



Hirschmanniella maritima n. sp. (Nematoda: Pratylenchidae) – a New Nematode Species Parasitizing Baltic Plants in Poland

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Nematodes from the genus *Hirschmanniella* Luc and Goodey, 1964 are obligate, migratory endoparasites of plants growing mainly in moist or aquatic niches. They have been isolated from the soil, root zones and roots of many crops, wild plants and weeds (Bridge et al., 2005; Maung et al., 2010; Prior et al., 2010; Uematsu et al., 2015; Takagi et al., 2016; Nguyen, 2017; Pervez et al., 2017; Shokoohi et al., 2019; Habteweld et al., 2019; Hassan, 2020).

Abstract

Hirschmanniella maritima n. sp. was recovered from the roots of *Potamogeton perfoliatus* L. plants collected in several locations along the Baltic coast (from the Puck Bay to the Vistula Lagoon) in Poland. *H. maritima* n. sp. is characterized by a long ($L = 1787 \mu\text{m}$ – $3085 \mu\text{m}$) body; anteriorly rounded or slightly flattened lip region with four to six lip annuli; medium stylet ($19.5 \mu\text{m}$ – $24.5 \mu\text{m}$) with large, oval knobs directed approximately laterally; excretory pore located posterior to pharyngo-intestinal junction; intestine partially overlapping rectum; medium-sized spicules ($34.0 \mu\text{m}$ – $43.2 \mu\text{m}$); gubernaculum slightly arcuate, narrow with hook-shaped distal part; elongate-conoid tail ($c' = 4.7$ – 8.1); and tail end irregular in size and shape, without annulations, but with a pointed terminus up to an axial mucro-like projection. Inside the roots of *P. perfoliatus*, the nematodes were found in intercellular spaces surrounded by degraded cortex cells. Morphological and 28S rDNA-based phylogenetic molecular analyses showed *H. maritima* n. sp.'s similarity to *H. santarosae* and *H. pomponiensis*, with a strong sister relationship between these three species and the clade encompassing *H. diversa* and *H. halophila*. The mtCOI-based phylogenetic analysis confirmed a close relationship between *H. maritima* n. sp. and *H. diversa*.

Keywords

cytochrome c oxidase subunit I, diagnostics, phylogenetic analysis, *Potamogeton perfoliatus*, ribosomal DNA, taxonomy, Tylenchina

Several species of *Hirschmanniella* are known to be harmful to commercially important crops, including cabbage (*Brassica oleracea* L.), maize (*Zea mays* L.), taro (*Colocasia esculenta* L.), tomato (*Solanum lycopersicum* L.), sugar cane (*Saccharum officinarum* L.), and rice (*Oryza sativa* L.) (Bridge et al., 1983, 1990; Duan et al., 1996; Youssef & Eissa, 2013; Takagi et al., 2019; Indarti et al., 2020; Beesa et al., 2021; Eche et al., 2021; Mwamula et al., 2022; Prasad &

Somasekhar 2022, 2024). It has been calculated that *Hirschmanniella* infection can reduce rice yields by up to several dozen percent (Indarti et al., 2020). Since the 1990s, the negative impact of *Hirschmanniella diversa* Sher, 1968 on the growth and development of Indian lotus (*Nelumbo nucifera* Gaertn.) has been observed in the Ibaraki, Tokushima and Chiba Prefectures of Japan, where it is most intensively cultivated (Takagi et al., 2019). Blackening disease of tubers caused by this nematode leads to a decrease in commercial value of lotus estimated at about US \$1 million per year (Kurashita et al., 2021). The harmful effects of *Hirschmanniella* nematodes have also been observed in tall fescue (*Festuca arundinacea* Schreb.) (Prior et al., 2010), aquatic plants *Diplanthera wrightii* (Sher, 1968), as well as *Hydrilla verticillata* and *Ceratophyllum demersum* (Gerber & Smart, 1987).

Host plant tissues are inhabited by all developmental stages of *Hirschmanniella* sp. As early as three days after infection, nematode feeding increases expression levels of oxidative stress-related genes and metabolic disorders, and activates programmed cell death processes (Kyndt et al., 2012; Bauters et al., 2020). Nematode feeding and movement in the air spaces of tissues causes damage to plants and may lead to a serious reduction in plant growth and development. Moreover, tissues disrupted by the nematodes become a natural path to secondary microbial infections, which promote the formation of necrosis in infected plant organs (Babatola & Bridge, 1979). At all developmental stages, *Hirschmanniella* sp. is able to survive unfavourable environmental conditions by remaining in the soil or in plant debris (Takakura & Yamamoto 1970; Bridge et al., 2005).

The detection of *Hirschmanniella* specimens in imported plants and concerns about their potential harms induced the relevant European Union institutions to subject these nematodes to legal regulations (EPPO, 2009, 2022; Ryss & Karnkowski, 2010; Jeger et al., 2018). Currently, all species belonging to this genus, except those that have been recorded in natural environments within the EU countries, are subjected to quarantine regulations. So far, five species have been observed in natural environments in Europe: *H. behningi* (Mikoletzky, 1923); *H. gracilis* (de Man, 1880); *H. halophila* Sturhan and Hallman, 2010; *H. loofi* Sher, 1968; and *H. zostericola* (Allgén, 1934) (EU 2021).

In Poland, studies on the occurrence of *Hirschmanniella* nematodes were initially conducted by Prejs (1977a, b, 1986). Examinations of the fauna associated with *Potamogeton* sp. plants inhabiting 31 lakes in northern Poland revealed the presence of *H.*

behningi and *H. gracilis*. The presence of *H. gracilis* in moist soils was also indicated by Skwiercz (1987) and Brzeski (1998). Studies by Brzeski (1998) showed the additional presence of *H. loofi*.

Currently, the genus *Hirschmanniella* includes 35 valid species that can be found all over the world (Uematsu et al., 2020). The first revision of the genus was proposed by Sher (1968), while the last identification key, with a summarized history of research on the genus, was prepared by Khun et al. (2015).

The aim of the paper is to present a new species named *Hirschmanniella maritima* n. sp., which has been found in the roots of *Potamogeton perfoliatus* L. collected along the Baltic coast from the Puck Bay to the Vistula Lagoon (Poland). *H. maritima* n. sp.'s description is provided based on morphological, morphometric and molecular data, including DNA sequences obtained from the large ribosomal subunit (28S rDNA) and the mitochondrial cytochrome c oxidase subunit I (mtCOI). The phylogenetic positioning of the species is also provided.

Materials and Methods

Nematode extraction, preservation, and morphological studies: *Potamogeton perfoliatus* L. plants were pulled out of the Baltic seabed from a depth of 1 to 3 meters by hand or using a fyke net set. They were also collected on the beach after an autumn storm (Table 1). Nematodes were separately extracted from the chopped roots of *P. perfoliatus* plants that had been collected at five locations on the Baltic coast (Table 1) on a 100-µm sieve using the Baermann method. For morphological studies, isolated nematodes were fixed in a TAF (triethanolamine formalin water solution) and mounted in permanent microscopic slides using the method of Hooper and Evans (1993). Females and males destined for molecular analyses were preserved in a DESS mixture (Yoder et al., 2006). Afterwards, they were transferred to anhydrous glycerol and mounted on temporary slides for microscopic observations and photographic documentation. After taking pictures, selected DESS-treated specimens were assigned for molecular studies. Measurements and photographs were performed with a Leica DFC 500 camera on a Leica DM 5000B light microscope (Leica Microsystems, Germany).

DNA extraction, PCR and sequencing: DNA was isolated from single nematode individuals using a QIAamp DNA Micro Kit (Qiagen, Germany) following the modified protocol of Burgermeister et al. (2005). Multiple rDNA fragments were acquired from over

Table 1: Description of *Hirschmanniella maritima* n. sp. populations isolated from *Potamogeton perfoliatus* L. plants with GenBank accession numbers of sequences acquired from the large subunit ribosomal DNA (28S rDNA) and the cytochrome c oxidase subunit I (mtCOI).

