MOLECULAR BASED-PHYLOGENETIC RELATIONSHIPS IN THE SUPERFAMILY CRICONEMATOIDEA USING ITS1-rDNA

Marco A. Cordero[1], Robert T. Robbins[1] and Allen L. Szalanski[2]

[1]Department of Plant Pathology, Cralley/Warren Research Laboratory, 2601 N. Young Ave., University of Arkansas, Fayetteville, AR 72704; [2] Department of Entomology, University of Arkansas, Fayetteville, AR 72701; Corresponding author:mcordero@uark.edu

ABSTRACT

Cordero, M, R. T. Robbins, and A. L. Szalanski. 2013. Molecular based-phylogenetic relationships in the superfamily Criconematoidea using ITS1-rDNA. Nematropica 43:145-151.

Populations of nematodes of the superfamily Criconematoidea were obtained and identified morphologically from different geographical areas in the continental United States. This previous information was used to analyze their phylogenetic relationship based on the first internal transcribed spacer ribosomal region. Phylogenetic analysis of the ITS1-rDNA region showed monophyletic and paraphyletic groups in Criconematidae: A) species of *Mesocriconema* and *Criconemoides*; and B) *Bakernema, Criconema Hemicriconemoides* and *Xenocriconemella* species. Subfamily Hemicycliophorinae, (*Caloosia, Hemicaloosia* and *Hemicycliophora*) and family Tylenchulidae (*Paratylenchus* and *Gracilacus*) were not resolved because of the high variation of the marker Molecular phylogenetic analysis using the ITS1-rDNA marker rejects the hypothesis of a common ancestor for criconematids with double sheath or cuticle among others characters. This molecular phylogenetic study showed that ITS1-rDNA sequences are too variable to study phylogenetics of Criconematoidea. However, ITS1-rDNA is useful to identify and to characterize species within families.

Key words: Bayesian analysis, Criconematoidea, genetic variation, ITS1-rDNA, internal transcribed spacer 1, phylogeny, ribosomal DNA.

RESUMO

Cordero, M., R. T. Robbins, and A. L. Szalanski. 2013. Relaciones filogeneticas moleculares en la superfamilia Criconematoidea usando ITS1-rADN. Nematropica 43:145-151.

Poblaciones de nematodos pertenecientes a la superfamilia Criconematoidea fueron obtenidos e identificados morfológicamente desde diferentes áreas geográficas de los Estados Unidos. Esta información previa fue usada con el fin de analizar relaciones filogenéticas usando la región ribosomal ITS1. El análisis filogenético mostro la posibilidad de grupos monofileticos and parafileticos en los grupos pertenecientes a: A) especies de *Mesocriconema* y *Criconemoides*; y B) especies de *Bakernema, Criconema Hemicriconemoides* y *Xenocriconemella*. Sin embargo, el marcador ITS1-rDNA no definió completamente las relaciones entre los miembros de la subfamilia Hemicycliophorinae, (*Caloosia, Hemicaloosia* and *Hemicycliophora*) y la familia Tylenchulidae (*Paratylenchus* and *Gracilacus*) posiblemente a la alta variación genetica del marcador. El análisis filogenético molecular usando el marcador ribosomal ITS1 rechazó la hipótesis de la existencia de un ancestro común para criconematidos con doble cutícula, entre otros caracteres. El presente estudio mostró que el marcador ITS1- rDNA es demasiado variable para estudiar las relaciones filogenéticas de Criconematoidea. Sin embargo, ITS1-rDNA es útil para identificar y caracterizar molecularmente las especies pertenecientes a diferentes familias dentro del grupo.

Palabras clave: análisis bayesiano, Criconematoidea, DNA ribosomal, filogénesis, ITS1-rDNA, variación genética, primer espaciador interno.

