First report and molecular characterization of the dagger nematode, *Xiphinema oxycaudatum* (Nematoda, Dorylaimidae) from South Africa

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Abstract

Plant-parasitic nematodes of the genus *Xiphinema* Cobb, 1913 comprise a complex group of nematode species, some of which are important vectors of plant viruses. During a field survey to determine the soil health of an abandoned honeybush (*Cyclopia genistoides*) monoculture, a high density of the dagger nematode, *Xiphinema oxycaudatum* Lamberti & Bleve-Zacheo, 1979 (Nematoda, Dorylaimidae), was observed in soil around the roots of honeybush plants in an abandoned farmland at Bereaville, an old mission station in the Western Cape province of South Africa. Soil samples were taken from the rhizosphere of plants and nematodes were extracted from the soil using a modified extraction tray method. Specimen of the dagger nematodes were processed for scanning electron microscopy, morphological and molecular analysis. Molecular profiling of the nematode species was done in order to give an accurate diagnosis and to effectively discriminate the nematode from other species within the *Xiphinema americanum* group. Phylogenetic analysis based on the D2D3 expansion segment of the 28S gene supported a close relationship of species within the *americanum* group, however, the protein-coding cytochrome oxidase (*coxI*) of the mitochondrial gene provided a useful tool for distinguishing the nematode from other species within the group. This study represents the first report of *X. oxycaudatum* from South Africa.

Keywords

*coxI*, D2D3, honeybush, molecular identification
Introduction

Dagger nematodes, belonging to the *Xiphinema americanum*-group, are economically important nematodes that may cause damage to agricultural crops, by means of direct feeding on plant roots and in transmitting plant viruses. *Xiphinema oxycaudatum* Lamberti & Bleve-Zacheo, 1979 (Nematoda, Dorylaimidae) is a polyphagous and cosmopolitan nematode, which was first described from the rhizosphere of oil palm, *Elaes guineensis* in Nigeria (Lamberti and Bleve-Zacheo 1979). A high population of this nematode species were found in soil around honeybush (*Cyclopia genistoides*), in an abandoned farmland (-34.0516, 19.5174) at Bereaville in the Western Cape province, South Africa.

Although many nematode species in the *X. americanum*-group are widespread in distribution, *X. oxycaudatum* is localized in Africa with a few reports from Asia and South America (Lamberti et al. 2000; Fadaei et al. 2003; Oliveira et al. 2003; Chen et al. 2005). In South Africa, *Xiphinema* species have been listed as one of the most common and abundant plant parasitic nematodes causing damage on grapevines and woody plants (Fourie et al. 2017). However, only a few species belonging to the *Xiphinema americanum*-group have been reported in the country, some of these include; *X. americanum*, *X. brevicolle*, *X. diffusum*, *X. incognitum*, and *X. pachtaicum* (Lamberti et al. 1995, 2002).

Honeybush is an exclusive African herbal tea with a distinctive honey aroma and it is a rich source of compounds with antimutagenic properties (Kokotkiewicz and Luczkiewics 2009). There is an increasing demand for honeybush production in South Africa, due to increased awareness of the health benefits obtainable from this unique tea (SAHTA 2011).

In this study, the dagger nematodes found in soil around honeybush were identified with a combination of traditional morphological characterization and molecular techniques, based on the D2D3 expansion segment of the 28S gene and the protein-coding cytochrome oxidase (*coxI*) of the mitochondrial gene.

