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On morphology and habitat of *Longidorus andalusicus* Gutiérrez-Gutiérrez et al., 2013 (Nematoda: Longidoridae) recovered in the North Frisian wadden sea island Hooge, Germany

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Abstract

A *Longidorus* population, recovered in the small island Hooge close to the coast of the German Bight in 1993 for the first time, had initially been identified as a representative of a still undescribed species. Morphological studies and molecular analyses now revealed species identity with *Longidorus andalusicus* recently described from southern Spain. The Hooge population occurred in the rhizosphere of *Halimione portulacoides* and other halophilic plants at a site often inundated by sea water, together with brackish water and marine nematodes. Morphological characters supplementing the original description of *L. andalusicus* are presented and an emended species diagnosis is proposed. The potential origin of the species and the means of dispersal to northern Europe are discussed.

Keywords Biogeography | dispersal | *Halimione portulacoides* | saline soil | North Sea

1. Introduction

Soil samples collected by the author in June 1993 at several sites of Hallig Hooge, a small offshore island in the wadden sea at the west coast of Schleswig-Holstein, Germany, unexpectedly revealed the presence of a species of the terrestrial dorylaim genus *Longidorus* in the rhizosphere of halophilic plants in a saline biotope. Further samplings in June 1999, October 2001, June and September 2003, September 2007 and May 2009 confirmed presence of this nematode at the same site; in some of the soil samples high population densities of this nematode species were encountered. The *Longidorus* species could not be attributed to any known species of the genus and was thus considered as being new to science and new to the nematode fauna of Germany.

When I finally worked on preparing the description of the new species, three new *Longidorus* species were described from southern Spain, among others *L. andalusicus* Gutiérrez-Gutiérrez, Cantalapiedra-Navarrete, Montes-Borrego, Palomares-Rius & Castillo, 2013. In its morphological characteristics this species closely agrees with the *Longidorus* population recovered in the North Sea island Hooge. Molecular data obtained for the Hooge population, which were deposited in the GenBank with the accession number of the D23 of 28S rRNA gene sequence under KF242336, and comparison with the molecular characters of the two populations of *L. andalusicus* from Andalusia confirmed species identity (Subbotin, pers. comm.).

The geographically remote recovery of *L. andalusicus* in northern Europe, the habitat 'unusual' for a *Longidorus* species and apparently different from that at the two collection sites in southern Spain, and morphological studies supplementing the original species description and increasing our knowledge of the intraspecific variation, appear to justify the present publication. Moreover, the recent finding is stimulating a discussion on nematode dispersion and possible means of dispersal.

The genus *Longidorus* Micoletzky, 1922 is among the best studied genera of soil-inhabiting, plant-parasitic nematodes. Main morphological characteristics are a

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long needle-like mouth stylet used for sucking on root cells and body lengths attaining up to 12 mm. Members of this genus can cause direct damage to a wide variety of cultivated plants, and several species are of particular economic significance as vectors of plant viruses (Hunt 1993, Taylor & Brown 1997). A correct identification of the species involved is generally required, in particular of species, for which phytosanitary regulations exist. More than 150 nominal *Longidorus* species are known worldwide and more than 70 species have been recorded for Europe so far, with most of the species known from the southern European region.

2. Materials and methods

Soil samples were taken from the rhizosphere of *Halimione portulacoides* (L.) and a few other halophilic plants in the island Hooge. Nematodes were isolated from the soil in the laboratory of the Biologische Bundesanstalt, Institut für Nematologie und Wirbeltierkunde (now: Julius Kühn-Institut) in Münster, mostly by using the centrifugation-flotation technique with $MgSO_4$, which is commonly applied for soil-inhabiting nematodes, but in part also by a sieving-decanting method with final extraction of the nematodes using a Baermann funnel equipped with cotton filter. The nematode suspensions obtained were fixed with hot TAF (triethanolamine-aqua dest.-formaline) or 4% formaldehyde and subsequently processed to dehydrated glycerine by a slow evaporation method. Selected nematode specimens were mounted on permanent microscopical slides for morphological studies. A few viable *Longidorus* specimens were hand-picked from the nematode suspensions in 2001 for subsequent molecular studies. Fixed nematode material and microscopical slides of *L. andalusicus* are deposited in the German Nematode Collection (DNST) at Julius Kühn-Institut, Münster, Germany.

