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Description of *Anaplectus deconincki* n. sp. from South Africa

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Research Paper

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Abstract

During a survey of soil nematodes in Kirstenbosch National Botanical Garden in Cape Town, a population of plectid nematodes belonging to the genus *Anaplectus* was recovered and proved to be a species new to science. *Anaplectus deconincki* n. sp. is characterized by female body length (612–932 µm), b = 4.6–5.2, c = 12.8–18.0, c' = 2.6–3.1, V = 51–54, and tail length (43–63 µm). Males are characterized by body length (779–956 µm), b = 4.8–5.6, c = 13.9–16.7, c' = 2.2–2.5, spicule length 33–39 µm, gubernaculum length 10–12 µm, and tail length (56–65 µm). Discriminant analysis clearly separated *A. deconincki* n. sp. from the other related species of *Aanaplectus.* The phylogenetic analysis placed *Anaplectus deconincki* n. sp. in a clade with 1.00 posterior probability values with other *Anaplectus.* Partial sequences of the 18S and 28S regions of the ribosomal DNA gene were amplified for *Anaplectus deconincki* n. sp., and 18S rDNA showed 99% similarity with an unidentified *Anaplectus* (AJ966473) and *A. porosus* (MF622934) from Belgium. In addition, 28S rDNA showed a 93% similarity with *A. porosus* from Belgium (MF622938) and a 98% similarity with *A. granulosus* from Germany (MF325171). Measurements, illustrations, and light microscopy pictures for *Anaplectus deconincki* n. sp. are given.

Introduction

The family Plectidae was established by Örley in 1880. Members of this family are regarded as bacterial feeders (Yeates *et al.* 1993). *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933, was placed in the subfamily Pakirinae Inglis, 1983, for species having a crown of four cephalic setae and a set of preanal tubuli in the males. Later, *Anaplectus* was placed by Holovachov (2006) in the family Plectidae along with *Arctiplectus* Andrássy, 2003; *Perioplectus* Sanwal in Gerlach & Riemann, 1973; *Plectus* Bastian, 1865; *Ceratoplectus* Andrássy, 1984; *Tylocephalus* Crossman, 1933; *Ereptonema* Anderson, 1966; *Neotylocephalus* Ali, Farooqui & Tejpal, 1969; and *Wilsonema* Cobb, 1913. In addition, he raised the rank of Pakirinae to family level. Brezeski (1963) indicated the "widen prostom hexagonal in cross-section" as a distinct characteristic from *Plectus.* Holovachov (2016) indicated the transverse amphid opening in *Anaplectus* as a distinguishing characteristic versus a unispiral amphid in *Plectus.* Several authors synonymized the genus *Anaplectus* with *Plectus* Bastian, 1865 (Schneider 1939; Goodey 1951, 1963). However, most authors considered *Anaplectus* a valid taxon (Chitwood & Chitwood 1937; Maggenti 1961; Brzeski 1963; Killick 1964). The first extensive study of the genus *Anaplectus* was made by Allen and Noffsinger (1968), followed by others who added more information about the taxonomy of the genus along with a key to its species (Andrássy 1984, 2005; Truskova 1972; Holovachov *et al.* 2004, 2016).

The genus *Anaplectus* is one of the most widely distributed genera within family Plectidae followed by the genus *Plectus* (Holovachov *et al.* 2004; Holovachov 2016; Jahan *et al.* 2020). *Anaplectus* is currently represented by fifteen valid species (Holovachov *et al.* 2004; Jahan *et al.* 2020), including *A. granulosus* (Bastian, 1865) De Coninck and Schuurmans Stekhoven, 1933; *A. atubulatus* Andrássy, 1987; *A. brzeskii* Holovachov, Boström, Winiszewska, and Háněl, 2004; *A. eurycerus* (Massey 1964) Andrássy, 1984; *A. grandepapillatus* (Ditlevsen, 1928) Andrássy, 1973; *A. labiosulcus* Jahan, Khan, Mahboob and Tahseen, 2020; *A. magnus* Brzeski, 1963; *A. octo* Zullini, 1973; *A. parasimilis* Truskova, 1978; *A. porosus* Allen & Noffsinger, 1968; *A. similis* Allen and Noffsinger, 1968; *A. subgranulosus* Truskova, 1978; *A. sudhausi* Jahan, Khan, Mahboob and Tahseen, 2020; *A. tortus* Andrássy, 1986 and *A. varicaudatus* Allen and Noffsinger, 1968. Furthermore, Holovachov *et al.* (2004) emended the diagnostic characteristics for *Anaplectus*, which facilitates accurate identification of the genus.

Detailed phylogenetic analysis of this group was performed by Holovachov (2006), supplemented with new data on morphology and development of the superfamily Plectoidea Örley, 1880. Afterward, the monophyletic origin of the family Plectidae was verified based on SSU rDNA (Meldal *et al.* 2007; van Megen *et al.* 2009; Shokoohi *et al.* 2013). Meldal *et al.* (2007) considered Plectida and Rahbditida to be sister groups by using 18S rDNA. Recently, the genetic

study of the whole mitochondrial genome of the two species of *Plectus* supported the close relationship of the mentioned groups (Kim *et al.* 2017).

The present paper deals with the description of a new species of the genus *Anaplectus,* namely *A. deconincki* n. sp., from Kirstenbosch National Botanical Garden of Cape Town, South Africa, isolated from natural grass. In addition, molecular analysis is performed and the phylogenetic position of the *Anaplectus deconincki* n. sp. based on 28S rDNA is discussed.

Material and methods

Nematode isolation and morphological observation

The soil sample having *Anaplectus deconincki* n. sp. was taken from Kirstenbosch National Botanical Garden of Cape Town, South Africa. Nematodes were extracted from a *Stenotaphrum secundatum* (Buffalo grass) soil sample by Baermann's (1917) funnel technique, fixed with a hot 4% formaldehyde solution and processed to anhydrous glycerin by the method of De Grisse (1969). Female and male specimens were extracted from the exact soil samples and preserved permanently on glass slides. Measurements (Table 1) were taken directly using a Zeiss Lab A1 microscope (Jena, Germany) equipped with digital camera, and drawings were made using the light microscopy (LM) photographs taken by a digital camera.

Statistical analysis

Discriminant analysis (DA) was performed on morphometric parameters derived from fixed specimens of the present study and the morphometrics that are available in the database for *Anaplectus* spp. (Allen & Noffsinger 1968; Holovachov *et al.* 2004; Jahan *et al.* 2020). The features used for DA included body length, a, b, c, c', V, G1 (% length of the anterior female gonad in relation to body length), G2 (% length of the posterior female gonad in relation to body length), stoma length, amphid location, nerve ring position from anterior end, excretory pore from anterior end, pharynx length, and tail length (Table 2). Using a stepwise model, the above-mentioned characteristics were used for DA (Addinsoft 2007).

