than one-third the distance between the vulva and the anus

3 = no post-vulval sac

C. Tail shape

1 = conoid: cone-shaped, with both sides of the tail surface tapering at an equal angle to the tail tip. Total length not exceeding five times the anal body width (Figure 12(B) (a)).

2 = elongate conoid: an elongated cone, with a length five times or more the anal body width (Figure 12(B) (b-c))

3 = dorsally convex conoid: at first appearance this tail shape is curved ventrally. The dorsal side of the tail is curved in a convex manner before it joins the ventral surface. The ventral surface is usually concave, but from some viewpoints may appear straight. The tail may be any length (Figure 12(B) (d-e)).

4 = subcylindroid: both sides of the tail appear to run parallel for most of its length, and they end in a hemispherical or subhemispherical tail tip (Figure 12(B) (f))

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 (Figure 12(C) (a))

2 = excretory pore level with the nerve ring (Figure 12(C) (b))

3 = excretory pore posterior to or opposite the posterior level of the nerve ring (Figure 12(C) (c))

The set of codes obtained should be compared with those set out in Table 4, which will allow a provisional diagnosis to be made. A positive diagnosis is made when the value of most of the codes matches the reference species.

Table 4. Polytomous codes of selected *Aphelenchoides* species

Species	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. fujianensis†	1	2	1	1/3	1	1/2
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. subtenuis	2	2	4	1	2	2/3
A. ritzemabosi	4	2	2	1	1	3
A. fragariae	2/4	2	2	1	3	2/3

Source: EPPO (2004).

Notes:

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

The assignment of more than one code for a particular feature for a species is based on variation noted in published data or seen in practice.

† The codes for *A. fujianensis* were assigned by Zuo *et al.* (2010).

4.3 Morphological identification of *Aphelenchoides fragariae*

4.3.1 Morphological characteristics

Allen (1952), Siddiqi (1975) and Hunt (1993) all provide detailed descriptions of *A. fragariae* (see Figure 13). This description was modified from Hunt (1993).

Female. Body slender (a = 45–70), straight to arcuate ventrally when relaxed. Cuticle finely annulated, lateral field with two incisures. Cephalic region almost continuous with body, appears smooth under the microscope, and four to five annuli visible by scanning electron microscopy (Khan *et al*., 2007, 2008). Stylet slender, about 8–14 µm long, often 10–11 µm; conus and shaft nearly equal in length; basal knobs minute but distinct. Pharynx typical of the genus, metacorpus oval and highly muscular with central valve plates, pharyngeal gland lobe with dorsal overlap of the intestine, two to four body widths long. Nerve ring encircling isthmus near its base, about one body width behind metacorpus. Excretory pore level with or close behind nerve ring. Genital tract monoprodelphic, outstretched, with oocytes in a single row, never reaching pharynx. Post-vulval sac long, extending more than half the distance between the vulva and the anus. Tail elongate conoid with a single simple spike or minute mucro at tail tip.

Male. Abundant. Essentially similar to female in general morphology. Tail arcuate through 45 to 90 degrees when relaxed, not sharply curved like a hook, with a simple terminal spine. Three pairs of caudal papillae present. Spicules rose thorn-shaped with moderately developed apex and rostrum, dorsal limb 10–19 µm long.

The key diagnostic features to distinguish *A. fragariae* from the other known species of *Aphelenchoides* are:

- body 0.4–1.0 mm long, very slender (a = 45–70) (Figure 13(D–F))
- stylet slender, about 8–14 µm long, with distinct basal knobs (Figure 13(A, B))
- tail elongate conoid with a single simple spike or mucro at tail tip (Figure 13(G, I, M, P, Q))

- post-vulval sac extending more than half the distance between the vulva and the anus (Figure 13(F))
- excretory pore level with or close behind nerve ring
- lateral field with generally two incisures (Figure 13(H, O))
- males common, spicules on dorsal limb 14–17 µm long (Figure 13(J–L)).

4.3.2 Comparison with similar species

A. fragariae is similar to *A. arachidis*, *A. helophilus*, *A. resinosi* and *A. rhytium*, but can be distinguished from all other species described in *Aphelenchoides* by its more slender body (a = 45–70), lateral field with generally two incisures and tail terminus with a single mucro. *A. fragariae* can be distinguished from these similar species using the key given in Table 6. A diagnostic compendium of *A. fragariae* and similar species and bud and leaf nematodes of the genus is presented in Table 6, which provides details to help to determine the identity of these similar species.

Table 5. Dichotomous key to distinguish *Aphelenchoides fragariae* from morphologically similar species

1	Female tail more than 30 µm long, conoid to elongate conoid	**2**
	– Female tail shorter, 22–28 µm long, subcylindroid with bluntly rounded tip	***A. arachidis***
2	Female body length less than 1.0 mm, tail mucro offset; male spicules less than 25 µm long	**3**
	– Female body 0.8–1.3 mm, tail mucro not offset; male spicules 26 µm long	***A. helophilus***
3	Post-vulval sac length less than half the distance between the vulva and the anus	**4**
	– Post-vulval sac length more than half the distance between the vulva and the anus	***A. fragariae***
4	Female tail less than 40 µm long, two lateral incisures; spicules 13–15 µm long	***A. resinosi***
	– Female tail 56.2 µm long, lateral incisures absent; spicules 22.9 µm long	***A. rhytium***

Table 6. Morphological characteristics of *Aphelenchoides fragariae* compared with similar species

Species	L (mm)	A	B	C	c´	Tail (µm)	Tail shape[†]	Terminal mucro shape[1]	V	PVS/VA[‡]	Stylet (µm)	LL[§]	Spicules (µm)	References
A. arachidis	0.51–1.0	39–50	11–18	25–42	2–3	22–28	Subcylindroid	Single central spine	67–74	Approximately half	11–12	2	15–25	Bridge and Hunt (1985)
A. besseyi	0.66–0.75	32–42	10.2–11.4	17–21	3.5–5.0	36–42	Conoid	Star	68–70	Less than one-third	10–12	4	18–21	Franklin and Siddiqi (1972); Andrássy (2007)
A. blastophthorus	0.68–0.95	28–50	9.0–12.8	15–28	2.3–5.0	42–48	Conoid	Single central spine	62–74	Approximately half	15–19.5	4	24–32	Hooper (1975); Shahina (1996)
A. fragariae	0.45–0.80	36–63	8–15	12–20	4.9	38–42	Elongate conoid	Single central spine	64–71	More than half	10–11	2 [¶]	14–17	Siddiqi (1975); Shahina (1996)
A. helophilus	0.80–1.30	43–78	12–14	14–20	5.5	>40	Elongate conoid	Single central spine	65–79	Unknown	12	Unknown	26	Shahina (1996); Andrássy (2007)
A. resinosi	0.40–0.80	29–53	7–13	12–19	3–4	33.7	Conoid	Single central spine	66–79	Less than half	10–11	2	13–15	Kaisa *et al*. (1995)
A. rhytium	0.78–0.94	43–48	11.7–13.4	16–21		56.2	Elongate conoid	Single central spine	67	Less than half	11	Absent	22.9	Massey (1974); Shahina (1996)
A. ritzemabosi	0.77–1.20	40–45	10–13	18–24	4–5	47	Elongate conoid	Peg with two to four minute processes	66–75	More than half	12	4	20–22	Allen (1952); Siddiqi (1974); Andrássy (2007)
A. saprophilus	0.45–0.62	26–33	8–12	12–18	2.5–3.0	32	Conoid	Ventral peg	66–70	Approximately half	11	4	22–23	Shahina (1996); Andrássy (2007)
A. subtenuis	0.87–1.15	44–57	12–17	24–28	2.78–3.27	42.4	Subcylindroid	Single ventral spine	69–71	More than half	11	3 or 4	18–23	Allen (1952); Deimi *et al.* (2006)

Source: Adapted from EPPO (2004).

[†] Shape of female tail and terminal mucro are presented in Figure 14.

[‡] Post-vulval sac (PVS) length divided by the distance between the vulva and the anus (VA).

[§] Number of lateral lines (LL).

[¶] Specific populations of *A. fragariae* can have more than two lateral fields.

4.4 Morphological identification of *Aphelenchoides ritzemabosi*

4.4.1 Morphological characteristics

Details and views are provided in Figure 15. Morphological characteristics are from Siddiqi (1974).

Female. Body slender, 0.77–1.20 mm long; annules 0.9–1.0 µm wide, distinct; lateral fields one-sixth to one-fifth as wide as body, with four incisures. Lip region hemispherical, set off by a constriction, slightly wider than adjacent body, no annulations visible under a light microscope; framework hexaradiate, weakly sclerotized. Stylet about 12 µm long, with distinct basal knobs and sharply pointed anterior. Procorpus slender; metacorpus large, somewhat oval in shape, highly muscular, with prominent internal cuticular thickening and orifices of dorsal and subventral pharyngeal glands. Nerve ring in neotype 1.5 body widths behind bulb. Excretory pore 0.5–2 body widths posterior to nerve ring. Three pharyngeal glands forming a lobe extending about four body widths over intestine dorsally. Pharyngo-intestinal junction about 8 µm behind metacorpus, indistinct and valve not discernible. Intestine with small spherical granules and a distinct lumen throughout. Vulva slightly protruding, transverse slit. Post-vulval uterine sac extending for more than half the distance between the vulva and the anus, often containing sperm. Ovary single anteriorly outstretched, oocytes in multiple rows. Tail elongate conoid, bearing a terminal peg which has two to four minute processes pointing posteriorly giving it a paintbrush-like appearance.

Male. Common. Posterior end of body usually curved through 180 degrees upon relaxation. Lip region, stylet and pharynx similar to that in female. Testis single, outstretched. Three pairs of ventro-submedian caudal papillae: first pair adanal, second midway on tail, third near tail end. Spicules smoothly curved, rose thorn-shaped, lacking a dorsal or ventral process at the proximal end; dorsal limb 20–22 µm long. Tail peg with two to four processes, of variable shape.

Measurements. ♀♀: L = 0.77–1.20 mm; a = 40–45; b = 10–13; c = 18–24; V = $^{48–33}$66– 75$^{14–17}$. Neotype ♀: L = 0.85 mm; a = 42; b = 12; c = 18; V = ^{35}68^{17}; stylet = 12 µm. ♂♂: L = 0.70–0.93 mm; a = 31–50; b = 10–14; c = 16–30; T = 35–64.

