



New records of plant-parasitic nematodes from Iceland

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Abstract

The plant-parasitic nematodes of Iceland are poorly understood. To address this, a study of the nematodes of the families Criconematidae and Hemicycliophoridae was performed in 2015. Soil samples were taken from underneath various host plants in different locations in Iceland. The identification was performed on the basis of the general morphology and subsequently confirmed by molecular markers (D2-D3 28S rDNA). The study revealed the presence of nematode specimens belonging to four species of the family Criconematidae: *Criconemoides amorphus*, *Criconema demani*, *Mesocriconema xenoplax* and *Mesocriconema curvatum*, as well as one species *Hemicycliophora conida* of the family Hemicycliophoridae. To our knowledge, this is the first record of the occurrence of these nematode species in Iceland. The species identified are economically important plant-parasitic nematodes of likely interest to—among others—Icelandic plant protection professionals. This report broadens our knowledge of Iceland's nematode biodiversity; moreover, morphological analyses and molecular data may contribute to better understanding the origin of nematode species on the island of Iceland.

Keywords New geographic record · *C. amorphous* · *C. demani* · *M. curvatum* · *M. xenoplax* · *H. conida* · Iceland

Introduction

Iceland is a Nordic island country which consists of several small islands and a larger one—the main island of Iceland. It is located in the North Atlantic Ocean at its confluence with the Greenland Sea (part of the Arctic Ocean). Although Iceland forms part of the Arctic region, the country's landscape chiefly reflects its volcanic origins and is significantly human altered, with large unvegetated or sparsely vegetated areas. The vegetated areas account for 44% of the total area of Iceland. 2.3% of Iceland's total area is covered by grasslands which largely replaced the natural birch woodland as a result of human activity pursued since the Viking settlement in the ninth century (Aradóttir et al. 2013; Sigurmundsson

et al. 2014). The importance of grasslands for Iceland's farming remains undisputed with permanent grass fields representing the vast majority (around 90%) of the country's cultivated areas (Helgadóttir et al. 2013). Iceland's wildlife is characterized by a relatively low number of endemic plants and animals (Buckland and Dugmore 1991). Up to date there are 490 species of vascular plants known from this island (Svanberg and Ægisson 2012). Most (if not all) of these species could probably be the food source and form host plants to plant-parasitic nematodes. Currently, there are about 4000 plant-parasitic nematodes known from the world, which is approximately 15% of known phylum species (Decraemer and Hunt 2013). Many of them are of significant economic importance as major pests of cultivated plants (e.g., Singh et al. 2013). Nevertheless, only several species of plant-parasitic nematodes have been recorded from Iceland so far, as for example *Globodera rostochiensis*, *Meloidogyne arenaria*, *Anguina radicola* and *Ditylenchus dipsaci* (Siggeirsson and Riel 1975). One of the economically important plant-parasitic nematode groups are species belonging to the families Criconematidae and Hemicycliophoridae. They inhabit terrestrial soil and are obligatory root-feeding ectoparasites of numerous plant species (Siddiqi 2000; Andrassy 2007). Representatives of Criconematidae are known from all continents (Geraert 2010; Ryss et al.

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2014) and Hemicycliophoridae are found worldwide on all continents except the Antarctic (Andrássy 2007; Subbotin et al. 2014).

To the best of our knowledge, representatives of Hemicycliophoridae have never been recorded from Iceland. As for Criconematidae, the only record of this family comes from the island of Surtsey, which is located off the southern coast of Iceland (Ilieva-Makulec et al. 2015). In this study, however, specimens were identified only down to the family level. Here we present new geographic records on five species of the families Criconematidae and Hemicycliophoridae from Iceland, data on their morphometrics and D2–D3 28S rDNA molecular markers.

