

Research Note

Observation on *Hemicriconemoides brachyurus* (Loos, 1949) Chitwood & Birchfield, 1957 associated with grass in South Africa

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Summary

Hemicriconemoides brachyurus was identified morphologically, using body annuli number (103-105), stylet length (49-52 μm), tail conical with small rounded lobe, spermatheca with round sperm, and VL/VB (1.3). Molecular analysis was also undertaken, based on the 18S rDNA region, and the obtained DNA sequence data confirmed the present population from South Africa as *Hemicriconemoides*. Phylogenetic analysis using the Bayesian inference method places this population close to *H. fujianensis* from China (MH444620-21; MH444626-28). To the best of our knowledge, this is the first report of the 18S rDNA sequence for *H. brachyurus*.

Keywords: South Africa; grass; *Hemicriconemoides*; morphology; 18S rDNA

Introduction

Biodiversity influences the stability of grasslands facing disturbances, such as in climate change (Le Roux *et al.*, 2011). Grasslands are one of the most critical biomes in South Africa, which cover the northern parts of the Western Cape Province. *Hemicriconemoides* species were recovered from rhizosphere soil samples collected from natural grass growing in Cape Town, South Africa.

The genus *Hemicriconemoides* belongs to the family Criconeematidae Taylor, 1936 (Thorne, 1949) and comprises over 52 valid species (Geraert, 2010). *Hemicriconemoides wessoni* Chitwood & Birchfield, 1957 is the type species (Geraert, 2010). Members of *Hemicriconemoides* are commonly known as sheath nematodes. The genus is constituted by ectoparasites, and the member species tend to cause yield losses only at high densities (Nguyen *et al.*, 2020). However, their ecological behavior has not yet been studied well. During a survey on nematodes of the natural areas of South Africa, *H. brachyurus* (Loos, 1949) Chitwood & Birchfield,

1957 was recovered from the natural grass in Cape Town. Specimens were collected at the Kirstenbosch National Botanical Garden in Cape Town (S: -33° 59' 13.19"; E 18° 25' 29.39") from the rhizosphere of grass plants. The species is herein characterized molecularly, and to our knowledge, this is the first report of 18S rDNA for *H. brachyurus* from South Africa.

Materials and Methods
Nematode extraction, processing, and LM pictures

The specimens were extracted using the tray method and were fixed with a hot 4 % formaldehyde solution and transferred to anhydrous glycerin using the De Grisse (1969) method. The classification provided by Geraert (2010) was used for the taxonomical study of *Hemicriconemoides*. Pictures were taken with a Nikon Eclipse 80i light microscope provided with differential interference contrast optics (DIC) and a Nikon Digital Sight DS-U1 camera (Nikon, Tokyo, Japan). The measurements and morphological