4.4.2 Comparison with similar species

Aphelenchoides species are morphologically very similar and can be easily confused. Molecular identification (section 4.5) may contribute to identification when there is any uncertainty with morphological identification. More information for comparison with similar species can be found in Table 6.

4.5 Molecular identification of *Aphelenchoides* species

Several molecular tests for the identification of *Aphelenchoides* species have been developed and are now in use (McCuiston *et al.*, 2007; Rybarczyk-Mydłowska *et al.*, 2012). Polymerase chain reaction (PCR) with species-specific primers can be used for diagnosis of nematodes isolated from plant material (section 3.2.2). Any development stage can be subjected to the molecular tests.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols 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.

The final volume of the PCR is based on the descriptions in the source papers. PCR could also be carried out in a volume that accords with the instructions of the Taq DNA polymerase enzyme being used.

4.5.1 DNA extraction

DNA may be extracted from single or mixed sexes and life stages (adults, juveniles and eggs) of nematodes. There is a risk of false negatives if DNA is not extracted properly, so a negative molecular

test result does not exclude the possibility of positive identification by morphological methods. For each PCR test described below, the DNA extraction methods refer to the source paper for the original specific procedure used. DNA may also be extracted using other methods suitable for nematodes. Alternatively, commercial kits for DNA isolation are available.

4.5.2 Real-time PCR for four foliar nematode species

Rybarczyk-Mydłowska *et al.* (2012) designed a small subunit (SSU) ribosomal (r)DNA-based species-specific PCR for four foliar nematode species: *A. besseyi*, *A. fragariae*, *A. ritzemabosi* and *A. subtenuis.* The species-specific primers were designed based on the full-length SSU rDNA sequences of these four *Aphelenchoides* species, and they were used for real-time PCR to rapidly identify one or more foliar nematode species isolated from plant material and soil.

The primer pair (designed with locked nucleic acid (LNA), designated by * below) recommended for the detection of *A. besseyi* is:

1770: 5′-GCG GGA TTC GTG GTT C*T-3′
1772: 5′-CGA CAT GCC GAA ACA GAG-3′

The primers specific for *A. fragariae* (located in the SSU rDNA sequence) used in this PCR are:

1469: 5′-CTT ATC GCA CGA CTT TAC G-3'
1472: 5′-TCA AAG TAA TCC GCA TCC AAT-3'

The primer pair (designed with LNA, designated by * below) recommended for the detection of *A. ritzemabosi* is:

1496: 5′-CGC TGG TGG GTT TCG A-3′
1499: 5′-CCC GCT AAG AAA TGA TCA C*C-3′

The exact melting temperature of the specific fragment produced by each primer pair is not provided in Rybarczyk-Mydłowska *et al.* (2012) but can be estimated from the graphs included as approximately 85 °C for *A. besseyi* (primers 1770/1772), approximately 84 °C for *A. fragariae* (primers 1469/1472) and approximately 87 °C for *A. ritzemabosi* (primers 1496/1499). Nevertheless, these values should be confirmed under the specific conditions of each laboratory.

Each real-time PCR is performed according to the conditions described in Table 7.

Table 7. Real-time PCR master mix composition, cycling parameters and amplicons (Rybarczyk-Mydłowska *et al.*, 2012)

Reagent	Final concentration
PCR-grade water	–[†]
PCR buffer Absolute qPCR mix SYBR Green[1] (Thermo Scientific)	1×
Specific forward primer	0.2 µM
Specific reverse primer	0.2 µM
DNA (volume)	3 µl
Cycling parameters	
Initial denaturation	95 °C for 15 min
Number of cycles	60
Denaturation	95 °C for 30 s
Annealing	63 °C for 60 s
Elongation	72 °C for 30 s
Expected amplicons	

Size	325 bp for *Aphelenchoides besseyi* 470 bp for *A. fragariae* 347 bp for *A. ritzemabosi*	

† For a final reaction volume of 25 µl.

bp, base pairs; PCR, polymerase chain reaction.

4.5.3 PCR for *Aphelenchoides fragariae*

This internal transcribed spacer (ITS)1-PCR was described by McCuiston *et al.* (2007) as a diagnostic test for early detection and identification of *A. fragariae* directly in host plant material using the species-specific primers given below. These specific primers amplify DNA from *A. fragariae* and do not amplify DNA from other plant-parasitic nematode species (*Meloidogyne incognita*, *Heterodera schachtii*, *Pratylenchus penetrans*, *Caenorhabditis elegans*, *D. dipsaci*, *A. besseyi* and *A. ritzemabosi*). The PCR is sensitive, detecting a single nematode in a background of plant material extract. The test has accurately detected *A. fragariae* in more than 100 naturally infected samples, including 50 ornamental plant species. Total DNA was extracted from infected plant material using the DNeasy Plant Mini Kit[1] (Qiagen).

The primers (within the rDNA-ITS1 region) used in this PCR are:

AFragF1 (forward): 5'-GCA AGT GCT ATG CGA TCT TCT-3'

AFragR1 (reverse): 5'-GCC ACA TCG GGT CAT TAT TT-3'

Each real-time PCR is performed according to the conditions described in Table 8.

Table 8. ITS1 rDNA conventional PCR master mix composition, cycling parameters and amplicons (McCuiston *et al.*, 2007)

Reagent	**Final concentration**
PCR-grade water	–†
PCR buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl)	1×
$MgCl_2$	1 mM
dNTPs	0.2 mM
Primer AFragF1 (forward)	0.4 µM
Primer AFragR1 (reverse)	0.4 µM
DNA polymerase (GoTaq Flexi DNA Polymerase[1] (Promega))	1.25 U
DNA (volume)	2 µl
Cycling parameters	
Initial denaturation	94 °C for 2 min
Number of cycles	40
Denaturation	94 °C for 1 min
Annealing	53 °C for 40 s
Elongation	72 °C for 1 min
Final elongation	72 °C for 10 min
Expected amplicons	
Size	169 bp

† For a final reaction volume of 25 µl.

bp, base pairs; ITS, internal transcribed spacer; PCR, polymerase chain reaction; rDNA, ribosomal DNA.

4.5.4 Controls for molecular tests

For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For PCR a positive nucleic acid control and a negative amplification control (no template control) are the minimum controls that should be used.

Positive nucleic acid control. This control is used to monitor the efficiency of the test method (apart from the extraction), and specifically the amplification. Pre-prepared (stored) nematode nucleic acid may be used.

Negative amplification control (no template control). This control is necessary for conventional and real-time PCR to rule out false positives due to contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage instead of DNA solution.

4.5.5 Interpretation of results from PCR

Conventional PCR

The pathogen-specific PCR will be considered valid only if the positive control produces an amplification product of the expected size for the target nematode and the negative control produces no amplification product of the expected size for the target nematode.

Real-time PCR

The real-time PCR will be considered valid only if the positive control produces an amplification curve with the pathogen-specific primers and the negative control produces no amplification curve.

If internal control primers are also used, the positive control and each of the test samples should produce an amplification curve.

5. Records

Records and evidence should be retained as described in ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*)) and where the nematode is found in an area for the first time, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability:

- the original sample of the infested material
- description and photographs of the symptoms and damage
- measurements and drawings or photographs of the nematode
- permanent slides or culture of the nematode
- if relevant, DNA extracts and PCR amplification products, stored at −80 °C and −20 °C, respectively.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), 389 Oyster Point Blvd, Suite 2, South San Francisco, CA 94080, United States (Fengru Zhang; e-mail: fzhang@aphis.usda.gov; tel.: +1 650 876 9098; fax: +1 650 876 0915).

Laboratory of Plant Nematology and Research Center of Nematodes of Plant Quarantine, College of Natural Resources and Environment, South China Agricultural University, Wushan Street, Guangzhou City, Guangdong Province 510642, China (Hui Xie; e-mail: xiehui@scau.edu.cn; tel.: +86 020 3829 7432; fax: +86 020 3829 7286).

Directorate Inspection Services, Department of Agriculture, Forestry and Fisheries, Private Bag X5015, Stellenbosch 7599, South Africa (Rinus Knoetze; e-mail: RinusK@daff.gov.za; tel.: +27 021 809 1621).

Nematology Unit, Fera Science Limited, Sand Hutton, York YO1 1LZ, United Kingdom (Sue Hockland; e-mail: sue.hockland@plantparasiticnematodes.com).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

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The information for *A. besseyi* is adapted from the EPPO Diagnostic Protocol for *Aphelenchoides besseyi* (PM 7/39(1)) (see EPPO, 2004), which was produced by S. Hockland with the cooperation of the members of the EPPO Panel for Diagnostics in Nematology, the EPPO Secretariat, David Hunt and Mischa Aalten.

8. References

The present annex refers to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispms.

Allen, M.W. 1952. Taxonomic status of the bud and leaf nematodes related to *Aphelenchoides fragariae* (Ritzema Bos, 1891). *Proceedings of the Helminthological Society of Washington*, 19: 108–120.

Ambrogioni, L. & Greco, N. 2014. Nematodi parassiti degli organi epigei delle piante e delle fungaie. Fam. Aphelenchoididae. *In* L. Ambrogioni, F.P. d'Errico, N. Greco, A. Marinari Palmisano & P.F. Roversi, eds. *Nematologia agraria generale e applicata*, pp. 262–270. Florence, Italy, Società Italiana di Nematologia, Tipografia Coppini. 475 pp.

Andrássy, I. 2007. Free-living nematodes of Hungary (*Nematoda errantia*), Vol. II. *In* C. Csuzdi & S. Mahunka, eds. *Pedozoologica Hungarica No. 4*, pp. 18–22. Budapest, Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences. 496 pp.

Atkinson, K. 1964. *Aphelenchoides ritzemabosi* Schwartz on *Crassula coccinea*. *Plant Pathology*, 13: 137.

Baermann, G. 1917. Ein einfache Methode zur Auffindung von Anklyostomum (Nematoden) Larven in Erdproben. *Geneeskundig Tijdschrift voor Nederlandsch-Indië*, 57: 131–137. (in German)

Baranovskaya, I.A. 1981. [*Nematodes of soil and plants (aphelenchoidids and seinurids).*] Moscow, Nauka. 233 pp. (in Russian).

