Molecular characterization of root-knot nematodes (*Meloidogyne* spp.) from Arkansas, USA

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Root-knot nematodes (*Meloidogyne* spp.) are the most common major pathogens of many crops throughout the world, impacting both the quantity and quality of marketable yields. In this study, a total of 244 root-knot nematode populations from various hosts from 39 counties in Arkansas were tested to determine the species diversity. Molecular characterization was performed on these populations by DNA sequencing of the ribosomal DNA 18S-ITS-5.8S, 28S D2/D3 and a mitochondrial DNA fragment flanking cytochrome oxidase gene subunit II - the intergenic spacer. Five species were identified, including *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 from soybean, cotton, corn and various vegetables (232 samples); *M. hapla* Chitwood, 1949 from rose (1 sample); *M. haplanaria* Eisenback, Bernard, Starr, Lee & Tomaszewski, 2003 from okra, tomato, peanut, Indian hawthorn, ash, willow and elm trees (7 samples); *M. marylandi* Jepson & Golden in Jepson, 1987 from grasses (3 samples); and *M. partityla* Kleynhans, 1986 from pecan (1 sample) through a combined analysis of DNA sequencing and PCR by species-specific primers. *Meloidogyne incognita* is the most abundant species that was identified in 95% samples and was the only species in field crops including soybean and cotton, except for one population of *M. haplanaria* from soybean in Logan County (TK201). Species-specific primers were used to verify *M. incognita* through PCR by species-specific primers. Unlike historical data, *M. arenaria*, *M. javanica* and *M. graminis* were not detected from any of the samples collected during this study. This result is essential for effective and sustainable management strategies against root-knot nematodes in Arkansas.

Root-knot nematodes (RKN) are microscopic worms that live in soil and feed on the roots of many crops and weeds. The nematode gets its name because its feeding causes galls to form on the roots of infected plants. They are sedentary endoparasitic nematodes that depend on the induction of a permanent feeding site in living roots to complete their life cycle. RKN are the most widespread and serious plant-parasitic nematode pests, damaging a very wide range of crops throughout the world1. They are scientifically classified in the genus *Meloidogyne* (Tylenchida: Meloidogynidae) with over 100 species described2.

The southern RKN, *M. incognita* (Kofoid & White) Chitwood, 1949, is the most important nematode parasite of cotton in Arkansas3 and it has replaced the soybean cyst nematode as the premier nematode pest of soybean4. RKN are also commonly found in corn and grain sorghum fields and are associated with various horticultural and ornamental crops and turf grasses in the state. Because of the significance of the agricultural production to Arkansas’ economy5, understanding the *Meloidogyne* species associated with crops in the state is vital to formulation of effective and sustainable management strategies.

Previous surveys of RKN in Arkansas were conducted by using classical morphological methods. In a few surveys from soybean4, cotton7, wheat8 and blueberry8, RKN were found but species identification was not attempted. *Meloidogyne graminis* (Sledge & Golden, 1964) Whitehead, 1968 was first found in 1967 by R. D. Riggs on *Zoysia* spp. in Arkansas10. *Meloidogyne hapla* Chitwood, 1949 was reported on black locust (*Robinia pseudoacacia*) near the Mississippi River in Arkansas11. Norton et al.12 documented the occurrence of *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. hapla*, and *M. incognita* in Arkansas. Wehunt et al.13 reported *M. incognita*, *M. hapla*, *M. arenaria*, *M. graminis*, and *M. javanica* from soybean fields near the Mississippi river. Elm et al.14,15 recorded *M. marylandi* Jepson & Golden in Jepson, 1987 from tall fescue. Walters and Barker16 reported *M. hapla*, *M. incognita*, *M. arenaria*, and *M. javanica* (Treub, 1885) Chitwood, 1949 in Arkansas. In a recent survey from

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106 soil and root samples, *M. incognita*, *M. marylandi*, *M. haplanaria*, *M. hapla*, *M. arenaria* and *M. partityla* Kleynhans, 1986 were identified through molecular diagnosis and *M. incognita* was the most abundant species.

Development of resistant varieties that suppress nematode growth and reproductions is the most desirable, cost-effective and environmentally sustainable strategy for managing plant-parasitic nematodes. Host plant resistance is effective against certain species or races; thus, accurate identification of RKN species is critical to the success of the use of host resistance or rotation. Species of RKN has been traditionally identified based on female perineal pattern, second-stage juvenile and male morphology and morphometrics, isozyme analysis, and host differential test. The traditional methods are always challenging due to highly conserved and similar morphology across species, lack of certain life stages, high intraspecies variability, potential hybrid origin and polyploidy. In the past 20 years, molecular tools have been progressively developed to identify RKN species using polymerase chain reaction (PCR), Restriction Fragment Length Polymorphism (RFLP), and DNA sequencing, because they are usually fast, sensitive, less subjective and applicable to any life stages of a population. The objective of this study was to collect RKN samples from field crops and natural sites in the state of Arkansas and to characterize the DNA sequences of RKN on the ribosomal DNA 18S-ITS-5.8S, 28S D2/D3 and mitochondrial DNA cytochrome oxidase gene subunit II-the intergenic spacer (CoxII-IGS) to determine the species and their distribution.

### Results

**RKN problem in Arkansas.** RKN are common in field samples submitted to the Arkansas Nematode Diagnostic Laboratory. Infected roots have typical gall formation and RKN females, juveniles and egg masses could be recovered from the galled tissues (Figs 1 and 2). *Meloidogyne marylandi* does not produce galls on turf-grasses, and only semi-penetrates the roots (Fig. 3A). The female is lemon-shaped, with a much harder cuticle.

![Nematode gall](image1.png)

**Figure 1.** Photographs of root galls and females of southern root-knot nematode (*Meloidogyne incognita*) from tomato in Pulaski, Arkansas (RT122).
Figure 2. Photographs of the infested potato and the juveniles of southern root-knot nematode (*Meloidogyne incognita*) from potato in Van Buren County, Arkansas (RT139).

