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Morphology, Oviposition and Embryogenesis in an Australian Population of *Acrobeloides nanus*

ALAN F. BIRD,[1] PAUL DE LEY,[2] AND JEAN BIRD[3]

Abstract: A population of *Acrobeloides nanus* in Australia is described and illustrated, based on light and scanning electron microscopy. Embryogenesis from egg laying to hatching is followed over a wide range of temperatures. At 15 C, hatching occurs in about 125 hours and at 35 and 37.5 C after about 40 hours. At 40 C, egg development ceases early in cleavage. The capacity of *A. nanus* to develop over such a range of temperatures, and its anhydrobiotic capabilities, are discussed in relation to its survival and wide distribution in Australia.

Key words: Acrobeloides nanus, Cephalobidae, description, egg laying, embryogenesis, hatching, light microscopy, morphology, nematode, oviposition, scanning electron microscopy.

An Australian population of *Acrobeloides nanus* was isolated and cultured on both Gram-negative and Gram-positive bacteria (5). Under certain conditions this nematode reduced the numbers of a bacterial biocontrol agent in the soil (see note added in proof). Descriptions of *A. nanus* have already been published from material originating in Canada (1) and Sweden (7). Although *A. nanus* is widely distributed in Australian soils (9), no description has been published of any Australian population. Furthermore, we are not aware of any studies on embryogenesis at different temperatures for *A. nanus*. Accordingly, in this paper we describe the morphology of an Australian population of *A. nanus*, its egg-laying behavior, and its rates of embryogenesis and hatching at different temperatures.

Materials and Methods

Acrobeloides nanus was isolated from soil from Wagga Wagga, New South Wales, Australia and reared on *Pseudomonas corrugata* growing on plates of 4% malt extract agar (Oxoid CM 59) at 22–23 C. The nematodes were washed from these plates with sterile distilled water (SDW) and examined alive in either sealed or sitting drop slides. To study rates of hatching and embryogenesis, eggs of similar development were collected from young, gravid females, which has been transferred to a sitting drop slide. After a minimum of six eggs had been laid, the females were removed, the eggs were grouped together to facilitate viewing, and the drop was sealed with a coverslip. Observations of living material were made with a Vanox AHBT research microscope equipped with both bright field and interference contrast optics. Photographs were taken of still objects using Ilford Pan F film and of moving objects using Ilford Delta 400 film.

For morphological studies by light microscopy, nematodes were first killed and fixed in suspension by adding an equal volume of boiling double strength FA 4:1 (20 ml 40% formaldehyde and 2 ml glacial acetic acid in 78 ml SDW). Specimens then were processed to pure glycerol as described by Seinhorst (11), mounted in desiccated glycerol on slides, and sealed by molten paraffin and a coverslip (8). For scanning electron microscopy, nematodes were fixed in phosphate-buffered (pH 7.3, 0.1M) 4% paraformaldehyde at 0 C, followed by repeated washes with SDW between immersions in solutions of 1% osmium tetroxide and freshly made, filtered, saturated aqueous thiocarbohydrazide (5). Washed nematodes were dried either directly on the aluminium surface of an SEM stub or on a glass coverslip attached to an SEM stub, and then gold coated. The nematodes were examined in a Cambridge S 250 Mk 3 SEM operated at 20 kV.

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[1] CSIRO Division of Soils, PB 2, P.O. Glen Osmond, South Australia 5064, Australia.

[2] Instituut voor Dierkunde, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000, Gent, Belgium.

[3] Zoology Department, The University of Adelaide, South Australia 5005, Australia.

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Results

Description

(Note: measurements given [Table 1] are of 15 ♀♀, unless specified otherwise.)

Acrobeloides nanus (de Man, 1880) Anderson, 1968.

