

# Mononchid nematodes from Spain. *Iotonchus parageminus* sp. n. and its relationships with *I. geminus* Heyns & Lagerwey, 1965 and *I. rinae* Coetzee, 1967

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**Summary** – *Iotonchus parageminus* sp. n. is described and illustrated with specimens found in an uncultivated area in southern Spain. It is characterized by having a medium size (1.72-2.43 mm), slender body ( $a = 35-53$ ), lip region offset by a constriction, buccal cavity  $18-27 \times 27-40 \mu\text{m}$ , dorsal tooth with apex situated at 25-39 % from base of buccal cavity,  $V = 63-70\%$ , muscles between *vas deferens* and *ductus ejaculatorius* taking the form of outstretched circular band, male ventral body region not contracted at beginning the supplement series, ejaculatory glands in tandem, spicules  $53-67 \mu\text{m}$ , lateral guiding pieces with chelate-forked extremity, 9-13 ventromedian supplements, tail conical with rounded terminus and spinneret opening subterminally. Univariate and principal component analyses based on female and male morphometric characters are used to test the differences that explain the separation of *I. parageminus* sp. n. from most related species.

**Résumé** – *Nématodes Mononchides d'Espagne. Iotonchus parageminus* sp. n. et ses relations avec *I. geminus* Heyns & Lagerwey, 1965 et *I. rinae* Coetzee, 1967 – *Iotonchus parageminus* sp. n. est décrit et illustré à partir de spécimens provenant de zones non cultivées du sud de l'Espagne. Il est caractérisé par : une taille moyenne (1,73-2,43 mm), un corps mince ( $a = 35-53$ ), une région labiale séparée par une constriction, une cavité buccale mesurant  $18-27 \times 27-40 \mu\text{m}$ , une dent dorsale dont l'apex est situé à 25-39 % de la base de la cavité buccale,  $V = 63-70$ , muscles situés entre le *vas deferens* et le *ductus ejaculatorius* ayant l'aspect d'une large bande circulaire, la région ventrale du mâle non contractée au niveau du commencement de la ligne des suppléments, des glandes éjaculatoires disposées en tandem, des spicules longs de  $53-67 \mu\text{m}$ , des pièces-guides à extrémités fourchues, 9 à 13 suppléments ventro-médians, une queue conique à extrémité arrondie et filières à ouverture subterminale. Des analyses univariées et en composantes principales basées sur les données morphométriques des femelles et des mâles ont été utilisées pour tester les différences expliquant la séparation de *I. parageminus* sp. n. d'avec les espèces les plus proches.

**Key-words** : Taxonomy, description, *Iotonchus parageminus* sp. n., Nematoda, Spain.

The genus *Iotonchus* Cobb, 1916 contains few species from Europe in contrast to the great number of them, about fifty, recorded in the rest of the world. However, it is suspected that other rare and undescribed species exist in that continent. Thus, recently two new species were reported from Romania (Popovici, 1990) and Spain (Peña Santiago & Jiménez Guirado, 1991), respectively. In addition, this paper deals with *I. parageminus* sp. n., recently collected in southern Spain, which is described and illustrated. Its morphometric differentiation from related species is analyzed.

The nematodes were extracted by the Flegg's method, killed by heat, fixed in F.G. 4 : 1, processed in hot lactophenol and mounted in pure glycerin by the method of Siddiqi (1964).

Material of the following related species was examined : *I. geminus* Heyns & Lagerwey, 1965 (four female and four male paratypes from the Nematode Collection of the Plant Protection Research Institute, Pretoria,

South Africa, slides n° 3164 and 3165; one female and two males from Argentina lent by Dr. E. Chaves) and *I. rinae* Coetzee, 1967 (female holotype and five female paratypes from the Nematode Collection of the Plant Protection Research Institute, Pretoria, South Africa, slides n° 4937-4940).

## *Iotonchus parageminus* sp. n. (Figs 1, 2 & 3)

### MEASUREMENTS

See Table 1.

### DESCRIPTION

*Female* : Slender nematodes of medium size, about 2 mm long. Body cylindrical, truncate anteriorly and tapering clearly toward posterior end. Habitus ventrally curved when fixed and relaxed, frequently G to J-shaped. Cuticle with fine transverse striations generally obscure except on the anterior and caudal regions. Lat-

