

Contribution to the knowledge of *Distolabrellus veechi* Anderson, 1983 (Nemata : Rhabditida)

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Summary — *Distolabrellus veechi* Anderson, 1983 was found associated with necrotic portions of the stems of cotton seedlings in Córdoba, Argentina. From the specimens recovered from the seedlings, two populations were developed: one on Nigon's medium and the other on *Galleria mellonella* L. larvae. The variability of morphological characters and measurements of the species was analyzed. The significance of differences between mean values of measurements of specimens developed under different conditions was studied. No significant morphological differences were observed between the type population and those obtained in Córdoba. Significant differences were detected in the latter populations in certain measurements in males and females. *D. veechi* is found for the second time. Because the species can develop on a medium that favours the development of bacteria as well as be associated with insect larvae, it may be more widely distributed than presented by these two records.

Résumé — *Contribution à la connaissance de Distolabrellus veechi* Anderson, 1983 (Nemata : Rhabditida) — *Distolabrellus veechi* Anderson, 1983 a été trouvé dans des portions nécrosées de la tige de plantules de cotonnier, à Córdoba, Argentine. A partir de spécimens isolés, deux populations ont été développées, l'une sur milieu de Nigon, l'autre en tant que parasite de larves de *Galleria mellonella* L. Une analyse de la variabilité morphologique et des mensurations de ces populations a été faite et la signification des différences entre moyennes étudiée. Les caractères morphologiques ne montrent pas de différences importantes entre les individus de la population type et ceux des populations développées à Córdoba. Entre ces dernières, des différences significatives ont été décelées dans certaines mensurations des mâles et des femelles. La capacité de cette espèce à se développer grâce à des bactéries ou à parasiter des larves d'insecte, suggère que sa répartition pourrait être très vaste.

Key-words : Nematodes, *Distolabrellus*, insect parasites.

The analysis of necrotic portions of stem of cotton seedlings (*Gossypium hirsutum* var. *Guazuncho*, INTA) developed on solid artificial agar medium, showed the presence of the species *Distolabrellus veechi* Anderson, 1983. Because this was the second time the species was found and a subsequent observation showed that this nematode could eventually parasitize insect larvae, a detailed morphological and morphometric study of populations developed under laboratory conditions was carried out.

Materials and methods

Two populations were developed from the specimens recovered from cotton seedlings. Part of the nematodes obtained were reproduced on agar medium (Nigon, 1949). The remainder was placed in contact with larvae of *Galleria mellonella* L., as a part of the laboratory routine task performed with various rhabditids. It was observed that some insect larvae died and the nematodes reproduced in the cadaver. A second population was then

developed by placing specimens in Petri dishes with three layers of humid filter paper and with larvae of *G. mellonella*. The experiments were conducted at laboratory temperature (20 °C-25 °C). Each population was replicated ten times. Subsequently, portions of Nigon's medium and dead insect larvae were removed and placed separately on a filter paper mounted on a screen (1 mm openings) in a glass filled with water. After 8 hours, representatives of all stages of growth were recovered from the water, killed and fixed in hot fixative (Netscher & Seinhorst, 1969) and processed to glycerin (Seinhorst, 1959). Observations of the labial structure were made on anterior portions of specimens mounted in glycerinated jelly (Anderson, 1958). Measurements were made with an ocular micrometer and drawings with a camera lucida. For SEM observations, fixed specimens were dehydrated in a graded series of alcoholic solutions, critical point dried from CO₂, coated with a 200 Å layer of gold. The spicules were liquid dissected in lactic acid and processed for SEM observations (Eisenback, 1985). The specimens were ex-

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amed with a Jeol SM - U 3 microscope, working at an accelerating voltage of 15 kV.

The significance of the differences between the mean values of measurements was assessed by means of non-parametric Mann - Whitney U test (Sokal & Rohlf, 1980).

***Distolabrellus veechi* Anderson, 1983**

(Figs 1-2)

MEASUREMENTS

See Tables 1 and 2.