Locality and habitat	28S rDNA		mtCOI	
	# individuals from which a sequence was acquired	corresponding GenBank number	# individuals from which a sequence was acquired	corresponding GenBank number
The Gulf of Gdańsk, Jelitkowo Gdańsk, from a 3-m depth, 17.09.2011. Collected with a fyke net set. Type population.	2*	KC507798	1	PV031916
The Gulf of Gdańsk, Sopot, estuary of the Swelina River, from a 1-m depth, 1.07.2012. The plants were pulled out by hand.	4		-	
The Puck Bay, Rewa, the Beka reserve. Plants were washed ashore after an autumn storm. 28.10.2009.	24**		11	PV031913
			3	PV031914
			1	PV031915
The Puck Bay, Władysławowo, from a 3-m depth, 12.07.2012. Collected with a fyke net set.	2		-	
Vistula Lagoon, Kąty Rybackie, from a 1-m depth, 28.06.2011. The plants were pulled out by hand.		-	1	PV031914

*The corresponding mtCOI sequence was also acquired from one of these individuals.

**Corresponding mtCOI sequences were also acquired from 13 of these individuals.

30 nematode representatives of five *H. maritima* n. sp. populations (Table 1). The first population from Rewa was the most numerous, and thus over 20 derived nematode individuals from this population were analyzed molecularly. Four individuals were analyzed from the Sopot population, two from Gdańsk, two from Władysławowo, and one from the Kąty Rybackie. The 28S rDNA was generated with the HIR28SL (5' CAAAGCACTTTGAAGAGAGAG 3') and HIR28SH (5' CTCAGGAATAGTTCACCATCTT 3') primers (this study). The PCR reaction mix consisted of 1 µl of the isolated DNA; 1.6 µl of each primer (10 µM); 20 µl REDTaq ReadyMix (Sigma Aldrich, USA); and 15.8 µl of PCR water. The following PCR thermal profile was used: 94° C for 3 min, followed by 45 cycles of 30 s at 94° C for denaturation, 30 s at 50° C for the annealing temperature, and 60 s at 72° C for elongation. Cycles ended with a final elongation at 72° C for 5 min.

mtCOI fragments were acquired from three *H. maritima* n. sp. populations (Table 1) using the M2F primer (Rybarczyk-Mydlowska et al., 2019) combined with JB5R (Derycke et al., 2010), or the M3.5F primer combined either with the M6.9R or M8aR primers (Rybarczyk-Mydlowska et al., 2019). The PCR reaction mix contained 12.5 µl JumpStart Taq Ready Mix (Sigma-Aldrich, USA); 1 µl of each of the corresponding primers (5 µM); 3 µl DNA template; and H₂O up to a total volume of 25 µl. PCR profile consisted of 3 min at 94° C, 55 cycles (30 s at 94° C; 30 s at 45° C or 50° C; and 60 s at 72° C) and 5 min at 72° C.

After visualization on agarose gel, the obtained PCR products were purified using Clean Up Kit (A&A Biotechnology, Poland) and sequenced on a capillary sequencer — a 3500xL Genetic Analyzer (Applied Biosystems, USA) — according to the manufacturer's protocol.

Sequence analysis and phylogeny: The obtained sequences were analyzed using the BioEdit program (Hall, 1999; v.7.2.5). The final 28S rDNA dataset for phylogenetic study included the new *H. maritima* n. sp. sequences, as well as other *Hirschmanniella* spp. sequences available from GenBank. A *Globodera rostochiensis* 28S rDNA sequence was used as an outgroup species. The final multiple-sequence alignment consisted of 681 characters.

As there were substantially fewer mtCOI sequences from *Hirschmanniella* sp. available in GenBank, the representative sequences of mtCOI from other closely related Tylenchida species of cyst-forming, root-knot or root-lesion nematodes, were included into multiple alignment (699 characters) of the acquired mtCOI fragments. *Aphelenchoides ritzemabosi* was used as an outgroup species.

The Bayesian rDNA phylogenies were constructed with the program MrBayes v. 3.1 (Ronquist & Huelsenbeck, 2003). GTR substitution models with a proportion of invariable sites and gamma distribution were used for all data sets. In each case, two independent runs were performed with four Markov chains per run for 1×10^6 generations. Sample frequency was set at 100 generations. The sampled trees from each run were combined into a single 50% majority-rule tree. Stabilization of the likelihood and parameters were checked with the Tracer program (Rambaut et al., 2014; v. 1.6).

Light and transmission electron microscopy of infected plant: The roots of *P. perfoliatus* L. plants pulled out at the Sopot location (Table 1) were examined under a stereo microscope; samples with brownish spots or visually-recognizable nematodes inside were dissected and immersed in a modified Karnowsky's fixative. The collected samples were processed, embedded in epoxy resin, sectioned, and examined in light and transmission electron microscopes, as described by Dąbrowska-Bronk et al. (2015).

Results

Hirschmanniella maritima n. sp.

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(Table 2, Figs. 1-3)

Morphometric data are provided in Table 2.

Description of adults: Body almost straight to slightly curved when heat-relaxed, tapering towards both ends. Cuticle had well-marked annulation; annulus width at midbody region was $2.0 \mu\text{m}$ ($1.4 \mu\text{m}$ – $2.6 \mu\text{m}$). The lateral field occupies 26.8% (16.5%–35.6%) of the maximal body diameter and is marked

by four regular lines, which are sometimes thickened and have the appearance of ribs. In one of the females from the Gdańsk type population, thickened outer lines gave the impression of two additional lines appearing in the lateral field. In most of the examined specimens, the outer bands of the lateral field were wider and smooth. Inner bands were wider in only three males from the Rewa population; in two females from the Gdańsk population, the outer bands were partially areolated.

The lip region of females and males was anteriorly rounded to flattened with rounded edges. Lips were not separated from the body contour. The average ratio of head width to height was 2.4 (1.8–3.0) in both sexes. A well-developed cephalic framework extended for two to three annuli from the basal plate. The stylet was relatively long, about twice (1.8–2.5 times) as long as the head diameter. The stylet cone was 1.3 (0.9–1.8) times as long as the shaft, or 50% (46%–56%) of the total stylet length. Stylet knobs were large and oval, directed more or less laterally. The orifice of the dorsal pharyngeal gland was located $5.3 \mu\text{m}$ ($4.0 \mu\text{m}$ – $6.8 \mu\text{m}$) behind the base of the stylet. The pharynx had an oval median bulb measuring $23.8 \mu\text{m}$ ($18.1 \mu\text{m}$ – $29.0 \mu\text{m}$) in length and $17.0 \mu\text{m}$ ($14.8 \mu\text{m}$ – $19.8 \mu\text{m}$) in width, with a 1.4 (1.1–1.7) ratio of length to width. The excretory pore was located posteriorly to pharyngo-intestinal junction (PIJ). The pharyngeal glands formed a lobe overlapping the intestine ventrally. The hemizonid was 3.1 (2–4) annuli long and located 3.5 (1–7) annuli anterior to the excretory pore. The intestine was partially overlapping the rectum. The tail was elongate-conoid and similar in both sexes. The tail end was irregular in size and shape, lacked annulations, and had a pointed tip extending to a mucro-like projection about $3.9 \mu\text{m}$ ($1.4 \mu\text{m}$ – $10.5 \mu\text{m}$) long.

Females: Genital branches were opposed and outstretched. Spermatheca were oval and axial, filled with rounded sperm cells. The vagina had straight walls $18.9 \mu\text{m}$ ($15.6 \mu\text{m}$ – $23.7 \mu\text{m}$) long that were perpendicular to the body axis and occupied 50.8% (43.3–61.3%) of the corresponding body diameter. Vulva was approximately $16.1 \mu\text{m}$ ($14.4 \mu\text{m}$ – $19.4 \mu\text{m}$) wide. Distinct, pore-like phasmids were located $64.0 \mu\text{m}$ ($53.8 \mu\text{m}$ – $84.0 \mu\text{m}$) anteriorly to the tail end or 31 (25–38) annuli from the tail terminus. In a female from the Władysławowo population, two eggs, with dimensions of 89.4 by $28.0 \mu\text{m}$ and 95.0 by $28.7 \mu\text{m}$, respectively, were found.