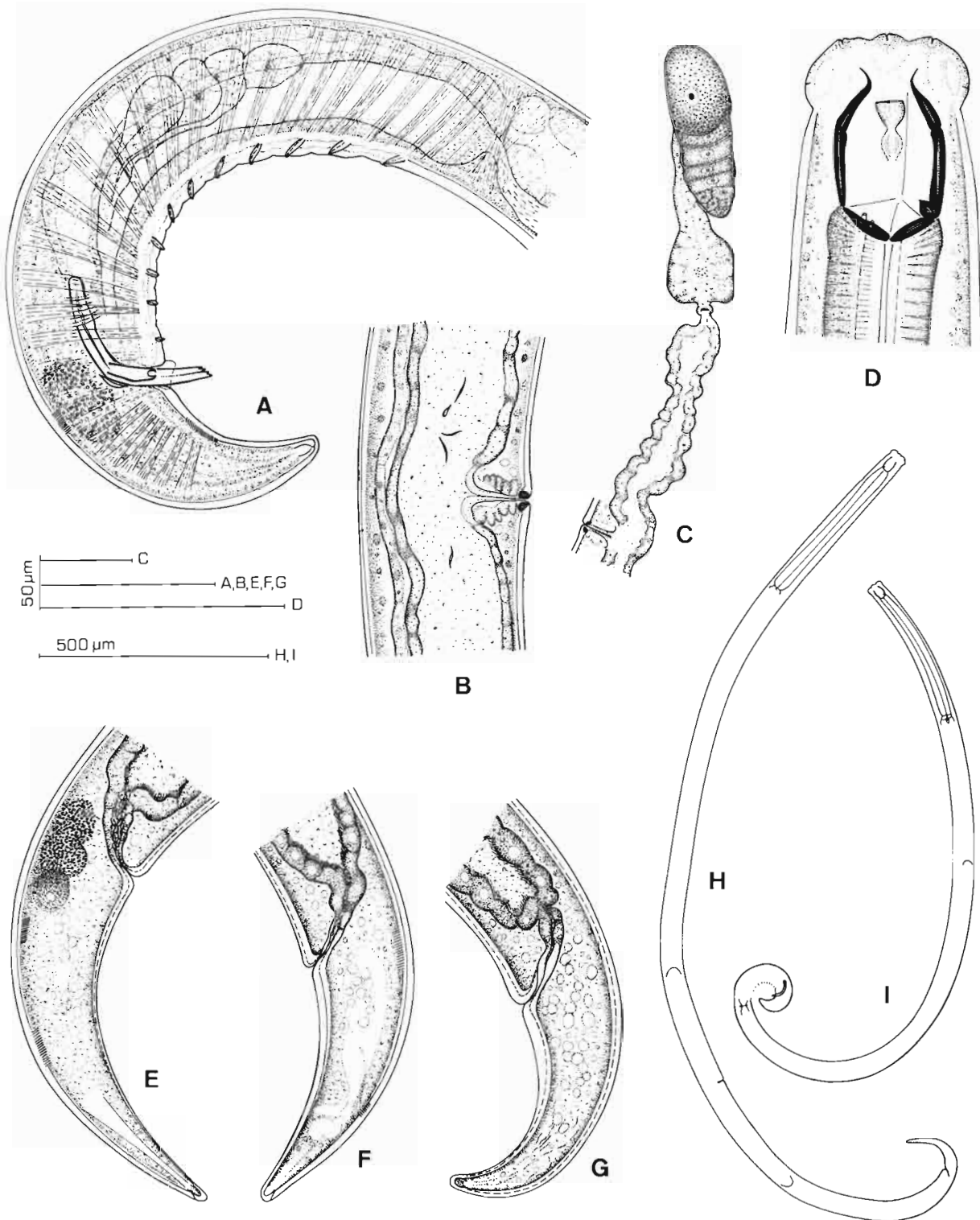
**Table 1.** Morphometric data of *Iotonchus parageminus* sp. n. (all measurements in  $\mu\text{m}$ , except L in mm).

	Holotype	Paratypes	
		Females	Males
n		30	20
L	2.09	2.12 $\pm$ 0.17 (1.79-2.43)	1.91 $\pm$ 0.13 (1.72-2.17)
a	47.9	45.7 $\pm$ 3.5 (39.2-52.6)	44.7 $\pm$ 4.5 (35.5-52.5)
b	5.6	5.7 $\pm$ 0.3 (5.1-6.2)	5.6 $\pm$ 0.2 (5.1-6.1)
c	20.	21 $\pm$ 2 (17-24.9)	28.2 $\pm$ 2.2 (24.4-32.3)
c'	3.7	3.3 $\pm$ 0.4 (2.5-4.3)	2 $\pm$ 0.1 (1.6-2.2)
V/T	68.8	66.3 $\pm$ 1.7 (63-69.3)	42.9 $\pm$ 2.7 (39-50)
G <sub>1</sub>	13	13.2 $\pm$ 1.8 (10.5-20.6)	-
G <sub>2</sub>	12.2	12.6 $\pm$ 1.4 (10.5-15.7)	-
Cuticle head	2.5	2 $\pm$ 0.5 (1.5-3)	2 $\pm$ 0.6 (1.5-3.5)
Cuticle midbody	1.5	2.5 $\pm$ 0.5 (1.5-3.5)	2.2 $\pm$ 0.5 (1.5-3)
Cuticle tail	3	3.1 $\pm$ 0.8 (1.5-5)	3 $\pm$ 0.5 (2-4)
Lateral chord	15.5	16.3 $\pm$ 3 (10-22)	15.8 $\pm$ 2 (11-20)
Lip region width	35.5	35.7 $\pm$ 1.8 (32.5-39)	32.2 $\pm$ 1.5 (30-34.5)
Lip region height	11.5	12.4 $\pm$ 0.9 (10.7-14.1)	11.4 $\pm$ 1.2 (10-14.5)
Amphid	6.5	6 $\pm$ 0.3 (5.5-6.5)	5 $\pm$ 0.6 (4.5-6.5)
Bucc. cav. length	34	35 $\pm$ 2.3 (29-40)	30.5 $\pm$ 1.7 (27-34)
Bucc. cav. width	20	23 $\pm$ 1.2 (20-27)	19.5 $\pm$ 0.9 (18.5-21.5)
Ant. end to n. ring	136	136.4 $\pm$ 16 (100-166)	130 $\pm$ 13.5 (105-158)
Ant. end to excr. p.	145	151.2 $\pm$ 14.3 (114-175)	145.6 $\pm$ 14.5 (121.5-172.5)
Pharynx length	335	332.6 $\pm$ 22.8 (268-369)	307.7 $\pm$ 19.8 (274-339)
Vagina length	20	17.6 $\pm$ 1.9 (13.5-22)	-
Tail	105	101.5 $\pm$ 13 (78-132)	68 $\pm$ 5.5 (61.5-84.5)
Spicules	-	-	59.1 $\pm$ 3.4 (53.5-67)
Gubernaculum	-	-	12.1 $\pm$ 1.2 (10.5-14)
Lat. guiding pieces	-	-	11.5 $\pm$ 1.2 (10-14)
Supplements	-	-	10.8 $\pm$ 1 (9-13)
Sperm	-	-	7.1 $\pm$ 0.8 (5.5-9)
Egg length	-	112.2 $\pm$ 8.1* (105-123)	-
Egg width	-	41.5 $\pm$ 1.6* (40-44)	-