3. Morphology

Morphometrics of females, males and the four juvenile stages from the Hooge population are given in Tables 1 and 2. The original text description of *L. andalusicus* by Gutiérrez-Gutiérrez et al. (2013) is detailed. Some morphological data supplementing this description are given below, including characters slightly differing from those of the Spanish populations.

Adults: Body C-shaped to almost circular when relaxed, posterior end of males more strongly curved to ventral side and often even hook-like. Cuticle at mid-body 2.5–3.5 µm thick, only slightly reinforced towards lip region and strongly thickened at posterior end (8–10 µm at tail terminus); inner cuticle layer markedly thinner than outer layers along body, at tail end inner (striated) layer thicker than outer layers (Fig. 1D, E). Along each body side numerous lateral pores present, 6–11 pores in region of pharynx with the first pore within range of guiding ring and 3 within range of odontostyle. Dorsal and ventral pores usually less distinct; 1–4 ventral pores and 1–2 dorsal pores observed within range of the anterior pharynx portion. Tail in both sexes with 2–3 pores in sublateral position. Lip region mostly slightly offset by a weak depression (as figured in the original description) with diameter slightly less than half body diameter at level of guiding ring (Fig. 1A, B). Amphidial lobes generally extending to 4/5 the distance from anterior end to guiding ring, rarely up to the guiding ring and lobes only exceptionally being slightly asymmetrical (Fig. 1C). Slender part of pharynx usually with a mucro of 1.5–6 µm length in the pharyngeal tissue, in variable position posterior to stylet base. Pharyngeal bulb measuring about ¼ of the total pharynx length; nucleolus of the dorsal gland nucleus mostly slightly smaller than the nucleoli of the subventral pharyngeal gland nuclei.

Females: Vagina extending over more than half body diameter at vulva. Both uteri in most females filled with roundish sperms. Eggs in uteri measured 165–215 µm in length and 52–55 µm in diameter. One female found with three eggs in each uterus. Females with developing eggs were found in samples taken in May and June.

Males: Spicules thicker and more robust (11–12 µm maximum width) than figured in the original description of *L. andalusicus* (Fig. 1F); lateral guiding pieces 15–17.5 µm long. Supplements 14–17, equally developed and arranged at almost even distances; the most posterior 5–7 supplements usually more or less in an irregular double row and in a ventro-sublateral position; 5–6 of the supplements situated within range of the spicules.

Juveniles: Body of heat-relaxed J1 specimens almost straight to slightly C-shaped, the next juvenile stages becoming increasingly more C-shaped.

Emended diagnosis of *L. andalusicus*: Body 3.4–5.5 mm long, assuming a circular or closed C-shape, not very slender (a = 61–97); body tapering to the anterior end, lip region rounded, 10–15 µm in diameter, continuous with body contour (often slightly offset by a weak depression); amphidial pouches symmetrically bilobed at base and mostly extending to 4/5 of the distance from anterior end to guiding ring; guiding ring 23–33 µm from oral opening which corresponds to more than two lip region diameter; odontostyle 64–86 µm long; pharyngeal bulb measuring about ¼ of the total

Table 1. Morphometrics of females and males of *Longidorus andalusicus* (measurements in µm, except for L).

