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Morphological and molecular identification of potato and cereal cyst nematode isolates from Algeria and their phylogenetic relationships with other populations from distant theirgeographical areas

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Abstract A nematode survey conducted in 2013 in Algeria, revealed that potato cyst nematodes (PCN) and cereal cyst nematodes (CCN) are widely distributed in several potato and cereal growing regions of the country. Sixteen PCN populations from five localities and five CCN populations from four of these localities were collected and characterized at the morphological and molecular levels. The PCN populations were identified as *Globodera rostochiensis* and *G. pallida* occurring separately or in mixed populations. Two species of CCN were detected. *Heterodera avenae* was found in four localities, whereas *H. hordecalis* only in one locality in association with *H. avenae*. The morphological and morphometric identification of PCN and CCN was confirmed by diagnostic ITS-RFLP profiles and sequencing. Phylogenetic analysis of the ITS, D2-D3 expansion domains of the 28S rRNA gene and 18S rRNA gene was made for PCN and CCN populations. *Globodera pallida* and *G. rostochiensis* from Algeria show great similarity with European and South American populations. Because of the high divergence among Algerian populations of *G. pallida* and *G. rostochiensis* it can be assumed that they were multi-introduced in Algeria. The most divergent population of *G. pallida*, that formed a well-separated group with some populations from Chile and Peru, suggests a later or independent introduction of this population into Algeria. *Heterodera avenae* and *H. hordecalis* formed a well-supported cluster with the corresponding populations.

Introduction

Cyst nematodes, *Heterodera* spp. and *Globodera* spp. are important obligate parasites and an economically relevant group of harmful plant parasitic nematodes which are found world-wide (Subbotin et al. 2010a, b). In particular, the potato cyst nematodes (PCN) *G. pallida* Stone, 1973 and *G. rostochiensis* (Wollenweber 1923) Skarbilovich, 1959 are a serious threat for the potato industry in many countries (Scurrah et al. 2005) and subject to strict quarantine regulations (Hafez et al. 2008). They are mainly associated with potato production essentially in temperate areas (Mugnièry 1984), but they occur also in the Mediterranean countries were host plants are grown from mid-autumn to spring. The origin of the two

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species is in the Andean mountains of South America from where they were spread all over the world, possibly together with potato tubers (Plantard et al. 2008). In North Africa, PCN have been reported in Algeria, Egypt, Libya, Tunisia and Morocco (Schlùter 1976; Mai 1977; Hlaoua et al. 2008). In Algeria, PCN have been recorded for the first time in 1953 following the introduction of potato seeds of British origin at the end of the Second World War (Frezal 1954). By 1961, the infested area had increased greatly in 33 townships around Algiers (Scotto La Massese 1961). Thereafter, the plant protection service reported these nematodes in several potato producing regions, the most important of which are Ain Defla, Tipaza, Chlef, Mascara and Sétif.

Cereal cyst nematodes (CCN) are considered as major pest of cereals throughout the world (McDonald and Nicol 2005). The main species parasitizing cultivated cereals are *Heterodera avenae* Wollenweber, 1924, *H. filipjevi* Madzhidov, 1981, *H. hordecalis* Andersson, 1975 and *H. latipons* Franklin, 1969. *Heterodera avenae* is the most damaging pest that limits cereals production in the major cereal-producing regions of the world including North Africa (Rammah 1994; McDonald and Nicol 2005; Namouchi-Kachouri and B'Chir 2005; Mokrini et al. 2009). In Algeria, *H. avenae* was first reported by Scotto La Massese in 1962 and by Lamberti et al. (1975). More recently, this species was recorded in different cereal-producing regions of Algeria (Mokabli et al. 2001; Mokabli 2002; Haddadi et al. 2013).

Heterodera filipjevi occurs in Mediterranean and European countries (Subbotin et al. 2003; Holgado et al. 2004). *Heterodera latipons* appears as a typical nematode of the Mediterranean region but occurs also in several European countries and elsewhere (Subbotin et al. 2010b). Mokabli (2002) reported this nematode also in Algeria. *Heterodera hordecalis* is spread less than *H. avenae* (Subbotin et al. 2010b) and has never been found in Algeria.

In Algeria, precise information on the species of nematodes affecting potatoes and cereals, their relative distribution and population densities, their association with different crops and potential damage in different environmental conditions is needed. This information is instrumental to suggest appropriate integrated management of these nematodes.

The PCN and CCN species can be distinguished by morphological characters, but sometimes, these characters overlap in different populations of these species (Baldwin and Mundo-Ocampo 1991; Subbotin et al. 2000; Manduric et al. 2004; Subbotin et al. 2010a, b). Thus, the morphological identification is not always reliable and, therefore, it must be validated by using molecular approaches (Ibrahim et al. 2001; Abrantes et al. 2004; Subbotin et al. 2011).

Different molecular markers are used for the identification of *Globodera* and *Heterodera* species damaging potato and cereals (Abrantes et al. 2004; Thiéry and Mugniéry 1996; Subbotin et al. 2000; Grenier et al. 2001; Skantar et al. 2007; Madani et al. 2008; Quader et al. 2008; Abidou et al. 2005; Subbotin et al. 2011).

In Algeria, there is also little information on the molecular characteristics of these cyst nematodes. Therefore, the aims of this study were: (i) to investigate the occurrence and distribution of PCN and CCN species in Algeria; (ii) to provide an accurate identification of PCN and CCN by using morphological traits and molecular markers (ITS, 28 S and 18 S rDNA gene sequences); (iii) to investigate intraspecific variation between populations of *G. rostochiensis* and *G. pallida* and between *Heterodera avenae* group species; and (iv) to explore the phylogenetic relationships between potato and cereal cyst nematode populations collected in the Ain Defla region, in Algeria, and those occurring in other countries.

Material and methods

Area surveyed

The survey was conducted in the Ain Defla region (Algeria) in localities where potato was rotated with cereals. The Ain Defla region is located in the Midwest of Algeria and is characterized by a semi-arid Mediterranean climate; fine textured soils are predominant (80 %) and more than 45 % of them are represented by clay soils. In Ain Defla, the arable land is around 235,611 ha representing 51.85 % of the total area, with 731 million of kg of potatoes (DSA, Ain Defla 2013); the region ranks second in Algeria in term of potato production.

The Ain Defla region ranked twelfth in Algeria in terms of cereal production in 2013 with 187 million of kg.

Nematode sampling and extraction procedures

Soil samples were collected with a shovel or sampling tube from potato and cereal fields located in different sites in northern-central provinces of the Ain Defla region (Fig. 1). Cyst nematodes were extracted from soil samples by Fenwick's apparatus. Specimens were preserved in permanent slides for morphological analysis or heat-killed and kept dry in an Eppendorf tube.

Morphological analyses

Identification of PCN and CCN populations was based on slides of permanent mounts of the perineal and vulval cone regions of cysts and of the measurements of main diagnostic characters of second stage juveniles (J2) according to Hooper (1970) and Wouts and Sturhan (1995). Results of morphological analysis of the cysts were compared with those of the J2 contained in the same cysts. Morphometric characters used were selected according to Golden (1986). Observations were made

from live and mounted specimens using a Leitz Diaplan optical microscope equipped with differential interference contrast optics and Leica® DFC 425 camera. Measurements of specimens were made using LAS (Leica Application Suite) Version 3.6.0 software.

Statistical analysis was performed using the STATISTICA program (version 6.1, 2010). Analysis of variance (ANOVA) was applied to assess significant variation in morphological characters among the different populations of CCN and PCN. Two morphometric characters for cysts (Granex's ratio and number of cuticular ridges between vulva and anus) and one for J2 (stylet length) of PCN were subjected to hierarchical classification and principal component analysis. These three characters were selected for their important diagnostic relevance for the identification of PCN species (OEPP/OEPP 2009, 2013). The morphological characters of CCN vulval cones listed in Table 4 (except presence of bullae and dimensions of the underbridge) were subjected to the same analyses.
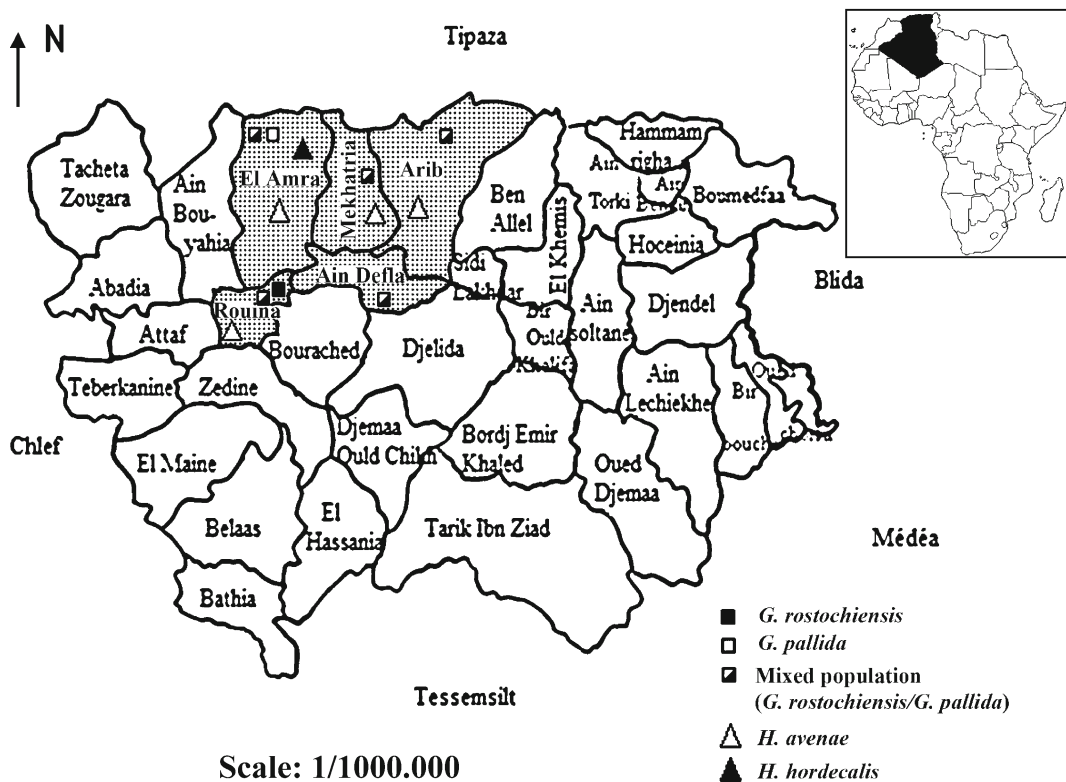