Methods

**Sampling, nematode isolation, and processing**

Soil samples were collected from three plots on the honeybush farmland, with five composite samples taken from each plot. Samples were taken from the rhizosphere of the plants, a depth of about 8 cm into the soil. Nematodes were extracted from the soil using a modified Whitehead and Hemming (1965) tray method and examined under a high-power compound microscope. Nematode specimens from a previously identified population of *Xiphinema americanum* Cobb, 1913 from a grapevine farm in the Western Cape was also included in the study. Nematodes were counted using a stereomicroscope and specimens collected for morphological study, scanning electron microscopy (SEM), and for molecular characterization of nematode species.
**Light and scanning electron microscope observations**

For light microscopy, nematode specimens were mounted on glass slides and observed under a compound microscope. Morphological characters were measured and light micrographs were taken with a Zeiss Axioskop 40 compound microscope equipped with a drawing tube. Adult females and juveniles were observed. Some of the morphometric features that were measured include total body length, oesophageal length, body diameter, stylet lengths (odontostyle and odontophore), lip region diameter, distance of basal guide ring from anterior, distance from anterior end to the vulva, width at vulva, and the tail length (Table 2). These measurements were used to calculate the characters; a, b, c, c’ and V.

**Table 1.** Primer combination.

| Primer code | Direction | Sequence (5’–3’) | Amplified gene | References |
|-------------|-----------|------------------|----------------|------------|
| D2A         | Forward   | ACA AGT ACC GTG AGG GAA AGT TG | 28S rRNA | Nunn 1992 |
| D3B         | Reverse   | TCG GAA GGA ACC AGC TAC TA |          | Nunn 1992 |
| ITS1        | Forward   | TTAGTACGTCCCTGGCCCTTT | ITS rRNA | Vrain et al. 1992 |
| P28S        | Reverse   | TTTCACCGCGGTACTAAGG-G- | COI | Vrain et al. 1992 |
| CO1F        | Forward   | GATTTTTGGKCATCCWGARG | COI | He et al. 2005 |
| CO1R        | Reverse   | CWACATAAAGTACATCG | COI |          |
| XIPHR1      | Reverse   | ACAATTCCAGTTAATCCTCACC | COI | Lazarova et al. 2006 |
| XIPHR2      | Reverse   | GTACATAATGAAAATGTGCCAC | COI | Lazarova et al. 2006 |

**Table 2.** Morphometrical data of *Xiphinema oxycaudatum* from South Africa. Measurements are in µm, except where stated otherwise, in the form of: mean ± standard deviation (range).

|                         | Female | Pre-adult | Stage before pre-adult |
|-------------------------|--------|-----------|------------------------|
| n                       | 11     | 6         | 1                      |
| L (mm)                  | 1.80 ± 10.52 (1.60–1.94) | 1.42 ± 8.79 (1.33–1.52) | 128.5                |
| a                       | 46.87 ± 4.37 (39.9–55.3) | 37.46 ± 2.90 (33.6–40.9) | 42.8                 |
| b                       | 6.14 ± 0.56 (4.8–6.9) | 4.93 ± 0.78 (3.5–5.7) | 5.2                  |
| c                       | 50.34 ± 2.96 (45.4–55.3) | 37.28 ± 4.48 (29.9–42.2) | 37.3                 |
| c’                      | 1.43 ± 0.08 (1.3–1.6) | 1.58 ± 0.10 (1.4–1.7) | 1.6                  |
| V                       | 49.82 ± 1.44 (47.8–52.4) | – | –                    |
| Odontostyle length      | 78.41 ± 5.12 (71–84) | 64.9 ± 2.84 (61–68) | 55                   |
| Odontophore length      | 56.14 ± 5.4 (46–66) | 47.5 ± 0.84 (47–49) | 43                   |
| Total stylet length     | 135.55 ± 5.41 (129.5–149.5) | 112 ± 3.33 (111–117) | 98                   |
| Replacement odontostyle length | – | 78.78 ± 3.87 (74–85) | 59.5                |
| Anterior to guide ring  | 67.36 ± 2.84 (64–73) | 55.5 ± 2.89 (50.5–58–73) | 51.5               |
| Tail length             | 35.82 ± 2.74 (31–41) | 37.00 ± 4.70 (31–44.5) | 34.5               |
| h (hyaline portion of tail; also J) | 12.91 ± 1.61 (10.5–15.5) | 9.92 ± 1.02 (9–11) | 11           |
| h % (hyaline portion/tail length) | 36.08 ± 3.85 (29–40.3) | 26.93 ± 1.95 (23.7–29) | 31.9               |
| Lip region diameter     | 12.86 ± 0.87 (11.5–13.5) | 11.5 ± 0.54 (11–12) | 11                   |
| Lip region height       | 5.86 ± 0.32 (5.5–6.5) | 5.42 ± 0.38 (5–6) | 4.5                  |
| Body diameter at guide ring | 28.64 ± 1.80 (26–31.5) | 25.75 ± 2.95 (23.5–31.5) | 24                  |
| Body diameter at base of pharynx | 36.20 ± 2.52 (33–42) | 34.67 ± 4.01 (33–41) | 29                  |
| Body diameter at vulva or mid-body for juvenile | 39.18 ± 2.57 (36.5–44) | 37.90 ± 5.19 (31–44) | 30                  |
| Body diameter at anus    | 25.18 ± 1.97 (20.5–27.5) | 23.42 ± 2.25 (20–26) | 21                  |
| Body diameter at beginning of hyaline portion of tail | 13.50 ± 1.22 (11.5–16) | 10.67 ± 0.61 (10–11.5) | 9                  |
| Pre-rectum length       | 103.85 ± 47.37 (47–214) | 55; 70 | –                      |
| Rectum length           | 20.14 ± 4.61 (15–31.5) | 23.13 ± 7.49 (17–34) | –                    |
| Vagina length           | 14.68 ± 1.01 (12.5–16) | – | –                      |
Specimen samples for SEM were handpicked, fixed overnight in 2% Glutaraldehyde and dehydrated in increasing concentrations of ethanol. The nematode specimens were chemically dried with Hexamethyldisilizane (HMDS) in a fume hood and kept in a desiccator overnight. Nematodes were mounted on double-sided carbon tapes on Al stubs and were sputter coated with Pd/Au at a thickness of 100 Å layer for 10 min.

