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Phylogenetic relationships among populations of the *Nacobbus aberrans* (Nematoda, Pratylenchidae) complex reveal the existence of cryptic species

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The plant-parasitic nematode *Nacobbus aberrans sensu lato* is an agricultural pest of quarantine importance. Due to the morphometric, physiological and genetic variability observed within the species, there is no agreement on the taxonomy of this nematode. The objective of this study was to analyse the ITS rDNA region and the D2–D3 expansion segments of 28S rDNA in 10 Argentine populations and one from Ecuador and to establish their phylogenetic relationship with other known sequences from South and North America. Phylogenetic trees of the ITS gene showed seven statistically well-supported clades; the high and significant F_{ST} values obtained among these groups confirmed this partitioning. The Argentine populations here considered were separated into three clades: one comprising a population from the Andean region and two grouping nematodes from lower altitudes. Three other clades were distinguished for South American populations, which included known sequences of individuals from Peru, Bolivia and north of Argentina. The other clade included sequences from Mexico, Ecuador and two Argentine populations of unknown origin. The important degree of genetic divergence observed among Andean populations suggests that the Andes may have played a crucial role in speciation of *Nacobbus*, which would have originated in this region. Although D2–D3 segments exhibited lower variation, they were useful for establishing phylogenetic relationships among the Argentine populations considered in this work. As there are no other GenBank sequences available for these segments, it was not possible to make comparisons with other populations from South and North America. The considerable genetic differentiation observed in ITS rDNA region among *Nacobbus* populations showed evidence of cryptic species within the *N. aberrans s.l.* complex. Integration of morphological and morphometric studies and molecular analyses considering other genes may aid in the identification of species and their phylogenetic relationships within this genus.

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Introduction

The genus *Nacobbus* is native to South and North America and includes sedentary endoparasites that induce galls in the host's roots. It was initially reported to comprise four species: *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944; *N. dorsalis* Thorne & Allen, 1944; *N. batatiformis* Thorne & Schuster, 1956; and *N. serendipiticus* Franklin, 1959; and the subspecies *N. serendipiticus bolivianus* Lordello, Zamith & Boock, 1961. Sher (1970) synonymised the latter two species and the subspecies with *N. aberrans* because of a lack of consistent morphological differences. At present, the genus would comprise the species: *N. dorsalis* and *N. aberrans* (Siddiqi 2000). The identification of these species is based on characteristics of adult stages, especially of the immature female (Manzanilla-López *et al.* 2002).

Nacobbus dorsalis has limited geographical distribution, having being found occasionally in a few fields in California (USA) (Sher 1970), whereas *N. aberrans* has been detected in Argentina, Bolivia, Chile, Ecuador, USA, Mexico and Peru (CABI 2002). The latter species parasitises several crops, native plants and weeds. It is the principal pathogen affecting potato production in the Andean region of South America, with losses ranging between 10.9–61.5% (Franco 1994). *Nacobbus aberrans* is an agricultural pest of quarantine importance (EPPO 2009). In Argentina, this nematode was found for the first time in 1977 in the province of Tucumán, infecting potato, wild potato and other horticultural crops (Costilla *et al.* 1977). At present, the species is widely distributed in the country, encompassing very different natural and agricultural environments and presenting a wide host range (Doucet & Lax 2005).

