

## PM 7194 (2) *Hirschmanniella* spp.

**Specific scope:** This Standard describes a diagnostic protocol for the genus *Hirschmanniella*.

This Standard should be used in conjunction with PM 7176 *Use of EPPO diagnostic protocols*.<sup>1</sup>

Terms used are those in the EPPO Pictorial Glossary of Morphological Terms in Nematology<sup>2</sup>

**Specific approval and amendment:** Approved in 2009–09. Revised in 2021–11

Authors and contributors are given in the Acknowledgements section.

### 1 | INTRODUCTION

The genus *Hirschmanniella* includes at present about 30 species (Khun et al., 2015). More than half of them have been recorded on rice and can be found in tropical and subtropical regions of the world, including the southern USA, Iran, Ecuador, Egypt, India, Myanmar, China and Japan (Ibrahim et al., 2010; Mukesh & Naveen, 2010; Koyama et al., 2013; Katsuta et al., 2016; Trivino Gilces et al., 2016). The non-rice species are found in the same region and in the northern temperate zone (fewer than 10 species). All members are obligate migratory endoparasites of roots and are adapted to a broad range of moist habitats with a preference for graminaceous roots (Loof, 1991). In addition, some dicotyledonous plants such as cotton, black pepper, sunflower and banana are hosts, including some weeds (Khan & Hasan, 2010; Anwar et al., 2011; Chau, 2017; Pervez et al., 2017). *Hirschmanniella* has also been detected on the roots and leaves of aquatic plants and in soil associated with bonsais (Ryss & Karnkowski, 2010). On rice, it is common to detect several species of *Hirschmanniella*. The genus *Hirschmanniella*, revised by Sher in 1968, is notorious for having multiple dubious or easily confused species. A study by Tandingan De Ley et al. (2007) on a few Californian *Hirschmanniella* species suggests that this genus harbours one or more complexes of species which have slightly diverged morphologically.

The following five *Hirschmanniella* species have been described from Europe: *H. behningi* (Micoletzky,

1923), *H. gracilis* (de Man, 1880), *H. halophila* Sturhan & Hallmann, 2010, *H. loofi* Sher, 1968 and *H. zostericola* (Allgén, 1934). *Hirschmanniella behningi* (USSR, Sweden), *H. halophila* (Germany), *H. loofi* (the Netherlands, Belgium, Italy and Poland) and *H. zostericola* (Sweden) have a limited European distribution. The species *H. gracilis* has been regularly detected in Europe and North America, particularly on *Phragmites* reeds, but also on rice in India, Vietnam and China (the later records need confirmation).

The genus *Hirschmanniella* spp., with the exception of *Hirschmanniella behningi*, *Hirschmanniella gracilis*, *Hirschmanniella halophila*, *Hirschmanniella loofi* and *Hirschmanniella zostericola*, is included in the EU legislation (EU, 2019). This protocol includes elements to identify the genus *Hirschmanniella* spp. and the species within *Hirschmanniella*.

### 2 | IDENTITY

**Name:** *Hirschmanniella* Luc & Goodey, 1964.

**Synonyms:** *Hirschmannia* Luc & Goodey, 1962 nec *Hirschmannia* Elofson, 1941.

**Taxonomic position:** Nematoda: Tylenchina:<sup>3</sup> Pratylenchidae.

**EPPO Code:** HIRSSP

**Phytosanitary categorization:** EU A1 Quarantine pest II A (as *Hirschmanniella* spp. Luc & Goodey [HIRSG], except: *Hirschmanniella behningi* (Micoletzky) Luc & Goodey [HIRSBE], *Hirschmanniella gracilis* (de Man) Luc & Goodey [HIRSGR], *Hirschmanniella halophila* Sturhan & Hallman, *Hirschmanniella loofi* Sher [HIRSLO] and *Hirschmanniella zostericola* (Allgén) Luc & Goodey [HIRSZO]).

### 3 | DETECTION

#### 3.1 | Symptoms

The genus *Hirschmanniella* includes some of the largest nematodes within the order Tylenchida. All juvenile and adult stages migrate through the root cortex and feed on cells. The life cycle takes about one month.

<sup>1</sup>Use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

<sup>2</sup>[https://www.eppo.int/media/uploaded\\_images/RESOURCES/eppo\\_publications/TD\\_1056\\_EPPO\\_Glossary\\_Nem\\_2020-04.pdf](https://www.eppo.int/media/uploaded_images/RESOURCES/eppo_publications/TD_1056_EPPO_Glossary_Nem_2020-04.pdf)

<sup>3</sup>Recent development combining a classification based on morphological data and molecular analysis refer to “Tylenchomorpha” (De Ley & Blaxter, 2004)

*Hirschmanniella* is often found in aquarium plants imported from Asian countries to Europe (Ryss & Karnkowski, 2010).

The symptoms of *Hirschmanniella* spp. are not specific. On rice, retardation of growth, decrease in culm height, reduction in weight of dry matter, delayed tillering, fewer shoots and discolouration of the older leaves have been reported (Siddiqi, 1973). Soil sampling and extracting roots is the best method for detecting adult *Hirschmanniella* males and females.

### 3.2 | Extraction procedures

*Hirschmanniella* species are migratory endoparasites that can be detected by extraction from soil and roots. For extraction from soil, useful methods are Baermann funnel, Oostenbrink elutriator and sieving/centrifugation. Methods to be used for the extraction of *Hirschmanniella* from root material include Baermann funnel, maceration<sup>4</sup>/filtration, maceration/centrifugal-flotation and mistifier technique. To improve detection efficacy, roots have to be cut into small pieces and the bigger roots have to be sliced longitudinally. For submerged aquatic plants, the whole plant may be used (with same procedure as for roots). Detailed descriptions of extraction equipment and procedures can be found in EPPO PM 7/119 *Nematode extraction* (EPPO, 2013).