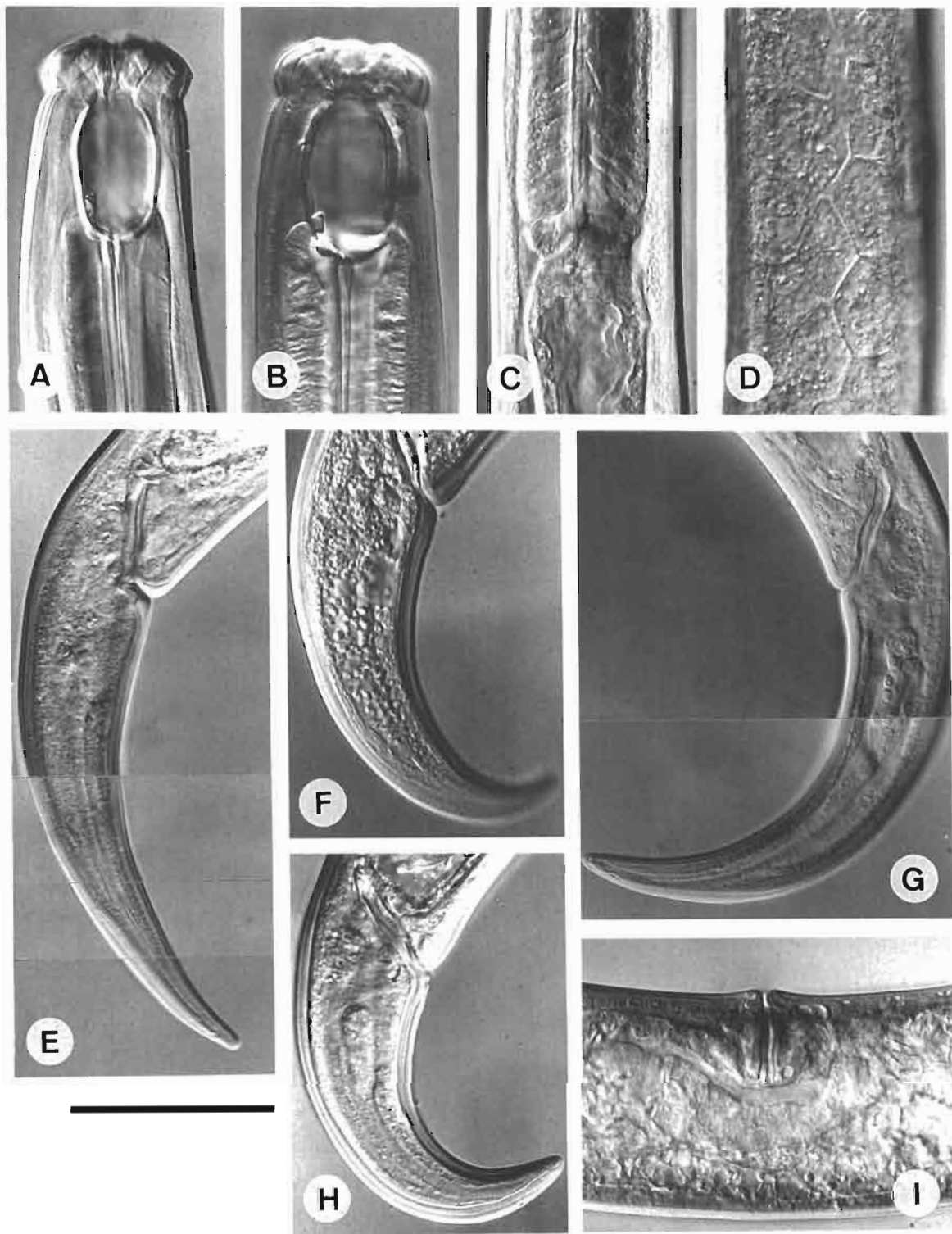
\* n = 3

eral chord occupying  $36 \pm 7\%$  (23-48) of midbody width. Lip region set off by a strong constriction,  $2.9 \pm 0.23$  times (2.5-3.3) as wide as high. Lips moderately separated and rounded. Labial and cephalic papillae prominent and interfering with the head contour. Amphids cup-shaped, just at level of the labial constriction; their opening occupying  $16 \pm 1\%$  (14-19) of the head width. Buccal cavity subrectangular, flattened at base, with relatively thick walls and  $1.53 \pm 0.12$  times (1.25-1.75) as long as wide. Dorsal tooth near basal, forward directed, moderately sized and its apex located at  $31 \pm 3.7\%$  (25-37) of the stoma length measured from base. Ventrosublateral foramina visible in the basal plates. Pharynx cylindrical, muscular and surrounding the basal part of the buccal cavity. Nerve ring located at  $36.7 \pm 3.0\%$  (28-41) of the neck region measured from the anterior body end. Excretory pore small and sometimes poorly visible. Pharynx-intestine junction tuberculate with tubercles relatively prominent and conical organ generally rounded. Intestine with six to eight granular polygonal cells in transverse section. Bacillary layer present, especially visible at the anterior and posterior regions. Genital system didelphic-amphidelphic. Ovary short, not generally reaching the oviduct-uterus junction. Oocytes few in number. Oviduct consisting of a slender region and a developed *pars dilatata*. Sphincter present at the oviduct-uterus junction with an inner moderately sclerotized part surrounded by a muscle. Uterus relatively developed, not especially elongated and without special differentiations. Vagina cylindrical, extending inwards  $37.5 \pm 5.3\%$  (27-52) of the corresponding body width. Small to medium sized sclerotized pieces in the vagina-vulva junction. Vulva a short transverse slit. Vulval papillae absent. Intra-uterine eggs elliptical, about 2.5-3 times as long as wide. Tail conical, ventrally curved and with rounded terminus; frequently it is elongated and with more narrow tip. Caudal glands and duct present but poorly visible or indistinct in some specimens. Spinneret opens subterminally, more clear dorsally in the elongate tails; in specimens with shorter tail it is nearly terminal. Caudal pores obscure.

