Zootaxa 3827 (2): 101–146
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3827.2.1
http://zoobank.org/urn:lsid:zoobank.org:pub:89E105BF-5126-41B9-9D40-80F9E551CA46

COI haplotype groups in *Mesocriconema* (Nematoda: Criconematidae) and their morphospecies associations

T.O. POWERS[1,3], E.C. BERNARD[2], T. HARRIS[1], R. HIGGINS[1], M. OLSON[1], M. LODEMA[1], P. MULLIN[1], L. SUTTON[1] & K.S. POWERS[1]

[1] *Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722, USA. E-mail: tpowers1@unl.edu*

[2] *Entomology & Plant Pathology, University of Tennessee, 2505 E.J. Chapman Drive, 370 Plant Biotechnology, Knoxville, TN, USA, 37996-4560. E-mail: ebernard@utk.edu*

[3] *Corresponding author*

Table of contents

Abstract

Without applying an *a priori* bias for species boundaries, specimen identities in the plant-parasitic nematode genus *Mesocriconema* were evaluated by examining mitochondrial COI nucleotide sequences, morphology, and biogeography. A total of 242 specimens that morphologically conformed to the genus were individually photographed, measured, and amplified by a PCR primer set to preserve the linkage between specimen morphology and a specific DNA barcode sequence. Specimens were extracted from soil samples representing 45 locations across 23 ecoregions in North America. Dendrograms constructed by neighbor-joining, maximum likelihood, and Bayesian Inference using a 721-bp COI barcode were used to group COI haplotypes. Each tree-building approach resulted in 24 major haplotype groups within the dataset. The distinctiveness of these groups was evaluated by node support, genetic distance, absence of intermediates, and several measures of distinctiveness included in software used for the exploration of species boundaries. Five of the 24 COI haplotype groups corresponded to morphologically characterized, Linnaean species. Morphospecies conforming to *M. discus*, *Discocriconemella inarata*, *M. rusticum*, *M. onoense*, and *M. kirjanovae* were represented by groups composed of multiple closely related or identical COI haplotypes. In other cases, morphospecies names could be equally applied to multiple haplotype groups that were genetically distant from each other. Identification based on morphology alone resulted in *M. curvatum* and *M. ornatum* species designations applied to seven and three groups, respectively. Morphological characters typically used for species level identification were demonstrably variable within haplotype groups, suggesting caution in assigning species names based on published compendia that solely consider morphological characters. Morphospecies classified as *M. xenoplax* formed a monophyletic group composed of seven genetically distinct COI subgroups. The spe-

Accepted by K. Davies: 5 Jun. 2014; published: 3 Jul. 2014

cies *Discocriconemella inarata* is transferred to *Mesocriconema inaratum* based on its phylogenetic position on the COI tree as well as previous phylogenetic analyses using 18S, ITS1, and cytochrome b nucleotide sequences. This study indicates that some of the species considered cosmopolitan in their distribution are actually multispecies polyphyletic groupings and an accurate assessment of *Mesocriconema* species distributions will benefit from molecular determination of haplotype relationships. The groups revealed by COI analysis should provide a useful framework for the evaluation of additional *Mesocriconema* species and will improve the reliability of designating taxonomic units in studies of nematode biodiversity.

Key words: DNA barcode, identification, North America, plant-parasitic nematode, taxonomy

Introduction

Nematodes are frequently cited as a major source of undiscovered biodiversity on earth (Creer *et al*. 2010; Wilson 2007). The vivid imagery evoked by N.A. Cobb (1915) of a world covered by a film of nematodes has permeated popular science literature and reinforces the impression of remarkable nematode abundance and diversity (White 1998). Yet in spite of the popular perception, there are surprisingly few described species when compared to other groups of invertebrates (Bik *et al.* 2012). A recent accounting of described species in the phylum Nematoda at 24,783 is conservatively believed to represent 5–10% of extant species (Hodda 2011). One contributing factor to this relatively low number of described species may be the actual process of species delimitation typically used in the description of a new nematode species. A large percentage of nematode species have been characterized solely on the basis of a comparatively small set of morphological characters and measurements. Species delimited in this fashion have been called morphospecies. The delimitation process in nematology is seldom articulated, although it generally involves the search for discriminating morphological characters. Discriminating or diagnostic characters often are identified by the authors as those that exceed the known range of a morphometric variable, or combine measurements in a novel form to satisfy implicit criteria of diagnosability. If no morphologically diagnosable characters are identified, the species is assumed to be conspecific with an existing nominal species. Given the limited set of available morphological characters easily resolved by light microscopy, this approach has resulted in the recognition of a large number of cosmopolitan species (Artois *et al*. 2011).