DESCRIPTION

Female : On specimens killed by gentle heating, habitus straight or slightly ventrally curved. Body stout with anterior end truncate, posterior end conical. Cuticle finely annulated, annules about 2 μm wide at midbody with regularly spaced rows of minute pores. Lateral field delimited by two lines taking up 2-4 % of body width and with two longitudinal rows of well marked punctuations. Labial area truncate, not separated from rest of body, 11-13 μm wide, bearing six lips of two different shapes : three rounded lips alternating with three wedge shaped lips; the inner end of the latter partly overlapping oral opening. Prostoma tubular, 22-25 μm long, 2-4 μm wide with walls generally parallel, sometimes irregular contour in its distal part. Metarhabdions slightly bulboid, bearing three cuticularized denticles. Oesophageal collar evident, 8-10 μm long. Oesophagus comprised of three parts whose length varies according to the origins of the specimens. Oesophagus - intestinal valve conical - rounded. Hemizonid prominent, equivalent to two-three annules, located anterior to excretory pore. Excretory pore located close to base of isthmus, connected to two well defined renette glands in ventral position by a somewhat tortuous duct. Intestine straight, showing an internal enlargement in its anterior portion full of bacteria. Rectum visible, straight. One anterior, genital branch; ovary length variable depending on the origin of the specimens, reflexed posteriorly; oviduct tubular, straight; spermatheca distinct, elongated (irregular contour observed only in seven specimens from Nigon's medium; this structure was neither observed in the remaining specimens nor in those from *G. mellonella*). Eggs 60-70 \times 27-33 μm , present in progressive stages of development (from not segmented to post-embryonic) along the rest of the reproductive system. Vagina straight, walls of uniform thickness. Vulva often covered by a translucent mass in which sperms with a rounded or subtriangular contour can be observed. Portion between vulva and anus gradually becoming thinner towards the posterior region. Rectum well defined. Tail conical, tapering in its posterior half. Phasmids not seen.

Male : On specimens killed by gentle heating, habitus similar to that of females. Labial area same as that of females, width 10-12 μm in specimens from Nigon's

medium and 11-14 μm in specimens from *G. mellonella*. Prostoma, metarhabdions and oesophageal collar as in females. Portions of oesophagus with different measurements depending on origin of specimens. Testis reflexed; measurements similar in specimens from both cultures. Elongated *vas deferens* with two saccate cement glands in lateral position, distally attached. Spicules straight, proximal end cephalated, distal end fused in 50-60 % of its length. Gubernaculum straight, distal end attenuated, flexible. Bursa peloderan, margin slightly crenate. Nine bursal papillae arranged in a 2 () 1 + 4 + 2 series; in a few cases it is observed : 2 () 1 + 4 + 3.

Juveniles : On specimens killed by gentle heating, habitus straight. Cuticle design identical to that of adults. External morphology of anterior region similar to that of adults. Labial area 4-5 μm wide (J2), 6-8 μm (J3), 9-10 μm (J4) in specimens from both populations. The tail differs in the different stages : in J2 it is conical-elongated, in J3 conical and in J4 those of future females (gradually conical) differ from those of future males (strangulation marked at posterior half). In regard to the reproductive system the following differences are observed between stages. In J2 the genital primordium is represented by two cells, which form an oval 6-8 μm wide, 10-14 μm long. In J3 a structure composed of many cells is observed, also with oval contour, 12-15 μm wide, 30-45 μm long. In J4 there is a tubular structure well defined of variable length; position of vagina in future females and spicular primordium in future males are clearly observed.