Figure 3. Photographs of females of Maryland root-knot nematode (*Meloidogyne maryandi*) from Sedge like grass in Washington County, Arkansas (RT106). (A) Female on the root. (B) Female.
| No. | DNA ID | Species     | Host        | County      | 18S + ITS GenBank Accession No. | 28S D2/D3 GenBank Accession No. | CoxII-IGS GenBank Accession No. |
|-----|--------|-------------|-------------|-------------|---------------------------------|---------------------------------|----------------------------------|
| 1   | RT70   | *M. incognita* | Tomato      | Pulaski     | MK102787                        | MK102799                        |
| 2   | RT73   | *M. incognita* | Cucumber on Tomato | Pulaski     | MK102787                        | MK102799                        |
| 3   | RT75   | *M. incognita* | Soybean     | Drew        | MK102787                        | MK102798                        |
| 4   | RT76   | *M. haplanaria* | Ash         | Washington  | MK102773                        | MK102784                        | MK102794                        |
| 5   | RT77   | *M. incognita* | Cucumber    | Pulaski     | MK102776                        | MK102799                        |
| 6   | RT78   | *M. incognita* | Tomato      | Sebastian   | MK102776                        | MK102799                        |
| 7   | RT79   | *M. incognita* | Okra        | Pulaski     | MK102776                        | MK102799                        |
| 8   | RT80   | *M. incognita* | Tomato      | Pulaski     | MK102778                        | MK102799                        |
| 9   | RT81   | *M. incognita* | Pocket melon | Pulaski     | MK102775                        | MK102798                        |
| 10  | RT82   | *M. haplanaria* | Okra        | Van Buren   | MK102773                        | MK102784                        | MK102799                        |
| 11  | RT83   | *M. hapla*    | Knockout rose | Craighead   | MK102780                        | MN475814                       | MK102792                        |
| 12  | RT84   | *M. incognita* | Carrot      | Washington  | MK102778                        | MK102799                        |
| 13  | RT85   | *M. haplanaria* | Tomato      | Baxter      | MK102778                        | MK102799                        |
| 14  | RT97   | *M. marylandi* | Italian rye grass | Logan       | MK102774                        | MK102798                        |
| 15  | RT98   | *M. incognita* | Tomato      | Logan       | MK102776                        | MK102787                        |
| 16  | RT99   | *M. incognita* | Soybean     | Woodruff    | MK102776                        | MK102787                        | MK102799                        |
| 17  | RT100  | *M. incognita* | Soybean     | Saline      | MK102778                        | MK102797                        |
| 18  | RT101  | *M. haplanaria* | Peanut      | Saline      | MK102775                        | MK102785                        | MK102794                        |
| 19  | RT102  | *M. incognita* | Fig         | Pulaski     | MK102776                        | MK102787                        |
| 20  | RT106  | *M. marylandi* | Sedge like grass | Washington  | MK102782                        |
| 21  | RT118  | *M. incognita* | Holy basil  | Montgomery  | MK102778                        | MK102790                        | MK102799                        |
| 22  | RT120  | *M. incognita* | Pinto bean  | Conway      | MK102787                        | MK102799                        |
| 23  | RT121  | *M. incognita* | Tomato      | Pope        | MK102776                        | MK102798                        |
| 24  | RT122  | *M. incognita* | Tomato, okra | Pulaski     | MK102778                        | MK102787                        |
| 25  | RT126  | *M. incognita* | Soybean     | Woodruff    | MK102776                        |
| 26  | RT127  | *M. incognita* | Zucchini    | Washington  | MK102776                        |
| 27  | RT128  | *M. partityla* | Pecan       | Logan       | MK102783                        | MK102796                        |
| 28  | RT129  | *M. marylandi* | Bermuda grass | Hempstead   | MK102781                        |
| 29  | RT130  | *M. haplanaria* | Willow, elm | Washington  | MK102772                        | MK102795                        |
| 30  | RT131  | *M. incognita* | Tomato      | Bradley     | KU948024                        |
| 31  | RT132  | *M. incognita* | Squash      | Cleburne    | KU948016                        |
| 32  | RT133  | *M. incognita* | Tomato      | Columbia    | KU948016                        |
| 33  | RT134  | *M. haplanaria* | Indian hawthorn | Faulkner    | KU948026                        |
| 34  | RT135  | *M. incognita* | Okra        | Garland     | KU948025                        |
| 35  | RT136  | *M. incognita* | Soybean     | Logan       | KU948016                        |
| 36  | RT137  | *M. incognita* | Squash, cucumber | Phillips   | KU948021                        |
| 37  | RT138  | *M. incognita* | Soybean     | Yell        | KU948016                        |
| 38  | RT139  | *M. incognita* | Potato      | Van Buren   | MK102778                        | MK102798                        |
| 39  | TK1    | *M. incognita* | Soybean     | Lonoke      | MK102777                        | MK102797                        |
| 40  | TK2    | *M. incognita* | Corn        | Desha       | MK102776                        | MK102787                        |
| 41  | TK3    | *M. incognita* | Corn        | Desha       | MK102787                        |
| 42  | TK4    | *M. incognita* | Corn        | Desha       | MK102787                        |
| 43  | TK5    | *M. incognita* | Cotton      | Desha       | MK102778                        | MK102787                        |
| 44  | TK6    | *M. incognita* | Soybean     | Lincoln     | MK102787                        | MK102799                        |
| 45  | TK7    | *M. incognita* | Soybean     | Lincoln     | MK102778                        | MK102787                        |
| 46  | TK8    | *M. incognita* | Soybean     | Desha       | MK102778                        | MK102787                        |
| 47  | TK9    | *M. incognita* | Soybean     | Desha       | MK102778                        | MK102787                        | MK102799                        |
| 48  | TK10   | *M. incognita* | Soybean     | Desha       | MK102787                        |
| 49  | TK11   | *M. incognita* | Soybean     | Mississippi | MK102778                        | MK102798                        |
| 50  | TK12   | *M. incognita* | Soybean     | Mississippi | MK102797                        |
| 51  | TK13   | *M. incognita* | Corn        | Mississippi | MK102777                        | MK102787                        |
| 52  | TK14   | *M. incognita* | Soybean     | Mississippi | MK102787                        |
| 53  | TK15   | *M. incognita* | Soybean     | Mississippi | MK102787                        |
| 54  | TK16   | *M. incognita* | Soybean     | Mississippi | MK102778                        | MK102799                        |
| 55  | TK17   | *M. incognita* | Soybean     | Mississippi | MK102787                        |
| 56  | TK18   | *M. incognita* | Corn        | Mississippi | MK102787                        |