INTRODUCTION

The history of the superfamily Criconematoidea Taylor, 1936 began in 1882-1883 at the international expedition to Hoste Island, Chile from which a juvenile of *Criconema giardi* (Certes, 1889) Micoletsky, 1925 was described, representing the earliest specimen described of the superfamily which became the type species of *Criconema* Hoffmänner & Menzel, 1914 (Taylor, 1936; Raski *et al*., 1984; Raski and Luc, 1985). Following years of new species descriptions, two systems of classification for this group have been proposed. In one system, the superfamily Criconematoidea was raised to the suborder Criconematina by Siddiqi (1980, 2000) with three superfamilies Criconematoidea, Hemiciclyophoroidea and Tylenchuloidea. On the other hand, Raski and Luc (1987) proposed the superfamily Criconematoidea consisting of only two families Criconematidae and Tylenchulidae. The morphological character that characterizes the superfamiliy Criconematoidea is the typical criconematoid oesophagus characterized by having a median bulb or metacorpus enormously developed, with a large median valvular apparatus, metacorpus and a broad procorpus are amalgamated and surrounded the basal region of the stylet. At postcorpus, the isthmus could be long and off set from the basal bulb or short and broad and fused with a small basal bulb, containing three oesophageal glands (Raski and Luc, 1987). However, the group shows diverse degrees of variation on morphological characters among the species which frequently makes their identification difficult (Geraert, 2010; Raski and Luc, 1987; Siddiqi, 2000).

Currently, molecular phylogeny is an excellent method to determine relationships among taxa based on the information resulting from different molecular markers as well as morphological identification. The nuclear ribosomal genes, 18S and 28S, have low variability (i.e. low rate of evolution) with the 28S gene less conserved than 18S. These two important genetic markers are currently used to study phylogenetic studies on different organisms in the same taxa that diverged long ago. Conversely, the ITS1 and ITS2 regions of rDNA have a high rate of evolution because of frequent mutational events however; some similarities in the ITS sequence regions tend to be greater within species than among species, with exception of *Meloidogyne* species which intraspecific variation is too high that the use of this marker is not reliable for species discrimination (Gasser, 2001; Powers, 2004; Blaxter, 2001; Subbotin and Moens, 2006). In recent years, evidence of intra-specific and intra-individual variation in nematode nuclear ribosomal DNA sequences, including ITS, has been reported (Cutillas *et al*., 2004; Hugall *et al*., 1999; Mes and Cornelissen, 2004; Porazinska *et al*., 2010).

Because of their low intraspecific variation, nuclear rDNA transcriber regions have been used as markers for species identification in several nematodes, representing useful information in order to develop tools for diagnostic purposes based on PCR reactions (Gasser, 2001). Recently, a phylogenetic analysis in Criconematoidea based on the D2-D3 expansion identified as a segment which is a less conserved region of the 28S-rDNA gene, named divergent domains, supported monophyly of the genera *Mesocriconema*, *Hemicriconemoides* and *Criconema* (Subbotin, 2005). In addition, a single origin of criconematids with single or double cuticle was rejected showing the usefulness of this marker to discriminate among characters that result from common ancestry versus those that are homoplasious. (Subbotin, 2005).

Lately, in a study by Powers *et al*., (2010), sequences of ITS1-rDNA region were obtained from *Discocriconemella inarata* Hoffman, 1974; *M. curvatum* (Raski, 1952) Loof & De Grisse, 1989; *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989 and *M. xenoplax* (Raski, 1952) Loof & de Grisse, 1989 which showed evidence for the paraphyly of *Discocriconemella* and *D. inarata* was related with species of *Mesocriconema*.