REMARKS

In the populations analyzed, the morphological characters are much more distinct and visible in the specimens reared on Nigon's medium. There are no significant differences in morphological characteristics between the populations studied and the type population (Anderson, 1983). In the females, the postvulvar region narrows gradually *vs* a post-vulvar constriction of body. In males, the posterior end is straight *vs* strongly hamate.

observed in some characters between the populations from Córdoba and the type population. In females, there are major differences in tail length : 58-89 *vs* 76-113 μm . Slight differences are observed in relation to spicules length : 50-79 *vs* 57-73 μm . In both sexes, the position of the excretory pore also shows slight differences; in females : 140-185 *vs* 161-202 μm and in males : 137-171 *vs* 131-184 μm .

In the populations developed under two different conditions in Córdoba, significant differences in various morphometric characters are observed in males as well as in females. These differences are well marked in features of specific importance such as tail length (in females), oesophagus length (in both sexes), spicules and

Table 1. Measurements of adults of *Distolabrellus veechi* Anderson, 1983.

Characters	Females			Males				
	Córdoba's populations		Significance of (1) difference	Type population (n = 20)	Córdoba's populations		Significances of (1) difference	Type population (n = 15)
	Pop. Nigon's med. (n = 20)	Pop. from <i>Galleria</i> (n = 20)			Pop. Nigon's med. (n = 20)	Pop. from <i>Galleria</i> (n = 20)		
Body length (mm)	1.14 ± 0.06 (1.04-1.31) 6.29	1.11 ± 0.07 (0.96-1.23) 6.81	NS	1.33 (1.15-1.47) —	0.89 ± 0.04 (0.83-1.00) 5.39	0.90 ± 0.07 (0.76-1.06) 7.97	NS	0.82 (0.72-0.99) —
Body diameter	72 ± 10.32 (56-97) 14.33	64 ± 6.77 (52-79) 10.58	**	74 (64-89) —	51 ± 3.35 (46-58) 6.57	43 ± 3.41 (35-49) 7.94	***	—
a	16.21 ± 2.02 (11.96-20.08) 12.46	17.70 ± 1.25 (15.92-19.64) 7.08	*	18 (16-21) —	17.71 ± 1.02 (15.74-19.47) 5.76	21.31 ± 1.22 (19.77-23.47) 5.75	***	20 (17-21) —
Length of corpus and metacarpus	115 ± 4.50 (106-121) 3.95	123 ± 6.00 (112-133) 4.82	***	129 (119-125) —	108 ± 4.50 (98-116) 4.15	116 ± 6.00 (104-127) 4.98	***	—
Isthmus	34 ± 2.38 (29-37) 7.00	52 ± 5.37 (45-69) 10.33	***	53 (43-69) —	34 ± 2.99 (29-40) 8.82	58 ± 4.63 (42-58) 9.27	***	—
Length of valvular basal bulb	36 ± 2.59 (33-42) 7.20	33 ± 2.53 (29-37) 7.68	***	37 (31-45) —	32 ± 1.38 (29-35) 4.33	30 ± 3.22 (25-35) 10.76	**	—
Diameter of valvular basal bulb	30 ± 1.77 (27-35) 5.91	27 ± 1.42 (24-29) 5.29	***	—	24.5 ± 1.23 (22-27) 5.02	23 ± 1.66 (20-27) 7.23	**	—
Oesophagus length	187 ± 8.00 (175-200) 4.28	212 ± 9.74 (196-232) 4.59	***	220 (210-229) —	175 ± 7.61 (157-189) 4.34	198 ± 9.69 (182-221) 4.89	***	—
b	6.13 ± 0.28 (5.82-6.81) 4.56	5.28 ± 0.37 (4.61-5.91) 7.01	***	6.1 (5.2-6.9) —	5.12 ± 0.21 (4.70-5.51) 4.27	4.59 ± 0.30 (4.11-5.18) 6.72	***	4.2 (3.7-5.3) —
Tail length	75 ± 7.85 (62-89) 10.47	68 ± 5.99 (58-79) 8.81	***	101 (76-113) —	27 ± 1.86 (23-32) 6.90	28 ± 2.98 (25-33) 10.64	NS	—
c	15.46 ± 1.46 (13.24-17.85) 9.48	16.49 ± 1.65 (13.98-19.81) 10.06	NS	13 (11-21) —	33.36 ± 1.62 (31.27-36.65) 4.08	32.24 ± 2.82 (28.58-39.16) 8.74	NS	21 (18-26) —
Rectum length	33 ± 4.23 (25-40) 18.82	35 ± 4.72 (27-44) 13.51	NS	45 (39-50) —	—	—	—	—
Body diameter at anus	22 ± 2.32 (19-29) 10.57	25 ± 1.79 (21-28) 7.16	***	—	25 ± 2.78 (22-33) 11.32	26 ± 2.41 (21-30) 9.28	NS	—
c'	3.39 ± 0.49 (2.31-4.35) 14.54	2.79 ± 0.26 (2.48-3.25) 9.61	***	3.9 (2.3-4.6) —	1.07 ± 0.11 (0.88-1.31) 10.46	1.11 ± 0.10 (0.92-1.34) 9.12	NS	1.7 (1.5-2) —
V ₀	87 ± 1.50 (85-90) 1.73	87 ± 1.55 (82-89) 1.78	NS	86 (84-89) —	—	—	—	—

