HAYATI
Journal of Biosciences
Vol. 30 No. 4, July 2023 757-769
DOI:10.4308/hjb.30.4.757-769
ISSN: 1978-3019
EISSN: 2086-4094

First Records of Male and Swollen Females of Two Species of *Achlysiella* (Nematoda: Pratylenchidae)

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ARTICLE INFO

Article history:
Received January 2, 2022
Received in revised form March 18, 2023
Accepted April 12, 2023

KEYWORDS:
Achlysiella,
nematodes,
plant parasites,
Pratylenchidae,
Radopholus,
taxonomy

ABSTRACT

Males and swollen mature females of *Achlysiella trilineata* and *Achlysiella magniglans* were discovered for the first time in Australia. These life stages confirmed that both species show well-marked sexual dimorphism in the anterior region of the body, with the males having reduced cephalic frameworks, stylets, and oesophagi. Males of *A. magniglans* displayed an extreme reduction in the style such that only the cone could be seen, and the rest of the esophagus was barely visible. In both species, the swollen females had postmedian vulvae, and the anterior branch of the ovary was longer than the posterior branch. In *A. magniglans*, the disparity in length was more pronounced, and the vulva was more posterior. The finding of swollen females confirmed the classification of both species in *Achlysiella* rather than *Radopholus*. Vermiform females of both species have large dorsal oesophageal glands and short ovaries. Justifiably, other species of *Radopholus* with these latter features (*R. brevicaudatus, R. capitatus,* and *R. vacuus*) should also be moved to *Achlysiella*.

1. Introduction

Radopholus has been recognized as one of the most economically important genera of plant parasitic nematodes, mainly because of its widely distributed species, *Radopholus similis* (Duncan and Moens 2006). For decades, however, the taxonomy of the genus has received little attention. One recurring taxonomic problem was the placement of some of its species in *Radopholus* or *Achlysiella*.

What became the type species of *Achlysiella* was originally described by Siddiqi (1964) as *Radopholus williamsi* Siddiqi, 1964, a parasite of sugar cane. The species showed all the distinguishing characteristics of *Radopholus*, particularly the low sclerotized head, dorsal oesophageal gland overlapping the intestine, and sexual dimorphism in the anterior region. Later, Hunt *et al.* (1989) found a swollen female of *R. williamsi* with egg-sacs in the root of diseased sugar cane and males and immature females in the surrounding soil. Subsequently, several morphological differences from *Radopholus* were discovered, including immature ovaries and very long oesophageal gland lobes in vermiform females. The morphological differences, together with biological differences, such as a gelatinous egg-sac containing large eggs, juveniles, adult males and vermiform females, and non-feeding juveniles with superimposed molts, led to the formal proposal of a new genus *Achlysiella* Hunt *et al.*, 1989 with *Achlysiella williamsi* (Siddiqi, 1964) Hunt *et al.*, 1989 as the type species.

Hunt *et al.* (1989) also pointed out that five other species of *Radopholus*—namely *R. brevicaudatus* Colbran, 1971; *R. capitatus* Colbran, 1971; *R. magniglans* Sher, 1968; *R. trilineatus* Sher, 1968, and *R. vacuus* Colbran, 1971—possibly belonged to *Achlysiella* as well because the oesophageal gland lobe was elongated and the ovaries in vermiform females were immature in all these species. However, two diagnostic features of *Achlysiella*, i.e., swollen mature females and sexual dimorphisms in the anterior region, were lacking in these five species, preventing their formal placement in *Achlysiella*. In describing *R. magniglans* and *R. trilineatus*, Sher (1968) speculated that the vermiform females may undergo further development in the root of plants, but he did not provide any data.

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Despite lacking the necessary data, Ebsary (1991) transferred the five *Radopholus* species to *Achlysiella* without providing any new data or explicitly explaining the reason for his action. This species revision was accepted by Siddiqi (2000) but rejected by others (Ryss 2003; Ryss and Wouts 1997). No further work has been done on this, so the disagreement remains.

During field sampling in New South Wales, the Australian Capital Territory, and Victoria, Australia, populations of *R. trilineatus* and *R. magniglans* have been found in natural vegetation. Vermiform females have been recovered from soils; males and swollen banana- or sausage-shaped females have been obtained from roots. The new records of swollen mature females may clarify the placement of these species in *Achlysiella*.

2. Materials and Methods

Field samples were collected by taking soils from the rhizosphere of plants along with some fine roots. In the laboratory, the roots were separated from the soil. Nematode extraction from soils was carried out using the modified Whitehead and Hemming tray method described in Hooper (1986) and Bloemers and Hodda 1995. Nematodes were also extracted from roots using a variation of the root immersion technique (Hallmann and Subbotin 2018).

Recovered nematodes were killed and fixed in a culture dish using hot formaldehyde (5% v/v) immediately, followed by a similar volume at room temperature (). The suspension was covered and left for at least two weeks, after which specimens were transferred to anhydrous glycerol by the slow method (Hooper 1986). Finally, the specimens were mounted on permanent slides.

Morphological examinations of the mounted specimens were performed using a Zeiss Axioscope compound microscope. Identifications were made by comparing specimens with type specimens, original publications, and published keys. Character states were obtained by direct observation, measurement using an eyepiece graticule, or using a camera lucida.

3. Results

3.1. Re-Description of *Achlysiella trilineata* (Sher, 1968) Ebsary, 1991

3.1.1. Vermiform Immature Females

The cephalic region set off, rounded, with 2, sometimes indistinct, annules. Stylet robust, length about twice head diameter or 3.0–3.8% of total body length, cone slightly longer than the shaft, basal knobs rounded. Excretory pore at the level of posterior or anterior to the esophagus-intestinal junction. Hemizonid 2-3 annules long, 1 annule from the excretory pore. Dorsal oesophageal glands are continuous and wide, mostly filling the corresponding body diameter, anterior part to the level of the first nucleus, with texture appearing rougher than the rest. Cuticle annulated. Lateral field with 3 incisures, not areolated. Vulva post-median, lips flat or slightly protuberant. The genital system is didelphic, and the anterior branch is usually slightly longer than the posterior. Ovaries are immature, with 1–10 developing oocytes. Spermathecae ovoid-rounded, filled with rod-like sperm. The tail was slightly tapering, with an annulated rounded terminus (Figure 1 and Table 1).

3.1.2. Mature Females

Head region rounded, set off, usually not annulated, but some specimens with 2 indistinct annules. The body is swollen, with the neck region narrower. Stylet robust, cone slightly longer than the shaft, basal knobs rounded or slightly pointed anteriorly. Hemizonid is 2 annules long, 1 annule from the excretory pore. Oesophago-intestinal junction was anterior to or at the level of excretory pore. Cuticle is annulated, and incisures of the lateral field are not visible. Vulva post-median, lips flat or slightly protuberant. Genital system amphi-didelphic, fully developed with numerous oocytes, anterior branch slightly longer than posterior. Tail with annulated rounded terminus (Figure 2 and Table 1).