The addition of DNA characters to the discovery process has revealed that many of these cosmopolitan species may actually consist of multiple genetically distinct subgroups (Kiontke *et al.* 2011; Nadler, S.A. & Pérez-Ponce De León, G. 2012). In this study we examined North American species in the plant-parasitic nematode genus *Mesocriconema* Andrássy, 1965. Worldwide this genus has 90 valid species (Geraert 2010). Approximately 24 species are reported from North America. Among the North American species are five morphospecies that could be considered worldwide in distribution. These species, *M. curvatum* (Raski, 1952) Loof & DeGrisse, 1989, *M. ornatum* (Raski, 1958) Loof & DeGrisse, 1989, *M. rusticum* (Micoletzky, 1915) Loof & DeGrisse, 1989, *M. sphaerocephalum* (Taylor, 1936) Loof & DeGrisse, 1989, and *M. xenoplax* (Raski, 1952) Loof & DeGrisse, 1989 are predominantly associated with agricultural plant hosts, and it is this association that is assumed responsible for their present-day cosmopolitan distribution (Wouts 2006). These species, however, are not confined to agricultural hosts, and surveys of native plant communities also record their presence. Another potential factor contributing to their perceived cosmopolitan distribution is the relatively early dates of their descriptions. All five species were described prior to 1952, a time when only 21 species were listed as belonging to the genus *Criconemoides* Taylor, 1936. Early records of occurrence leading to present-day perception of global distributions may actually reflect the limited options available in early keys, "fuzzy" species boundaries, or assumptions of identity biased by plant host association. For example, a criconematid species associated with peach in the southeastern U.S. might automatically be assumed to be *M. xenoplax*, based on that species' role in the disease complex called peach tree short-life (Nyczepir *et al*. 1985).

Our study encompasses collections from within 23 of the 118 North American World Wildlife Federation (WWF) ecoregions (Olson *et al*. 2004) including both native plant communities and cultivated soils (Table 1). From these soils we have extracted and analyzed specimens morphologically conforming to the genus *Mesocriconema*. Each specimen is measured, photographed, and then processed for PCR by primers that permit nucleotide sequencing of a 721-bp portion of the mitochondrial COI gene. This approach preserves the link between specimen morphology and COI haplotype, and allows us to link morphologically distinct juvenile and difficulties in identification and the recognition of species boundaries. Obvious examples include the variation in labial plates in SEM face views, the degree of crenation on annule margins of juveniles, the size of the projections in the anterior lip of the vulva, and the interpretation of the shape of the vagina. These diagnostic morphological characters need to be systematically re-evaluated within the context of molecularly derived groupings in order to fully realize the information content of the taxonomic units. That realization will, in turn, allow nematode taxonomy to better integrate with and contribute to larger questions of global biodiversity.

Acknowledgements

This research was supported by the National Science Foundation, Proposal DEB-1145440. We would like to thank the following people for contributing specimens: Kathy Lawrence, Auburn University; Gary Lawrence, Mississippi State University; Tim Todd, Kansas State University; Jim Smolik and Larry Osborne, South Dakota State University; Steve Thomas and Jackie Beacham, New Mexico State University; Deb Neher, University of Vermont; Dave Wedin and Tara Wood, University of Nebraska; Bob Robbins, University of Arkansas; Andy Nyczepir, USDA Southeastern Fruit and Nut Research Laboratory; Paula Agudelo, Clemson University; Tad Weaver, Montana State University; Glen Dappen, Nebraska Wesleyan University; Erik Oberg, National Park Service; Alejandro Esquivel, National University of Costa Rica; Rich Henderson, Prairie Enthusiasts of Wisconsin; Al Swain, Friends of Blue Mound State Park. Thanks also to Spring Creek Prairie Audubon Center, Denton, NE; Organization of Tropical Studies; George Washington Memorial Parkway, study#GWMP-00090 and Great Smoky Mountains National Park, study#GRSM-01076.

