

Diagnosics Diagnostic

Hirschmanniella spp.

Specific scope

This standard describes a diagnostic protocol for the genus *Hirschmanniella*¹.

Specific approval and amendment

Approved in 2009–09.

Introduction

The genus *Hirschmanniella* includes at present about 35 species (Siddiqi, 2000). 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, China and Japan. The non-rice species are found in the same region and in the northern temperate zone (less 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 are hosts, including some weeds. *Hirschmanniella* has also been detected on roots and leaves of aquatic plants and in soil associated with bonsais. On rice, it is common to detect several species of *Hirschmanniella*. The genus *Hirschmanniella*, revised by Sher (1968), is notorious for having multiple dubious or easily confused species. A recent study by 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 four *Hirschmanniella* species have been described from Europe: *H. behningi* (Micoletzky, 1923), *H. gracilis* (de Man, 1880), *H. loofi* Sher, 1968 and *H. zostericola* (Allgén, 1934). *Hirschmanniella behningi* (USSR, Sweden), *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 outside the normal area on rice in India, Vietnam and China (the later records need confirmation).

The genus *Hirschmanniella* spp., with the exception of *Hirschmanniella gracilis* has been added to the EU legislation after

several interceptions had been recorded on soil associated with bonsai plants. This was an indication that the requirements regarding soil attached to plants were not fulfilled. This protocol includes elements to identify the genus *Hirschmanniella* spp. and the species *Hirschmanniella gracilis*.

Identity

Name: *Hirschmanniella* Luc & Goodey, 1964.

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

Taxonomic position: Nematoda: Tylenchina²: Pratylenchidae.

EPPO code: HIRSSP

Phytosanitary categorization: EU Annex designation: I/A1 (as *Hirschmanniella* spp., other than *Hirschmanniella gracilis* (de Man) Luc & Goodey).

Detection

Symptoms

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 discoloration 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.

Identification

Identification of *Hirschmanniella* at genus and at species level is based in general on a combination of morphological

¹Use 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.

²Recent development combining a classification based on morphological data and molecular analysis refer to 'Tylenchomorpha' (De Ley & Blaxter, 2004).

& 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 phase microscope. Molecular methods for the identification of the genus *Hirschmanniella* or the species *H. gracilis* and the mentioned other European species are not yet available.

Extraction procedures

Extraction from roots

Roots have to be cut in small pieces, the bigger roots have to be sliced longitudinally. For submerged aquatic plants it is also recommended to extract the leaves (same procedure as for roots). The cut root parts are submerged in water contained in Petri dishes, Baermann funnels etc. at a temperature of about 20°C. Minimum extraction time is 24 hours. Extraction may be continued up to two weeks. Other techniques such as maceration/filtration technique, maceration/centrifugal-flotation technique,

mistifier technique (Southey, 1986) may also be used for extraction of the nematode from root material. The maceration/centrifugal-flotation technique allows recovery of nematodes within a few hours. The mistifier technique will enable recovery of nematodes in good condition.

Extraction from soil

Baermann funnel, Oostenbrink funnel and Whitehead techniques and sieving/centrifugation (Southey, 1986) are useful for the extraction of *Hirschmanniella* from soil.

Morphology

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. Both keys can be found in Appendix 1. Alternatively the dichotomous key for subfamily and genus identification produced by Siddiqi (2000) can be used.