DNA extraction, PCR, and phylogenetic analysis

DNA extraction was done using the Chelex method (Straube & Juen 2013). Three specimens of the species were hand-picked with a fine-tipped needle and transferred to a 1.5 ml Eppendorf tube containing 20 µl of 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 mixed to the microcentrifuge tube containing the crushed nematodes. The microcentrifuge tube with the nematode lysate was incubated at 56°C for 2 h and then set at 95°C for 10 min to deactivate the proteinase K and finally spin for 2 min at 16000 rpm (Shokoohi 2022). The supernatant was extracted from the tube and stored at –20°C. Following this step, the forward and reverse primers, 988F (5-CTCAAAGATTAAGCCATGC-3) and 1912R (5-TTTACGGTCAGAACTAGGG-3) (Holterman *et al.* 2006) and D2A (5'–ACAAGTACCGTGAGGGAAAGTTG–3'), D3B (5'–TCGGAAGGAACCAGCTACTA–3') (De Ley *et al.* 1999), were used in the polymerase chain reactions (PCRs) for partial amplification of the 18S and 28S rDNA regions, respectively. PCR was conducted with 8 µl of the DNA template, 12.5 µl of 2X PCR Master Mix (NEB, Ipswich, USA), one µl of each primer (10 pmol μl^{-1}), and ddH_2O, for a final volume of 30 µl. The amplification was done using a BioRad master cycler (Hercules, California, USA) with the following program: initial denaturation for 3 min at 94°C, 37 cycles of denaturation for 45 s at 94°C; 54°C and 56°C annealing temperature for 18S and 28S rDNA, respectively; extension for 1 min at 72°C, and finally, an extension step of 6 min at 72 °C followed by a temperature hold at 4°C. After DNA amplification, 4 µl of PCR product was loaded on a 1% agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid, and 1 mM EDTA) for evaluation of the DNA bands. The bands were stained with a Safe-View (Applied Biological Materials, Richmond, BC, Canada) and visualized and photographed on a UV transilluminator. The amplicon of the gene was stored at –20°C. Finally, Inqaba Biotech (Pretoria, South Africa) purified the PCR product for sequencing. The ribosomal DNA sequence was analysed and edited with BioEdit (Hall 1999) and aligned using CLUSTAL W (Thompson *et al.* 1994). The phylogenetic tree was generated using the Bayesian inference method as implemented in the program Mr Bayes 3.1.2 (Ronquist & Huelsenbeck 2003). The GTR + I + G model was selected using jModeltest 2.1.10 (Guindon & Gascuel 2003; Darriba *et al.* 2012). Analysis was initiated with a random starting tree and run with Markov Chain Monte Carlo (MCMC) simulations for 10^6 generations. The tree was visualized with the TreeView program. Outgroup, *Rhabditis blumi* Sudhaus, 1974 (MT012150, MT043860 for 18S rDNA; KM233155, KM233156 for 28S rDNA) were selected following Holovachov *et al.* (2013). The original partial 18S and 28S rDNA sequences of *Anaplectus deconincki* n. sp. were deposited in GenBank under the accession numbers OQ743744 and OM905072, respectively.

Results

Anaplectus deconincki *n. sp.*

Measurements and morphology of *Anaplectus deconincki* n. sp are shown in Table 1 and Figures 1-3.

Description

Female. Description is based on seven females in a good state of preservation. Body small, cylindrical, ventrally arcuate upon heat fixation. Cuticle with fine transverse striations, annulus 0.8–0.9 µm wide at mid-body. Lateral field with two ridges (alae) (Figure 1I), 3–4 µm wide at mid-body, occupying 14–15% of the corresponding body width. Head regions narrow, continuous with body contour. Lip region offset from body contour, truncated, twice as wide as high. The lip region consists of six separate lips (Figure 1D) with shorter interspersed 'liplets.' Labial sensilla indistinct. Cephalic sensilla setiform, originating on the fourth or fifth body annule (4–6 µm from the anterior end), 2–4 µm long. Somatic setae absent, except for caudal setae. Amphidial openings transverse (Figure 1C), 2–3 µm width, located at level with anterior part of stegostom. Stoma plectoid, cylindrical, 2.0–2.6 times longer than lip region diameter, cheilostom short, gymnostom well cuticularised, wide, slightly arched, stegostom narrower. Hypodermal glands arranged in four (ventral, dorsal, and sublateral) rows and open to the outside via pores, first sublateral gland 7–15 µm from anterior end (Figure 1H), 17 glands from anterior end to the end of pharynx. Glands in females along one side of the entire body 91–109. Pharynx 117–176 µm long, essentially cylindrical; basal bulb spheroid, 15 × 31 µm in size, 1.7–3.1 times cardia length. Basal bulb with a grinder, in anterior part, haustrulum narrow. Post-bulbar extension 7–8 µm long. Cardia 10–17 µm long, one coelomocyte presents

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Table 1. Measurements of *Anaplectus deconincki* n. sp. from Cape Town, South Africa. All measurements are in μm and the format: mean±standard deviation (range).