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| No. | DNA ID | Species | Host       | County   | 18S + ITS GenBank Accession No. | 28S D2/D3 GenBank Accession No. | CoxII-IGS GenBank Accession No. |
|-----|--------|---------|------------|----------|---------------------------------|---------------------------------|---------------------------------|
| 57  | TK19   | M. incognita | Soybean   | Mississippi | MK102778                        | MK102787                        |                                  |
| 58  | TK20   | M. incognita | Soybean   | Mississippi | MK102778                        | MK102787                        |                                  |
| 59  | TK21   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 60  | TK22   | M. incognita | Soybean   | Lonoke    | MK102776                        | MK102787                        | MK102799                        |
| 61  | TK23   | M. incognita | Soybean   | Lonoke    | MK102776                        | MK102787                        |                                  |
| 62  | TK24   | M. incognita | Soybean   | Lonoke    | MK102776                        | MK102787                        | MK102799                        |
| 63  | TK25   | M. incognita | Soybean   | Lonoke    | MK102777                        | MK102787                        |                                  |
| 64  | TK26   | M. incognita | Soybean   | Pulaski   | MK102776                        | MK102787                        | MK102799                        |
| 65  | TK27   | M. incognita | Corn      | Randolph | MK102776                        | MK102787                        | MK102799                        |
| 66  | TK28   | M. incognita | Soybean   | Randolph | MK102776                        | MK102787                        |                                  |
| 67  | TK29   | M. incognita | Soybean   | Chicot    | MK102777                        | MK102787                        |                                  |
| 68  | TK30   | M. incognita | Soybean   | Chicot    | MK102777                        | MK102787                        | MK102799                        |
| 69  | TK31   | M. incognita | Soybean   | Chicot    | MK102777                        | MK102787                        | MK102799                        |
| 70  | TK32   | M. incognita | Soybean   | Mississippi | MK102777                        | MK102787                        | MK102799                        |
| 71  | TK33   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 72  | TK34   | M. incognita | Grain Sorghum | Mississippi | MK102779                        | MK102787                        |                                  |
| 73  | TK35   | M. incognita | Grain Sorghum | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 74  | TK36   | M. incognita | Grain Sorghum | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 75  | TK37   | M. incognita | Grain Sorghum | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 76  | TK38   | M. incognita | Grain Sorghum | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 77  | TK39   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 78  | TK40   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 79  | TK41   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 80  | TK42   | M. incognita | Grain Sorghum | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 81  | TK43   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 82  | TK44   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 83  | TK45   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 84  | TK46   | M. incognita | Soybean   | Mississippi | MK102778                        | MK102787                        | MK102799                        |
| 85  | TK47   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 86  | TK48   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 87  | TK49   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 88  | TK50   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 89  | TK51   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 90  | TK52   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 91  | TK53   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 92  | TK54   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 93  | TK55   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 94  | TK56   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 95  | TK57   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 96  | TK58   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 97  | TK59   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 98  | TK60   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 99  | TK61   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 100 | TK62   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 101 | TK63   | M. incognita | Soybean   | Drew      | MK102779                        | MK102787                        | MK102799                        |
| 102 | TK64   | M. incognita | Soybean   | Drew      | MK102779                        | MK102787                        | MK102799                        |
| 103 | TK65   | M. incognita | Soybean   | Drew      | MK102779                        | MK102787                        | MK102799                        |
| 104 | TK66   | M. incognita | Corn      | Drew      | MK102779                        | MK102787                        | MK102799                        |
| 105 | TK67   | M. incognita | Corn      | Drew      | MK102779                        | MK102787                        | MK102799                        |
| 106 | TK68   | M. incognita | Soybean   | Mississippi | MK102778                        | MK102787                        | MK102799                        |
| 107 | TK69   | M. incognita | Soybean   | Prairie   | MK102779                        | MK102787                        | MK102799                        |
| 108 | TK70   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 109 | TK71   | M. incognita | Soybean   | Mississippi | MK102776                        | MK102787                        | MK102799                        |
| 110 | TK72   | M. incognita | Soybean   | Craighead | MK102779                        | MK102787                        | MK102799                        |
| 111 | TK73   | M. incognita | Soybean   | Mississippi | MK102779                        | MK102787                        | MK102799                        |
| 112 | TK74   | M. incognita | Soybean   | Craighead | MK102779                        | MK102787                        | MK102799                        |
| 113 | TK75   | M. incognita | Soybean   | Craighead | MK102776                        | MK102787                        | MK102799                        |

Continued
| No. | DNA ID | Species   | Host     | County | 18S + ITS GenBank Accession No. | 28S D2/D3 GenBank Accession No. | CoxII-IGS GenBank Accession No. |
|-----|--------|-----------|----------|--------|---------------------------------|---------------------------------|---------------------------------|
| 114 | TK76   | M. incognita | Soybean  | Craighead | MK102778 | MK102787 | MK102799 |
| 115 | TK77   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 116 | TK78   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 117 | TK79   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 118 | TK80   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 119 | TK81   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 120 | TK82   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 121 | TK83   | M. incognita | Soybean  | Mississippi | MK102779 | MK102787 | MK102799 |
| 122 | TK84   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 123 | TK85   | M. incognita | Soybean  | Mississippi | MK102779 | MK102787 | MK102799 |
| 124 | TK86   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 125 | TK87   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 126 | TK88   | M. incognita | Soybean  | Mississippi | MK102787 | MK102799 | MK102799 |
| 127 | TK89   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 128 | TK90   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 129 | TK91   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 130 | TK92   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 131 | TK93   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 132 | TK94   | M. incognita | Soybean  | Mississippi | MK102776 | MK102787 | MK102799 |
| 133 | TK95   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 134 | TK96   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 135 | TK97   | M. incognita | Soybean  | Mississippi | MK102776 | MK102787 | MK102799 |
| 136 | TK98   | M. incognita | Soybean  | Mississippi | MK102776 | MK102787 | MK102799 |
| 137 | TK99   | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 138 | TK100  | M. incognita | Corn     | Desha    | MK102779 | MK102787 | MK102802 |
| 139 | TK101  | M. incognita | Soybean  | Craighead | MK102779 | MK102787 | MK102799 |
| 140 | TK102  | M. incognita | Soybean  | Craighead | MK102779 | MK102787 | MK102799 |
| 141 | TK103  | M. incognita | Soybean  | Craighead | MK102778 | MK102787 | MK102799 |
| 142 | TK104  | M. incognita | Soybean  | Craighead | MK102776 | MK102787 | MK102799 |
| 143 | TK105  | M. incognita | Soybean  | Lonoke   | MK102778 | MK102787 | MK102799 |
| 144 | TK106  | M. incognita | Soybean  | Lonoke   | MK102776 | MK102787 | MK102799 |
| 145 | TK107  | M. incognita | Soybean  | Cross    | MK102778 | MK102787 | MK102799 |
| 146 | TK108  | M. incognita | Soybean  | Cross    | MK102778 | MK102787 | MK102799 |
| 147 | TK109  | M. incognita | Soybean  | Jackson  | MK102787 | MK102799 | MK102799 |
| 148 | TK110  | M. incognita | Soybean  | Jackson  | MK102787 | MK102799 | MK102799 |
| 149 | TK111  | M. incognita | Soybean  | Jackson  | MK102778 | MK102787 | MK102799 |
| 150 | TK112  | M. incognita | Soybean  | Jackson  | MK102787 | MK102799 | MK102799 |
| 151 | TK113  | M. incognita | Soybean  | Pope     | MK102778 | MK102787 | MK102799 |
| 152 | TK114  | M. incognita | Soybean  | Woodruff | MK102778 | MK102787 | MK102801 |
| 153 | TK115  | M. incognita | Soybean  | Jefferson | MK102791 | MK102799 | MK102799 |
| 154 | TK116  | M. incognita | Soybean  | Woodruff | MK102778 | MK102787 | MK102801 |
| 155 | TK117  | M. incognita | Soybean  | Craighead | MK102778 | MK102787 | MK102799 |
| 156 | TK118  | M. incognita | Soybean  | Lafayette | MK102778 | MK102799 | MK102799 |
| 157 | TK119  | M. incognita | Corn     | Lafayette | MK102776 | MK102787 | MK102799 |
| 158 | TK120  | M. incognita | Corn     | Lafayette | MK102778 | MK102787 | MK102799 |
| 159 | TK121  | M. incognita | Corn     | Lafayette | MK102778 | MK102787 | MK102799 |
| 160 | TK122  | M. incognita | Soybean  | Desha    | MK102787 | MK102799 | MK102799 |
| 161 | TK123  | M. incognita | Soybean  | Desha    | MK102778 | MK102799 | MK102799 |
| 162 | TK124  | M. incognita | Corn     | Desha    | MK102777 | MK102787 | MK102799 |
| 163 | TK125  | M. incognita | Soybean  | Desha    | MK102790 | MK102799 | MK102799 |
| 164 | TK126  | M. incognita | Soybean  | Desha    | MK102779 | MK102799 | MK102799 |
| 165 | TK127  | M. incognita | Soybean  | Lincoln  | MK102778 | MK102799 | MK102799 |
| 166 | TK128  | M. incognita | Soybean  | Lincoln  | MK102778 | MK102799 | MK102799 |
| 167 | TK129  | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 168 | TK130  | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 169 | TK131  | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |
| 170 | TK132  | M. incognita | Soybean  | Mississippi | MK102778 | MK102787 | MK102799 |