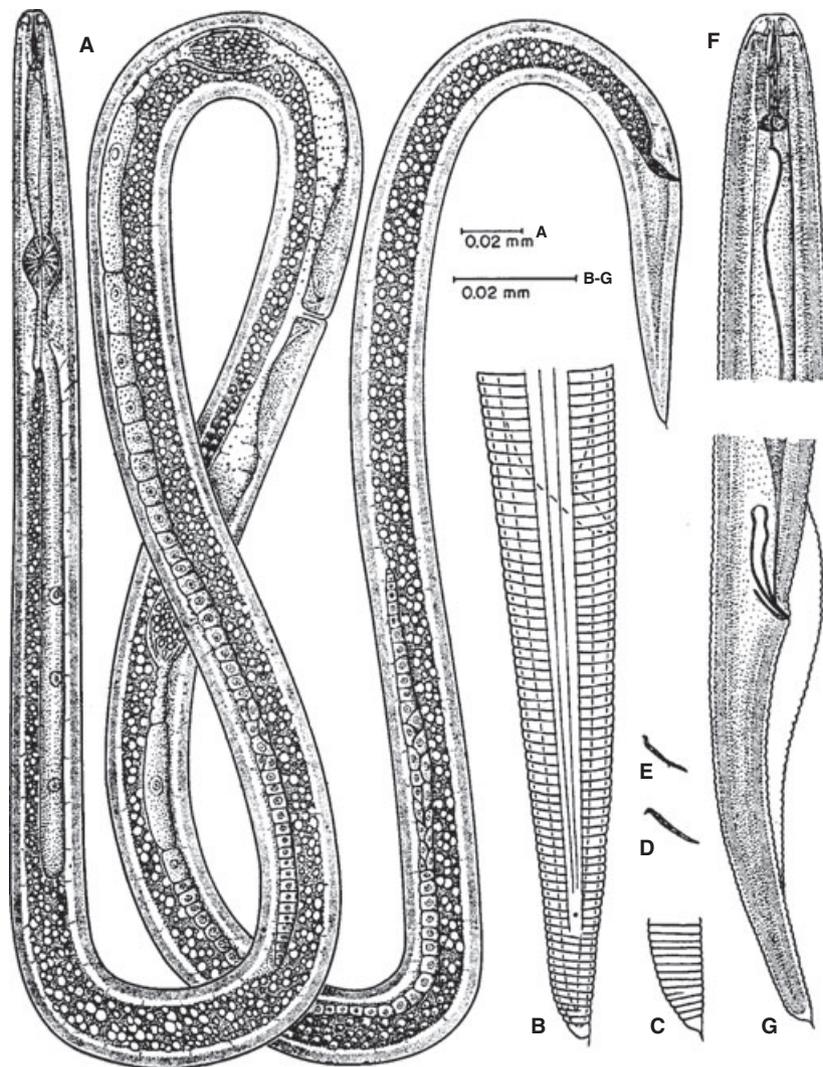


Fig. 1 *Hirschmanniella oryzae*. (A) Whole female. (B) Female tail. (C) Female terminus. (D,E) Variation in gubernaculum shape. (F) Male anterior end. (G) Male tail (after Sher, 1968).

Morphological characteristics of Hirschmanniella spp.

Long (1 to 4 mm) and slender nematodes. 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 3 to 7 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 shows a ventral notch just anterior to terminus. Male tail with crenate, subterminal bursa lacking phasmidial pseudoribs.

Identification of Hirschmanniella species

The lip region shape and stylet length are important identification characters, others are body length, presence of males (sperma-

theca filled or empty), tail terminus shape, number of annules between phasmids and terminus, and presence or absence of a ventrosubterminal notch on the tail terminus. Other characters are of less value due to high intraspecific variation.

A dichotomous identification key towards the *Hirschmanniella* species can be found in Appendix 2. Identification keys at species level were also given by Razjivin *et al.* (1981), Ebsary & Anderson (1982), Sivakumur & Khan (1983) & Ryss (1988).

Drawings of male and female *Hirschmanniella* (*H. oryzae*) are presented in Fig. 1. Drawings of different *Hirschmanniella* female tails can be found in Fig. 4.

Morphological and morphometrical characteristics of 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 μm . 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 μm ; spicules = 27–38 μm ; gubernaculum = 9–15 μm (after Loof, 1991).