Body of specimens, fixed in cold 4% buffered paraformaldehyde and transferred into glycerol, straight or nearly straight. Cuticle 1 μm thick, divided into smooth annules that are 1.5–2.4 μm wide at midbody. Lateral field with five incisures from cardia to rectum, the incisures disappearing anteriorly in the neck region and posteriorly on the tail, except for the middle incisure, which continues to the tail tip (Fig. 1E,F). Lip region not or only weakly offset, 8–9 μm wide, with small slit-like amphidial apertures and 6 + 4 papillae (Figs. 1D,2B). Lips low-rounded, partly fused, the lateral lips slightly smaller than the others. Labial probolae low, blunt, and ridge-shaped, connected by tangential ridges (Fig. 2B).

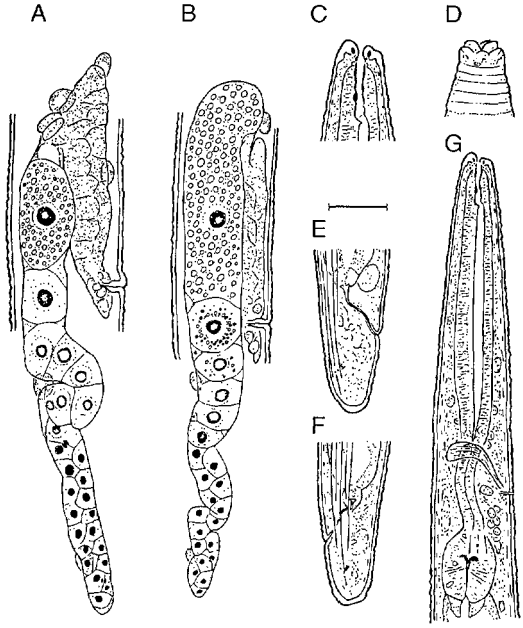

FIG. 1. A,B) Female reproductive system of *Acrobeloides nanus* with accompanying coelomocytes. C) Anterior end in median view. D) Lip region in surface view. E,F) Tails. G) Neck region. Scale bar = 10 μm for C, D and 15 μm for A, B, E, F, G.

TABLE 1. Measurements (in μm) of 15 females of *Acrobeloides nanus* fixed and mounted in glycerol.

	Mean	SD	Range	CV
Length	417	22.7	359–452	5.4
Body width	27	2.2	24–31	7.9
Pharynx length	118	3.9	110–126	3.3
Tail length	17	1.4	15–21	8.3
Anal width	14.5	0.9	13–16	6.2
a	15	1.2	14–18	7.8
b	3.5	0.17	3.3–3.9	4.7
c	25	1.6	22–28	6.4
c′	1.2	0.07	1.1–1.3	6.1
Stoma	13	0.7	12–15	5.7
Corpus	61	2.0	57–64	3.2
Isthmus	25	1.8	22–30	7.3
Bulbus	18	1.3	15–20	7.4
Nerve ring	79	3.8	71–85	4.8
Excretory pore	87	3.9	79–94	4.5
Deirid	98	4.8	89–107	4.9
n.r. (% pharynx)	67	2.2	64–71	3.3
e.p. (% pharynx)	74	2.8	69–79	3.9
deirid (% pharynx)	84	3.7	75–90	4.5
R_{ep}†	41	1.8	37–45	4.4
R_{dei}†	47	1.5	45–51	3.2
V (%)	66	1.0	65–69	1.6
G (%)	32	2.5	26–35	7.7
Rectum	16	2.3	10–19	14.8

† R_{ep} = ventral annules between lip region and excretory pore; R_{dei} = ventral annules between lip region and deirid.

Stoma cephalobid, with at least six regions; cheilorhabdia granule-shaped (Fig. 1C). Corpus fusiform, 2.4–2.9 times as long as isthmus; isthmus anterior with prominent muscle fibers. Nerve ring at anterior end of isthmus, somewhat anterior to secretory-excretory pore (Fig. 1G). Deirids lying three to eight annules posterior to secretory-excretory pore, i.e., near isthmus–bulbus junction. Cardia small-rounded, usually fixed in open condition, 4–6 μm long.