A Zeiss Merlin FESEM (Carl Zeiss Microscopy, USA) was used to generate electron images at 3kV accelerating voltage using InLens SE and SE2 detection and a probe current of 100–150 pA. Images were captured in TIF format using a pixel averaging noise reduction algorithm.

DNA extraction, PCR, and sequencing

DNA was extracted from single adult female nematodes using a modified method of Nguyen (2007). The polymerase chain reaction (PCR) to confirm the identity of the nematode specie was carried out by the amplification of the internal transcribed spacer (ITS) region, the D2D3 expansion segment of the 28S gene of the ribosomal DNA, and the portion of the cytochrome oxidase (coxi) gene of the mitochondrial DNA. PCR of the ITS region was carried out as described by Chen et al. 2005 using KAPA2G 40 Robust HotStart ReadyMix (KAPA Biosystems) with the primer combination of S-ITS1 (5'-TTGATTACGTCCCTGCCCTTT-3') and 28S (5'-TTTCACTCGCCGTTACTAAGG-3'). Amplification was carried out in a thermal cycler with the following cycling condition; 1 cycle at 94 °C for 4 min, followed by 30 cycles at 94 °C for 30 sec, 52 °C for 30 sec, and 72 °C for 2 min 30 sec, and ending with one cycle at 72 °C for 7 min and finally kept at 4 °C. PCR amplification of the D2-D3 expansion segments of the 28S rDNA gene was carried out with the primer set D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') with the cycling condition of 4 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min 30 sec at 72 °C, and a final extension at 72 °C for 10 min (Orlando et al. 2016). The portion of the partial coxi of the mitochondrial gene was amplified using a primer combination of the forward primer, COIF (5'-GATT TTTT TGGKCATCCWGARG-3') with the reverse primer, XIPHR2 (5'-GTACATAATGAAAATGTGCCAC-3') as described by Lazarova et al. (2006). The thermal condition includes 1 cycle of 94°C for 1 min, 50 °C for a further 1 min and 72 °C for 2 min. This was followed by 40 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 2 min. PCR was ended with a final extension phase of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 5 min.