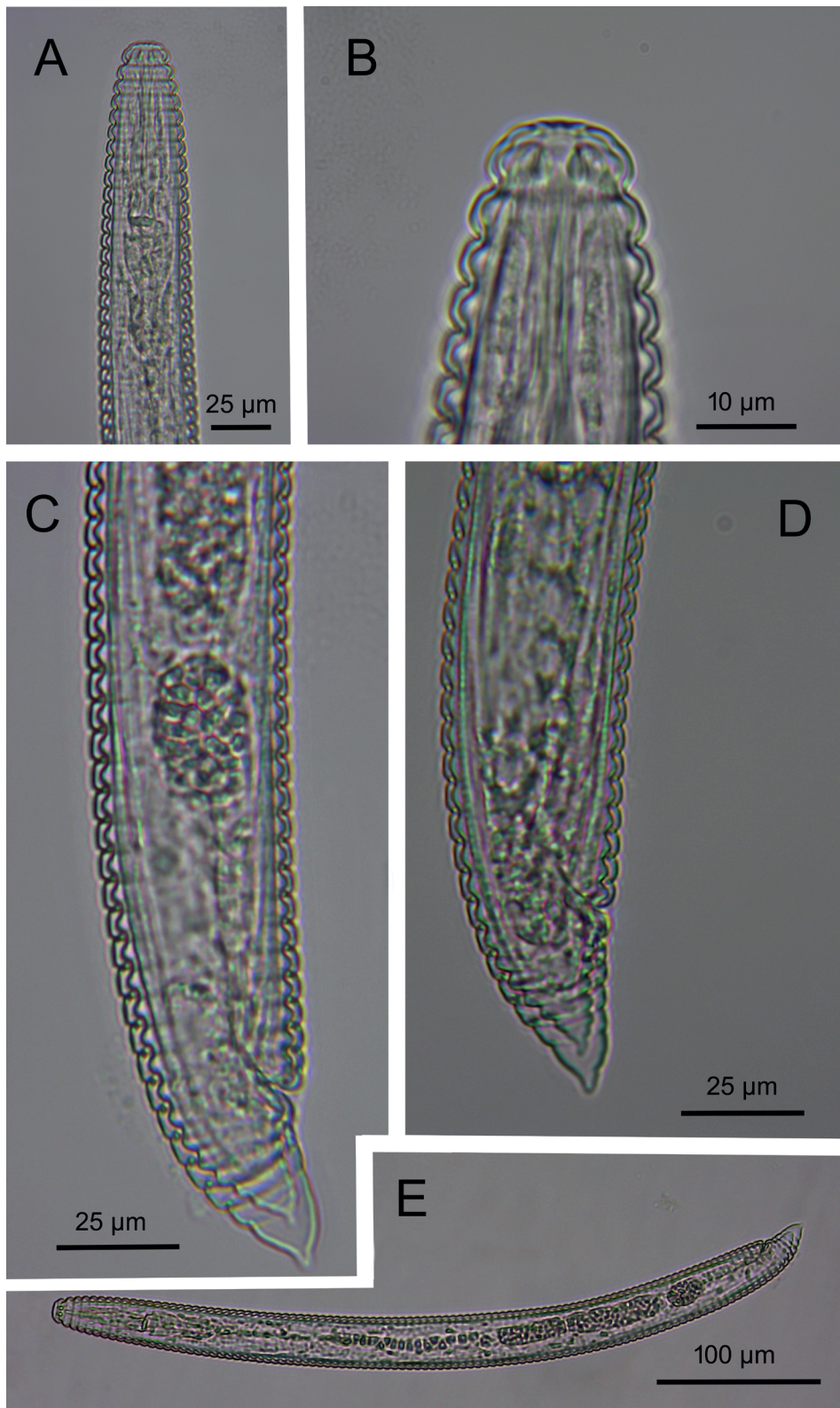


Fig. 1. *Hemicriconemoides brachyurus* (Loos, 1949) Chitwood & Birchfield, 1957. (A) anterior end; (B) lip region; (C) posterior end including spermatheca; (D) female posterior end; (E) entire female body.

Table 1. Measurements of females of *Hemicriconemoides brachyurus* from South Africa. All measurements are in μm and in the form: mean \pm SD (range), except for the ratio.