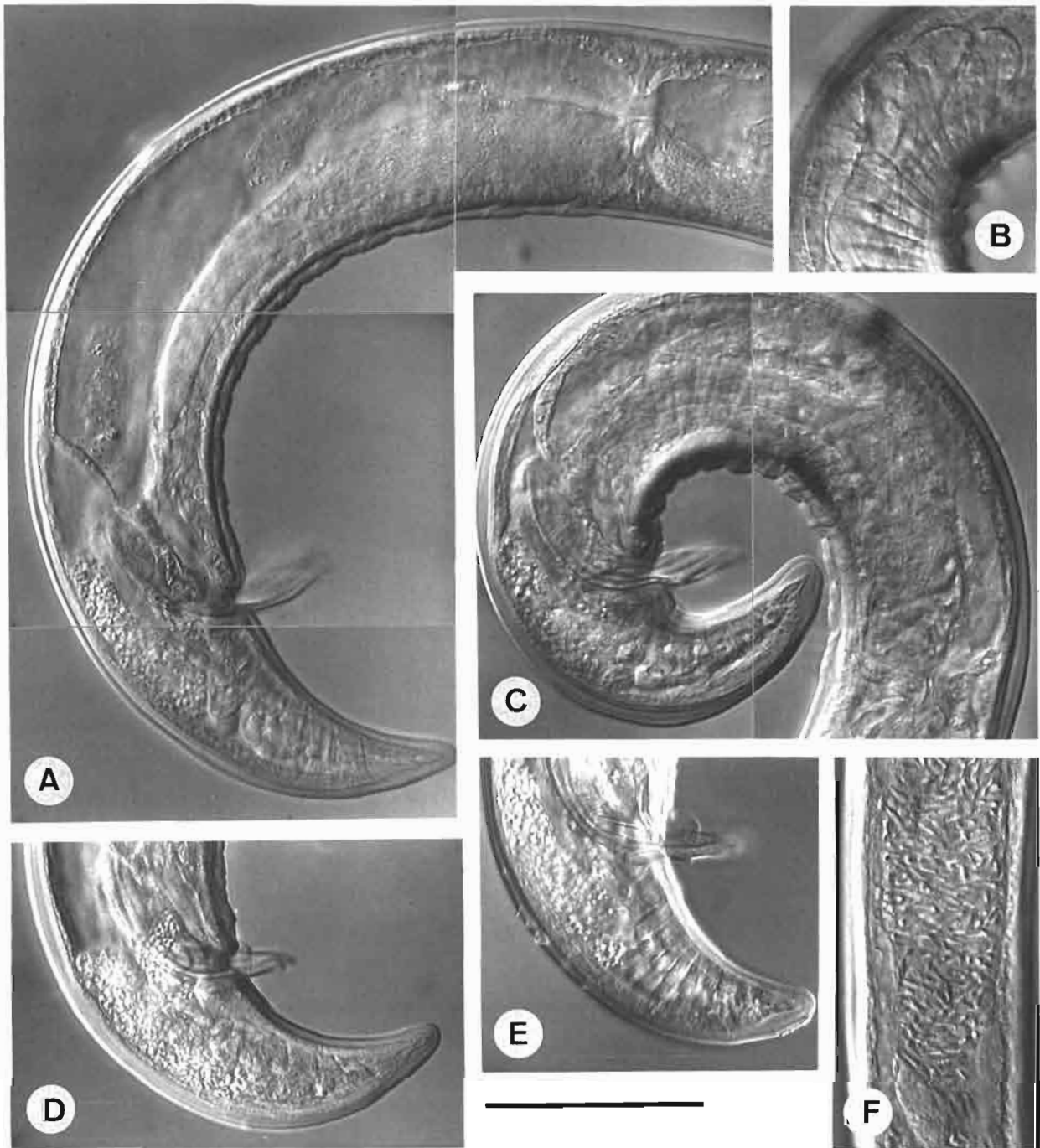
*Male*: General morphology similar to female but the body is slightly shorter and the posterior region more ventrally curved. Genital system diorchid. Testes opposed with spindle-shaped to cylindrical spermatozoa. *Vas deferens* and *ductus ejaculatorius* separated by a well developed constriction with associated muscles which form an outstretched circular band located just in front of the supplement series. The ventral body contour is not contracted in this body region. Ejaculatory glands in tandem, generally distinct; the rectal glands are poorly visible. Nine to thirteen similar ventromedian supplements present more or less regularly spaced. Spicules moderately slender, ventrally curved,  $1.7 \pm 0.15$  times (1.5-2) as long as anal body width, measured along axis. Gubernaculum moderately developed and lateral guiding pieces with chelate-forked extremity. Tail like female



**Fig. 1.** *Iotonchus parageminus* sp. n. A : Male posterior region; B : Vulval region; C : Female genital branch; D : Head; E-G : Female tail; H, I : Body habitus.