Materials and methods

38 soil samples from the root zones of various host plants were collected in 2015. The samples were obtained from different geographical locations, both in the south and north of Iceland. Nematodes were extracted from the soil by the centrifugal flotation method (Van Bezooijen 2006) and observed alive under the dissecting microscope. Out of each sample 1–5 *Criconemoides amorphus*, *Criconema demani*, *Mesocriconema curvatum*, *Mesocriconema xenoplax* and *Hemicycliophora conida* specimens were preserved in DESS for the purposes of molecular studies (Yoder et al. 2006). Remaining specimens were gently heat killed and preserved in TAF solution and submitted for morphological analysis (Courtney et al. 1955). From TAF, nematodes were transferred to glycerine (Seinhorst 1959) and subsequently mounted on microscopic slides. Morphological observations, measurements and photographs were obtained using a Leica light microscope with the Nomarski differential interference contrast. Single nematode individuals from DESS,

after washing in sterilized milli-Q water, were used for DNA extraction. DNA was isolated from specimens according to the nematode lysis procedure, as described by Holterman et al. (2006). The obtained single nematode lysate (crude DNA extract) was used immediately as a DNA template for a PCR reaction or stored at $-20\text{ }^{\circ}\text{C}$. PCR reactions were performed in Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Both PCR and sequencing were performed using the D2A and D3B primers (Nunn 1992). Amplicons were visualized under UV illumination after Simply Safe (EURx, Gdańsk, Poland) gel staining and gel electrophoresis. The 28S rDNA regions were sequenced by the Sanger method on ABI 3500L genetic analyser (Applied Biosystems, Foster City, CA, USA). The quality of obtained chromatograms was assessed in Chromas Lite 2.1.1 (<http://www.techneesium.com.au>). Final sequences were determined by assembling sequences obtained from opposite directions in BioEdit (Hall 1999) and deposited in GenBank.

Results and discussion

Overall, in our study, species of Criconematidae and Hemicycliophoridae families have been found only in samples from the region of South Iceland. The geographic coordinates of sampling locations are presented in Table 1. Outline maps indicating the regional setting of our study on the island of Iceland, and more broadly, in the Arctic, are given in Online Resource 1. Seven samples were found to carry nematodes of Criconematidae family, whereas in one sample nematodes of Hemicycliophoridae family were found (Table 1). Analyses of the investigated females confirmed their affiliation to four species of the family Criconematidae: *Criconema demani* Micoletzky 1925, *Criconemoides*

Table 1 Plant-parasitic nematode species found on plants in Iceland with habitat location coordinates

Nematode species	Plant species	Sample reference number	Approximate geographical coordinates/height above sea level
<i>Criconema demani</i>	<i>Vicia cracca</i> L.	I	63°50'00"N, 20°07'03"W/96 m a.s.l (all populations were in close proximity to one another)
<i>Criconemoides amorphous</i> (three populations)	<i>Lathyrus japonicus</i> Wild.	II	
	<i>Lotus corniculatus</i> L.	III	
	<i>Vicia cracca</i> L.	I	
<i>Mesocriconema curvatum</i>	<i>Lotus corniculatus</i> L.	III	
<i>Mesocriconema xenoplax</i> (five populations)	<i>Trifolium pratense</i> L.	IV	
	<i>Vicia cracca</i> L.	I	
	<i>Vicia sepium</i> L.	V	
	two populations on <i>Leymus arenarius</i> (L.) Hochst	VI, VII	63°32'38"N, 20°06'27"W/4 m a.s.l
	<i>Hemicycliophora conida</i>	<i>Vicia sepium</i> L.	VIII