Vulva inconspicuous, located at two-thirds of body length. Vagina 8–10 μm long (n = 6), i.e., about one-third of corresponding body width. Female reproductive system cephalobid (Fig. 1A,B). Postvulval sac rudimentary, 7–12 μm long (n = 9), consisting of small cells merging with the ventral chord. Spermatheca 8–18 μm long, never with sperm. Oviduct short, with unclear number of cells, usually reflexed but occasionally outstretched along-

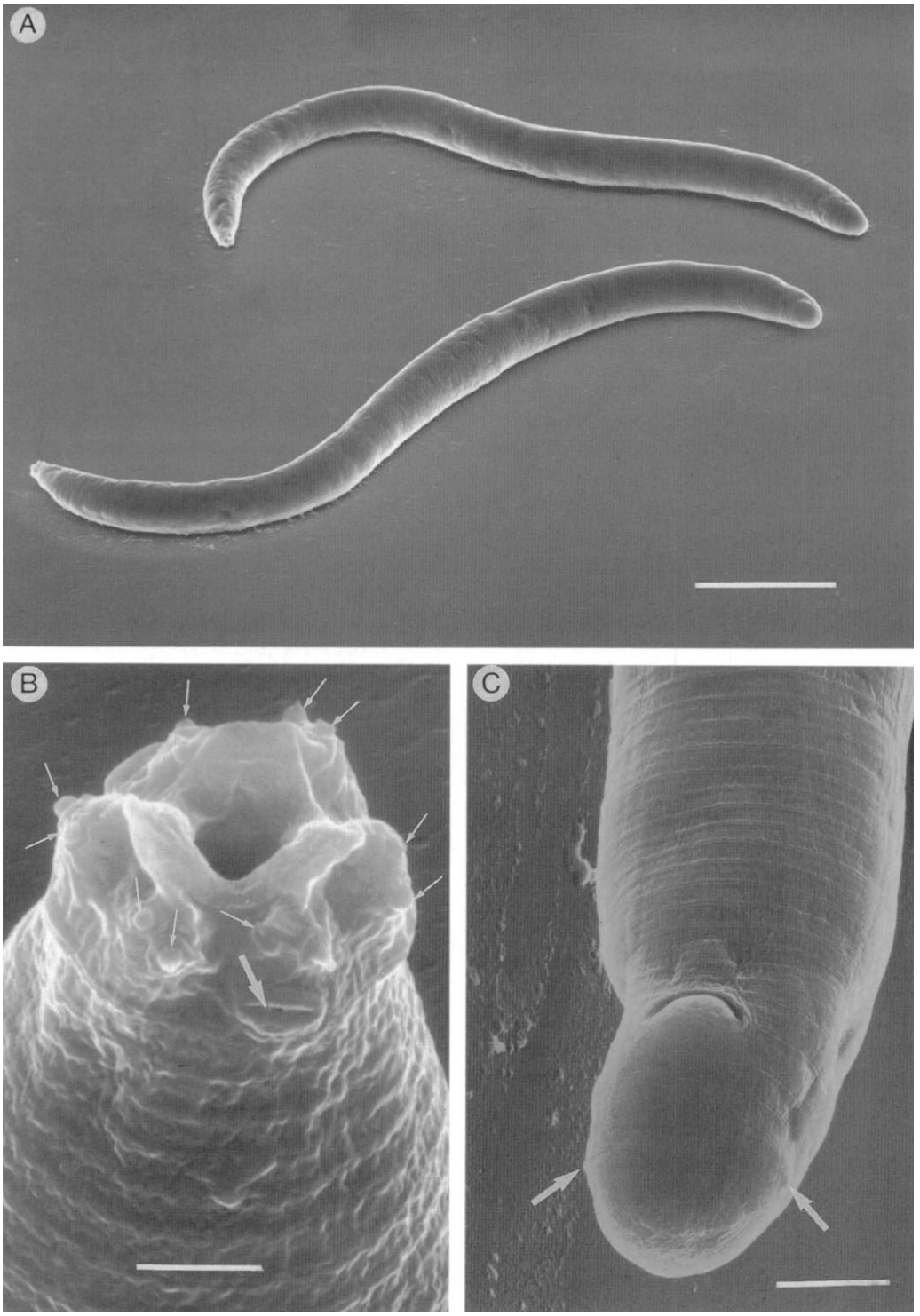

FIG. 2. Scanning electronmicrographs of *Acrobeloides nanus*. A) Two young females illustrating the characteristic slug-like shape and rounded tails. Scale bar = 50 μm. B) Head at higher magnification showing characteristic labial probolae, the six lips with cephalic probolae and labial and cephalic papillae (small arrows), and one of the two amphid apertures (large arrow). Scale bar = 2 μm. C) Tail showing the crescent-shaped anus and the two phasmids (arrows). Scale bar = 5 μm.