Table 1 (continued)

Characters	Females				Males			
	Córdoba's populations			Type population	Córdoba's populations			Type population
	Pop. Nigon's med. (n = 20)	Pop. from <i>Galleria</i> (n = 20)	Significance of (1) difference	(n = 20)	Pop. Nigon's med. (n = 20)	Pop. from <i>Galleria</i> (n = 20)	Significances of (1) difference	(n = 15)
Distance between anterior end and excretory pore	164 ± 9.06 (146-177) 5.52	166 ± 10.66 (140-185) 6.42	NS	183 (161-202)	151 ± 6.84 (140-166) 4.53	155 ± 9.11 (137-171) 5.87	NS	148 (131-184)
Distance between anterior end and nerve ring	132 ± 5.97 (123-146) 5.42	149 ± 6.69 (135-162) 4.49	***	—	123 ± 5.00 (116-131) 4.06	137 ± 5.56 (126-150) 4.06	***	—
Ovary length	579 ± 40.19 (500-665) 6.94	417 ± 59.23 (280-520) 14.20	***	—	—	—	—	—
Ovary % of body length)	51 ± 4.86 (43-60) 9.54	37 ± 5.22 (24-45) 14.13	***	60 (51-65)	—	—	—	—
Body diameter at vulva	48 ± 2.89 (40-52) 6.02	45 ± 3.70 (40-52) 8.22	**	—	—	—	—	—
Distance between vulva and anus	77 ± 6.93 (62-89) 9.00	75 ± 5.05 (67-87) 6.73	NS	—	—	—	—	—
Number of eggs	30 ± 7.26 (27-35) 33.00	11 ± 7.39 (0.00-34) 67.20	***	—	—	—	—	—
Total length of testis	—	—	—	—	650 ± 52.40 (582-811) 8.06	665 ± 78.48 (530-807) 11.80	—	—
Length of reflexed portion of testis	—	—	—	—	111 ± 14.05 (89-139) 12.66	110 ± 18.52 (87-116) 16.83	—	—
Spicules	—	—	—	—	68 ± 4.30 (60-79) 6.33	56 ± 3.75 (50-63) 6.70	***	62 (57-73)
Gubernaculum	—	—	—	—	44 ± 3.28 (39-51) 7.46	37 ± 1.95 (32-41.50) 5.27	***	38 (33-43)

(1) According U Mann-Whitney test. * P < 0.05; ** P < 0.01; *** P < 0.001.

All measurements are in µm, except body length. The disposition of the measurements corresponds to the following arrangement : arithmetic mean ± standard deviation (range) coefficient of variation.

gubernaculum length. The range of certain measurements varies according to the conditions in which the nematodes were reared although they come from the same original population. Thus, we consider — in spite of the differences observed — the population found in Córdoba a variant of *D. veechi*.