This study followed the classification system for the superfamily Criconematoidea of Raski and Luc (1987) and Maggenti *et al*., (1988). The genera *Mesocriconema* Andrássy, 1965 and *Criconemoides* Taylor, 1936 are used in accordance with their re-establishment and validation, respectively (Loof and De Grisse, 1967; Loof and De Grisse, 1989). Previously, a taxonomy study and ITS1-rDNA were used to identify and to characterize *Mesocriconema* and *Criconemoides* species (Cordero *et al*., 2012a), spines nematodes like *Bakernema, Criconema, Ogma, Xenocriconemella* and *Hemicriconemoides* (Cordero *et al*., 2012b) and *Hemicaloosia, Hemicycliophora, Gracilacus* and *Paratylenchus* (Cordero *et al*., 2013). The objective of this study was to use this previous data obtained by the authors to analyze phylogenetic relationships of the superfamily Criconematoidea using ITS1-rDNA.

MATERIALS AND METHODS

The following methodology was previously used to identify and characterize different species of Criconematoidea and to report GenBank accession numbers of known and new species (Cordero *et al*., 2012a; Cordero *et al*., 2012b; Cordero *et al*., 2013). Additional sequences downloaded from GenBank (Table 1) used previously are cited here as reference.

Nematodes were collected from undisturbed natural locations in Arkansas, USA from 2008 to 2011 and a handheld global positional system device (GPS) (Etrex Garmin, Olathe, KS) was used to identify and record the location. Additional populations of nematodes were received in 1M NaCl from California, Florida, Kansas, Missouri, North Carolina and Tennessee. Nematodes collected in Arkansas were extracted from soil by Cobb sieving and flotation-centrifugation methods (Cobb, 1918; Jenkins, 1964). Specimens of each

Table 1. Supplemental ITS1-rDNA sequences of Criconematoidea obtained from GenBank for the Bayesian analysis.

	GenBank number	Host	Origin
Caloosia longicaudata	GU989621	Unknown	Hawaii, USA
Discocriconemella inaratus	HM116055	Tallgrass prairies	USA
Gracilacus aculenta	EU247526	Bamboo	Taiwan
Gracilacus bilineata	EU247525	Bamboo	Taiwan
Helicotylenchus sp.	AB602604	Bermuda grass	Japan
Hemicaloosia_graminis	JQ446376	Turfgrass	USA
Hemicriconemoides californianus	EU180057	tea	Taiwan
Hemicriconemoides kanayaensis	EF126179	tea	Taiwan
Hemicriconemoides parasinensis	EU664601	Grape	Taiwan
Hemicriconemoides stricthatecatus	GQ354786	Unknown	Taiwan
Hemicycliophora lutosa	GQ406237	Fallow soil	South Africa
Hemicycliophora typica	GQ406239	Sugar cane	South Africa
Mesoscriconema curvatum	HM116066	Tallgrass prairies	USA
Mesoscriconema xenoplax	HM116057	Tallgrass prairies	USA
Mesoscriconema xenoplax	HM116073	Tallgrass prairies	USA
Ogma decalineatum	HM116075	Tallgrass prairies	USA
Paratylenchus lepidus	EF126178	tea	Taiwan
Paratylenchus minutus	EF126180	tea	Taiwan
Scutellonema brachyurum	DQ316097	Unknown	Taiwan

population were prepared in two copies: 1) nematodes for morphological identification and 2) nematodes for molecular analysis. For morphological identification, nematodes were fixed in hot 3% formaldehyde for one week and later infiltrated with glycerine using the modified slow method of Seinhorst (Seinhorst, 1959; Seinhorst 1962). A range of 5 to 10 nematodes for molecular work were used for each population. Nematodes were crushed individually in 5µl of PCR water (BDH Chemicals, Chester, PA) and store at -80oC until use.