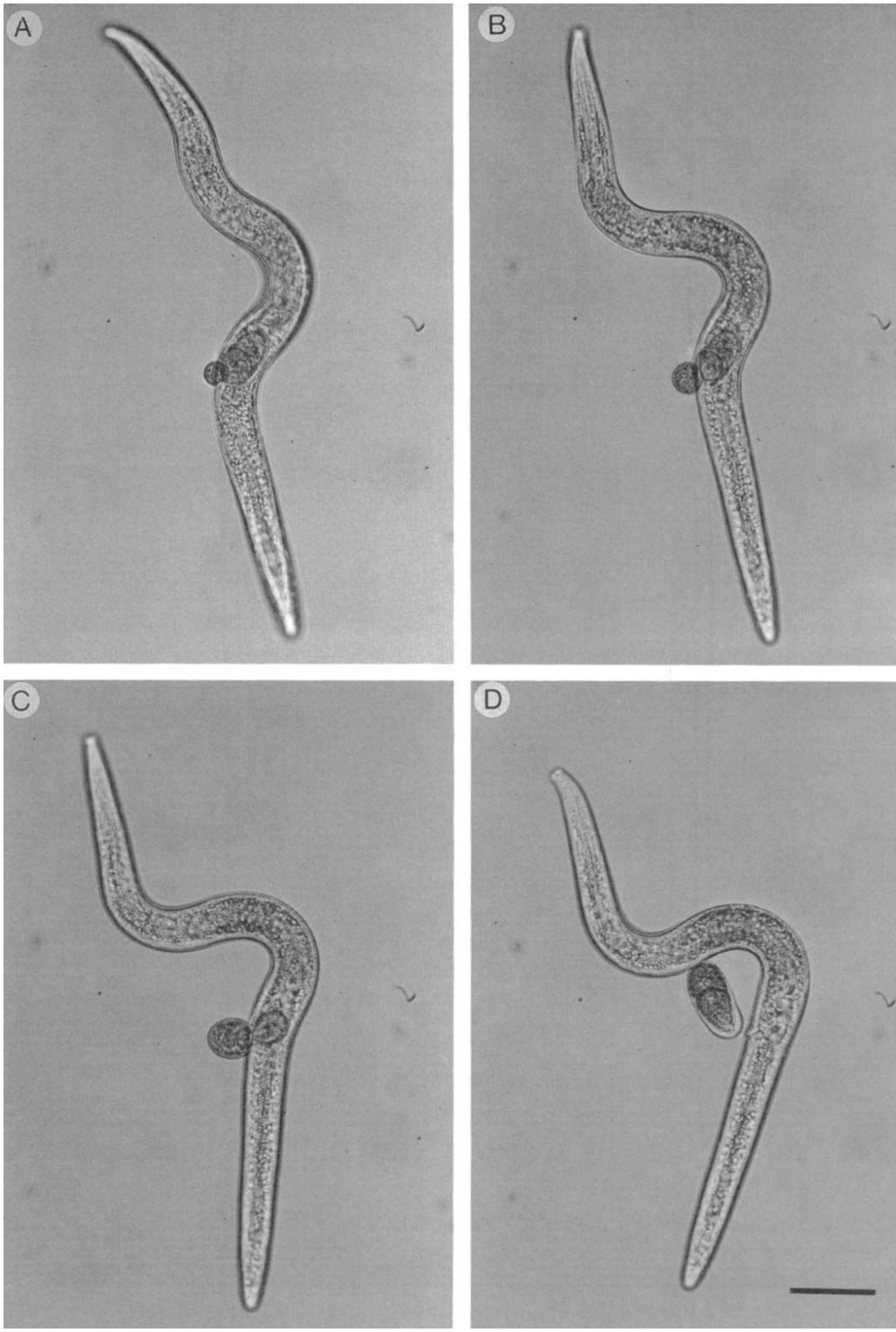

FIG. 3. Stages of egg laying in a specimen of *Acrobeloides nanus*. This process takes only a few seconds. Scale bar = 50 μm.