Males: The general morphology was similar to that of the female, with the exception of the reproductive system. The male genital system was monorchic when outstretched. The spicules

Table 2: Morphometric characteristics of females and males of *Hirschmanniella maritima* n. sp. Measurements are provided in μm and in the form of mean $\pm$ standard deviation, with the range given in parentheses and the coefficient of variation (CV) in italics after the parentheses. All abbreviations are used according to Brzeski (1998).

Character	Gdańsk			Rewa			Władysławowo			All populations	
	Holotype female	Paratype females	Paratype males	Females	Males	Females	Females	Males	Females	Males	
n	1	14	12	20	22	6	8	41	42		
L	3009	2355 $\pm$ 266.4 (1893-2708) 11.3	2122 $\pm$ 198.3 (1787-2441) 9.3	2688 $\pm$ 228.2 (2218-3010) 8.5	2619 $\pm$ 205.8 (2065-3085) 7.9	2531 $\pm$ 201.6 (2146-2682) 8.0	2248 $\pm$ 93.3 (2100-2356) 4.2	2559 $\pm$ 284.3 (1893-3010) 11.1	2406 $\pm$ 294.1 (1787-3085) 12.2		
a	62	67.0 $\pm$ 7.2 (59.2-81.8) 10.8	64.5 $\pm$ 5.2 (57.3-75.3) 8.1	68.6 $\pm$ 5.6 (59.5-79.7) 8.2	69.7 $\pm$ 6.6 (55.3-81.2) 9.5	64.5 $\pm$ 3.5 (60.4-70.0) 5.4	62.6 $\pm$ 4.3 (58.2-69.8) 6.9	67.3 $\pm$ 6.0 (59.2-81.8) 8.9	66.9 $\pm$ 6.5 (55.3-81.2) 9.8		
b	18.9	15.6 $\pm$ 1.4 (13.1-17.8) 8.7	13.8 $\pm$ 1.0 (11.6-15.4) 7.5	17.1 $\pm$ 1.5 (14.5-19.7) 9.0	17.2 $\pm$ 1.0 (14.9-18.8) 5.8	15.9 $\pm$ 1.0 (15.0-17.4) 6.4	14.2 $\pm$ 0.7 (13.2-15.2) 4.9	16.4 $\pm$ 1.6 (13.1-19.7) 9.6	15.7 $\pm$ 1.9 (11.6-18.8) 12.0		
b'	6.8	5.6 $\pm$ 0.6 (4.8-6.5) 11.2	4.9 $\pm$ 0.3 (4.5-5.7) 7.0	7.9 $\pm$ 0.8 (6.1-9.2) 10.5	5.7 $\pm$ 0.8 (4.5-7.0) 13.5	5.5 $\pm$ 0.4 (5.0-5.9) 7.9	5.3 $\pm$ 0.6 (4.5-6.3) 11.4	6.7 $\pm$ 1.3 (4.8-9.2) 20.0	5.3 $\pm$ 0.6 (4.5-7.0) 12.3		
c	14.6	15.1 $\pm$ 2.0 (12.6-17.8) 13.2	15.5 $\pm$ 1.2 (13.7-17.9) 7.7	14.5 $\pm$ 1.2 (12.8-17.4) 8.1	17.6 $\pm$ 1.5 (14.2-20.6) 8.7	14.0 $\pm$ 0.5 (13.4-14.8) 3.9	15.3 $\pm$ 1.5 (13.5-17.4) 9.5	14.6 $\pm$ 1.5 (12.6-17.8) 9.9	16.6 $\pm$ 1.8 (13.5-20.6) 10.6		
c'	6.6	5.8 $\pm$ 0.5 (5.1-6.5) 8.2	6.0 $\pm$ 0.4 (5.4-6.7) 7.0	6.7 $\pm$ 0.7 (5.2-8.1) 11.0	6.1 $\pm$ 0.7 (4.7-7.7) 11.2	6.6 $\pm$ 0.6 (6.2-7.7) 8.8	5.9 $\pm$ 0.6 (4.7-6.6) 10.6	6.4 $\pm$ 0.7 (5.1-8.1) 11.7	6.0 $\pm$ 0.6 (4.7-7.7) 10.0		
V	46.8	52.8 $\pm$ 1.7 (50.0-55.4) 3.3	-	49.8 $\pm$ 1.9 (46.8-53.7) 3.9	-	52.2 $\pm$ 1.7 (49.3-53.5) 3.2	-	51.1 $\pm$ 2.3 (46.8-55.4) 4.6	-		
G ₁	19.2	24.0 $\pm$ 2.8 (18.8-28.9) 11.8	-	22.3 $\pm$ 4.8 (14.7-29.8) 21.4	-	32.3 $\pm$ 3.4 (27.8-36.8) 10.4	-	24.9 $\pm$ 5.2 (14.7-36.8) 20.8	-		
G ₂	16.8	23.8 $\pm$ 2.6 (18.8-28.8) 10.9	-	22.7 $\pm$ 5.1 (14.7-28.5) 22.4	-	30.7 $\pm$ 2.9 (26.1-34.2) 9.4	-	24.6 $\pm$ 4.8 (14.7-34.2) 19.3	-		
T	-	-	44.3 $\pm$ 2.8 (38.9-48.0) 6.4	-	39.7 $\pm$ 4.5 (29.2-51.2) 11.3	-	44.9 $\pm$ 3.1 (40.4-49.5) 7.0	-	42.2 $\pm$ 4.4 (29.2-51.2) 10.5		
Lip region diameter	13.2	10.5 $\pm$ 0.6 (9.6-11.5) 5.8	10.3 $\pm$ 0.6 (9.7-11.8) 5.5	10.5 $\pm$ 0.5 (9.5-11.8) 5.0	10.3 $\pm$ 0.4 (9.5-10.8) 3.5	10.9 $\pm$ 0.7 (9.6-11.6) 6.3	10.2 $\pm$ 0.6 (9.2-11.1) 5.7	10.6 $\pm$ 0.7 (9.5-13.2) 6.6	10.3 $\pm$ 0.5 (9.2-11.8) 4.5		
Lip region height	5.7	4.6 $\pm$ 0.4 (3.8-5.2) 9.6	4.7 $\pm$ 0.6 (3.9-5.9) 12.0	4.5 $\pm$ 0.4 (3.8-5.2) 9.3	4.0 $\pm$ 0.4 (3.4-4.9) 9.3	4.7 $\pm$ 0.2 (4.5-5.1) 5.1	4.7 $\pm$ 0.3 (4.4-5.2) 5.6	4.6 $\pm$ 0.4 (3.8-5.7) 9.5	4.4 $\pm$ 0.5 (3.4-5.9) 12.5		

(Continued)