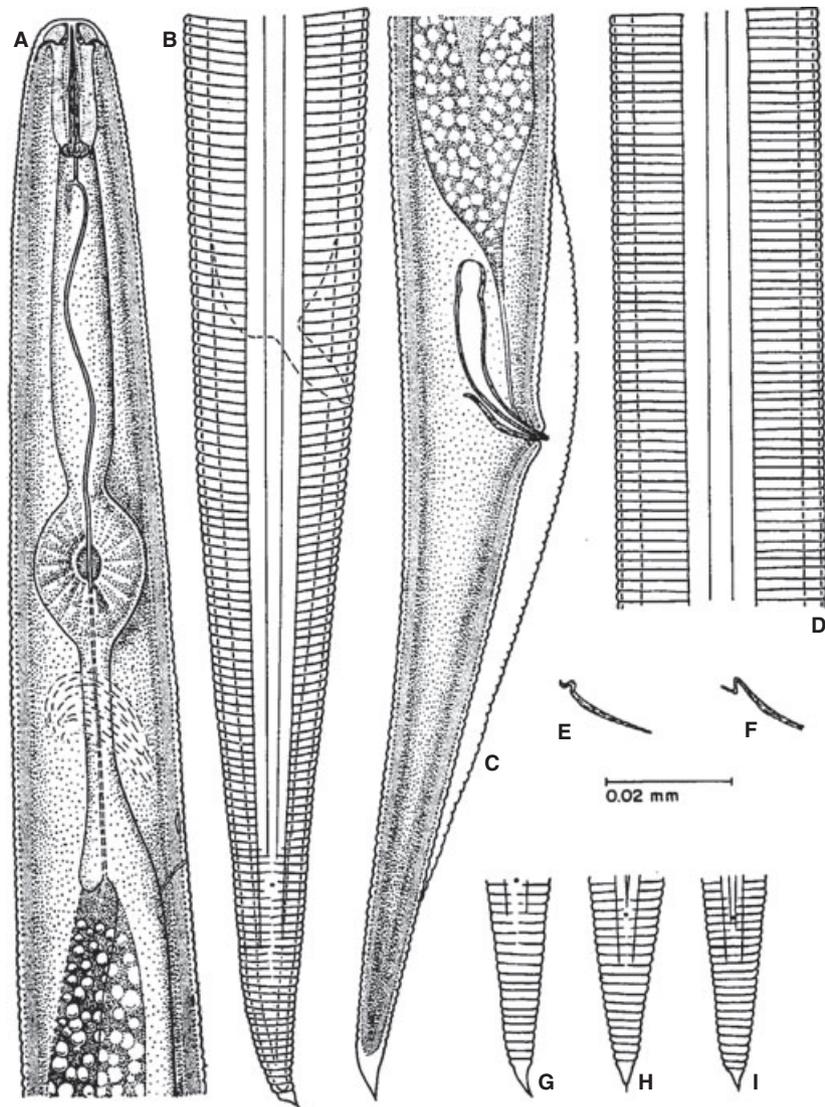


Fig. 2 *Hirschmanniella gracilis*. (A) Female anterior part. (B) Female tail. (C) Male tail. (D) female lateral field near mid-body. (E,F) Variation in gubernaculum shape. (G–I) Female tail shape variation (after Sher, 1968).

Long and straight nematode when relaxed. Lip region composed of 3 to 5 annules; flattened lip region shape, about 9–12 μm wide. Cephalic framework weakly refractive, extends for about 2–3 annuli. Stylet with approximately equal length of cone and shaft, knobs rounded, about 5 μm across. Dorsal pharyngeal glands opens 2.5–6 μm 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).

Drawings of male and female *Hirschmanniella gracilis* are presented in Fig. 2. Drawings of male and female of two other European species (*H. loofi* and *H. behningi*) are presented in Fig. 3.

Reference material

Can be obtained at the Plant Protection Service, Wageningen (NL) and Julius Kühn Institute (JKI), Institut für Epidemiologie und Pathogen Diagnostic, Toppheideweg 88, 48161 Münster (DE).

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM 7/77 (1) *Documentation and reporting on a diagnosis*.

Further information

Further information on these organisms can be obtained from: Karssen G, Plant protection Service, P.O. Box 9102, 6700 HC Wageningen (NL) and A Y. Ryss, Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1, 199034 St. Petersburg (RU).

Acknowledgement

This protocol was originally drafted by: Karssen G, Plant protection Service, Wageningen (NL).