Location	Kirstenbosch, Cape Town		
n	holotype 1 female	paratypes 10 females	paratypes 5 males
Body length	895	830.3 ± 108.9 (612–932)	885.3 ± 62.2 (779–956)
a	33.1	29.5 ± 2.4 (25.5–33.1)	34.5 ± 1.5 (33.4–36.2)
b	4.8	4.9 ± 0.2 (4.6–5.2)	5.3 ± 0.4 (4.8–5.6)
c	16.0	15.6 ± 1.8 (12.8–18.0)	15.3 ± 1.4 (13.9–16.7)
c’	2.9	2.8 ± 0.2 (2.6–3.1)	2.4 ± 0.2 (2.2–2.5)
V	50.6	52.2 ± 1.2 (51–54)	–
G1	26.4	22.9 ± 3.0 (18–26)	–
G2	21.8	21.7 ± 2.9 (17–25)	–
Labial region diameter	9	7.9 ± 0.8 (7–9)	7.8 ± 0.3 (7–8)
Stoma length (STL)	20	18.8 ± 2.0 (16–22)	21.5 ± 2.3 (20–21)
Stoma diameter (STD)	3.6	2.9 ± 0.7 (2–4)	2.3 ± 0.1 (2–3)
STL/STD	5.6	7.0 ± 1.5 (5–10)	9.2 ± 1.2 (8–10)
Amphid location	8	6.1 ± 2.2 (4–8)	7.5 ± 0.6 (7–8)
Nerve ring from anterior end	93	89.4 ± 9.4 (73–100)	88.7 ± 5.9 (82–93)
Excretory pore from anterior end	111	98.5 ± 10.5 (80–111)	96.7 ± 2.5 (94–99)
Pharynx	165	151.6 ± 19.2 (117–176)	144.5 ± 2.3 (143–147)
Neck	185	171.5 ± 20.7 (134–200)	166.0 ± 3.5 (162–176)
Cardia length	17	12.1 ± 3.9 (7–17)	12.6 ± 1.5 (11–14)
Cardia width	2.3	4.3 ± 1.5 (2–6)	3.7 ± 0.6 (3–4)
Annuli width	1	1.2 ± 0.2 (1.0–1.5)	1.3 ± 0.2 (1.2–1.5)
Cuticle	1.4	1.5 ± 0.2 (1.3–1.9)	1.6 ± 0.2 (1.4–1.7)
Body diameter at neck	24	26.1 ± 2.4 (23–28)	25.7 ± 2.5 (23–28)
Body diameter at mid–body	27	28.0 ± 2.6 (24–32)	25.8 ± 2.6 (23–29)
Body diameter at anus	19	18.7 ± 0.9 (17–20)	24.3 ± 2.1 (22–26)
Vagina length	8	9.1 ± 1.5 (7–13)	–
Vagina/body diameter	0.3	0.3 ± 0.1 (0.3–0.4)	–
Spermatheca length	25	21.8 ± 6.2 (15–32)	–
Spermatheca width	19	15.0 ± 3.6 (10–19)	–
Rectum length	17	15.2 ± 1.6 (13–17)	19.0 ± 1.0 (18–20)
Tail length	56	53.9 ± 5.5 (43–63)	57.7 ± 2.9 (56–65)
Vulva anterior end	453	436.8 ± 51.9 (323–494)	–
Vulva-anus distance	386	343.5 ± 49.0 (241–386)	–
Anterior genital branch	236	198.0 ± 41.5 (113–238)	–
Posterior genital branch	195	185.1 ± 35.7 (91–245)	–
Spicule	–	–	34.0 ± 1.7 (33–39)
Gubernaculum	–	–	10.5 ± 0.5 (10–12)

anterior to cardia. Intestine without granules. Rectum 0.8–0.9 anal body diameter long. Nerve ring at 48–54% of neck length. Secretory-excretory pore just posterior to nerve ring, at 58–60% of neck length. Reproductive system didelphic, amphidelphic with reflexed ovaries. Uterus tubular, ovaries reflexed dorsally. Both genital branches equally developed; entire reproductive tract (reproductive branches plus reflexed ovaries) 7–9 times longer than the mid-body diameter. Vulva protruded (Figure 1E) at 51–54% of body length from anterior end. Spermatheca containing spherical to ovoid sperm, 6–7 μm dimension. Tail 43–63 μm long, conoid in

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Table 2. Morphometric characters used for discriminant analysis (DA) for different species of *Anaplectus*.

Reference	Present study	Allen and Noffsinger 1968					Holovachov *et al.* 2004			Jahan *et al.* 2020		
Characteristic*	*A. deconincki*	*A. granulosus*	*A. similis*	*A. porosus*	*A. varicaudatus*	*A. magnus*	*A. granulosus*	*A. atubulatus*	*A. brzeskii*	*A. sudhausi*	*A. labiosulcus*	*A. granulosus*
Body length	830.3	1225.0	1600.0	1650.0	850.0	2000.0	902.0	942.3	986.5	833.0	772.0	838.0
a	29.5	30.8	48.0	34.5	29.0	39.0	26.1	26.5	29.1	25.4	21.3	22.0
b	4.9	5.1	7.0	5.7	6.0	7.0	5.1	4.9	5.3	4.4	4.1	4.9
c	15.6	17.5	18.0	23.0	18.0	19.0	18.8	17.7	18.1	18.9	15.7	15.7
c’	2.8	2.3	2.7	2.4	2.2	2.0	2.3	2.4	2.5	2.0	1.9	2.2
V	52.2	50.8	55.0	53.5	50.5	47.0	52.8	51.3	50.9	53.7	54.9	52.2
G1	22.9	–	–	–	–	–	26.2	15.5	16.3	16.1	23.5	22.4
G2	21.7	–	–	–	–	–	16.9	15.7	17.4	15.8	23.9	19.8
Stoma length	18.8	24.8	35.0	24.0	24.0	30.0	17.3	29.3	24.8	24.4	26.0	22.5
Amphid location	6.1	9.3	13.0	8.5	5.5	16.0	7.9	7.8	9.2	8.6	9.3	8.5
Nerve ring from anterior end	89.4						95.0	103.0	98.7	110.0	94.0	96.0
Excretory pore from anterior end	98.5	128.8	172.0	162.0	91.5	149.0	104.0	114.0	106.2	117.0	79.0	103.0
Pharynx	151.6	232.6	228.6	280.7	144.3	285.7	178.0	194.5	192.2	187.0	183.0	171.0
Rectum length	15.2	–	–	–	–	–	–	–	–	18.0	20.0	21.0
Tail length	53.9	70.2	88.9	71.8	47.5	105.3	48.0	54.0	55.0	46.0	49.0	53.0

*Average recovered from original works of literature; some of them rounded.