Up to date, there is no agreement on the taxonomy of *N. aberrans*. Variability in morphometric characters and some morphological traits of populations of different geographical origin has been observed (Doucet 1989; Doucet & Di Rienzo 1991; Manzanilla-López *et al.* 1999; Lax *et al.* 2007a). The difficulties in partitioning morphological and morphometric variation within *N. aberrans sensu* Sher have been attributed to cryptic or sibling species (Manzanilla-López 2010). In addition, variability in other aspects has been revealed for populations of this species, which has generated further controversy: (i) host range, indicating the existence of physiological 'races/biotypes/groups' (Inserra *et al.* 1985; Costilla 1990; Castiblanco *et al.* 1999; Manzanilla-López *et al.* 2002; Lax *et al.* 2011); (ii) reproductive potential of a single population on different plants (Toledo *et al.* 1992, 1993; Lax *et al.* 2006, 2011); (iii) number of chromosomes within and among populations of South America (Anthoine & Mugniéry 2005a); (iv) isoenzyme phenotypes (Doucet & Gardenal 1992; Ibrahim *et al.* 1997; Doucet *et al.* 2002). Moreover, genetic variability was observed using molecular markers, such as RAPD-PCR (Ibrahim *et al.* 1997) and ISSR (Lax *et al.* 2007b).

Molecular approaches using ribosomal DNA (rDNA) sequences from ITS regions have proven to be a useful diagnostic tool for identification and phylogenetic studies within plant-parasitic nematode species (Palomares-Rius *et al.* 2010; Cantalapiedra-Navarrete *et al.* 2013). This region has also been useful to reveal sequence divergence between populations of several species (Tanha Maafi *et al.* 2003; Madani *et al.* 2004, 2007). The ITS gene revealed the presence of different groups, supporting the hypothesis that *N. aberrans sensu lato* would be a species complex (Reid *et al.* 2003; Anthoine & Mugniéry 2005b; Vovlas *et al.* 2007; Ramirez-Suarez 2011).

The large subunit (LSU) of rDNA contains highly variable regions called divergent domains (D) or expansion segments. The D2-D3 expansion segments of the 28S rDNA have been used to infer phylogenetic relationships among species of different genera of plant-parasitic nematodes, such as *Longidorus* (Palomares-Rius *et al.* 2010), *Meloidogyne* (Castillo *et al.* 2003), *Pratylenchus* (Al-Banna *et al.* 1997), *Rotylenchus* (Cantalapiedra-Navarrete *et al.* 2013) and *Xiphinema* (Gutiérrez-Gutiérrez *et al.* 2011). Up to the present, there are only four partial sequences of the D3 domain published in GenBank, three corresponding to an *N. aberrans* population from Argentina (Vovlas *et al.* 2007) and one from USA (Al-Banna *et al.* 1997).

The objective of this study was to analyse the phylogenetic relationships among populations of *N. aberrans s.l.* based on ITS and D2-D3 sequences.

Materials and methods

Nematode populations

Ten Argentine *N. aberrans s.l.* populations of different geographical origin were considered for this study; a population from Ecuador was also included (Table 1). Some of the populations studied were previously characterised: El Pucará del Aconquija (Catamarca Province), Río Cuarto (Córdoba Province), Tunuyán (Mendoza Province) (Lax *et al.* 2007a), Lules and Tafí del Valle (Tucumán Province) (Doucet & Di Rienzo 1991) and Coronel Baigorria (Córdoba Province) (Doucet 1989). The remaining populations were identified as *N. aberrans s.l.* (EPPO 2009) according to the description of Sher (1970). Juveniles (third and fourth stages), males and immature females were obtained from infected host roots. Specimens from Jujuy Province were extracted from the skin of Andean potato using the technique proposed by Costilla (1985). Nematodes were individually transferred to a sterile 0.5-mL tube containing 5 μL of double distilled water.