Scale: 1/1000.000

Fig. 1 Map of Algeria showing the collection sites of PCN and CCN in Ain Defla

Molecular analyses

DNA extraction, PCR amplification and sequencing

Individual cysts were crushed with a sterile micro-spatula under a stereo-microscope and the second stage juveniles were collected. Genomic DNA was extracted from fifteen individual nematodes as described by De Luca et al. (2004). The crude DNA isolated from each individual nematode was directly amplified. The ITS1–5.8S-ITS2 regions were amplified using the forward primer TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and the reverse primer AB28 (5′-ATATGCTTAAGT-TCAGCGGGT −3′) (Joyce et al. 1994); the 18S rDNA was amplified using the 18SnF (5′-TGGATAACTGTGGTAATTCTAGAGC-3′) and 18SnR (5′-TTACGACTTTTGCCCGGTTC-3′); the D2A-D3B expansion segments of 28S rRNA gene using the primers D2A (5′-ACAAGTACCGTGGG-GAAAGTTG-3′) and the D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Nunn 1992). PCR cycling conditions used for all amplification reactions were: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 50 s, annealing at 55 °C for 50 s and extension at 72 °C for 1 min and a final step at 72 °C for 7 min. The size of the amplification products was determined by comparison with the molecular weight marker ladder 100 (Fermentas, St. Leon-Rot, Germany) following electrophoresis of 10 µl on a 1 % aga-rose gel. PCR products of the ITS containing region, the 18S rRNA gene and the D2-D3 expan-sion domains from three individual nematodes were purified using the protocol suggested by the manufac-turer (High Pure PCR elution kit, Roche, Germany). Purified DNA fragments were cloned (pGEM-T Easy Vector System II, Promega) and sent for sequencing, in both directions, at MWG-Eurofin in Germany.

Phylogenetic analysis

A BLAST (Basic Local Alignment Search Tool) search at NCBI (National Center for Biotechnology Information) was performed in order to confirm their nematode origins and species (Altschul et al. 1997). The newly obtained sequences for ITS containing region, the partial 18S gene and the D2-D3 expansion domains of 28S gene were aligned using ClustalW with default parameters with the corresponding published gene sequences of *Globodera* and *Heterodera* species. Sequence alignments were manually edited using BioEdit in order to improve the multi-alignment (Hall 1999). Outgroup taxa for each dataset were chosen according to the results of previously pub-lished data (Skantar et al. 2007; De Luca et al. 2013). Phylogenetic trees, obtained for ITS dataset, the partial 18S gene and the D2-D3 expansion domains were performed with Neighbour-Joining (NJ), Minimum Evolution (ME), Maximum Likelihood (ML) and Maximum Parsimony (MP) methods using MEGA version 6 software (Tamura et al. 2013). No significant conflict in branching order and support level among methods was observed and, therefore, only the ML tree is shown for each marker. The phylograms were bootstrapped 1000 times to assess the degree of support for the phylogenetic branching indicated by the optimal tree for each method. The newly obtained sequences were submitted to GenBank with the following accession numbers and shown in Tables 1 and 2.

RFLP analysis

Ten µl of the PCR product obtained using ITS primers for *G. rostochiensis* and *G. pallida* were digested with the following restriction enzymes: *Alu* I (Roche) and *Rsa* I (Roche) (5 U of enzyme for each digestion) at 37 °C overnight. For *Heterodera avenae* and *H. hordecalis* the five restriction enzymes: *Alu* I, *Hae* III, *Hinf* I, *Pst* I and *Rsa* I were used. The digested DNA fragments were loaded onto 2.5 % agarose gel and visualized by GelRed (Biotium) fluorescent stain. All gel images were stored digitally.

Results

Nematode sampling

Sixteen populations of PCN were found, mainly occur-ring in five localities (Table 1, Fig. 1). Five populations of CCN were also found in the same localities (except in Ain Defla) (Table 2, Fig. 1).

Morphological analysis

Measurements of cysts and J2 s for PCN and CCN are reported in Tables 3 and 4.

Table 1 Codes and origin of potato cyst nematode populations used in this study and crop systems adopted in the sampled fields

Population code	Species	Origin	Host plant/ variety at sampling	Previous crop	GenBank accession number		
					ITS rDNA	D2-D3 28S rDNA	18S rDNA
ADG1	*Globodera pallida*	Ain Defla	Potato (Spunta)	Potato (Spunta)	-	-	-
ADG2	*G. pallida*	Ain Defla	Potato (Bartina)	Potato	-	-	-
ADG3*	*G. pallida*	Ain Defla	Potato (Spunta)	Potato	LT159834-LT159835	LT159820-LT159821	LT159811
ADG4	*G. pallida*	Ain Defla	Potato (Spunta)	Potato (Spunta)	-	-	-
ADG5	*G. pallida*/*G. rostochiensis*	Ain Defla	Potato (Spunta)	Cereals	-	-	-
ADG6	*G. pallida*/*G. rostochiensis*	Ain Defla	Potato	Cereals	-	-	-
EAG1	*G. pallida*/*G. rostochiensis*	El Amra	Potato	Cereals	-	-	-
EAG2*	*G. pallida*	El Amra	Potato	Cereals	LT159831-LT159832	LT159818-LT159819	LT159812
EAG3	*G. pallida*/*G. rostochiensis*	El Amra	Potato	Cereals	-	-	-
EAG4	*G. pallida*/*G. rostochiensis*	El Amra	Potato	Cereals	-	-	-
RG1	*G. pallida*/*G. rostochiensis*	Rouina	Potato (Spunta)	Potato	-	-	-
RG2*	*G. rostochiensis*	Rouina	Potato (Spunta)	Cereals	LT159837-LT159838	-	LT159813
RG3*	*G. rostochiensis*	Rouina	Potato (Spunta)	Watermelon	LT159839-LT159840	-	LT159814
RG4	*G. pallida*/*G. rostochiensis*	Rouina	Potato (Spunta)	cereals (wheat)	-	-	-
MEG*	*G. pallida*/*G. rostochiensis*	Mekhatria	Potato (Spunta)	cereals (wheat)	LT159833	-	LT159817
ARG*	*G. pallida*/*G. rostochiensis*	Arib	Potato	cereals	LT159830-LT159836	-	LT159815-LT159816

*Populations molecularly characterized

PCN identification

Morphological and morphometric analysis of the sixteen PCN populations collected from five localities in Ain Defla, identified two nematode species: *G. pallida* and *G. rostochiensis* in single or mixed populations (Table 3). Six (ADG3, EAG2, RG2, RG3, MEG and ARG) out of these 16 populations, listed in Table 1, were characterized at the morphological and molecular levels.

Cyst perineal region

Morphometrics of cyst perineal region (Table 3) revealed clear differences between populations of *G. rostochiensis* and those of *G. pallida*. The populations from Rouina

Table 2 Codes and origin of cereal cyst nematode populations used in this study and crop systems adopted in the sampled fields

Population code	Species	Origin	Host plant/variety at sampling	Previous crops	GenBank accession number		
					ITS rDNA	D2-D3 28S rDNA	18S rDNA
EAH (*Ha*)	*Heterodera avenae*	El Amra	Potato (spunta)	Cereals	LT159843-LT159844	LT159822-LT159823	LT159804
EAH (*Hh*)	*H. hordecalis*	El Amra	Potato (spunta)	Cereals	LT159841-LT159842	LT159828-LT159829	LT159808-LT159809
RH	*H. avenae*	Rouina	Potato (spunta)	Cereals	-	LT159824-LT159827	LT159807
MEH	*H. avenae*	Mekhatria	Potato (spunta)	Cereals (wheat)	-	-	LT159806
ARH	*H. avenae*	Arib	Potato (spunta)	Cereals	-	-	LT159805

Table 3 Measurements of potato cyst nematode populations from Algeria (All measurements are in μm, presented as mean ± standard deviation, range)