TABLE 2. Measurements (in μm) of living eggs of *Acrobeloides nanus*.

Number	Stage	Mean	SD	Range	CV
	Freshly laid				
28	Length	54.7	3.6	44–62	6.6
28	Width	25.4	1.5	23.3–27.9	5.9
	Late larval				
17	Length	57.3	3.4	49.6–62	5.9
17	Width	31.1	2.1	27.9–34.1	6.6

side spermatheca by the ripest oocyte in the ovary (Fig. 1B). Ovary straight or with multiple flexures, with 19–29 oocytes, of which 10–24 in double file. Two females gravid, dimensions of two eggs: 51–53 μm by 22–25 μm.

Anus crescentic (Fig. 2C). Rectum 0.7–1.3 anal body widths long. Phasmids located at 5–10 μm from anus, i.e., at 33–52% of tail length. Tail short and bluntly rounded, with four to seven annules ventrally (Fig. 2A,C).

Males not found.

Egg laying

Egg laying was observed in about 10 nematodes in sitting drop slides. The vulva becomes more conspicuous and the nematode flexes itself in a characteristic manner as egg laying takes place. From the commencement of egg emergence, the whole process takes only a few seconds (Fig. 3A–D). The eggs are usually laid when they are at the one- or two-celled stage.

Freshly laid eggs (Table 2) had mean dimensions of 55 μm by 25 μm (n = 28), compared with similar eggs that were close to hatching, which had mean dimensions of 57 μm by 31 μm (n = 17). These changes are doubtless brought about by the activity of the larva within the egg. Independent t tests were used to test differences between the mean length and mean width of eggs at different stages of development. There was a significant difference between the length of the egg in the freshly laid and larval stages ($P < 0.02$). There was also a significant difference ($P < 0.001$) between the width of egg in the freshly laid and larval stages. Clearly the stage that egg development has reached is important if measurements are made.

Embryogenesis

Embryogenesis was observed in sitting drop slides over a range of temperatures (Table 3). In all instances from 15 to 37.5 C, larvae hatched from the eggs. At 40 C, development did not proceed beyond a few cell divisions. Development at 35 and 37.5 C was similar, and it seems likely that the most rapid rate of embryogenesis would occur close to 36 C. However, only a few hours separate the rates from laying to hatching in the 30 to 37.5 C range. This population of *A. nanus* clearly develops most rapidly at high temperatures whilst retaining the ability to continue to develop and to hatch out at much lower temperatures. The various stages of development at 37.5 C are shown in three out of six eggs observed (Fig. 4A–N). At this temperature, the L_1 that hatched became immobile within 4 hours compared with L_1 hatched at 35 C and the others at lower temperatures, all of which remained mobile.

First-stage larva

The L_1 (Fig. 5) differs from the other stages in having a characteristic spike at

TABLE 3. Stages of embryogenesis in *Acrobeloides nanus* at different temperatures.

Temperature (C)	Multicell (hours)	Gastrulation (hours)	Tadpole (hours)	Elongation (hours)	Hatch (hours)
15	20	28	54	68	125
23	8	18	26	32	55
30	6	—	20	28	43
35	4	14	18	20	40
37.5	6	15	18	20	40
40	a few divisions and then no development				