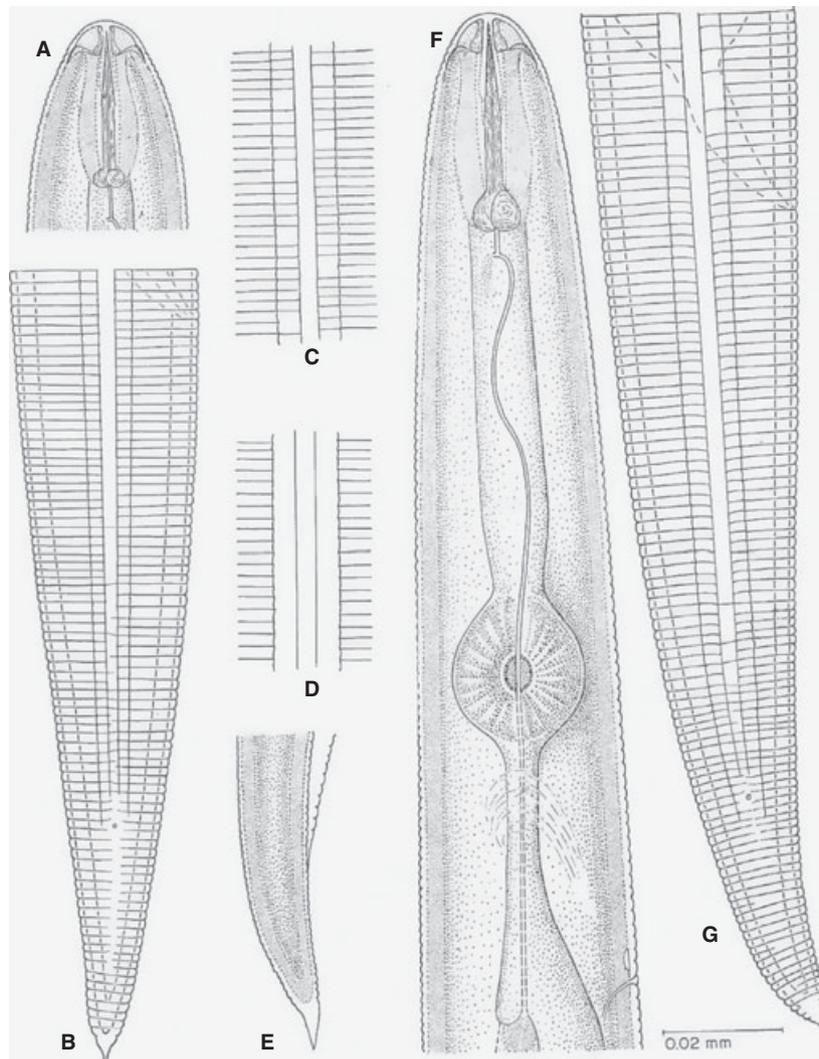


Fig. 3 *Hirschmanniella behningi*. (A) Female anterior part. (B) Female tail. (C) Female lateral field at mid-body. *Hirschmanniella loofi*. (D) Female lateral field near mid-body. (E) Male tail. (F) Female anterior region. (G) Female tail (After Sher, 1968).

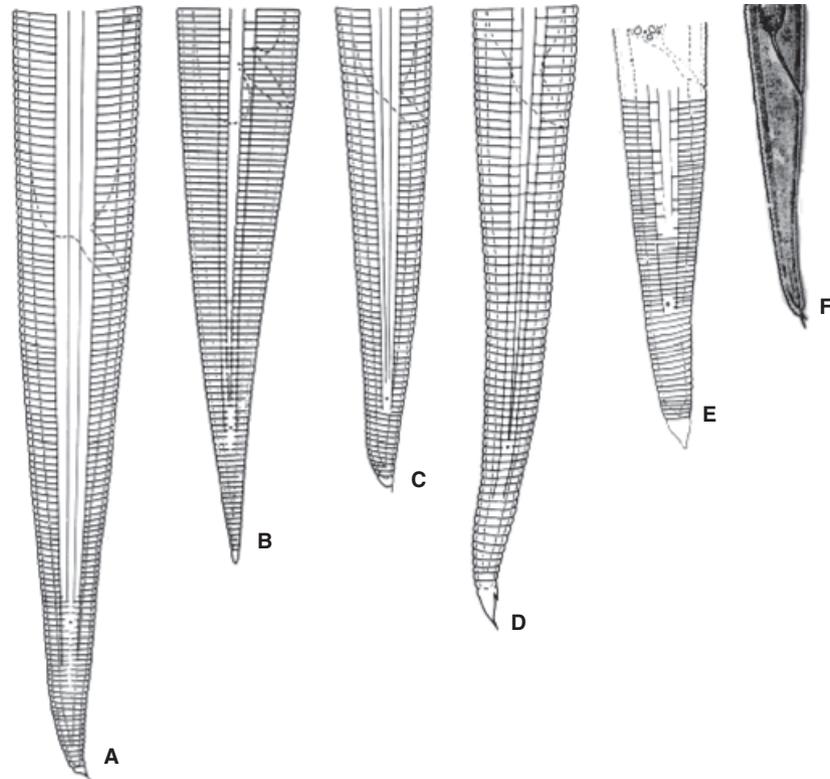


Fig. 4 *Hirschmanniella* female tails. (A) *H. gracilis*, with axial mucro. (B) *H. spinicaudata*, no mucro. (C) *H. oryzae*, with ventral mucro. (D) *H. caudacrena*, axial mucro plus ventral notch. (E) *H. miticausa*, tip narrowed but no real mucro. (F) *H. shamimi*, two mucrons. [Figure adapted after Loof, 1991].