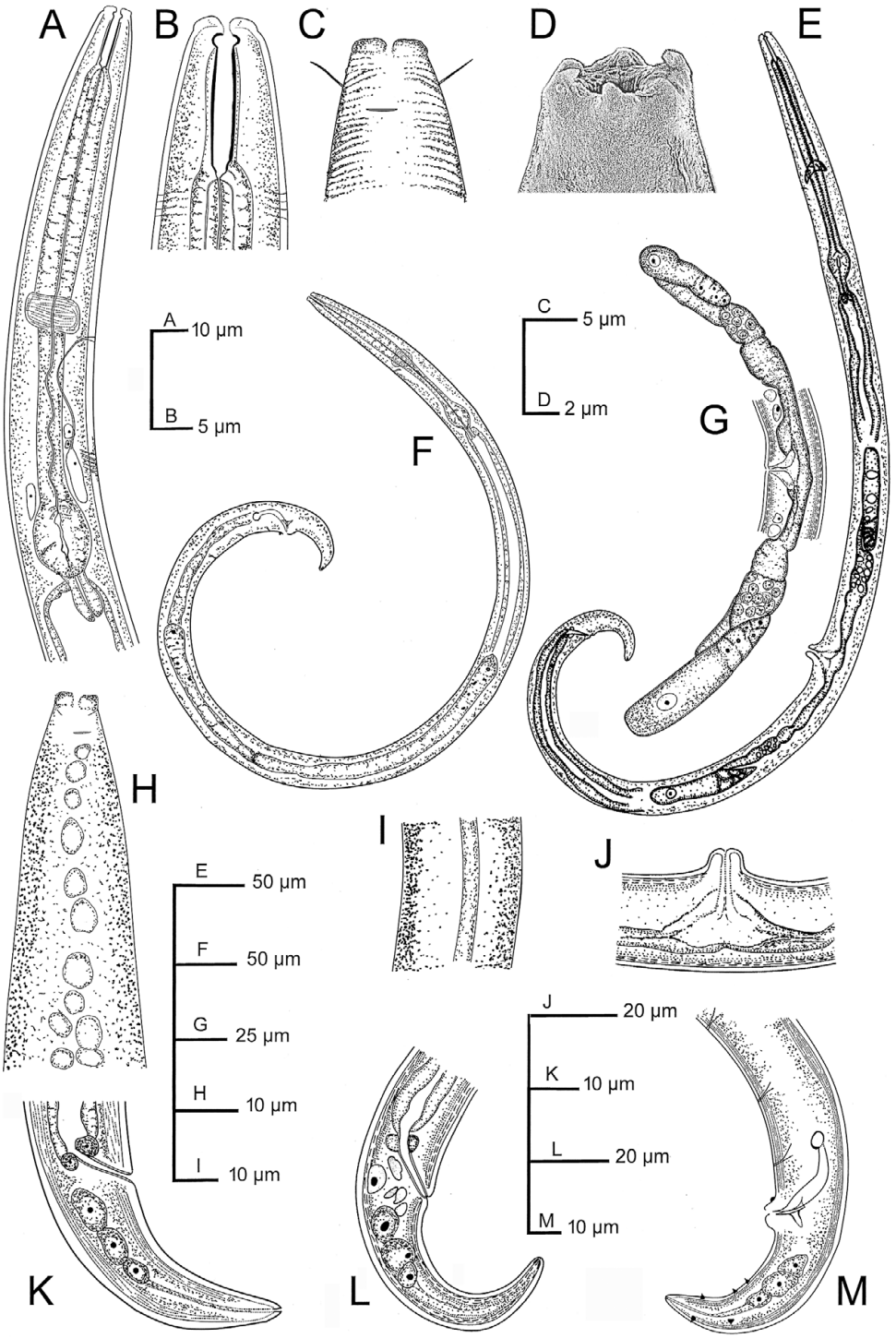

Figure 1. *Anaplectus deconincki* n. sp. A: Neck; B: Anterior region; C: Amphid; D: Lip region; E: Entire female; F: Entire male; G: Female reproductive system; H: Anterior lateral gland; I: Lateral field; J: Vulva region; K, L: Female posterior end; M: Male posterior end.

its anterior part and cylindrical in its posterior part, ventrally curved in the posterior half. One pair of very short ventral setae located 10–11 µm from the tail end, and one dorsal very short located 30 µm from the tail end. Spinneret present. Three caudal glands are present (Figure 1K,L), arranged in tandem.

Male. Generally similar to female in morphology. Reproductive system diorchic, anterior testis outstretched, posterior testis reflexed. Anterior testis 136–237 µm long, along with vas deferens on right-hand side and posterior testis 78–133 µm long (Figure 1F), on left-hand side of intestine. Three sclerotized preanal tubular supplements or tubuli present of the first one opening at 11–15 µm, the second one at 31–39 µm and the third one at 57–72 µm anterior to cloacal opening (Figure 1M, Figure 3H). Tail short, ventrally arcuate. Spicules arcuate, with oval or round manubrium slightly wider than adjoining calamus. Gubernaculum enveloping one third of spicules length distally. Gubernaculum plate-like with a dorsal

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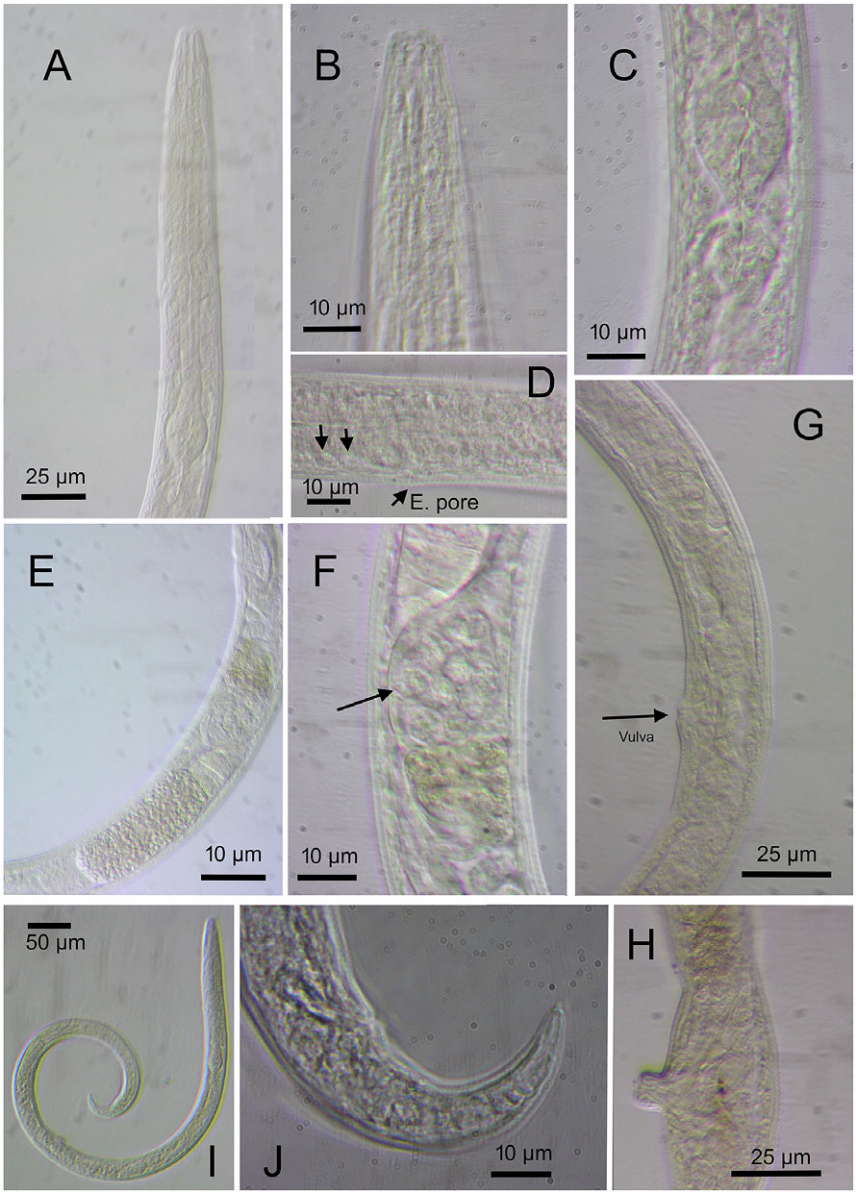

Figure 2. *Anaplectus deconincki* n. sp. (LM). A: Anterior end; B: Stoma; C: Pharyngeal-intestinal junction; D: Excretory pore and glands (arrows); E: Anterior genital branch; F: Spermatheca; G, H: Vulval region; I: Entire female; J: Female posterior end.

triangular projection perpendicular to corpus, 11–12 µm long. One ventral papillae 4–5 µm anterior to cloacae. Five pairs of post cloacal papillae with four ventral and one subdorsal close to tail tip.