Continued
| No. | DNA ID | Species | Host | County | 18S + ITS GenBank Accession No. | 28S D2/D3 GenBank Accession No. | CoxII-IGS GenBank Accession No. |
|-----|--------|---------|------|--------|---------------------------------|----------------------------------|---------------------------------|
| 171 | TK133  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 172 | TK134  | M. incognita | Soybean | Mississippi | MK102779 | MK102787 | MK102799 |
| 173 | TK135  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 174 | TK136  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 175 | TK137  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 176 | TK138  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 177 | TK139  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 178 | TK140  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 179 | TK141  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 180 | TK142  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 181 | TK143  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 182 | TK144  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 183 | TK145  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 184 | TK146  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 185 | TK147  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 186 | TK148  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 187 | TK149  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 188 | TK150  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 189 | TK151  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 190 | TK152  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 191 | TK153  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 192 | TK154  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 193 | TK155  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 194 | TK156  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 195 | TK157  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102801 |
| 196 | TK158  | M. incognita | Soybean | Crittenden | MK102778 | MK102787 | MK102801 |
| 197 | TK159  | M. incognita | Soybean | Crittenden | MK102778 | MK102787 | MK102799 |
| 198 | TK160  | M. incognita | Soybean | Crittenden | MK102778 | MK102787 | MK102799 |
| 199 | TK161  | M. incognita | Soybean | Crittenden | MK102778 | MK102787 | MK102799 |
| 200 | TK162  | M. incognita | Soybean | Greene | MK102777 | MK102777 | MK102798 |
| 201 | TK163  | M. incognita | Soybean | Clay | MK102778 | MK102787 | MK102799 |
| 202 | TK164  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102801 |
| 203 | TK165  | M. incognita | Soybean | Clay | MK102778 | MK102787 | MK102799 |
| 204 | TK166  | M. incognita | Soybean | Clay | MK102778 | MK102787 | MK102799 |
| 205 | TK167  | M. incognita | Soybean | Conway | MK102778 | MK102787 | MK102799 |
| 206 | TK168  | M. incognita | Soybean | Lawrence | MK102778 | MK102787 | MK102799 |
| 207 | TK169  | M. incognita | Soybean | Conway | MK102778 | MK102787 | MK102799 |
| 208 | TK170  | M. incognita | Soybean | Lawrence | MK102778 | MK102787 | MK102799 |
| 209 | TK171  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 210 | TK172  | M. incognita | Soybean | Craighead | MK102778 | MK102787 | MK102799 |
| 211 | TK173  | M. incognita | Soybean | Lawrence | MK102778 | MK102787 | MK102799 |
| 212 | TK174  | M. incognita | Soybean | Lawrence | MK102778 | MK102787 | MK102799 |
| 213 | TK175  | M. incognita | Soybean | Lawrence | MK102778 | MK102787 | MK102799 |
| 214 | TK176  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 215 | TK177  | M. incognita | Soybean | Mississippi | MK102778 | MK102787 | MK102799 |
| 216 | TK178  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 217 | TK179  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 218 | TK180  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 219 | TK181  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 220 | TK182  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 221 | TK183  | M. incognita | Soybean | Desha | MK102778 | MK102787 | MK102799 |
| 222 | TK184  | M. incognita | Soybean | Woodruff | MK102778 | MK102787 | MK102799 |
| 223 | TK185  | M. incognita | Soybean | Miller | MK102778 | MK102787 | MK102799 |
| 224 | TK186  | M. incognita | Soybean | Miller | MK102778 | MK102787 | MK102799 |
| 225 | TK187  | M. incognita | Soybean | Miller | MK102778 | MK102787 | MK102799 |
| 226 | TK188  | M. incognita | Soybean | Miller | MK102778 | MK102787 | MK102799 |
| 227 | TK189  | M. incognita | Soybean | Miller | MK102778 | MK102787 | MK102799 |

Continued
Table 1. Species and isolates of root-knot nematodes (*Meloidogyne* spp.) sequenced in the present study.