Characters	ADG3	EAG2	RG2	RG3	MEG	ARG
Cyst (n = 30)						
Body length	580.3 ± 54.5	532.1 ± 72.7	590.3 ± 76.6	559.9 ± 71.7	555.9 ± 87.3	514.9 ± 90.3
	(472.5–742.5)	(405.0–652.5)	(427.5–742.5)	(391–693)	(405.0–742.5)	(323.5–740.4)
Body width	569.7 ± 61.3	517.9 ± 78.8	568.9 ± 80.5	542.0 ± 88.4	537.4 ± 79.1	487.2 ± 85.8
	(450–765)	(360.0–652.5)	(405.0–697.5)	(390.5–697.9)	(360.0–697.5)	(336.5–660.4)
Neck length	85.4 ± 28.7	100.7 ± 35.3	70.2 ± 25.2	100.9 ± 35.1	97.2 ± 31.6	105.2 ± 26.2
	(45–135)	(45–180)	(33.8–135)	(31.3–157.6)	(45–180)	(56.7–167)
Perineal region (n = 20)						
Distance (anus–vulva)	58.5 ± 13	56.9 ± 16.9	75.5 ± 20.4	78.2 ± 19.8	71.1 ± 23.6	58.1 ± 23.0
	(37.1–88.9)	(28.1–89.0)	(48.1–116.8)	(47.2–110.6)	(32.2–114.5)	(24.3–128.7)
Fenestra length	25.7 ± 5.5	21.3 ± 3.8	19.2 ± 4.0	20.6 ± 5.5	23.2 ± 5.8	22.0 ± 6.7
	(19.2–43.6)	(13.0–28.2)	(13.4–27.3)	(14.1–35.7)	(14.4–35.0)	(15.8–35.7)
Number of cuticular ridges	12.3 ± 2.6	13.4 ± 2.8	19.3 ± 3.5	20.4 ± 4.1	15.9 ± 4.4	14.6 ± 4.8
	(9–16)	(8–18)	(16–26)	(16–29)	(8–25)	(7–27)
Granek's ratio	2.3 ± 0.5	2.7 ± 0.6	4.0 ± 1.3	4.0 ± 1.3	3.1 ± 0.9	2.8 ± 1.2
	(1.5–3.3)	(1.4–3.6)	(2.4–8.0)	(2.5–6.7)	(2.0–5.5)	(1.3–6.0)
Second stage juveniles (n = 10)						
Stylet length	23.1 ± 0.4	23.3 ± 0.7	21.5 ± 0.7	21.0 ± 0.7	23.4 ± 0.6	23.6 ± 1.4
	(22.8–23.9)	(22.2–24.6)	(20.0–22.8)	(20.0–22.5)	(22.5–24.2)	(21.1–26.5)
Stylet knobs shape observed	1	1	2	2	1, 2	1, 2
Body length	417.4 ± 46.9	457.7 ± 22.0	422.5 ± 40.9	424.8 ± 32.8	462.2 ± 46.2	435.7 ± 34.7
	(382.6–508.2)	(434.0–502.5)	(371.1–502.5)	(388.3–502.5)	(399.7–525.3)	(394.0–502.5)
Body width	18.0 ± 1.3	20.4 ± 1.0	19.4 ± 1.4	18.9 ± 1.0	19.9 ± 1.3	19.5 ± 1.0
	(16.0–20.0)	(18.8–22.8)	(16.2–21.7)	(17.1–20.5)	(17.7–21.7)	(18.2–21.7)
Body width at anus	11.6 ± 1.2	12.4 ± 0.5	12.0 ± 0.6	10.8 ± 0.6	12.4 ± 1.0	12.4 ± 0.4
	(9.7–13.1)	(11.4–13.1)	(10.5–12.5)	(9.7–11.7)	(10.3–13.4)	(11.4–12.8)
Tail length	44.3 ± 5.0	49.7 ± 3.7	45.8 ± 4.9	41.5 ± 3.7	50.9 ± 3.9	43.8 ± 5.1
	(39.9–57.0)	(43.3–57.0)	(37.0–54.7)	(35.3–47.9)	(46.1–57.6)	(35.3–49.6)
Hyaline tail length	23.4 ± 2.2	30.6 ± 3.8	27.1 ± 2.8	24.8 ± 1.7	31.7 ± 2.9	25.0 ± 2.3
	(19.9–26.8)	(23.4–37.0)	(22.8–31.9)	(22.2–27.4)	(28.5–36.5)	(21.7–28.5)
a	23.2 ± 2.9	22.5 ± 1.3	21.9 ± 2.4	22.5 ± 2.3	23.2 ± 2.4	22.4 ± 2.6
	(19.7–27.9)	(20.0–24.5)	(18.6–26.0)	(19.5–27.5)	(20.3–28.4)	(19.7–27.5)
c	9.5 ± 1.2	9.3 ± 0.8	9.3 ± 1.0	10.3 ± 1.4	9.1 ± 1.0	10.0 ± 1.6
	(8.5–12.7)	(8.2–10.6)	(8.1–11.4)	(8.6–12.6)	(7.4–10.4)	(8.4–14.1)
c'	3.8 ± 0.5	4.0 ± 0.2	3.8 ± 0.3	3.8 ± 0.4	4.1 ± 0.3	3.5 ± 0.3
	(3.2–4.7)	(3.7–4.3)	(3.2–4.4)	(3.2–4.5)	(3.7–4.7)	(2.8–3.9)

1: type *G. pallida* (anteriorly pointed); 2: type *G. rostochiensis* (rounded)

RG2 and RG3 showed the highest values of vulva-anus distance, number of cuticular ridges between vulva and anus and Granek's ratio, fitting well within ranges of *G. rostochiensis* (Subbotin et al. 2010a), whereas populations from Ain Defla ADG3 and El Amra EAG2 were rather close to those of *G. pallida* (Subbotin et al. 2010a). Mean values of Granek's ratio and number of cuticular ridges of the populations from Arib ARG and Mekhatria MEG were closer to *G. pallida* although some overlap occurred with the ranges of *G. rostochiensis*, indicating

Table 4 Measurements of cereal cyst nematode populations from Algeria. (All measurements are in μm, presented as mean ± standard deviation, range)

Characters	EAH (*Hh*)	EAH (*Ha*)	RH	MEH	ARH
Vulval cones	*n* = 12	*n* = 6	*n* = 6	*n* = 10	*n* = 7
Fenestra length	66.0 ± 6.3	45.5 ± 5.3	48.0 ± 12.1	46.8 ± 4.2	47.3 ± 2.2
	(59.3–78.7)	(39.3–55.3)	(45.7–51.5)	(40.0–51.5)	(42.9–49.3)
Semi fenestra width	22.9 ± 2.4	20.2 ± 2.3	19.9 ± 1.5	18.6 ± 3.1	20.4 ± 1.5
	(18.6–27.2)	(17.7–24.5)	(17.2–21.4)	(13.6–22.9)	(18.6–22.9)
Vulval bridge width	18.8 ± 6.4	5.0 ± 0.7	8.4 ± 1.1	10.1 ± 1.5	7.5 ± 1.7
	(12.9–30)	(4.6–6.3)	(7.1–10.0)	(8.6–12.9)	(5.0–10.0)
Vulval slit length	14.6 ± 4.8	5.7 ± 0.8	8.5 ± 1.4	8.4 ± 2.3	9.2 ± 0.6
	(9.3–27.2)	(4.8–6.8)	(6.8 ± 10.8)	(5.7–12.2)	(8.6–10.0)
Underbridge length	111.5 ± 13.7	Absent	Absent	Absent	Absent
	(84.4–134.4)				
Underbridge width	25.7 ± 4.2	Absent	Absent	Absent	Absent
	(17.2–32.2)				
Bullae	Present few	Present heavy	Present heavy	Present heavy	Present heavy
Second stage juveniles	*n* = 10	-	*n* = 10	*n* = 10	*n* = 10
Stylet length	24.6 ± 0.6	-	26.5 ± 0.4	26.5 ± 0.6	27.7 ± 1.3
	(23.9–25.9)		(25.9–27.1)	(25.6–27.9)	(26.2–30.2)
Body length	477.8 ± 19.1	-	510.9 ± 35.4	473.3 ± 35.7	502.0 ± 12.0
	(446.5–508)		(465.5–576.6)	(425–529.4)	484–523
Body width	22.1 ± 0.5	-	22.1 ± 0.9	21.2 ± 1.3	22.2 ± 0.6
	(21.1–22.7)		(21–23.5)	(19–23.5)	(21.2–23.6)
Body width at anus	16.0 ± 0.4	-	16.3 ± 0.7	15.7 ± 0.6	16.7 ± 0.5
	(15.2–16.4)		(15.5–17.2)	(14.5–16.3)	(16.2–18)
Tail length	54.6 ± 1.5	-	61.5 ± 5.0	61.3 ± 5.1	60.3 ± 4.1
	(52.6–56.7)		(55.2–70.3)	(54–74.5)	(56–69.2)
Hyaline tail length	34.5 ± 1.8	-	43.3 ± 5.8	39.7 ± 4.4	39.1 ± 3.8
	(31.4–36.7)		(35–51.2)	(34.8–49)	(34–47)
a	21.6 ± 1.2	-	23.1 ± 1.6	22.3 ± 1.2	22.6 ± 0.8
	(19.8–23.4)		(21.1–25.5)	(20.8–24.5)	(21.2–23.7)
c	8.8 ± 0.4	-	8.3 ± 0.7	7.7 ± 0.7	8.3 ± 0.5
	(8.1–9.2)		(7.4–9.5)	(6.7–8.8)	(7.3–8.8)
c'	2.5 ± 0.1	-	3.8 ± 0.4	3.9 ± 0.3	3.6 ± 0.3
	(2.3–2.7)		(3.2–4.4)	(3.4–4.6)	(3.2–4.1)
No. of lines in lateral fields	4	-	4	4	4