3.1.3. Males

The body ventrally curved when heat-relaxed, forming an open C-shape or slightly coiled. The cephalic region set off, knob-like, not as high as broad with a height less than 50% of the diameter, smooth, without distinct annules, framework much reduced. Stylet slender, with a cone slightly longer than the shaft, knobs merging into a single round base. Oesophagus reduced, median bulb small. Oesophageal glands are short, only slightly overlapping the intestine. The excretory pore is posterior to the oesophago-intestinal junction. Hemizonid prominent. Cuticular annulation is much less pronounced than in females. Lateral field with 4 equally spaced incisures, sometimes indistinct. Spicules are arcuate. Gubernaculum stout, more than

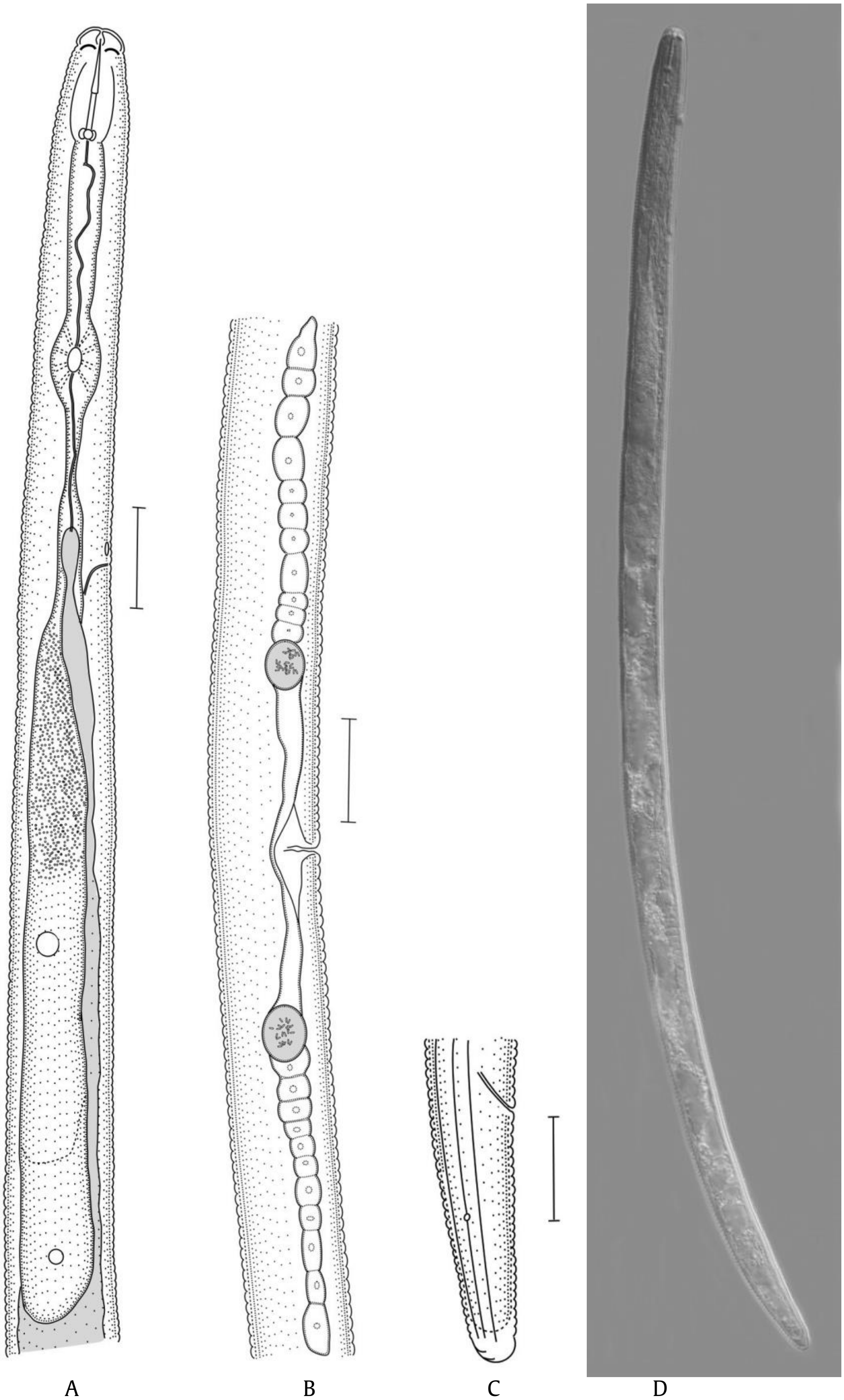

Figure 1. *Achlysiella trilineata* immature female. (A) anterior part, (B) Mid body, showing gonads, (C) Posterior part, (D) whole body. Bars correspond to 20 µm