The above result shows the existence of certain variability in some morphometric characters of this species. For most measurements the coefficient of variation (CV) is lower than 10 %. A character in females — the position of the vulva in relation to the total body length — of great taxonomic importance — shows a CV less than

Table 2. Measurements of juveniles of *Distolabrellus veechi* Anderson, 1983 from Córdoba, Argentina.

Characters	J2		J3		J4			
	Pop. Nigon's med.	Pop. from <i>Galleria</i>	Pop. Nigon's med.	Pop. from <i>Galleria</i>	Pop. Nigon's med.		Pop. from <i>Galleria</i>	
	(n = 14)	(n = 15)	(n = 7)	(n = 12)	future female (n = 9)	future male (n = 9)	future female (n = 8)	future male (n = 7)
Body length (mm)	0.24 ± 0.01 (0.22-0.26) 6.25	0.32 ± 0.03 (0.27-0.37) 10.85	0.52 ± 0.01 (0.51-0.54) 2.11	0.61 ± 0.04 (0.55-0.69) 6.72	0.75 ± 0.07 (0.59-0.87) 10.26	0.67 ± 0.04 (0.63-0.76) 6.41	0.83 ± 0.06 (0.75-0.99) 7.46	0.79 ± 0.04 (0.72-0.85) 6.20
Body diameter	15 ± 1.15 (12-16) 7.67	17 ± 1.35 (15-21) 7.97	24 ± 1.51 (22-27) 6.29	29 ± 1.83 (26-32) 6.31	39 ± 3.48 (31-42) 8.92	35 ± 1.48 (33-37) 4.23	40.5 ± 3.11 (36-45) 7.69	38 ± 3.14 (33-42) 8.98
a	17.09 ± 1.72 (15.56-22.25) 10.11	18.79 ± 1.55 (16.64-20.88) 8.29	21.49 ± 1.36 (20.0-24.27) 6.35	21.65 ± 1.52 (19.40-24.88) 7.03	19.5 ± 2.45 (14.82-23.16) 12.56	19.39 ± 1.15 (18.25-21.13) 5.96	20.6 ± 0.87 (19.27-21.87) 4.23	21.12 ± 0.99 (19.47-22.84) 4.72
Oesophagus length	85 ± 3.19 (82-93) 3.76	100 ± 5.79 (89-109) 5.79	134 ± 6.14 (127-142) 4.58	142 ± 6.95 (132-157) 4.90	155 ± 8.41 (139-164) 5.42	150 ± 9.84 (143-169) 6.56	183 ± 14.20 (164-204) 7.76	183 ± 16.01 (161-200) 8.75
b	2.92 ± 0.19 (2.55-3.25) 6.78	3.25 ± 0.21 (2.59-3.49) 6.70	3.90 ± 0.17 (3.61-4.01) 4.35	4.36 ± 0.29 (3.98-4.76) 6.76	4.86 ± 0.48 (3.68-5.33) 9.97	4.54 ± 0.10 (4.40-4.74) 2.33	4.56 ± 0.14 (4.34-4.76) 3.24	4.36 ± 0.19 (4.09-4.68) 4.35