**Fig. 2.** *Iotonchus parageminus* sp. n. A, B: Head; C: Pharynx-intestine junction; D: Intestinal cells; E-H: Female tail; I: Vulval region. (Scale bar = 50  $\mu$ m).



**Fig. 3.** *Iotonchus parageminus* sp. n. *A, C* : Male posterior region; *B* : Ejaculatory glands; *D, E* : Male tail; *F* : Sperm. (Scale bar = 50  $\mu$ m).

**Table 2.** Morphometric data on remeasured specimens of *I. geminus* and *I. rinae* (all measurements in  $\mu\text{m}$ , except *L* in mm).

	<i>I. geminus</i>				<i>I. rinae</i>
	Pop. Argentina		Pop. South Africa		Pop. South Africa
	females	males	females	males	females
n	1	2	2	4	4
L	1.75	1.96, 1.72	2.03, 1.98	1.83 (1.69-1.89)	1.80 (1.72-1.94)
a	33.5	44.1, 32.9	33.2, 32.2	30.9 (27.6-32.8)	33.6 (30.6-35.8)
b	4.5	4.5, 4.4	4.3	4.4 (4-4.5)	4.70 (4.5-4.9)
c	15.2	20.7, 19.4	13.2	16.6 (16-17)	14.7 (14-16.5)
c'	3.4	2.0, 2.2	3.4, 3	2.2 (2-2.4)	3.8 (3.5-4)
V/T	64.7	39.9, 40.5	65.1, 67	45.7 (42.5-50)	69.4 (68.3-70.7)
G <sub>1</sub>	11.8	–	14.2, 16.5	–	11.5 (9.5-14)
G <sub>2</sub>	9.8	–	14.6, 13.6	–	9.7 (9-10.3)
Cuticle head	2	2.5	2.5, 2	2.4 (2-2.5)	1.7 (1.5-2)
Cuticle midbody	2.5	3.5, 2.0	2.5, 2	2.5 (2-3.5)	2.4 (2-2.5)
Cuticle tail	6	3.5, 2.5	3, 4	4 (3-5.5)	2.9 (2.5-3.5)
Lat. chord	21.5	21, 23	23	20.2 (14-27)	19.4 (17.5-20.5)
Lip region width	38.	38, 35	47, 45	39.7 (35.5-43)	33.7 (32.5-35.5)
Lip region height	14	14.5	16, 15	14 (12.5-16.5)	10.3 (9.5-11)
Amphid	–	7, 8	7, 6.5	6.3 (5.5-7.5)	5.1 (5-5.5)
Bucc. cav. length	38	41, 34	45, 44	41.1 (39-43)	34.1 (33.5-35.5)
Bucc. cav. width	25	23, 22	31, 32	26.5 (25.5-27.5)	24.3 (23.5-25)
Dorsal tooth apex	26	28, 27.6	25, 29	27.1 (25.2-30.4)	36.3 (33.1-39.8)
Ant. end to n. ring	163	163, 135	167, 163	137 (132-141)	130 (120-135.5)
Ant. end to excr. p.	172	185, 155	193, 189	160 (158-162)	144.6 (140.5-150.5)
Pharynx length	350	399, 348	418, 417	370 (363-376)	344 (331-355)
Vagina length	20.5	–	21.5, 28	–	17.6 (17-18.5)
Tail	115	94, 89	154, 150	110 (105-116)	122 (117-129)
Spicules	–	79, 74.5–	–	87.4 (82.5-94)	–
Gubernaculum	–	24, 19	–	25.5 (23-27)	–
Lat. guiding pieces	–	13, 11	–	16.9 (14-18)	–
Supplements	–	12	–	14	–
Sperm	–	9, 8	–	6.1 (5.6-6.5)	–

but shorter and with more rounded terminus. Caudal glands apparently reduced and spinneret opens subterminally or terminally. Caudal pores not clearly visible.

#### TYPE HABITAT AND LOCALITY

Soil on granitic rocks with unidentified grasses in Jándula riverside, Andújar, province of Jaén, Spain. Soil data: pH 6.4, organic matter 1.4%, C/N 9.9, sand 58.8%, silt 30.5% and clay 10.7%.

#### TYPE MATERIAL

*Holotype*, twenty-three female and fourteen male *paratypes* in collection of the Departamento de Biología Animal, Universidad de Córdoba, Spain. Two female and two male *paratypes* in the following collections: Departamento de Biología Animal, Universidad de Granada, Spain; Laboratoire de Biologie Parasitaire, Muséum National d'Histoire Naturelle, Paris, France and Instituut voor Dierkunde, Rijksuniversiteit Gent, Belgium.

#### DIAGNOSIS AND RELATIONSHIPS

*I. parageminus* sp. n. is characterized by medium size (body length 1.72-2.43 mm) and slender body ( $a = 35-53$ ), lip region offset by a strong constriction, buccal cavity  $18-27 \times 27-40 \mu\text{m}$  or 1.2-1.8 times as long as wide, dorsal tooth basal in the corresponding vertical plate with apex at 25-39% of the total buccal cavity length from base,  $V = 63-70\%$ , muscles between *vas deferens* and *ductus ejaculatorius* forming an outstretched circular band, ventral body contour not contracted at beginning of supplement series, ejaculatory glands in tandem, spicules  $53-67 \mu\text{m}$ , chelate-forked end in lateral guiding pieces, 9-13 ventromedian supplements, conical ventrally curved tail with rounded terminus and caudal glands present with spinneret opening subterminally.