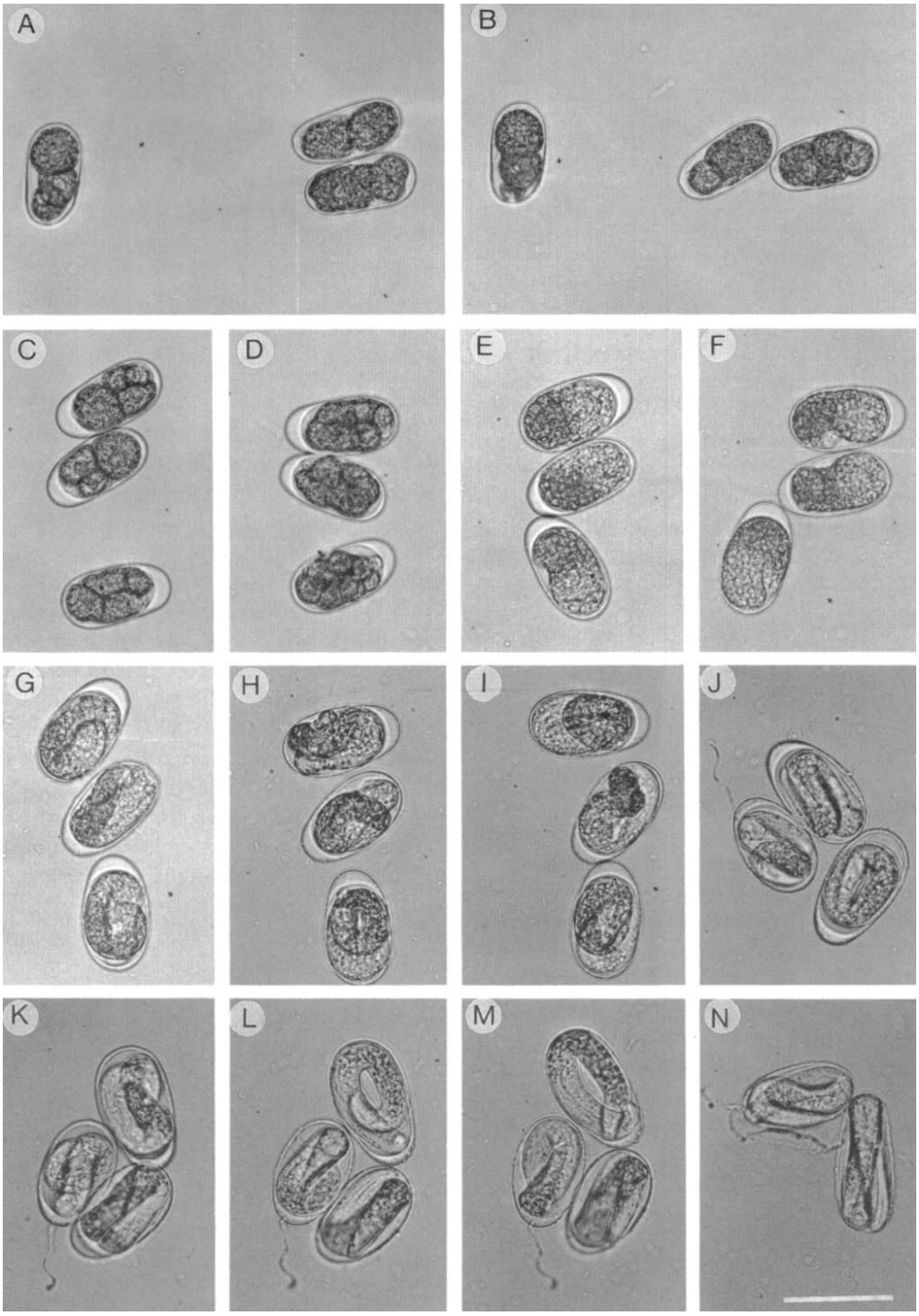

FIG. 4. Stages of embryogenesis at 37.5 C in three freshly laid eggs of *Acrobeloides nanus*. A) 1–4 cell stages a few minutes after laying. B) Eggs with several cells after 1 hour. C) After 3 hours, divisions continue. D) After 6½ hours, multicell stages. E) After 15½ hours, gastrula stage developing into tadpole showing some movement. F) After 18 hours, all in tadpole stage. G) After 20 hours, elongating. H) After 22 hours, elongated. I) After 24 hours, development within elongated larvae. J) After 27 hours, development continuing. K) After 30 hours, L_1 developing. L,M) After 33 hours, photographs taken within a few seconds of each other to illustrate degree of larval movement. N) After 40 hours, one L_1 has hatched. Scale bar = 50 μm.