Tail length	36 ± 4.13 (30-42) 11.47	45 ± 3.69 (40.53-53) 8.20	62 ± 7.89 (51-73) 12.72	71 ± 4.50 (65-76) 6.34	80 ± 9.59 (62-92) 11.99	52 ± 4.13 (46-59) 7.95	64.5 ± 7.34 (50-73) 11.39	62 ± 4.38 (57-67) 7.07
c	7.05 ± 0.63 (6.05-8.10) 8.93	7.24 ± 0.57 (6.07-8.66) 7.87	8.61 ± 1.18 (7.31-10.38) 13.73	8.76 ± 0.57 (8.28-9.97) 6.58	9.56 ± 1.27 (7.14-10.91) 13.36	13.17 ± 1.20 (11.60-14.66) 9.14	13.02 ± 0.98 (11.86-15.0) 7.56	12.78 ± 0.63 (11.73-13.86) 0.04
Rectum length	8 ± 0.70 (7-9) 8.83	11 ± 1.32 (8-13) 12.04	16 ± 1.70 (14-18) 10.66	18 ± 2.61 (17-23) 14.55	23 ± 1.66 (21-25) 7.24	27 ± 2.94 (23-31) 10.9	29 ± 2.37 (27-32) 8.17	—
Body diameter at anus	9 ± 0.60 (8-10) 6.74	10 ± 1.06 (9-13) 10.65	14 ± 1.49 (11-16) 10.68	17 ± 0.73 (16-19) 4.33	20 ± 1.26 (17-21) 6.34	23 ± 0.92 (21-24) 4.02	19 ± 2.91 (16-23) 15.34	25 ± 2.0 (22-27) 8.0
c'	3.99 ± 0.49 (3.33-4.87) 12.38	4.40 ± 0.40 (3.23-4.88) 9.15	4.57 ± 0.29 (3.25-4.76) 23.58	4.12 ± 0.29 (3.82-4.41) 7.03	3.97 ± 0.53 (3.10-4.64) 13.40	2.27 ± 0.18 (2.0-2.56) 8.06	3.28 ± 0.45 (2.94-4.06) 13.96	2.49 ± 0.19 (2.18-2.81) 7.83
Distance between anterior end and excretory pore	63 ± 4.29 (57-68) 6.80	78.5 ± 6.61 (65-89) 8.42	103 ± 4.50 (97-108) 4.37	112 ± 5.93 (104-118) 5.24	131 ± 6.88 (118-139) 5.25	122 ± 6.0 (114-129) 4.92	146.5 ± 11.42 (131-158) 7.79	146 ± 8.79 (130-151) 6.02
Distance between anterior end and nerve ring	55 ± 2.34 (51-59) 4.25	69 ± 4.51 (57-75) 6.54	89 ± 2.63 (85-92) 2.96	97 ± 4.04 (92-106) 4.17	106 ± 6.48 (97-116) 6.12	101 ± 6.46 (93-110) 6.39	129 ± 12.91 (112-147) 10.01	122 ± 7.32 (116-130) 6.0
Position of vulvar region (in °)	—	—	—	—	80 ± 2.18 (75-82) 2.73	—	83 ± 1.40 (81-85) 0.01	—
Distance between vulvar region and anus	—	—	—	—	66 ± 4.0 (62-71) 6.06	—	67 ± 5.64 (58-74) 8.42	—