that these populations could be mixed. The greatest differences, and to some extent conflicting, were observed in the range of the cuticular ridges between anus and vulva within populations of *G. pallida* that were 9–16 (ADG3), 8–18 (EAG2), 8–25 (MEG) and 7–27 (ARG). For the MEG and ARG populations, rather large ranges of the Granek's ratio were observed (2.0–5.5 and 1.3–6.0, respectively) that overlap that for *G. rostochiensis*. This observation would suggest that they were mixed populations. The MEG population of *G pallida* had a mean hyaline tail region length of 31.7 ± 2.9 μm (range 28.5–36.5 μm) longer than that of the other populations of *G pallida* reported in this study and in the literature (Subbotin et al. 2010a).
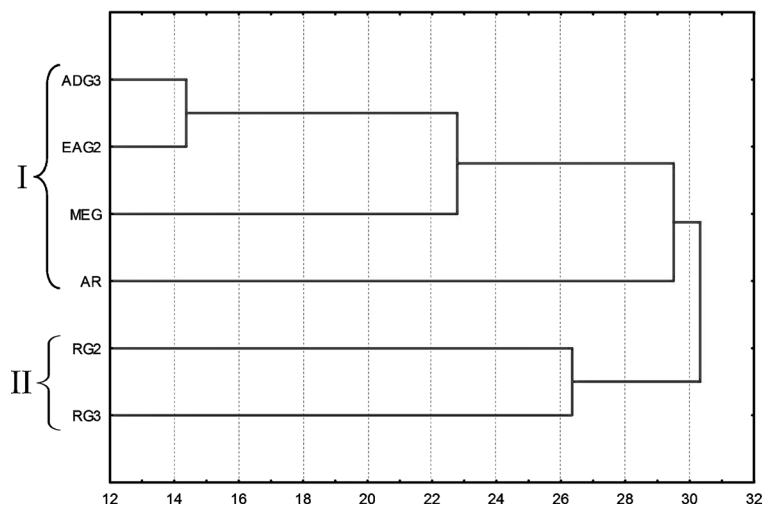
J2

There were clear differences between morphological characters of J2 belonging to different populations. Analysis of variance showed significant differences for J2 body length (0.024) and c' ratio ($p = 0.018$), highly significant for body width of J2 ($p = 0.000588$), very high meaningful differences for stylet length, body width at anus, tail length, hyaline tail length and c ratio, but for the ratios a and c, the differences were not significant ($p = 0.75$; $p = 0.057$ respectively).

Main morphometrics (stylet length and stylet knobs shape) of J2, listed in Table 3 for the populations examined were in agreement with data obtained from cysts and corresponded with data for *G. pallida* and *G. rostochiensis* reported in literature (Baldwin and Mundo-Ocampo 1991; EPPO/OEPP 2009).

Two distinct clusters were displayed by the ascending hierarchical classification based on minimal jump method and using the three most important diagnostic characters of *Globodera* genus (Fig.2). The cluster I is formed by the populations of *G. pallida* collected in Ain Defla, El Amra, Arib and Mekhatria, the last two localities contained both species. The cluster II contained the two populations of *G. rostochiensis* from Rouina.

The principal component analysis separated the two populations of *G. rostochiensis* from Rouina (RG2 and RG3) from those of *G. pallida* collected from Ain Defla (ADG3) and El Amra (EAG2) (Fig. 3). The mixed populations from Arib (ARG) and Mekhatria (MEG) grouped in the same cluster with *G. pallida* because of dominance of *G. pallida*.

CCN identification

Morphology of the cyst vulval cones and J2 of the CCN populations from Rouina, Mekhatria, Arib and El Amra, revealed the presence of *H. avenae* in all sites sampled (Table 2). However, mixed populations of *H. avenae* and *H. hordecalis* were detected at El Amra. The populations ARH, EAH (*Ha*), EAH (*Hh*), MEH and RH were further characterized at the morphological and molecular levels.

Cyst vulval cones

Morphology of vulval cones of populations from Rouina, Arib, El Amra and Mekhatria showed typical features of *H. avenae* such as heavy bullaes, short vulval slit and absence of underbridge. Measurements of fenestral lengths and semi fenestral widths determined from all populations were close to each other. Variations were observed in the width of the vulval bridge of Rouina, Arib and Mekhatria populations (mean values 8.4, 7.6 and 10.1 μm, respectively) (Table 4). Specimens of the mixed population from El Amra fitting the morphology of *H. avenae* had the smallest width of vulval bridge (5 μm). Other specimens in this mixed population showed much larger fenestra length, larger vulval bridge and the presence of a strong under-bridge enlarged in the middle portion indicating that they were belonging to the species *H. hordecalis* (Table 4).

The analysis of variance showed very highly significant differences for fenestra length, vulval bridge width

Fig. 2 Dendrogram of hierarchical classification of six Algerian PCN populations based on three morphological characters of cysts and second stage juveniles (Cluster1: *G. pallida* from Ain Defla ADG3, *G. pallida* from El Amra EAG2, mixed populations from Mekhatria MEG and mixed populations from Arib ARG; Cluster 2: two populations of *G. rostochiensis* from Rouina RG2 and RG3)

Fig. 3 Distribution of populations of PCN inferred by the principal component analysis. *Globodera rostochiensis* populations RG2 and RG3 cluster in a different quadrant from that of the *G. pallida* populations ADG3 from Ain Defla, ARG from Arib; EAG2 from El Amra and MEG from Mekhatria
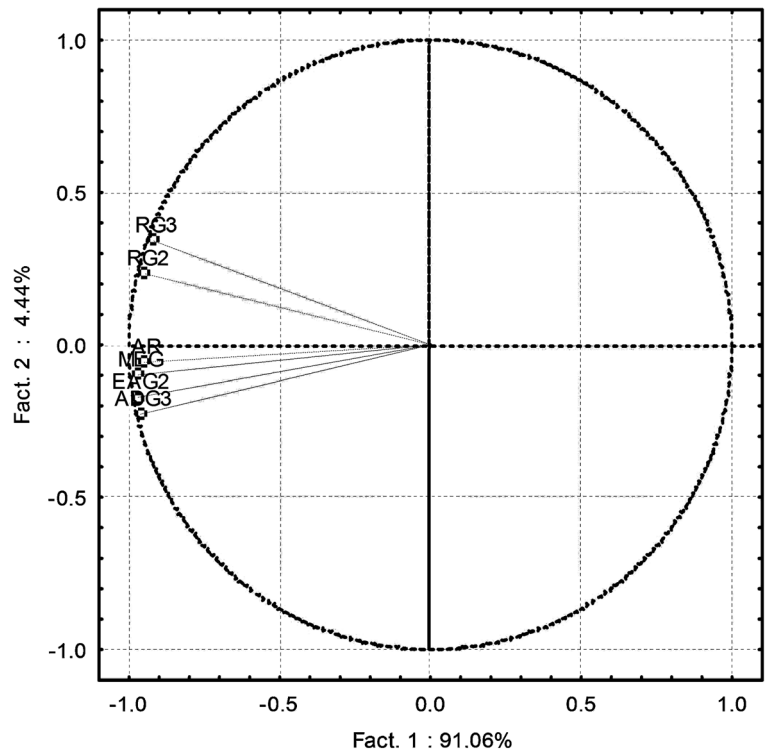
and vulval slit length ($p < 0.0005$) and highly significant difference for semi fenestra width ($P = 0.0037$) between *H. avenae* populations and *H. hordecalis* (Fig. 4).

CCN populations were distributed into two clusters by the ascending classification based on minimal jump using selected morphological characters of vulval cones (Fig. 4). Cluster I included *H. avenae* (EAH (*Ha*), RH, MEH and ARH) populations, whereas cluster II

contained the single population of *H. hordecalis* EAH (*Hh*). Similar results were also obtained using the principal component analysis, which discriminated the *H. avenae* populations from that of *H. hordecalis* (Fig. 5). The morphological identification of the population of *H. hordecalis* was confirmed by the results of the molecular analyses reported in the following sections.