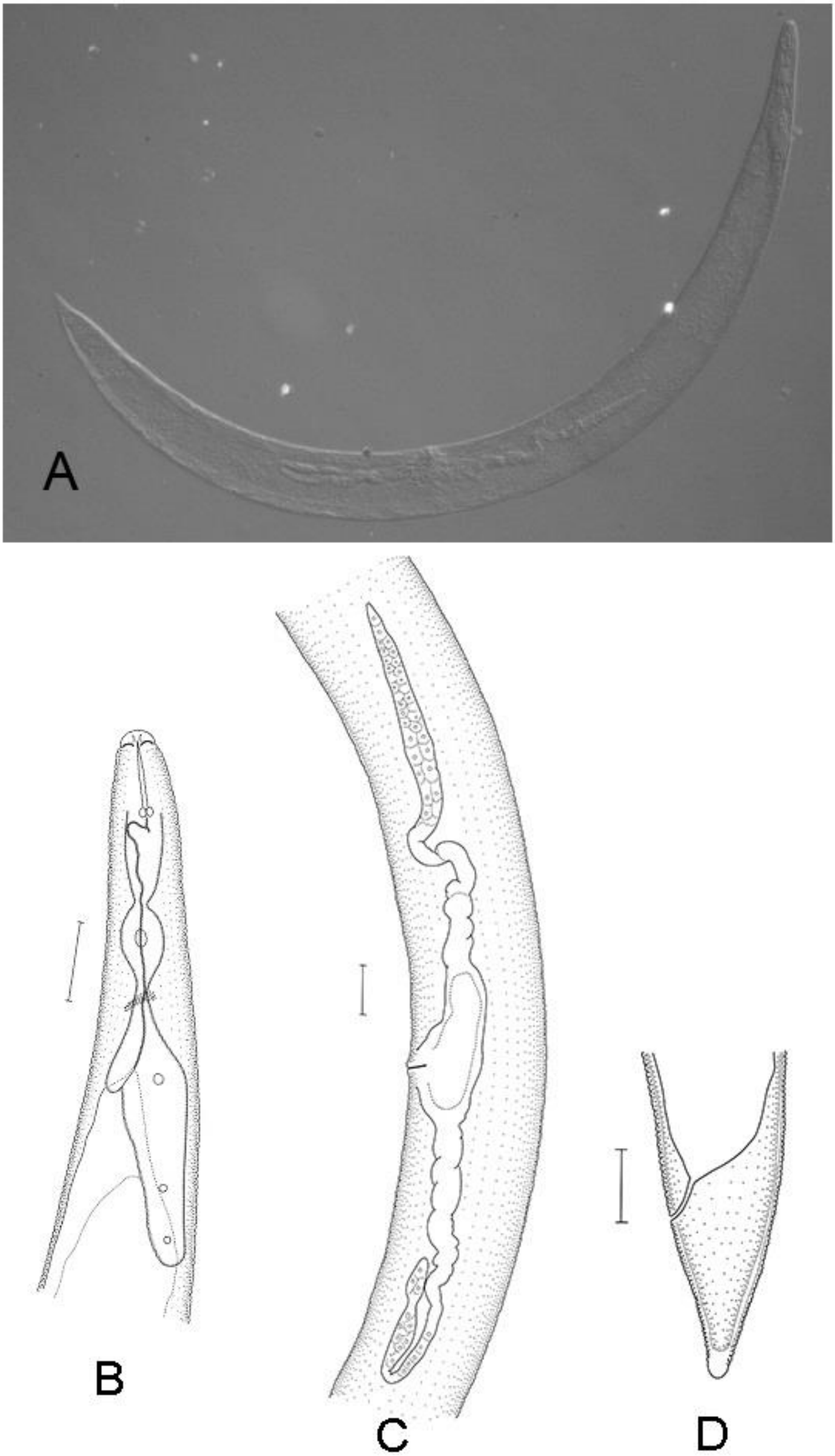

Figure 2. *Achlysiella trilineata* mature female. (A) whole body, (B) anterior region, (C) mid body showing gonad, (D) tail. Bars correspond to 20 µm

half spicule in length, with a hook at the proximal end. Genital tract with rod-like sperm. Phasmid anterior to mid-tail. Bursa areolated, reaching the tail tip. Tail with arcuate digitiform terminus (Figure 3 and Table 1).

3.1.4. Materials Examined

Collected from the entrance to Ben Boyd National Park (-36.9882, 149.9228), Yambulla State Forest (-37.1991, 149.6408), and Yurammie State Forest (-36.9102, 149.7867) New South Wales, Australia.

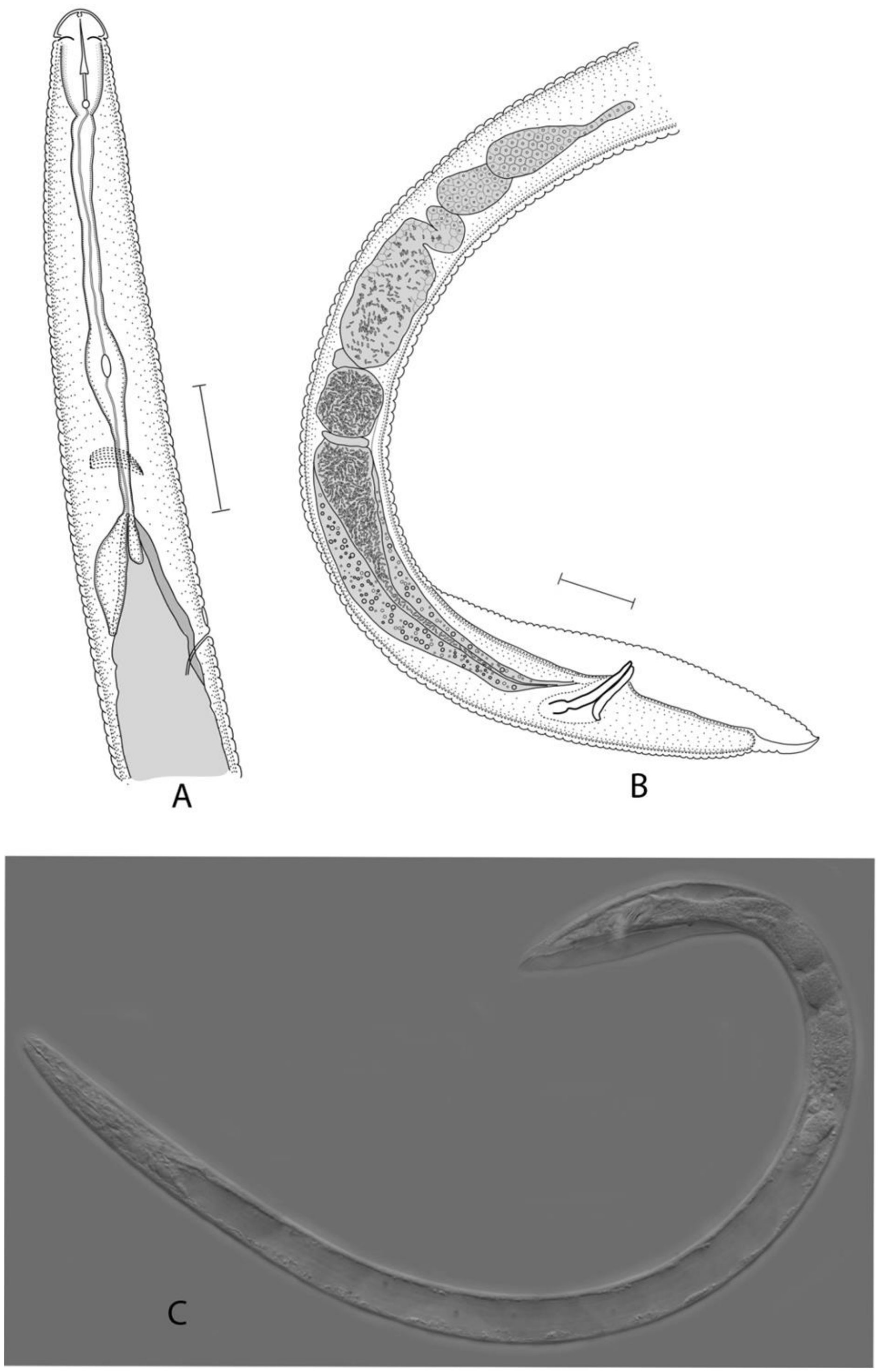

Figure 3. *Achlysiella trilineata* male. (A) anterior part, (B) posterior part, (C) whole body. Bars correspond to 20 μm