## 4 | IDENTIFICATION

Identification of *Hirschmanniella* at genus and at species level is based in general on a combination of morphological and morphometrical characters of the adult stages. For light microscopical identification it is recommended to examine specimens mounted in fixative on microscope slides with a differential interference contrast microscope. Molecular tests for the identification of the genus *Hirschmanniella* or of European species are not yet available.

### 4.1 | Identification on the basis of morphological features

#### 4.1.1 | Morphological identification of the genus *Hirschmanniella*

The genus *Hirschmanniella* is a member of the Pratylenchidae Thorne, 1949 family. Loof (1991) produced a useful dichotomous and tabular key for the identification of genera within the family Pratylenchidae. Alternatively, the dichotomous key for subfamily and

genus identification produced by Siddiqi (2000) can be used. Both keys can be found in Appendix 1.

In summary, members of *Hirschmanniella* have: a body length of 1–4 mm; habitus straight and slender; no sexual dimorphism in lip region and pharynx region. Cuticle with distinct transverse striae. Lateral field with four incisures areolated towards extremities. Deirids absent. Phasmids pore-like, near tail tip. Lip region either low, flattened with rounded edges, or higher, hemispheric, composed of three to seven fine annules, not offset from the neck. Stylet strong (15–46 µm) with large stylet knobs. Metacarpus well developed. Pharyngeal glands in tandem, forming a long ventrally overlapping lobe. Two branches of female reproductive organs equally developed. Spermathecae round to oval, axial. Tails similar between sexes, elongate-conoid, usually with a terminal mucro; some species show a ventral notch just anterior to terminus. Male tail with crenate, subterminal bursa lacking phasmidial pseudoribs.

#### 4.1.2 | Morphological identification of *Hirschmanniella* species

The lip region shape and stylet length are important identification characters, others are body length, the presence of males (spermatheca filled or empty), tail terminus shape, number of annules between phasmids and tail terminus, and the presence or absence of a ventro-subterminal notch on the tail terminus. Other characters are of less value owing to the high level of intraspecific variation. A dichotomous identification key for the *Hirschmanniella* species can be found in Appendix 2. A polytomous key to the species of the genus *Hirschmanniella* can be found in Appendix 3.

Identification keys at species level were also given by Razjivin et al. (1981), Ebsary and Anderson (1982), Sivakumur and Khan (1983) and Ryss (1988). Drawings of male and female *Hirschmanniella* (*H. oryzae*) are presented in Figure 1. Drawings of different *Hirschmanniella* female tails can be found in Figure 2.

#### 4.1.3 | Morphological identification of species present in Europe

##### 4.1.3.1 | *Hirschmanniella behningi*

Females: L = 2.23–2.85 mm; a = 54–78; c = 15–20; c' = 4.6–6.9; V = 48–56; stylet = 24–28 µm. Males: spicules = 36–43 µm; gubernaculums = 9–14 µm (after Micoletzky, 1923) (Figure 3).

Female body mostly straight or slightly arcuate ventrally. Lip region continuous with body contour, almost hemispherical, with six to nine faint annules. Cephalic framework heavily sclerotized, extends for about two annules from basal plate. Strong stylet, stylet knobs slightly or acutely indented anteriorly, occasionally more or less

<sup>4</sup>If maceration with a blender is used, be aware that larger specimens might get damaged.

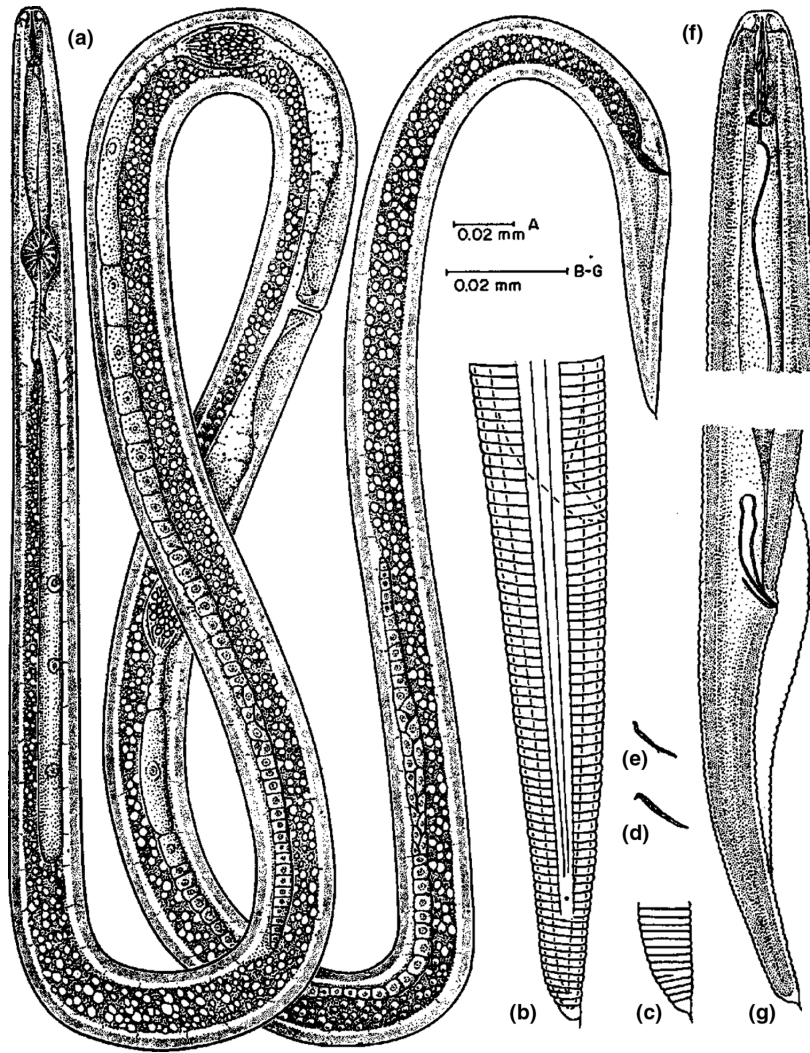


FIGURE 1 *Hirschmanniella oryzae*. (a) Whole female; (b) female tail; (c) female terminus; (d, e) variation in gubernaculum shape; (f) male anterior end; and (g) male tail (after Sher, 1968)