Fig. 4 Dendrogram of hierarchical classification of five Algerian CCN populations based on morphological characters and morphometrics of vulval cones and second stage juveniles (Cluster 1: *H. avenae* from Rouina, *H. avenae* from Arib, *H. avenae* from El Amra (*Ha*), *H. avenae* from Mekhatria; Cluster 2: *H. hordecalis* from El Amra EAH (*Hh*))
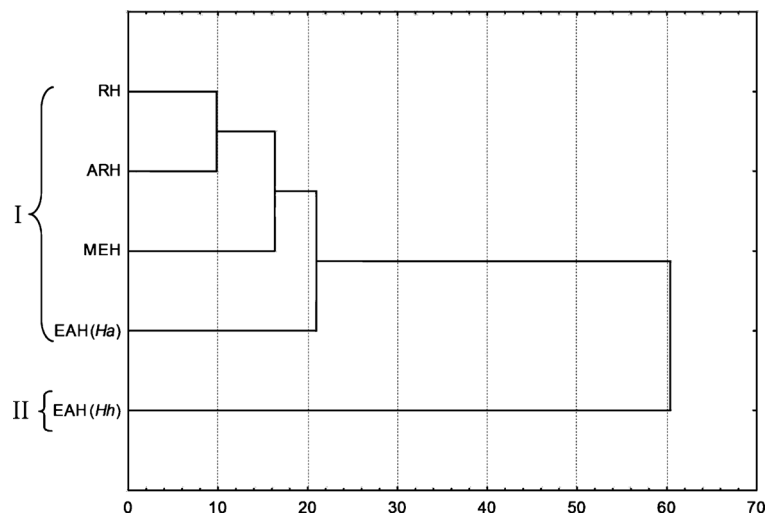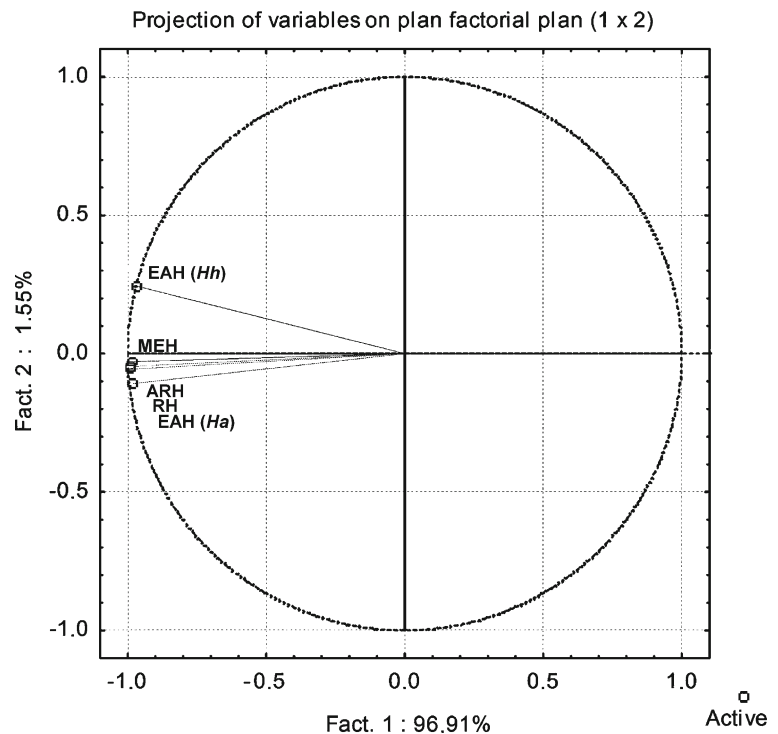
Fig. 5 Distribution of populations of cereal cyst nematodes (MEH, ARH, RH and EAH) inferred by the principal component analysis. The population EAH (*Hh*) of *H. hordecalis* from El Amra clusters in a different quadrant from that of *H. avenae* MEH, ARH, RH and EAH (*Ha*) populations

Projection of variables on plan factorial plan (1 x 2)

J2

Among populations identified as *H. avenae* (Table 2), J2 from Mekhatria (MEH) showed the smallest body length, width, and c ratio whereas RH and ARH J2 had very similar measurement ranges.

Specimens of J2 in the mixed population from El Amra (EAH) had the shortest stylet, tail, terminal hyaline portion and c' ratio. These characters were fitting those reported for of *H. hordecalis* rather than *H. avenae* (Table 4).

Molecular analyses

PCR-RFLP diagnostics of PCN and CCN populations

Amplification of the ITS containing region, the 18S rRNA gene and the D2-D3 expansion segments of the 28S gene of *G. pallida* and *G. rostochiensis* yielded single fragments of 1188 bp, 1628 bp and 700 bp, and 1190 bp, 1628 bp and 700 bp, respectively. Diagnostic PCR-ITS-RFLP profiles for *G. pallida* and *G. rostochiensis* from Algeria are given in Fig. 6. The two diagnostic enzymes produced RFLP patterns that allowed discriminating the two *Globodera*

species from Algeria, even for mixed populations. The *Alu* I (lanes 1, 2, 5 and 7; fragment sizes 505 and 383 bp) digestion of Algerian *G. pallida* populations showed identical restriction profiles among geographical populations and were identical to those of *G. pallida* from Peru, UK (York) and USA (Idaho) (Skantar et al. 2007, 2011; Subbotin et al. 2011).
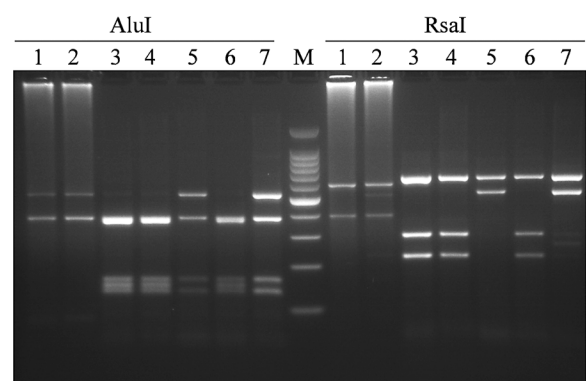

Fig. 6 PCR-RFLP profiles for *Globodera pallida* and *G. rostochiensis* populations from Algeria digested by using the *Alu* I and *Rsa* I enzymes. M: 100 bp ladder DNA; lane 1: *G. pallida* from Ain Defla; lane 2: *G. pallida* from El Amra; lane 3: *G. rostochiensis* from Rouina 1; lane 4: *G. rostochiensis* from Rouina 2; lane 5: *G. pallida* from Arib; lane 6: *G. rostochiensis* from Arib; lane 7: *G. pallida* from Mekhatria

Rsa I digestion (lanes 1, 2, 5 and 7; fragment sizes 587 and 385 bp) produced a restriction profile for *G. pallida* populations from Ain Defla and El Amra (lanes 1and 2) identical to that of *G. pallida* populations from Europe, Asia, North and South America and Oceania, but populations from Arib and Mekhatria showed a polymorphism at *Rsa* I site resulting in missing restriction sites (lanes 5 and 7).

The *Alu* I (lanes 3, 4 and 6) and *Rsa* I (lanes 3, 4 and 6) profiles for Algerian *G. rostochiensis* populations were identical each other but different from those of *G. rostochiensis* published in the literature.

PCR amplicons of the the ITS region, the D2-D3 expansion segments of the 28S rDNA and the partial 18S rRNA gene of *H. avenae* and *H. hordecalis* produced single fragments of 1057 bp, 700 bp, and 1600 bp and 1142 bp, 700 bp and 1600 bp, respectively. Five restriction enzymes generated ITS-RFLP profiles for *H. avenae* and *H. hordecalis* allowing for differentiation of these species even when present as mixed populations (El Amra). Intraspecific polymorphism was not observed within *H. avenae* and *H. hordecalis* populations. However, Algerian *H. avenae* populations contain ITS belonging to the type B because it was restricted by *Alu* I and *Rsa* I enzymes (Fig. 7). Furthermore, the restriction profiles with *Hae* III and *Hinf* I enzymes of *H. avenae*, characterized in this study, are identical to those of *H. avenae* from Algeria reported by Rivoal et al. (2003). These profiles were also typical of *H. avenae* populations from India, Syria, Turkey, Italy, France and Oregon, but different from those obtained from Tunisian populations.
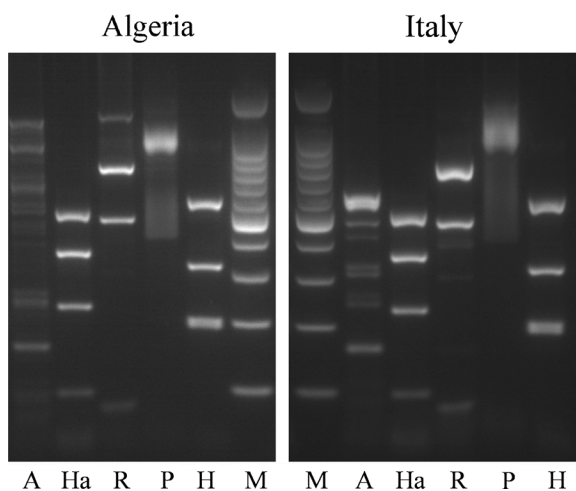
ITS-RFLP of *H. hordecalis* from Algeria showed identical profiles of *H. hordecalis* populations from Scotland and Italy (Subbotin et al. 1999; Madani et al. 2004) but different from those of a population of *H. hordecalis* from Iran by Tanha Maafi et al. (2003) (Fig. 8).

Phylogenetic analysis

Intraspecific variations among Algerian populations were as follows: *G. pallida* (six sequences) 7–22 nucleotides; *G. rostochiensis* (five sequences) 11–20 nucleotides. The most variable populations of *G. pallida* and *G. rostochiensis* were those from Mekhatria and Rouina RG3, respectively.