Literature cited

Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19 (6), 716–723. http://dx.doi.org/10.1109/tac.1974.1100705

Andrássy, I. (2007) *Free-living nematodes of Hungary. Pedozoologica Hungarica. No 4.* Hungarian Natural History Museum, Budapest, 496 pp.

Artois, T., Fontaneto, D., Hummon, W.D., McInnes, S.J., Todaro, M.A., Sørensen, M.Y. & Bullini, A. (2011) Ubiquity of microscopic animals? Evidence from the morphological approach in species identification. *In*: Fontaneto, D. (Ed.), *Biogeography of Microscopic Organisms. Is Everything Small Everywhere? The Systematics Association Special Vol. 79.* Cambridge University Press, Cambridge, pp. 244–283.

Bik, H.M., Porazinska, D.L., Creer, S., Caporaso, J.G., Knight, R. & Thomas, W.K. (2012) Sequencing our way towards understanding global eukaryotic biodiversity. *Trends in Ecology & Evolution,* 27, 233–243. http://dx.doi.org/10.1016/j.tree.2011.11.010

Brzeski, M.W., Choi, Y.E. & Loof, P.A.A. (2002a) Compendium of the genus *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae). *Nematology*, 4, 325–339. http://dx.doi.org/10.1163/156854102760199178

Brzeski, M.W., Choi, Y.E. & Loof, P.A.A. (2002b) Compendium of the genus *Mesocriconema* Andrássy, 1965 (Nematoda: Criconematidae). *Nematology*, 4, 341–360. http://dx.doi.org/10.1163/156854102760199187

Callejón, R., Nadler, S., De Rojas, M., Zurita, A., Petrášová, J. & Cutillas, C. (2013) Molecular characterization and phylogeny of whipworm nematodes inferred from DNA sequences of *cox1* mtDNA and 18S rDNA. *Parasitology Research*, 112, 3933–3949. http://dx.doi.org/10.1007/s00436-013-3584-z

Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecule of Drosophila yakuba: nucleotide sequence, gene organization, and genetic code. *Journal of Molecular Evolution*, 22, 252–271. http://dx.doi.org/10.1007/bf02099755

Cobb, N.A. (1915) Nematodes and their relationships. *Yearbook of the Department of Agriculture for 1914*. Washington, D.C., 457–490.

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

Creer, S., Fonseca, V.G., Porazinska, D.L., Giblin-Davis, R.M., Sung, W., Power, D., Packer, M., Carvalho, G., Blaxter, M., Lambshead, J. & Thomas, W.K. (2010) Ultra-deep 454 sequencing of the meiofaunal biosphere: practice, promises, and pitfalls. *Molecular Ecology*, 19 (1) (Supplement), 4–20. http://dx.doi.org/10.1111/j.1365-294x.2009.04473.x

Decraemer, W. & Hunt, D. (2006) Structure and classification. *In* Perry, R.N. & Moens, M. (Eds.), *Plant Nematology*. CABI, Wallingford, UK, pp. 3–32.

De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology,* 565, 879–886.

DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: Taxonomy, species delimitation, and DNA barcoding. *Philosophical Transactions of the Royal Society B*, 360, 1905–1916.
http://dx.doi.org/10.1098/rstb.2005.1722

Ebsary, B.A. (1982) *Bakernema yukonense* n.sp. (Nematoda: Criconematidae) with keys to the species of *Criconemella* and *Discocriconemella*. *Canadian Journal of Zoology,* 60, 3033–3047.
http://dx.doi.org/10.1139/z82-386

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

Gibbs, J. (2009) Integrative taxonomy identifies new (and old) species in the *Lasioglossum* (*Dialictus*) *tegulare* (Robertson) species group (Hymenoptera, Halictidae). *Zootaxa*, 2032, 1–38.

Graham, A. (1999) *Late Cretaceous and Cenozoic history of North American vegetation: north of Mexico*. Oxford University Press, New York, 370 pp.