Table 2: Continued

Character	Gdańsk		Rewa		Władysławowo		All populations	
	Holotype female	Paratype females	Paratype males	Females	Males	Females	Males	Females
Lip annules number	5	5.3±0.5(5-6) 8.9	5.2±0.4 (5-6) 7.5	4.8±0.4 (4-5) 8.7	4.7±0.5 (4-5) 9.6	5.2±0.4 (5-6) 7.9	5.4±0.5(5-6) 9.6	5.1 ± 0.5 (4-6) 10.0
Stylet length	23.2	21.3 ± 1.8 (19.3-24.5) 8.6	20.9 ± 1.3 (19.4-23.4) 6.2	21.5 ± 0.9 (20.0-23.4) 4.3	21.3 ± 0.6 (20.4-22.8) 2.8	21.6 ± 0.4 (20.9-22.1) 1.9	21.5 ± 1.0 (20.2-23.3) 4.5	21.5 ± 1.3 (19.3-24.5) 5.8
Conus length	10.8	10.6 ± 0.9 (9.4-12.1) 8.4	10.4 ± 0.8 (9.2-12.0) 8.1	11.0 ± 1.0 (10.0-12.9) 8.7	10.9 ± 0.6 (9.8-11.8) 5.7	10.7 ± 0.2 (10.4-11.0) 1.9	10.6 ± 0.6 (9.4-11.2) 6.0	10.8 ± 0.8 (9.4-12.9) 7.9
Shaft length	9.5	8.3 ± 0.8 (7.0-9.7) 9.6	7.9 ± 0.5 (7.3-8.8) 6.6	7.8 ± 0.9 (6.7-10.4) 11.2	10.4 ± 0.7 (9.0-12.0) 6.4	8.1 ± 0.3 (7.6-8.4) 3.7	8.3 ± 0.4 (7.7-9.2) 5.2	8.0 ± 0.8 (6.7-10.4) 10.3
Stylet knob height	2.9	2.5 ± 0.3 (2.1-3.1) 13.5	2.6 ± 0.2 (2.2-3.0) 8.5	2.9 ± 0.4 (2.3-3.8) 13.0	2.8 ± 0.3 (2.3-3.5) 11.3	2.8 ± 0.2 (2.7-3.1) 5.8	2.6 ± 0.3 (2.3-3.0) 10.5	2.8 ± 0.4 (2.1-3.8) 13.9
Stylet knob width	5.8	5.0 ± 0.5 (4.1-5.9) 10.6	4.8 ± 0.3 (4.4-5.3) 5.7	4.8 ± 0.6 (3.6-5.8) 12.2	4.5 ± 0.3 (4.0-5.2) 7.1	5.1 ± 0.3 (4.7-5.5) 6.3	4.6 ± 0.2 (4.3-5.0) 5.0	4.9 ± 0.5 (3.6-5.9) 11.0
Anterior end to median bulb valve distance	108.8	102.9 ± 6.1 (94.1-115.0) 6.0	103.1 ± 5.6 (95-111) 5.4	108.5 ± 4.8 (102.1-119.0) 4.4	111.2 ± 5.0 (102.1-120.3) 4.5	108.8 ± 3.7 (104.4-112.5) 3.4	108.5 ± 3.3 (103-112) 3.0	106.5 ± 5.7 (94.1-119) 5.4
Anterior end to pharyngo-intestinal junction (PIJ) distance	159	151.1 ± 12.9 (132-172) 8.5	153.8 ± 8.4 (136-165) 5.5	157.8 ± 8.4 (135-170) 5.3	152.4 ± 8.6 (138-173) 5.6	159.3 ± 12.5 (143-177) 7.8	157.9 ± 4.6 (151-167) 2.9	155.8 ± 10.9 (132-177) 7.0
Pharyngeal overlap length	286	273.6 ± 40.1 (228-377) 14.6	277.1 ± 39.1 (226-357) 14.1	185.7 ± 31.4 (159-284) 16.9	304.9 ± 65.8 (215-423) 21.6	302.5 ± 36.3 (259-359) 12.0	270.3 ± 53.1 (203-324) 19.7	238.2 ± 60.5 (159-377) 25.4
Anterior end to nerve ring distance	143.6	134 ± 10.4 (120-155) 7.8	130.0 ± 8.3 (116-145) 6.4	136.5 ± 6.1 (126-143) 4.5	141.9 ± 6.2 (133-149) 4.4	139.3 ± 8.2 (127-147) 5.9	136.3 ± 4.7 (128-141) 3.5	136.5 ± 8.5 (120-155) 6.2
Anterior end to excretory pore (EP) distance	180.8	167.8 ± 12.8 (144-186) 7.6	170.0 ± 9.4 (154-184) 5.5	174.8 ± 9.3 (155-190) 5.3	176.7 ± 11.7 (159-197) 6.6	181.8 ± 11.1 (163-196) 6.1	182.1 ± 8.5 (174-200) 4.6	173.4 ± 11.8 (144-196) 6.8
EP to PIJ distance	21.8	16.7 ± 7.1 (4-25) 42.4	16.3 ± 4.4 (7-22) 27.0	16.3 ± 6.9 (3.0-30.0) 42.2	24.3 ± 10.0 (6.8-46.1) 41.4	22.9 ± 3.6 (19.5-28.9) 15.9	22.9 ± 5.0 (16.8-30.9) 22.1	17.7 ± 6.8 (3-30) 38.2
Max. body diameter	48.5	35.2 ± 2.7 (31.3-40) 7.8	33.0 ± 2.4 (29.5-38.2) 7.3	39.3 ± 3.2 (34.0-45.2) 8.2	37.8 ± 3.3 (31.7-43.8) 8.7	39.4 ± 4.5 (32.6-44.4) 11.5	36.1 ± 2.9 (32.2-40.5) 8.2	38.1 ± 4.1 (31.3-48.5) 10.7

(Continued)

Table 2: Continued

Character	Gdańsk		Rewa		Władysławowo		All populations	
	Holotype female	Paratype females	Paratype males	Females	Males	Females	Males	Females
Female anterior genital branch length	578	564.3 ± 91.6 (418-729) 76.2	-	604.4 ± 105.8 (414-764) 17.5	-	814.0 ± 74.1 (709-939) 9.1	-	626.0 ± 130.3 (414-939) 20.8
Female posterior genital branch length	505	560.9 ± 84.6 (424-727) 75.1	-	619.9 ± 121.5 (413-761) 19.6	-	774.8 ± 82.6 (666-906) 10.7	-	619.5 ± 124.8 (413-906) 20.1
Anterior spermatheca length	54.7	45.6 ± 12.2 (32.5-74.0) 26.7	-	76.0 ± 4.4 (69.5-80.4) 5.8	-	54.3 ± 3.2 (51.2-58.8) 5.8	-	55.3 ± 15.1 (32.5-80.4) 27.2
Anterior spermatheca diameter	28.8	22.7 ± 5.0 (13.2-28.8) 22.1	-	34.1 ± 4.7 (26.9-38.3) 13.8	-	23.3 ± 3.2 (20-27) 13.6	-	25.9 ± 6.5 (13.2-38.3) 25.0
Posterior spermatheca length	47.1	43.9 ± 9.3 (32-59) 21.3	-	83.1 ± 3.7 (78.6-87.3) 4.5	-	55.0 ± 4.3 (47.7-59.2) 7.8	-	54.7 ± 16.8 (32.0-87.3) 30.7
Posterior spermatheca diameter	31.5	22.1 ± 5.3 (11.8-27.8) 24.1	-	36.8 ± 3.3 (33.2-40.4) 8.9	-	24.3 ± 2.0 (22.1-26.7) 8.2	-	26.3 ± 7.2 (11.8-40.4) 27.3
Cloaca to most anterior part of testis distance	-	-	937.9 ± 94.3 (812-1103) 10.1	-	1042.7 ± 137.5 (798-1382) 13.2	-	1007.8 ± 69.8 (879-1074) 6.9	1002.2 ± 120.0 (798-1382) 12.0
Anal body diameter	31.3	27.1 ± 2.1 (24.5-30.4) 7.7	23.0 ± 2.2 (20.6-25.9) 9.5	27.8 ± 2.3 (24.0-31.8) 8.4	24.8 ± 2.3 (20.6-28.9) 9.2	27.6 ± 3.9 (20.9-31.7) 14.1	25.2 ± 1.6 (22.7-28.0) 6.2	27.6 ± 2.5 (20.9-31.8) 9.1
Rectum/ cloaca length	24.7	25.3 ± 2.6 (20.6-29.6) 10.3	33.5 ± 2.9 (31.4-38.4) 8.8	24.5 ± 4.2 (19.4-35.0) 17.0	27.8 ± 3.2 (24.0-35.0) 11.4	29.8 ± 1.1 (28.5-31.3) 3.8	33.4 ± 2.3 (31.5-36.0) 7.0	25.8 ± 3.7 (19.4-35.0) 14.2
Spicule length	-	-	38.4 ± 1.1 (36.4-39.4) 2.9	-	38.5 ± 2.3 (34.0-43.2) 6.0	-	40.8 ± 1.8 (38.4-42.2) 4.4	38.7 ± 2.2 (34-43.2) 5.6
Gubernaculum length	-	-	13.1 ± 1.0 (12.0-14.5) 7.3	-	12.1 ± 1.0 (10.4-14.6) 8.4	-	12.1 ± 0.7 (11.4-12.7) 5.4	12.3 ± 1.0 (10.4-14.6) 8.3
Tail length	205.6	157 ± 9.0 (150-178) 5.7	136.8 ± 9.0 (120-150) 6.6	186.6 ± 18.3 (153-215) 9.8	149.8 ± 14.3 (115-178) 9.6	180.8 ± 13.0 (160-196) 7.2	147.5 ± 12.6 (126-159) 8.6	176.1 ± 20.3 (150-215) 11.6