| No. | DNA ID | Species     | Host   | County | 18S + ITS GenBank Accession No. | 28S D2/D3 GenBank Accession No. |coxII-IGS GenBank Accession No. |
|-----|--------|-------------|--------|--------|---------------------------------|-------------------------------|---------------------------------|
| 228 | TK190  | *M. incognita* | Soybean | Miller | MK102778                        | MK102799                      | MK102789                        |
| 229 | TK191  | *M. incognita* | Soybean | Miller | MK102778                        | MK102799                      | MK102789                        |
| 230 | TK192  | *M. incognita* | Soybean | Miller | MK102778                        | MK102799                      | MK102789                        |
| 231 | TK193  | *M. incognita* | Soybean | Desha  | MK102778                        | MK102789                      | MK102799                        |
| 232 | TK194  | *M. incognita* | Soybean | Desha  | MK102778                        | MK102789                      | MK102799                        |
| 233 | TK195  | *M. incognita* | Soybean | Desha  | MK102778                        | MK102789                      | MK102799                        |
| 234 | TK196  | *M. incognita* | Soybean | Desha  | MK102778                        | MK102789                      | MK102799                        |
| 235 | TK197  | *M. incognita* | Soybean | Ashley | MK102779                        | MK102789                      | MK102799                        |
| 236 | TK198  | *M. incognita* | Soybean | Ashley | MK102779                        | MK102789                      | MK102799                        |
| 237 | TK199  | *M. incognita* | Soybean | Desha  | MK102778                        | MK102789                      | MK102799                        |
| 238 | TK200  | *M. incognita* | Soybean | Randolph | MK102778                      | MK102789                      | MK102799                        |
| 239 | TK201  | *M. haplanaria* | Soybean | Logan  | MK102771                        | MK102784                      | MK102793                        |
| 240 | TK202  | *M. incognita* | Soybean | Randolph | MK102778                      | MK102789                      | MK102799                        |
| 241 | TK203  | *M. incognita* | Soybean | Logan  | MK102779                        | MK102789                      | MK102799                        |
| 242 | TK204  | *M. incognita* | Soybean | Johnson | MK102778                        | MK102789                      | MK102798                        |
| 243 | TK205  | *M. incognita* | Soybean | Clay    | MK102776                        | MK102789                      | MK102799                        |
| 244 | TK206  | *M. incognita* | Soybean | Lincoln | MK102776                        | MK102789                      | MK102799                        |

**Table 2.** Primers used for polymerase chain reaction and DNA sequencing.
and a slightly protruding vulva-anus region (Fig. 3B), that is different from the pear-shaped female and rounded vulva-anus region in other common RKN living inside the galls (Fig. 1).

**RKN identification.** Five RKN species were identified including *M. incognita*, *M. hapla*, *M. haplanaria*, *M. marylandi* and *M. partityla*; the results are presented in Table 1. Species identification in this study was based on the combined analysis of DNA sequencing on the rDNA 18S-ITS-5.8S, 28S D2/D3 and CoxII-IGS (Table 1) and PCR by species-specific primers (Table 2). *Meloidogyne incognita*, the most prevalent species, was found in 232 samples (95%) from soybean, cotton, corn and various vegetables in 36 of the 39 counties from which samples were collected (Ashley, Bradley, Chicot, Clay, Cleburne, Columbia, Conway, Craighead, Crittenden, Cross, Desha, Drew, Garland, Greene, Jackson, Jefferson, Johnson, Lafayette, Lawrence, Lincoln, Logan, Lonoke, Miller, Mississippi, Montgomery, Phillips, Pope, Prairie, Pulaski, Randolph, Saline, Sebastian, Van Buren, Washington, Woodruff, and Yell) (Fig. 4).

*Meloidogyne hapla* was found in only one sample from rose in Craighead County (Fig. 5).

*Meloidogyne haplanaria* was found in seven samples from okra, tomato, peanut, Indian hawthorn, ash, willow and elm trees in Baxter, Faulkner, Logan, Saline, Sebastian, Van Buren, Washington, Woodruff, and Yell) (Fig. 4). *Meloidogyne hapla* was found in only one sample from rose in Craighead County (Fig. 5). *Meloidogyne haplanaria* was found in seven samples from okra, tomato, peanut, Indian hawthorn, ash, willow and elm trees in Baxter, Faulkner, Logan, Saline, Sebastian, Van Buren, Washington, Woodruff, and Yell) (Fig. 4).

*Meloidogyne marylandi* was found in three samples from grasses in Hempstead, Logan, and Washington counties (Fig. 7). *Meloidogyne partityla* was found in only one sample from pecan in Logan County (Fig. 8). There were no samples with mixtures of species found.

**DNA sequencing.** The rDNA 18S-ITS-5.8S (182 sequences), 28S D2/D3 (226 sequences) and CoxII-IGS (197 sequences) were deposited in GenBank and their GenBank accession numbers are presented in Table 1. Although attempts were made to perform DNA sequencing on all three genes for each sample, not all PCR or DNA sequencing was successful. However, at least one gene was sequenced from all RKN populations except for one population (TK42). One hundred forty-two samples (58.2%) have all three genes sequenced. Many of the sequences from different populations are identical, thus their sequences were assigned the same accession number. Minor DNA sequence variations within the same species were observed in each gene among some populations.

DNA sequences of MK102775 (1,980 bp), MK102776 (2,296 bp), MK102777 (1,227 bp), MK102778 (968 bp) and MK102779 (1,815 bp) are different regions of 18S-ITS-5.8S and have more than 99% identity with many sequences of *M. incognita*, *M. javanica* and *M. arenaria* from GenBank. MK102771 (2,180 bp), MK102772 (2,216 bp) and MK102773 (2,180 bp) matched with two sequences of *M. haplanaria* (AY919178, 637 bp and AY757867, 637 bp) with 100% identity in aligned region. These three sequences are 98–99% identical to many
tropical species sequences including *M. incognita*, *M. javanica* and *M. arenaria* from GenBank. The 2,215-bp DNA sequence of 18S-ITS-5.8S (MK102780) is 99–100% identical to DNA sequences of *M. hapla* from the GenBank (KP901065, KJ636268, AY268119, AY593892, EU669941, EU669942, AY942628, MH011983, EU669943 and KJ636267). DNA sequences of MK102774 (2,015 bp) and MK102781 (790 bp) are 100% identical to *M. marylandi* (KP901041) and 99% identical to *M. marylandi* (KP901049 and KP901043).

The DNA sequence of 28S D2/D3 (MK102787, 1,006 bp) of *M. incognita* is fairly conserved; no sequence variation was observed among most Arkansas populations. It has minor nucleotide differences with other Arkansas sequences of *M. incognita* (MK102786, 1,006 bp, MK102788, 643 bp, MK102789, 641 bp, MK102790, 643 bp, and MK102791, 643 bp). Blast search of these sequences revealed 97–100% identity with many tropical species sequences including *M. incognita*, *M. javanica* and *M. arenaria* from GenBank (KP901082, KP901083, KP901078, etc.). The DNA sequences of 28S D2/D3 (MK102784, 1,003 bp and MK102785, 935 bp) on *M. haplanaria* are 95–96% identical to many tropical species sequences including *M. incognita*, *M. javanica* and *M. arenaria* from GenBank (KP901082, KP901083, KP901078, etc.). No 28S DNA sequence of *M. haplanaria* from GenBank is available to compare with the study populations. The 1,042-bp DNA sequence (MK102780) of *M. hapla* is 99–100% identical to DNA sequences of *M. hapla* from GenBank (GQ130139, KU180679, KP306534, KP306532, KU587712, KP901086, DQ145641, KJ598136 and KJ598153). The 678-bp DNA sequence (MK102782) of *M. marylandi* is close to many sequences of *M. marylandi* (KP901066 etc.) with 99–100% identity. The 667-bp DNA sequence of *M. partityla* (MK102783) is 94% identical to *M. ethiopica* (KY882483), *M. hispanica* (EU443606) and *M. luci* (LN626951). No 28S DNA sequence of *M. partityla* from GenBank is available to compare with the study population.