Population	**Hooge, Germany (original)**		**Type locality, Spain (Gutiérrez-Gutiérrez et al. 2013)**		**San Fernando, Spain (Gutiérrez-Gutiérrez et al. 2013)**	
Character	Females	Males	Females	Males	Females	Males
n	18	10	15	10	8	5
L (mm)	4.89 (4.20–5.51)	4.73 (4.12–5.50)	4.39 (3.38–4.99)	4.24 (3.40–4.77)	4.38 (3.76–5.10)	3.77 (3.42–4.05)
a	73.6 (66–83)	83.1 (71–97)	75.3 (60.7–86.6)	76.0 (64.2–83.8)	78.1 (69.9–95.0)	76.3 (68.5–87.9)
b	12.7 (9.9–15.3)	11.8 (9.8–13.4)	12.4 (9.4–20.0)	12.3 (9.0–21.1)	13.5 (11.2–16.2)	11.0 (9.6–12.6)
c	148 (132–162)	136 (104–162)	141.2 (112.6–177.6)	143.9 (113.3–194.1)	141.5 (121.0–164.8)	119.6 (110.4–134.8)
c’	0.77 (0.7–0.8)	0.87 (0.7–1.0)	0.8 (0.7–1.0)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.9 (0.82–0.97)
Odontostyle length	78.2 (71–84)	79.5 (75–83)	74.0 (67.0–80.0)	72.4 (64.0–80.0)	82.5 (78.0–86.0)	77.3 (72.0–86.0)
Lip region diameter	13.7 (12.2–14.7)	12.9 (13.1–15.0)	10.9 (10.0–12.0)	11.0 (10.5–12.0)	11.7 (10.5–12.5)	11.8 (11.5–12.0)
Oral aperture-guiding ring	29.2 (27–31)	31.3 (30–33)	28.5 (26–31.5)	29.1 (26–32)	25.8 (25–27)	26.1 (23–28)
Tail length	32.8 (29–36)	35.5 (31–40)	31.2 (22.5–36.0)	29.9 (22.0–36.0)	31.3 (25.0–38.0)	31.6 (30.0–34.0)
V	51.3 (48.5–53.5)	- -	49.5 (46.5–51.5)	- -	47.9 (44–50)	- -
Spicule length	- -	55.0 (50–58)	- -	61.6 (55–68)	- -	59.6 (56–65)

Table 2. Morphometrics of the four juvenile stages of *Longidorus andalusicus* from Hooge island (measurements in µm, except for L).

Stage	J1	J2	J3	J4
n	12	11	15	13
Character				
L (mm)	1.28 (1.10–1.50)	1.91 (1.47–2.30)	2.51 (2.21–3.05)	3.33 (2.41–4.10)
a	46.1 (42–49)	53.5 (47–65)	59.6 (57–67)	66.1 (57–75)
b	4.6 (4.5–6.6)	5.4 (6.7–9.0)	7.9 (6.7–10.0)	9.1 (6.5–11.1)
c	49 (44–57)	56 (51–59)	82 (72–98)	98 (73–117)
c’	1.3 (1.1–1.4)	1.2 (1.1–1.3)	0.95 (0.7–1.2)	0.9 (0.8–1.0)
Odontostyle length	44.4 (40–48)	51.5 (49–58)	59.1 (55–64)	68.6 (68–72)
Replacement odontostyle	50.3 (47–53)	60.3 (54–66)	68.5 (64–76)	82.7 (74–100)
Oral aperture–guiding ring	16.5 (14–18)	20.1 (19.5–21.5)	22.2 (20–25)	26.8 (25–28)
Tail length	28 (25–31)	28.5 (25–33)	30 (27–33)	33 (30–38)

pharynx length, with subventral nuclei usually slightly posterior to middle of bulb and dorsal nucleus less than one bulb diameter anterior, nucleoli of almost the same diameter (dorsal gland nucleolus mostly slightly smaller); tail of females hemispherical to bluntly conoid, with c' = 0.7–1.0; males common, spicules 50–68 µm long, 14–17 supplements present; all four juvenile stages with broadly rounded tail terminus.

The alphanumerical code proposed by Chen et al. (1997) for the identification of *Longidorus* species is slightly changed from that presented for *L. andalusicus* by Gutiérrez-Gutiérrez et al. (2013) to: A23 - B12 - C23 - D12 - E2 - F23 - G12 - H1(2) - I2.

4. Locality and habitat

Longidorus andalusicus was found in Hallig Hooge, an offshore marsh island at the eastern edge of the German Bight, Schleswig-Holstein, Germany. The 5.78 km² small North Frisian Island is unprotected by dikes and regularly inundated by sea water in hibernal floodings (except the settlements built on man-made hills). The collection site is a small area with halophilic vegetation at the northern side of the island, at the north-western edge of a small lagoon connected to the small island harbour, where insular surface water is continuously mixing with sea water (54°34'28.8''N, 8°32'5.2''E).