Bridge, J. & Hunt, D.J. 1985. *Aphelenchoides arachidis*. CIH descriptions of plant-parasitic nematodes, Set 8, No. 116. St Albans, UK, Commonwealth Institute of Helminthology (CIH). 3 pp.

Bridge, J., Luc, M. & Plowright, R.A. 1990. Nematode parasites of rice. *In* M. Luc, R.A. Sikora & J. Bridge, eds. *Plant parasitic nematodes in subtropical and tropical agriculture,* 1st edn, pp. 69–108. London, CABI International Institute of Parasitology. 643 pp.

Brown, D.J.F., Dalmasso, A. & Trudgill, D.L. 1993. Nematode pests of soft fruits and vines. *In* K. Evans, D.L. Trudgill & J.M. Webster, eds. *Plant parasitic nematodes in temperate agriculture,* pp. 427–462. Wallingford, UK, CABI. 656 pp.

CABI. 2006. *Aphelenchoides besseyi*. Crop Protection Compendium, 2006 Eedition. CABI, Wallingford, UK. (software accessible Available at http://www.cabi.org/cpc/datasheet/6378, (last accessed on the 26 January /01/2016).

CABI. n.d.. *Aphelenchoides besseyi*. Crop Protection Compendium,. Wallingford, UK, CABI. Available at http://www.cabi.org/cpc (last access 05 March 2016).

CABI/EPPO. 2000. *Aphelenchoides ritzemabosi*. Distribution Maps of Plant Diseases, Map No. 808. Wallingford, UK, CABI.

Chaves, N., Cervantes, E., Zabalgogeazcoa, I. & Araya, C. 2013. *Aphelenchoides besseyi* Christie (Nematoda: Aphelenchoididae), agente causal del amachamiento del frijol común. *Tropical Plant Pathology*, http://dx.doi.org/10.1590/S1982-56762013005000009.

Chizov, V.N., Chumakova, O.A., Subbotin, S.A. & Baldwin, J.G. 2006. Morphological and molecular characterization of foliar nematodes of the genus *Aphelenchoides*: *A. fragariae* and *A. ritzemabosi* (Nematoda: Aphelenchoididae) from the Main Botanical Garden of the Russian Academy of Science, Moscow. *Russian Journal of Nematology,* 14: 179–184.

Cobon, J. & O'Neill, W. 2011. *Aphelenchoides fragariae (Ritzema Bos, 1890) Christie, 1932*. Australasian Plant Pathology Society Pathogen of the Month, August. Available at http://www.appsnet.org/publications/potm/pdf/Aug11.pdf (last accessed 7 March 2016)

Coolen, W.A. & D'Herde, C.J. 1972. *A method for the quantitative extraction of nematodes from plant tissue.* Ghent, Belgium, Ministry of Agriculture, State Agricultural Research Centre. 77 pp.

Crosse, J.E. & Pitcher, R.S. 1952. Studies in the relationship of eelworms and bacteria to certain plant diseases. I. The etiology of strawberry cauliflower disease. *Annals of Applied Biology*, 39: 475–484.

Dastur, J.F. 1936. A nematode disease of rice in the Central Provinces. *Proceedings of the Indian Academy of Sciences, Section B,* 4: 108–121.

Deimi, A.M., Maafi, Z.T., Rius, J.E.P. & Castillo, P. 2006. *Aphelenchoides subtenuis* (Cobb, 1926) Steiner & Buhrer, 1932 (Nematoda: Aphelenchoididae) from Iran with morphological and morphometric characterization. *Nematology*, 8(6): 903–908.

EPPO (European and Mediterranean Plant Protection Organization). 2004. *Aphelenchoides besseyi.* PM 7/39(1). *EPPO Bulletin*, 34: 303–308.

EPPO (European and Mediterranean Plant Protection Organization). n.d. EPPO Global Database Available at https://gd.eppo.int (last accessed 7 March 2016).

EPPO (European and Mediterranean Plant Protection Organization). 2013a. Nematode extraction. PM 7/119(1). *EPPO Bulletin*, 43: 471–495.

EPPO (European and Mediterranean Plant Protection Organization). 2013b. *Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology.* EPPO Technical Document No. 1056 (Rev. 4). Paris, EPPO. Available at http://www.eppo.int/QUARANTINE/diag_activities/EPPO_TD_1056_Glossary.pdf.

EPPO/CABI (I.M. Smith, D.G. McNamara, P.R. Scott & M. Holderness, eds). 1997. *Quarantine pests for Europe*, 2nd edn. Wallingford, UK, CABI. 1425 pp.

Escuer, M. & Bello, A. 2000. Nematodos del género *Aphelenchoides* de interés fitopatológico y su distribución en España. *Boletin de Sanidad Vegetal – Plagas*, 26: 47–63.

Esser, R.P. 1966. *Nematodes attacking plants above the soil surface: Crimp (foliar nematodes on strawberry).* Nematology Circular No. 5. Gainesville, FL, Florida Department of Agriculture and

Consumer Services, Division of Plant Industry. 2 pp. Available at http://www.freshfromflorida.com/content/download/10810/141151/nem005.pdf.

Fortuner, R. 1970. On the morphology of *Aphelenchoides besseyi* Christie, 1942 and *A. siddiqii* n. sp. *Journal of Helminthology,* 44: 141–152.

Fortuner, R. & Williams, K.J.O. 1975. Review of the literature on *Aphelenchoides besseyi* Christie, 1942, the nematode causing "white tip" disease in rice. *Helminthological Abstracts,* 44: 1–40.

Franklin, M.T. 1978. *Aphelenchoides* and related genera. *In Plant nematology,* GD1, pp. 172–187. London, Ministry of Agriculture, Fisheries and Food, HMSO. 440 pp.

Franklin, M.T. & Siddiqi, M.R. 1972. *Aphelenchoides besseyi*. CIH descriptions of plant-parasitic nematodes, Set 1, No. 4. Wallingford, CABI. St Albans, UK, Commonwealth Institute of Helminthology (CIH). 3 pp.

Hesling, J.J. & Wallace, H.R. 1960. Susceptibility of varieties of chrysanthemum to infestation by *Aphelenchoides ritzemabosi* (Schwartz). *Nematologica,* 5: 297–302.

Hockland, S. & Eng, L. 1997. *Capsicum annuum v. longum:* A new host record for the rice white tip nematode, *Aphelenchoides besseyi*. *International Journal of Nematology,* 7: 229.

Hooper, D.J. 1975. *Aphelenchoides blastophthorus*. CIH Descriptions of plant-parasitic nematodes, Set 5, No. 73. Wallingford, CABI. St Albans, UK, Commonwealth Institute of Helminthology (CIH). 4 pp.

Hooper, D.J. & Cowland, J.A. 1986. Fungal hosts for the chrysanthemum nematode, *Aphelenchoides ritzemabosi*. *Plant Pathology,* 35(1): 128–129.

Hooper, D.J. & Evans, K. 1993. Extraction, identification and control of plant-parasitic nematodes. *In* K. Evans, D.L. Trudgill & J.M. Webster, eds. *Plant parasitic nematodes in temperate agriculture*, pp. 1–59. Wallingford, UK, CABI. 656 pp.

Hooper, D.J., Hallmann, J. & Subbotin, S. 2005. Methods for extraction, processing and detection of plant and soil nematodes. *In* M. Luc, R.A. Sikora & J. Bridge, eds. *Plant parasitic nematodes in subtropical and tropical agriculture*, 2nd edn, pp. 53–86. Wallingford, UK, CABI. 871 pp.

Hunt, D.J. 1993. *Aphelenchida, Longidoridae and Trichodoridae: Their systematics and bionomics.* Wallingford, UK, CABI. 352 pp.

Ibrahim, S.K., Perry, R.N., Burrows, P.R. & Hooper, D.J. 1994a. Differentiation of species and populations of *Aphelenchoides* and of *Ditylenchus augustus* using a fragment of ribosomal DNA. *Journal of Nematology,* 26: 412–421.

Ibrahim, S.K., Perry, R.N. & Hooper, D.J. 1994b. Use of esterase and protein patterns to differentiate two new species of *Aphelenchoides* on rice from other species of *Aphelenchoides* and from *Ditylenchus augustus* and *D. myceliophagus*. *Journal of Nematology,* 40: 267–275.

Jagdale, G.B. & Grewal, P.S. 2006. Infection behavior and overwintering survival of foliar nematodes, *Aphelenchoides fragariae*, on Hosta. *Journal of Nematology,* 38(1): 130–136.

Johnson, C.S. 1998. Tobacco. *In* K.R. Barker, G.A. Pederson & G.L. Windham, eds. *Plant and nematode interactions,* pp. 487–522. Agronomy Monograph No. 36. Madison, WI, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America 771 pp..

Kaisa, T.R., Harman, A.L. & Harman, D.M. 1995. Two new species of Aphelenchoididae (Nemata) from declining red pines in Maryland. *Journal of Nematology,* 27(2): 213–221.

Khan, Z., **Son, S., Moon, H., Kim, S., Shin, H., Jeon, Y. & Kim, Y.** 2007. Description of a foliar nematode, *Aphelenchoides fragariae* (Nematoda: Aphelenchida) with additional characteristics from Korea. *Journal of Asia-Pacific Entomology,* 10(4): 313–315.

Khan, Z., Son, S.H., Shin, H.D. & Kim, Y.H. 2008. First report of a foliar nematode *Aphelenchoides fragariae* (Aphelenchidae) on *Stachys riederi* var. *japonica*, a medicinal plant, in Korea. *Plant Pathology Journal,* 24(1): 97–100.

Knight, K.W.L., Hill, C.F. & Sturhan, D. 2002. Further records of *Aphelenchoides fragariae* and *A. ritzemabosi* (Nematoda: Aphelenchida) from New Zealand. *Australasian Plant Pathology,* 31: 93–94.

Kohl, L.M. 2011. Astronauts of the nematode world: An aerial view of foliar nematode biology, epidemiology, and host range. *APSnet Features,* doi:10.1094/APSnetFeature-2011-0111.

Liu, W., Lin, M., Li, H. & Sun, M. 2008. Dynamic development of *Aphelenchoides besseyi* on rice plant by artificial inoculation in the greenhouse. *Agricultural Sciences in China,* 7(8): 970–976.