Phylogenetic relationships of *G. pallida* and *G. rostochiensis* from Algeria were inferred from analyses of the ITS, 18S and D2-D3 expansion domains sequences of the closest species of cyst nematodes by using ML method. Sequence alignment of the ITS regions included 64 sequences, 11 (six for *G. pallida* and five for *G. rostochiensis* from Algeria) of which were newly obtained in this study (Fig. 9). *Globodera artemisiae* and *G. capensis* were used as outgroups for the ITS dataset. The ML tree as inferred by the analyses

Fig. 7 PCR-RFLP profiles of *Heterodera avenae* from Algeria (left) and from Italy (right) digested by five enzymes. M: 100 bp ladder; A: *Alu* I; Ha: *Hae* III; R: *Rsa* I; P: *Pst* I; H: *Hinf* I
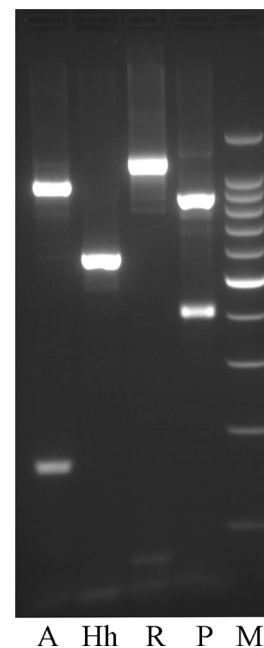

Fig. 8 PCR-RFLP profiles of *Heterodera hordecalis* from Algeria. M: 100 bp ladder; A: *Alu* I; Ha: *Hae* III; R: *Rsa* I; P: *Pst* I; H: *Hinf* I

Fig. 9 Phylogenetic tree of ITS containing region describing the evolutionary relationships among different geographical populations of PCN (*Globodera pallida* and *G. rostochiensis*) using Maximum Likelihood (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Numbers at nodes indicate bootstrap values
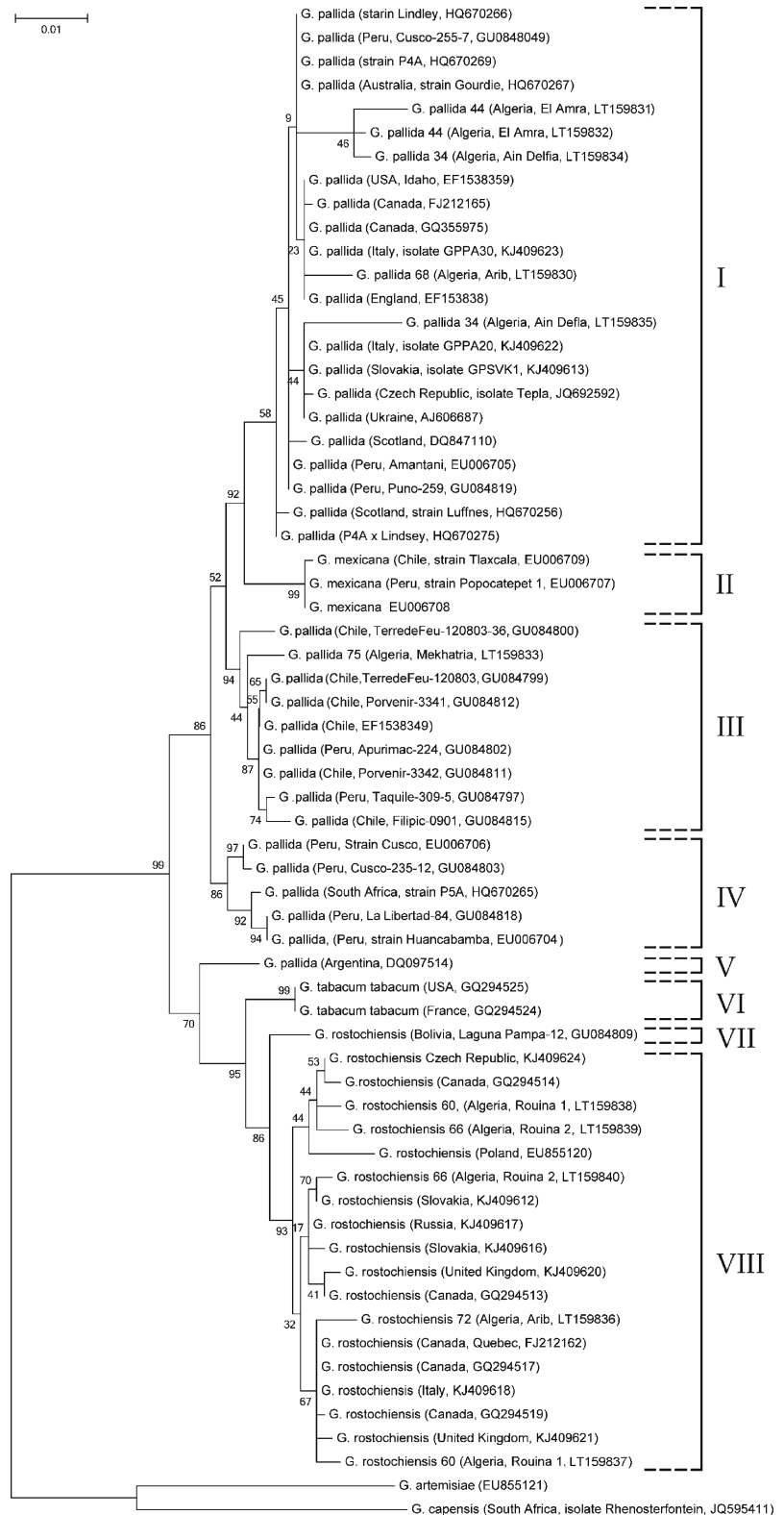
of the ITS rRNA gene sequences shows the phylogenetic relationship of the PCN populations studied. All *G. pallida* and *G. mexicana* isolates clustered in a well-supported clade, which contained four groups (I-IV). All *G. rostochiensis* and *G. tabacum* isolates clustered also in a separated well-supported clade containing four groups (V-VIII). *Globodera artemisiae* and *G. capensis* formed a small clade at the bottom of the tree. The four groups in the *G. pallida* and *G. mexicana* clade included: I) *G. pallida* sequences from Algeria (Ain Defla, Arib and El Amra) that fitted into a highly supported cluster (92 % support) along with all sequences of populations from Australia, Canada, Europe, Peru, and USA (Idaho); II) *G. mexicana* group as sister species of group I; III) *G. pallida* sequences from Mekhatria (Algeria), Peru, and Chile; IV) *G. pallida* from Peru and South Africa isolates at basal position (86 % support) of all *G. pallida* isolates used in this analysis. The four groups in the *G. rostochiensis* and *G. tabacum* clade included: V) a population of *G. pallida* from Argentina confirming that this isolate could represent a hybrid or a naturally occurring variant closely related to *G. rostochiensis*; VI) *G. tabacum* closely related to *G. rostochiensis* (95 % support); VII) *G. rostochiensis* from Bolivia, which grouped as sister species of all *G. rostochiensis* (86 % support); VIII) *G. rostochiensis* isolates from Algeria, Europe and Canada.

Sequence alignments of the 18S gene included 31 sequences, seven of which were obtained in this study. The phylogenetic tree of the 18S rRNA gene revealed no clear phylogenetic relationships among the different isolates of *G. pallida* and *G. rostochiensis* (unpublished data).

The phylogenetic tree inferred by the analysis of the D2-D3 of the 28S rRNA gene sequences of CCN and a selected number of PCN populations revealed that all sequences of PCN populations clustered in a clade separated from that of the CCN populations (Fig. 11). The *G. pallida* populations from Algeria grouped with the corresponding sequences of *G. pallida* from distant geographical areas with high support, and resulted to be closely related to those of *G. rostochiensis* group (Fig. 11).
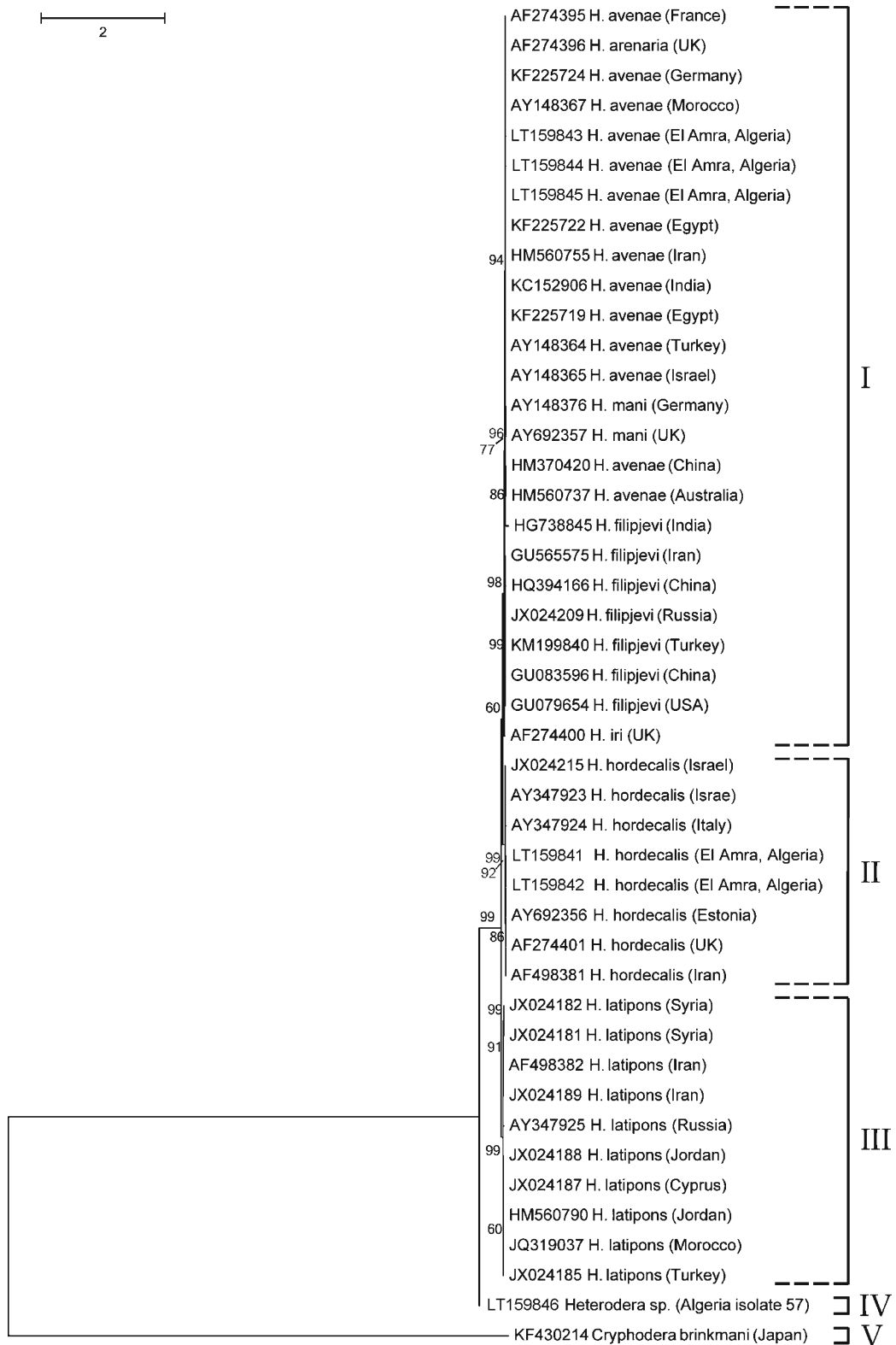
Phylogenetic relationships of *H. avenae* and *H. hordecalis* from Algeria were inferred from analyses of the ITS rRNA, the 18S and the D2-D3 of the 28S rRNA gene sequences of the closest species of cyst nematodes by using the ML method. *Cryphodera brinkmani* was used as outgroup.