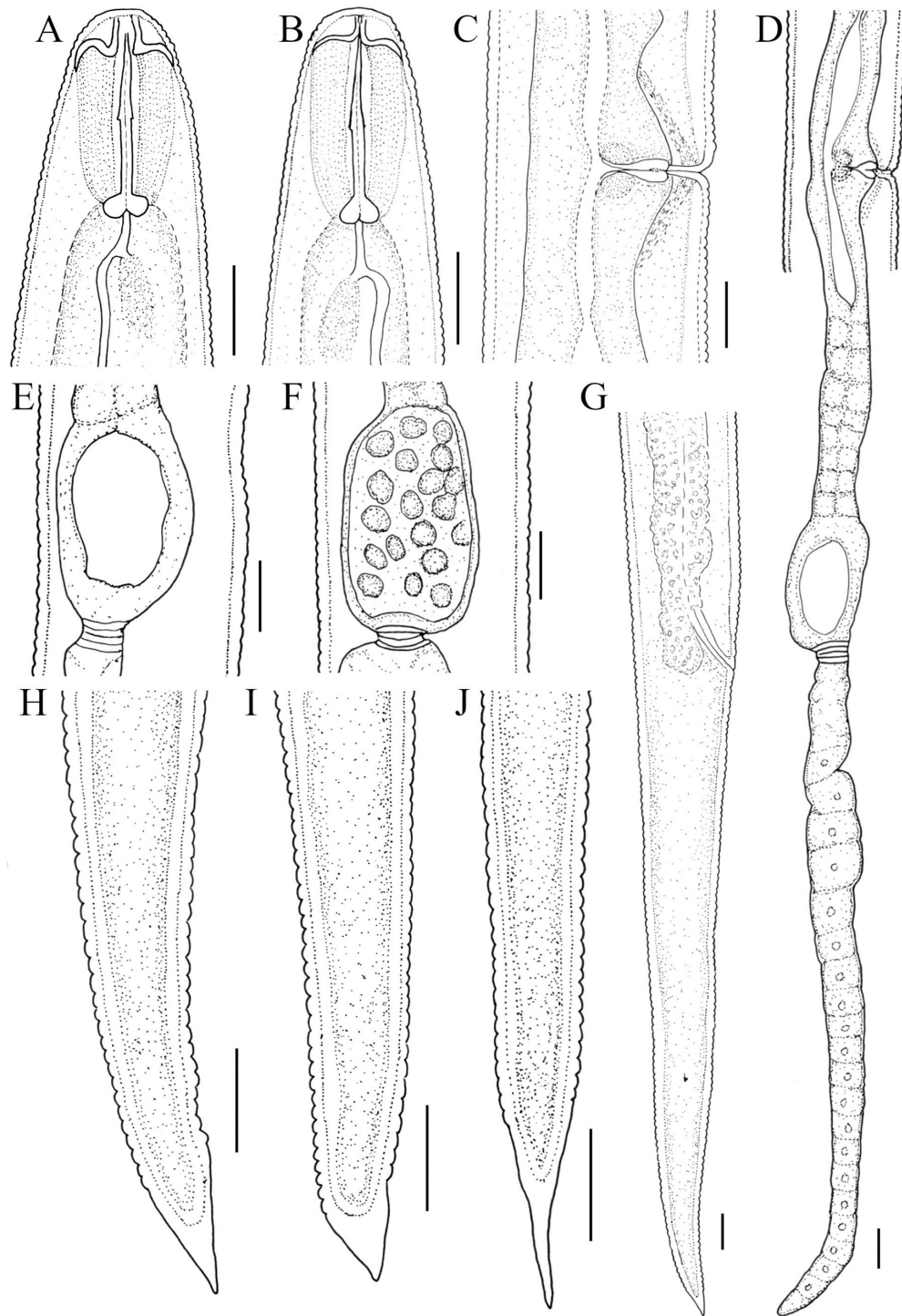


Figure 1: Line drawings of *Hirschmanniella maritima* n. sp. Female: A, B: Anterior end: A: holotype, B: Rewa population. C: Vulval region, holotype. D: posterior genital branch, paratype. E, F: Spermatheca, paratypes: E: empty, F: filled with sperm. G: Posterior part of body, holotype. H - J: Distal part of tail: H: holotype, I: paratype, J: Rewa population. Scale bar = 10 μ m.