Table 1 List of *Nacobbus aberrans sensu lato* populations used in the study

Code	Origin	Altitude	Host	GenBank accession number	
				ITS	D2-D3
	Argentina				
LOL	Buenos Aires (Lisandro Olmos)	29 m	Tomato (*Lycopersicon esculentum*)	KF254308–KF254310	KF178900
PUC	Catamarca (El Pucará del Aconquija)	1722 m	Potato (*Solanum tuberosum*)	KF254313–KF254315	KF178903–KF178904
SMP	Catamarca (Santa María)	1890 m	Pepper (*Capsicum annuum*)	KF254316–KF254318	KF178905
CBQ	Córdoba (Coronel Baigorria)	525 m	Quinoa (*Chenopodium album*)	KF254319–KF254322	KF178906–KF178907
RCT	Córdoba (Río Cuarto)	430 m	Tomato	KF254307	KF178899
TUN	Mendoza (Tunuyán)	883 m	Tomato	KF254311–KF254312	KF178901–KF178902
SFE	Santa Fe (Recreo)	20 m	Sugarbeet (*Beta vulgaris*)	KF254301–KF254303	KF178895–KF178896
LUL	Tucumán (Lules)	419 m	Tomato	KF254304–KF254306	KF178897–KF178898
TAF	Tucumán (Tafí del Valle)	2062 m	Potato	KF254323–KF254325	KF178908–KF178909
JUJ	Jujuy	3440 m	Andean potato (*S. tuberosum* subsp. *andigenum*)	KF254326–KF254328	KF178910–KF178912
	Ecuador				
ECU	Pichincha (El Quinche)	2500 m	*Gypsophila* sp.	KF254329	–

DNA extraction, PCR and sequencing

Nematode DNA was extracted from single individual, and protocols for PCR were performed as described by Lax *et al.* (2007b). Oligonucleotides used for ITS region were the universal 18S-28S primers spanning the 3′ end of the 18S rDNA to the 5′ end of the 28S rDNA, including the ITS1 and ITS2 regions and the 5.8S rDNA (Vrain *et al.* 1992). The D2-D3 expansion segments of 28S rDNA were amplified using the forward D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and reverse D3B (5′-TCGGAAGGAACCAGCTACTA-3′) primers (Al-Banna *et al.* 1997). PCR amplifications were performed in a Mastercycler Eppendorf thermal cycler programmed for an initial denaturation at 94 °C for 3 min, followed by 39 cycles of 30 sec at 93 °C, 90 sec at 48 °C, 1 min at 72 °C and a final extension of 10 min at 72 °C. Negative controls were added in all assays to discard contamination. Amplification products were separated by electrophoresis on 1% agarose gel in 0.5X TBE buffer. A molecular weight marker of 100-bp DNA Ladder (Promega, Madison, WI, USA) was used. Gels were stained with ethidium bromide and photographed with a Kodak-DC digital camera under a UV transilluminator. PCR amplifications from three or four different specimens of each population were purified and sequenced in both directions by Macrogen Korea Inc using the same primers mentioned above. The newly obtained sequences were submitted as *Nacobbus* sp. to the GenBank database under accession numbers indicated in bold on the phylogenetic trees.

Phylogenetic analyses

Analyses were performed with all the ITS sequences obtained in the present study and data of *Nacobbus* spp. available from GenBank; accession numbers of previously published sequences are given in Fig. 1. A sequence of *Pratylenchus vulnus* (FJ713011) was selected as outgroup. Regarding the D2-D3 expansion segments, only the results of the present study were considered in the analyses because of the lack of information in the database.

Nucleotide alignments were produced using ClustalX 2.0 (Larkin *et al.* 2007) with default parameters. Sequence alignments were manually edited using BioEdit (Hall 1999). Phylogenetic analyses of the data set of both markers were performed with maximum likelihood (ML) based on the Tamura–Nei model (Tamura *et al.* 2004) using the Molecular Evolutionary Genetics Analysis 5 (MEGA 5) software (Tamura *et al.* 2011). The estimation of the support for each node was assessed by bootstrap analysis with 1000 replicates.

ITS data set was also analysed using Bayesian inference (BI) with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The best fitted model of DNA evolution was obtained using jModelTest 0.1.1 (Posada 2008) with the Akaike information criterion. The SYM + G model was selected. Two independent runs were performed simultaneously on the data, each one using one cold and three heated chains. After 9 million generations, the average standard deviation of split frequencies between the two independent runs at completion was 0.007. After discarding 25% of burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities are given on appropriate nodes. Trees were visualised using TreeView (Page 1996).