Sequence and phylogenetic analysis

PCR products were purified using the Nucleo-Fast Purification System (Macherey Nagel, Waltham, Massachusetts, USA). Sequencing of the purified DNA was performed in both directions with the Big Dye Terminator V1.3 sequencing kit, followed
by the use of electrophoresis on the 3730× 1DNA Analyser (Applied Biosystems) at the DNA Sequencing Unit (Central Analytical Facilities, Stellenbosch University). The Software CLC Main Workbench 7.3 (http://www.clcbio.com) was used for sequence assembly and editing. Newly obtained partial cox1 sequences of *X. oxycaudatum* and *X. americanum* were deposited on the GenBank database with accession numbers MK211480 and MK956813 respectively. DNA sequences obtained for the D2D3 expansion segment of *X. oxycaudatum* was also deposited with accession numbers MK947997, MK966417, and MK988554.

The newly obtained DNA sequences were used for BLASTN (Altschul et al. 1997) comparison against GenBank sequences. DNA sequences from the top BLASTN matches, and other nematode sequences, were downloaded from GenBank and aligned using Multiple Alignment using Fast Fourier Transform (MAFFT).

The evolutionary history of the cox1 region of the mitochondrial gene and D2D3 expansion segment of the 28S gene was inferred using the maximum parsimony (MP). The most parsimonious tree is shown. Evolutionary analyses were conducted in MEGA X version 10.0.5 (Kumar et al. 2018) and the confidence intervals for the various branching patterns in the trees were measured using bootstraps (Felsenstein 1985) with 1000 replicates. Estimates of the evolutionary divergence between sequences was done using pairwise distance analysis.

**Results**

*Xiphinema oxycaudatum* was observed in high numbers from samples taken from the abandoned honeybush farmland with a mean population density of about 510/250 cm³ soil.

Observations with SEM provided detailed information on some intrinsic features of the nematode such as the stirrup-shaped amphidial pouch, slit-like aperture, caudal pores and vagina opening (Fig. 1).

The morphological features of the nematodes are similar to those described from Nigeria (Lamberti and Bleve-Zacheo 1979; Bos and Loof 1983). Both adult females and juveniles were observed. The habitus of the nematodes are spiral or C-shaped with a head that is slightly offset. Adult females are between 1600–1800 µm long. They are more ventrally curved at the posterior end than the anterior. The vulva is located slightly above 50% of the body length; the ovary is amphi-didelphic with long oviduct and short uteri. The tail is conoid with bluntly rounded terminus. The juvenile stages are similar to adult females, but with a smaller body size. They also possess more pointed and sharper conoid tails. No male was found.

**Description**

Female: Body strongly curved ventrally into close C-shape. Cuticle 2.7 µm wide at mid-body, 6.5 µm at dorsal side of tail, radial striations visible on tail end. Lip region demarcated from body by slight depression (Fig. 2). Position of pharyngeal gland nuclei
Figure 1. Scanning electron micrographs of *Xiphinema oxycaudatum* A–C head region with stirrup-shaped amphidial pouch and slit-like aperture, vulva opening and tail showing a caudal pore. Scale bars: 2 μm.
**Figure 2.** Light microscopy of *Xiphinema oxycaudatum* A–D head region, female reproductive system with didelphic ovary, tail region and vulva. Scale bars: 10 µm (A, C, D), 20 µm (B).
and outlets (as percentage of bulb length): DO = 9.21 (6.5–11); DN = 15.05 (10.1–18.2); DN–DO = 5.83 (3.6–7.3); SN1 = 59.07 (49.3–67.8); SO = 64.54 (49.3–74.3); SN1–SO = 5.42 (0–11); SN2 = 62.86 (59.1–69.3); SN2–SO = 11.25 (5–18.3). Neck region 288.75 ± 20.16 (265–310) µm long; cardia small, hemispherical to conoid in shape. Female reproductive system typical of X. americanum lineage (ovaries with symbionts, long oviducts, short uteri), each branch about two corresponding vulva diameters long. Tail conoid, dorsally convex, ventrally slightly arcuate with rounded terminus, two caudal pores on each lateral side.