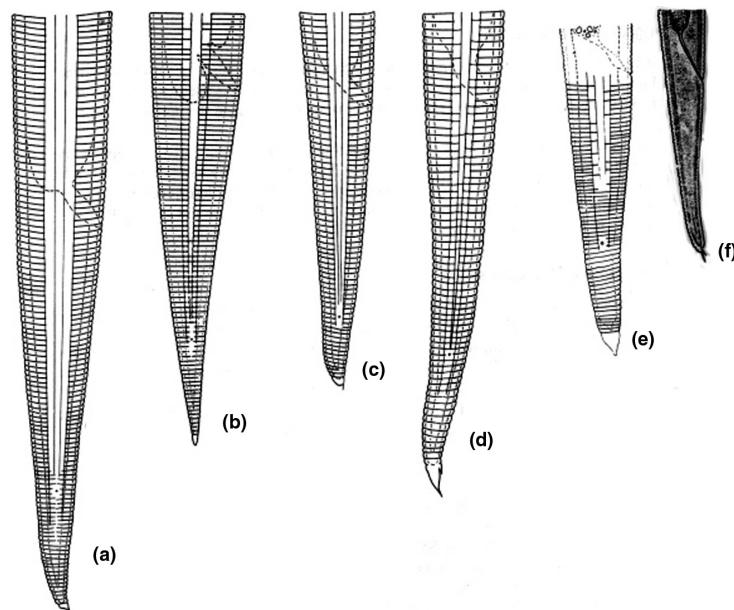
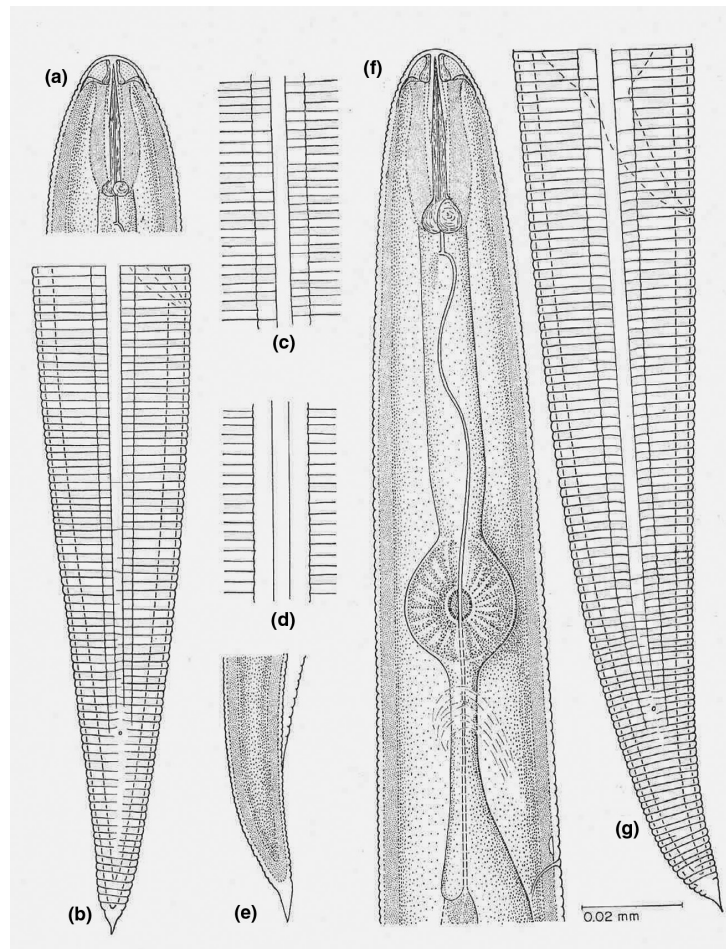


FIGURE 2 (a–f) *Hirschmanniella* female tails. (a) *H. gracilis*, with axial mucro; (b) *H. spinicaudata*, no mucro.; (c) *H. oryzae*, with ventral mucro; (d) *H. caudacrena*, axial mucro plus ventral notch; (e) *H. miticausa*, tip narrowed but no real mucro; (f) *H. shamimi*, two mucrons. (Figure adapted after Loof, 1991.)





**FIGURE 3** *Hirschmanniella behningi*: (a) female anterior part; (b) female tail; and (c) female lateral field at mid-body. *Hirschmanniella loofi*: (d) female lateral field near mid-body; (e) male tail; (f) female anterior region; and (g) female tail (after Sher, 1968)

rounded. Stylet base 5.5–6  $\mu\text{m}$  wide and 4–4.5  $\mu\text{m}$  high. Pharynx with elongate medium bulb and 6–7.5  $\mu\text{m}$  long metacorporal plates. Pharyngo-intestinal junction at the level of excretory pore. Pharyngeal gland lobe extends 6–10 body widths posterior to the pharyngo-intestinal junction. Both gonads outstretched and equally developed. Spermatheca more or less rounded, filled with approximately 4  $\mu\text{m}$  roundish sperm cells. Intestine not overlapping rectum. Tail conical with a ventral subterminal notch, ending in an axial mammillate terminus which is often only slightly set off and occasionally even totally lacking. Spicules slightly arcuate, cephalated, distal end blunt. Gubernaculum slightly arcuate with a thin proximal extension (adapted after Sturhan & Hallmann, 2010).