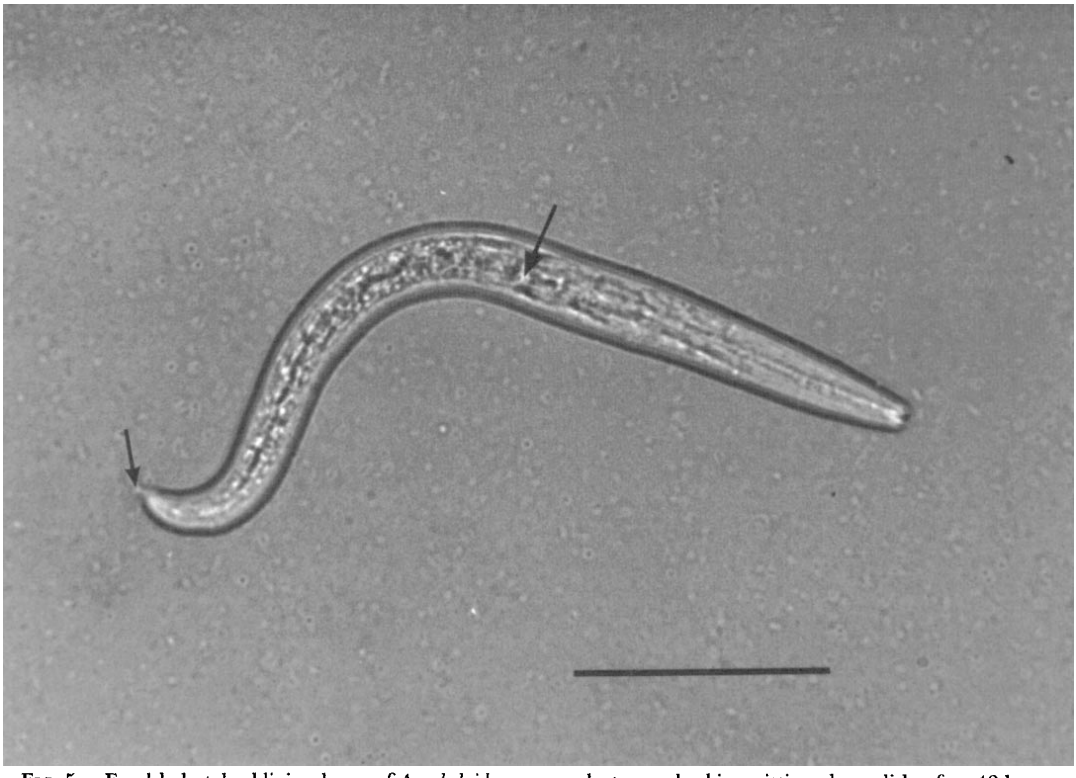

FIG. 5. Freshly hatched living larva of *Acrobeloides nanus*, photographed in a sitting-drop slide after 43 hours at 30 C and showing junction of pharynx and intestine (large arrow) and spike at end of tail (small arrow). Note that living forms are slightly longer and wider than the glycerol mounts measured in Table 4. Scale bar = 50 μm.

the tip of its tail. It measures approximately 180 μm in length and 14 μm in width (Table 4) and has proportionally a much longer pharynx compared with its total length, i.e., ratio b = 2.3 (Table 4), than in the adult, where ratio b = 3.5 (Table 1).

DISCUSSION

Identification of the Australian population of *A. nanus* is based mainly on comparison with the descriptions of that species by Anderson (1) and Boström and Gydemo (7). Our specimens agree rather well with *A. nanus* as described from Canada but tend to have somewhat shorter tails (c = 22–28 vs. 14–23 and tail length 15–21 μm vs. 14–30 μm), with slightly fewer ventral annules (4–7 vs. 5–12 counted on Figs. 3G, 4R–Z in Anderson's paper) (1). Compared with the description of *A. nanus* from Sweden by Boström and Gydemo (7),

TABLE 4. Measurements (in μm) of first-stage larvae of *Acrobeloides nanus* fixed and mounted in glycerol.