Madej, T., Janowicz, K. & Baszkowski, J. 2000. The diseases of ornamental plants caused by *Aphelenchoides ritzemabosi* in association with fungi. *Archives of Phytopathology and Plant Protection,* 33(2): 141–148.

Massey, C.L. 1974. *Biology and taxonomy of nematode parasites and associates of bark beetles in the United States.* Agriculture Handbook No. 446. Washington, DC, US Department of Agriculture Forest Service. 233 pp.

McCuiston, J.L., Hudson, L.C., Subbotin, S.A., Davis, E.L. & Warfield, C.Y. 2007. Conventional and PCR detection of *Aphelenchoides fragariae* in diverse ornamental host plant species. *Journal of Nematology,* 39(4): 343–355.

Moretti, F., Cotroneo, A., Tacconi, R., Santi, R. & De Vincentis, F. 1999. [Damage from *Aphelenchoides besseyi* on rice and nematode extraction methods from rice seeds.] *Informatore Fitopatologico,* 3: 39–41 (in Italian).

Perry, R.N. & Moens, M. 2006. Plant nematology. Wallingford, UK, CABI Publishing. 568 pp.

Remeeus, P.M. & Pelazza, N. 2014. *Detection of* Aphelenchoides besseyi *on* Oryza sativa. International Rules for Seed Testing Annex to Chapter 7: Seed Health Testing Methods, 7-025. Version 1.2, 2014-01-01. Bassersdorf, Switzerland, International Seed testing Association (ISTA). 5 pp.

Richardson, P.N. & Grewal, P.S. 1993. Nematode pests of glasshouse crops and mushrooms. *In* K. Evans, D.L. Trudgill & J.M. Webster, eds. *Plant parasitic nematodes in temperate agriculture,* pp. 501–544. Wallingford, UK, CABI. 656 pp.

Ritzema Bos, J. 1891. Zwei neue Nematodenkrankheiten der Erdbeerpflanzen. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz,* 1:1–16.

Ritzema Bos, J. 1893. Neue Nematodenkrankheiten bei Topfpflanzen. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz,* 3: 69–82.

Rybarczyk-Mydłowska, K., Mooyman, P., van Megen, H., van den Elsen, S., Vervoort, M., Veenhuizen, P., van Doorn, J., Dees, R., Karssen, G., Bakker, J. & Helder, J. 2012. Small subunit ribosomal DNA-based phylogenetic analysis of foliar nematodes (*Aphelenchoides* spp.) and their quantitative detection in complex DNA backgrounds. *Phytopathology,* 102(12): 1153–1160.

Sánchez-Monge, A., Flores, L., Salazar, L., Hockland, S. & Bert, W. 2015. An updated list of the plants associated with plant-parasitic *Aphelenchoides* (Nematoda: Aphelenchoididae) and its implications for plant-parasitism within this genus. *Zootaxa,* 4013(2): 207–224.

Sanwal, K.C. 1961. A key to the species of the nematode genus *Aphelenchoides* Fischer, 1894. *Canadian Journal of Zoology,* 39: 143–148.

Shahina, F. 1996. A diagnostic compendium of the genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchida) with some new records of the group from Pakistan. *Pakistan Journal of Nematology,* 14: 1–32.

Shepherd, J.A. & Barker, K.R. 1990. Nematode parasites of tobacco. *In* M. Luc, R.A. Sikora & J. Bridge, eds. *Plant parasitic nematodes in subtropical and tropical agriculture,* 1st edn, pp. 493–517. Wallingford, UK, CABI. 643 pp.

Siddiqi, M.R. 1974. *Aphelenchoides ritzemabosi*. Commonwealth Institute of Helminthology (CIH) descriptions of plant-parasitic nematodes, Set 3, No. 32. St Albans, UK, Commonwealth Institute of Parasitology (CIP). 4 pp.

Siddiqi, M.R. 1975. *Aphelenchoides fragariae*. CIH descriptions of plant-parasitic nematodes, Set 5, No. 74. St Albans, UK, Commonwealth Institute of Helminthology (CIH). 4 pp.

Society of Nematologists. 1980 Society of Nematologists slide set, Available at http://plpnemweb.ucdavis.edu/nemaplex/Taxadata/G011S1.htm#Damage: (last access 5 March 2016).

Southey, J.F. 1993. Nematode pests of ornamental and bulb crops. *In* K. Evans, D.L. Trudgill & J.M. Webster, eds. *Plant parasitic nematodes in temperate agriculture*, pp. 463–500. Wallingford, UK, CABI. 656 pp.

Stokes, D.E. & Alfieri Jr, S.A. 1969. A foliar nematode and a *Phytophthora* parasitic to gloxinia. *Proceedings of the Florida State Horticultural Society* 1968, 81: 376–380.

dan Supriadi, D. 2008. Determination of parasitic nematode *Aphelenchoides* sp. causing leaf blotch disease of sambiloto *(Andrographis paniculata). Jurnal Littri*, 14(2): 61–66. (in Indonesian)

Swart, A., Lesufi, M.M. & Tiedt, L.R. 2007. Report on the chrysanthemum leaf nematode, *Aphelenchoides ritzemabosi* from South Africa. *South African Journal of Plant and Soil*, 24: 253.

Tamura, I. & Kegasawa, K. 1959. Studies on the ecology of the rice nematode *Aphelenchoides besseyi* Christie. III. The injured features of the rice plant and the population density of nematodes found in the unhulled rice grain with special reference to the type of the nursery bed. *Japanese Journal of Ecology*, 9(1): 1–4.

Thomas, P.R. 1968. *Aphelenchoides ritzemabosi* on Saintpaulia. *Plant Pathology*, 17: 94–95.

Thorne, G. 1961. *Principles of nematology*. New York, NY, McGraw-Hill Book Co., Inc. 553 pp.

Todd, E.H. & Atkins, J.G. 1958. White tip disease of rice. I. Symptoms, laboratory culture of nematodes, and pathogenicity tests. *Phytopathology*, 48: 632–637.

Uebayashi, Y., Amano, T. & Nakanishi, I. 1976. Studies on the abnormal rice kernel "Kokutenmai". V. Mechanism of the symptoms development. *Japanese Journal of Nematology*, 6: 67–72.

Wager, V.A. 1972. *Records of diseases of ornamental plants not previously reported in South Africa.* Technical Communication No. 100. Pretoria, Department of Agricultural Technical Services, South Africa.

Wang, X., Wang, P., Gu, J.F., Wang, J.L. & Li, H.M. 2013. Description of *Aphelenchoides xui* sp. n. (Nematoda: Aphelenchoididae) in packaging wood from South Africa. *Nematology*, 15: 279–289.

Yoshii, H. & Yamamoto, S. 1950. A rice nematode disease "Senchu Shingare Byo". I. Symptom and pathogenic nematode. *Journal of the Faculty of Agriculture, Kyushu University*, 9: 209–222.

Yoshii, H. & Yamamoto, S. 1951. [On some methods for the control of the rice nematode disease.] *Science Bulletin of the Faculty of Agriculture, Kyushu University*, 12: 123–131 (in Japanese).

Yu, P.C. & Tsay, T.T. 2003. Occurrence of a foliar nematode disease of fern in Taiwan. *Plant Pathology Bulletin*, 13: 35–44.

Zhen, F., Agudelo, P. & Gerard, P. 2012. A protocol for assessing resistance to *Aphelenchoides fragariae* in hosta cultivars. *Plant Disease*, 96: 1438–1444.

Zuo, K., Cui, R.Q., Ye, W.M., Luo, M., Wang, H.H., Hu, X.N. & Liao, J. 2010. Morphological and molecular characterisation of *Aphelenchoides fujianensis* n. sp. (Nematoda: Aphelenchoididae) from *Pinus massoniana* in China. *Zootaxa*, 2509: 39–52.

9. Figures

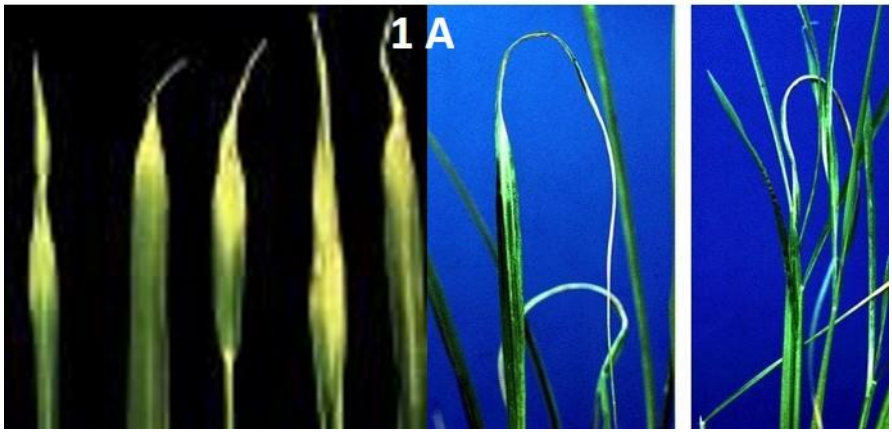


Figure 1(A). Symptoms caused by *Aphelenchoides besseyi* on *Oryza sativa* leaves: white tip (left and middle) and necrotic patches and crinkled leaves (right).
Photo courtesy Society of Nematologists (1980) (left) and CABI (2006) (middle and right).

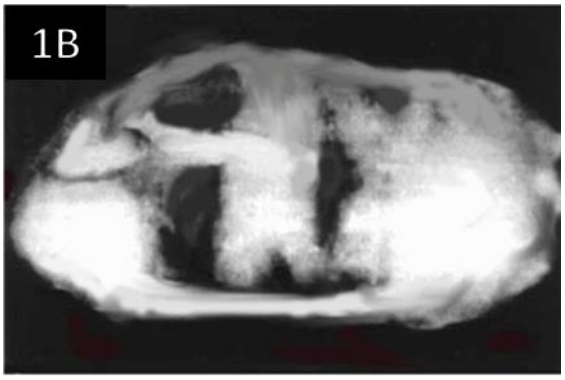


Figure 1(B). Necrotic lesions caused by *Aphelenchoides besseyi* in the endosperm of a rice kernel.
Photo courtesy Bridge et al. *(1990)*.

Figure 1(C). Symptoms caused by *Aphelenchoides besseyi* on strawberry.
Photo courtesy Jeffrey Lotz, Florida Department of Agriculture and Consumer Services, Gainesville, FL, United States.