#### 4.1.3.2 | *Hirschmanniella gracilis*

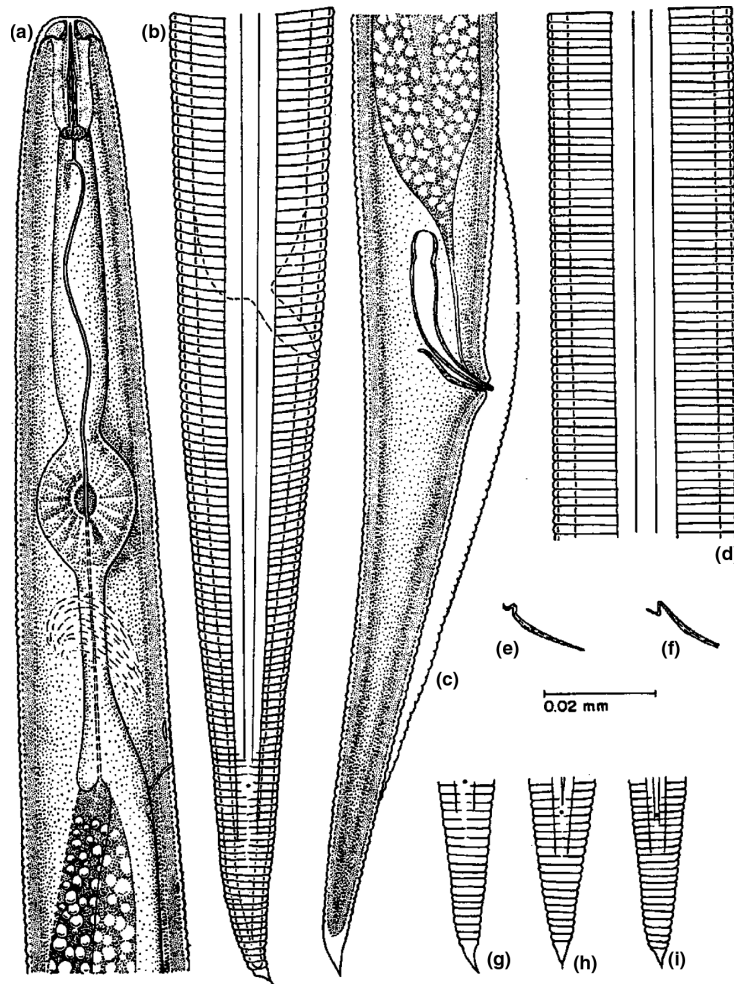
Females: L = 1.48–2.22 mm; a = 50–65; b = 11–17; b' = 5.2–8.7; c = 14–21; c' = 4.0–6.1; V = 48–55; stylet = 20–24  $\mu\text{m}$  (Figure 4). Males: L = 1.38–2.02 mm; a = 45–66; b = 12–15; b' = 4.5–7.3; c = 15–22; c' = 3.8–6.1; stylet = 20–23  $\mu\text{m}$ ; spicules = 27–38  $\mu\text{m}$ ; gubernaculums = 9–15  $\mu\text{m}$  (after Loof, 1991) (Figure 4).

Long and straight nematode when relaxed. Lip region composed of three to five annules; flattened lip region shape, about 9–12  $\mu\text{m}$  wide. Cephalic framework weakly refractive, extends for about two to three annules. Stylet with approximately equal length of cone and shaft, knobs rounded, about 5  $\mu\text{m}$  across. Dorsal pharyngeal glands opens 2.5–6  $\mu\text{m}$  posterior to stylet knobs. Pharyngeal gland lobe extends 5–20 body widths posterior to pharyngo-intestinal junction. Vulva with double epiptygma. The tail is tapering to acute terminus, often with needle-shaped ventral or axial mucro. Gubernaculum sinuate, often proximally hook-shaped (adapted after Brzeski, 1998).

#### 4.1.3.3 | *Hirschmanniella halophila*

Females: L = 1.26–1.75 mm; a = 46.5–66.6; b = 11.0–14.4; b' = 3.63–4.55; c = 12.0–17.5; c' = 5.0–6.8; V = 50.0–61.0 (Figure 5); stylet = 16.8–19.0  $\mu\text{m}$ . Males: L = 1.05–1.50 mm; a = 49.2–74.5; b = 10.5–14.6; b' = 3.50–4.65; c = 13.7–18.3; c' = 4.1–7.0; stylet = 16.0–17.6  $\mu\text{m}$ ; spicules = 26.3–30.4  $\mu\text{m}$ ; gubernaculums = 8.0–12.5  $\mu\text{m}$  (after Sturhan & Hallmann, 2010) (Figure 5).

Body long and straight, usually not twisted around their own axis. Lip region continuous, three to five lip