Relationship

The specimens from South Africa agree well with the type description of X. oxycaudatum (Table 2) but are slightly longer (1.6–1.94 mm vs 1.5–1.7 mm); the vulva is situated more anterior in one specimen (47.8% vs 51–54%) and have a wider head region (11.5–13.5 µm vs 9–10 µm). However, the South African specimens are closer to the description of X. oxycaudatum from Iran (Fadaei et al. 2003) especially in the body length (1.6–1.9 mm in Iranian specimens) and more anterior position of vulva in some females (45.5–54% in Iranian specimens). The wider head region in the South African specimens are considered to be an intraspecific variation. The pre-adult stage juvenile from South Africa agrees well with the description of this stage described from Iran (Fadaei et al. 2003). One juvenile was found, which apparently falls in a stage before the pre-adult juvenile. It can be distinguished from the pre-adult stage, by the shorter replacement odontostyle (59 µm vs 74–84 µm in pre-adult juvenile). The specimens from South Africa are also near X. peruvianum Lamberti & Bleve-Zacheo, 1979, but can be distinguished by the shorter odontostyle (71–84 µm vs 85–92 µm and the shape of the tail (gradually tapered, conoid vs not so gradually tapered, almost subdigitate).

The phylogenetic relationships within the X. americanum-group species inferred from the analysis of D2D3 expansion segments of 28S and the partial mitochondrial coxl gene using MP are given in Figures 3 and 4 respectively. The D2D3 alignment was 710 base pairs long and included 59 X. americanum-group sequences with two outgroup sequences (X. index and Longidorus crataegi). Phylogenetic analysis of the D2D3 expansion region revealed a high similarity of almost 100% with some species in the americanum-group. Nearly identical sequences were obtained from the studied species, with interspecific divergence ranging from 0 to 0.25%. The MP tree showed two supported clades. Clade I (72%) includes: X. pachtaicum, X. incertum, X. pachydermum, X. parapachydermum, Xiphinema sp., X. simile, X. browni, and other nematode species. Clade II (100%) comprised of X. brevicolles species complex (Orlando et al. 2016): X. citricolum, X. americanum, X. californicum, X. rivesi, X. laevistriatum, and other species. Relationship within this clade was not well resolved. Intra-specific variation with about 2–3 indel events was also observed in the X. oxycaudatum sequences. The genetic relationship of the newly obtained sequences with reference sequences obtained from the National Centre for Biotechnology Information (NCBI) is illustrated in Figure 3.
Figure 3. Phylogenetic relationship within species of the *Xiphinema americanum*-group, based on analysis of the D2D3 regions with maximum parsimony (MP) using *Xiphinema index* and *Longidorus crataegi* as outgroups. Newly obtained sequence is indicated by bold letters.
Figure 4. Phylogenetic relationship within species of the *Xiphinema americanum*-group, based on analysis of the *coxI* regions with maximum parsimony (MP), using *Pratylenchus bolivianus* and *Caenorhabditis elegans* as outgroups. Newly obtained sequences are indicated by bold letters.
Species delimitation of X. oxycaudatum within the X. americanum group was achieved by analysing the coxI sequence alignment which comprised of 66 X. americanum group sequences and two other sequences, Pratylenchus bolivianus and Caenorhabditis elegans as outgroups. The alignment length was 298 base pairs long. Although there was no available sequence of the partial coxI gene of X. oxycaudatum on the NCBI database for comparison, the sequence showed a similarity of 86.19% and 82.48% with X. peruvianum and X. rivesi respectively. The pair-wise distance of X. oxycaudatum to the closely related Brazilian population of X. peruvianum is 245 base pairs differences (Table 3). Newly obtained X. americanum sequence showed a high similarity of 98.84% to the South African isolate (AM086690) with only four nucleotide differences. Estimates of the evolutionary divergence between the newly obtained sequence and some closely related ones is shown in Table 3. The number of base differences per sequence from between sequences are indicated.