Forty-five ITS sequences of the CCN populations were aligned. These populations belonged to the *H. avenae* group and included those (three of *H. avenae*, two of *H. hordecalis* and one *Heterodera* sp. isolate 57) identified in this study. The phylogenetic tree, inferred by the analysis of these ITS rRNA gene sequences contained a major clade with all the CCN populations studied and a small clade with the outgroup *Cryphodera brinkmani* (Fig. 10). The major clade was clearly divided into four groups. Group I included all *H. avenae* populations along with those from Algeria, *H. filipjevi*, *H. iri*, and *H. mani*. Group II included all sequences of *H. hordecalis* along with those from Algeria. Group III included all *H. latipons* populations. Group IV included, at the basal position of the major clade, the sequence of an unidentified *Heterodera* sp. isolate 57 from Algeria. In the 18S phylogenetic tree, 25 sequences of CCN, belonging to the *H. avenae* group, were aligned with *C. brinkmani* used as outgroup. Two subgroups were observed: subgroup I included all *H. avenae* populations, *H. filipjevi*, *H. mani* and *H. hordecalis* determined in this study. Subgroup II included all *Heterodera* sequences from the database including one sequence of *H. hordecalis* and *Heterodera* sp. from Algeria (isolate 57) confirming that this isolate does not belong to the *H. avenae* group (data not shown).

Forty-four sequences of the D2-D3 of 28S rRNA gene obtained from CCN and selected PCN populations were aligned. Twelve of these sequences (six identified as *H. avenae*, two as *H. hordecalis* and four as *G. pallida*) were newly obtained in this study. The D2-D3 phylogenetic tree showed a separation of CCN clade from that containing the PCN populations (Fig. 11). The populations of *Heterodera avenae* group (CCN) clustered in different groups, which fitted those reported in the literature (Fu et al. 2011; De Luca et al. 2013). In particular, *H. avenae* and *H. hordecalis* from Algeria clustered with all Asian, African and European *H. avenae* group populations (Fig. 11).

Discussion

This survey demonstrates that the major potato growing areas in Algeria are commonly infested by *G. pallida* and *G. rostochiensis*. Fields in the same areas, previously cropped with cereals, were infested with *H. avenae*, in particular in El Amra area *H. avenae* was associated with *H. hordecalis* in mixed populations.

AF274395 H. avenae (France)
AF274396 H. arenaria (UK)
KF225724 H. avenae (Germany)
AY148367 H. avenae (Morocco)
LT159843 H. avenae (El Amra, Algeria)
LT159844 H. avenae (El Amra, Algeria)
LT159845 H. avenae (El Amra, Algeria)
KF225722 H. avenae (Egypt)
94 HM560755 H. avenae (Iran)
KC152906 H. avenae (India)
KF225719 H. avenae (Egypt)
AY148364 H. avenae (Turkey)
AY148365 H. avenae (Israel)
AY148376 H. mani (Germany)
96 AY692357 H. mani (UK)
77
HM370420 H. avenae (China)
86 HM560737 H. avenae (Australia)
HG738845 H. filipjevi (India)
GU565575 H. filipjevi (Iran)
98 HQ394166 H. filipjevi (China)
JX024209 H. filipjevi (Russia)
99 KM199840 H. filipjevi (Turkey)
GU083596 H. filipjevi (China)
60 GU079654 H. filipjevi (USA)
AF274400 H. iri (UK)

I

JX024215 H. hordecalis (Israel)
AY347923 H. hordecalis (Israe)
AY347924 H. hordecalis (Italy)
99 LT159841 H. hordecalis (El Amra, Algeria)
92 LT159842 H. hordecalis (El Amra, Algeria)
99 AY692356 H. hordecalis (Estonia)
86 AF274401 H. hordecalis (UK)
AF498381 H. hordecalis (Iran)

II

99 JX024182 H. latipons (Syria)
91 JX024181 H. latipons (Syria)
AF498382 H. latipons (Iran)
JX024189 H. latipons (Iran)
AY347925 H. latipons (Russia)
99 JX024188 H. latipons (Jordan)
JX024187 H. latipons (Cyprus)
HM560790 H. latipons (Jordan)
60 JQ319037 H. latipons (Morocco)
JX024185 H. latipons (Turkey)

III

LT159846 Heterodera sp. (Algeria isolate 57)   IV
KF430214 Cryphodera brinkmani (Japan)   V

2

◀ **Fig. 10** Phylogenetic tree of ITS containing region describing the evolutionary relationships among different geographical populations of CCN belonging to the *Heterodera avenae* group using Maximum Likelihood (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Numbers at nodes indicate bootstrap values

Our study confirmed that both PCN and CCN are present in regions with temperatures in the higher 20 °C in the high summer. Furthermore our data showed for the first time a mixed occurrence of *G. pallida* and *G. rostochiensis* in the same locality in Algeria, with the predominance of one species for each locality. *Globodera pallida* is dominant in Ain Defla and El Amra, whereas *G. rostochiensis* is dominant in the Rouina region. In Mekhatria and Arib regions both species were present as mixed populations. The occurrence of mixed populations of *G. pallida* and *G. rostochiensis* in the same field was already reported in South America (Evans et al. 1975), in New Zeland (Marshall 1993), in Northern Ireland (Turner 1996), in England and Wales (Ibrahim et al. 2001) and recently in Slovakia (Douda et al. 2014). This finding reinforces the hypothesis by Den Nijs (1992) of the cross-hybridization between these two nematode species that might result in a generation of new genotypes. The incidence of mixed populations of these two *Globodera* species complicates control measures because of the lack of resistant potato cultivars for *G. pallida*, in contrast for *G. rostochiensis* nearly all contemporary potato cultivars are resistant or tolerant to this species.

Perineal patterns of cysts and J2 stylet characteristics distinguished PCN species, but sometimes the diagnostic characters may overlap among various populations of the different species (Baldwin and Mundo-Ocampo 1991). To confirm the identification of PCN based on the morphology, molecular analyses were carried out to specifically identify mixed populations and to get precise information about polymorphism of *G. pallida* and *G. rostochiensis* populations from Algeria. ITS-RFLP analysis revealed species-specific profiles for *G. pallida* and *G. rostochiensis* from Algeria. The comparison of the ITS-RFLP profiles of *G. pallida* populations from Algeria by using *Rsa* I and *Alu* I enzymes with those obtained in other studies revealed the presence of additional bands (ITS haplotypes) that may result from a higher level of rRNA gene heterogeneity or from the presence of a natural hybrid between species occurring

in the same area. In our study, *G. pallida* from Mekhatria and Arib localities showed identical restriction profiles by using *Rsa* I but different from those of *G. pallida* from Ain Defla and El Amra regions. Heterogeneity in ITS of Peruvian *G. pallida* populations was already reported by RFLP analysis and several restriction enzymes discriminate European and Peruvian populations (Grenier et al. 2001). *Globodera rostochiensis* populations from Algeria showed identical restriction profiles but differed from other populations of *G. rostochiensis* reported in literature (Subbotin et al. 2000). The heterogeneity found in *G. pallida* and *G. rostochiensis* from Algeria may be due to an incomplete process of homogenization among the different ribosomal repeats and thus the rDNA repeats in their genomes are present as a mixture of haplotyes with different sequences.