The Arlequin Package version 3.11 (Excoffier *et al.* 2005) was used to calculate pairwise F_{ST} among the groups defined in the phylogenetic analysis. The Tamura and Nei nucleotide substitution model (Tamura & Nei 1993) was applied with 1023 permutations. To estimate the degree of genetic similarity among goups, we first calculated the

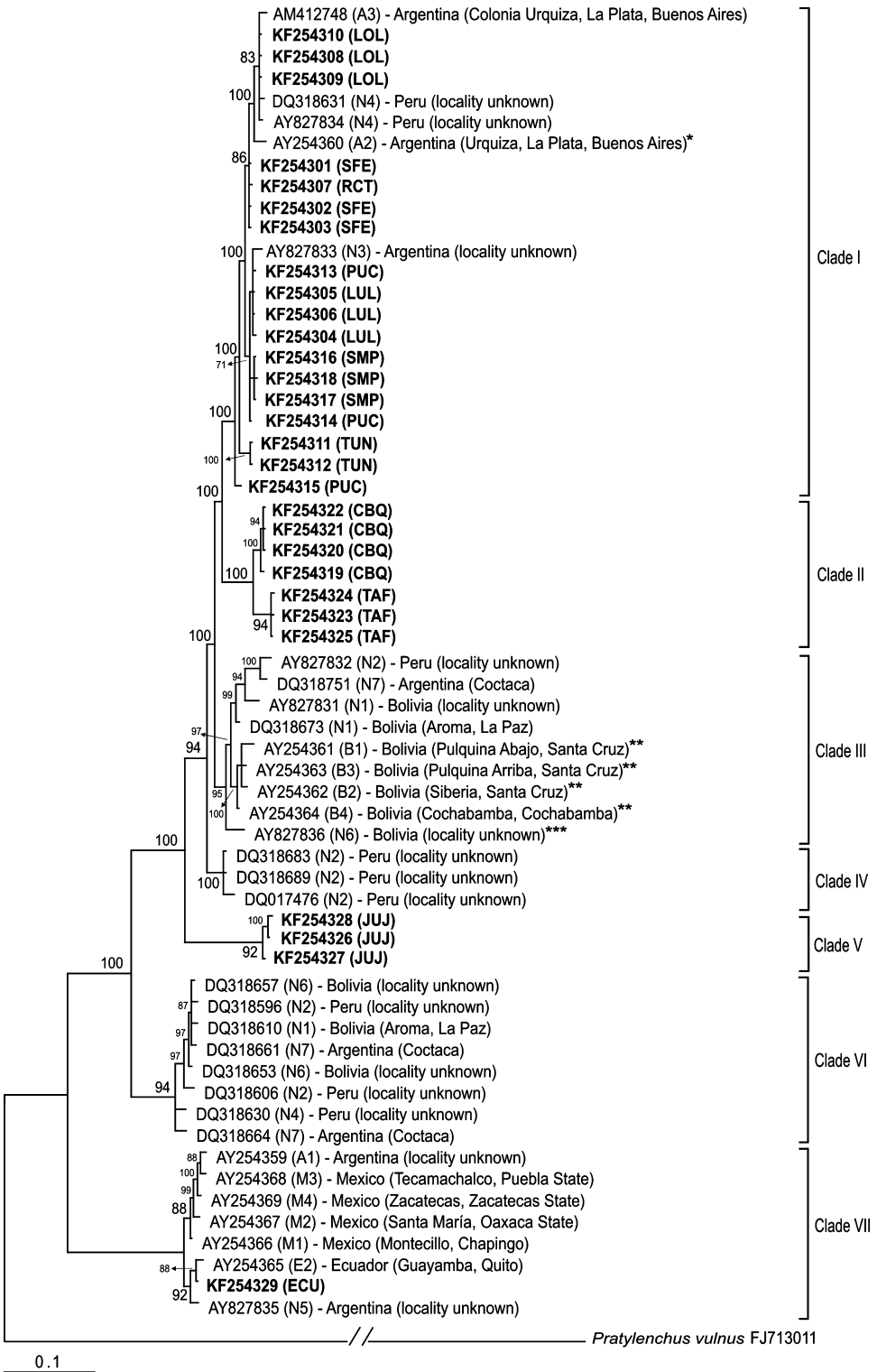

Fig. 1 The 50% majority-rule consensus tree from Bayesian analysis generated from the ITS rDNA gene data set with the SYM + G model; indication of seven highly divergent clades. Posterior probabilities more than 65% are given for appropriate clades. Newly obtained sequences are in bold letters. Sequence uploaded as *Nacobbus* sp.(*) and *N. bolivianus* (**); (***) Argentina was indicated as the country of origin in the GenBank; however, Bolivia was indicated as the origin in the publication by Anthoine & Mugniéry (2005b).

percentages of similarity values among pairs of all ITS and D2-D3 sequences using the BioEdit program (Hall 1999); then, we considered the range of variation of those values for comparison between group pairs.

Results

ITS sequences obtained in the present work (29 in total) varied *ca* 885–905 bp (Argentine populations) and 913 bp (Ecuadorian population) in length. For phylogenetic analyses, 62 ITS sequences of *Nacobbus* spp. from different geographical regions and hosts were considered. The majority-rule consensus tree of the BI revealed the presence of seven clades (Fig. 1), with high support values, defined mainly by their geographical location, with some exceptions. Clade I was composed of most of the Argentine populations considered in the present work (LOL, SFE, RCT, LUL, SMP, PUC and TUN). Sequences of other populations of the same country, published in GenBank, were also included in this group (AY254360, AY827833 and AM412748) and sequences from Peru (AY827834 and DQ318631). Clade II was