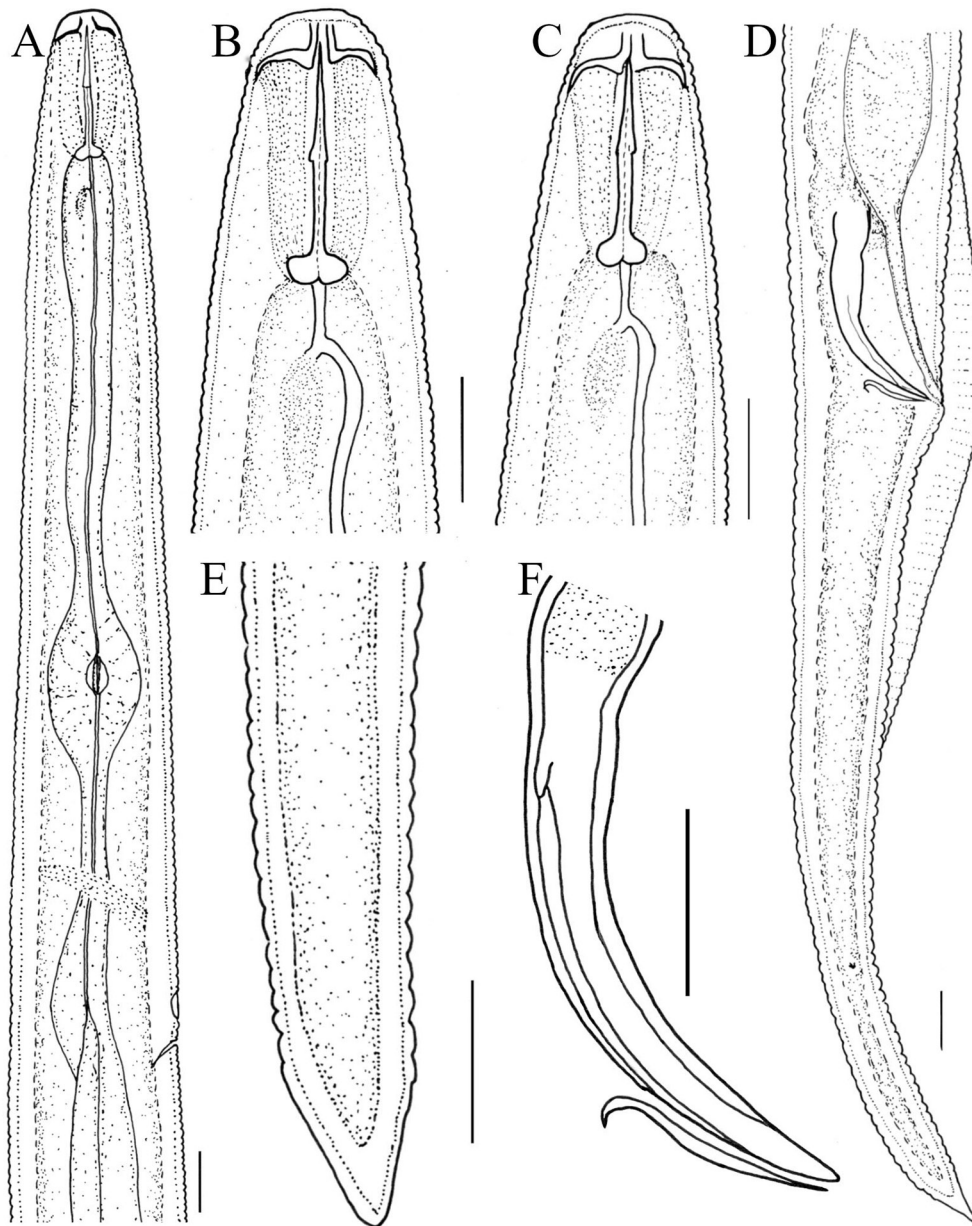


Figure 2: Line drawings of *Hirschmanniella maritima* n. sp. Male, paratype : A: Anterior part of body. B, C: Anterior end. D: Posterior part of body. E: Distal part of tail. F: Male copulatory apparatus. Scale bar = 10 μ m.

were of medium size, slightly curved ventrally, and cephalated. The gubernaculum was slightly arcuate and narrow, with a hook-shaped distal part occupying about 31.8% (25.6%–40.0%) of the total spicules' length. Hypoptygma was present. The bursa leptoderan was 123.1 μ m (81.5 μ m–143.9 μ m) long and 11.8 μ m (8.7 μ m–15.4 μ m) wide, with slightly crenate margins. The bursa started 45.3 μ m (33.4 μ m–57.8 μ m) anteriorly and ended 77.9 μ m (48.1 μ m–102.0 μ m) posteriorly relative to the

cloacal opening. Phasmids were distinct, pore-like, and located 52.5 μ m (44.3 μ m–60.9 μ m) anteriorly to the end of the tail, or 29 (22–36) annuli from the tail terminus.

Type locality: *Hirschmanniella maritima* n. sp. was detected in the roots of *Potamogeton perfoliatus* L. plants growing at a depth up to 3 m in the Baltics Sea's Gulf of Gdańsk, near Gdańsk, Poland. Geographic coordinates of the sampling point are N54.4278, E18.5964.

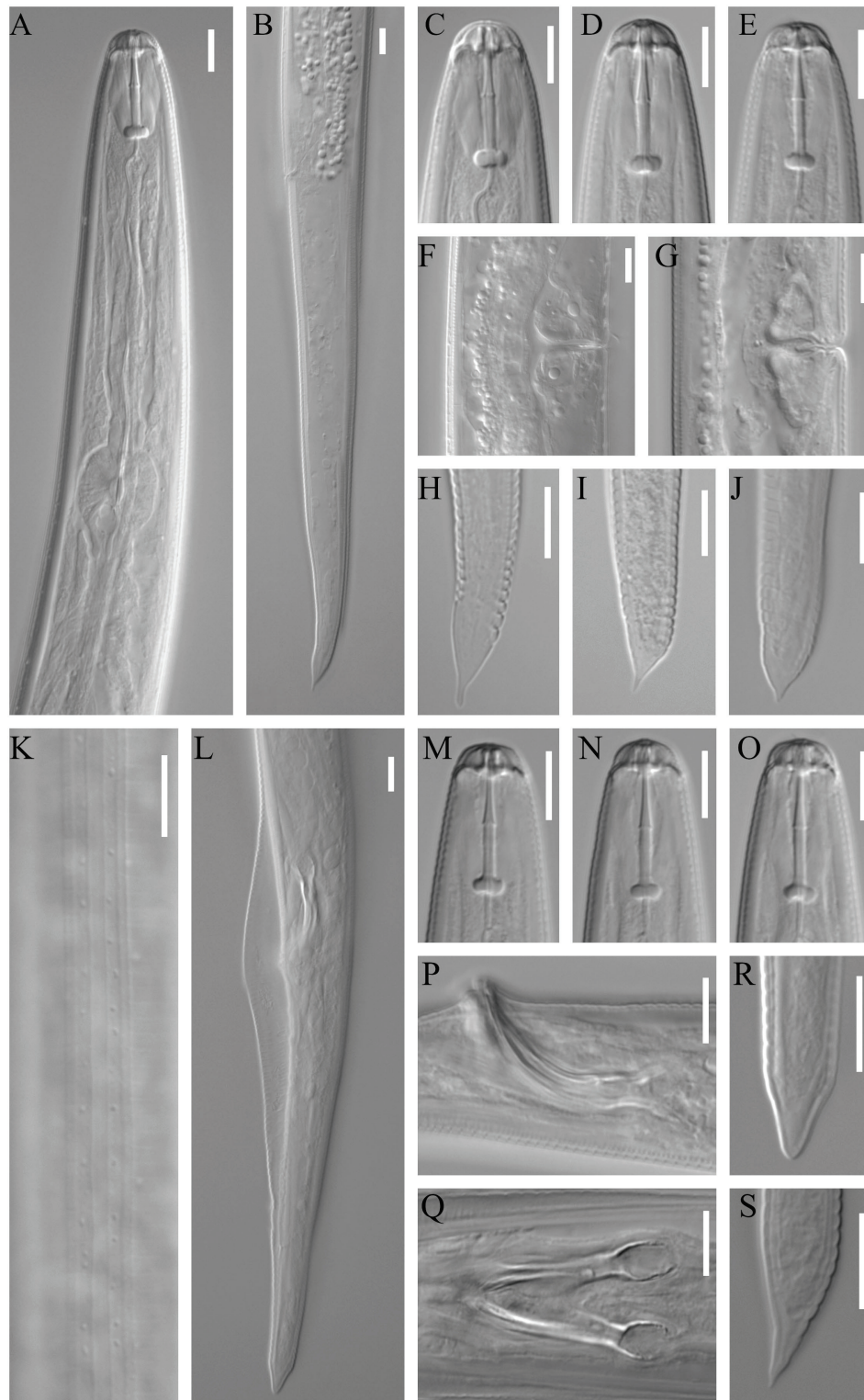


Figure 3: Light micrographs *Hirschmanniella maritima* n. sp. A - J. Female. K - S. Male. A: Anterior part of body, holotype. B: Posterior part of body, holotype. C - E: Anterior end, paratypes. F, G: Vulval region: F: holotype, G: paratype. H - J: Distal part of tail, paratypes. K: Lateral field. L: Posterior part of body. M - O: Anterior part of body. P, Q. Male copulatory apparatus. R, S. Distal part of tail. Scale bar = 10 µm.

Other localities: From the same host, found in locations listed in Table 1.

Etymology: Latin, *maritima* = maritime, referring to the sea habitat of this species.

Type specimens: Holotype female and paratypes (12 female and 10 male) deposited in the Nematode Collection at the Museum and Institute of Zoology Polish Academy of Sciences in Warsaw; two female and two male paratypes deposited at the National Plant Protection Organization, Wageningen Nematode Collection (WaNeCo), Wageningen, The Netherlands.

Polytomous key code: According to Khun et al. (2015), matrix codes for the new species are A2, B3, C1, D1, E3, F222, G224, H324, I324, J223, K1.

Differential diagnosis: *Hirschmanniella maritima* n. sp. is an amphimictic species characterized by a long ($L = 1787\ \mu\text{m}$ – $3085\ \mu\text{m}$) body with an anteriorly rounded or slightly flattened lip region consisting of four to six lip annuli. Stylet is medium-long ($19.5\ \mu\text{m}$ – $24.5\ \mu\text{m}$) with large, oval knobs directed slightly laterally. Excretory pore is located posteriorly to PIJ. Intestine partially overlapping the rectum. Spicules are medium sized ($34.0\ \mu\text{m}$ – $43.2\ \mu\text{m}$). Gubernaculum is slightly arcuate and narrow, with a hook-shaped distal part. The tail is elongate-conoid ($c' = 4.7$ – 8.1). Tail end, without annulations, is irregular in size and shape, with a pointed terminus up to an axial mucro-like projection. Phasmids are distinct and located 22–38 annuli from the tail terminus.