Location	Cape Town
n	8 females
Body Length	442.5 \pm 21.4 (429-474)
a	15.1 \pm 0.4 (14.6-15.3)
b	4.6 \pm 0.2 (4.5-4.8)
c	28.5 \pm 3.6 (24.3-30.6)
V	93.4 \pm 0.5 (93-94)
o	7.7 \pm 0.0 (7.7-7.8)
dgo	3.9 \pm 0.1 (3.8-4.0)
G1	43.1 \pm 1.6 (41-44)
First lip annuli width	4.5 \pm 0.5 (3.8-5.0)
Second annuli width	2.1 \pm 0.5 (1.8-2.8)
Lip region width	14.1 \pm 0.6 (14-15)
Stylet length	54.3 \pm 8.6 (49-67)
Stylet knob width	7.3 \pm 2.3 (5-10)
Stylet knob length	3.6 \pm 1.0 (3-5)
Metenchium length	40.9 \pm 1.0 (40-42)
Telenchium length	8.8 \pm 0.2 (8.7-9.0)
m	81.8 \pm 0.9 (81-82)
Median bulb to the anterior end	61.3 \pm 2.3 (60-64)
Nerve ring to the anterior end	73.7 \pm 0.6 (73-74)
Excretory pore to anterior end	102.0 (n = 1)
Pharynx	101.3 \pm 13.3 (92-121)
Body diameter at neck	28.7 \pm 1.2 (28-30)
Body diameter at mid-body	30.3 \pm 0.6 (30-31)
Body diameter at anus	14.0 \pm 1.7 (13-16)
Annuli width	4.3 \pm 0.3 (4-5)
Vagina	6.7 \pm 0.6 (6-7)
Anterior genital branch	208.0 \pm 43.5 (181-273)
Spermatheca length	27.5 \pm 2.5 (24-30)
Spermatheca width	16.0 \pm 2.7 (14-20)
First body length	432.0 \pm 5.2 (429-438)
Second body length	425.3 \pm 4.0 (423-430)
Body annuli number	104.3 \pm 1.2 (103-105)
Rst	13.0 \pm 1.4 (11-14)
Rph	22.7 \pm 0.6 (22-23)
Rex	24.0 (n = 1)
Rv	6.3 \pm 0.0 (5-7)
Ran	5.3 \pm 5.0 (5-6)
Rvan	3.7 \pm 0.6 (3-4)
VL/VB	1.3 \pm 0.1 (1.2-1.4)
Tail Length	15.3 \pm 2.3 (14-18)
St%L	11.6 \pm 0.3 (11-12)
Vulva-anus distance	12.3 \pm 1.2 (11-13)
Vulva anterior end	414.5 \pm 21.8 (402-447)
Vulva to tail end	30.8 \pm 5.2 (27-38)
sperm size	4.1 \pm 0.7 (3.4-5.0)

analysis were done using microscopic facilities of Aquaculture Research Unit, University of Limpopo. Micrographs were edited using Adobe® Photoshop® CS.

DNA extraction, PCR, and phylogenetic analysis

DNA extraction was done using the Chelex method (Straube & Juen, 2013). Two specimens of the species were hand-picked with a fine tip needle and transferred to a 1.5 ml Eppendorf tube containing 20 μl double distilled water. The nematodes in the tube were crushed with the tip of a fine needle and vortexed. Thirty microliters of 5 % Chelex® 50 and 2 μL of proteinase K were added to each of the microcentrifuge tubes that contained the crushed nematodes and mixed. These separate microcentrifuge tubes with the nematode lysate were incubated at 56 °C for two hours and then incubated at 95 °C for 10 minutes to deactivate the proteinase K and finally spun for 2 min at 16000 rpm (Shokoohi, 2021). The supernatant was then extracted from each of the tubes and stored at -20 °C. Following this step, the forward and reverse primers, 988F (5'-CTCAAAGATTAAGCCATGC-3') and 1912R (5'-TTTACGGT-CAGAACTAGGG-3') (according to Carta & Li, 2018), were used in the PCR reactions for partial amplification of the 18S rDNA region. PCR was conducted with eight μl of the DNA template, 12.5 μl of 2X PCR Master Mix Red (Promega, USA), one μl of each primer (10 pmol μl^{-1}), and ddH₂O for a final volume of 30 μl . The amplification was processed using an Eppendorf master cycler gradient (Eppendorf, Hamburg, Germany), with the following program: initial denaturation for 3 min at 94 °C, 37 cycles of denaturation for 45 s at 94°C; 54 °C annealing temperatures for 18S rDNA; extension for 45 s to 1 min at 72 °C, and finally an extension step of 6 min at 72 °C followed by a temperature on hold at 4 °C. After DNA amplification, four μl of product from each tube was loaded on a 1 % agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid, and one mM EDTA) for evaluation of the DNA bands. The bands were stained with RedGel and visualized and photographed on a UV transilluminator. The amplicons were stored at -20 °C. Finally, the PCR products were purified for sequencing by Inqaba Biotech (South Africa). The ribosomal DNA sequences were analyzed and edited with BioEdit (Hall, 1999) and aligned using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were generated using the Bayesian inference method as implemented in the program Mr Bayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The HKY+ Γ (gamma distribution of rate variation with a proportion of invariable sites) model was selected using jModeltest 2.1.10 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). Analysis using the GTR+G+I model was initiated with a random starting tree and ran with the Markov chain Monte Carlo (MCMC) for 10⁶ generations for 18S rDNA. The trees were visualized with the TreeView program. Also, as outgroups, *Criconemoides myungsugae* (MH444644) was selected based on Nguyen *et al.* (2020). The original partial 18S rDNA sequence of *H. brachyurus* was deposited in GenBank under the accession numbers OM914954.