composed of individuals from the Argentine localities of Córdoba (CBQ) and Tucumán (TAF), whereas clades III and VI comprised GenBank sequences of individuals from Peru, Bolivia and one population from northern Argentina. Clades IV and V included sequences of individuals from Peru and from the Andean region of Argentina (JUJ), respectively. Clade VII comprised known sequences of specimens from Mexico and Ecuador, including a sequence obtained in the present work (ECU) and two Argentine sequences of unknown origin (AY827835 and AY254359). An ML analysis of the total ITS sequences produced a tree with the same topology.

The phylogenetic analysis (ML) based on D2-D3 expansion segments of 28S rDNA including the 18 sequences obtained in this work (754–755 bp in length) showed three highly supported groups (Fig. 2). The tree topologies were congruent and showed a similar clustering to that obtained for ITS region for the same Argentine populations. One group was composed of most of the populations included in this work (Clade I). Furthermore, the sequences of individuals from CBQ and TAF were grouped together (Clade II) and Clade V (according to the denomination using ITS) comprised specimens from the north of Argentina associated with Andean potato (JUJ).

Based on the seven groups defined by phylogenetic analyses (BI and ML) considering the ITS region, a matrix of pairwise F_{ST} and P values is presented in Table 2. F_{ST} values comparing pairs of groups ranged between 0.590–0.959, showing significant differences between them. Similarity percentages from ITS region among the different clades are also presented in Table 2. When the D2-D3 expansion segments were considered, the three groups defined in the phylogenetic analyses also showed significant differences, with F_{ST} values ranging between 0.644–0.864 (Table 3). Similarity percentages obtained from this gene varied between 97–99% (Table 3).