The DNA sequences of mitochondrial DNA CoxII-IGS of *M. incognita* (MK102798, 912 bp, MK102799, 879 bp, MK102800, 909 bp, MK102801, 771 bp, MK102802, 831 bp) are comprised of 138-bp CoxII and the rest IGS. The CoxII sequences are highly conserved and identical which encode a polypeptide GQCSEICGINHSFMPILVEITLFDFFNLWLYFCWKS. However, there are five types of IGS sequences that showed four significant gaps, six mutations and one insertion/deletion as shown in Fig. 9. Blast search of these sequences revealed 99–100% identity to many tropical species sequences including *M. incognita*, *M. javanica* and *M. arenaria* from GenBank (MH152335, MF043913, LN864824, etc.). The DNA sequences of CoxII-IGS (MK102793, 660 bp, MK102794, 541 bp and MK102795, 541 bp) on *M. haplanaria* are 99% identical to sequences of *M. haplanaria* (KT783539, KM881682, AY757895 and AY757906). The 470-bp DNA sequence (MK102792) of *M. hapla* is 99% identical to DNA sequences of *M. hapla* from the GenBank (KJ598134, AY757887, AY757888, AY757899, KP681265, KM881684 and KP993633). The 533-bp DNA sequence

Figure 5. Distribution of Texas peanut root-knot nematode (*Meloidogyne haplanaria*) in Arkansas.
(MK102797) of *M. marylandi* is identical to sequence of *M. marylandi* (JN241917, KM881683 and KC473862). The 511-bp DNA sequence of *M. partityla* (MK102796) is 99% identical to *M. partityla* (AY672412, AY757908, AY672413 and KM881686).

**Molecular phylogenetic relationships.** A phylogenetic tree based on the rDNA 18S-ITS-5.8S is presented in Fig. 10 with two *Pratylenchus* species as outgroup taxa. This tree placed the study populations in three distinct groups. *Meloidogyne incognita* populations are in a clade with other tropical RKN species including *M. incognita*, *M. arenaria*, *M. javanica*, *M. floridensis* and *M. morocccensis* with 100% support. *Meloidogyne haplanaria* is sister to this clade with 100% support. *Meloidogyne enterolobii* is basal to this clade with 93% support. *Meloidogyne marylandi* and *M. graminis* are very closely related and are in a clade with *M. spartinae* with 100% support. *Meloidogyne partityla* from this study was not sequenced successfully.

A phylogenetic tree based on the rDNA 28S D2/D3 sequences is presented in Fig. 11 with two *Pratylenchus* species as outgroup taxa. This tree placed Arkansas RKN in four distinct groups. *Meloidogyne hapla* population RT83 (MN475814) is in a clade with *M. hapla* (KP901086). This clade is in a monophyletic clade with *M. dunensis* (EF612712) with 84% support. *Meloidogyne incognita* (MK102786-MK102791) and *M. haplanaria* (MK102784 and MK102785) are in a monophyletic clade with *M. arenaria*, *M. javanica*, *M. incognita*, *M. konaensis*, *M. paranaensis*, *M. thailandica*, *M. enterolobii*, *M. hispanica*, *M. ethiopica* and *M. inornata* with 100% support. *Meloidogyne partityla* is sister to this clade with 82% support. *Meloidogyne marylandi* (MK102782) is in a clade with *M. marylandi* (JN157852 and KP901066) and *M. graminis* (JN019331, KP901076 and KP901077) with 99% support.

A phylogenetic tree based on the mitochondrial DNA CoxII-IGS sequences is presented in Fig. 12 rooted with *M. partityla* (MK102796) based on the multiple sequence alignment whose sequence is most distinct from the other sequences. No outgroup species was included in the analysis because of the large sequence divergency. This tree placed Arkansas RKN in five distinct groups. *Meloidogyne partityla* (MK102796) is at the basal position. *Meloidogyne hapla* population RT83 (MK102792) is in a clade with other *M. hapla* (AY757887, AY757888, KP681265, KM881668, KF993633 and AY757899). *Meloidogyne haplanaria* (MK102793-MK102795) is in a clade with other *M. haplanaria* (KT783539, KM881662, AY757905 and AY757906). This clade is sister to *M. enterolobii* with 100% support. *Meloidogyne marylandi* (MK102797) is in a clade with two other *M. marylandi* (JN241917 and...
Meloidogyne incognita (MK102798- MK102802) is in a monophyletic clade with M. incognita, M. arenaria, M. javanica, M. luci, M. ethiopica, M. arabicida, M. lopezi, M. paranaensis, and M. izalcoensis with 98% support. This clade is sister to M. arenaria, M. morocciensis, M. thailandica and M. incognita with 100% support.

**PCR by species-specific primers.** The species identification of M. incognita was confirmed using PCR by M. incognita-specific SCAR primers Inc-K14-F/Inc-K14-R which produced a 399-bp DNA fragment (Fig. 13a) or Finc/Rinc which produced a 1200-bp PCR fragment (Fig. 13b). Only one population (RT83) is positive to primers MH0F/MH1R which were M. hapla-specific with 960-bp amplicon (Fig. 13b). None of these study samples were positive to primers Fjav/Rjav and Far/Rar which are species-specific to M. javanica and M. arenaria respectively. One population TK42 failed to get any good DNA sequencing results on three genes, but it is positive for M. incognita when using PCR by M. incognita-specific SCAR primers (Fig. 13).