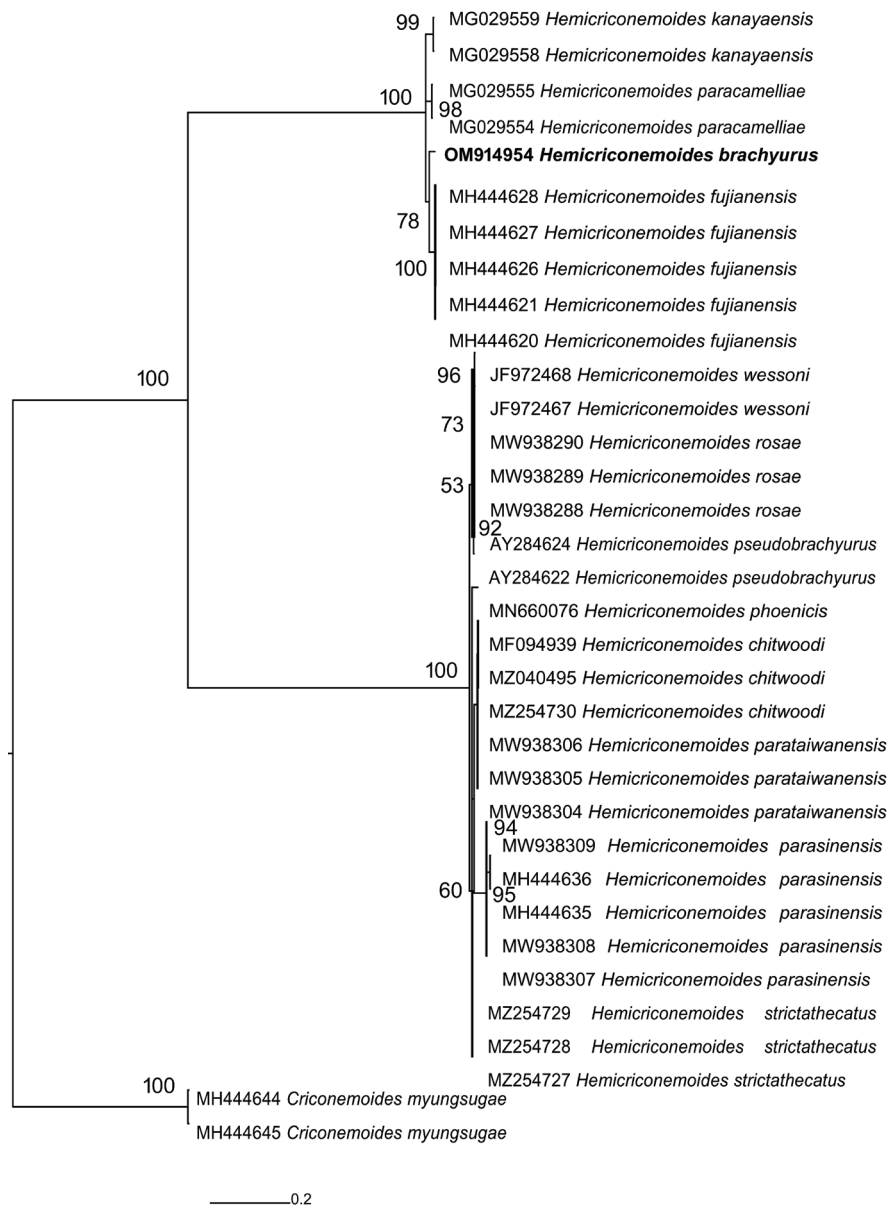


Fig. 2. 18S rDNA Bayesian tree inferred from known and newly sequenced *Hemicriconemoides brachyurus* from South Africa.