Discussion

Nacobbus aberrans s.l. is widely distributed in Argentina, occurring in sites located up to 4500 m asl and encompassing different phytogeographical regions. Up to the present, sequences of the ITS region of six Argentine populations have been published in GenBank database; here, we add 10 new populations from this country

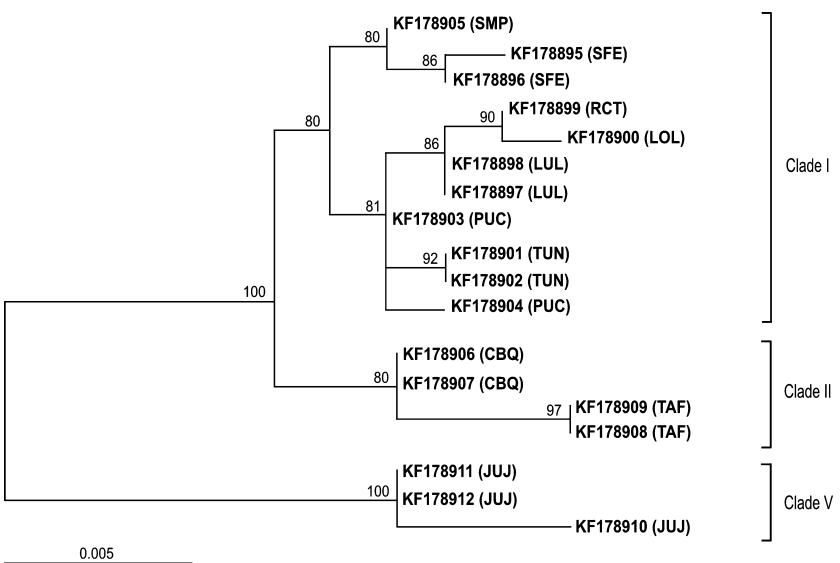

Fig. 2 Maximun likelihood phylogenetic tree based on D2–D3 expansion segments of 28S rDNA gene of *Nacobbus* populations from Argentina; indication of the three divergent clades, according to the same denomination used in ITS. Bootstrap values are given in nodes.

Table 2 Genetic differentiation and similarity values (%) among clades of the *Nacobbus aberrans* complex obtained through Maximum likelihood and Bayesian Inference of the ITS region (F_{ST} values: below diagonal; similarity values: above diagonal)

Clade	1	2	3	4	5	6	7
1	–	90–92	89–92	91–93	86–87	83–85	73–75
2	0.787*	–	88–93	90–91	87	83–84	72–75
3	0.677*	0.669*	–	92–94	86–89	82–85	72–76
4	0.755*	0.825*	0.590*	–	87–88	84–85	72–74
5	0.905*	0.918*	0.827*	0.959*	–	84–85	73–75
6	0.901*	0.885*	0.850*	0.878*	0.920*	–	76–79
7	0.945*	0.938*	0.912*	0.945*	0.957*	0.928*	–

*Significant differences after 1023 permutations ($P < 0.05$).

Table 3 Genetic differentiation and similarity values (%) among clades the *Nacobbus aberrans* complex obtained through Maximum Likelihood of the D2–D3 expansion segments (F_{ST} values: below diagonal; similarity values: above diagonal)

Clade	1	2	5
1	–	98–99	98
2	0.644*	–	97–98
5	0.819*	0.864*	–

*Significant differences after 1023 permutations ($P < 0.05$).

associated with different hosts and covering a broad geographical distribution.

Phylogenetic studies of the ITS rDNA region showed seven statistically well-supported clades within *N. aberrans s.l.* The high and significant F_{ST} values obtained among these groups confirmed this partitioning. Genetic similarity percentages among clades (between 72 and 94%) were comparable to those reported for different species of plant-parasitic nematodes considering the same gene (Cantalapiedra-Navarrete *et al.* 2013).