Type locality and habitat

The specimens examined were found in Kirstenbosch National Botanical Garden of Cape Town, South Africa (GPS coordinate: S: 33°59'17.0"; E: 18°25'52.1"), associated with the rhizosphere of the lawn *Stenotaphrum secundatum* (Buffalo grass).

Type material

Four slides including 10 females and 5 males were deposited in the Nematology collection of the Aquaculture Research Unit of the University of Limpopo, South Africa. One slide contains two specimens that were deposited in the laboratory of the Virginia Tech University, USA.

Differential diagnosis and relationship

A. deconincki n. sp. is characterized by 612–932 µm long body in females, hypodermal glands present along the body, 16–22 µm long stoma, lip region offset from the body contour, bearing six separated lips, 7–9 µm in diameter, amphids openings transverse slits located in the middle part of stoma or 4–8 µm from anterior end, two lateral incisures, 117–176 µm long pharynx, amphidelphic female reproductive system (V = 51–54), vulva 323–494 µm from anterior end, tail elongate-conoid (43–63 µm, c = 12.8–18.0, c' = 2.6–3.1 in females; 56–65 µm, c = 13.9–16.7, c' = 2.2–2.5 in males) with rounded terminus and functional spinneret, bearing one setae on the ventral and one visible setae on the dorsal side of females tail.

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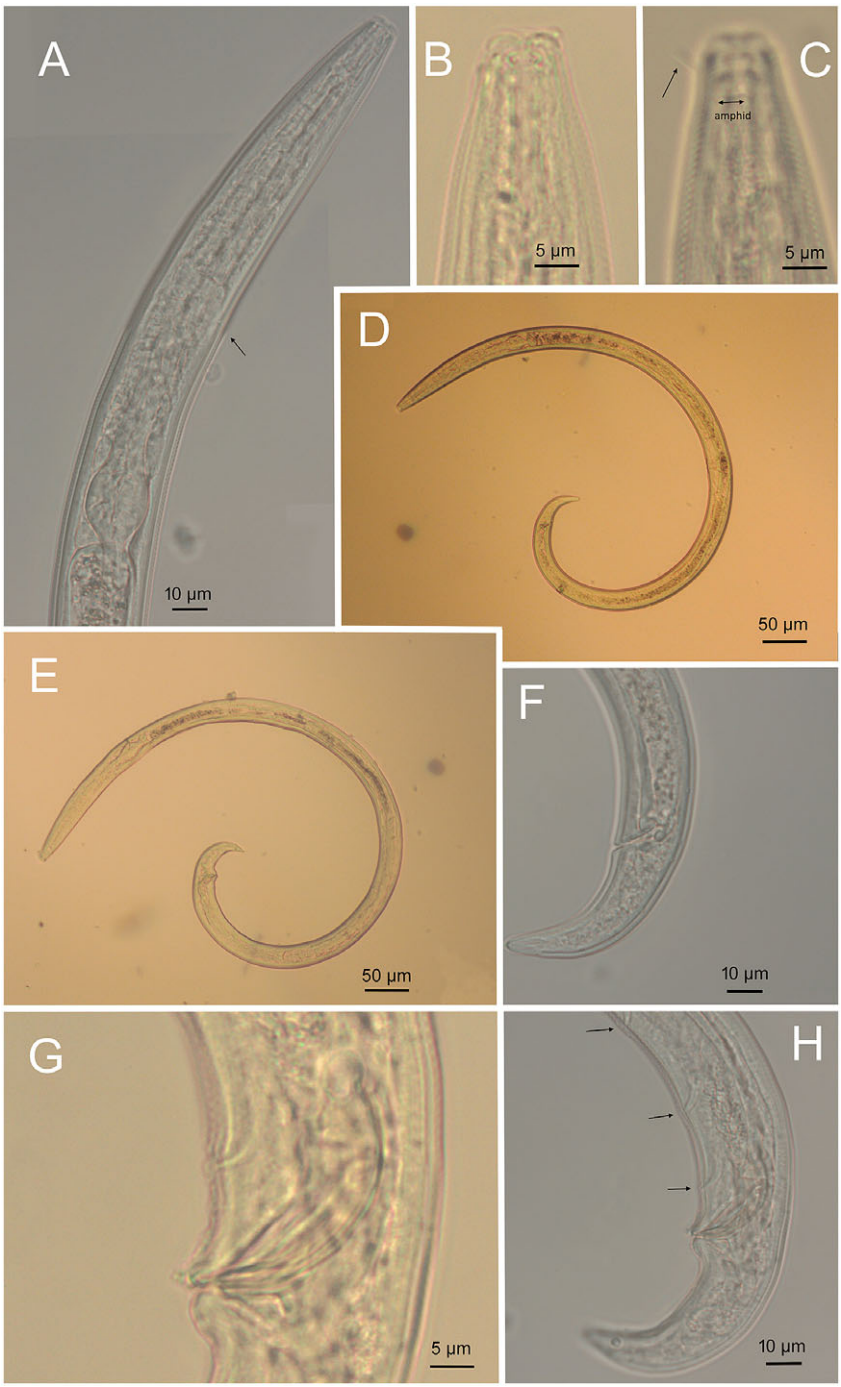

Figure 3. *Anaplectus deconincki* n. sp. (LM). A: Anterior end (arrow indicates excretory pore); B: Stoma; C: Amphid and setae (arrow indicates setae); D: Entire female; E: Entire male; F: Female posterior end; G: Spicule; H: Male posterior end (arrows indicate supplementary organs).

Males with body length of 779–956 µm, spicule 33–39 µm long, gubernaculum 10–12 µm long.