Phylogenetic analysis of the aligned sequences revealed five major subclades within the studied americanum-group. They include: X. americanum, X. californicum, Xiphinema sp., X. brevicolle complex, and X. pachtaicum. Xiphinema oxycaudatum was closely related to Xiphinema sp. (Iran) and the Brazilian population of X. peruvianum. Within the 50% majority rule consensus MP tree, no significant difference was obtained in the two closely related species. However, sequences obtained from the coxI mitochondrial gene clearly discriminates X. oxycaudatum from other species within the X. americanum-group. The genetic relationship of this sequence with reference sequences obtained from the NCBI is illustrated in Figure 4.

**Discussion**

Precise identification of nematode species and knowledge of their distribution is important for effective phytosanitary and management options. Species identification of nematodes within the Xiphinema americanum group is often difficult and complicated due to overlapping of morphological features and phenotypic plasticity. The taxonomy of this group of nematodes is often regarded as controversial and subjective (Luc et al. 1998; Orlando et al. 2016), and there is a possibility to confuse and misidentify species within the group.

Some key morphological features that have been frequently used as diagnostic keys for differentiating between species within the Xiphinema americanum group include the lip region, odontostyle length, position of C, tail shape, and length (Lamberti and Bleve-Zacheo 1979; Lamberti and Carone 1991). However, in more recent times, identification has been done in combination with molecular tools with indications of mitochondrial marker cytochrome oxidase subunit 1 (coxI) as a barcode for species identification and a tool for resolving the complexity in identifying cryptic americanum species (Palomares-Rius et al. 2017).

Although the molecular analysis, based on the D2D3 region of the nematodes species in the present study revealed low interspecific variation in the nematodes within the X. americanum group, two distinct clades were evident from the phylogenetic tree.
Table 3. Pairwise distances of COI regions between *Xiphinema oxycaudatum* and some closely related sequences within the *Xiphinema americanum* group. The number of base differences per sequence from between sequences are shown.