**Discussion**
This study characterized DNA sequences on ribosomal DNA 18S-ITS-5.8S, 28S D2/D3 and a mitochondrial DNA CoxII-IGS on 244 RKN populations from various hosts, collected from 39 counties in Arkansas. Five species were identified, including M. incognita, M. hapla, M. haplanaria, M. marylandi and M. partityla through a combined analysis of DNA sequencing and PCR by species-specific primers. The phylogenetic relationships agreed broadly, i.e. sequences analysed were grouped into clades as reasonably expected with no contradictions irrespective of the three loci sequenced. Although DNA sequencing can determine M. hapla, M. haplanaria, M. marylandi and M. partityla by any of the three genes, it is impossible to determine M. incognita because these genes are too conserved among other closely related RKN as shown in blast search and phylogenetic trees. PCR by species-specific primers is needed for the identification of M. incognita. Unlike earlier surveys of the state, M. arenaria, M. javanica and M. graminis were not detected from any of the samples. One RKN population with the second-stage juveniles having very short tails was found in a sample collected at the Lon Mann Cotton Research Station near Brinkley, Arkansas. This sample was found below an oak tree in a mixture of grasses and dicot weeds. Several attempts to find females failed and no DNA study was ever performed. There were some RKN samples forwarded to the second author by the Arkansas Nematode Assay Service and by the Arkansas Plant Health Clinic that contained soil with little or no roots, thus only the second-stage juveniles were available. These second-stage juveniles were reared in a greenhouse using tomato and bermudagrass as possible hosts. While some success in producing
a population of RKN resulted, most testing resulted in failure. This failure was disappointing in that two samples identified with the second-stage juveniles appeared to be *M. arenaria*\(^1\). Another failure was not establishing a RKN population when finding males along with the second-stage juveniles in grass samples in experimental plots from the main University Experiment Station in Fayetteville.

*Meloidogyne incognita* (Southern RKN) is the most abundant species and was identified in 95% samples. It was the only species found in field crops including soybean and cotton, except for one population of *M. haplanaaria* from soybean in Logan County (TK201). This species has worldwide distribution and numerous hosts and is the most damaging species throughout the tropics and warmer regions of the world. *Meloidogyne incognita* is predominantly found in warmer climates, at latitudes between 35°S and 35°N\(^2\). This study revealed *M. incognita* is the most common and widespread species in field crops in Arkansas.

*Meloidogyne hapla* (Northern RKN) is widely distributed, particularly in temperate regions and the cooler, higher altitude areas of the tropics. Taylor & Buhrer\(^27\) reported that in the USA, *M. hapla* was most common north of 39°N. It is polyphagous and affects over 550 crops and weeds\(^28\) including many agricultural and horticultural plants (vegetables, fruits, ornamentals), but few grasses or cereals\(^28\). From the current and previous study\(^17\), this species was found from knockout rose, oak, elm and poke weed (*Phytolacca americana*) from three northern counties including Craighead, Logan, and Washington (Fig. 5), but not from any field crops.

*Meloidogyne haplanaria* (Texas peanut RKN) was originally found attacking peanut in Texas\(^29\) and was also reported from Arkansas\(^17\) and *Mi*-resistant tomato in Florida\(^30\). Host range studies revealed that it can parasitize several legumes and crucifer crops\(^29\) and infect *M. arenaria*-susceptible cultivars of peanut, garden pea and radish\(^31\). Although watermelon, cotton, corn, tobacco and wheat are nonhosts for *M. haplanaria*, peper, eggplant, soybean and common bean are moderate hosts for this nematode\(^29,31\). In our study, this species was found on ash, tomato, peanut, willow, elm, Indian hawthorn and soybean from six counties including Baxter, Faulkner, Logan, Saline, Van Buren and Washington (Fig. 6). It’s worthy to note that only one soybean field (TK201) had *M. haplanaria*. This species is distinct by mitochondrial DNA CoxII-IGS, but similar to *M. incognita*, *M. arenaria* and *M. javanica* in ribosomal DNA 18S-ITS and 28S D2/D3.

*Meloidogyne marylandi* (Maryland RKN) was first described by Jepson & Golden\(^32\) on bermudagrass (*Cynodon dactylon*) in College Park, Maryland, USA. It has been reported from Arkansas\(^17\), Texas\(^33\), Florida\(^34\), Oklahoma\(^35\), North Carolina, South Carolina\(^36\), Arizona, California, Nevada, Utah and Hawaii\(^37\). Outside USA, *M. marylandi* has been found in Japan\(^38\), Israel\(^39\), and Costa Rica\(^40\). From current and previous study\(^17\), this species was found from grasses from six counties including Craighead, Drew, Hempstead, Logan, Perry and Washington (Fig. 7).
Another closely related species, *M. graminis*, is native to USA. It was first described infecting St. Augustine grass (*Stenotaphrum secundatum*) in Winter Haven, Florida, in 1964. This species has been reported on cultivated grasses from Florida to California and Hawaii, as far north as New England, on native grasses in the Konza Prairie in Kansas, North Carolina, and South Carolina. The *M. graminis* from grass reported in 1974 by Grisham et al. and in 1982 by Robbins was believed to be *M. marylandi* which was described much later in 1987. Before *M. marylandi* was described in 1987, no DNA analysis was available and species found from grass in Arkansas was assigned as *M. graminis*. Thus, no *M. graminis* is really confirmed in Arkansas.

*Meloidogyne partityla* (pecan RKN) is a plant pathogenic nematode infecting pecan. It was first described in pecan trees in South Africa by Kleynhans (1986). It is thought to have been introduced into South Africa by pecan seedlings that came from USA in 1912, 1939 and 1940. Today, this nematode is seen infecting pecan trees in Arizona, Arkansas, Florida, Georgia, New Mexico, Oklahoma, South Carolina and Texas. In addition to pecans, they also infect the California black walnut (*Juglans hindsii*), English walnut (*J. regia*), shag-bark hickory (*Carya ovata*), post oak (*Quercus stellate*), water oak (*Quercus nigra*) and laurel oak (*Q. laurifolia*). The health of infested trees continues to decline every year. In this study, only one sample from pecan in Logan County was identified as *M. partityla* (Fig. 8).

*Meloidogyne enterolobii* (Guava RKN) is a recent emerging and highly pathogenic RKN species in the USA. It was originally described from China in 1983 and later reported in Florida in 2004, North Carolina in 2013, Louisiana in 2019 and South Carolina in 2019 attacking field crops, vegetables, ornamental plants, guava tree and weeds. *Meloidogyne enterolobii* is considered as a tropical species; due to its limited distribution and high damage impact, it was added to the European and Mediterranean Plant Protection Organization A2 Alert list and became a regulated nematode in South Korea, Costa Rica and USA (Florida, Louisiana, Mississippi, North...
Carolina). Fortunately, *M. enterolobii* was never detected in our survey and thus it is listed as a regulated species to prevent its dispersal.

In this study, DNA sequencing and PCR by species-specific primers were employed successfully to characterize and identify RKN from a wide range of plants from 39 counties in Arkansas. The results revealed the presence of five RKN species with *M. incognita* being the most predominant. Their hosts, distribution, DNA sequences of three genes and phylogenetic relationships were investigated. This study provides basic information for future management of these economically important species in Arkansas.