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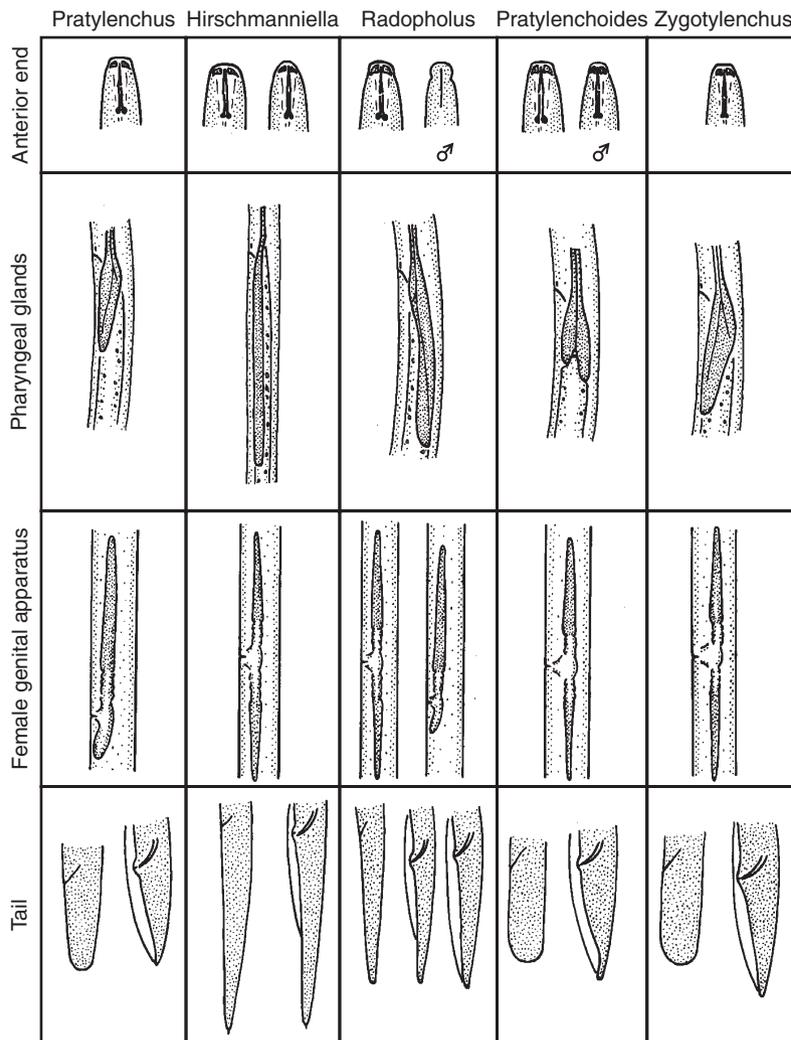
Appendix 1

Dichotomous keys to the genera of the family Pratylenchidae
[adapted by Karssen & Loof (1991)]

1. Females monoprodelf	2
Females didelph	6
2. Young adult female free in soil with long dorsal pharyngeal overlap and undifferentiated, short genital tubes; later they become sessile, immobile, swollen and induce root-galls	<i>Nacobbus</i>
Adult female with fully developed genital tubes, vermiform, motile and at most slightly swollen	3
3. Short ventral pharyngeal overlap, low lip region, female with short rounded tail	<i>Pratylenchus</i>
Long dorsal pharyngeal overlap	4
4. Lateral field with three lines; female tail subacute; lip region conoid; stylet knobs tulip-shaped	<i>Hoplotylus</i>
Lateral field with four (rarely three) lines; knobs not tulip-shaped	5