PCR

Polymerase chain reaction (PCR) was performed using 5 µl of a DNA extraction in a 50-µl PCR reaction mixture. Primers used to amplified the entire ribosomal ITS1 region (ITS1-rDNA) and the 5' end of the 5.8S rDNA gene were rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') (Vrain *et al*., 1992) and rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA- 3') (Cherry *et al*., 1997). The PCR mixture contained 4 µl of dNTP-mixture (0.2 mM each final concentration) (Qiagen, Valencia, CA), 1 µl of each primer (0.4 µM), 0.4 µl (2 units) *Taq* DNA polymerase (New England Biolabs, Ipswich, MA) and 5 µl 10 X ThermoPol reaction buffer (New England Biolabs, Ipswich, MA). PCR was conducted using a Hybaid Express thermal cycler [Thermo Hybaid, Middlesex, UK] with the follow parameters: denaturation at 94°C for 2 minutes, then 40 cycles of denaturation at 94°C for 45 seconds, annealing at 52 and 56°C for 45 seconds and extension at 72°C for 60 seconds. A final extension for 5 minutes at 72°C was performed. Visualization of PCR product was performed using a 5 µl of PCR product and 100 bp DNA ladder (Promega, Madison, WI) subjected to electrophoresis on a 1% agarose gel stained with ethidium bromide. An UV transluminator (BioDoc-it™ system, UVP, Upland, CA) was used to visualize PCR products.

Sequencing

PCR products were purified using Nanosep centrifuge tubes 100 k (Pall, Port Washington, NY) in a refrigerated centrifuge at 15°C for 20 minutes at 13,000 rev. Samples were sequenced in both directions using an Applied Biosystems Model 3100 genetic analyzer by the DNA sequencing core facility at the University of Arkansas Medical School, Little Rock, AR. Pairwise alignment of forward and reverse sequences was performed to obtain consensus sequences of ITS1 amplicon using BioEdit alignment software (Hall,

1999). Alignment of all ITS1 sequences earlier obtained was performed using MAFFT (Katoh *et al*., 2002)

Molecular phylogenetic study

The model of base substitution was evaluated using JModeltest 2.1.1 based on Akaike Information Criterion (AIC) parameters (Dariba *et al*., 2012; Posada and Crandall, 1998; Posada, 2012). The identity matrix and the Bayesian analysis were obtained using MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001) with Geneious Pro 5.6.6 created by Biomatters (http://www.geneious.com). Bayesian analysis was initiated with a random starting tree, running the chain for 1 x 10^6 generations and setting the "burn in" at 100,000. The Markov Chain Monte Carlo method (MCMC) was used to estimate the posterior probability of the phylogenetics trees using 50% majority rule (Larget and Simon, 1999). Sampling in the Markov chain was made with a frequency of 200 generations.

RESULTS

Phylogenetic Study of ITS1 Region

The length of the ITS1-rDNA PCR amplicon ranged between 450 to 640 bp. After alignment of all the consensus sequences, the ITS1 size used for phylogenetic analysis was 549 bp. JModel test estimated the GTR+G model as the best fit (-Ln likelihood = 15770.6970; AIC= 31867.3939; K=163; freqA =0.2323; freqC=0.2740; freqG=0.2758; freqT=0.2179; R(a) [AC]=0.8263; R(b)[AG]=1.7400; R(c)[AT]=1.2319; R(d)[CG]=0.9083; R(e)[CT]=2.2622; R(f)[GT]=1.000; Gamma shape=2.3370) for the analysis (Fig. 1).

The ITS1-rDNA marker data showed the probability of monophyly and paraphyly of *Mesocriconema* and *Criconemoides* species and among spine nematodes *Bakernema, Criconema, Ogma, Xenocriconemella* species and sheathoid nematodes, *Hemicriconemoides*. Monophyly and paraphyly of this species should be clarified adding more sequences in future studies. Also, not all specimens of *Hemicycliophora* were clustered together because the high genetic divergence of three species of *H. zuckermani* and *H. shepherdi. Paratylenchus* and *Gracilacus* were clustered in two groups but their monophyly was not confirmed. A population of *M. xenoplax* which showed the highest genetic divergence (57%) among the rest of the *M. xenoplax* group was clustered along with the most dissimilar species: *M. onoense, M. surinamense* and *Criconemoides informis*. The position of *Hemicaloosia graminis* and *Caloosia longicaudata* were not resolved in this study.