All measurements are in µm, except Body length. The disposition of the measurements corresponds to the following arrangement : arithmetic mean ± standard deviation (range) coefficient of variation.

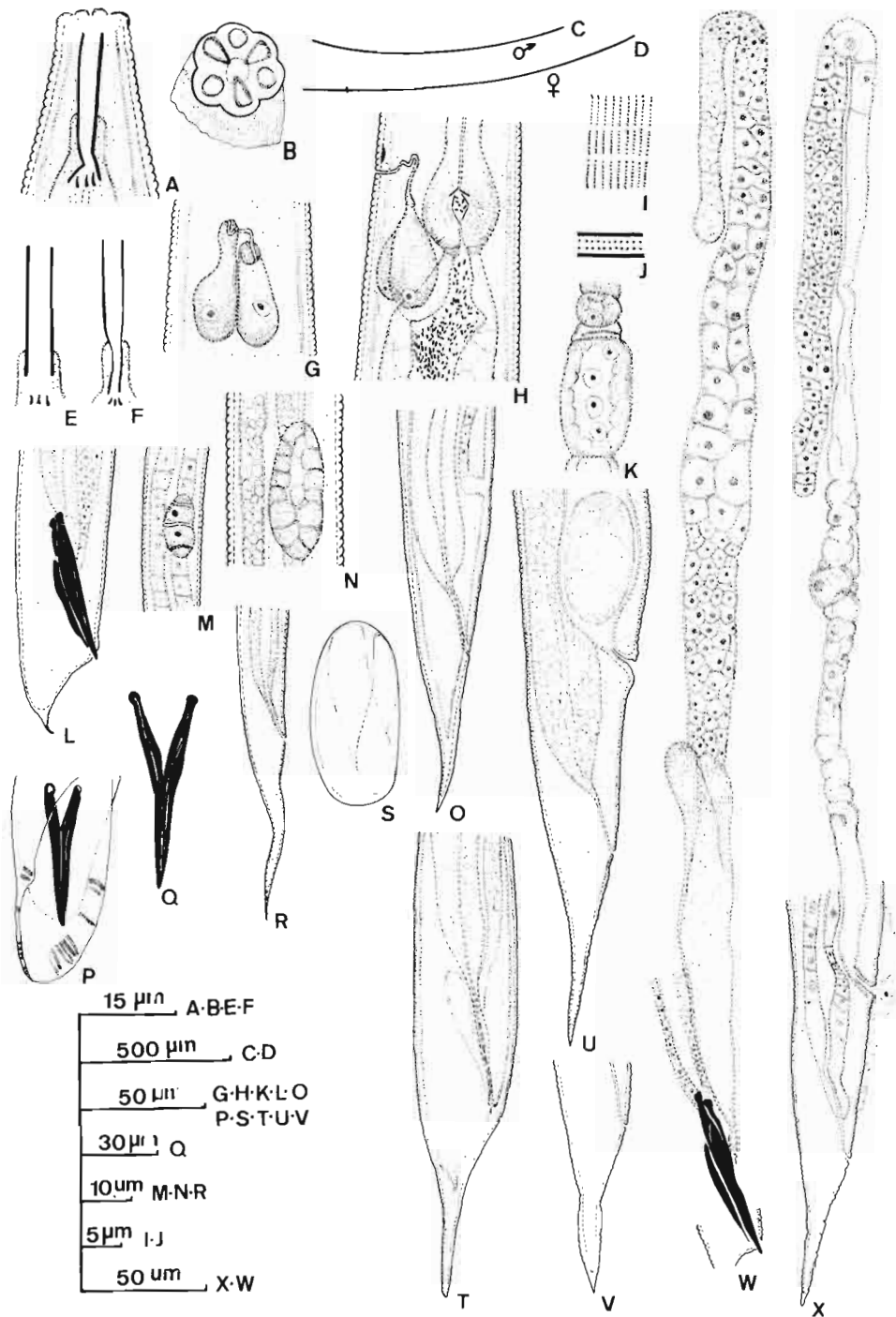


Fig. 1. *Distolabrellus veechi* Anderson, 1983 from Córdoba, Argentina. — Female. A : Anterior region; B : Lip region (en face view); D : Habitus; E, F : Prostoma and beginning of oesophagus; G : Renette glands (ventral view); H : Oesophago-intestinal region; I : Punctuations of the cuticle; J : Lateral field; K : Spermatheca; S : Egg; U : Posterior region (lateral view); V : Tail; X : Genital branch. — Male. C : Habitus; L : Posterior region (lateral view); P : Posterior region (ventro-lateral view); Q : Spicules (ventral view); W : Genital branch. — Juveniles. M : Genital primordium of J2; N : Genital primordium of J3; O : J4 future female (posterior region); R : J2 (posterior region); T : J4 future male (posterior region).