**Methods**

**Nematode sample collection.** A total of 244 RKN populations from various hosts from 39 counties in Arkansas were sampled in this study from 2014 to 2018 (Table 1) (Fig. 1). These samples were collected during the growing season. No specific permissions were required in sampling for plant-parasitic nematodes and no endangered or protected species were involved. Two hundred and six RKN samples (TK1-TK206) were initially
collected from soil samples that were taken by Arkansas Cooperative Extension Service agents as a part of a statewide nematode survey sponsored in part by the Arkansas Soybean Promotion Board. Samples were collected during the period from September 1 – November 1 in 2014–2016 and were from fields that were either in soybean in the year they were sampled, or they were cropped to corn, grain sorghum, or cotton as a rotation crop with soybean. Samples were stored and transported to the Arkansas Nematode Diagnostic Laboratory in Hope, Arkansas in plastic bags inside insulated coolers. Samples were stored no longer than two weeks prior to assay. When RKN was extracted through routine elutriation and sugar flotation of a sub-sample, the remaining soil was placed into a 15-cm-diameter clay pot filled with 50:50 mixture of fine builders’ sand and sandy loam topsoil. A single...

Figure 11. Bayesian consensus tree inferred from rDNA 28S D2/D3 under TVM + I + G model (−\ln L = 5664.7959; AIC = 11347.5918; freqA = 0.2548; freqC = 0.1889; freqG = 0.2676; freqT = 0.2888; R(a) = 0.6653; R(b) = 3.0047; R(c) = 1.7303; R(d) = 0.3041; R(e) = 3.0047; R(f) = 1; Pinva = 0.2636; Shape = 0.6053). Posterior probability values exceeding 50% are given on appropriate clades.
tomato seedling (*Solanum lycopersicon* L var. *lycopersicum*, cv. ‘Rutgers’) at the age of three to four week old from germination was grown in the soil in a greenhouse. Tomato plants were then removed from the soil and the root systems were washed to remove excess soil at harvest. Root galls on tomato were collected after 60–70 days of inoculation and shipped to Nematode Lab at Agronomic Division in North Carolina Department of Agriculture. Thirty-eight other populations were collected by the second author. Galls or dissected females were shipped to NCDA without rearing nematodes on tomato.

**DNA extraction.** RKN females were dissected in water in a 9-cm petri dish under Zeiss Stemi 2000-C microscope (Gottingen, Germany). A single female was pipetted into 10-µl 1X TE buffer (10 mM Tris-Cl, 1 mM EDTA; pH 9.0) on a glass microscope slide (7.5 cm × 2.5 cm). The nematodes were then macerated with a pipette tip into pieces, collected in 50-µl 1X TE buffer and stored at −20 °C. Three DNA replicates per sample were prepared for.
any samples with females. If only the second-stage juveniles were available, 1–10 juveniles were macerated with a pipette tip into pieces and put in one tube as DNA template in 50-µl 1X TE buffer.

**DNA amplification, cleaning and sequencing.** The primers used for ribosomal and mitochondrial DNA PCR and DNA sequencing are shown in Table 2 as previously described. These primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). The 25-µl PCR was performed using 12.5-µl 2X Apex Taq red master mix DNA polymerase (Genesee Scientific Corporation, San Diego, CA, USA), 9.5-µl water, 1-µl each of 10-µM forward and reverse primers, and 1 µl of DNA template according to the manufacturer's protocol in a Veriti® thermocycler (Life Technologies, Carlsbad, CA, USA). The thermal cycler program for PCR was as follows: denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. PCR products were cleaned using ExoSap-IT (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. DNA sequencing was performed using PCR primers for direct sequencing by dideoxynucleotide chain termination using an ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Life Technologies, Carlsbad, CA, USA) in an Applied Biosystems 3730 XL DNA Analyzer (Life Technologies) by the Genomic Sciences Laboratory (North Carolina State University, Raleigh, NC, USA). The molecular sequences were compared with other nematode species available at the GenBank sequence database using the BLASTn homology search program.

**Phylogenetic analyses.** DNA sequences were edited with ChromasPro1.5 2003–2009 (Technelysium Pty Ltd, Helensvale, Australia) and were aligned by Mega7.0.14 using default settings. The model of base substitution in the DNA sequence data was evaluated using MODELLTEST version 3.06. The Akaike-supported model, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses using DNA sequence data. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0, running the chain for 1,000,000 generations and setting the ‘burnin’ at 2,500. Markov Chain Monte Carlo (MCMC) methods were used within a Bayesian framework to estimate the posterior probabilities (pp) of the phylogenetic trees using the 50% majority-rule. The λ² test for homogeneity of base frequencies and phylogenetic trees was performed using PAUP* version 4.0 (Sinauer Associates, Inc. Publishers, Sunderland, MA, USA).
Species identification using PCR by species-specific primers. The species identification of *M. incognita* was confirmed using PCR by species-specific SCAR primers Inc-K14-F/Inc-K14-R which produce a 399-bp DNA fragment. Another set of *M. incognita*-specific SCAR primers was a 1200-bp PCR fragment amplified by Finc/Rinc, Fjav/Rjav, Far/Rar, and MHOF/MH1R. The other species-specific primers to *M. javanica*, *M. arenaria*, and *M. hapla* which produced 670-bp, 420-bp, and 960-bp DNA fragment respectively. The 25-µl PCR was performed using 12.5-µl 2X Apex Taq DNA polymerase, 7.5-µl water, 1-µl each of 10-µM forward and reverse primers, and 1-µl of DNA template. The PCR condition is the same as described above.

Received: 6 August 2019; Accepted: 11 October 2019; Published: xx xx xxxx

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Acknowledgements
The authors thank Katie Sullivan, the lab supervisor at the Arkansas Nematode Diagnostic Laboratory, University of Arkansas, who provided samples from the agronomist crops and built up the root-knot nematode populations in the greenhouse and Dr. Churamani Khanal currently at the Nematology lab, University of Florida for providing some nematode samples from his master degree project in University of Arkansas. The authors also thank Sherrie Smith, the director of the Arkansas Plant Health Clinic in University of Arkansas, for her efforts in recruiting root-knot samples for us in her monthly reports to county agricultural agents and master gardeners of Arkansas. This work was supported in part by the Arkansas Soybean Promotion Board. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Any opinion, finding, conclusion or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view and policies of the funding source.

Author contributions
Conceived and designed the experiments: W.Y., R.T.R. and T.K. Performed the experiments: W.Y., R.T.R. and T.K. Analyzed the data: W.Y. Contributed reagents/materials/analysis tools: W.Y., R.T.R. and T.K. Wrote the paper: W.Y., R.T.R. and T.K.

Competing interests
The authors declare no competing interests.

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