In several soil samples collected, *L. andalusicus* was exclusively found in samples from the rhizosphere of sea purslane (*Halimione portulacoides*), with highest population densities of >100 specimens per ca. 250 ml of soil from pure stands of this obligatory halophyte plant and almost regularly flooded at high tide. The nematode was less common in samples a few metres apart, with other maritime plants such as *Suaeda maritima* (L.), *Plantago maritima* L., *Spergularia marina* (L.), *Limonium vulgare* Mill., *Triglochin maritimum* L., *Puccinellia maritima* (Huds.), *Armeria maritima* (Mill.), *Salicornia europaea* L.; it was absent at more sandy sites also very close but not inundated with tides. The soil was wet and muddy and containing many fragments of shells. Sampling at the pure *H. portulacoides* stands growing along a small tidal creek was mostly possible only at low tide. *Longidorus andalusicus* could not be encountered from samples taken at other sites of the island, including two salt marsh sites with *H. portulacoides*. Only *L. elongatus* (de Man, 1876), the most common and widely occurring *Longidorus* species in Germany, was found at dryer grassy sites in the island.

In the samples containing *L. andalusicus* various brackish water or marine nematodes were found, such as *Adoncholaimus* sp., *Eurystomina terricola* (de Man, 1907), *Haliplectus* sp., *Deontolaimus papillatus* de Man, 1880, *Sphaerolaimus* sp., various Desmoscolecida, *Calyptronema maxweberi* (de Man, 1922) and *Enoploides* sp. Also several plant-parasitic Tylenchida species known from saline or brackish habitats were present: *Hemicycliophora thornei* Goodey, 1963, *Pratylenchus pratensis* (de Man, 1880), *Tylenchorhynchus striatus* Allen, 1955 (first and only record of this species for Germany), *Nagelus* sp. (an obviously still undescribed species of the genus) and *Sphaeronema* sp., a species new to science, living as semi-endoparasite on the roots of *H. portulacoides* and included in previous morphological studies on phasmids in Tylenchulidae (Sturhan & Geraert 2005).

5. Discussion

Measurements and ratios of the adults are close to the data published for the Spanish populations and are generally extending the known range of the intraspecific variation of *L. andalusicus* adults and the four juvenile stages. Deviations in measurements of the lip region diameter may be a result of slight differences in determining the length of the lip region in the conoid anterior end. Differences in spicule length may be due to the fact that the chord has been measured in the German population. Differences in morphometrics are more obvious in the juvenile stages, which are based on a total of 51 specimens from the Hooge population, whereas only 12 specimens from Andalusia were measured.

In most general morphological characters the Hooge population closely agrees with the original description of *L. andalusicus.* But the slender part of the pharynx is generally not outstretched, when the stylet is in a 'resting' position (likewise in the type specimens re-examined), the spicules are significantly thicker than shown in Fig. 6G of the original description and there appear to be more supplements within the range of the spicules than is evident from the original description by Gutiérrez-Gutiérrez et al. (2013). Two paratype males re-examined had thicker spicules, with 12–13 µm maximum diameter; the posterior supplements were in a staggered line with the last two arranged as a pair, and 5 and 6 supplements were present within range of the spicules. A mucro in the pharyngeal tissue was observed also in the paratype specimens re-examined.

The comparative morphological and molecular data obtained for the Andalusian populations and the Hooge population of *L. andalusicus* are considered as another good example of congruence between both methods applied. General morphological characteristics are certainly more reliable for correct species identification than morphometrics, which apparently show a wider range of variation depending on the ecological conditions of the habitat and the geographical origin. The value and significance of calculating standard deviation of means of morphometrics of several individual populations and the justification of determining such statistical data in cases, where only a few specimens are available, should generally be critically checked. Data obtained from populations of different geographical origin and from different habitats will generally provide more reliable data for a correct identification of a species. Minor differences in measurements, which are currently often used for the distinction of species, should be used with caution only. The diagnosis of a species should not be confined to data of the type population, but include also characters of other reliably identified populations or specimens studied. An impressive example confirming the potential huge influence of environmental factors on the morphometric variability of a single nematode species has been documented by a recent elaborate study of Fonderie et al. (2013).