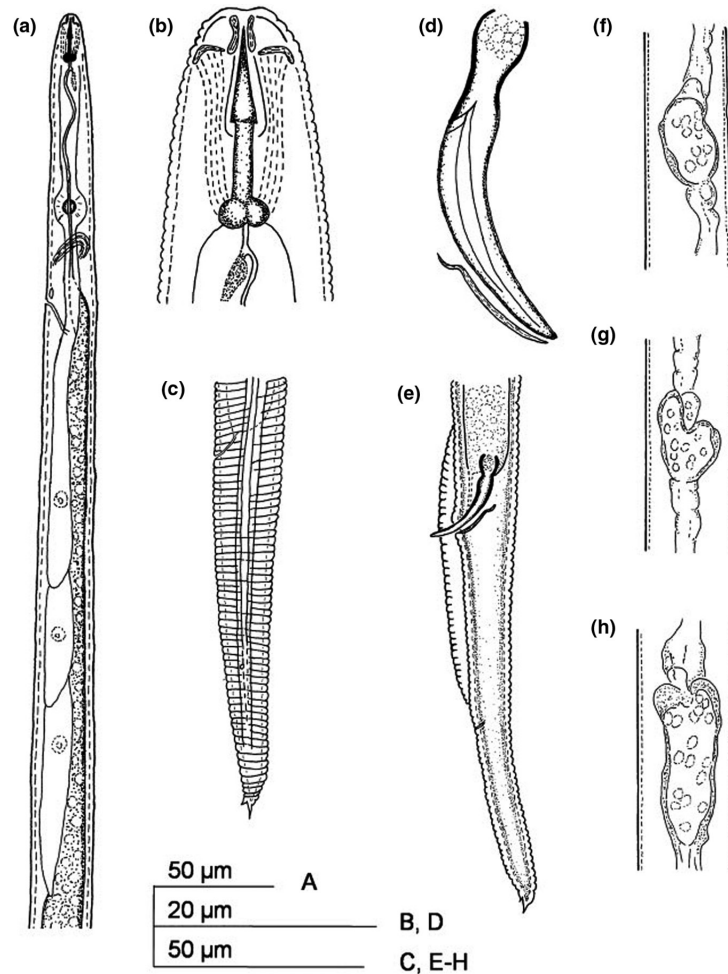
**FIGURE 4** *Hirschmanniella gracilis*: (a) female anterior part; (b) female tail; (c) male tail; (d) female lateral field near mid-body; (e, f) variation in gubernaculum shape; (g–i) female tail shape variation (after Sher, 1968)

annules. Lip region 8–10  $\mu\text{m}$  wide and 3–5  $\mu\text{m}$  high, in lateral view anteriorly flattened, in dorsoventral view almost hemispherical. Cephalic framework moderate, extending posteriorly for one to two annules from basal plate. Stylet robust, conus and basal part of equal length. Stylet knobs rounded, slightly directed laterally, occasionally slightly inclined or concave. Dorsal gland orifice 3–3.5  $\mu\text{m}$  behind stylet base. Medium bulb oval, 16–18  $\mu\text{m}$  long and 11–14  $\mu\text{m}$  wide with 4–5  $\mu\text{m}$  long metacarpal plates. Pharyngo-intestinal junction generally slightly anterior to excretory pore. Pharyngeal gland lobe extends 8–13 body width posterior to pharyngo-intestinal junction. Vulva slit 9–11  $\mu\text{m}$  wide. Both gonads equally developed. Spermatheca rounded to oval, mostly filled with rounded to oval sperms of 2–3  $\mu\text{m}$  in diameter. The tail is tapering to subcylindrical, usually with pointed ventral mucro and one or two notches 2–4  $\mu\text{m}$  anterior of terminal tail mucro. Males similar to females. Spicules slightly curved, capitulum set off with indistinct ornamentation. Gubernaculum slightly arcuate with slender and flexible proximal extension (adapted after Sturhan & Hallmann, 2010).

#### 4.1.3.4 | *Hirschmanniella loofi*

Females: L = 1.81–2.58 mm; a = 43–66; b = 11–16; b' = 4.8–7.2; c = 14–20; c' = 4.1–6.9; V = 50–58; stylet = 34–37  $\mu\text{m}$  (Figure 3). Males: L = 1.80–2.14 mm; a = 48–60; b = 13–14; b' = 4.8–7.3; c = 15–19; c' = 3.9–4.9; stylet = 31–34  $\mu\text{m}$ ; spicules = 38–44  $\mu\text{m}$ ; gubernaculums = 12–16  $\mu\text{m}$  (after Sher, 1968) (Figure 3).

Body mostly straight. Lip region continuous or with slight depression, hemispherical, with six to nine faint annules. Strong cephalic framework with outer margins extending three to four annules from basal plate. Stylet strong with conical part slightly shorter than cylindrical part. Stylet knobs massive, extending anteriorly and occasionally showing pointed tips. Stylet base pear-shaped, 7.0–8.5  $\mu\text{m}$  in width. Dorsal gland orifice 4–5  $\mu\text{m}$  behind stylet base. Hemizonid three to four annules long, located two to four annules anterior to excretory pore. Excretory opening close to pore wide, excretory canal usually distinct until region of dorsal gland. Median bulb in pharynx well developed, about half body diameter and with 7.5–8  $\mu\text{m}$  long metacarpal plates. Pharyngo-intestinal junction at about excretory



**FIGURE 5** *Hirschmanniella halophila*: (a) Female anterior part; (b) female head; (c) female tail; (d) spicule and gubernaculum; (e) male tail; and (f–h) variation in shape of spermatheca (after Sturhan & Hallmann, 2010)

level. Pharyngeal glands extend for 9–15 body widths posterior to pharyngo-intestinal junction. Vulva slit about 17 µm wide. Both gonads outstretched and equally developed. Spermatheca axial, rounded to oval, up to twice as long as wide. Sperm cells 3–4 µm in diameter. Intestine not overlapping rectum. Tail conical, with an axially or ventrally placed slender and pointed mucro of 2.5–9 µm length. No ventral notch or subterminal spines present. Male similar to female in general characteristics. Spicules slightly arcuate, cephalated, with small ventral velum and rounded tip. Gubernaculum with a slender dorsal extension of 5–6 µm in length. Small hypopygmata present (adapted after Sturhan & Hallmann, 2010).

#### 4.1.3.5 | *Hirschmanniella zostericola*

Female: L = 2.25 mm; a = 75; b = 11–16; pharynx = 225 µm; c = 13.2; c' = 9; V = 49; stylet = 15 µm. Males: L = 1.75 mm; a = 65; b = 9.75; c = 15.9; spicules = 25 µm; gubernaculum = 12 µm (after Allgén, 1934).