Statistical analysis

To evaluate the morphological variations between the nematodes isolated in this study and the nematodes species, including *H. brachyurus* and *H. promissus* Vovlas, 1980 as the close species of the genus *Hemicriconemoides*, principal component analyses (PCA) and Discriminant Analysis (DA) with different morphological traits were conducted. PCA analyses were carried out in XLSTAT (Addinsoft, 2007). Various morphometric features were obtained from fixed nematodes, including an average of body length, *a*, *b*, *c*, *V*, stylet length, total body annuli (*R*), *Rex* (annuli number from anterior end to the excretory pore), *RV* (annuli number from anterior

or end to the vulva), *Rst* (annuli number from anterior end to stylet knobs), *Rph* (annuli number from anterior end to pharynx), *Ran* (annuli number from tail terminus to anus), *VL/VB* (distance from terminus to vulva divided by body diameter at vulval position) were included in the PCA and DA analyses. The morphometric measurements of other species were taken from their original descriptions (Van den Berg & Heyns, 1977; Geraert, 2010; Vovlas, 1980; Vovlas *et al.*, 2006). The measures were normalized using XLSTAT software before their analysis (Addinsoft, 2007). The scores values were determined for each species based on each of the principal components, and the scores for the first two components

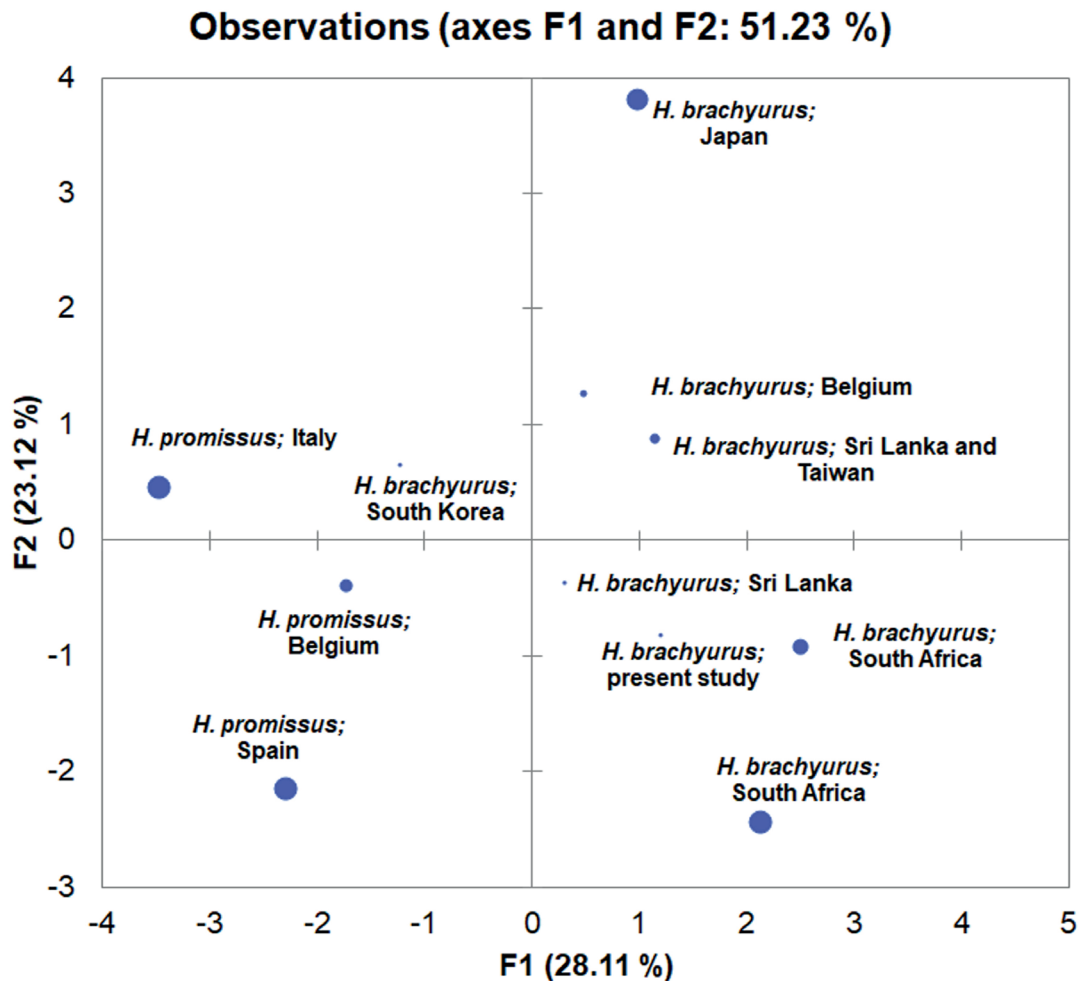


Fig. 3. PCA analysis of different populations of *Hemicriconemoides brachyurus*.