DISCUSSION

Molecular phylogenetic analysis using the ITS1-rDNA region rejects the hypothesis of single origin for genera with a double cuticle or double cuticular sheath in *Hemicycliophora* and *Hemicriconemoides* as this two species were clustered separately, even though, this analysis did not show monophyly of *Hemicycliophora* because the high genetic divergence of ITS1 sequences of 3 species previously mentioned, the monophyly of *Hemicycliophora* was previously reported by Subbotin *et al*., (2005) using the D2-D3 expansion fragment in the ribosomal gene 28S.

Additionally, rejection of one ancestor hypothesis could includes other important characters as presence or absence of sub-median lobes, variations on the criconematoid oesophagus as length of isthmus and size of basal glands in postcorpus, absence of phasmids; regular ectoparasitism, ectoparasitism with sedentary obese females (Family Paratylenchinae: *Paratylenchus, Gracilacus*) and sedentary obese females with or without presence of immature females showing endo or semi-endoparasitism in their life cycle (Family Tylenchulinae: *T. semipenetrans, Trophotylenchus*). (Geraert, 2010; Raski and Luc, 1987; Siddiqui, 2000).

The position of *Mesocriconema sphaerochepala* with a genetic divergence ranged between 42-56% with others species of *Mesocriconema* was not resolved. The uncertainty of the position of *M. sphaerochepala* as sister species of the group is in agreement with the results of the analysis of the D2-D3 expansion segments of the 28S-rRNA by Subbotin *et al*., (2005). Furthermore, *Discocriconemella inarata* was placed as sister species with the spines nematodes in this group. However, Powers *et al*. (2010) found decisive arguments to establish the position of this species as part of *Mesocriconema* group, using ITS1 and cytochrome b.

The presence of some low support values and extremely long branches, i.e., high variation on ITS1-rDNA sequences among families of Criconematoidea, revealed that this marker is not reliable for a phylogenetic study of this group of nematodes. However, the ITS1-rDNA was useful to identify and characterize specimens to species level of Criconematoidea (Cordero *et al*., 2012a; Cordero *et al*., 2012b; Cordero *et al*., 2013). Therefore, others populations of Criconematoidea species should be incorporated along with markers such as mitochondrial DNA (e.g., COI and COII) to have a complete understanding of the phylogenetic relationships based on morphology, biology and molecular information to determine monophyletic and/or paraphyletic groups within subfamilies (Powers, 2004).

In general, the ITS1-rDNA marker has been used successfully with different groups of plant parasitic nematodes to study their phylogenetic relationships. Species of *Ekphymatodera thomasoni* and *Bilobodera flexa*, non-forming cyst nematodes, were clustered with cyst forming nematodes when 18S and ITS1 rDNA were used to study their phylogenetic relationships (Ferris *et al*., 2004). Tanha Maafi *et al*. (2003) using ITS1 rDNA obtained defined group of cyst nematodes: *Avenae, Sacchari, Schatchtii, Humuli, Cyperi* and *Goettingiana* groups based on morphological and molecular