Handoo, Z.A., Carta, L.K., Skantar, A.M. & Chitwood, D.J. (2012) Description of *Globodera ellingtonae* n. sp. (Nematoda: Heteroderidae) from Oregon. *Journal of Nematology*, 44, 40–57.

Hoda, M. (2011) Phylum Nematoda Cobb 1932. *Zootaxa*, 3148, 63–95.

Hoffmann, J.K. (1974) Morphological variation in species of *Bakernema*, *Criconema*, and *Criconemoides* (Criconematidae: Nematoda). *Iowa State Journal of Research*, 49, 137–153.

Hollis, J.P. (1977) Loss in yield of rice caused by the ring nematode *Criconemoides onoensis* revealed by elimination of yellow nutsedge, *Cyperus esculentus*. *Nematologica* , 23, 71–78.
http://dx.doi.org/10.1163/187529277x00246

Hunt, D.J., Luc, M. & Manzanilla-Lopez, R.H. (2005) Identification, morphology, and biology of plant parasitic nematodes. *In*: Luc, M., Sikora, R.A. & Bridge, J. (Eds.), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CABI, Wallingford, UK, pp. 11–52.

Hunt, D.J. (2008) Book review of Wouts, 2006. *Nematology,* 10, 781.

Jenkins, W.R. (1964) A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, 48, 692.

Kanzaki, N., Ragsdale, E.J., Herrmann, M., Mayer, W.E. & Sommer, R.J. (2012) Description of three *Pristionchus* species (Nematoda: Diplogastridae) from Japan that form a cryptic species complex with the model organism *P. pacificus*. *Zoological Science*, 29, 403–417.
http://dx.doi.org/10.2108/zsj.29.403

Kiontke K.C., Félix, M., Ailion, M., Rockman, M.V., Braendle, C., Pénigault, J-B. & Fitch D.H.A. (2011) A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evolutionary Biology*, 11, 339.
http://dx.doi.org/10.1186/1471-2148-11-339

Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics,* 25, 1451–1452.
http://dx.doi.org/10.1093/bioinformatics/btp187

Loof, P.A.A. (1965) Zur Taxonomie von *Criconemoides rusticus* (Micoletzky) und C. *informis* (Micoletzky). *Mitteilungen aus dem Museum für Naturkunde in Berlin. Zoologisches Museum und Institut für Spezielle Zoologie (Berlin)*, 41, 183–192.
http://dx.doi.org/10.1002/mmnz.4830410206

Loof, P.A.A. (1974) *Macroposthonia curvata*. *C.I.H. Description of Plant-parasitic Nematodes*. Set 4, No. 58.

Loof, P.A.A. & De Grisse, A. (1973) Interrelationships of the genera of Criconematidae (Nematoda: Tylenchida). *Mededelingen van de Fakulteit der Landbouwwetenschappen Gent,* 38, 1303–1328.

Loof, P.A.A. & De Grisse, A. (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. (1970) Contribution à l'étude du genre *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae). *Cahiers ORSTOM, Série Biologie,* 11, 69–131.

Masters, B.C., Fan, V. & Ross, H.A. (2011) Species delimitation- a geneious plugin for the exploration of species boundaries. *Molecular Ecology Resources,* 11, 154–157.
http://dx.doi.org/10.1111/j.1755-0998.2010.02896.x

Mulawarman & Geraert, E. (1997) The genera *Basiria* Siddiqi, 1959; *Filenchus* Andrássy, 1954 and *Criconemella* De Grisse & Loof, 1965 (Nemata: Tylenchida) from South Sumatra, Indonesia. *Biologisch Jaarboek Dodonaea,* 64, 125–137.

Nadler, S. (2002) Species delimitation and nematode biodiversity: Phylogenies rule. *Nematology*, 4, 615–625.
http://dx.doi.org/10.1163/15685410260438908

Nadler, S.A. & Pérez-Ponce De León, G. (2012) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. *Parasitology-Cambridge*, 138, 1688.
http://dx.doi.org/10.1017/s003118201000168x

Norton, D.C., Donald, P., Kimpinski, J., Myers, R.F., Noel, G.R., Noffsinger, E.M., Robbins, R.T., Schmitt, D.P., Sosa-Moss, C. & Vrain, T.C. (1984) *Distribution of Plant-Parasitic Nematode Species in North America*. Nematode Geographical Distribution Committee, Society of Nematologists, Lakeland, FL, 205 pp.