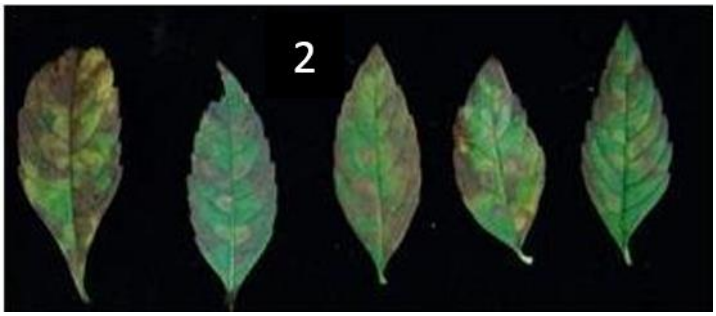


Figure 2. Symptoms caused by *Aphelenchoides fragariae* on *Stachys riederi* var. *japonica.*
Photo courtesy Khan et al. *(2008)*.

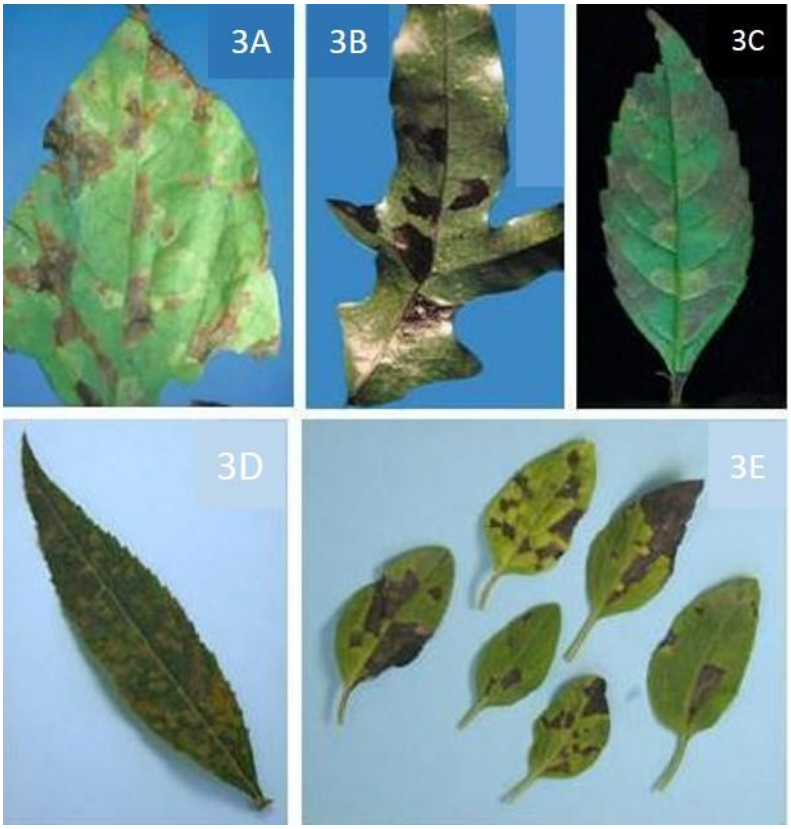


Figure 3. Symptoms of *Aphelenchoides fragariae* attack in: (A) *Convolvulus arvensis*; (B) *Phymatodes diversifolium*; (C) *Stachys riederi*; (D) *Buddleja* sp.; and (E) *Salvia* sp.
Photo courtesy (A, B) Knight et al. *(2002); (C) Khan* et al. *(2008); and (D, E) Kohl (2011)*.

Figure 4. Different degrees of symptom severity on *Hosta* leaves caused by *Aphelenchoides fragariae.*
Photo courtesy Zhen et al. *(2012)*.

Figure 5. Leaf blotch symptoms on plants infested with *Aphelenchoides fragariae*: (A) ferns; (B) *Pteris cretica*; and (C) *Stenochlaena tenuiflolia.*
Photo courtesy (A) Cobon and O'Neill (2011) and (B, C) Chizov et al. *(2006)*.

Figure 6. Leaf blotch symptoms on plants infested by *Aphelenchoides fragariae:* (A) *Begonia* sp. and (B) *Andrographis paniculata.*
Photo courtesy (A) Department of Crop Sciences, University of Illinois at Urbana–Champaign, Champaign, IL, United States, and (B) dan Supriadi (2008).

Figure 7. *Fragaria* spp. plants infested with *Aphelenchoides fragariae:* (A) tight aggregation of crown with malformed leaves; (B) abnormal plant growth with stunting and deformation; (C) an uninfested plant; and (D) malformed leaves.
Photo courtesy (A–C) Cobon and O'Neill (2011) and (D) Adam Szczygieł, formerly Institute of Pomology and Floriculture, Experimental Research Station at Brzezna, Poland.

Figure 8. Symptoms (interveinal necrosis) caused by *Aphelenchoides ritzemabosi* on *Chrysanthemum* leaves.
Photo courtesy J. Bridge, CABI BioScience.

Figure 9. *Aphelenchoides* spp.: (A) female; (B) male; (C) female anterior end; (D) lateral field; (E) female tail; (F) male tail; (G) female tail terminal mucro; and (H) male tail terminal mucro.
Photo courtesy (A, B, E) Wang et al. *(2013); (D, G) Deimi* et al. *(2006); (H) Yu and Tsay (2003); and (C, F) Z. F. Yang and H. Xie, South China Agricultural University, Guangzhou, China)*.

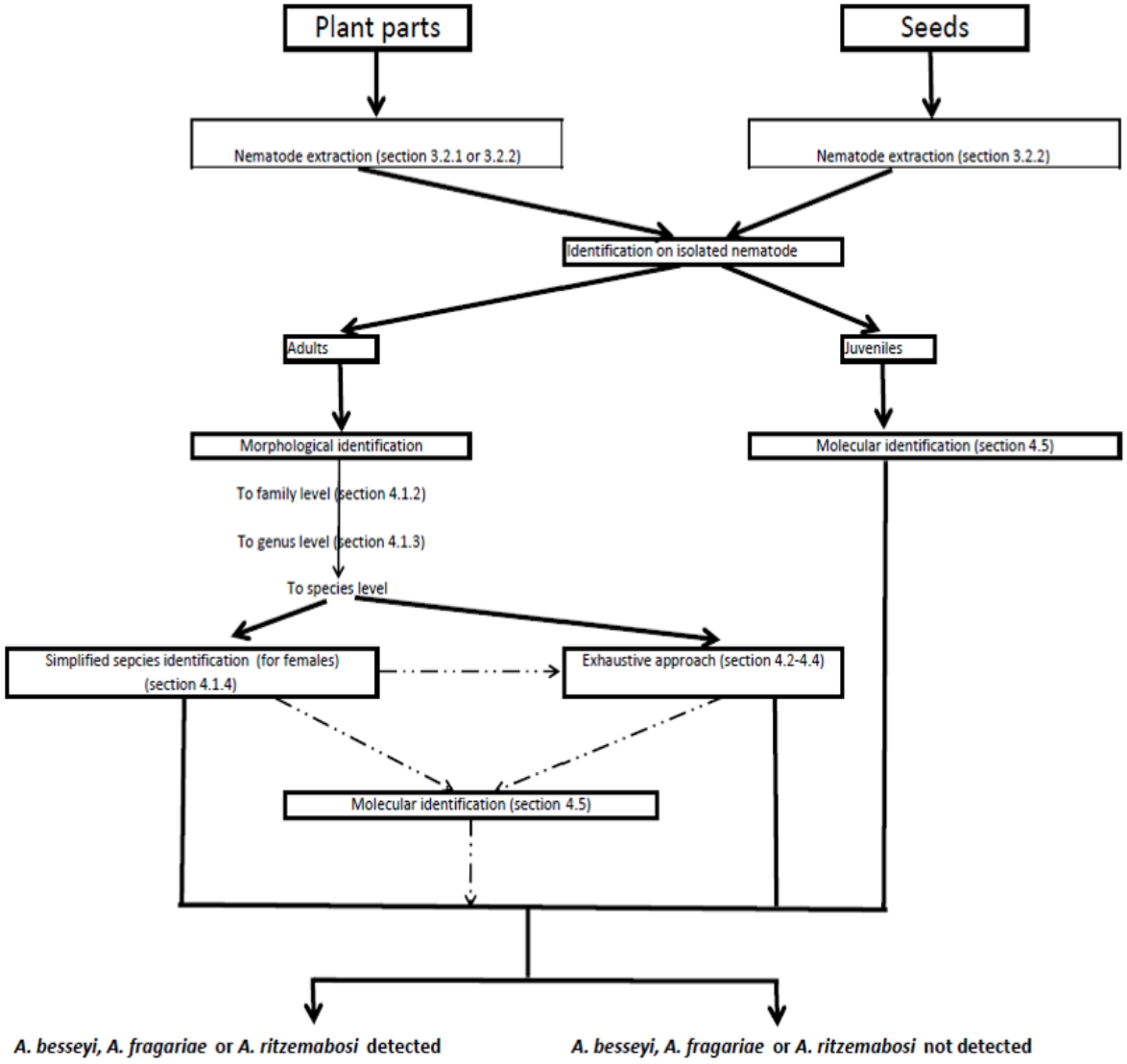


Figure 10. Flow diagram of the process to identify *Aphelenchoides* species.