5. Lip region low in both sexes; male stylet not reduced, male pharynx slightly reduced	<i>Apratylenchoides</i>
Lip region in female flattened, in male knob-shaped; male stylet and pharynx strongly reduced	<i>Radopholoides</i>
6. Adult female swollen to become sausage-shaped	<i>Achlysiella</i>
Adult female not swollen	7
7. Body length well over 1 mm; tail very long, terminus often mucronate	<i>Hirschmanniella</i>
Body length less than 1 mm; tail terminus not mucronate	8
8. Deirids present	<i>Pratylenchoides</i>
Deirids absent	9
9. Pharyngeal gland overlap ventrally	<i>Zygotylenchus</i>
Pharyngeal gland overlap dorsally	10
10. Lip region low in both sexes; male stylet not reduced	<i>Zygradus</i>
Lip region in female flattened, in male knob-shaped; male stylet and pharynx reduced	<i>Radopholus</i>

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



Appendix 2

Dichotomous key to the species of the genus *Hirschmanniella* (adapted by Karssen after Loof, 1991) Species in bold are those present in Europe.

1.	Spermatheca empty, males rare or unknown	2		11.	$c' > 4.7$	<i>H. diversa</i>
	Spermatheca filled with sperm, males common	3			$c' < 4.3$	<i>H. areolata</i>
2.	Stylet = 20–22 μm ; phasmids about 20 annules from the terminus; tail with a ventral, often needle-shaped mucro		<i>H. belli</i>	12.	Phasmids 24 annules from terminus	<i>H. behningi</i>
	Stylet = 23–25 μm ; phasmids 30–42 annules from the terminus; tail with axial mucro		<i>H. pisquidensis</i>		Phasmids 19 or less annules from terminus	13
3.	Lip region hemispheric; stylet knobs large, high, stylet length 22–50 μm	4		13.	Phasmids 4–12 annules from terminus; stylet length 27–30 μm	<i>H. thornei</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			Phasmids 12–19 annules from terminus; stylet length 22–29 μm	<i>H. mucronata</i>
4.	Stylet length 34 μm or more	5		14.	$c' = 9$; stylet length 15 μm	<i>H. zostericola</i>
	Stylet length 32 μm or less	9			$c' = 7.5$ or less; stylet length 16–25 μm	15
5.	Stylet length 40 μm or more; body length over 3 mm	6		15.	Tail with two mucros or two peg-like projections	16
	Stylet length 39 μm or less; body length under 3 mm	7			Tail with 0–1 mucro or zero one peg-like projections	17
6.	Intestine overlaps rectum; lateral field at least partly areolated		<i>H. spinicaudata</i>	16.	Tail with two needle-shaped mucros	<i>H. shamimi</i>
	Intestine does not overlap rectum; lateral field not areolated		<i>H. obesa</i>		Tail with two lateral peg-like projections	<i>H. caribbeana</i>
7.	$c' = 2.5$ or less; tail truncate		<i>H. truncata</i>	17.	Tail with ventral subterminal notch	18
	$c' > 3$; tail not truncate	8			Tail without notch	20
8.	Tail with ventral notch; phasmids 18 annules from terminus		<i>H. furcata</i>	18.	Phasmids 10 annules from terminus	<i>H. mexicana</i>
	Tail without ventral notch; phasmids 20–25 annules from terminus		<i>H. loofi</i>		Phasmids 18 or more annules from terminus	19
9.	Tail with ventral mucro	10		19.	Stylet length 22–25 μm ; $c' < 4$; marine species	<i>H. marina</i>
	Tail with axial mucro, or without mucro	12			Stylet length 18–22 μm ; $c' > 4$; freshwater species	<i>H. caudacrena</i>
10.	Stylet length 29–32 μm ; phasmids 17–18 annules from terminus		<i>H. imamuri</i>	20.	Tail with ventral, needle shaped, sharply offset mucro; stylet length 16–20 μm	<i>H. oryzae</i>
	Stylet length 23–25 μm ; phasmids 9–14 annules from terminus	11			Tail with axial mucro	21
					Tail without mucro, but with one small peg-like projection	22
				21.	Stylet length $\geq 19 \mu\text{m}$	<i>H. gracilis</i>
					Stylet length $< 19 \mu\text{m}$	<i>H. anchoryzae</i>
				22.	Lip region composed of 3 annules	<i>H. microtyla</i>
					Lip region composed of 5 to 6 annules	23
				23.	S-E pore anterior to pharyngo-intestinal junction	<i>H. santarosae</i>
					S-E pore posterior to pharyngo-intestinal junction	24
				24.	$c' \geq 5$	<i>H. pomponiensis</i>
					$c' < 5$	<i>H. miticausa</i>

Note: *Hirschmanniella* female tails are presented in Fig. 4