Based on the dichotomous key by Karssen after Loof (1991; EPPO, 2022) and the polytomous key by Khun et al. (2015), *H. maritima* n. sp. is closely related to *H. miticausa* Bridge, Mortimer and Jackson, 1983; *H. santarosae* Tandingan De Ley et al., 2007; and *H. pomponiense* Abdel-Rahman and Maggenti, 1987. *H. maritima* n. sp. differs from *H. miticausa* by its longer body ($1787\ \mu\text{m}$ – $3085\ \mu\text{m}$ vs. $1520\ \mu\text{m}$ – $1860\ \mu\text{m}$), the shape of the stylet knobs (oval vs. rounded), its longer and narrower tail ($c' = 4.7$ – 8.1 vs. 3.4 – 4.9), and its intestine partially overlapping its rectum (vs. intestine not overlapping rectum).

It differs from *H. santarosae* by its longer body ($1787\ \mu\text{m}$ – $3085\ \mu\text{m}$ vs. $1380\ \mu\text{m}$ – $1960\ \mu\text{m}$); the shape of the stylet knobs (oval vs. pumpkin-shaped); position of the excretory pore (ES) in relation to the PIJ (ES posterior to PIJ vs. ES anterior or at same level as PIJ); longer spicules ($34.0\ \mu\text{m}$ – $43.2\ \mu\text{m}$ vs. $27.0\ \mu\text{m}$ – $33.0\ \mu\text{m}$); longer gubernaculum ($10.4\ \mu\text{m}$ – $14.6\ \mu\text{m}$ vs. $6.0\ \mu\text{m}$ – $11.0\ \mu\text{m}$); the shape of the distal part of gubernaculum (hook-shaped vs. straight), and its intestine partially overlapping the rectum (vs. not overlapping).

Based on morphological and morphometric characters, *H. maritima* n. sp. is very close to *H.*

pomponiense, from which it differs by the shape of the stylet knobs (oval vs. rounded), and its longer tail in both females ($150\ \mu\text{m}$ – $215\ \mu\text{m}$ vs. $99\ \mu\text{m}$ – $189\ \mu\text{m}$) and males ($115\ \mu\text{m}$ – $177\ \mu\text{m}$ vs. $97\ \mu\text{m}$ – $133\ \mu\text{m}$). These species can, however, be clearly distinguished on the basis of molecular markers.

H. maritima n. sp. is the second species of the genus *Hirschmanniella* occurring in the Baltic Sea. The first, *H. zostericola*, was described based on two specimens (a single female and male) collected from Hallands Väderö (Sweden) by Allgén in 1934. *H. maritima* n. sp. can be distinguished from *H. zostericola* by its longer stylet ($19.3\ \mu\text{m}$ – $24.5\ \mu\text{m}$ vs. $15\ \mu\text{m}$), the shape of the stylet knobs (oval vs. rounded), its longer spicules ($34.0\ \mu\text{m}$ – $43.2\ \mu\text{m}$ vs. $25\ \mu\text{m}$), and its intestine partially overlapping rectum (vs. intestine not overlapping rectum).

Comparison of the obtained 28S sequences with the sequences of other *Hirschmanniella* species available in GenBank showed close relationships between *H. maritima* n. sp. and *H. santarosae*, as well as *H. pomponiense* (despite described above morphological and morphometric features differentiating these species). Close relationships were also found with *H. diversa* Sher, 1968; *H. gracilis* (de Man, 1880); *H. halophila* Sturhan and Hallmann, 2010; and *H. imamuri* Sher, 1968.

Among these species, *H. maritima* n. sp. can also be distinguished also by the morphological and morphometric characters described below. It differs from *H. diversa* by shape of the stylet knobs (oval vs rounded); its intestine partially overlapping the rectum (vs. intestine not overlapping rectum); the number of ventral annuli between the phasmids and tail terminus (22–38 vs. 9–14); and shape of the distal part of gubernaculum (hook-shaped vs. straight). From *H. gracilis*, it is distinguishable by its longer body ($1787\ \mu\text{m}$ – $3085\ \mu\text{m}$ vs. $1380\ \mu\text{m}$ – $2220\ \mu\text{m}$); the shape of the stylet knobs (oval vs. rounded); and its intestine partially overlapping rectum (vs. intestine not overlapping rectum). It diverges from *H. halophila* by its longer body ($1787\ \mu\text{m}$ – $3085\ \mu\text{m}$ vs. $1050\ \mu\text{m}$ – $1500\ \mu\text{m}$); longer stylet ($19.3\ \mu\text{m}$ – $24.5\ \mu\text{m}$ vs. $16\ \mu\text{m}$ – $19\ \mu\text{m}$); the shape of the stylet knobs (oval vs. rounded); its longer spicules ($34.0\ \mu\text{m}$ – $43.2\ \mu\text{m}$ vs. $26.3\ \mu\text{m}$ – $30.4\ \mu\text{m}$); and its tail with a pointed terminus up to an axial mucro-like projection (vs. tail with mostly terminal mucro and one or two pegs or notches). From *H. imamuri*, it differs by its shorter stylet ($19.3\ \mu\text{m}$ – $24.5\ \mu\text{m}$ vs. $29\ \mu\text{m}$ – $32\ \mu\text{m}$); the shape of the stylet knobs (oval vs. rounded); the position of the ES in relation to the PIJ (ES posterior to PIJ vs. ES anterior to PIJ); and its intestine partially overlapping the rectum (vs. intestine not overlapping rectum).

Microscopic examinations of nematode-infected roots: The uninfected roots of *P. perfoliatus* usually had degraded epiblem that had been replaced by exodermis composed of two cell layers (Fig. 6A). The cortex was created by several layers of cells. The cells in the outer cortex region below the exodermis tended to be arranged in files separated by extensive intercellular spaces. In contrast, the inner layers of the cortex were arranged in regular rings, with only small intercellular spaces (Fig. 6A). In infected roots, the nematodes were usually present in intercellular spaces, which increased in size (Fig. 6B). Portions of broken cell walls occurred frequently next to the nematodes (Fig. 6C). On some occasions, the nematodes were present inside the cell lumen, and debris of degraded protoplast was present in their vicinity (Fig. 6D). Nematodes found next to the inner

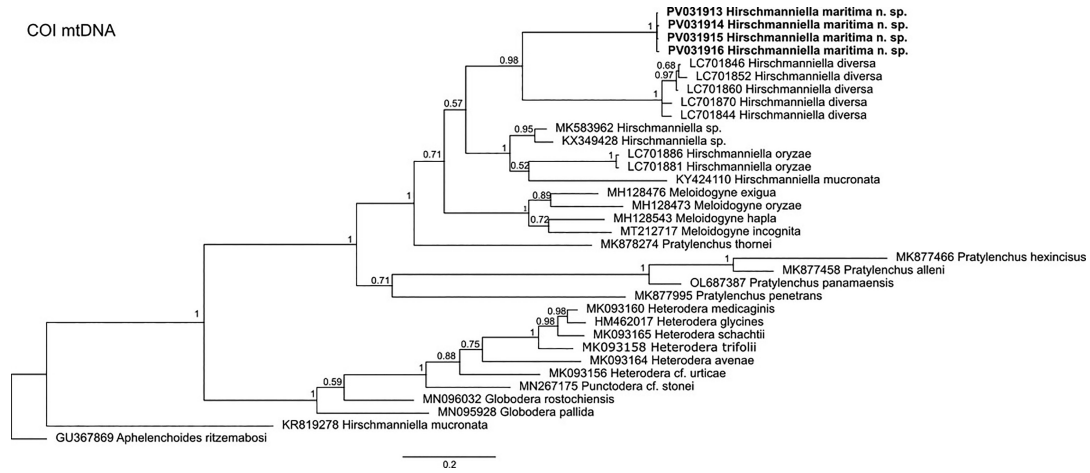


Figure 5: mtCOI-based Bayesian phylogeny of the genus *Hirschmanniella* and the most closely related genera. The new *H. maritima* n. sp. sequences are indicated in bold. Numbers near nodes indicate posterior probabilities.