The phylogenetic relationships by using ITS sequences revealed that all European *G. pallida* isolates and those from Ain Defla, Arib and El Amra clustered in the subgroup I together with some Peruvian isolates (P4A, Cusco, Puno and Amantani). The *G. pallida* isolate from Mekhatria resulting the most divergent isolate of *G. pallida* from Algeria, grouped instead in the subgroup III together with isolates of *G. pallida* from different areas of Chile and Peru. As the two groups containing Algerian *G. pallida* isolates are well supported, each related to different South American populations (Peru and Chile), a multi-introduction origin of Algerian *G. pallida* from Europe and South America could be hypothesized. In the phylogenetic tree, the position of *G. pallida* isolates from Mekhatria, in the group III, and Arib, in the group I, is intriguing because although morphometric values are in agreement with those of *G. pallida*, some overlap occurs with *G. rostochiensis*. The morphometrics and morphology of the J2 of both populations fit well within the ranges known for *G. pallida*. The phylogenetic analysis revealed that Mekhatria and Arib populations could represent a naturally occurring variants that diverged from the European and some Peruvian *G. pallida* isolates or, in the case of Mekhatria, could represent a genetically intermediate population. Our data confirm that *G. pallida* may represent a species complex as already reported (Madani et al. 2010; Subbotin et al. 2011) and the possible existence of a third PCN species regarding some *G. pallida* populations from South America, characterized by higher sequence variability compared with other *G. pallida* populations, included in the subgroups III and IV.
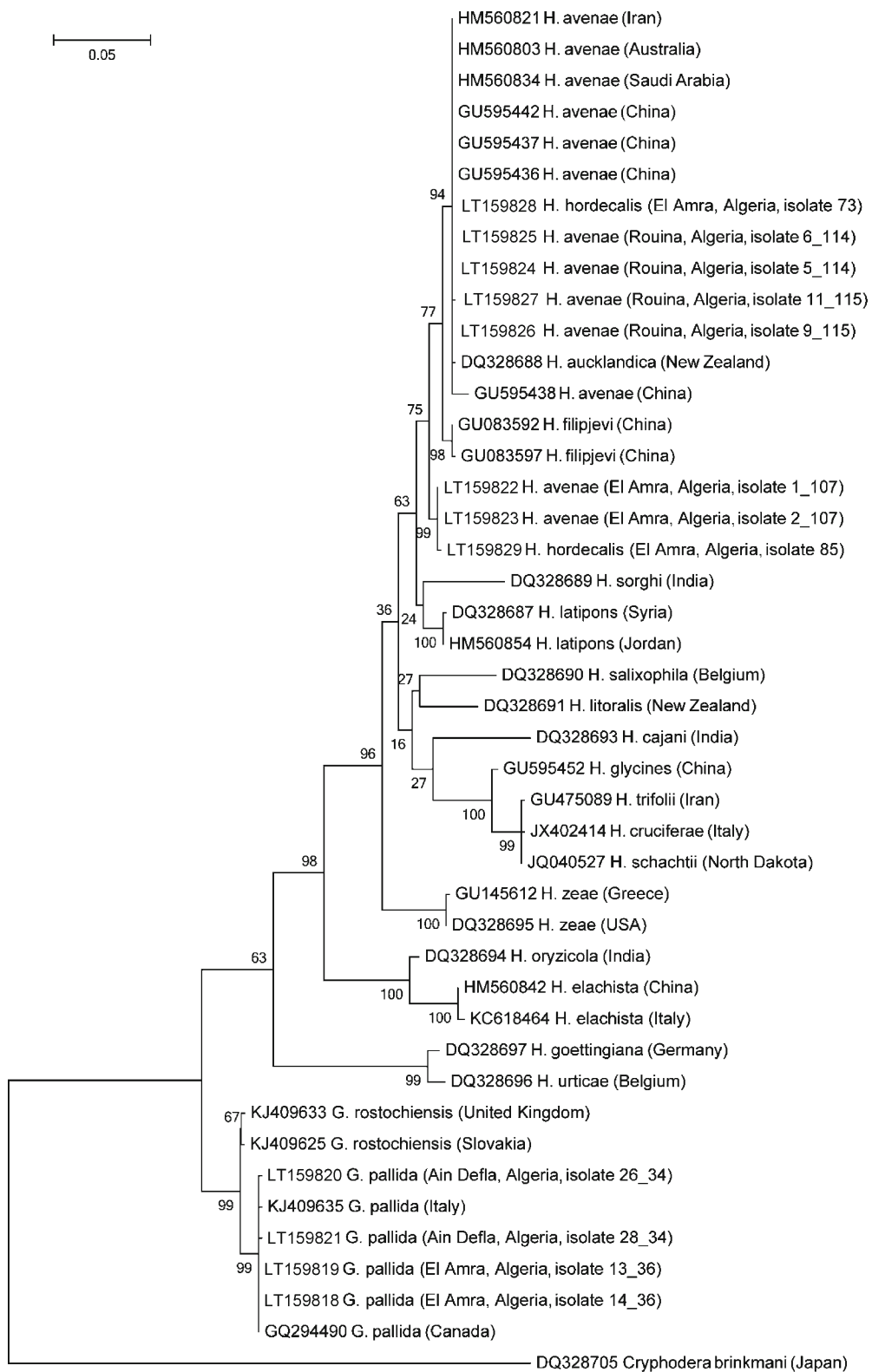
◀ **Fig. 11** Phylogenetic tree of D2-D3 expansion domains describing the evolutionary relationships among different geographical CCN (*Heterodera avenae* group) and selected PCN (*Globodera pallida* and *G. rostochiensis*) populations using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Numbers at nodes indicate bootstrap values

In contrast to *G. pallida*, a lower variability was displayed by ITS sequence diversity among *G. rostochiensis* populations from Algeria and from all over the world. In the phylogenetic tree, all populations of *G. rostochiensis* from Europe, Canada and Algeria formed a supported cluster (with 93 % support) with four subgroups, even some relationships among *G. rostochiensis* sequences were unresolved. *Globodera rostochiensis* populations from Algeria contain a mixture of ITS haplotypes that clustered in different groups suggesting that the process of homogenization among repeats is still incomplete as shown by RFLP analysis. This finding also suggests that the presence of *G. rostochiensis* in Algeria is the result of multiple introductions of European isolates rather than of direct import of infected potatoes from Latin America. The phylogenetic tree of the 18S rRNA gene revealed that the origin of both species in Algeria was consistent with a bush-like radiation as no clear phylogenetic relationships was revealed between the different haplotypes of *G. pallida* and *G. rostochiensis*.

A previous survey of CCN conducted by Mokabli (2002) recorded the occurrence of *H. avenae* and *H. latipons* in other regions of Algeria such as Setif, Béjaia, Relizane, Dar El Beida and Mascara. Our survey reveals that *H. avenae* is more spread than previously reported and *H. hordecalis* is recorded for the first time in Algeria and at present only in El Amra region. The use of the enzyme *Rsa* I in the PCR-RFLP analyses clearly identified *H. avenae* from Algeria as type B also reported from Iran (Tanha Maafi et al. 2007), from Turkey (Abidou et al. 2005), from Italy (De Luca, unpublished data). Furthermore *Alu* I profile revealed heterogeneity of the ITS region among several specimens from different Algerian *H. avenae* populations as reported for Iranian *H. avenae* (Tanha Maafi et al. 2010). The *Alu* I, *Pst* I and *Rsa* I patterns of the Algerian *H. hordecalis* population were identical to those from the Northern Europe and Iran but different from those of the Italian population reported by Subbotin et al. (2000) and Tanha Maafi et al. (2003). Furthermore a high level

of sequence divergence between those populations of *H. hordecalis* was already observed (Madani et al. 2004). Species identification of *H. avenae* and *H. hordecalis* based on combinations of *Hinf I*, *Hae* III and *Rsa* I enzymes and sequencing of the ITS, 18S gene and D2-D3 expansion domains was congruent with the morphological and morphometrical data of these species (Subbotin et al. 2000; Tanha Maafi et al. 2010) and resulted useful for species identification. The ML tree based on the ITS sequences revealed that both *H. avenae* and *H. hordecalis* from Algeria grouped with populations of the same species from different geographical areas. However, *H. hordecalis* from Algeria formed a well-supported group close to that of *H. latipons* confirming that these species constitute a species complex within the *H. avenae* group as suggested by Rivoal et al. (2003). The *Heterodera* sp. isolate 57 from Algeria grouped at basal position of the phylogenetic tree revealing that this is a different species related to the *H. avenae* group, suggesting further investigation for its correct identification.

The D2-D3 sequences were obtained for the first time for *H. hordecalis*. Phylogenetic relationships inferred using the D2-D3 expansion domains showed clades similar to those of the ITS tree. *Heterodera hordecalis* from Algeria formed a well-supported group closely related to *H. avenae* group sensu stricto, while *H. latipons* populations grouped together at basal position of the *H. avenae* group and *H. hordecalis*.

In conclusion, the correct and rapid identification of PCN and CCN is essential for their control as they can be present as mixtures. This study has elucidated the geographical distribution and the genetic variation of *Heterodera* and *Globodera* species in Algeria and has provided accurate molecular tools for the identification of PCN and CCN in the country. Furthermore, the occurrence of *Heterodera* and *Globodera* species in the same localities in Algeria is reported for the first time so far, suggesting that the cultural conditions, especially rotation type based on cereals-potato in these localities may be the cause of the presence of both nematode species as mixture in the same fields.

Cereal cyst nematodes, especially *H. avenae*, are very damaging in rather finely structured soils and mild climates. In Algeria these conditions occur in the regions bordering the Mediterranean sea and, therefore, as reported from Tunisia (Namouchi-Kachouri and B'Chir 2005), severe yield losses of cereals must be expected whenever the soil population densities of these

nematodes exceed the tolerance of the host crop, esti-mated in 1.3 eggs/g soils. Potato cyst nematodes are well adapted to the same environmental conditions al-though they would tolerate also less finely structured soils. These nematodes are expected to severely affect potato when the soil population densities exceed 1.9 eggs/g soil, as reported in Italy (Greco et al. 1982). The control of cyst nematodes should be based mainly on the use of environment friendly methods, such as the use of appropriate crop rotations by intercropping a leguminous crop between cereal and potato and by adding resistant cultivars if available. This requires information on the soil population densities of different nematodes, precise identification to species level and identification of the race/pathotype. Haddadi et al. (2013) reported the reaction to some Algerian populations of *H. avenae* of several cereal host plants of the international host test range used to identify races of the nematode. This study should be extended also to populations from other Algerian regions, because there is no information on the pathotypes of the potato cyst nematode populations. Such information is necessary to select potato cultivars carrying the correct resistance genes for nematode control.

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