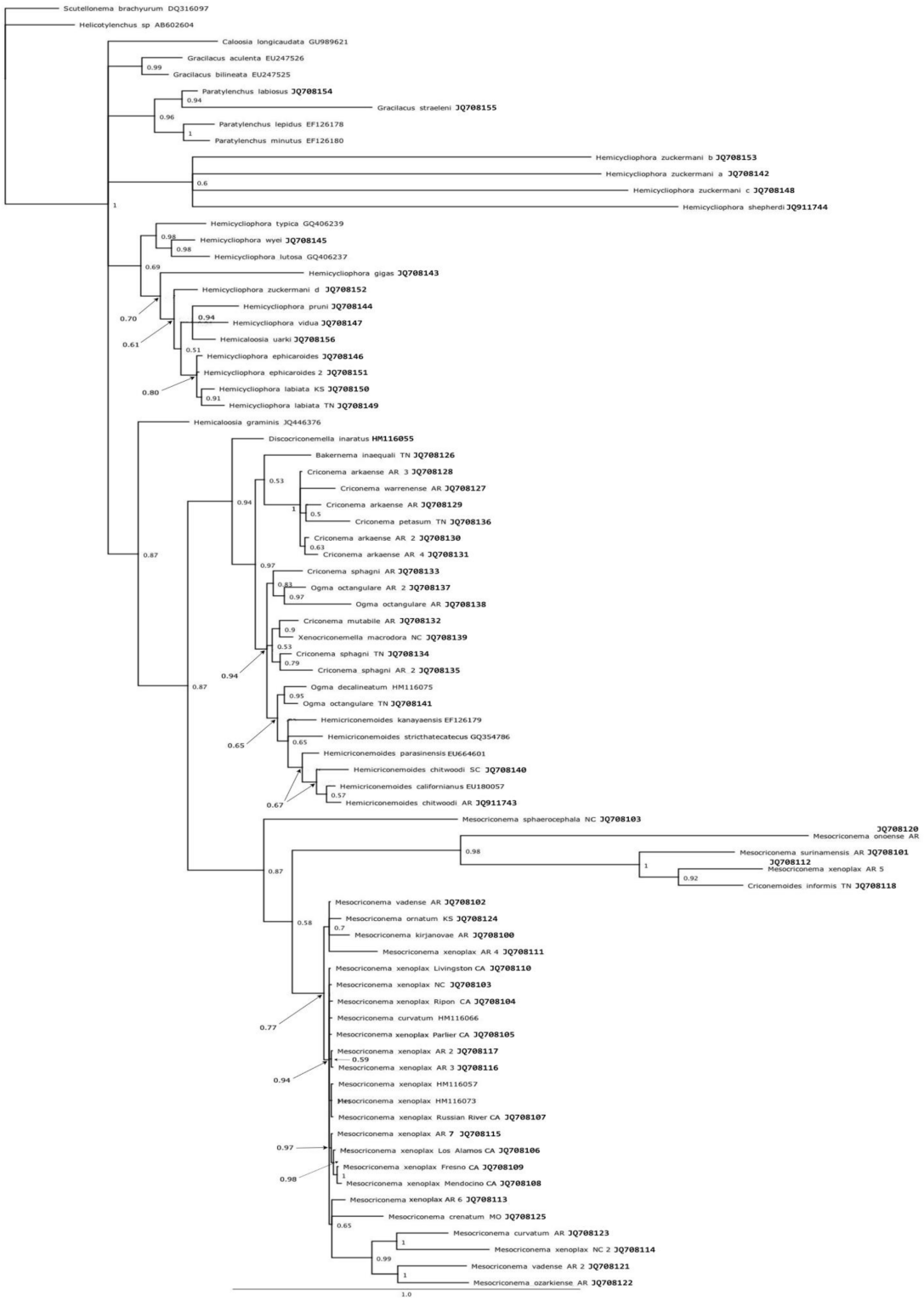

Fig 1. Bayesian inference 50% majority rule consensus tree of ITS1-rDNA region under the GTR+G model (-Ln likelihood = 15770.6970; AIC= 31867.3939; K=163; freqA =0.2323; freqC=0.2740; freqG=0.2758; freqT=0.2179; R(a)[AC]=0.8263; R(b)[AG]=1.7400; R(c)[AT]=1.2319; R(d)[CG]=0.9083; R(e)[CT]=2.2622; R(f)[GT]=1.000; Gamma shape=2.3370). A (Adenine); T (Thymine); C (Cytosine); G (Guanine). Numbers at nodes are posterior probability values

information. Powers *et al*., (2010) using ITS1-rDNA information showed that *Discocriconemella inarata* which morphologically showed lack of submedian lobes and an anterior vulval lip with two small lobes is closely related to *Mesocriconema* species and distantly related to species of the genus *Discocriconemella*.