were used to form a two-dimensional plot (F1 and F2) of each isolate based on the eigenvalues given by the software XLSTAT.

Ethical Approval and/or Informed Consent

The author confirms that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered. The author confirms that the conducted research is neither related to human nor animals use.

Results

Hemicriconemoides brachyurus (Loos, 1949) Chitwood & Birchfield, 1957

Morphological characterization

The morphological analyses confirmed that the species was *H. brachyurus*. Measurements of *H. brachyurus* in this study are in

agreement with the measurement of *H. brachyurus* in Geraert (2010), and Van den Berg and Heyns (1977) (Table 1). Females of *H. brachyurus* are characterized by having body slightly curved ventrally (Fig. 1E); lip region with two annuli, slightly flattened labial disc (Fig. 1B); vulva having flap (Fig. 1C), stylet robust, knobs 5 – 10 µm wide, and 3 – 5 µm high (Fig. 1A); vulva located near the posterior end; tail conical with small rounded lobe, in all specimens (Fig. 1D). Male not found.

Molecular analysis

The forward 988 and reverse 1912 primers of 18S rDNA for *H. brachyurus* isolated 940 base pairs long. The nBlast test of 18S rDNA showed 98 % similarity of the test population with the Chinese population of *H. fujianensis* (MH444620). Compared with *H. kanayaensis* (MG029559) from China, it shows 15 (98 % identity) base pairs differences.