*I. parageminus* sp. n. is a didelphic species most closely related to *I. geminus* Heyns & Lagerwey, 1965 (see Fig. 4 for male specimens from Argentina) and to *I. rinae* Coetzee, 1967. These two species described from South Africa are different in some morphological features, but as well they have important morphometric differences (see below). The new species differs by the lip region shape (set off by a depression in *I. geminus* and *I. rinae*), absence of vulval papillae (*vs* presence), disposal of ejaculatory glands (grouped in *I. geminus*), muscles between *vas deferens* and *ductus ejaculatorius* (forming a not outstretched band in *I. geminus* and *I. rinae*), ventral body contour at beginning of supplement series (contracted in *I. geminus* and *I. rinae*), tail terminus (subtruncate in *I. geminus* and *I. rinae*) and spinneret (fully terminal in *I. geminus* and *I. rinae*).

#### Morphometric study

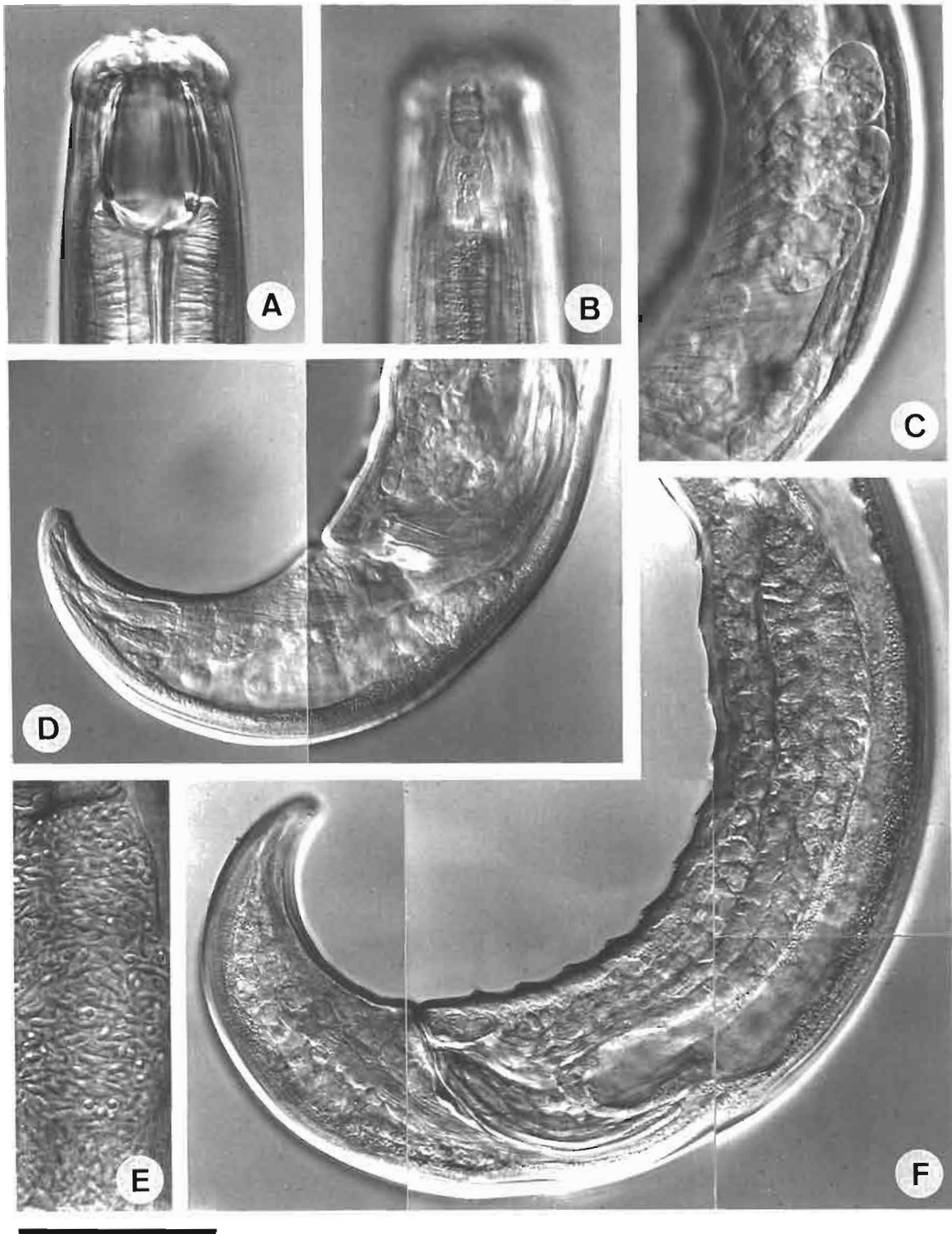
The three species were subjected to morphometric analysis based on selected characters or variables, seventeen for females and eighteen for males. The specimens of *I. geminus* and *I. rinae* were remeasured for morphometric purposes (Table 2), taking into consideration that they have become flattened and, therefore, the indices  $a$  and  $c'$  were corrected. The one-way analysis of the variance (ANOVA) for each of these variables has shown a significant difference ( $P < 0.01$ ) in fifteen and fourteen of them, for females and males respectively (Table 3). *A priori*, as a result of the univariate analysis, a great number of characters separate the species. In order to confirm this separation a principal component analysis (PCA) based on the variance-covariance matrix was performed with the last variables after log-transforming the data.