To conclude, authors are in agreement with the opinion of several researchers (Luc *et al*., 2010) that DNA sequence data from a study involving molecular diagnostics or molecular phylogenetics should be integrated with morphological identification to avoid confusion when morphology and biology relationships need to be studied. Further research is needed to have a clearer idea about the relationships between taxonomic and molecular identification and the phylogeny of Criconematoidea.

LITERATURE CITED

Blaxter, M. 2001. Molecular analysis of nematode evolution Pp 1-82 *in* Kennedy, M. and W. Harnett eds. Parasitic nematodes. Molecular biology, biochemistry and immunology. UK. CAB international.

Cherry, T., A. T. Szalanski, T. C. Todd, and T. O. Powers. 1997. The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). Journal of Nematology 29:23-29.

Cobb, N. A. 1918. Estimating the nematode population of the soil. Agricultural Technical Circular Bureau Plant Industries. United States Department of Agriculture. No. 1. 48 p.

Cordero, M., R. Robbins, and A. Szalanski. 2012a. Taxonomic and molecular identification of species of *Mesocriconema* and *Criconemoides* (Nematoda: Criconematidae). Journal of Nematology 44:399-426

Cordero, M., R. Robbins, and A. Szalanski. 2012b. Taxonomic and molecular identification of species of *Bakernema, Criconema, Hemicriconemoides, Ogma* and *Xenocriconemella* (Nematoda: Criconematidae). Journal of Nematology 44:427-446

Cordero, M., R. Robbins, and A. Szalanski. 2013. Taxonomic and molecular identification of species of *Hemicaloosia, Hemicycliophora, Gracilacus* and *Paratylenchus* (Nematoda: Criconematidae). Journal of Nematology (In press).

Cutillas, C., R. Oliveros, M. de Rojas, and D. C. Guevara. 2004. Determination of *Trichuris skrjabini* by sequencing of the ITS1-5.8S-ITS2 segment of the ribosomal DNA: Comparative molecular study of different species of Trichurids. Journal of Parasitology 90:648-652.

Dariba D, G. L. Taboada, R. Doallo, and D Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

Ferris, V. R., A. Sabo, J. G. Baldwin, M. Mundo-Ocampo, R. N. Inserra, and S. Sharma. 2004. Phylogenetic relationships among selected *Heteroderoidea* based on 18S and ITS1 ribosomal DNA. Journal of Nematology 36:202-206.

Gasser, R. B. 2001. Identification of parasitic nematodes and study of genetic variability using PCR approaches Pp 53-82 *in* Kennedy, M. and W. Harnett eds. Parasitic nematodes. Molecular biology, biochemistry and immunology. UK. CAB international.

Geraert, E. 2010. The Criconematidae of the world: Identification of the family Criconematidae (Nematoda). Gent, Academia Press. 615 pp.

Hall, A. H. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98

Hugall, A., J. Stanton, and C. Moritz. 1999. Reticulate evolution and the origins of ribosomal internal spacer diversity in apomitic *Meloidogyne*. Molecular Biology and Evolution 16:157-164.

Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755

Jenkins, W. R. 1964. A rapid centrifugal flotation technique for extracting nematodes from soil. Plant Disease Reporter 48:692.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059-3066.

Larget, B. and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetics trees. Molecular Biology and Evolution 16:750-759

Loof, P. A. A and A. De Grisse. 1967. Re-establishment of genus *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae) Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent.32:466-475.

Loof, P. A. A. and A. De Grisse. 1989. Taxonomic and nomenclatorial observations on the genus *Criconemella* De Grisse & Loof, 1965 sensu Luc & Raski, 1981. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. 54:53-74.