Table 1. Morphometric characters of immature females, mature females, and males of *Achlysiella trilineata*

	Immature females					Mature females		Males	
	Yambulla SF	Yurammi SF	Benboyd NP	Measure (n = 12)	Range	Measure (n = 6)	Range	Measure (n = 5)	Range
L	615.3±33.9	565.3±30.2	605.0±16.1	595.2±33.8	529-666	817.7±138.7	655-1,050	644.2±30.3	595.0-678.0
a	29.7±1.5	28.7±2.2	29.2±1.3	29.2±1.6	26.5-31.7	15.6±2.7	11.3-18.6	27.0±3.3	23.8-32.3
b	6.3±0.4	6.1±0.5	6.2±0.1	6.2±0.4	5.45-6.67	7.8±1.2	6.2-9.4	8.1±0.3	7.6-8.4
b'	2.6±0.1	2.2±0.1	2.5±0.1	2.4±0.2	1.97-2.67	4.9±1.4	3.6-7.2	6.1±0.6	5.1-6.8
c	12.9±0.4	13.6±1.8	12.8±0.6	13.1±1.1	11.5-15.9	17.7±2.5	15.1-21.0	12.8±0.5	11.9-13.3
c'	2.9±0.2	3.1±0.4	3.1±0.1	3.0±0.3	2.57-3.54	1.8±0.4	1.2-2.0	2.9±0.1	2.8-3.0
V	61.7±1.2	63.2±3.8	57.5±2.9	60.8±3.6	53.2-68.4	60.9±3.1	58.1-66.6	n.a.	n.a.
V'	0.7±0.0	0.7±0.0	0.6±0.0	0.7±0.0	0.58-0.75	64.6±3.5	61.3-71.1	n.a.	n.a.
G1	16.0±1.1	14.4±1.1	14.5±0.7	15.0±1.2	13.3-17.3	30.8±5.5	22.7-39.0	n.a.	n.a.
G2	14.6±0.9	13.2±1.3	14.4±1.0	14.0±1.2	12-15.5	24.5±3.3	20.2-28.4	n.a.	n.a.
Stylet length	19.8±0.5	19.8±0.5	20.0±0.0	19.8±0.4	19-20	19.8±0.4	19.0-20.0	13.0±1.6	11.0-15.0
Stylet cone length	10.8±0.5	10.8±0.5	11.0±0.0	10.8±0.4	10-11	10.7±0.5	10.0-11.0	8.2±1.3	7.0-10.0
Stylet shaft length	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0	9-9	9.2±0.4	9.0-10.0	4.8±0.4	4.0-5.0
Stylet shaft width	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2-2	2.0±0.0	2.0-2.0	1.5±0.1	1.3-1.7
Stylet length/head width	2.1±0.1	2.2±0.1	2.1±0.1	2.1±0.1	2-2.22	2.1±0.1	2.0-2.2	2.0±0.2	1.8-2.2
Stylet/L %	3.2±0.2	3.5±0.2	3.3±0.1	3.3±0.2	3-3.78	2.5±0.4	1.9-2.9	79.6±3.6	78.0-86.0
Anterior to OIJ	97.3±5.0	93.5±5.4	97.3±1.7	96.0±4.4	86-101	105.3±4.7	100.0-112.0	107.2±11.2	100.0-127.0
Anterior to OG end	236.5±9.7	262.3±4.9	241.5±16.1	246.8±15.5	226-268	171.8±33.2	135.0-220.0	27.6±12.9	14.0-49.0
OIJ to OG end	139.3±9.5	168.8±3.2	144.3±16.5	150.8±16.8	127-171	66.5±33.2	30.0-112.0	50.4±0.5	50.0-51.0
Tail length	47.8±3.8	42.0±4.9	47.5±2.9	45.8±4.5	36-53	46.3±6.3	36.0-53.0	4.0±0.0	4.0-4.0
Head length	3.8±0.5	3.5±0.6	4.0±0.0	3.8±0.5	3-4	4.0±0.6	3.0-5.0	8.8±0.4	8.0-9.0
Head width	9.5±0.6	9.0±0.0	9.8±0.5	9.4±0.5	9-10	9.7±0.5	9.0-10.0	18.4±0.5	18.0-19.0
Anterior to DGO	27.8±1.0	26.0±1.4	28.0±0.0	27.3±1.3	24-29	26.3±1.9	23.0-28.0	4.4±0.5	4.0-5.0
Stylet base to DGO	6.0±0.8	5.0±0.0	6.5±0.6	5.8±0.8	5-7	4.7±1.4	2.0-6.0	98.4±4.0	95.0-104.0
Anterior to EP	102.8±3.5	100.0±4.7	94.8±6.1	99.2±5.6	89-107	114.2±5.4	109.0-122.0	17.4±0.5	17.0-18.0
Anal body width	16.5±0.6	13.6±0.8	15.3±0.5	15.1±1.4	13-17	27.0±6.1	21.0-38.0	24.1±2.7	21.0-27.5
Max body width	20.8±0.5	19.8±0.5	20.8±1.0	20.4±0.8	19-22	53.5±11.4	38.0-72.0	13.0±1.6	11.0-15.0
Anterior to vulva	379.5±15.3	356.3±10.2	348.5±26.2	361.4±21.6	310-401	496.7±78.9	401.0-640.0	n.a.	n.a.
Ovary anterior length	98.5±6.2	81.0±3.2	87.5±5.6	89.0±8.9	77-104	253.1±64.3	148.5-348.0	n.a.	n.a.
Ovary posterior length	90.0±7.6	74.3±5.1	86.8±4.3	83.7±8.8	67-98	200.4±59.1	132.0-285.0	n.a.	n.a.
Anterior to Anal Orifice	567.5±30.3	523.3±30.8	557.5±14.2	549.4±30.9	483-613	771.3±135.3	619-1,000	593.8±30.0	545.0-627.0
Hyaline length	5.9±0.3	6.0±0.8	5.5±0.6	5.8±0.6	5-7	6.2±1.0	5-7	13.6±2.1	12.0-17.0
Spicule length	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23.2±0.8	22.0-24.0
Gubernaculum	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15.2±0.8	14.0-16.0
Spicule/L %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.6±0.1	3.5-3.7

Measurements in µm in the form: mean ± standard deviation. OG = oesophageal gland, OIJ = oesophago-intestinal junction, DGO = dorsal gland orifice, EP = excretory pore. L = total body length, a = body length/maximum body width, b = body length/OIJ, b' = body length/anterior to OG end, c = body length/tail length, c' = tail length/body width at anus, V = (anterior to vulva/body length) × 100, V' = (anterior to vulva/L') × 100, G1 = (ovary anterior/body length) × 100, G2 = (ovary posterior/body length) × 100