In females (Fig. 5), components I and II explain only the 41.3% and 19.0% respectively of the total variation; the cumulative percentage is 60.3%. The first component contrasts linear measurements (except body length)

**Table 3.** One-way ANOVA of the differences in character values, both for females and males, between the specimens of the three species of *Iotonchus*. Snedecor's *F* values are for significant differences ( $P < 0.01$ ). In males only *I. parageminus* sp. n. and *I. geminus* are compared.

$a = I. parageminus$ ,  $b = I. geminus$ ,  $c = I. rinae$ .

Character	Females		Males
	F. value	Significant differences between species	F value
1. L.	8.3	a-c	-
2. a.	38.8	a-b/a-c	25.3
3. b.	54.0	a-b/a-c	110.3
4. c.	34.6	a-b/a-c	108.6
5. $c'$	-	-	9.0
6. V/T	6.6	a-c/b-c	-
7. $G_1$	-	-	-
8. $G_2$	7.0	a-c/b-c	-
9. Lip region width	21.8	a-b/b-c	52.8
10. Lip region height	19.8	a-b/a-c/b-c	22.9
11. Bucc. cav. length	15.4	a-b/b-c	86.0
12. Bucc. cav. width	25.1	a-b/b-c	90.1
13. Dorsal tooth apex	6.4	a-b/a-c/b-c	-
14. Ant. end to n. r.	5.5	a-b/b-c	-
15. Pharynx length	9.9	a-b/b-c	48.7
16. Vagina length	10.0	a-b/b-c	-
17. Tail	14.7	a-b/a-c	123.6
18. Spicules	-	-	144.9
19. Gubernaculum	-	-	82.3
20. Lat. guiding pieces	-	-	20.9
21. Supplement number	-	-	26.4



**Fig. 4.** *Iotonchus geminus* (male from Argentina). A, B : Head; C : Ejaculatory glands; D, F : Posterior region; E : Sperm. (Scale bar = 50  $\mu$ m).



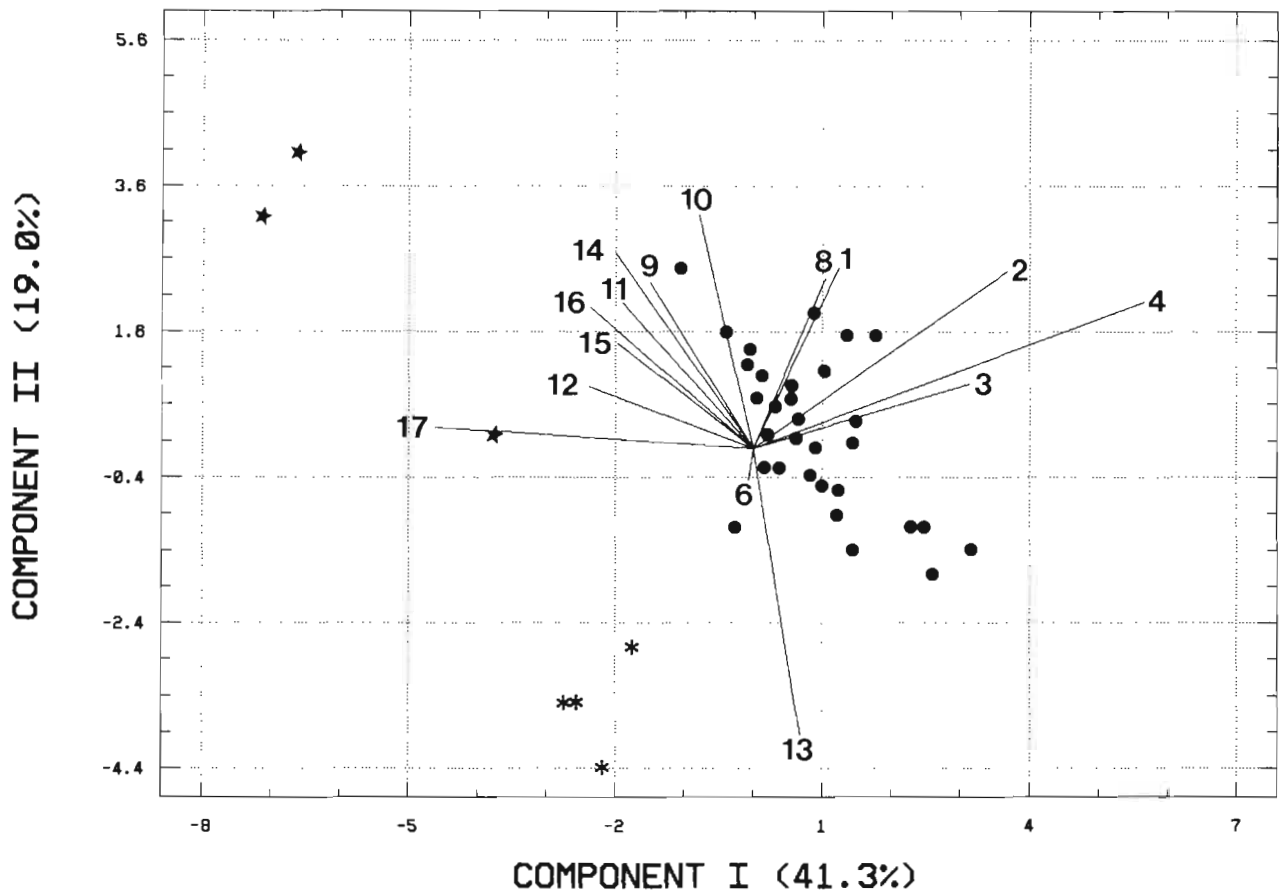


Fig. 5. Biplot of *Iotonchus* females and corresponding characters, resulting from PCA, along the two first axes. *I. geminus* (★); *I. rinae* (\*); *I. parageminus* (●). Character numbers as in Table 3.