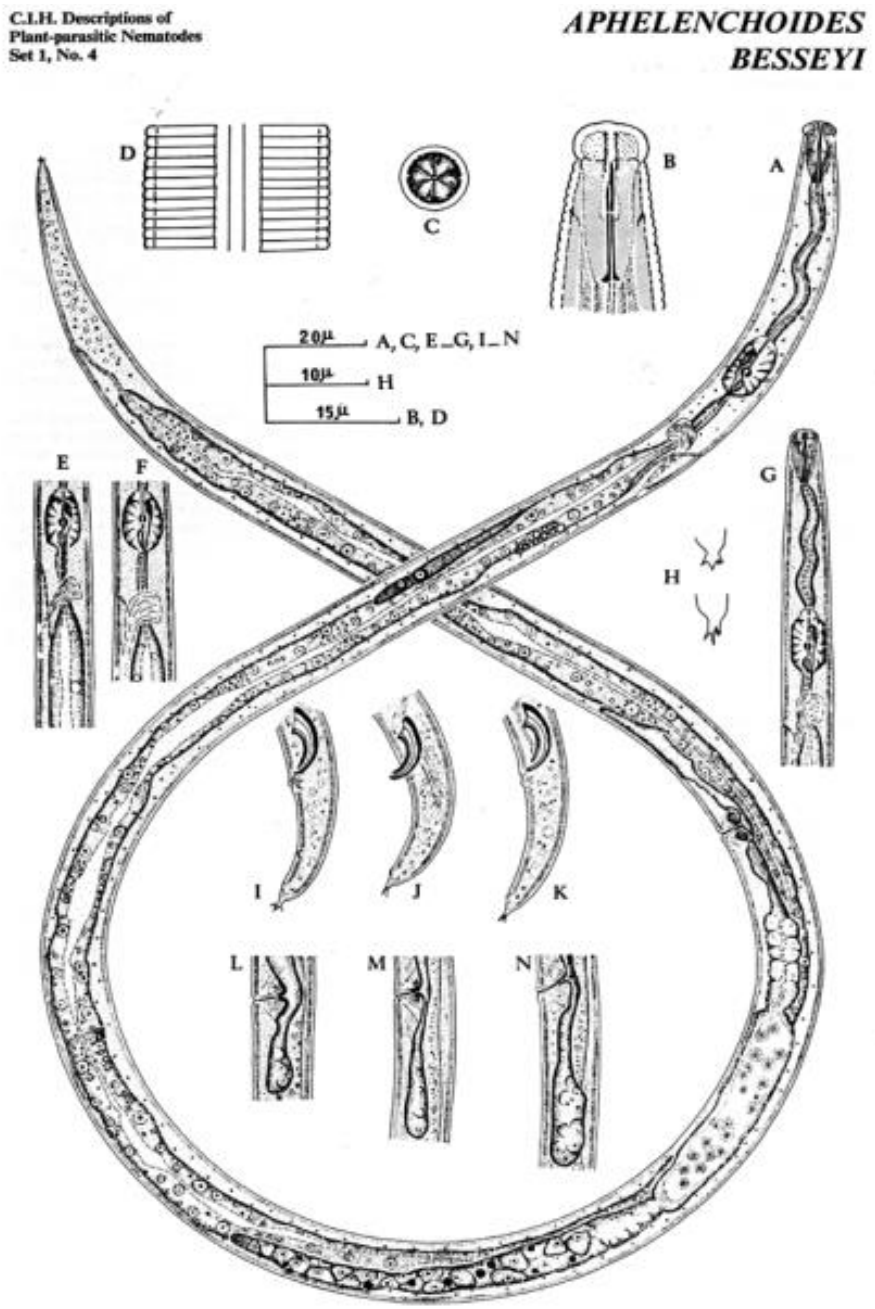
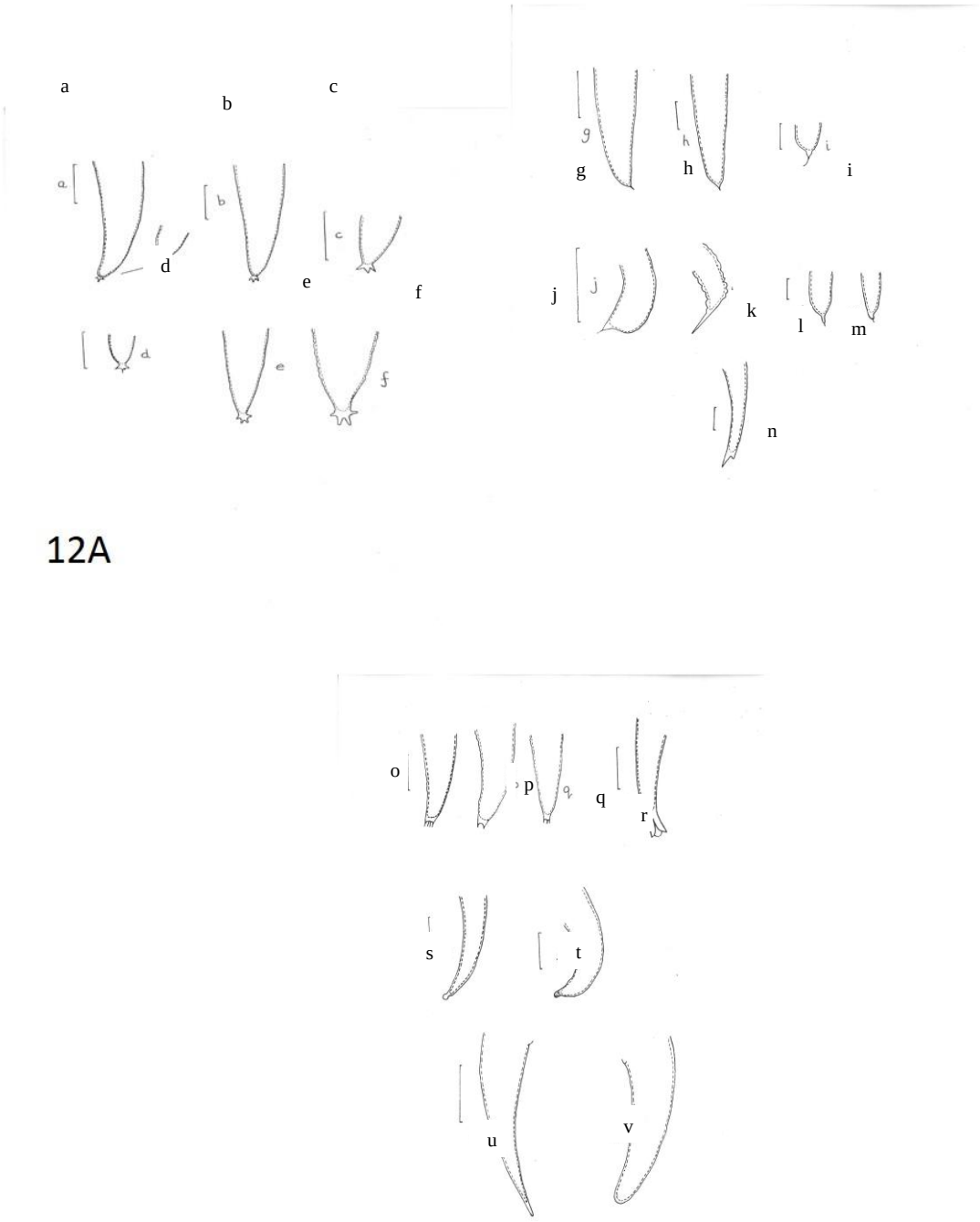


Figure 11. *Aphelenchoides besseyi*: **(**A) female; (B) female head end; (C) female *en face* view; (D) lateral field; (E, F) variation in female metacorpus and pharynx region and position of excretory pore with respect to nerve ring; (G) male anterior end; (H) female tail termini showing variation in shape of mucro; (I–K) male tail ends; and (L–N) variation in post-vulval sac.
Source: Fortuner (1970), except (D) Franklin and Siddiqi (1972).



12A

Figure 12(A). Tail terminus types of *Aphelenchoides* species (code numbers according to the polytomous key in section 4.2.2.2): (a–f) star shape: (a) *A. aligarhiensis*, (b) *A. asterocaudatus*, (c) *A. besseyi* and (d) *A. goodeyi* (all scale bars = 10 µm); (e, f) *A. nonveilleri* (×1 100 and ×2 200, respectively); (g–m) single terminal mucro: (g) *A. richardsoni*, (h) *A. nechaleos*, (i) *A. vaughani,* (j) *A. sp.* (k) *A. tsalolikhini* and (l, m) *A. submersus*; (n) bifurcate: *A. bicaudatus* (all scale bars = 10 µm); (o–t) other: (o–q) *A. ritzemabosi*, (r) *A. sphaerocephalus*, (s) *A. gynotylurus* and (t) *A. helicosoma* (all scale bars = 10 µm); and (u, v) no mucro: (u) *A. microstylus* (scale bar = 10 µm) and (v) *A. obtusus* (×1 250).
Drawing Sue Hockland, Fera Science Limited, York, United Kingdom.