This species is poorly known and not available for study. The original description is based on one female and one male specimen. Owing to its poor description

and since there are no further reports of this species, it is considered to be a species inquirenda. The lip region is anteriorly flattened with round edges, composed of five annules. Stylet knobs rounded. *H. zostericola* can be distinguished from other species within the genus by the short stylet and the long narrow tail.

## 4.2 | Molecular methods

Molecular tests for the identification of the genus *Hirschmanniella* or of European species are not yet available. A phylogenetic study (Majd Taheri et al., 2013) was conducted based on the 28S rRNA (D2–D3 region) and included one unknown *Hirschmanniella* species and *H. oryzae* from Myanmar. Koyama et al. (2013) developed real-time PCR tests targeting the ITS region for the detection of *H. diversa* and *H. imamuri*, which are major pests on lotus. Katsuta et al. (2016) developed a quantitative real-time PCR for the detection *H. oryzae*, targeting the D2–D3 region of the 28S rRNA gene. Sequences for *H. gracilis* and *H. loofi* are available in EPPO-Q-bank; however the protocols published



in PM 7/129 (EPPO, 2021) have not been validated for these species.

## 5 | REFERENCE MATERIAL

Reference material can be obtained at the National Plant Protection Organization, National Reference Centre, PO Box 9102, 6700 HC, Wageningen (the Netherlands) and Julius Kühn Institute, Institut für Epidemiologie und Pathogen Diagnostik, Messeweg 11/12, 38108 Braunschweig (Germany).

## 6 | REPORTING AND DOCUMENTATION

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

## 7 | PERFORMANCE CHARACTERISTICS

When performance characteristics are available, these are provided with the description of the test. Validation data is also available in the EPPO Database on Diagnostic Expertise (<http://dc.eppo.int>), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports).

## 8 | FURTHER INFORMATION

Further information on these organisms can be obtained from:

G. Karssen, National Plant Protection Organization, National Reference Centre, PO Box 9102, 6700 HC, Wageningen (the Netherlands).

A.Y. Ryss, Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St Petersburg (Russia).

J. Hallmann, Julius Kühn Institute Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38108 Braunschweig (Germany).

## 9 | FEEDBACK ON THIS DIAGNOSTIC STANDARD

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this

protocol that you wish to share, please contact [diagnostics@eppo.int](mailto:diagnostics@eppo.int).

## 10 | STANDARD REVISION

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

### ACKNOWLEDGEMENTS

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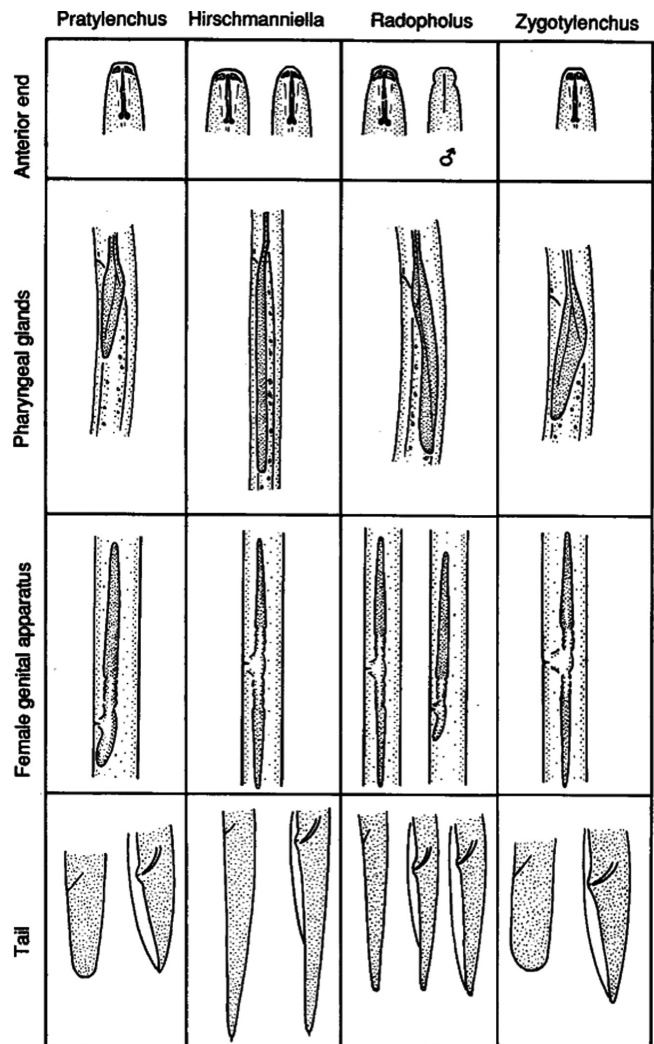
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**APPENDIX 1 – DICHOTOMOUS KEYS  
TO THE GENERA OF THE FAMILY  
PRATYLENCHIDAE (ADAPTED BY  
GERAERT, 2013)**