amorphous De Grisse 1967, *Mesocriconema xenoplax* (Raski 1952) Loof & De Grisse 1989, *Mesocriconema curvatum* (Raski 1952) Loof & De Grisse 1989 and one species *Hemicycliophora conida* Thorne 1955 of the family Hemicycliophoridae. Morphological observations of all identified species were in line with descriptions published by other authors (Brzeski 1998; Geraert 2010; Subbotin et al. 2014). Data on morphometrics of specimens of each species are presented in Online Resource 2. Molecular characteristics using LSU (28S rDNA) were also provided. Obtained sequences D2-D3 28S rDNA of each species were deposited in GenBank under the following accession numbers: *C. demani*: MN628432, *H. conida*: MN628433, *M. xenoplax*: MN628435, *C. amorphus*: MN628431 and *M. curvatum*: MN628434. BLAST search of obtained 28S rDNA GenBank revealed the following results: sequences of *C. demani* showed a 98% similarity to the sequences of *C. demani* number MH828126 and MH828128; *M. xenoplax* a 98–99% similarity with 24 sequences of *M. xenoplax* published by different authors, for example with sequence DQ077792 (De Ley et al. 2005) and AY780963 (Subbotin et al. 2005); *M. curvatum* showed 99.7% similarity to the *M. curvatum* MN720094 (Mwamula et al. 2020); *H. conida* a 99% similarity to the sequences of *H. conida* numbers FN433875 (unpublished), KF430448 and KF430447 (Subbotin et al. 2014). Finally, BLAST search of sequences from *C. amorphus* revealed similarity to other sequences from criconematids, however, not an important one.

The identification of five plant-parasitic nematode species, from the region of South Iceland, largely expands the list of species known from this island country. Species of the family Criconematidae were found on the following plants: *Vicia cracca* L., *Vicia sepium* L., *Lathyrus japonicus* Wild., *Leymus arenarius* (L.) Hochst., *Lotus corniculatus* L., *Trifolium pratense* L. and the family Hemicycliophoridae, a population of the single species, was found in the rhizosphere of *V. sepium* L. (Table 1). Additionally, at least some of the investigated species are of known economic importance as plant pests and could potentially cause crop yield losses in Iceland's farming sector. Below we present a short outline of each species' biology, its known geographic range and economic impact.

Criconema demani (Fig. 1a–c) was reported from many countries in Europe, including the European part of Russia (Bongers 1988; Escuer and Bello 1994; Brzeski 1998; Bert et al. 2003; Tabolin 2017), also from Korea (Choi and Geraert 1975), as well North (Bernard 1980; Luna-Guerrero et al. 2011) and South America (Crozzoli and Lamberti 2002). It is found mainly in agricultural crops (Bernard 1980; Crozzoli 2002). This species has been found to be co-responsible for the development of Peach Tree Short Life (PTSL) disease (Luna-Guerrero et al. 2011). With regards to Iceland, we found this species in patches of uncultivated

land, i.e., in one soil sample of the basalt sand on a lava flow originating in the nearby Mount Hekla volcano, later referred to as “Hekla”.

Criconemoides amorphus (Fig. 1d–f) was first described from Belgium (Luc 1970). This species is known to occur also in the Netherlands (Bongers 1988), Spain (Castillo and Gómez-Barcina 1993), Italy (Bello et al. 1988), Russia (Tabolin 2017) and Iraq (Stephan et al. 1985). It is found in intact as well agricultural habitats (Antoniou 1981; Bello et al. 1988; Budurova et al. 1996; Mateille et al. 2011; Renco and Baležentienė 2015) and is considered a major grapevine pest (Antoniou 1981; Stephan et al. 1985; Escuer and Bello 1994; Philis 1995). In wastelands it was found to occur on herbaceous and dune plants (Escuer and Bello 1996; Talavera and Navas 2002; Mateille et al. 2011). *Criconemoides amorphus* reported from Iceland occurred in two soil samples collected from a non-agricultural vegetated habitat, under a leguminous plant community, in the basalt sand on lava flow from Hekla.

Mesocriconema xenoplax (Fig. 1g–i) is a polyphagous cosmopolitan species (Wouts 2006). There are reports of its occurrence both in farmed land and in areas not in agricultural use, on a wide range of plants (Zehr et al. 1986; Winiszewska et al. 2012, 2017; Powers et al. 2014). The species is commonly found in vineyards worldwide where it has been observed to develop more rapidly and cause greater damage to vine than other species of the genus *Mesocriconema* (Walker 1995; McKenry and Anwar 2006; Wiśniewska et al. 2013; Skwiercz et al. 2015). It is a major factor in PTSL syndrome in the United States and other parts of the world (Gomes et al. 2000; Walters et al. 2008). In our study this species was found to be the most common as it occurred in five soil samples. Its presence was found on various plants in the basalt sand on lava from Hekla and in dunes located about 300–1000 m from Iceland's southern coast.