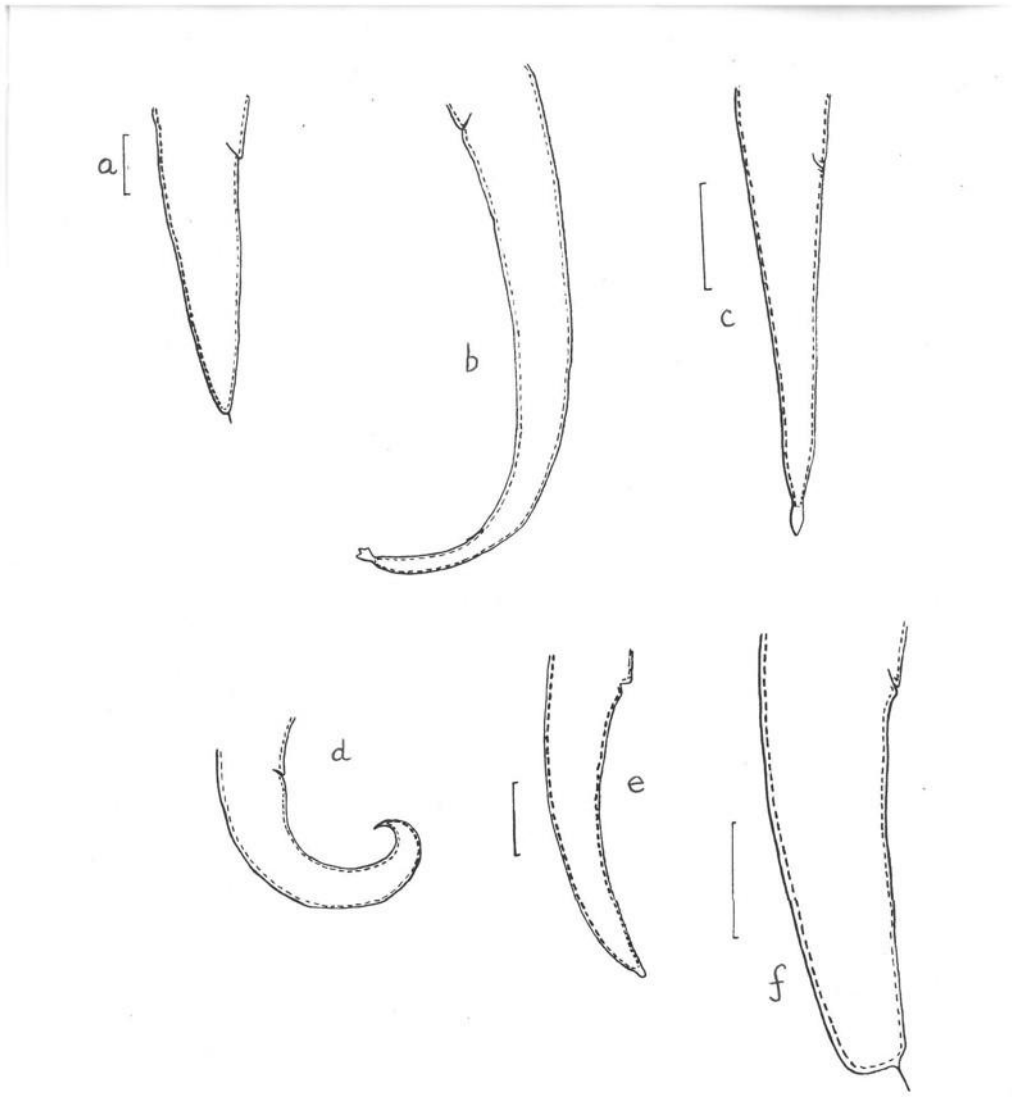
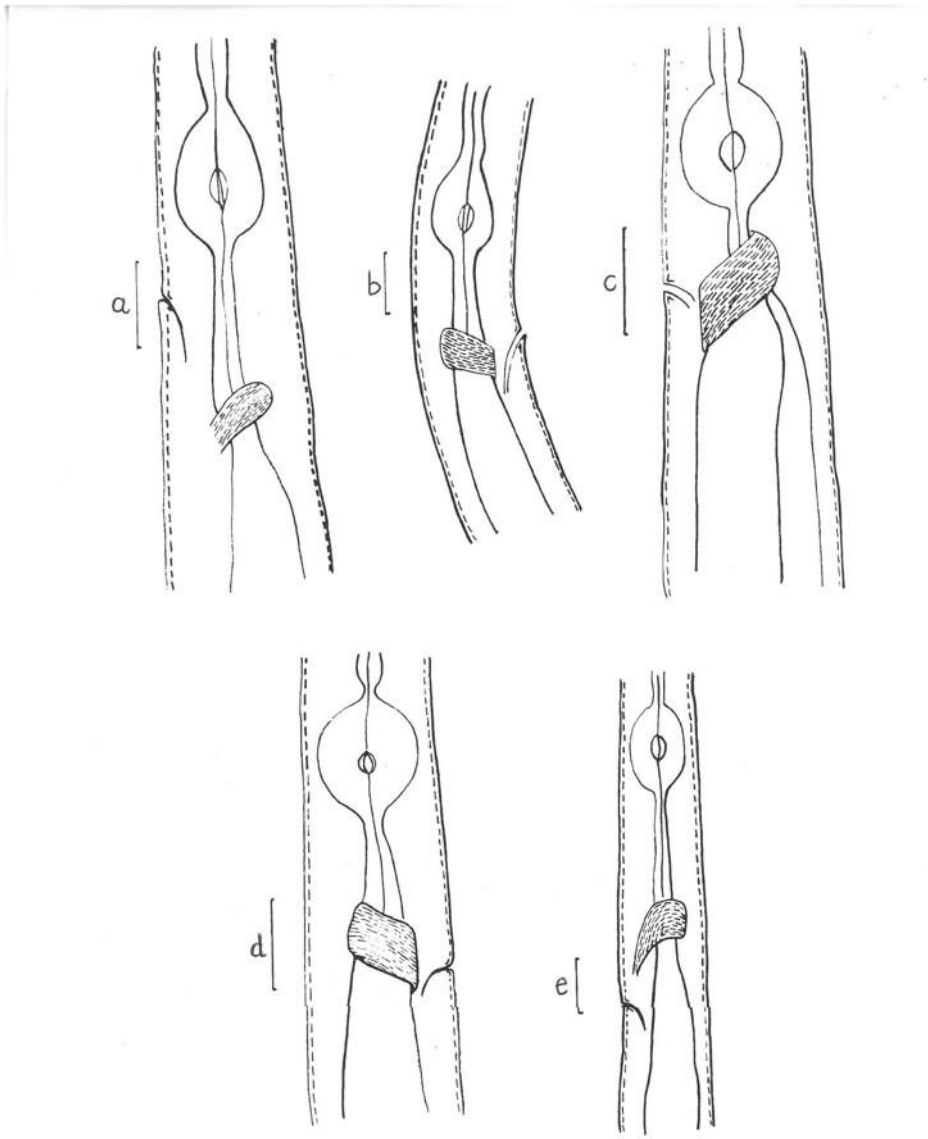


Figure 12(B). Tail shapes in *Aphelenchoides* species (scale bars = 10µm): conoid: (a) *A. blastophthorus*; elongate conoid: (b) *A. andrassyi* (no scale bar) and (c) *A. chalonus*; dorsally convex conoid: (d) *A. fluviatilis* (×1 100) and (e) *A. franklini*; and subcylindroid: (f) *A. subtenuis.*
Drawing Sue Hockland, Fera Science Limited, York, United Kingdom.



12C

Figure 12(C). Positions of the excretory pore relative to the nerve ring in *Aphelenchoides* species: excretory pore anterior to or level with the anterior edge of the nerve ring: (a) *A. longiurus* and (b) *A. blastophthorus*; excretory pore level with the nerve ring (from behind the anterior point to in front of the posterior point): (c) *A. cibolensis*; and excretory pore level with the posterior edge of the nerve ring: (d) *A. arcticus* or posterior to it: (e) *A. ritzemabosi* (all scale bars = 10 µm).
Drawing Sue Hockland, Fera Science Limited, York, United Kingdom.

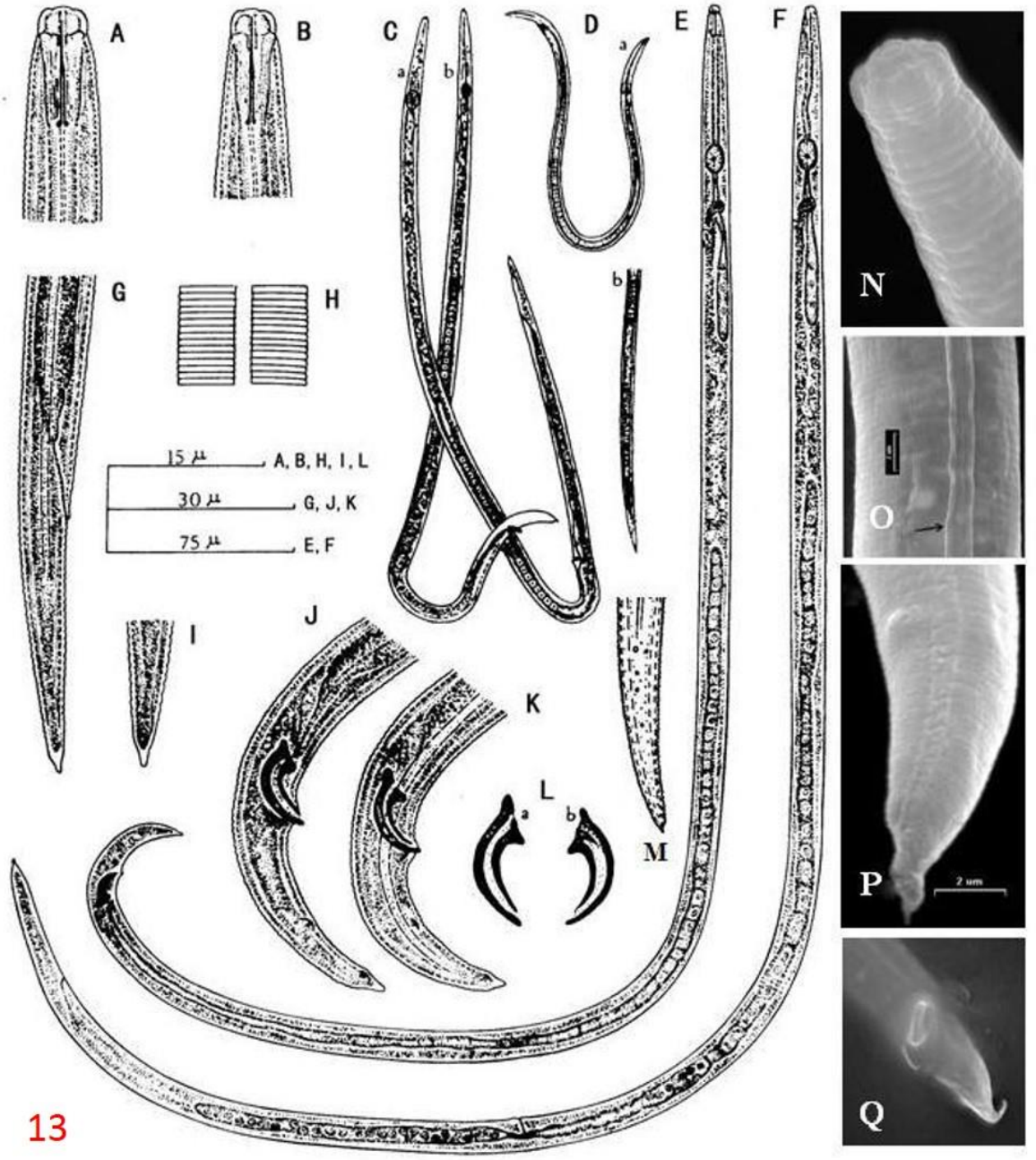


Figure 13. *Aphelenchoides fragariae*: **(**A, N) female anterior or lip region; (B) male anterior or lip region; (C) (a) female and (b) male of *A. olesistus* Ritzema Bos, 1893 (= *A. fragariae*); (D) (a) male and (b) posterior portion of female of *Aphelenchus fragariae* Ritzema Bos, 1891; (E) male; (F) female; (G) female tail; (H, O) lateral field; (I, M, P) female tail tip; (J, K, Q) male tails; and (L) spicules.
Photo courtesy (A, B, E–L) Siddiqi (1975); (C) Ritzema Bos (1893); (D) Ritzema Bos (1891); (M) Allen (1952); (N, Q) Kohl (2011); and (O, P) Khan et al. *(2008)*.