| Species | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1 X._ oxycaudatum_(South_Africa_M K211480) | 98 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2 X._rivesi_(USA_Florida_KX263104) |    | 98 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 3 Xiphinema _sp._(USA_Florida_KX263101) | 99 | 52 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 4 X._ tarjanense_( USA_Florida_AM086694) | 103 | 45 | 32 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5 Xiphinema _sp._(Iran_MK 202796) | 105 | 56 | 66 | 56 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 6 X._georgianum_(USA_Florida_AM086695) | 106 | 56 | 65 | 56 | 65 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 7 X._incognitum_(China_AM086705) | 107 | 68 | 63 | 58 | 65 | 72 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 8 X._brevicolle_(Russia_KX263107) | 107 | 76 | 68 | 62 | 62 | 72 | 55 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 9 X._rivesi_(Spain_KIZ90060) | 110 | 53 | 47 | 53 | 57 | 75 | 61 | 70 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 10 X._lambertii_(Czech_Republic_H M163208) | 112 | 77 | 74 | 79 | 72 | 75 | 63 | 62 | 73 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 11 X._brevicolle_(Brazil_AM086707) | 118 | 69 | 70 | 74 | 69 | 81 | 66 | 77 | 82 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 12 X._luci_(Spain_KY816627) | 120 | 65 | 70 | 63 | 67 | 63 | 78 | 81 | 74 | 82 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 13 X._taylori_(Slovakia_AM086703) | 120 | 69 | 66 | 70 | 61 | 69 | 71 | 43 | 72 | 71 | 81 | 71 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 14 X._citricolum_(USA_Florida_AM086693) | 122 | 61 | 58 | 59 | 57 | 67 | 68 | 67 | 71 | 79 | 59 | 73 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 15 X._florida_c_(USA_Florida_AM086696) | 127 | 69 | 72 | 64 | 72 | 64 | 82 | 81 | 78 | 87 | 85 | 64 | 81 | 63 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 16 X._rivesi_(USA_Arkansas_s_AM086697) | 128 | 69 | 70 | 65 | 70 | 66 | 84 | 79 | 74 | 85 | 85 | 62 | 81 | 69 | 6 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 17 X._diffusum_(China_AM086701) | 120 | 62 | 65 | 61 | 63 | 67 | 69 | 54 | 67 | 71 | 79 | 76 | 56 | 71 | 88 | 86 |    |    |    |    |    |    |    |    |    |    |    |    |
| 18 X._diffusum_(Brazil_AM086699) | 122 | 63 | 64 | 58 | 62 | 64 | 65 | 50 | 67 | 70 | 79 | 74 | 55 | 71 | 83 | 81 | 11 |    |    |    |    |    |    |    |    |    |    |    |    |
| 19 X._asta regiense_(Spain_KP268977) | 132 | 113 | 108 | 113 | 102 | 116 | 103 | 105 | 116 | 105 | 106 | 112 | 106 | 119 | 122 | 122 | 116 | 116 |    |    |    |    |    |    |    |    |    |    |    |    |
| 20 X._simile_(Slovakia_AM086708) | 135 | 94 | 105 | 106 | 84 | 108 | 97 | 102 | 98 | 94 | 108 | 99 | 99 | 101 | 104 | 103 | 101 | 106 | 123 |    |    |    |    |    |    |    |    |    |    |    |    |
| 21 X._peruvianum_(Brazil_AM086712) | 245 | 211 | 212 | 243 | 203 | 247 | 250 | 218 | 230 | 259 | 252 | 233 | 250 | 250 | 248 | 243 | 245 | 241 | 247 |    |    |    |    |    |    |    |    |    |    |    |
| 22 X._peruvianum_(USA_Georgia_AM086692) | 272 | 234 | 241 | 255 | 224 | 265 | 268 | 242 | 254 | 279 | 267 | 258 | 264 | 266 | 269 | 263 | 266 | 256 | 264 | 72 |    |    |    |    |    |    |    |    |    |    |
| 23 X._americanum_(South_Africa_M K956813) | 98 | 48 | 52 | 47 | 57 | 58 | 59 | 58 | 56 | 67 | 74 | 50 | 64 | 32 | 58 | 61 | 58 | 58 | 97 | 94 | 236 | 240 |    |    |    |    |    |    |    |    |    |
| Species                        | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|-------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 24 X._america num_(South_Africa_AM086690) | 119| 57 | 59 | 53 | 60 | 64 | 67 | 63 | 62 | 75 | 83 | 56 | 71 | 34 | 67 | 69 | 66 | 63 | 114| 98 | 248| 268| 4  |    |    |    |    |    |
| 25 X._americanum_(USA_Florida_AM086691)  | 273| 243| 244| 260| 254| 284| 279| 247| 266| 286| 282| 256| 274| 272| 272| 279| 277| 267| 278| 260| 291| 239| 276|    |    |    |    |    |
| 26 X._america num_(USA_California_KX263065) | 226| 238| 241| 216| 229| 235| 228| 239| 244| 243| 239| 236| 232| 228| 226| 226| 233| 232| 236| 233| 224| 246| 205| 232| 50 |    |    |    |    |    |
| 27 X._america num_(USA_California_KX263057) | 232| 247| 249| 221| 235| 247| 239| 245| 250| 253| 248| 242| 238| 236| 236| 243| 242| 245| 239| 224| 249| 213| 240| 6  | 47 |    |    |    |    |    |
| 28 X._america num_(USA_Alabama_KX263058)   | 244| 242| 247| 226| 230| 244| 242| 245| 244| 246| 246| 248| 243| 243| 246| 244| 244| 246| 245| 237| 65 | 33 | 223| 247| 271| 262| 263|    |    |    |    |    |
| 29 X._america num_(USA_California_KX263064) | 247| 237| 243| 227| 225| 245| 241| 243| 245| 259| 250| 249| 246| 244| 247| 245| 247| 249| 251| 236| 63 | 28 | 223| 248| 271| 257| 256| 35 |    |    |    |    |    |
| 30 X._america num_(USA_California_KX263060) | 250| 242| 247| 232| 230| 250| 248| 251| 250| 265| 252| 254| 249| 249| 252| 250| 250| 252| 250| 243| 65 | 34 | 229| 253| 274| 262| 263| 0  | 36 |    |    |    |    |    |
| 31 X._america num_(USA_California_KX263063) | 253| 242| 247| 235| 230| 253| 251| 251| 253| 268| 255| 257| 252| 252| 255| 253| 255| 252| 246| 65 | 35 | 232| 256| 277| 262| 263| 0  | 37 | 0  |    |    |    |    |    |
| 32 X._ina equale_(Czech_Republic_HM163207) | 264| 249| 258| 258| 233| 279| 270| 259| 286| 287| 278| 272| 272| 265| 267| 267| 269| 269| 283| 270| 259| 290| 241| 272| 76 | 69 | 67 | 279| 281| 282| 285|    |    |    |    |    |
**X. oxycaudatum** was separated in a group from other *Xiphinema* species with a strong statistical support. This was also evident from previous studies where low interspecific variation within the *X. americanum*-group has been reported (He et al. 2005; Orlando et al. 2016). They indicated that *X. americanum*-group species formed two highly supported clades, *X. americanum* and *X. pachtaicum* (sensu Lamberti and Ciano 1993). Oliveira et al. (2004) also obtained nearly identical result with analysis of the 18 rDNA sequences where species belonging to the *X. americanum*-group formed a single group separated from the other *Xiphinema* species. He however suggested that 18S rDNA does not provide a useful marker to discriminate *Xiphinema* in the *americanum* group at the species level. This was also confirmed by Zasada et al. (2014), who showed that 18S rDNA sequence data did not provide taxonomic clarity among some populations of *X. americanum*. In the present study, the sequences obtained from the ITS region were of poor quality and were not used for phylogenetic analysis.