with the indices a, b and c, indicating an opposite relation of absolute measurements to ratios. Its main elements are the variables index c and length of the tail, continued by the indices a and b. The three species are clearly separated in the plot. When the analysis is done with only the variables corresponding to positive values in the eigenvector, i.e. ratio variables and body length, the results of the analysis are similar. Contrary, when the same variables are removed from it, component I becomes a size vector but the separation of the species is not clear-cut. In summary, this component can be interpreted as a general size vector, although some of these variables are shape related. *I. parageminus* is different from the two other species by shorter tail, both in absolute and relative value, and by higher indices a and b. The second component separates the three species on the basis of an apparently head shape vector, the main elements corresponding to variables dorsal tooth apex and lip region height.

In males (Fig. 6), components I and II accounted for 80.7 % and 6.0 % of the variance and the cumulative percentage is 86.7 %. The first component is not different than obtained from the female PCA, but adds the variable gubernaculum to index c and length of the tail. The second component does not bring information on the separation between *I. parageminus* and *I. geminus*, depicting only intra-specific variation.

#### Acknowledgements

I am grateful to Dr. J. Heyns for arranging the loan of type specimens from Nematode Collection of the Plant Protection Research Institute, Pretoria, to Dr. E. Chaves for providing with specimens from Argentina and to Dr. P. Castillo for the assistance in the sampling. This work has been financially supported in part by the Project D.G.I.C.Y.T. PB89-0081 (Fauna Ibérica II).

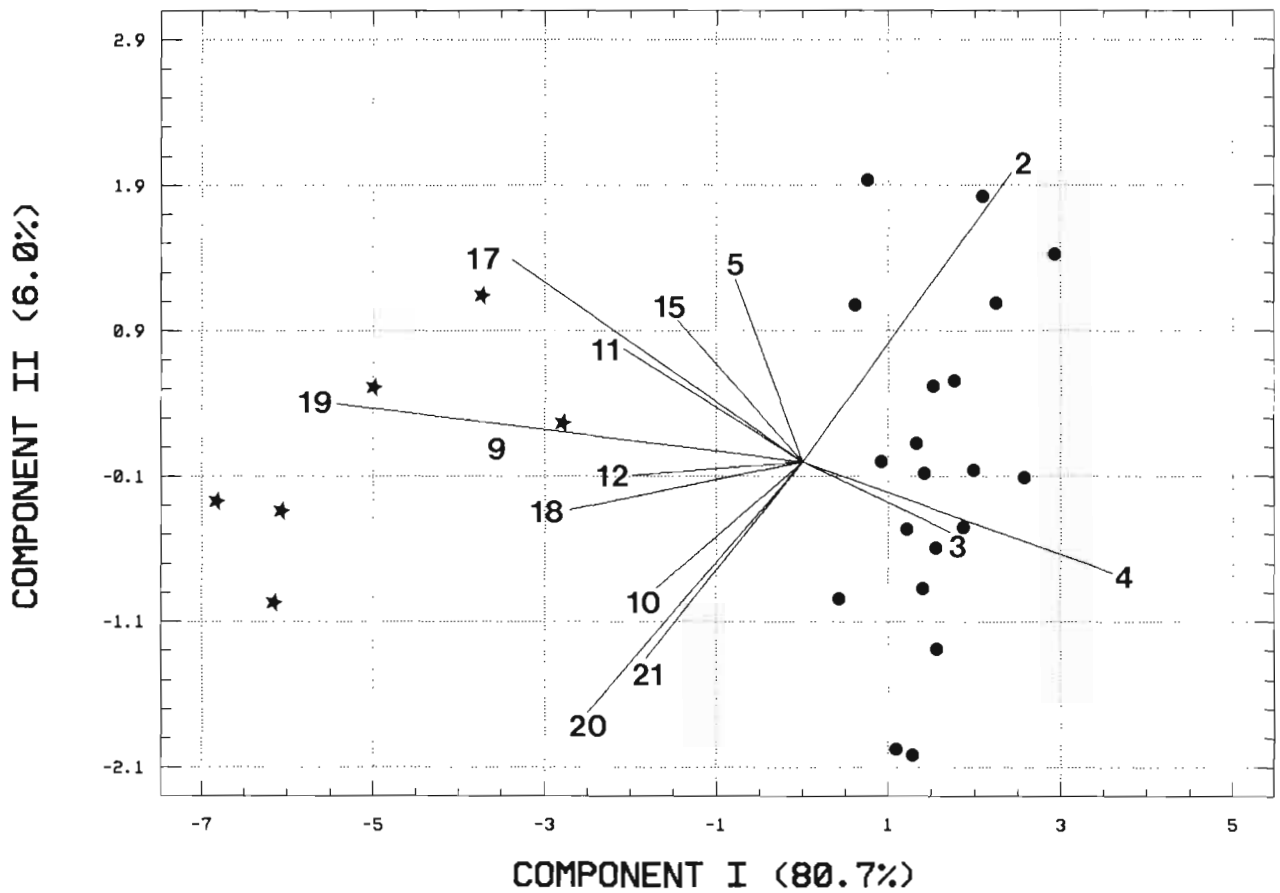


Fig. 6. Biplot of *Iotonchus* males and corresponding characters, resulting from PCA, along the two first axes. *I. geminus* (★); *I. parageminus* (●). Character numbers as in Table 3.

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