The identification of *L. andalusicus* from the German coast has increased the number of identified *Longidorus* species known from Germany to a total of 21 species (Sturhan 2012). According to observations by the author based on sampling throughout Germany, several more new and still unidentified species are present; moreover, the species status of some species described from Germany has still to be evaluated. *Longidorus andalusicus* is morphologically well distinguished from

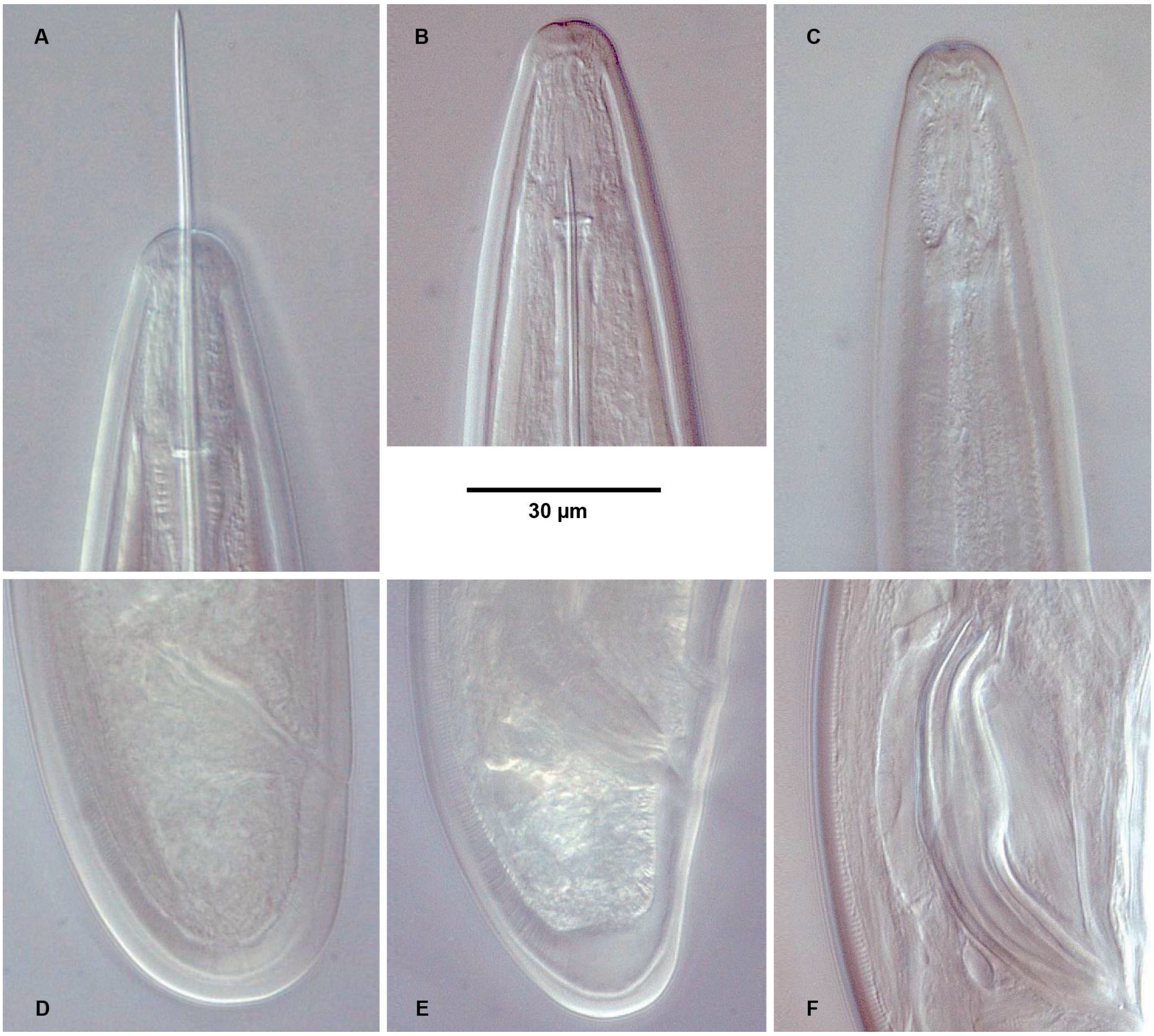

Figure 1. *Longidorus andalusicus* from Hooge, Germany. (**A**) Head end with projected odontostyle (male), (**B**) Head end showing guiding ring (female), (**C**) Head end in lateral position showing lobed amphidial pouch (male), (**D**) Female tail, (**E**) Male tail, (**F**) Spicule.

all other *Longidorus* species known from Germany and adjoining central and northern European countries (see emended diagnosis above).