Most of the Argentine populations formed a well-defined group (Clade I) that comprised sequences published in GenBank and others obtained in the present work, mostly of individuals from regions located between 29–1900 m asl: (i) Pampas (central-eastern region): provinces of Buenos Aires (LOL, A2: AY254360, A3: AM412748), Santa Fe (SFE) and Córdoba (RCT); (ii) Cuyo: province of Mendoza (TUN); (iii) north-western region: provinces of Catamarca (PUC and SMP) and Tucumán (LUL). This group also included a sequence known for other Argentine population (coded as N3, Catamarca Province) and individuals from Peru (N4; unknown locality) (Anthoine 2006). This clear separation of Argentine specimens from other South and North American populations was already observed in previous studies that considered populations A2, indicated as *Nacobbus* sp. (Reid *et al.* 2003), A3 (Vovlas *et al.* 2007) and N3 (Anthoine & Mugniéry 2005b). According to Reid *et al.* (2003) population A2 would represent a new taxon within the genus *Nacobbus*, which would also include the other sequences grouped in Clade I.

Clade II included sequences from other Argentine populations (CBQ, Córdoba and TAF, Tucumán) of nematodes naturally developing on quinoa and potato, respectively. In previous studies (Lax *et al.* 2011), these two populations were clearly distinguished from other Argentine populations by restriction patterns of the ITS region for the enzymes *Hin*fI and *Rsa*I (Reid *et al.* 2003; Anthoine & Mugniéry 2005b; Vovlas *et al.* 2007). In addition, the CBQ population showed different isoenzyme phenotypes (Doucet & Gardenal 1992) and high genetic divergence using ISSR markers (Lax *et al.* 2007b) with respect to other populations from the country. CBQ and TAF populations belong to different 'groups/races' according to their behaviour on differential hosts (Lax *et al.* 2011). The sampling sites are about 770 km apart and present different geographical characteristics, mainly altitude (CBQ: 525 m asl; TAF: 2065 m asl); the area of Tafí del Valle (Tucumán) is typically cultivated with seed potato. This nematode is able to infect also the tuber skin, a characteristic that would favour passive transport of the parasite among sites (Lax *et al.* 2008) and that could explain the high genetic similarity between TAF and CBQ populations.

Clades III, IV, V and VI included South American populations from the Andean region and others of unknown origin (N2, N6). Some of those populations were obtained from potato fields (Reid *et al.* 2003) or indicated as belonging to the 'potato race' (Anthoine & Mugniéry 2005b). Clade III was composed of GenBank sequences of specimens from northern Argentina (N7) and from unknown localities of Peru (N2) and Bolivia (N6). This group also included different Bolivian localities from the departments of La Paz (N1), Santa Cruz (B1, B2, B3) and Cochabamba (B4). The last four sequences were submitted to GenBank as *N. bolivianus* Lordello, Zamith & Boock, 1961 (Reid *et al.* 2003); the authors considered this species as valid based on the uniform RFLP banding patterns and also by the fact that the population B4 (Cochabamba, Cochabamba Department) came from the type locality of *N. s. bolivianus*. However, Lordello *et al.* (1961) mentioned that the type locality was located 85 km far from Cochabamba city (Bolivia), but did not provide the geographical coordinates. Based only on four populations, Reid *et al.* (2003) concluded that *N. bolivianus* is an apparently indigenous species widespread in Bolivia and also present in Peru. However, it is important to note that Clade VI included sequences from the same populations grouped in Clade III (N1, N2, N6 and N7), as well as one sequence from Peru (N4). Clade VI is characterised by encompassing short

sequences of the ITS region (917–923 bp), whereas the sequences of Clade III were longer (946–952 bp). Populations N1, N2, N4, N6 and N7 were amplified by Anthoine (2006) using the primers provided by Vrain *et al.* (1992); she reported this difference in length (approximately between 920 and 950 bp) and, according to the population, some individuals having one type, whereas others had the two types. Based on the presence of each ITS type sequence, the author concluded that these South American populations would be the result of two or more hybridisation events. These observations show the existence of a high genetic variability within what would be considered a 'population' from a single geographical locality. For this reason, and because Clade VI also comprises sequences from Bolivia of unknown locality (N6), it is not possible to ensure that Clade III or the populations B1, B2, B3 and B4 would correspond to *N. bolivianus*, as claimed by Reid *et al.* (2003). New nematode populations from Cochabamba Department (Bolivia) should be included in the phylogenetic analyses. In addition, the variation of the ITS sequences within the N2 population (Peru) was noticeable, being separated into three different clades: III, IV (948–952 bp) and VI (917–923 bp). All these observations highlight the need for analysing several specimens from the same geographical population for taxonomic and phylogenetic nematode studies. Intrapopulation variation of the ITS region was not observed in the Argentine populations here analysed or in previous studies (Reid *et al.* 2003; Vovlas *et al.* 2007).