Our phylogenetic analysis using 18S rDNA, placed the South Af-

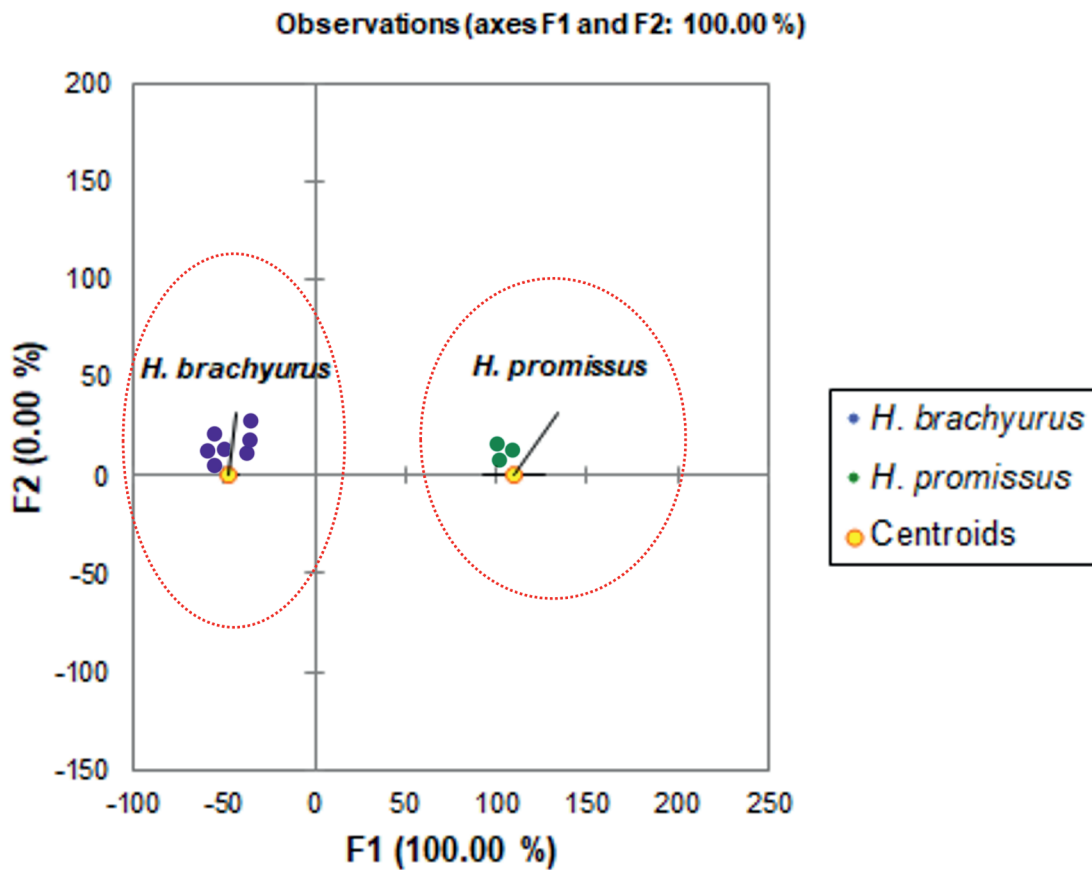


Fig. 4. Discriminant analysis of *H. brachyurus* and *H. promissus*.

rican *H. brachyurus* population in a clade together with other *H. fujianensis*, *H. kanayaensis*, and *H. paracamelliae* populations (Fig. 2). Findings in the current study were in agreement with the phylogenies of *Hemicriconemoides* species studied using 28S rDNA (Nguyen *et al.*, 2020). *Hemicriconemoides brachyurus* and *H. fujianensis* are placed together in a group. This is also supported by their morphological similarities. They are similar in terms of the stylet of the female (48 – 69 vs 56 – 70 μm), VL/VB (0.8 – 1.4 vs 1.2 – 1.9), and presence of male (as both have sperm-filled spermatheca) (see Geraert, 2010; Maria *et al.*, 2019; Mwamula *et al.*, 2020). However, these species can be distinguished appropriately by the absence of a lateral vulval flap and a blunt tail terminus in *H. fujianensis* vs lateral vulval flap present and terminus ending in a small rounded lobe in *H. brachyurus*. Two permanent microscope slides containing the females of *H. brachyurus* were deposited in the Nematology collection of the University of Limpopo, South Africa. According to the literature, this is the first record of 18S rDNA sequence for *H. brachyurus* in South Africa. In conclusion, the ecological behavior of this species needs to be studied to find out the economic importance of the pest.

Discussion

On the identity of *H. brachyurus* and *H. promissus*

Principal component analysis using morphometric features of *H. brachyurus* and *H. promissus* based on the female analysis showed that *H. brachyurus* has a morphometric variation (Fig. 3). The analyzed morphological characters allowed a clear separation between *H. brachyurus* and *H. promissus*.

An accumulated variability of 51.23 % was observed in female-based PCA, specifically, 28.11 % in the F1 and 23.12 % in the F2 (Fig. 3).

We observed in the PCAs that some populations of *H. brachyurus* were placed together. The previously reported South African *H. brachyurus* (Heyns, 1970; Van den Berg & Heyns, 1977) showed similar morphological characters. The PCA result also indicated that the Japanese population of *H. brachyurus* (Gotoh, 1965) differ morphometrically from the rest of the populations (Fig. 3). The results indicated that there is intraspecific morphological variation across *H. brachyurus*, and it depends on the nematode's geographical location (Fig. 3). Additionally, based on the DA, the populations of *H. brachyurus* and *H. promissus* grouped separately (Fig. 4). These two species are very similar in terms of morphology. However, they differ only on the basis of the vulval flap, which

is present in the *H. brachyurus* and absent in the *H. promissus* (Geraert, 2010). *Hemicriconemoides promissus* was described by Vovlas in 1980. Then its validity was studied using a population from Spain by Vovlas *et al.* (2006). The DA analysis revealed that these two species are valid and distinguished by their morphology. The result obtained agrees with the result obtained by Vovlas *et al.* (2006).

In conclusion, the vulval flap is a valuable taxonomic character in the species diagnosis of *Hemicriconemoides*. Besides, the ecological role of *H. brachyurus* needs to be investigated in the grassland quality of South Africa.

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