**Tabular key to some genera of the family Pratylenchidae  
(adapted after Loof, 1991)**

1	Pharyngeal glands extending over intestine mostly ventrally and ventro-laterally	2
	Pharyngeal glands extending over intestine mostly dorsally and dorso-laterally	7
2	Mature females are swollen	3
	Mature females are not swollen	5
3	Body ornamented by transverse rows of tubercles	<i>Apratylenchus</i>
	Body not ornamented by transverse rows of tubercles	4
4	Excretory pore located opposite anterior margins of median pharyngeal bulb or more anteriorly (except <i>M. silvicola</i> ); male tail lacking a well-developed bursa	<i>Meloinema</i>
	Excretory pore located opposite posterior margins of median pharyngeal bulb or more posteriorly; male tail with a well-developed bursa	<i>Bursadera</i>
5	Tails similar between sexes; phasmids near terminus	<i>Hirschmanniella</i>
	Tails dissimilar between sexes; phasmids not near terminus	6
6	One functional ovary	<i>Pratylenchus</i>
	Two functional ovaries	<i>Zygotylenchus</i>
7	Mature females are swollen	8
	Mature females are not swollen	9
8	Monodelphic with posterior vulva	<i>Nacobbus</i>
	Didelphic with vulva near mid-body	<i>Achlysiella</i>
9	Sexual dimorphism in anterior region prominent	10
	Sexual dimorphism in anterior region not prominent	12
10	Posterior ovary normally developed, functional	<i>Radopholus</i>
	Posterior ovary reduced or absent, non-functional	11
11	Female stylet 21–29 µm long, basal knobs tulip-shaped	<i>Hoplotylus</i>
	Female stylet under 20 µm, basal knobs not tulip-shaped	<i>Radopholoides</i>
12	Posterior ovary normally developed, functional	<i>Zygradus</i>
	Posterior ovary reduced, non-functional	<i>Apratylenchoides</i>



**APPENDIX 2 – DICHOTOMOUS KEY TO THE SPECIES OF THE GENUS *HIRSCHMANNIELLA* (ADAPTED BY KARSEN AFTER LOOF, 1991).**

Species in bold are those present in Europe

1	Spermatheca empty, males rare or unknown	2	12	Phasmids 24 annules from terminus	<i>H. behningi</i>
	Spermatheca filled with sperm, males common	3		Phasmids 19 or less annules from terminus	13
2	Stylet = 20–22 µm; phasmids about 20 annules from the terminus; tail with a ventral, often needle-shaped mucro	<i>H. belli</i>	13	Phasmids 4–12 annules from terminus; stylet length 27–30 µm	<i>H. thornei</i>
	Stylet = 23–25 µm; phasmids 30–42 annules from the terminus; tail with axial mucro	<i>H. pisquidensis</i>		Phasmids 12–19 annules from terminus; stylet length 22–29 µm	<i>H. mucronata</i>
3	Lip region hemispheric; stylet knobs large, high, stylet length 22–50 µm	4	14	c' = 9; stylet length 15 µm	<i>H. zostericola*</i>
	Lip region low, mostly flattened with distinct anterior surface and sides; stylet knobs more clearly offset, not conspicuously high, stylet length 15–25 µm	14		c' = 7.5 or less; stylet length 16–25 µm	15
4	Stylet length 34 µm or more	5	15	Tail with two mucros or two peg-like projections	16
	Stylet length 32 µm or less	9		Tail with 0–1 mucro or zero to one peg-like projections	17
5	Stylet length 40 µm or more; body length over 3 mm	6	16	Tail with two needle-shaped mucros	<i>H. shamimi</i>
	Stylet length 39 µm or less; body length under 3 mm	7		Tail with two lateral peg-like projections	<i>H. caribbeana</i>
6	Intestine overlaps rectum; lateral field at least partly areolated	<i>H. spinicaudata</i>	17	Tail with ventral subterminal notch	18
	Intestine does not overlap rectum; lateral field not areolated	<i>H. obesa</i>		Tail without notch	21
7	c' = 2.5 or less; tail truncate	<i>H. truncata</i>	18	Phasmids 10 annules from terminus	<i>H. mexicana</i>
	c' over 3; tail not truncate	8		Phasmids 18 or more annules from terminus	19
8	Tail with ventral notch; phasmids 18 annules from terminus	<i>H. furcata</i>	19	c' < 4	<i>H. marina</i>
	Tail without ventral notch; phasmids 20–25 annules from terminus	<i>H. loofi</i>		c' > 4	20
9	Tail with ventral mucro	10	20	a = 53 (46–62); stylet length 18–22	<i>H. caudacrena</i>
	Tail with axial mucro, or without mucro	12		a = 60.1 (46.5–66.6); stylet length 17–19	<i>H. halophila</i>
10	Stylet length 29–32 µm; phasmids 17–18 annules from terminus	<i>H. inamuri</i>	21	Tail with ventral, needle shaped, sharply offset mucro	22
	Stylet length 23–25 µm; phasmids 9–14 annules from terminus	11		Tail with axial mucro	23
11	c' > 4.7	<i>H. diversa</i>	22	Tail without mucro, but with one small peg-like projection	24
	c' < 4.3	<i>H. areolata</i>		Body length 1.14–1–63 mm; b = 8.8–12.1	<i>H. oryzae</i>
				Body length 1.52–2.05 mm; b = 3.7–7.8	<i>H. kwazuna</i>
			23	Stylet length ≥ 19 µm	<i>H. gracilis</i>
				Stylet length < 19 µm	<i>H. anchoryzae</i>
			24	Lip region composed of three annules	<i>H. microtyla</i>
				Lip region composed of five to six annules	25
			25	Excretory pore anterior to pharyngo-intestinal junction	<i>H. santarosae</i>
				Excretory pore posterior to pharyngo-intestinal junction	26
			26	c' ≥ 5	<i>H. pomponiensis</i>
				c' < 5	<i>H. miticausa</i>

Note: *Hirschmanniella* female tails are presented in [Figure 5](#).

\* Owing to its poor description, *H. zostericola* is considered a species inquirenda.