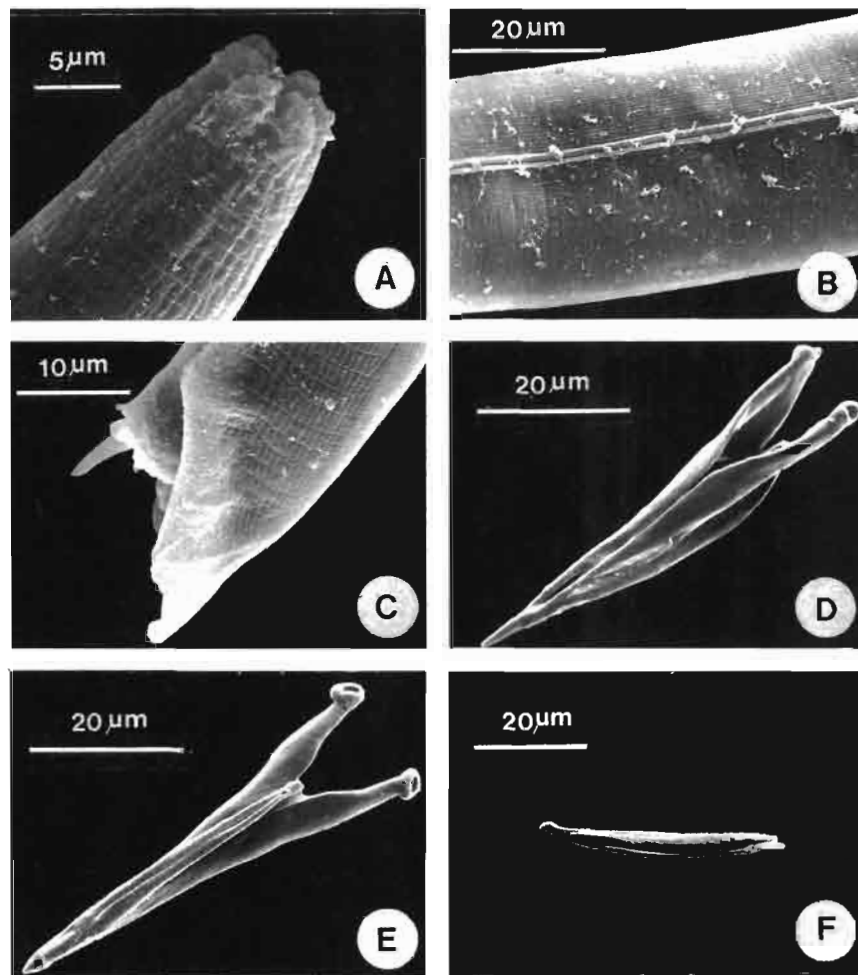


Fig. 2. *Distolabrellus veechi* Anderson, 1983 from Córdoba, Argentina. (SEM pictures). — Female. A : Anterior region (lateral view); B : Cuticle (lateral field). — Male. C : Tail (lateral view); D : Spicules (latero-ventral view); E : Spicules (dorsal view); F : Gubernaculum.

2 %. This confirms the reliability of ratio V in comparison with other ratios whose significance may be doubtful (Geraert, 1983).

The differences between measurements of specimens of the same species from different ecological conditions or of the same population developed in different mediums have already been pinpointed for some rhabditids (Kosfyowska & Mianowska, 1971; Mianowska, 1977; Boström & Gydemo, 1983). In regard to the latter, it is necessary to remark that total body length showed significant differences in the species *Chiloplacus minimus* (Thorne, 1925) Andrassy, 1959 when reared in soil and agar (Boström, 1985). However, no major differences were detected for this character in this study.

The variability among rhabditids seems to be important, to the extreme of generating confusion between

species of the same genus or showing a marked overlap between species belonging to different genera (Anderson & Hooper, 1970, 1971; Boström, 1989).

D. veechi appears as a bacteriophagous free-living nematode which also could parasitize insects. This characteristic has been reported for other rhabditids which although found on or within insects (as ecto-or endoparasites), can develop on an artificial medium without the need of a host (Ali *et al.*, 1972; Boström, 1989; Geraert *et al.*, 1990). Furthermore, the reproductive potential of *D. veechi* on Nigon's medium is greater than on *G. mellonella*.

Because the species can develop well in a nutritious medium for bacteria and also be associated to insect larvae it may have a wide world distribution.

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