3.1.5. Remarks

The discovery of swollen mature females has led to the conclusion that the specimens belonged to *Achlysiella*. The specimens were compared with the type specimens of *Achlysiella* deposited at the Queensland Museum, but the latter was very old and generally not satisfactory for close morphological observations. So, the conclusion was also based on the interpretation of the original descriptions and illustrations of the species of the genus (Sher 1968; Colbran 1971). Of all the described species of *Achlysiella, A. trilineata* is unique in having three incisures throughout the female body. The material studied generally corresponded to the original description of the immature female *A. trilineata* regarding a number of lateral lines, body length, morphometric index b, length of the hyaline portion of the tail, stylet length, and rod-like sperm shape. Differences from the original description concerning the ranges of the morphometric indices a, c, c', V, G1, and G2 were not considered to be significant due to the considerable overlap between the ranges in the specimens and the descriptions. Similarly, since some specimens examined had 2 head annules, and others showed no distinct head annulation, while the original description mentioned 2 distinct annules, the differences were considered an intraspecific variation. The only remarkable dissimilarity was in the range of morphometric index b' (2.0–2.7 versus 2.5–3.1), but this character still showed some overlap between the specimens and the original description.

3.2. Re-Description of *Achlysiella magniglans* (Sher, 1968) Ebsary, 1991

3.2.1. Vermiform Immature Females

Cephalic region rounded, set off, with 2 or 3 annules. Stylet robust, length about twice head diameter or 3.7–4.3% of total body length, cone as long as or slightly longer than the shaft, basal knobs rounded or slightly flattened anteriorly. Excretory pore at the level of posterior or anterior to the oesophago-intestinal junction. Hemizonid 2-3 annules long, immediately anterior to excretory pore. Dorsal oesophageal glands are continuous, wide, posterior parts filling the corresponding body diameter; the anterior gland with a larger nucleus and smoother texture than the two posterior glands. Cuticle annulated, annules 1.6-1.7 µm wide at mid-body. Lateral field with three incisures on anterior body anterior to the anterior oesophageal gland, then four incisures in mid-body, then coalescing to three incisures somewhere between vulva and anus or just posterior to phasmid; areolation absent, but outer lines crenate; inner lines usually closer together than outer ones, but some specimens show equally spaced lines in mid-body. Vulva post-median, lips not protuberant. Genital system didelphic, anterior branch longer than posterior. Spermathecae ovoid, posterior distinctly smaller than anterior, filled with rod-like sperm. Ovaries are immature with 3–11 developing oocytes, posterior smaller, shorter, and bearing fewer oocytes than anterior. Tail tapering, with annulated rounded terminus (Figure 4 and Table 2).

3.2.2. Mature Females

Head region set off, low, rounded, with 2 or 3 annules. The body is swollen, with the neck region narrower. Stylet robust; cone slightly longer than shaft; basal knobs rounded, flattened anteriorly, or slightly pointed anteriorly, making them appear somewhat anchor-shaped. Hemizonid 2 annules are long but hardly visible in some specimens. The esophagus-intestinal junction at the level of hemizonid or excretory pore. Cuticle is annulated, and incisures of the lateral field are not visible. Vulva post-median, lips protuberant in some specimens. Genital system amphi-didelphic, anterior branch distinctly longer than posterior. Tail with annulated rounded terminus (Figure 5 and Table 2).

3.2.3. Males

The body ventrally curved when heat-relaxed, forming an open C- or J-shape. Cephalic region set off, knob-like, widest at base, height more than 50% of the diameter, without distinct annules, framework weak. Stylet reduced so that only the cone is visible. Oesophagus degenerate, median bulb barely visible. Dorsal oesophageal glands are short, only slightly overlapping the intestine. Hemizonid present. The excretory pore is posterior to the oesophago-intestinal junction. Lateral field with 4 equally-spaced incisures at mid-body. Genital tract with rod-like sperm. Spicules are arcuate. Gubernaculum is more than half the spicule in length, with the hook at the proximal end. Bursa areolated, extending to the tail terminus. Phasmid anterior to mid-tail. Tail with arcuate digitiform terminus (Figure 6 and Table 2).