**APPENDIX 3 – POLYTOMOUS KEY  
TO THE SPECIES OF THE GENUS  
*HIRSCHMANNIELLA* (AFTER KHUN  
ET AL., 2015).**

Species in bold are those present in Europe

It should be noted that in this key one character may have several states; this intraspecific variation is expressed with several digits, e.g. for *H. anchoryzae* the excretory pore can be anterior to, or at the level of the pharyngeal–intestinal valve,<sup>5</sup> i.e. character states 1 or 2. This allows numerical sorting with a computerized version of the key (<http://www.nematodes.ugent.be/vce.html>). For morphometric data such as body length, stylet length, ratio *c'*, ventral annules from phasmids to tail terminus and spicule length (characters F–J), the first digit of the code represents the state associated with the mean value, followed by digits with the minimum and maximum value, respectively. If the character had only one state in a given species, the character code for this species was composed of the same repeated digit (characters F–J). In characters E and K, the first digit of the code was that of the most frequent state of the character in the species and the other digits in the code relate to decreasing frequency of occurrence. In some cases, when a state of a character rarely occurs in the species, this state is indicated between brackets, or in an additional column in the Excel file to allow sorting of the character states.

Characters used in the polytomous key and their codes:

A	1	Lip region hemispherical
	2	Lip region not hemispherical
B	1	Tail terminus pointed or round with a mucro
	2	Tail terminus pointed with subterminal notch
	3	Tail terminus pointed or round with or without a projection
C	1	No or single mucro or projection
	2	Two or more mucros or projections

D	1	Axial position of mucro or projection
	2	Ventral position of mucro or projection
E	1	Excretory pore anterior to pharyngeal–intestinal valve
	2	Excretory pore about same level as pharyngeal–intestinal valve
	3	Excretory pore posterior to pharyngeal–intestinal valve
F	1	Stylet = 18.5 µm or shorter
	2	Stylet = 18.6–25.0 µm
	3	Stylet = 25.1–32.0 µm
	4	Stylet = 32.1–39.9 µm
	5	Stylet = 40 µm or longer
G	1	Body length = 1600 µm or shorter
	2	Body length = 1601–2500 µm
	3	Body length = 2501–3000 µm
	4	Body length = 3001 µm or longer
H	1	<i>c'</i> = 3.0 or lower
	2	<i>c'</i> = 3.1–4.9
	3	<i>c'</i> = 5.0–6.9
	4	<i>c'</i> = 7.0–8.9
	5	<i>c'</i> = 9.0 or higher
I	1	14 annules from phasmids to tail or lower
	2	15–24 annules from phasmids to tail
	3	25–34 annules from phasmids to tail
	4	35 annules from phasmids to tail or higher
J	1	Spicule length = 27 µm or shorter
	2	Spicule length = 28–40 µm
	3	Spicule length = 41 µm or longer
K	1	Geographical distribution: Europe
	2	Geographical distribution: Africa
	3	Geographical distribution: North America
	4	Geographical distribution: South and Central America including the Caribbean
	5	Geographical distribution: Asia
	6	Geographical distribution: Oceania

<sup>5</sup>Mentioned as pharyngeal–intestinal junction in species descriptions.



Polytomous key to species of the genus *Hirschmanniella* (after Khun et al., 2015)

	A	B	C	D	E	F	G	H	I	J	K
<i>H. anchoryzae</i>	2	1	1	1	12	112	212	324	334	222	35
<i>H. areolata</i>	1	3	1	2	23	323	222	222	111	222	5
<b><i>H. behningi</i></b>	1	3	1	1	21	323	223	323	324	223	1
<i>H. belli</i>	2	1	1	2	1	212	212	323	222	222	3(5)
<i>H. brassicae</i>	2	1	1	2	3	111	111	223	222	112	5
<i>H. caribbeana</i>	2	3	2	2	1	112	112	223	222	222	4(5)
<i>H. caudacrena</i>	2	2	1	1	231	212	112	324	324	212	351
<i>H. diversa</i>	1	3	1	2	3	222	223	323	111	222	6(5)
<i>H. furcata</i>	1	2	1	1	1	444	333	222	222	-	4
<b><i>H. gracilis</i></b>	2	3	1	1(2)	213	222	212	223	324	223	1(5)
<b><i>H. halophila</i></b>	2	2	1	2	3	112	112	323	324	212	1
<i>H. inamuri</i>	1	3	1	2	1	333	223	323	223	323	5
<i>H. kwazuna</i>	2	1	1	2	1	212	212	213	213	222	2
<b><i>H. loofi</i></b>	1	3	1	2(1)	21	435	324	223	223	323	1(4)
<i>H. marina</i>	2	2	1	2	1	222	212	334	222	222	3(5)
<i>H. mexicana</i>	2	2	1	2	3	222	222	222	111	-	3(5)
<i>H. microtyla</i>	2	3	1	1	2	212	111	222	222	222	35
<i>H. miticausa</i>	2	3	1	2	21	222	212	222	223	222	6
<i>H. mucronata</i>	1	3(1)	1	2(1)	123	223	213	213	213	212	5
<i>H. obesa</i>	1	2	1	1	1	555	444	222	222	—	4
<i>H. oryzae</i>	2	1	1	2(1)	1	112	112	213	222	112	523(41)
<i>H. pisquidensis</i>	1	1(2)	1(2)	1	3	222	222	323	434	—	3
<i>H. pomponiensis</i>	2	3	1	1	32	212	222	324	333	222	3
<i>H. santarosae</i>	2	3	1	1	12	212	212	324	333	222	3
<i>H. shamimi</i>	2	1	2	2	3	112	111	223	233	111	5
<i>H. spinicaudata</i>	1	3	1	1	23	545	424	223	222	333	243(5)
<i>H. thornei</i>	1	3	1	1	2	333	223	222	111	222	5
<i>H. truncata</i>	1	3	1	1	1	444	222	111	222	—	4
<b><i>H. zostericola</i></b>	2	3	1	1	—	111	222	555	—	111	1

A rare or exceptional character is given in brackets; —, data not available.