Mesocriconema curvatum has a broad geographic distribution (Wouts 2006), covering almost all continents with reports of its occurrence from the United States (Zeng et al. 2012), Europe (Castillo and Gómez-Barcina 1993; Winiszewska et al. 2012, 2017), Asia (Choi and Geraert 1975; Mwamula et al. 2020) and Africa (Lamberti et al. 1991; De Waele et al. 1998). In general this species has agricultural associations; however, it was also found in uncultivated grasslands (Powers et al. 2014). We found only two specimens in the basalt sand on a lava flow from Hekla.

Hemicycliophora conida (Fig. 1j–l) was first described from Ireland by Thorne (1955). The species has been widely reported from European countries (Loof 1968; Brzeski 1998; Peña-Santiago et al. 2004), Iran (Loof 1984), the Americas (Subbotin et al. 2014), South Africa (Van den Berg 1981) and Svalbard, high Arctic tundra (Kerfahi et al. 2017). As an obligate ectoparasite of plants, it inhibits growth and con torts the shape of root systems in farm crops (Winiszewska

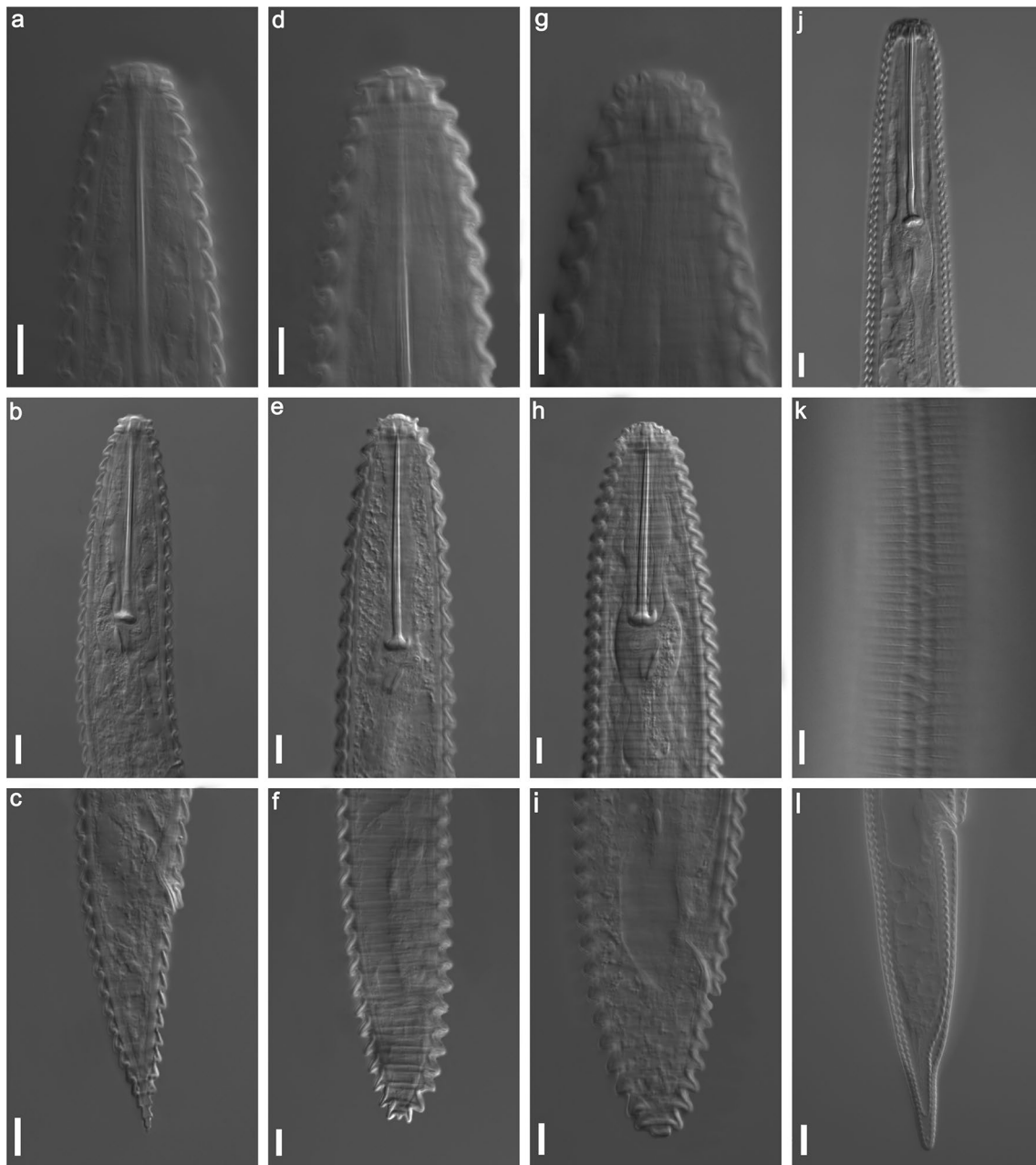


Fig. 1 *Criconema demani* Micoletzky 1925, female. **a** Shape of head; **b** anterior body region; **c** posterior body region. *Criconemoides amorphus* De Grisse 1967, female. **d** Shape of head; **e** anterior body region; **f** posterior body region. *Mesocriconema xenoplax* (Raski

1952) Loof & De Grisse 1989, female. **g** Shape of head; **h** anterior body region; **i** posterior body region. *Hemicycliophora conida* Thorne 1955, female. **j** Anterior body region; **k** lateral field; **l** posterior body region. Scale bars = 10 μ m

et al. 2012; Chitambar and Subbotin 2014; Van Den Berg et al. 2018). This species has been found to occur in Iceland in one location only in the basalt sand from the bank of the river Eystri-Rangá.

The study broadens our knowledge of the distribution of the species *C. amorphus*, *C. demani*, *M. xenoplax*, *M. curvatum* and *H. conida*. Aside from the potential threat to agriculture, the occurrence of plant-parasitic nematodes in