The new species, *A. deconincki,* resembles several species of *Anaplectus,* namely *A. porosus* based on hypodermal glands, and *A. granulosus* based on body length, tail length, and cuticularized spinneret. However, the new species differs from *A. porosus* in female body (612–932 vs 1600 µm), tail length (43–63 vs 71–75 µm), and spicule length (33–39 vs 47 µm) (see Allen & Noffsinger 1968). Compared with *A. granulosus*, the new species differs in the anterior gland (present vs absent), vulva (protruded vs not protruded), stoma length (16–22 vs 25–29 µm), G1 (18–26 vs 14–18), and G2 (17–25 vs 12–10) (see Holovachov *et al.* 2004). Compared with *A. granulosus* studied by Jahan *et al.* (2020), they differ in stoma length (16–22 vs 21–24 µm), and a value (25.5–33.1 vs 19.2–24.6). Besides, the new species differs from the population reported by Allen and Noffsinger (1968) in lower range of female tail (43–63 vs 58–65 µm), and body length (vs 700–1500 µm). Compared with *A. grandepapillatus* (described as *A. submersus* (Hirschmann, 1952) Maggenti, 1961), the new species differs in female body length (612–932 vs 1000–1700 µm) and anterior gland

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(present *vs* absent) (Allen & Noffsinger 1968). Compared with *A. similis*, the new species differs in body length (612–932 vs 1200–1600 µm), the position of the amphids (4–8 vs 7–13 µm), stoma length (16–22 vs 26–35 µm) and anterior gland (present vs absent) (Allen & Noffsinger 1968). Compared with *A. varicaudatus*, the new species differs in stoma length (vs 22–26 µm), anterior gland (present vs absent), and spinneret (cuticularized vs not cuticularised) (Allen & Noffsinger 1968). Compared with *A. magnus*, the new species differs in body length (612–932 vs 2000 µm), stoma length (16–22 vs 30 µm), and amphid position (4–8 vs 16 µm) (Allen & Noffsinger 1968). Compared with *A. atubulatus*, the new species differs in body length (612–932 vs 867–1000 µm), lip region (continues with body vs offset from the body), spinneret (present vs absent), and anterior gland (present vs absent) (Holovachov *et al.* 2004). Compared with *A. brzeskii*, the new species differs in body length (612–932 vs 863–1131 µm), lip region (continuous with the body contour vs offset from body contour), vulva (protruded vs depressed), anterior gland (present vs absent), and amphid location (4–8 vs 7–11 µm) (Holovachov *et al.* 2004). Compared with *A. sudhausi*, the new species differs in stoma length (16–22 vs 23–27 µm), G1 (18–25 vs 16–17), and male tail (56–65 vs 46–52 µm) (Jahan *et al.* 2020). Compared with *A. labiosulcus*, they differ in stoma length (16–22 vs 25–27 µm), a (25.5–33.1 vs 19.2–23.2), c' (2.6–3.1 vs 1.8–2.2) values, and caudal gland arrangement (tandem vs grouped) (Jahan *et al.* 2020). Compared with *A. eurycercus*, the new species differs in tail shape (conoid in its anterior part and cylindrical in its posterior part vs plump), and higher c' value (2.6–3.1 vs 2.0–2.5). Compared with *A. octo*, the new species differs in shape of stoma (arched anteriorly vs hourglass shaped) (Holovachov *et al.* 2004).

Etymology

The species is named after Prof. L.A.P. De Coninck for his excellent research on the Plectida nematodes.

Discriminant analysis of Anaplectus deconincki n. sp.

Based on 15 morphometric characters (Table 2), the comparative analysis of variation was made. Discriminant function analysis revealed six groups (Figure 4), including 1) *A. magnus*, 2) *A. grandepapillatus*, 3) *A. similis*, 4) *A. porosus*, 5) *A. deconincki*, and 6) *A. sudhausi*, *A. varicaudatus*, *A. granulosus*, *A. brzeskii*, *A. atubulatus*, and *A. labiosulcus*. The first two functions explain 76.15% of the total variation in the data, which is sufficient for the analysis. The result of discriminant analysis indicated that *A. deconincki* n. sp. differs from other *Anaplectus* species included in the analysis based on morphometric characters.

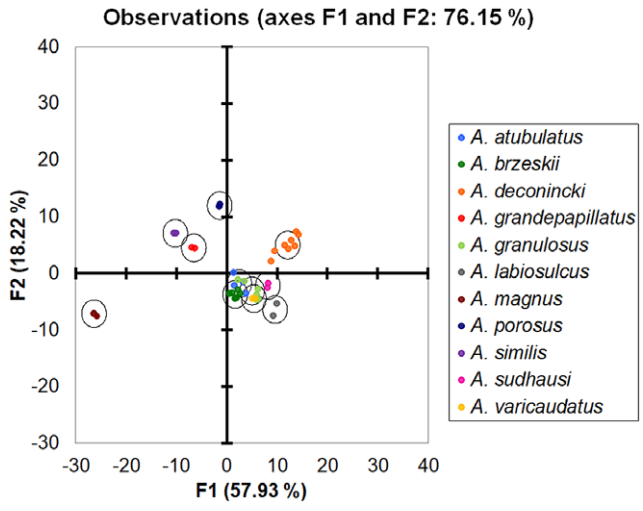

Figure 4. Discriminant analysis plot for *Anaplectus* species based on the important morphometric characteristics.

DNA characteristics

Nblast of the 18S rDNA of the new species indicated 99% similarity with an unidentified *Anaplectus* (AJ966473) and *A. porosus* (MF622934) from Belgium. Moreover, nblast of the 28S rDNA indicated 98% similarity with *A. granulosus* (MF325169; MF325170; MF325171; MF325172) from Germany. Furthermore, the new species showed 93% similarity with *A. porosus* (MF622938) from Belgium. Compared with an unidentified species of *Anaplectus* (MG994930) from the UK, it showed 93% similarity.

Discussion

Anaplectus is a bacterivorous nematode genus with 15 valid species (Holovachov *et al.* 2004; Jahan *et al.* 2020). This group of nematodes occupies various habitats, and the presence of hypodermal glands with conspicuous pores may provide an added advantage by presumably trapping bacteria in mucous cords (Tahseen 2012).