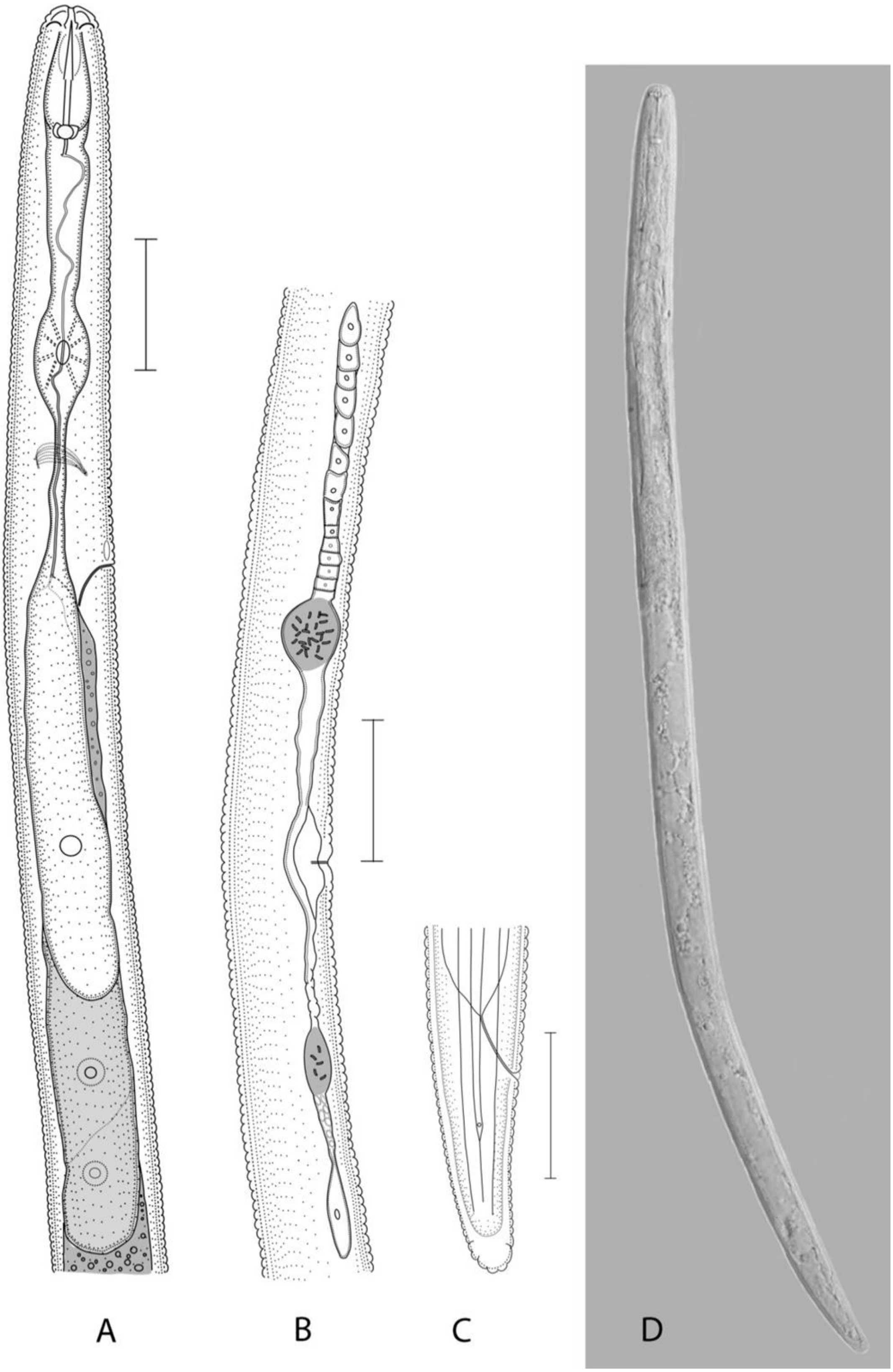

Figure 4. *Achlysiella magniglans* immature female. (A) anterior part, (B) mid body, showing gonads, (C) posterior part, (D) whole body. Bars correspond to 20 µm

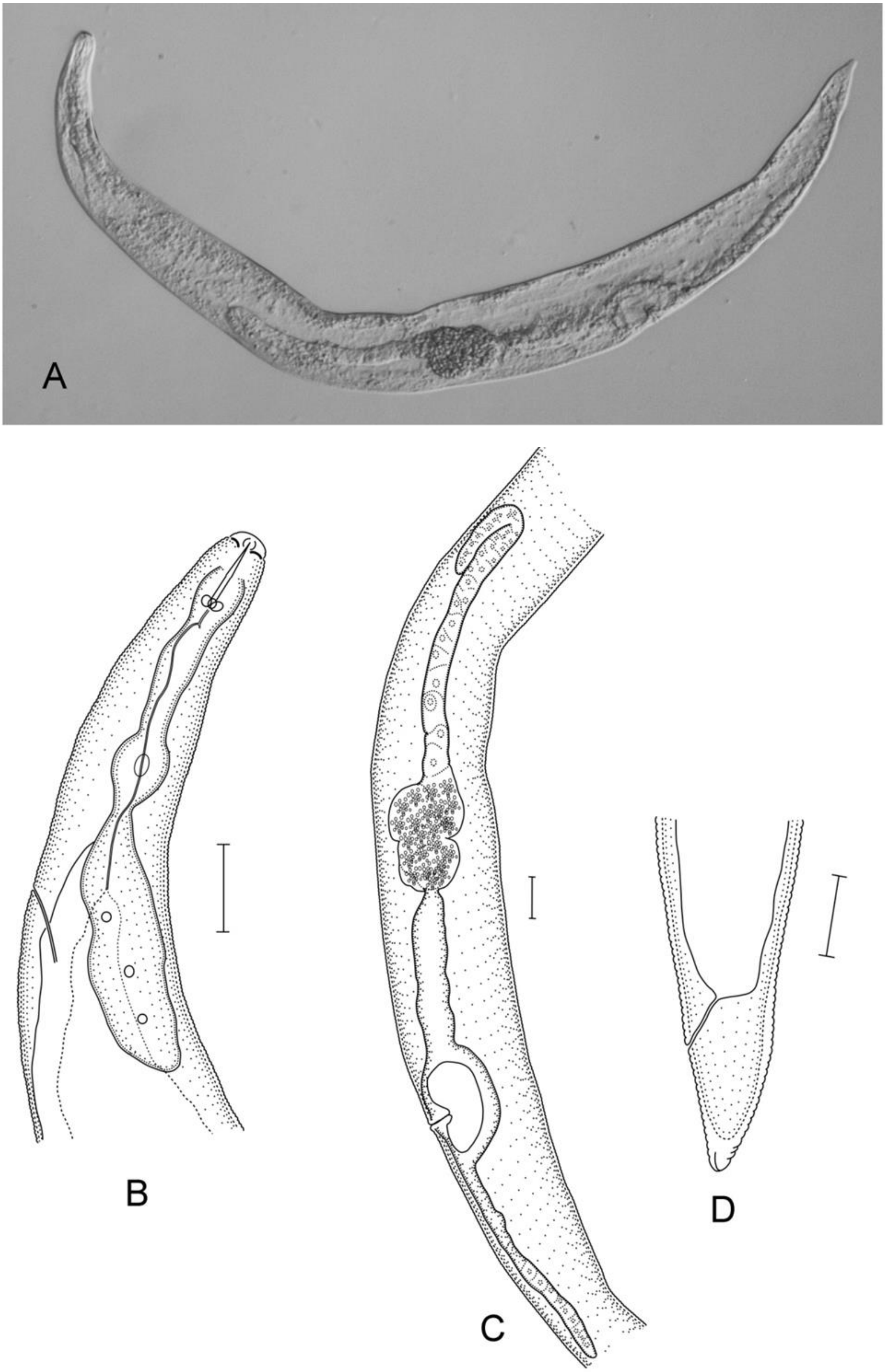

Figure 5. *Achlysiella magniglans* mature female. (A) whole body, (B) anterior region, (C) mid body showing gonad, (D) tail. Bars correspond to 20 μm