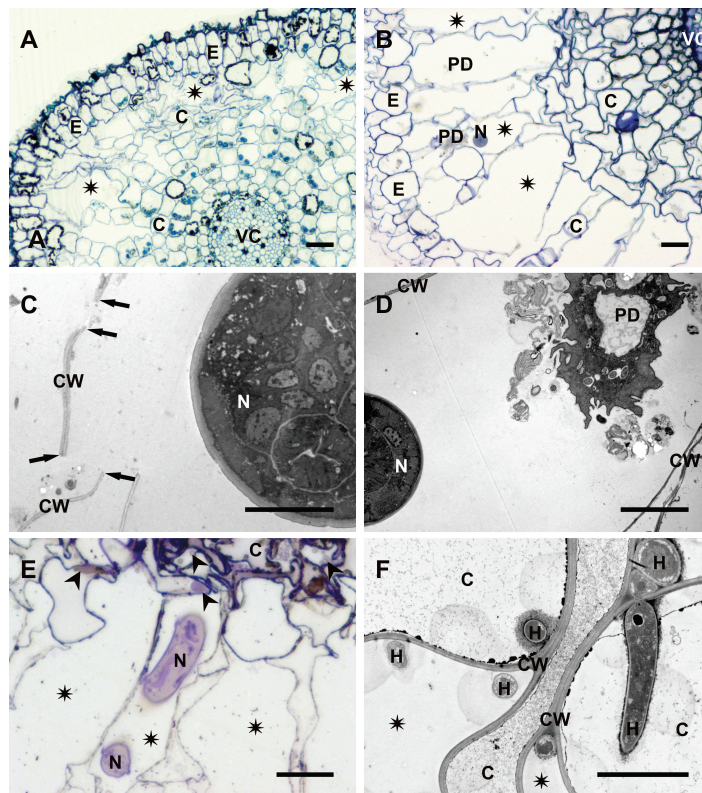


Figure 6: Micrographs of *P. perfoliatus* roots infected with *H. maritima* n. sp. Light (A, B, and E) and transmission electron (C, D, and F) microscopy images. A: Cross section of uninfected root. B: Cross section of infected root with nematode in outer cortex. C: Broken cell walls next to nematode. D: Plant cell protoplast debris next to nematode. F: Nematodes next to degraded inner cortex cells. E: Fungal hyphae in inner cortex of root. Abbreviations: C, cortex; CW, cell wall; E, exodermis; H, hypha; N, nematode; PD, protoplast debris; VC, vascular cylinder. Arrows point to broken cell walls (C); arrowheads point to hyphae (E); asterisks indicate intercellular spaces (A, B, E, and F). Scale bars: = 50 μ m (A, B, and E) and = 5 μ m (C, D, and F).

cortex induced extensive degradation in adjacent cells (Fig. 6E). The inner cortex layers and the vascular cylinder tissues were extensively colonized by fungal hyphae, and some of them had a degraded morphology (Figs. 6E and 6F). No structures related to the feeding of plant-parasitic nematodes — i.e., feeding plugs, feeding tubes, or a stylet inserted into the plant cell wall — were found (Wyss et al., 1979; Zunke, 1990).

Discussion

To date, out of 35 *Hirschmanniella* species, only 11 have been characterized molecularly. The phylogenetic trees of the genus *Hirschmanniella* reveal a split into two well-supported lineages (Figs. 4 and 5). The first clade (Clade III, following Feng et al., 2016) encompasses *H. diversa*, *H. gracilis*, *H. halophila*, *H. imamuri*, *H. pomponiensis* and *H. santarosae*. Our results indicate that *H. maritima* n. sp. was also positioned there, being the most closely related to *H. pomponiensis* and *H. santarosae*. The other *Hirschmanniella* lineage encloses *H. belli*, *H. kwazuna*, *H. mucronata* and *H. oryzae*. This clade was further divided into two smaller subclades that were annotated as Clades I and II by Feng et al. (2016). The exact positioning within the 28S rDNA trees and phylogenetic relationships remain unclear for species such as *H. mucronata* and *H. oryzae*, which appear to be polyphyletic. Additionally, the obtained phylogenetic data indicate the necessity for more extensive studies of this genus, as many of the *Hirschmanniella* sequences were submitted as unidentified species (Figs. 4 and 5).

Next to *H. diversa*, *H. oryzae* and some undescribed *Hirschmanniella* sp., our study provided new mitochondrial sequences within this genus. The positioning of mtCOI sequences from *H. maritima* n. sp. was in agreement with the positioning of the species within the 28S rDNA tree in close proximity to *H. diversa*.

The occurrence of *H. maritima* n. sp. was observed on *P. perfoliatus* plants growing at various depths along the south Baltic coast, from the Puck Bay through the Gulf of Gdańsk to the Vistula Lagoon. Depending on season, proximity to fresh water sources, and other factors, the salinity of water in this region oscillates between 0.5 and 7.65 psu (Chubarenko & Margoński, 2008; Kruk-Dowgiałło & Szaniawska, 2008). The occurrence of *H. maritima* n. sp. at these locations along the Baltic coast may indicate that it tolerates various degrees of water salinity.

H. maritima n. sp. has so far only been found in *P. perfoliatus* roots — a common plant in freshwater and brackish water bodies. It is an important component of lowland freshwater flora in Poland. *P. perfoliatus*

has a great value as a water-oxygenating plant. It creates underwater thickets, which are a habitat for fry and other small aquatic fauna, and is often used in waterholes, ponds and aquariums.

Some species of the *Hirschmanniella* genus, due to their negative impact on the growth and development of host plants, are considered harmful and subject to legal regulations (EPPO, 2009, 2022). In light of this, we examined the parasitic behavior of *H. maritima* n. sp. inside plant roots using light and transmission electron microscopy (Fig. 6). The range of its destruction was relatively restricted in comparison to cases of infection by *H. diversa* (Uematsu et al., 2020), but broken cell walls and collapsed plant cell protoplasts were still found along nematode bodies. *H. maritima* n. sp. was usually present in intercellular spaces between the cells of the outer layers of the cortex and have occasionally they were found in contact with cells of the inner layer of the root cortex, which reacted necrotically and collapsed.

Additionally, the inner cortex and vascular cylinder of these plants were colonized by hyphae of endophytic fungi, and some of the hyphae appeared degraded. This raises the question of whether *H. maritima* n. sp. is really a specialized plant pathogen, or if it can feed on both plants and fungi. Plant-parasitic nematodes are biotrophs, which withdraw their food in liquids form only from living cells, and are not able to ingest coagulated protoplast through their stylet (Sijmons et al., 1994). Structures commonly related to plant-parasitic feeding, such as stylet punctures, feeding tubes, zones of modified cytoplasm, or feeding plugs — described, e.g., by Wyss et al. (1979), Sijmons et al. (1994) or Zunke (1990) — were not found in our material. It could have been a matter of chance, or it could indicate, in a very simple and primitive way, that *H. maritima* n. sp. parasitism is restricted to stylet insertion and the quick ingestion of cytoplasm, followed by cell death. This will be a subject of future research.

In summary, our studies have shown that *H. maritima* n. sp. does not cause harmful symptoms in host plants and therefore there is rather no need to subject it to legal restrictions. Thus, the spread of this nematode, either naturally together with the host plant or through breeding activities (water ponds, aquariums), does not have to be monitored by phytosanitary services. Farther and more detailed examinations, however, should be continued.

Acknowledgements

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