The protein coding mitochondrial gene, cytochrome oxidase subunit I (**coxI**), has been described as a reliable and preferred molecular barcode and a useful tool for highlighting the intra-specie variation within some species of *X. americanum*-group (Lazarova et al. 2006; Gutiérrez-Gutiérrez et al. 2012; Lazarova et al. 2016; Orlando et al. 2016; Palomares-Rius et al. 2017). In the present study, **coxI** gene was used to reconstruct the phylogenetic relationship within the species; thus, in combination with morphological identification, it provided a useful tool for delimitation and discrimination of *X. oxycaudatum* from other species within the *americanum*-group.

This study represents the first report of *X. oxycaudatum* in association with honeybush in South Africa. The South African population is both morphometrically and genetically similar to *X. peruvianum*. Meza et al. (2011) indicated that a high homology exists between Chile population of *X. peruvianum* and *X. oxycaudatum* identified from Taiwan. The South African population are similar to *X. peruvianum* but are distinguished by their shorter odontostyle and tail shape. This nematode species has been reported in association with a wide range of cultivated plants from Nigeria, Kenya, Iran, Pakistan, Brazil, and Taiwan (Lamberti and Bleve-Zacheo 1979; Bos and Loof 1983; Coomans and Heyns 1997; Fadaei et al. 2003; Oliveira et al. 2003; Chen et al. 2005). Our record of *X. oxycaudatum*, in association with *Cyclopia* spp. from South Africa, will add a new record to this list.

Nematodes belonging to the *Xiphinema americanum*-group are cosmopolitan in their distribution and have phytopathological importance with some species being implicated as vectors of important plant viruses. High numbers of *X. oxycaudatum* that were recorded from the honeybush farmland in South Africa could have resulted from high multiplication rate of nematodes due to availability of a suitable host, presence of some attractants in the soil, and some edaphic factors. The occurrence of *X. oxycaudatum* in such high density recorded in this study is disturbing and suggests that a damage potential may exist, which could have future implications on the budding honeybush tea industry.

To our knowledge, this will be the first documented report of the occurrence of *X. oxycaudatum* in South Africa.
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