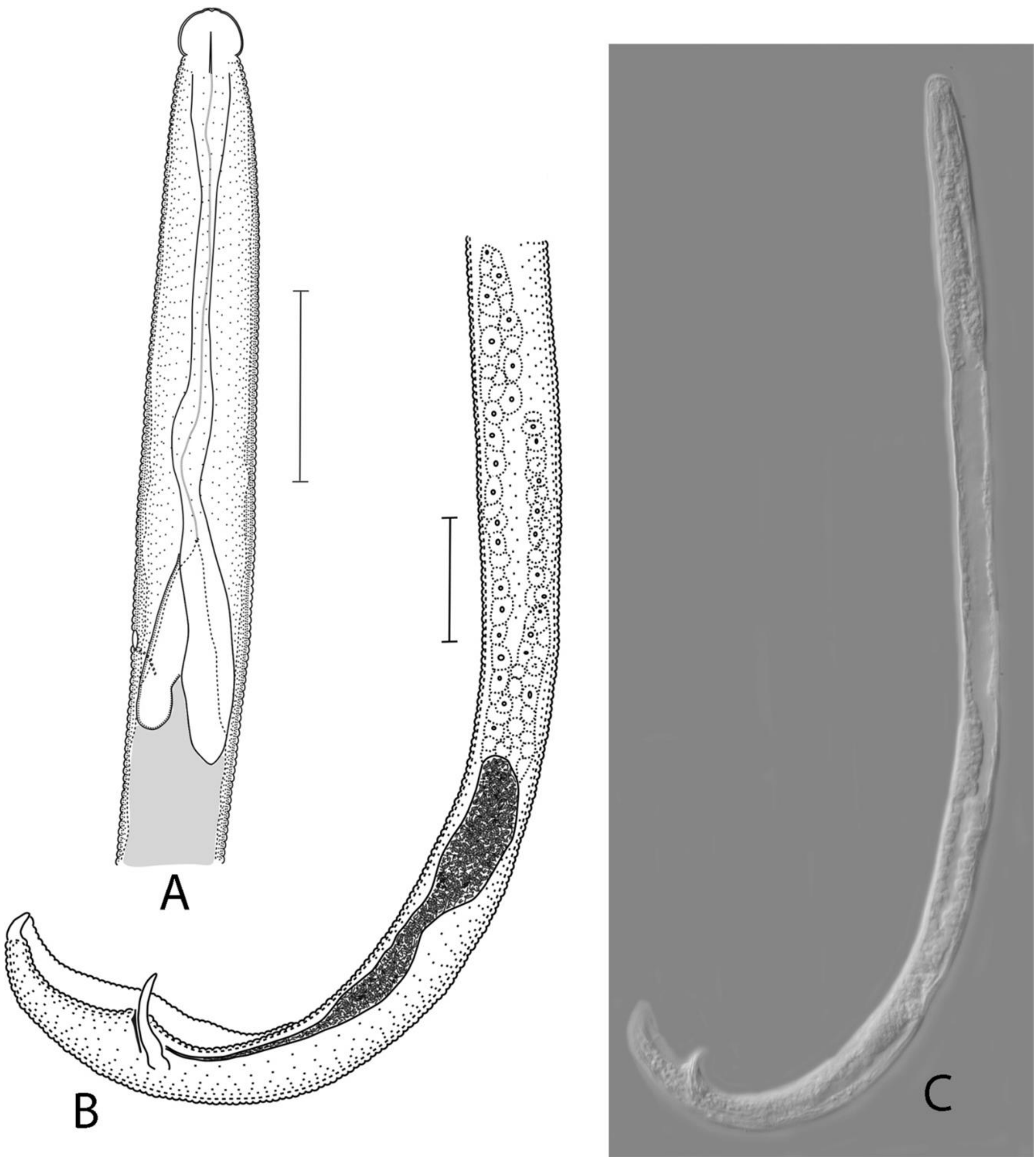

Figure 6. *Achlysiella magniglans* male. (A) anterior part, (B) posterior part, (C) whole body. Bars correspond to 20 µm

Table 2. Morphometric characters of immature females, mature females, and males of *Achlysiella magniglans*

	Immature females					Mature females		Males	
	Mournpall	Dasyurus	Black Mt	Measure (n = 10)	Range	Measure (n = 6)	Range	Measure (n = 5)	Range
L	482.0±15.9	509.0±18.3	495.0±1.0	496.7±17.5	470-535	771.8±45.4	709-815	378.0±31.1	356-400
a	25.5±1.4	27.2±2.1	27.3±0.4	26.7±1.6	23.8-29.7	13.1±1.9	10.9-14.8	29.1±0.8	28.6-29.7
b	5.6±0.3	5.7±0.3	6.0±0.3	5.8±0.3	5.3-6.2	7.5±0.8	7.0-8.1	6.8±0.6	6.4-7.3
b'	2.5±0.2	2.7±0.1	2.8±0.2	2.7±0.2	2.3-3.0	4.9±0.3	4.6-5.1	5.1±0.9	4.5-5.7
c	14.2±0.2	16.5±1.4	15.5±0.7	15.4±1.3	14.0-17.8	23.7±2.1	20.9-25.5	12.8±1.1	12.1-13.6
c'	2.6±0.1	2.6±0.1	2.5±0.1	2.6±0.1	2.4-2.8	2.4±0.6	1.6-3.1	2.7±0.0	2.7-2.7
V	66.1±1.0	67.7±1.3	65.5±2.9	66.6±1.9	63.1-68.9	67.3±5.7	59.3-72.7	n.a.	n.a.
V'	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.6-0.7	70.3±5.9	61.9-75.7	n.a.	n.a.
G1	16.4±1.6	16.7±0.7	14.6±2.2	16.0±1.6	12.1-18.1	39.5± 4.1	34.8-44.7	n.a.	n.a.
G2	12.8±0.8	11.3±1.7	7.5±4.1	10.6±3.2	3.4-13.7	17.3±2.1	15.0-19.6	n.a.	n.a.
Stylet length	20.0±0.0	19.5±0.6	20.3±0.6	19.9±0.6	19.0-21.0	19.8±1.3	18-21	7.5±0.7	7-8
Stylet cone length	10.3±0.6	10.5±0.6	10.3±0.6	10.4±0.5	10.0-11.0	10.3±1.0	9-11	n.a.	n.a.
Stylet shaft length	9.7±0.6	9.0±0.0	9.7±0.6	9.4±0.5	9.0-10.0	9.5±0.6	9-10	n.a.	n.a.
Stylet shaft width	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2.0-2.0	2.1±0.3	2.0-2.5	n.a.	n.a.
Stylet length/head width	2.2±0.0	2.1±0.1	2.2±0.1	2.2±0.1	2.0-2.3	2.2±0.1	2.0-2.3	1.0±0.2	0.9-1.1
Stylet/L %	4.2±0.1	3.8±0.1	4.1±0.1	4.0±0.2	3.7-4.3	2.6±0.3	2.2-2.8	2.0±0.0	2-2
Anterior to OIJ	86.7±3.1	88.9±4.9	83.0±3.6	86.5±4.4	80.0-94.0	101.0±1.4	100-102	55.5±0.7	55-56
Anterior to OG end	197.0±16.5	188.0±9.5	178.0±12.8	187.7±13.7	167-208	156.3±3.2	154-160	75.0±7.1	70-80
OIJ to OG end	110.3±19.3	99.1±11.1	95.0±13.0	101.3±14.3	83-122	56.5±4.9	53-60	19.5±6.4	15-24
Tail length	34.0±1.0	31.0±1.7	32.0±1.4	32.4±1.8	30-35	33.0±1.8	31-35	29.5±0.0	29.5-29.5
Head length	3.3±0.3	3.5±0.4	3.0±0.0	3.3±0.4	3-4	3.0±0.0	3-3	4.5±0.0	4.5-4.5
Head width	9.0±0.0	9.1±0.3	9.2±0.3	9.1±0.2	9.0-9.5	9.0±0.0	9-9	7.5±0.7	7-8
Anterior to DGO	27.0±0.0	24.8±0.5	26.3±0.6	25.9±1.1	24-27	25.3±2.1	23-27	n.a.	n.a.
Stylet base to DGO	5.7±0.6	4.3±0.5	5.2±0.3	5.0±0.8	4-6	5.5±0.7	5-6	n.a.	n.a.
Anterior to EP	80.3±2.1	84.1±6.3	82.3±5.0	82.5±4.8	75-89	98.5±9.2	92-105	n.a.	n.a.
Anal body width	13.0±1.0	12.0±1.0	13.0±0.0	12.6±0.9	11-14	14.3±3.4	11-19	11.0±0.0	11-11
Max body width	19.0±1.7	18.8±1.0	18.2±0.3	18.7±1.1	18-21	59.8±9.1	48-70	13.0±1.4	12-14
Anterior to vulva	318.7±5.7	344.5±14.1	324.3±14.0	330.7±16.2	313-360	524.6±43.9	482-585	n.a.	n.a.
Ovary anterior length	78.7±5.5	84.8±3.3	72.3±10.8	79.2±8.1	60-89	307.8±38.0	273-353	n.a.	n.a.
Ovary posterior length	61.7±2.9	57.8±10.2	37.0±20.5	52.7±15.8	17-66	135.5±23.2	106-160	n.a.	n.a.
Anterior to Anal Orifice	448.0±14.9	485.8±24.2	473.7±17.7	470.8±24.3	437-508	746.8±48.6	675-780	348.5±31.1	326.5-370.5
Hyaline length	8.0±1.0	6.0±0.8	9.5±0.5	7.7±1.7	5-10	6.2±1.3	5-8	5.3±1.1	4.5-6.0
Spicule length	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16.0±0.7	15.5-16.5
Gubernaculum	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	9.5±0.7	9-10
Spicule/L %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.2±0.2	4.1-4.4