Norton, D.C. & Ponchillia, P.E. (1968) Stylet-bearing nematodes associated with plants in Iowa prairies. *Iowa Academy of Sciences,* 75, 32–35.

Nyczepir, A.P., Bertrand, P.F., Miller, R.W. & Motsinger, R.E. (1985) Incidence of *Criconemella* spp. and peach orchard histories in short-life and non-short-life sites in Georgia and South Carolina. *Plant Disease,* 698, 74–877. http://dx.doi.org/10.1094/pd-69-874

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'Amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. & Kassem, K.R. (2004) Terrestrial ecoregions of the world: A new map of life on earth. *BioScience*, 51, 933–938.

Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxomony. *Frontiers in Zoology*, 7, 16. http://dx.doi.org/10.1186/1742-9994-7-16

Peneva, V., Neilson, R., Boag, B. & Brown, D.J.F. (2000) Criconematidae (Nematoda) from oak forests in two nature reserves in Russia. *Systematic Parasitology*, 46, 191–201. http://dx.doi.org/10.1023/a:1006338019502

Powers, T.O., Harris, T., Higgins, R., Sutton, L. & Powers, K.S. (2010) Morphological and molecular characterization of *Discocriconemella inarata*, an endemic nematode from North American native tallgrass prairies. *Journal of Nematology,* 42, 35–45.

Powers, T., Harris, T., Higgins, R., Mullin, P., Sutton, L. & Powers, K. (2011) MOTUs, morphology and biodiversity estimation: A case study using nematodes of the suborder Criconematina and a conserved 18S DNA barcode. *Journal of Nematology*, 43, 35–48.

Raski, D. (1952) On the morphology of *Criconemoides* Taylor, 1936, with descriptions of six new species. *Proceedings of the Helminthological Society,* 19, 85–99.

Rodrigo, A., Bertels, F., Heled, J., Noder, R., Shearman, H. & Tsai, P. (2008) The perils of plenty: what are we going to do with all these genes? *Philosophical Transactions of the Royal Society*, Series B, 363, 3893–3902. http://dx.doi.org/10.1098/rstb.2008.0173

Rosenberg, N.A. (2007) Statistical tests for taxonomic distinctiveness from observations of monophyly. *Evolution*, 61, 317–323. http://dx.doi.org/10.1111/j.1558-5646.2007.00023.x

Ross, H.A., Murugan, S. & Li, W.L.S. (2008) Testing the reliability of genetic methods of species identification via simulation. *Systematic Biology*, 57, 216–230. http://dx.doi.org/10.1080/10635150802032990

Schmitt, D.P. & Norton, D.C. (1972) Relationships of plant parasitic nematodes to sites in native Iowa prairies. *Journal of Nematology,* 4, 200–206.

Siddiqi, M.R. (2000) *Tylenchida: Parasites of plants and insects. 2nd Edition*. CABI Publishing, Wallingford, UK, 833 pp.

Srivathsan, A. & Meier, R. (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics, 28, 190–194. http://dx.doi.org/10.1111/j.1096-0031.2011.00370.x

Thorne, G. (1961) *Principles of Nematology*. McGraw-Hill Book Company, New York, 553 pp.

Thorne, G. & Malek, R.B. (1968) *Nematodes of the Northern Great Plains Part 1 Tylenchida [Nemata: Secernentea].* Agricultural Experiment Station South Dakota State University, Brookings, South Dakota, 111 pp.

White, B. (1998) *Quite a Year for Plums*. Alfred A. Knopf, New York, 220 pp.

Wilson, E.O. (2007) TED Talk: My wish: Build the Encyclopedia of Life. Available from: http://www.ted.com/talks/e_o_wilson_on_saving_life_on_earth.html (accessed 10 February 2014)

Wouts, W.M. (2006) Criconematina (Nematoda: Tylenchida). *Fauna of New Zealand,* 55, 1–228.

Zeng, Y., Ye, W., Tredway, L., Martin, S. & Martin, M. (2012) Taxonomy and morphology of plant-parasitic nematodes associated with turfgrasses in North and South Carolina, USA. *Zootaxa*, 3452, 1–46.