Grenier *et al.* (2010) suggested that geological events as the uplift of the Andes may have played a crucial role in speciation of cyst-forming nematode species, considering the Andes as a 'species factory'. The same process might have contributed to the speciation of *Nacobbus*, whose geographical origin would also be the Andean region (Bridge & Starr 2007), as suggested by the fact that the clades III, IV, V and VI recovered in the present work grouped sequences of specimens from that area. Among the Argentine populations here considered, we highlight the significant genetic divergence of JUJ in Clade V, probably as a consequence of a founder effect, with absence of gene flow with nearby populations. In the Andean region, the exchange of tubers among communities is a frequent activity; the passive transport of nematodes in seed potato and the cultivation of infected tubers from other region in contaminated soil might promote the formation of natural hybrids. Further phylogenetic and phylogeographical studies focusing on unexplored geographical regions in the Andes might reveal a greater genetic diversity than that currently known and allow us to infer colonisation patterns in the genus.

Clade VII included published sequences from central Mexico (M1-M4), two from Argentina (A1 and N5, unknown localities) and two from Ecuador. Anthoine & Mugniéry (2005a) obtained sterile, non-viable progeny from laboratory crosses between individuals of population N5 and specimens belonging to different South American populations (N1, N2, N3 and N4) and concluded that N5 might represent a different species. Crossing experiments with specimens from other populations included in clade VII would help to define their taxonomic status.

Besides the seven clades here detected within *N. aberrans s.l.* using the ITS region, there would be two other groups according to recent reports: one corresponding to individuals from Nebraska (USA), the type locality of *N. aberrans* and another group composed of sequences of specimens from northern Mexico (Ramirez-Suarez 2011). This fact reinforces the idea of a great genetic diversity in the genus *Nacobbus*, mainly in populations from South America.

Based on the ITS region, the Argentine populations considered in this work grouped into three different clades (I, II and V), which coincides with our present results using the D2-D3 expansion segments. As there are no other GenBank sequences available for the D2-D3 segments, it was not possible to make comparisons with other populations from South and North America. Although these segments exhibited lower genetic variation (97–99% similarity among clades), they showed to be useful for establishing the phylogenetic relationships among Argentine populations, since they are similar to those obtained using the ITS region. Percentages of genetic similarity within the same range values have been observed among different *Rotylenchus* spp. for the 28S gene (Cantalapiedra-Navarrete *et al.* 2013). Minimal sequence differences in rDNA genes might be the result of a recent speciation (Subbotin *et al.* 2008).

As nematodes of the genus *Nacobbus* generate great interest worldwide, researchers from different institutions and countries have exchanged biological material; this activity might generate errors in the identification of populations. For example, Argentine sequences AY254359 (A1) and AY827835 (N5), both of unknown origin, clustered with Mexican populations (Reid *et al.* 2003; Anthoine & Mugniéry 2005b); they might correspond to samples that have been wrongly labelled regarding their geographical origin. In addition, sometimes there are labelling problems generated during data submission to GenBank. Such is the case of the AY827836 sequence which, according to Anthoine & Mugniéry (2005b), was from Bolivia; however, Argentina was indicated as the origin in the database.

The significative degree of genetic divergence observed among *Nacobbus* populations from South and North America clearly indicates the existence of cryptic species within the *N. aberrans s.l.* complex. Integration of morphological and morphometric studies and molecular analyses considering

other genes may clarify the identification of species and their phylogenetic relationships within this genus.

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