Measurements in µm in the form: mean ± standard deviation. OG = oesophageal gland, OIJ = oesophago-intestinal junction, DGO = dorsal gland orifice, EP = excretory pore. The explanation of the L, a, b, b', c, c', V, V', G1, G2 refer to Table 1

3.2.4. Materials Examined

Collected at Black Mountain, Australian Capital Territory (-35.267368, 149.110151); Dasyurus Picnic Area, Monga National Park, New South Wales (-35.5606 Lat, 149.9219 Lon); Lake Mournpall, Kulkyne National Park, Victoria (-34.7130, 142.3560).

3.2.5. Remarks

The discovery of swollen mature females has led to the conclusion that the specimens belonged to *Achlysiella*. The specimens were compared with the type specimens of *Achlysiella* deposited at the Queensland Museum, but the latter was very old and generally not satisfactory for close morphological observations. Therefore, the conclusion was also based on interpreting the original descriptions and illustrations of species (Colbran 1971; Sher 1968). The specimens obtained in the present study fitted the original description of the immature female of *A. magniglans* in having 4 lateral lines, in body length, morphometric indices a, b, b', c, c,' V, and rod-like sperm shape. Differences from the description of *A. magniglans* included the ranges of stylet length and the morphometric indices G_1 and G_2. However, these were not considered significant due to large overlaps between the specimens and the descriptions. The specimens examined had 2 or 3 head annules, while the original description mentioned only 2 distinct annules; therefore, the difference is considered an intraspecific variation.

4. Discussion

When Sher (1968) described *Radopholus magniglans* and *Radopholus trilineatus*, no swollen females were found. However, all the vermiform females recovered had short ovaries, which led him to suspect that there might be further development in the roots of plants. The discovery of swollen mature females of *A. williamsi* from the roots of diseased plants (Hunt *et al.* 1989) has confirmed the further development of females in the roots. The presence of swollen females was then regarded as one of the main distinguishing features of the genus *Achlysiella* (Hunt *et al.* 1989). In the present study, based on the vermiform females, the populations in NSW and the ACT were initially identified as *Radopholus trilineatus* and *Radopholus magniglans*. However, the discovery of swollen females from these populations suggested that they belong to *Achlysiella*. Therefore, the results of the present study substantiated Sher's speculation that both species should be transferred to the genus *Achlysiella* as *A. magniglans* and *A. trilineata.*

In addition to *R. magniglans* and *R. trilineatus*, Ebsary (1991) has also transferred *R. brevicaudatus, R. capitatus*, and *R. vacuus* to *Achlysiella*. Morphologically, vermiform *R. brevicaudatus, R. capitatus*, and *R. vacuus* resemble more closely vermiform *A. magniglans* and *A. trilineata* than other species of *Radopholus*, particularly in having elongated oesophageal gland lobes and immature ovaries. Due to the generic status of *A. magniglans* and *A. trilineata* having been clarified, placing the three additional species into *Achlysiella* is justified. Further sampling directed at plant roots, using more efficient extraction methods for roots, such as ultrasonic techniques (Tangchitsomkid *et al.* 2015), or investigations using culturing, may lead to the discovery of mature female forms and definitive differentiating biological data for these species.

Ryss and Wouts (1997) described a new species *Radopholus nelsonensis*, from New Zealand. Somewhat swollen females were found. However, since swelling seemed minimal and without distortion of the general shape, plus the female did not swell until egg laying began, the authors placed the new species in *Radopholus* until biological data became available. In contrast to *R. nelsonensis*, some swollen females of *A. magniglans* and *A. trilineata* have shown developing ovaries with minimally developed eggs, indicating that in these two species, swelling occurs even before egglaying begins. Therefore, the present study suggested that *R. nelsonensis* is not *Achlysiella* and that its placement in the genus *Radopholus* instead of *Achlysiella* was justified.

In conclusion, the present study verified earlier actions (Ebsary 1991) of transferring *R. trilineatus* and *R. magniglans* to the genus *Achlysiella* as *Achlysiella trilineata* (Sher, 1968) Ebsary, 1991 and *Achlysiella magniglans* (Sher, 1968) Ebsary, 1991. Furthermore, the present study also supported that *R. brevicaudatus, R. capitatus*, and *R. vacuus* should be treated as *Achlysiella brevicaudata* (Colbran, 1971) Ebsary, 1991, *Achlysiella capitata* (Colbran, 1971) Ebsary, 1991, and *Achlysiella vacua* (Colbran, 1971) Ebsary, 1991.

Acknowledgements

We would thanks to Dr. John Truemann (ANU) for the discussions and support of the project and Dr.

David Rowell (ANU) for supporting the project. We also thank Dr. Mal Bryant (Queensland Museum), Dr. Zeng Zhao (New Zealand Arthropod Collection), Dr. David Hunt (CABI UK), Rebecca Lawson (FERA UK), and Dr. Zafar Handoo (Nematology Laboratory USDA) for providing specimens for comparison. This work was financed by the Australian Agency for International Development that awarded to the first author.

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