Measurement	Number	Mean	SD	Range	CV
Length	23	179.7	9.0	152–195	5.0
Body width	22	13.7	1.8	12–16	13.0
Pharynx length	20	79.8	3.8	71–84	4.8
Tail length	10	14.0	2.1	12–16	15.0
a	22	13.3	1.7	11.1–15.9	12.8
b	19	2.3	0.2	2.1–2.7	8.7
c	10	13.5	2.2	11.1–16.3	16.3

our specimens again have shorter tails (c = 22–28 vs. 13–21 and tail length 15–21 μm vs. 20–36 μm), with fewer ventral annules (4–7 vs. 8–15 counted from Fig. 1F, 2H–L in Boström's and Gydemo's paper) (7). Also, both Anderson (1) and Boström and Gydemo (7) found greater variability in numerous respects. This is to be expected, as these workers examined material cultured under different conditions as well as field populations, whereas our material was derived from a single soil source and cultured under uniform conditions. We are reasonably confident that our material is conspecific with the Canadian and Swedish populations.

Egg laying or oviposition in *A. nanus* resembles that of other nematodes that have been described (4) in that the process is rapid and the shell is compressed so that the egg emerges from the vulva as a spherical "bud," which enlarges as the egg cytoplasm streams into it; the egg immediately returns to its normal oval shape once it has been laid. In the past, this process, coupled with the movement of the nematode (Fig. 3A–D) could only be captured using ciné photomicrography. We have been able to capture this sequence using still photomicrography and modern high-speed, fine-grain black-and-white film.

During our analyses of the numerous photographs taken of eggs undergoing embryogenesis at different temperatures, we noticed that there was a significant difference in the size of freshly laid eggs compared with those that had developed to the late larval stage. We decided to see if this applied to other genera of nematodes where embryogenesis of individual eggs had been followed. Accordingly, we measured photographs of developing eggs of *Meloidogyne* (3, Fig. 2A,G) and similar photographs of *Anguina* (6, Figs. 2,8). Paired *t* tests were performed on these measurements, as each individual egg could be identified at both stages. The null hypothesis tested was that the mean of the population of differences between stages for each egg is zero. For the *Anguina* eggs, there was no significant difference between the lengths of eggs in the two stages, but there was a significant difference between the widths ($P < 0.001$). There were significant differences between stages for both length ($P < 0.01$) and width ($P < 0.01$) for the *Meloidogyne* eggs. These differences would appear to be due to a combination of larval movement and plasticity or stretching of the egg shell. It seems that because these dimensional changes occur in nematodes from widely separated taxa, they may be of universal occurrence. Accordingly, the stage of development of the egg should be taken into account if taxonomic measurements are being made.

The wide temperature range over which embryogenesis takes place in *A. nanus,* coupled with its ability to develop at relatively high temperatures and survive at high temperatures in the anhydrobiotic state, help explain its world-wide distribution, its ability to colonize newly formed volcanic islands such as Surtsey off the coast of Iceland (12) and Krakatau, Indonesia (10), and its wide distribution in Australia. In that country, it is found in arid areas 1500 Km apart (9), in various different soil types and locations in agricultural areas around Adelaide, South Australia (13), and on Kangaroo Island off the coast of South Australia. The ability of *A. nanus* to survive in a wide range of environments, coupled with its ability to consume a range of bacteria and its ease of culture, may give it potential as a biocontrol agent.

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Note Added in Proof

Information regarding reduction of bacterial numbers by *A. nanus* has been accepted for publication in December as follows: Ryder, M. H., and A. F. Bird. 1993. Effect of *Acrobeloides nanus* (Nematoda: Cephalobidae) upon the survival of *Pseudomonas corrugata* (Eubacteria) in pasteurized soil from Kapunda, South Australia. Tranactions of the Royal Society of South Australia 117, in press.