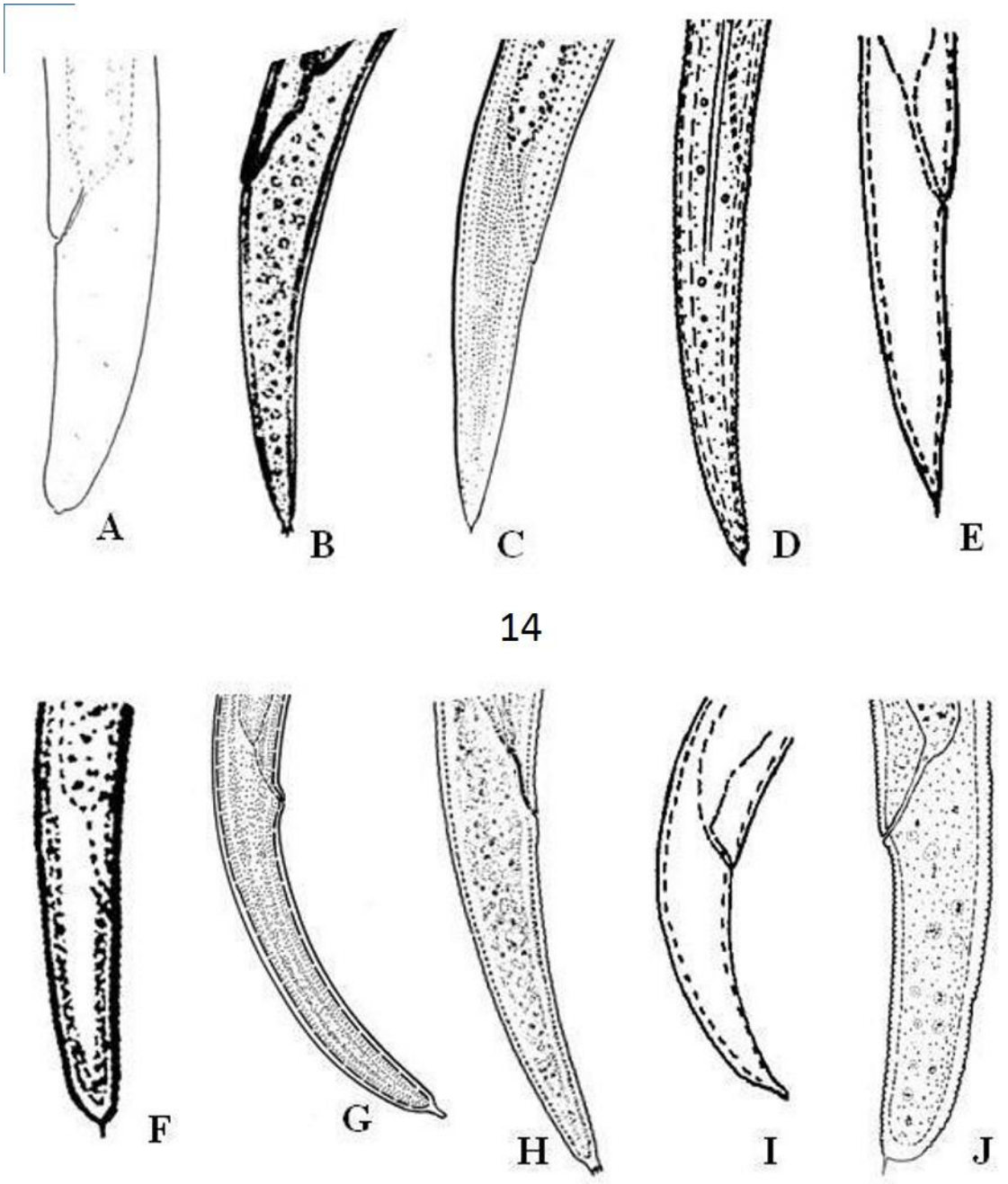


Figure 14. Tails of Aphelenchoides fragariae and related species of Aphelenchoides: (A) A. arachidis; (B) A. besseyi; (C) A. blastophthorus; (D) A. fragariae; (E) A. helophilus; (F) A. resinosi; (G) A. rhytium; (H) A. ritzemabosi; (I) A. saprophilus; and (J) A. subtenuis.
Photo courtesy (A) Bridge and Hunt (1985); (B) Franklin and Siddiqi (1972); (C) Hooper (1975); (D) Allen (1952); (E) Shahina (1996); (F) Kaisa et al. *(1995); (G) Massey (1974); (H) Siddiqi (1974); (I) Shahina (1996); and (J) Deimi* et al. *(2006).*

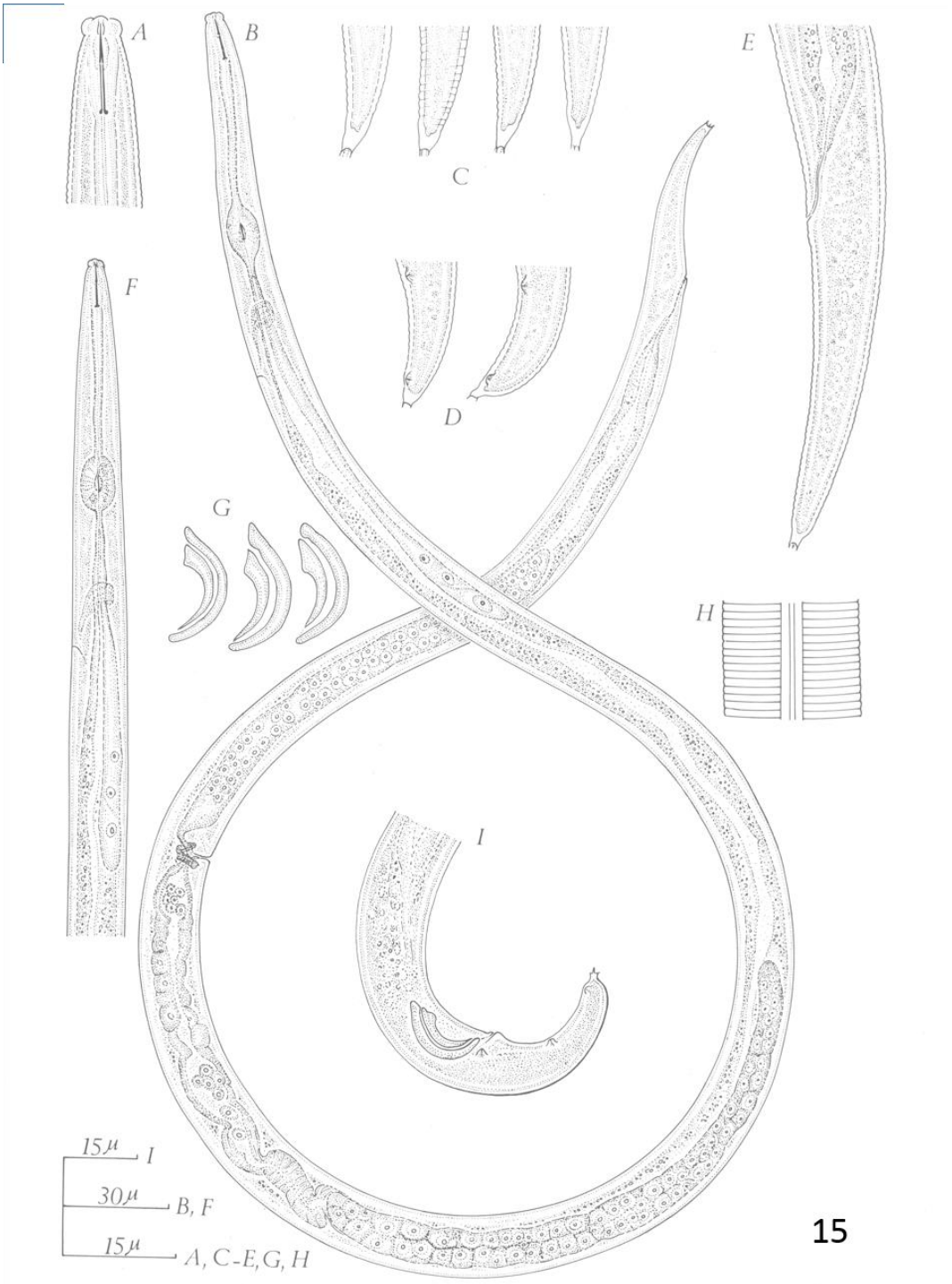


Figure 15. *Aphelenchoides ritzemabosi:* (A) female head end; (B) female; (C) female tail ends; (D) male tail ends; (E) female tail; (F) female pharyngeal region; (G) spicules; (H) lateral field; and (I) male tail region.
Photo courtesy Siddiqi (1974).

Publication history

This is not an official part of the standard.

2006-05 SC introduced original subject: *Aphelenchoides besseyi, A. fragariae and A. ritzemabosi* (2006-025).

2007-03 CPM-2 (2007) added subject under work programme topic: Nematodes (2006-008).

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2014-03 Expert consultation.

2015-03 TPDP e-decision for submission to SC.

2015-06 SC e-decision approval for submission to MC (2015_eSC_Nov_01).

2015-07 Member consultation.

2016-03 DP drafting group revised draft based on editor's comments.

2016-03 TPDP e-decision (2016_eTPDP_Mar_04).

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2016-08 SC adopted DP on behalf of CPM (with no objections received).

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IPPC

The International Plant Protection Convention (IPPC) is an international plant health agreement that aims to protect cultivated and wild plants by preventing the introduction and spread of pests. International travel and trade are greater than ever before. As people and commodities move around the world, organisms that present risks to plants travel with them.

Organization

- There are over 180 contracting parties to the IPPC.
- Each contracting party has a national plant protection organization (NPPO) and an Official IPPC contact point.
- Nine regional plant protection organizations (RPPOs) work to facilitate the implementation of the IPPC in countries.
- IPPC liaises with relevant international organizations to help build regional and national capacities.
- The Secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).

International Plant Protection Convention (IPPC)

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