The application of discriminant analysis previously showed that it is a helpful technique for species identification (Shokoohi & Moyo 2022). Similarly, Stock and Nadler (2006) analysed the *Panagrellus* and differentiated the species sufficiently using the same method. Results of the present study separated *A. deconincki* from other *Anaplectus*. Our discriminant analysis showed that *A. magnus*, *A. grandepapillatus*, *A. similis*, and *A. porosus* are morphologically different from the other species of *Anaplectus* selected for study. *Anaplectus magnus* has a longer body and tail than the other *Anaplectus* species (Allen & Noffsinger 1968; Holovachov *et al.* 2004). In contrast, *A. similis* and *A. grandepapillatus* differentiate based on the posteriormost tubular supplements (half of the spicule length vs equal to spicule length) (Holovachov *et al.* 2004). Additionally, *A. porosus* and *A. deconincki* n. sp., diverge from the other species of *Anaplectus*, which both have dorsal and ventral hypodermal glands and pores in the anterior part of the body (Allen & Noffsinger 1968; Holovachov *et al.* 2004). However, *A. deconincki* n. sp., has a shorter body and tail length. In addition, the lateral field bears two incisures compared with three in *A. porosus* (Allen & Noffsinger 1968). On the other hand, several species overlap with the morphometric characteristics, including *A. ganulosus*. Jahan *et al.* (2020), indicating morphometric variation within *A. ganulosus* may potentially imply a cryptic species.

On the phylogenetic position of the Anaplectus deconincki *n. sp.*

The phylogenetic analysis was based on 18S and 28S rDNA markers. The consensus tree inferred from 18S rDNA (Figure 5) revealed that within the family Plectidae, *Anaplectus* is a monophyletic group, consistent with the results of Holovachov *et al.* (2013). The phylogenetics resulted in four well-supported clades: I) *Plectus* species, *Hemiplectus muscorum* Zell, 1991; *Ceratoplectus* cf. *armatus* (Bütschli, 1873) Andrássy, 1984; *C.* cf. *assimilis*

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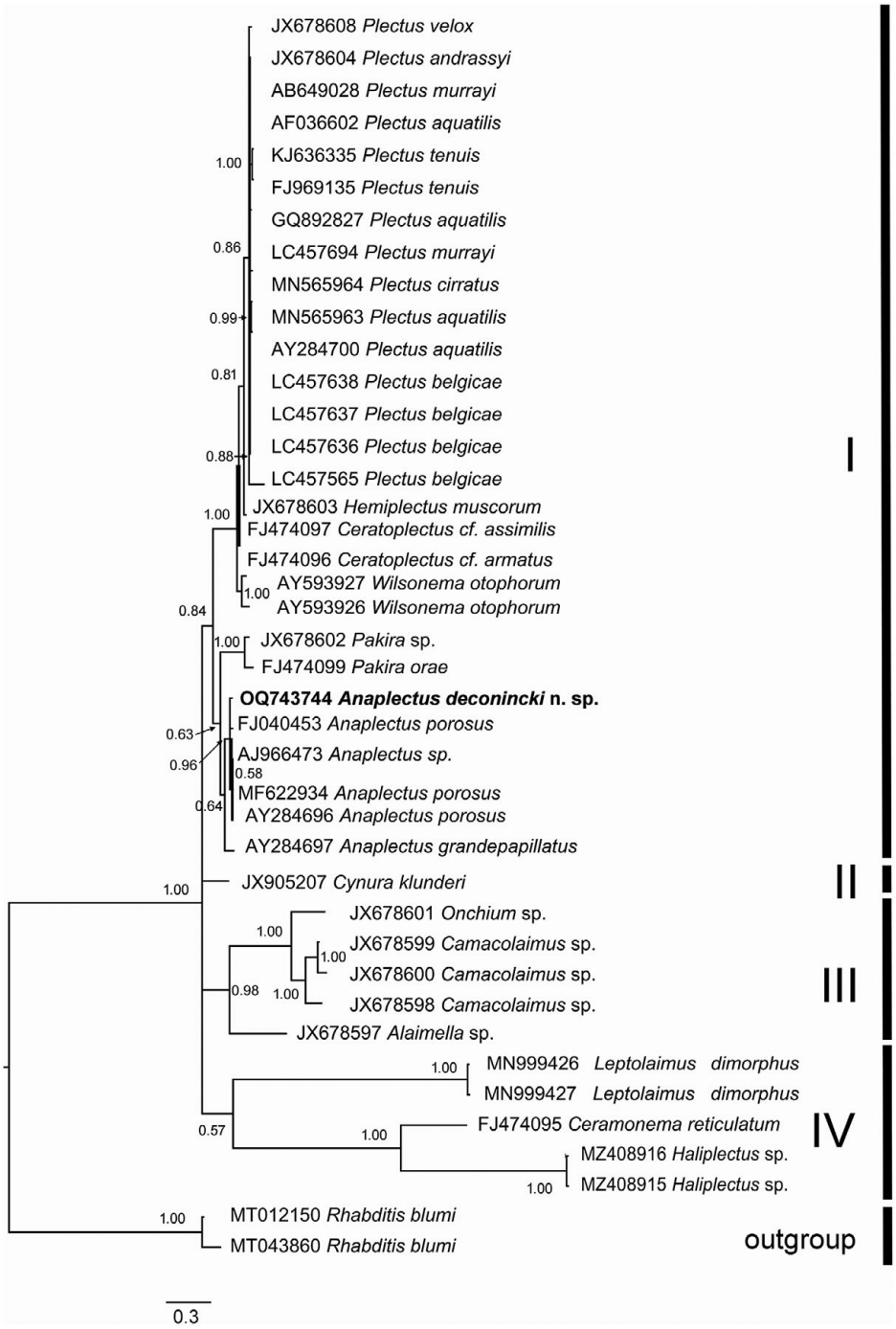

Figure 5. Bayesian tree inferred from 18S rDNA sequences in the genus *Anaplectus*, including *A. deconincki* n. sp. and closely related species.

(Bütschli, 1873) Andrássy, 1984; *Wilsonema otophorum* (de Man, 1880) Cobb, 1913; *Anaplectus* and *Pakira* Yeates, 1967 species with 0.84 posterior probability; II) *Cynura klunderi* Murphy, 1965 with 1.00 posterior probability; III) *Onchium* sp., *Camacolaimus* sp. and *Alaimella* sp. with 0.98 posterior probability, and IV) *Leptolaimus dimorphus* Gharahkhani, Pourjam, Holovachov & Pedram, 2020; *Ceramonema reticulatum* Chitwood, 1936, and *Haliplectus* sp. with 0.57 posterior probability. The result of 18S rDNA placed *Anaplectus* species close to *Pakira*, which is the same result obtained by Holovachov *et al.* (2013) and Gharahkhani *et al.* (2020). Two genera, including *Anaplectus* and *Pakira,* are similar in having lip region truncate, amphidial fovea a transverse slit, and females with didelphic reproductive system (Holovachov 2006; 2016). However, they differ in deirid (present in *Anaplectus* vs absent in *Pakira*), renette cell of excretory system (enveloping distal part of pharynx in *Anaplectus* vs enveloping the anterior part of intestine in *Pakira*), caudal gland (present in *Anaplectus* vs absent in *Pakira*), and male supplementary tubular organs (2–5 in *Anaplectus* vs 2 in *Pakira*) (Holovachov 2006; 2016). In addition, *Anaplectus* species form a clade by 0.96 posterior probability. The same result was obtained by Gharahkhani *et al.* (2020).