Iceland can become an interesting field of biogeographic research. For example it is not clear how the plant-parasitic nematodes survived the Pleistocene glaciations. Currently, there are two major concepts of species origin on the island of Iceland. The first one, “tabula rasa”, indicates that all species on the island arrived there after the end of the glaciation period. The second one, “glacial refugia”, assumes that species were able to survive the glacial period on the

island within refugias. In case of plant-parasitic nematodes, the survival of their hosts, the vascular plants, is probable (Rundgren and Ingólfsson 1999). That could enable the survival of the nematodes. It does not necessarily follow, however, that all plant-parasitic nematodes occurring in Iceland have survived the glaciation in refugias. Some nematode species might have been transferred to Iceland after the glaciation by either natural causes or human activity. To establish how those species arrived in Iceland, further studies will be necessary involving a larger number of Icelandic populations and their comparison with populations from mainland Europe and North America.

Conclusion

This report broadens our knowledge of nematode biodiversity of Iceland as well of the morphometry and molecular characteristics of the five economically important species *C. amorphus*, *C. demani*, *M. xenoplax*, *M. curvatum* and *H. conida*. The study yielded molecular data which may contribute to the further development of the concepts of species origin of nematodes on the island of Iceland. Our observations could draw the attention of, among others, Icelandic agricultural practitioners and plant protection experts to the interaction between the cropping system and the populations of nematodes newly investigated in Iceland. To our knowledge this is the first report of five species of the families Criconeematidae and Hemicyclophoridae from Iceland.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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