The recovery of a *Longidorus* species in a more or less marine biotope appeared quite 'unusual' for this genus and may even be unique. There is not much information about the exact origin and the habitat of the two Andalusian populations studied, which are reported as 'fallow soil samples' for the type population from Sanlúcar de Barrameda and 'purple glasswort' for the second population studied from Cádiz province (Gutiérrez-Gutiérrez et al. 2013). The occurrence of a halophyte plant (*Salicornia ramosissima* Woods) at the second collection site indicates a high salinity of the soil. *Halimione portulacoides* and *Salicornia* spp. are both members of the family Chenopodiaceae. Castillo (pers. comm.) confirmed origin of the Andalusian populations from saline habitats near the coast.

The remote occurrence of *L. andalusicus* in the Cádiz province in southern Spain and in northern Europe raises questions about the origin and the potential distribution area of this nematode species, as well as on the mode of dispersal. In this context an aspect not considered or discussed in various previous papers on distribution patterns of longidorids in Europe (Navas et al. 1990, Navas et al. 1993) is that there have been several glaciations within the past rather short geological period, which made the environmental conditions in northern and central Europe unfavourable for the existence of many animals and plants. Most likely the majority of nematode taxa currently known to occur in the northern parts of Europe could not have existed in this region during the glacial or postglacial periods, which were connected with fundamental changes in climatic conditions, in vegetation type etc. Only about 10.000 years ago the climate in the northern parts of Europe returned to a status similar to the present situation. Few authors only considered such aspects: Topham & Alphey (1985) related the distribution of longidorid species to geological changes and indicated the influence of quaternary glaciations, and McNamara & Flegg (1981) discussed the significance of the most recent glaciation on distribution of longidorids (in Britain), their origin, re-colonisation and potential means of spread northwards.

Most of the nematodes must have invaded and/or re-colonised central and northern Europe since the last ice age, in particular, species which require higher temperatures for permanent establishment. The dispersal mechanisms are largely unknown, but besides various natural means (active migration, accidental transport by running surface waters, wind, migrating animals etc.), the present distribution of many nematodes has certainly been much influenced by man. The *L. andalusicus* occurrence in the coastal region of northern Germany, at an obviously very restricted site and in a 'peculiar' biotope, could be due to an incidental phoresis by migrating birds of the coast. But more likely, the nematodes have been introduced to the North Sea island Hooge (or first to adjoining harbours at the coast) with cargo transported by ships and contaminated with soil or by ballast water or soil, which had commonly been used in the past for stabilisation of ships and was often discharged wherever cargo was loaded or unloaded. Both collection sites in the Cádiz province of Andalusia are located close to the coast.

The molecular analyses by Subbotin (pers. comm. and Subbotin et al., in press) and by Gutiérrez-Gutiérrez et al. (2013) indicate that *L. orientalis* Loof, 1983 appears to be the *Longidorus* species most close to *L. andalusicus*. This species is known so far from Saudi Arabia, Iraq, Iran and southern Spain (Loof 1983, Palomares-Rius et al. 2010). It is thus likely that *L. orientalis*, *L. andalusicus* and possibly other related species (in particular, *L. oleae* Gutiérrez-Gutiérrez et al., 2013, also described from Andalusia) have their centre of origin in the Mediterranean-Oriental region. The presence of *L. andalusicus* in northern Germany is possibly the result of a single quite recent introduction and further recoveries of this species in adjacent countries cannot necessarily be expected. Most probably also other nematodes, which were originally described from northern Europe and have their type locality situated within this area, did not originate in this region. It appears unlikely that new nematode species evolved in central or northern Europe since the end of the last glacial period.

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