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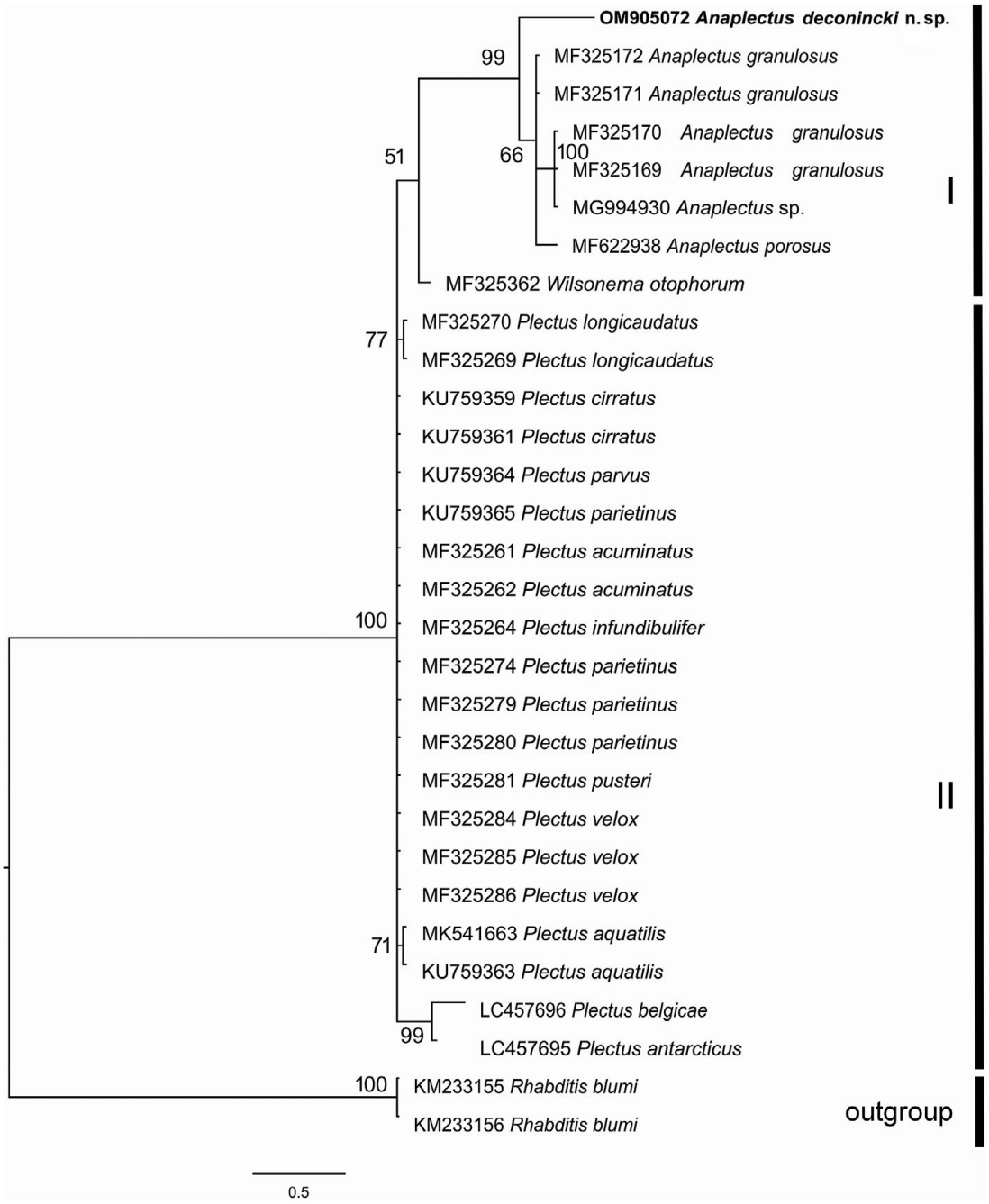

Figure 6. Bayesian tree inferred from 28S rDNA sequences in the genus *Anaplectus*, including *A. deconincki* n. sp. and closely related species.

The consensus tree inferred from 28S rDNA (Figure 6) revealed that within the family Plectidae, *Anaplectus* is a monophyletic group, aligning with results published Holovachov *et al.* (2013). The phylogenetics result grouped in two well-supported clades: I) *Plectus* species with 1.00 posterior probability; II) *Anaplectus* species and *Wilsonema otophorum* with 0.99 posterior probability. *Anaplectus* and *Wilsonema* have similar characteristics, such as deirids, rennet cells of the excretory system enveloping the posterior part of pharynx, and the female reproductive system, which is didelphic. However, *Wilsonema* differs in the unique lip region, which is complicated and expanded (Holovachov 2006; 2016).

The present analysis of the 18S and 28S rDNA sequences indicates a close relationship between *A. deconincki* n. sp. with *A. porosus* and *A. granulosus,* although, the new species is well separated from the other two species identified molecularly. Allen and Noffsinger (1968) indicated that *A. porosus* can be distinguished from other species by the presence of an anterior series of dorsal and ventral hypodermal pores. Despite the fact that this characteristic has been observed in the South African new species even posterior to the pharyngeal region, it is distinguished by shorter body length and tail length. Furthermore, the anterior gland was not reported previously by Allen and Noffsinger (1968) or Holovachov *et al.* (2004) for

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A. granulosus; therefore, it can be clearly distinguished from the other two species of *Anaplectus* identified molecularly.

Ethical standards. The paper reflects the authors' own research and analysis in a truthful and complete manner. All authors have been personally involved in substantive work leading to the manuscript and contributed to preparing the final draft of the manuscript.

Data and specimens' availability. This material is the authors' original work, which has not been previously published elsewhere and has no conflict of interest. Four slides of *Anaplectus deconincki* n. sp. were deposited in the Nematology collection of the Aquaculture Research Unit, University of Limpopo, South Africa. One slide was deposited in the Nematode Collection of Virginia Tech University.

Authors' contributions. ES collected the samples. ES identified the species, analysed the data, wrote and revised the manuscript. JE took LM of the species. ES conducted the statistical analysis. All authors revised the manuscript and contributed to the final draft of the manuscript.

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Competing interest. The authors declare that they have no conflicts of interest.

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