Luc, M., M. Doucet, R. Fortuner, P. Castillo, W. Decraemer, and P. Lax. 2010. Usefulness of morphological data for the study of nematode biodiversity. Nematology 12:495-504

Maggenti, A. R., M. Luc, D. J. Raski, R. Fortuner, and E. Geraert. 1988. A reappraisal of *Tylenchida* (Nemata). 11. List of generic and supra-generic taxa, with their junior synonyms. Revue de Nématologie 11:177-188.

Mes, T. H. S. and A. W. Cornelissen. 2004. Non-homogenized ITS regions in the parasitic nematode *Cooperia oncophora*. Parasitology 129:213-222.

Porazinska, D., W. Sung, R. Giblin-Davis, and W. K. Thomas. 2010. Reproducibility of read numbers in high-throughput sequencing analysis of nematode

community composition and structure. Molecular Ecology Resources 10:666-676.
Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818.
Posada, D. 2008. JModeltest: Phylogenetic model averaging. Molecular Biology and Evolution 25:1253-1256.
Powers, T. O. 2004. Nematodes molecular diagnostic: from bands to barcodes. Annual Review of Phytopathology 42:367-383
Powers, T. O., T. Harris, R. Higgins, L. Sutton, and K. Powers. 2010. Morphological and molecular characterization of *Discocriconemella inarata*, an endemic nematode from North American native tallgrass prairies. Journal of Nematology 42:35-45.
Raski, D., M. Luc, and A. Valenzuela. 1984. Redescription of *Criconema giardi* (Certes, 1889) Micoletzky, 1925, type species of the genus *Criconema* Hoffmänner & Menzel, 1914 (Criconematidae: Nematoda). Revue de Nematologie 7:301-314.
Raski, D. and M. Luc. 1985. A reappraisal of the genus *Criconema* Hoffmänner & Menzel, 1914 (Nematoda: Criconematidae). Revue de Nematologie 7:323-334
Raski, D. and M. Luc, 1987. A reappraisal of *Tylenchida* (Nemata). 10. The superfamily Criconematoidea Taylor, 1936. Revue de Nematologie 10:409- 444.
Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67-69.
Seinhorst, J. W. 1962. On the killing, fixation, and transferring to glycerin of nematodes. Nematologica 8:29-32.
Siddiqi, M. R. 1980. Taxonomy of plant nematode superfamily Hemicycliophoroidea, with a proposal for Criconematina, new suborder. Revue de Nematologie 3:179 -199.
Siddiqi, M. R. 2000. Tylenchida: Parasites of plants and insects. Wallingford, UK. CAB International. 805 pp.
Subbotin, S., N. Vovlas, R. Crozzoli, D. Sturhan, F. Lamberti, M. Moens, and J. Baldwin. 2005. Phylogeny of Criconematina Siddiqi, 1980 (Nematoda: Tylenchida) based on morphology and D2-D3 expansion segments of 28S-rDNA gene sequences with application of a secondary structure model. Nematology 7:927-944.
Subbotin, S. and M. Moens. 2006. Molecular taxonomy and phylogeny. Pp 33-58 *in* Perry, R and M. Moens eds. Plant Nematology. UK. CAB International.
Tanha Maafi, Z., S. Subbotin, and M. Moens. 2003. Molecular identification of cyst nematodes (Heteroderidae) from Iran and the phylogeny based on ITS1-rDNA sequences. Nematology 5:99-111.
Taylor, A. L. 1936. The genera and the species of the Criconematinae, a sub-family of the Anguillulinidae (Nematoda). Transactions of American Microscopical Society 4:39-421
Vrain, T. C., D. A. Wakarchuk, A. C. Levesque, and R.I. Hamilton. 1992. Intraspecific rDNA restriction fragment length (bp) polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15:563-573.

Received: 3/IV/2013
Recibido:
Accepted for publication: 12/IX/2013
Aceptado para publicación: