

Nematodes of the Order Rhabditida from Andalucía Oriental, Spain. The Genera *Protorhabditis* (Osche, 1952) Dougherty, 1953 and *Diploscapter* Cobb, 1913, with Description of *P. spiculocrestata* sp. n. and a Species *Protorhabditis* Key

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Abstract: A new species of the genus *Protorhabditis* is described from natural areas in the SE Iberian Peninsula. *Protorhabditis spiculocrestata* sp. n. is distinguished by its body length 387–707 μm in females and 375–546 μm in males, lip very low and flattened, stoma 14–22 μm long, female tail conical-elongate (48–100 μm , $c = 6.4\text{--}8.3$, $c' = 4.8\text{--}7.5$), phasmid near anus, male tail conical (20–27 μm , $c = 18.3\text{--}22.3$, $c' = 1.4\text{--}1.5$), bursa peloderan closed anteriorly and bears eight papillae (1+2+1+1+3), spicules 23–26 μm long, and gubernaculum 10–16 μm long. *Diploscapter coronatus* is also presented. Description, measurements and illustrations, including SEM photographs, are provided. A key to species of *Protorhabditis* is also given as well a compendium of their measurements.

Key words: description, *Diploscapter*, key, morphology, new species, *Protorhabditis*, Rhabditids, SE Spain, SEM, taxonomy.

Rhabditid nematodes are an interesting zoological taxon. They are very abundant in all types of soil and sediments of freshwater bodies and play important ecological roles mainly as primary consumers—their free-living forms display saprophagous or bacteriophagous feeding habits—but also as animal parasites, in particular entomopathogenic forms. From a systematic point of view, rhabditids are a difficult nematode group whose classification has been matter of long and strong discussions and whose diversity is far from being well known.

Several years ago, a research project was planned to study the Iberian rhabditid fauna, focused on both its morphological characterization and its taxonomy, since no monographic study on this matter was available.

The genera *Protorhabditis* (Osche, 1952) Dougherty, 1953 and *Diploscapter* Cobb, 1913 are two rhabditid taxa characterized by having a long stoma whose stegostome lacks glottoid apparatus. They were classified by Andrassy (1983) in separate families (Rhabditidae Örley, 1880 and Diploscapteridae Micoletzky, 1922, respectively) of the superfamily Rhabditoidea Örley, 1880, mainly due to morphology of lip region: six rounded lips with similar aspect in *Protorhabditis*; subdorsal and subventral lips hook-like and lateral lips membranous and rounded with dentate margin in *Diploscapter*. However, more recent evidence based on both morphological and molecular studies demonstrated a close relationship between both genera (Fitch, 2000; Sudhaus and Fitch, 2001).

Three species belonging to these two genera have been recorded from several localities in the Iberian Peninsula (Abolafia and Peña-Santiago, 2001): *P. filiformis*

(Bütschli, 1873) Sudhaus, 1976, *P. tristis* (Hirschmann, 1952) Dougherty, 1955 and *D. coronatus* (Cobb, 1893) Cobb, 1913. Nevertheless, no relevant morphological or taxonomical information on them was provided in the corresponding publications. In this contribution, part of the series on rhabditid species from Andalucía Oriental (SE Iberian Peninsula, Spain), *D. coronatus* and an undescribed species of *Protorhabditis* are reported.

MATERIALS AND METHODS

Nematodes were extracted from soil samples by Flegg's (1967) method and a somewhat modified Baermann's (1917) funnel technique. Nematodes obtained were later relaxed and killed by heat, fixed in 4% formaldehyde, and processed to anhydrous glycerine according to Siddiqi (1964). Measurements were taken using an ocular micrometer, and drawings were made using a drawing tube attached to a Leica microscope; LM pictures were made using a Nikon Eclipse 80i microscope provided with a digital video camera Nikon Digital Sight DS-5M. For SEM studies, fixed specimens were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried and coated with gold (Abolafia and Peña-Santiago, 2005), and observed with a JEOL JSM-5800 microscope. The terminology used for morphology of stoma and spicules follows the proposals by De Ley et al. (1995) and Abolafia and Peña-Santiago (2006), respectively.

In addition, the *D. coronatus* specimens examined were cultured in vitro in petri dishes (2% agar and 1.5% glucose) and maintained under laboratory conditions for several months. This culture is no longer available.

DESCRIPTION

Protorhabditis spiculocrestata sp. n. (Figs. 1–3)

The specific epithet refers to the presence of a subdorsal crest in the spicules, a remarkable morphological feature of this species.

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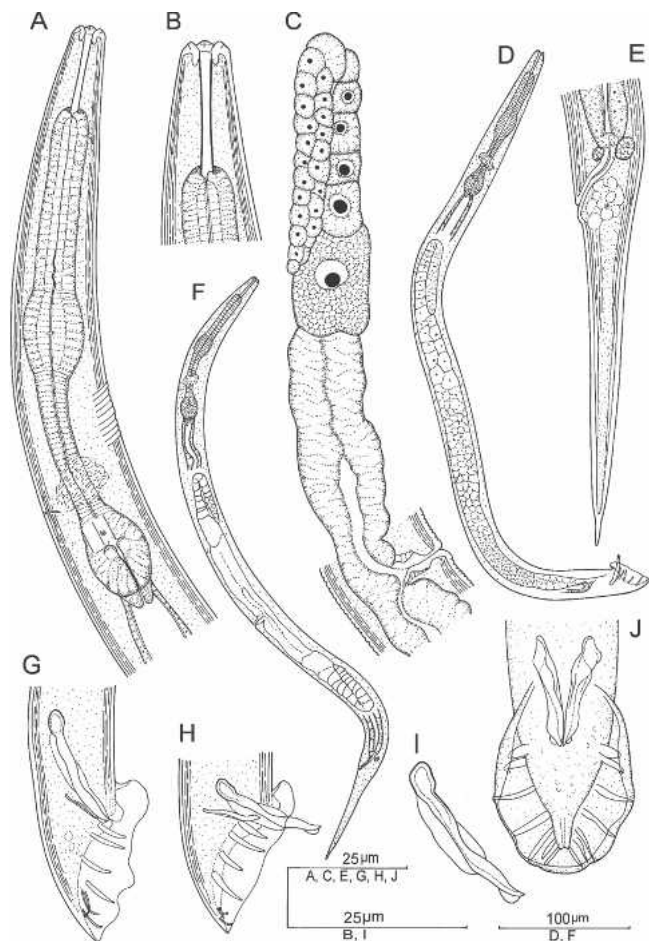


FIG. 1. *Protorhabditis spiculocrestata* sp. n. A: Neck. B: Anterior end. C: Female reproductive system. D: Entire male. E: Female posterior end. F: Entire female. G, H: Male posterior end (lateral side). J: Idem (ventral side). I: Spicule.

Measurements: Listed in micrometers in Table 1.

Type population collected near road from Fuente de Piedra to Alameda, province of Málaga

Female ($n = 7$): Small nematodes, 441–561 μm long. Body cylindrical, tapering towards both ends, more so posteriad. Habitus rather sigmoid after fixation. Cuticle with distinct transverse striations or annuli, about 1 μm wide at midbody. Lateral field with five incisions or four ridged wings at midbody, two at posterior region which fade out near anus. Lip region with six small lips, more or less rounded; papillae visible. Stoma rhabditoid, lacking glottoid apparatus. Cheilostom with weakly refractive walls. Buccal ring (membrane wedge ring) lower in longitudinal section. Buccal prism with straight walls, very long and narrow. Pharyngeal collar very short. Procorpus cylindrical. Metacorpus swollen, almost ovoid, with its inner walls more refractive. Isthmus almost as long as procorpus. Basal bulb small, ovoid or pyriform. Cardia conoid to hemispherical, surrounded by intestinal tissue. Intestine lacking distinct specializations, but a cardiac portion is sometimes differentiated at its anterior part. Nerve ring at 71–80% of neck length, surrounding the isth-

mus. Excretory pore at 82–92% of neck length, at level of isthmus base or basal bulb, behind the hemizonid. Deirid at basal bulb level, at 90–97% of neck length. Reproductive system didelphic-amphidelphic, with anterior branch dextral and posterior branch sinistral. Ovaries having a flexure. Uterus two times the corresponding body diameter long, having thicker walls at proximal portion and distinct lumen at distal one. Eggs 15–23 \times 37–39 μm . Vagina extending inwards to one-third of body diameter, with thicker walls at proximal portion. Rectum 1.1–1.3 times as long as anal body diameter. Tail conical-elongate, and terminus with acute mucro. Phasmid near anus.

Male ($n = 3$): Similar to female in general morphology. Habitus ventrad curved after fixation. Reproductive system monorchic. Testis reflexed ventrally anteriorly. Tail conical. Bursa pelodera, closed anteriorly, and with eight papillae (1+2+1+1+3). Spicules free: manubrium rounded; lamina bent at its middle, with well developed dorsal hump and ventral wing at its middle; terminus ventrad curved, with acute tip and a subdorsal crest. Gubernaculum sigmoid.

Other material examined (see Table 1)

Very similar to type population, but showing variations in body length, shorter in the population from Almirajara Mountain (387 μm in females and 395–405 μm in males), and longer in the populations from Almargin and Sierra de las Nieves (634 μm and 644–707 μm in females, respectively).

Diagnosis

Protorhabditis spiculocrestata sp. n. is distinguished by its body length 387–707 μm in females and 375–546 μm in males, lips very low and flattened, stoma 14–22 μm , female tail conical-elongate (48–100 μm , $c = 6.4$ –8.3, $c' = 4.8$ –7.5), phasmid near to anus, male tail conical (20–27 μm , $c = 18.3$ –22.3, $c' = 1.4$ –1.5), bursa peloderan closed anteriorly and bears eight papillae (1+2+1+1+3), spicules 23–26 μm long, and gubernaculum 10–16 μm long.

Relationships

The new species resembles *Protorhabditis oxyuroides* Sudhaus, 1974, although it can be distinguished from it by its having lips lower and reduced (vs. more prominent), stoma shorter (14–22 μm vs. 21–27 μm), and spicules with different morphology (vs. terminus lacking subdorsal crest).

Type locality and habitat

Los Carvajales, near the road from Alameda to Fuente de Piedra, province of Málaga, Spain; GPS coordinates: N 37°8.0', E 4°43.0'. Soil in a Mediterranean

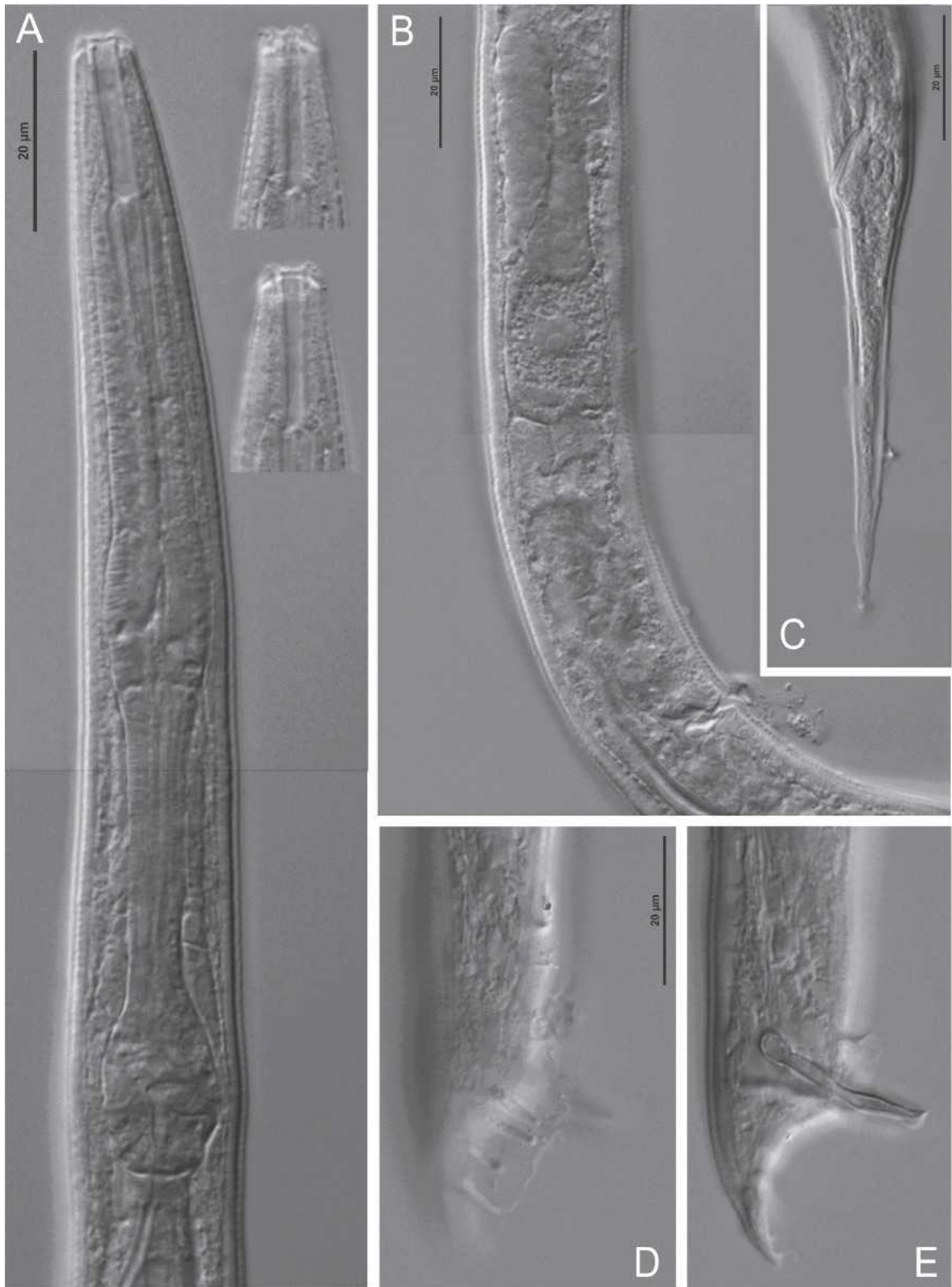


FIG. 2. *Protorhabditis spiculocrestata* sp. n. (LM). A: Neck. B: Female reproductive system (anterior branch). C: Female tail. D: Male posterior end (bursa view). E: Male posterior end (spicule view).

plant community whose dominant species are *Olea europaea* L. var. *sylvestris* Brot., *Daphne gnidium* L., *Rosmarinus officinalis* L., *Cistus monspeliensis* L., *Retama sphaero-*

carpa (L.) Boiss., *Phlomis purpurea* L., *Thymus* sp., *Quercus coccifera* L., and *Ulex parviflorus* Pourret. Collected on 19 October 1991.

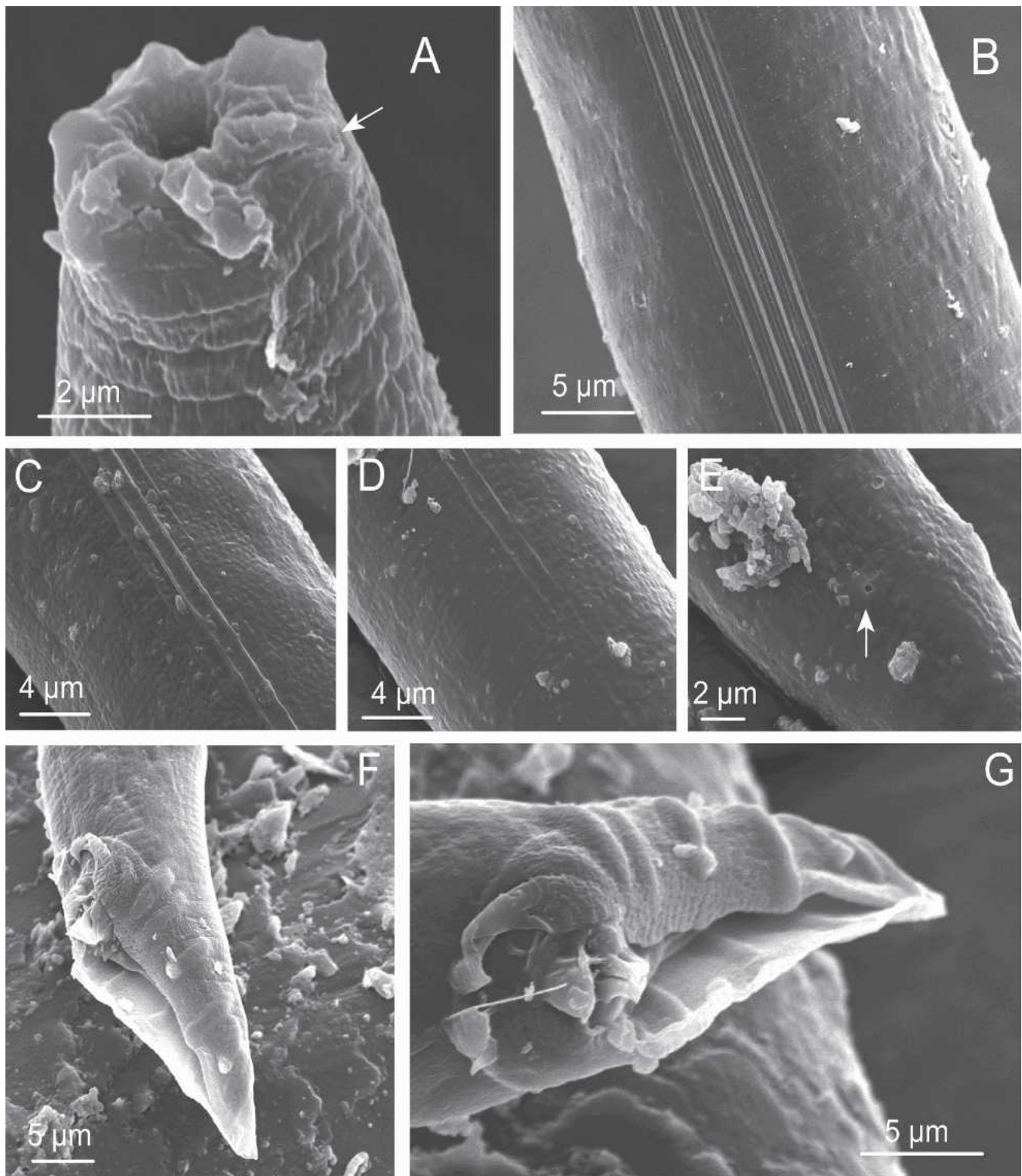


FIG. 3. *Protorhabditis spiculocrestata* sp. n. (SEM). A: Lip region (arrow indicates amphid opening). B: Lateral field at middle body. C: idem at posterior end. D: idem near to anus. E: Phasmid. F, G: Male posterior end.

Other localities and habitats

The species has also been collected from several localities in the SE Iberian Peninsula: Sierra de Guillimona (province of Granada); Sierras de Cazorla, Segura and Las Villas Natural Park (province of Jaén);

road to Canillas de Aceituno, Periana, road from Archidona to Villanueva del Trabuco, Sierra de Mijas, and Sierra de las Nieves Natural Park (province of Málaga). It has been found associated with the rhizosphere of several wild plant communities whose dominant species were *Pinus nigra* Arn. var. *salzmannii* (Dunal) Franco,

TABLE 1. Measurements (in μm) of females (n = 17) and males (n = 11) of *Protorhabditis spiculocrestata* sp. n. presented as mean \pm s.d. and (range).

Locality	Road from Fuente de Piedra to Alameda Málaga Brushwood			Road to Canillas de Aceituno Málaga Holm oak tree		Road from Archidona to Villanueva del Trabuco Málaga Walnut tree		Periana Málaga Eucalyptus		Almargen Málaga Pine		Sierra de las Nieves Málaga Brushwood	
	Holotype ♀	Paratypes 6♀♀	Paratypes 3♂♂	♀	2♂♂	4♀♀	♀	♂	♀	4♂♂	3♀♀	♂	
Body length	540	511.0 \pm 43.8 (441–561)	502.3 \pm 52.6 (444–546)	387	395, 405	528.3 \pm 47.6 (488–597)	562	375	634	450.5 \pm 37.8 (421–506)	644–707	518	
a	21.6	23.7 \pm 3.0 (19.2–27.9)	20.9 \pm 1.0 (20.2–21.7)	20.4	21.4, 24.5	26.4 \pm 1.4 (25.3–28.4)	22.5	20.3	?	22.4 \pm 1.5 (20.2–23.4)	22.3–24.8	24.7	
b	4.5	4.4 \pm 0.1 (4.3–4.4)	4.2 \pm 0.5 (3.9–4.6)	3.6	3.3, 3.9	4.6 \pm 0.6 (4.2–5.5)	4.6	?	5.1	4.2 \pm 0.1 (4.0–4.3)	4.4–5.2	4.4	
c	6.5	7.1 \pm 0.4 (6.4–7.6)	20.8 \pm 2.1 (19.3–22.3)	8.1	19.8, 19.8	7.2 \pm 0.5 (6.5–7.7)	8.0	18.3	8.3	21.1 \pm 0.7 (20.5–22.0)	6.9–8.0	21.1	
c'	6.0	5.9 \pm 0.4 (5.3–6.4)	1.4 \pm 0.1 (1.4)	4.8	1.4, 1.5	6.7 \pm 0.5 (6.3–7.5)	5.6	1.5	5.4	1.4 \pm 0.1 (1.4–1.5)	5.9–6.6	1.4	
V	53	54.1 \pm 1.8 (51–56)	—	56	—	53.4 \pm 4.3 (47–56)	53	—	56	—	52–54	—	
Lip region width	8	7.6 \pm 0.9 (7–9)	7.0 \pm 0.0 (7)	7	7	7.0 \pm 0.0 (7)	8	7	7	7.0 \pm 0.7 (7–8)	8–9	7	
Stoma	18	18.6 \pm 0.6 (18–20)	18.5 \pm 0.9 (18–20)	18	19, 20	18.1 \pm 0.6 (18–19)	17	15	14	16.5 \pm 1.1 (15–18)	20–22	19	
Pharyngeal corpus	52	53.3 \pm 2.6 (51–56)	50.3 \pm 2.3 (48–53)	48	48, 42	48.9 \pm 1.3 (48–50)	51	?	52	48.3 \pm 1.4 (48–50)	63–65	50	
Isthmus	31	30.1 \pm 3.3 (29–35)	31.0 \pm 0.9 (30–32)	30	32, 29	29.3 \pm 1.9 (27–32)	30	?	31	27.7 \pm 3.1 (25–31)	32–39	29	
Basal bulb	20	18.8 \pm 1.1 (18–21)	18.7 \pm 0.6 (18–19)	17	18	18.9 \pm 1.2 (18–21)	21	17	18	16.0 \pm 1.1 (15–18)	22–25	21	
Pharynx length	120	121.3 \pm 8.1 (114–130)	117.7 \pm 3.2 (114–120)	108	120, 104	114.3 \pm 7.5 (109–125)	122	?	125	109.7 \pm 6.7 (104–117)	135–146	119	
Nerve ring-ant. end	92	88.3 \pm 5.0 (84–96)	86.5 \pm 4.3 (82–91)	73	80, 79	81.4 \pm 2.9 (78–85)	87	72	81	78.9 \pm 4.3 (74–85)	105–110	86	
Excretory pore-ant. end	110	99.7 \pm 0.6 (99–100)	102.0 \pm 8.5 (96–108)	84	93, 87	?	96	78	?	95.3 \pm 11.0 (88–103)	120–126	?	
Deirid-ant. end	116	108 (n = 11)	116.0 (n = 1)	?	106, ?	?	108	?	?	90.0 (n = 1)	?	?	
Annuli width	1	1 \pm 0.0 (1)	1.0 \pm 0.0 (1)	1	1	1 \pm 0.0 (1)	1	1	1	1 \pm 0.0 (1)	?	?	
Cuticle thickness	1	1.0 \pm 0.0 (1)	1.0 \pm 0.0 (1)	1	1	1 \pm 0.0 (1)	1	1	2	1.0 \pm 0.0 (1)	1	2	
Body width; neck base	23	20.8 \pm 2.1 (18–24)	21.3 \pm 1.9 (20–24)	18	18, 16	18.8 \pm 1.0 (18–20)	23	18	24	19.0 \pm 2.0 (18–22)	25–27	22	
midbody	25	21.8 \pm 2.5 (18–24)	23.2 \pm 3.4 (21–27)	19	19, 17	20.0 \pm 1.1 (19–21)	25	19	?	20.3 \pm 3.2 (18–25)	26–31	21	
anus	14	12.3 \pm 0.9 (11–14)	16.8 \pm 1.0 (16–18)	10	14	11.0 \pm 0.8 (10–12)	13	14	14	14.8 \pm 1.0 (14–16)	14–15	18	
Vagina	10	8.6 \pm 0.8 (8–10)	—	7	—	7.4 \pm 0.8 (7–8)	8	—	?	—	10	—	
Anterior ovary/testis	51	47.3 \pm 5.6 (42–58)	77.5 \pm 22.3 (62–103)	40	74, 93	39.7 \pm 5.0 (34–44)	65	80	38	82.1 \pm 9.2 (70–91)	62–66	95	
Anterior genital branch	110	100.3 \pm 16.9 (85–125)	307.0 \pm 52.0 (255–359)	67	205, 222	98.0 \pm 7.0 (93–106)	110	205	82	271.8 \pm 31.7 (241–315)	138–157	308	
Posterior ovary	56	46.0 \pm 5.8 (35–50)	—	35	—	40.0 \pm 5.7 (36–44)	56	—	43	—	44–56	—	
Posterior genital branch	110	98.3 \pm 16.6 (77–121)	—	63	—	87.0 \pm 19.8 (73–101)	105	—	132	—	115–145	—	
Rectum	15	15.5 \pm 1.0 (14–17)	?	15	?	12.9 \pm 1.4 (12–15)	15	?	18	?	18–20	?	
Tail	84	72.3 \pm 5.9 (66–80)	24.8 \pm 2.0 (23–27)	48	20, 21	74.0 \pm 6.3 (67–82)	70	21	76	21.4 \pm 2.1 (20–25)	85–100	25	
Vulva-anterior end	286	277.2 \pm 30.7 (224–311)	—	216	—	280.8 \pm 8.6 (270–291)	298	—	353	—	348–379	—	
Vulva-anus/tail	2.0	2.2 \pm 0.1 (2.1–2.4)	—	2.6	—	2.3 \pm 0.4 (1.9–2.9)	2.8	—	2.7	—	2.3–2.8	—	
Spicules	—	—	24.7 \pm 1.5 (23–26)	—	23, 24	—	—	23	—	23.8 \pm 1.0 (23–25)	—	25	
Gubernaculum	—	—	11.8 \pm 0.8 (11–13)	—	10, 12	—	—	11	—	10 \pm 0.0 (10)	—	16	

Pinus pinaster Aiton, *Quercus rotundifolia* Lam., *Q. coccifera* L., *Juglans regia* L., *Retama sphaerocarpa* (L.) Boiss., *Juniperus oxycedrus* L., *Ulex* sp., *Cistus* sp., *Nerium oleander* L. and *Eucalyptus* sp.

Type material

Six females (holotype and paratypes) and three males (paratypes) deposited in Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Spain; and one female (paratype) deposited in the nematode collection of the Swedish Museum of Natural History, Stockholm (Sweden).

Diploscapter coronatus (Cobb, 1893) Cobb, 1913 syn. *Rhabditis coronata* Cobb, 1893; *Rhabditis bicornis* Zimmermann, 1898; *Diploscapter bicornis* (Zimmermann, 1898) Goodey, 1963; *Rhabditis cephaloides* Stefański, 1922; *Acrobeles armatus* Kreis, 1929.

(Figs. 4–6)

Measurements: Listed in micrometers in Table 2.

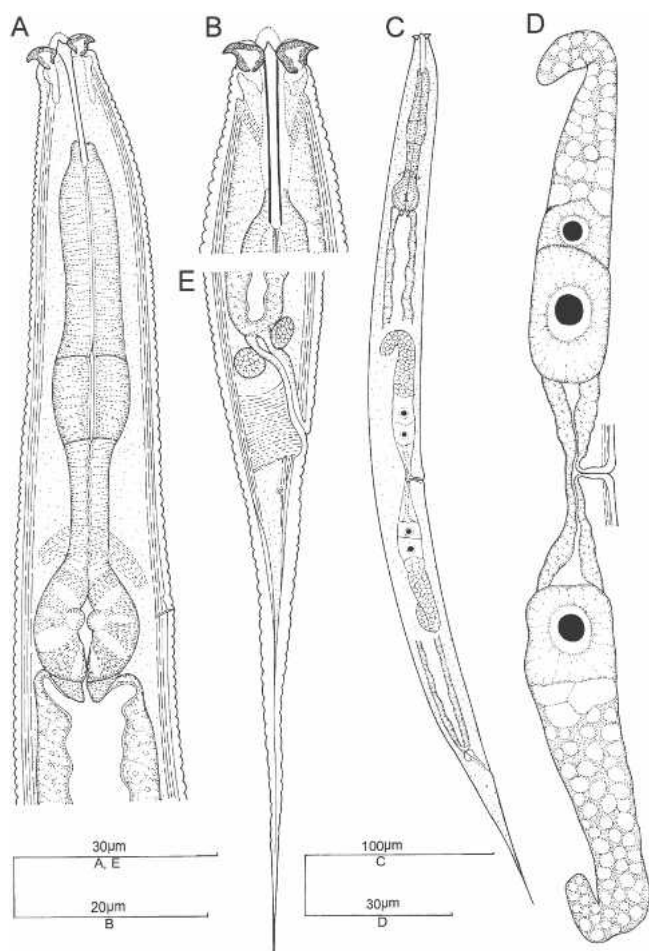


FIG. 4. *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 (female). A: Neck. B: Anterior end. C: Entire female. D: Reproductive system. E: Posterior end.

Population from agar culture comes from specimens collected in Carchuna, province of Granada

Female ($n = 20$): Small nematodes, 358–504 μm long. Habitus ventrad curved after fixation. Cuticle annulated; annuli 1–2 μm wide at midbody. Lateral field occupying 14–22% of the midbody diameter, with four incisures under LM and two separated wings under SEM which fade out before to anus level. Lip region with six lips: the subdorsal and the subventral are very refractive and hook-like (4–5 μm high); the lateral lips are membranous (termed lacinae), high and with dentate margin, not easily observed under LM since only the central triangular part is more refractive. Stoma rhabditoid. Buccal prism with straight walls, very long and narrow. Stegostom lacking glottoid apparatus. Pharyngeal corpus well demarcated, slightly longer than isthmus and basal bulb together; procorpus cylindrical and metacarpus swollen, almost as long as wide. Basal bulb spheroid, with well developed valves at its middle. Cardia hemispherical, surrounded by intestinal tissue. Intestine lacking any peculiar differentiation. Nerve ring at isthmus level, at 70–77% of neck length. Excretory pore at 77–92% of neck length, at level of posterior portion of isthmus or basal bulb. Deirid at basal bulb level, at 81–97% of neck length. Reproductive system didelphic-amphidelphic. Anterior genital branch dextral (on the right side of intestine) and posterior branch sinistral (on the left). Ovaries with a flexure, rarely straight. Uterus short, less than the corresponding body diameter long. Vagina with thin walls, extending inwards one-third of body diameter. Rectum 0.8–2.0 times anal body diameter long. Tail conical-elongate to filiform, ending in acute tip. Phasmid at 11–12% of tail length.

Male: Not found.

Other material examined (see Table 2)

Very similar to population from Carchuna, although having smaller body (273 μm long) and pharyngeal corpus shorter than isthmus-basal bulb.

Distribution

The species has been collected in six localities: (i) Vélez-Málaga, province of Málaga, in association with *Persea americana* Miller; (ii) La Vidriera, Guillimona Mountain, province of Granada, in association with *Quercus rotundifolia* Lam. and *Pinus* sp.; (iii) Santa Fé, province of Granada, in association with *Populus alba* L.; (iv) Desfiladero de los Gaitanes, province of Málaga, in association with *Citrus limon* (L.) Burm. fil.; (v) Carchuna, province of Granada, in association with *Lycopersicon esculentum* Miller.; and (vi) La Caldera pool, Sierra Nevada National Park (3,000 m height), province of Granada.



FIG. 5. *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 (LM). A: Neck. B: Female reproductive system. C: Lip region (lacinia level). D: Female tail. E: Lateral field.

Remarks

The material examined agrees well with the re-description by Eyualem et al. (1998) and previous de-

scriptions by Paetzold (1958), Loof (1964), Andrásy (1968) and Bongers (1988), although the specimen collected in Sierra de Guillimona is smaller (273 vs. 317–

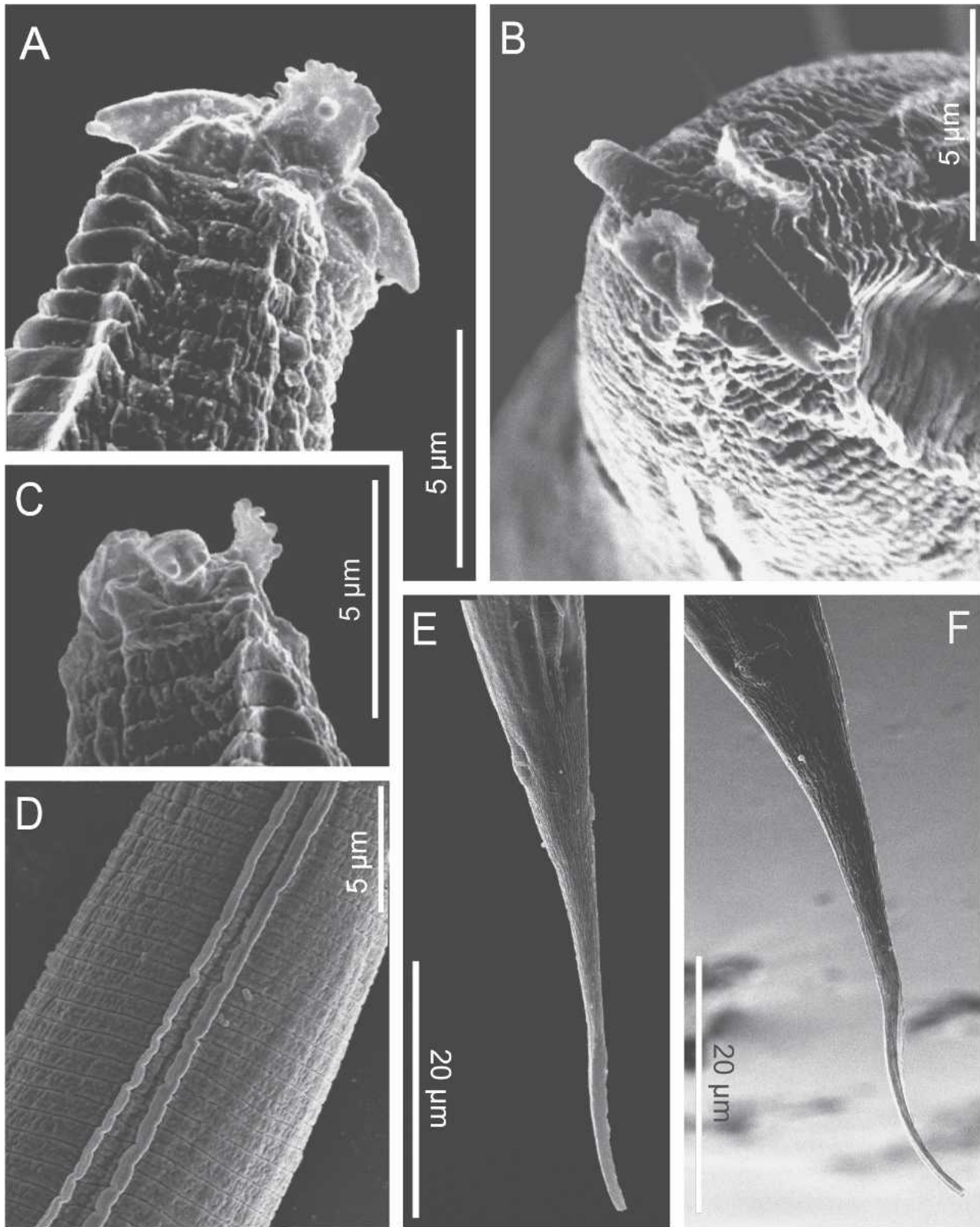


FIG. 6. *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 (SEM). A–C: Lip region (A: lateral view. B: frontal view. C: ventral view). D: Lateral field. E–F: Female tail (E: lateral view. F: ventral view).

480 µm). Our material is also very similar to populations studied by Tahseen et al. (2002), although having greater body length range (273–504 vs. 300–491 µm),

greater tail width range, *c* value, and *c'* value (54–80 µm, *c* = 5.1–7.4, *c'* = 4.4–6.8 vs. 42–65 µm, *c* = 5–8, *c'* = 5–8, respectively).

TABLE 2. Measurements (in μm) of females ($n = 21$) of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 presented as mean \pm s.d. and (range).

Locality Province Habitat n	Carchuna Granada Tomato	Guillimona Mountain Granada Holm oak tree
	20 ♀♀	♀
Body length	437.6 \pm 39.5 (358–504)	273
a	16.7 \pm 1.0 (14.9–18.0)	22.8
b	4.7 \pm 0.3 (4.0–5.3)	4.0
c	6.4 \pm 0.5 (5.7–7.4)	5.1
c'	5.8 \pm 0.7 (4.4–6.8)	6.8
V/T	50.7 \pm 1.9 (48–57)	53
Lip region: high	4.0 \pm 0.0 (4)	4
width	9.7 \pm 0.5 (9–11)	9
Stoma	18.3 \pm 1.4 (16–22)	18
Pharyngeal corpus	40.0 \pm 2.6 (35–47)	25
Isthmus	18.1 \pm 1.7 (15–22)	14
Basal bulb	17.0 \pm 1.0 (15–19)	12
Pharynx length	95.3 \pm 5.0 (90–109)	68
Nerve ring-ant. end	70.5 \pm 4.0 (66–85)	52
Excretory pore-ant. end	78.0 \pm 9.0 (69–100)	?
Deirid-ant. end	81.3 \pm 4.6 (75–89)	?
Annuli width	1.2 \pm 0.3 (1–2)	?
Cuticle thickness	1.0 \pm 0.1 (1–2)	1
Body width:		
neck base	24.3 \pm 3.1 (20–30)	14
midbody	26.3 \pm 3.1 (20–32)	12
anus	12.0 \pm 1.7 (9–17)	8
Lateral field	4.8 \pm 0.7 (4–6)	?
Vagina	7.3 \pm 0.8 (7–9)	?
Anterior ovary	59.1 \pm 11.5 (37–80)	?
Anterior genital branch	83.3 \pm 14.1 (57–106)	31
Posterior ovary	60.1 \pm 15.3 (37–83)	?
Posterior genital branch	84.5 \pm 18.6 (59–130)	33
Rectum	14.9 \pm 3.4 (10–24)	9
Tail	68.8 \pm 5.9 (55–80)	54
Vulva-anterior end	221.7 \pm 21.7 (182–260)	144
Phasmid-anus distance	8.3 \pm 0.4 (8–9)	?
Vulva-anus/tail	2.1 \pm 0.3 (1.6–2.6)	1.4

Comments on the genus *Protorhabditis* (Osche, 1952) Dougherty, 1953
syn. *Rhabditis* (*Protorhabditis*) Osche, 1952

Paradoxorhabditis Khera, 1971 op. Andr assy (2005)

Andr assy (2005) has provided an emended diagnosis of *Protorhabditis* as well a list of its species. Sudhaus (1991) transferred the type and only species of the genus *Paradoxorhabditis* Khera 1971, *P. paradoxa* Khera, 1971, to *Rhabditis*, but did not formally propose the synonymy of both genera, although very recently, Andr assy (2005) proposed its synonymy with *Protorhabditis*. *Paradoxorhabditis* is characterized by, among other features, the absence of glottoid apparatus and fits well the diagnosis of *Protorhabditis*, a fact that justifies the proposed synonymy by Andr assy. The presence of pseudopeloderan bursa in *P. paradoxa* is a peculiar feature of this species, which is herein regarded as intrageneric variability, but that should be incorporated in the diagnosis of *Protorhabditis*.

The genus includes hitherto 13 species plus two species inquirendae or incertae sedis (see Table 3):

Type species:

Protorhabditis xylocola (K rner in Osche, 1952) Dougherty, 1953
syn. *Rhabditis* (*Protorhabditis*) *xylocola* K rner in Osche, 1952

Other species:

P. cervi (Andr assy, 1985) Sudhaus, 1991
syn. *Caenorhabditis cervi* Andr assy, 1985
P. elaphri (Hirschmann, 1952) Dougherty, 1955
syn. *Rhabditis* (*Protorhabditis*) *elaphri* Hirschmann, 1952
P. filiformis (B utschli, 1873) Sudhaus, 1976
syn. *Rhabditis filiformis* B utschli, 1873
Rhabditis (*Choriorhabditis*) *filiformis* (B utschli, 1873) Osche, 1952
Rhabditis agilis von Linstow, 1876
Protorhabditis lengerkeni Paezotld, 1958
P. macrovelata Sudhaus, 1974
P. oxyuroides Sudhaus, 1974
syn. *Rhabditis oxyuris apud* B utschli, 1873, nec Claus, 1862
P. paradoxa (Khera, 1971) Andr assy, 2005
syn. *Paradoxorhabditis paradoxa* Khera, 1971
Rhabditis (*Rhabditis*) *paradoxa* (Khera, 1971) Sudhaus, 1991
P. parvovelata (K rner in Osche, 1952) Dougherty, 1955
syn. *Rhabditis* (*Protorhabditis*) *parvovelata* K rner in Osche, 1952
P. postneri (K rner in Osche, 1952) Dougherty, 1955
syn. *Rhabditis* (*Protorhabditis*) *postneri* K rner in Osche, 1952
P. ruehmi (K rner in Osche, 1952) Dougherty, 1955
syn. *Rhabditis* (*Protorhabditis*) *ruehmi* K rner in Osche, 1952

TABLE 3. Morphometrics of *Protorhabditis* species.

Species	Female body length (mm)	Male body length (mm)	a (female)	b (female)	c (female)	c' (female)	V	Stoma length (µm)	Pharyngeal medial bulb	Female tail (µm)	Bursa	Bursal papillae arrangement	Spicules (µm)
<i>P. cervi</i>	0.56-0.68	0.57-0.60	22-25	4.5-4.7	8.3-9.0	5.0-5.4	54-56	20-23	Yes	68-76	Pelodera (closed)	8 (1+4+3)	22-24
<i>P. elaphri</i>	0.63-0.92	0.54-0.69	20-28	5.7-7.1	4.0-9.0	10-14	43-51	20-22	No	86-175	Pelodera (open)	7 or 9 (3+3+3)	17-21
<i>P. filiformis</i>	0.44-0.96	0.35-0.59	22-35	4.2-7.1	3.2-4.3	10-13	42-48	15-17	Yes	125-233	Pelodera (closed)	8 (1+4+3)	10-16
<i>P. lepida</i>	0.46	—	30	4.8	5.5	7.5*	59	13	Yes	?	—	—	—
<i>P. macronelata</i>	—	0.49-0.57	—	—	—	3.0-4.0	?	22-25	Yes	?	Pelodera (open)	8 (2+4+2)	16-21
<i>P. oxyuroides</i>	0.59-0.87	0.32-0.72	17-21	4.3-5.9	6.7-10.8	5.0	53-56	21-27	Yes	?	Pelodera (closed)	8 (1+3+1+3)	13-28
<i>P. paradoxa</i>	0.90-0.97	0.64-0.67	24	5.0-5.1	3.7-4.0	15-20	42	21	Yes	?	Pseudopelodera (open)	7 (3+3+1)	25-27
<i>P. parvoelata</i>	0.58-0.87	0.52-0.64	20-24	4.0-5.9	11-30	1.5-4.0	56-62	18	Yes	?	Pelodera (open)	8 (2+2+2+2)	23-30
<i>P. postneri</i>	0.63-0.87	0.63-0.72	16-22	4.3-5.3	7.8-13.5	3.0-4.0	56-63	18*	Yes	?	Pelodera (open)	8 (2+3+3)	22-34
<i>P. ruzhmi</i>	0.60-0.75	0.54-0.66	17-21	4.4-4.9	8.0-9.0	3.0-4.0	54-58	18-20	Yes	?	Pelodera (open)	8 (2+1+2+3)	24-29
<i>P. spiculocrestata</i>	0.38-0.71	0.37-0.55	19-28	3.6-5.5	6.4-8.3	4.8-7.5	47-56	14-22	Yes	48-100	Pelodera (open)	8 (1+3+1+3)	23-26
<i>P. tristis</i>	0.45-0.55	0.27-0.30	22-27	4.4-6.2	3.3-4.5	12-14	46-48	17-20	Yes	?	Pelodera (open)	9 (2+4+3)	13
<i>P. virgo</i>	0.43-0.53	—	22-26	4.3-5.5	6-9	4.0-5.0	53-57	15	Yes	?	Pelodera (open)	—	—
<i>P. xylodora</i>	0.52-0.68	0.40-0.58	19-22	4.6-5.7	9.6-13.2	3.0-4.0	54-58	18-20	Yes	?	Pelodera (open)	8 (2+1+3+2)	21-29

* Measurements from drawings.

P. spiculocrestata sp. n.

P. tristis (Hirschmann, 1952) Dougherty, 1955
syn. *Rhabditis (Protorhabditis) tristis* Hirschmann, 1952

P. virgo (Körner in Osche, 1952) Dougherty, 1955
syn. *Rhabditis (Protorhabditis) virgo* Körner in Osche, 1952

Species inquirendae and/or incertae sedis:

P. lepida (Kreis, 1930) Sudhaus, 1976

syn. *Rhabditis lepida* Kreis, 1930

Rhabditis (Choriorhabditis) lepida Kreis, 1930 (Osche, 1952)

Rhabditis elegans apud Kreis, 1929, nec Maupas, 1899

P. minuta (Cobb, 1893) Dougherty, 1955

syn. *Rhabditis minuta* Cobb, 1893

Notes on some species

The material identified and described as *Rhabditis filiformis* by Cobb (1893) certainly does not belong to this species because of its rounded lips with acute setae, monodelphic-prodelphic female reproductive system and vulva very posterior close to anus. It better fits the diagnosis of *Mesorhabditis* (Osche, 1952) Dougherty, 1953, but further studies are needed to clarify its identity.

Some doubts persist on the true identity of *Rhabditis agilis* von Linstow, 1876. It was considered (Sudhaus, 1976; Andrassy, 1983) to be a junior synonym of *P. filiformis* (Bütschli, 1873) Sudhaus, 1976. However, there are some remarkable differences between both taxa, among others, shorter female tail ($c = 5.5-6.0$ vs. $c = 3.2-4.0$) and spicules (26 vs. 10 µm long), and they might not be identical.

P. lepida (Kreis, 1930) Sudhaus, 1976 (= *Rhabditis elegans* apud Kreis, 1929) is herein regarded as species inquirendae (cf. Andrassy, 1983), since the original description lacks many morphological details. Very recently, Andrassy (2005) considered it to be a valid species, but it is better to maintain it as species inquirendae.

The original description of *Rhabditis minuta* Cobb, 1893 is very poor in diagnostic features, and its true identity might not be established with accuracy. Thus, it should be maintained as species incertae sedis (cf. Andrassy, 1983).

Key to *Protorhabditis* species identification:

1. Female tail longer ($c' = 10-20$)..... 2
- Female tail shorter (c' less than 8)..... 5
2. Pharyngeal collar very long, occupying most of the length of the stoma; pharyngeal corpus cylindrical (metacarpus not swollen) *elaphri*
- Pharyngeal collar short or absent (shorter than stoma width); pharyngeal corpus with metacarpus swollen 3

3. Bursa bearing 9 pairs of papillae ----- *tristis*
 Bursa bearing 7–8 pairs of papillae ----- 4
4. Cuticle lacking longitudinal striae; female tail shorter ($c' = 10-12$); bursa anteriorly closed, pelodera ----- *filiformis*
 Cuticle longitudinally striated; female tail longer ($c' = 15-20$); bursa anteriorly open, pseudopelodera, with a short and fine terminal filament ----- *paradoxa*
5. Bursa anteriorly open ----- 6
 Bursa anteriorly closed ----- 11
6. Body length less than 500 μm ----- *virgo*
 Body length more than 500 μm ----- 7
7. Bursa with scarcely developed velum, in lateral view not exceeding the ventral margin of body - ----- *parovovelata*
 Bursa with well developed velum, in lateral view exceeding the ventral margin of body ----- 8
8. Lips having acute tines ----- *ruehmi*
 Lips lacking acute tines ----- 9
9. Spicule length 16–21 μm ----- *macrovelata*
 Spicule length 21–34 μm ----- 10
10. Inner (perioral) region of lips peculiarly elevated; cheilorhabdia divergent anteriorly ----- *postneri*
 Inner (perioral) region of lips as usual, not peculiarly elevated; cheilorhabdia not divergent anteriorly ----- *xylocola*
11. Pharyngeal collar well developed, occupying more than half of the stoma length ----- *cervi*
 Pharyngeal collar very short or absent (shorter than stoma width)
12. Lip region narrower than adjacent part of neck; stoma 14–22 μm long; spicules bearing subdorsal crest ----- *spiculocrestata*
 Lip region wider than adjacent part of neck; stoma 21–27 μm long; spicules lacking subdorsal crest ----- *oxyuroides*

LITERATURE CITED

- Abolafia, J., and Peña Santiago, R. 2001. Rhabditid species (Nematoda, Rhabditida) recorded in Peninsular Spain and Balearic Islands. *Graellsia* 57:113–131.
- Abolafia, J., and Peña-Santiago, R. 2005. Nematodes of the order Rhabditida from Andalucía Oriental: *Pseudacrobeles elongatus* (de Man, 1880) comb. n. *Nematology* 7:917–926.
- Abolafia, J., and Peña-Santiago, R. 2006. Nematodes of the order Rhabditida from Andalucía, Spain. The family Panagrolaimidae, with a compendium of species of *Panagrolaimus* and a key to their identification. *Journal of Nematode Morphology and Systematics* 8:133–160.
- Andrássy, I. 1968. Fauna Paraguayensis. 2. Nematoden aus den Galeriewäldern des Acaray-Flusses. *Opuscula Zoologica Budapest* 8: 167–312.
- Andrássy, I. 1983. A taxonomic review of the suborder Rhabditina (Nematoda: Secernentia). Paris: ORSTOM.
- Andrássy, I. 1985. A dozen new nematode species from Hungary. *Opuscula Zoologica Budapest* 19–20:3–39.
- Andrássy, I. 2005. Free-living nematodes of Hungary (Nematoda errantia). Vol. 1 Bp., Magyar Természettudományi Múzeum, Budapest.
- Baermann G. 1917. Eine einfache Methode zur Auffindung von *Ankylostomum* (Nematoden) Larven in Erdproben. *Geneeskunding Tijdschrift voor Nederlandsch-Indië* 57:131–137.
- Bongers, T. 1988. De nematoden van Nederland. Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging.
- Bütschli, O. 1873. Beiträge zur Kenntnis der freilebenden Nematoden. *Nova Acta der Kaiserlich-Leopoldinisch-Carolinische Deutschen Akademie der Naturforscher* 36:1–124.
- Claus, C. 1862. Über einige im Humus lebende Anguillulinen. *Zeitschrift für Wissenschaftliche Zoologie* 12:354–359.
- Cobb, N. A. 1893. Nematode worms found attacking sugar-cane. *Agricultural Gazette of New South Wales* 4:808–833.
- Cobb, N. A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. *Journal of Washington Academy of Sciences* 3:432–433.
- De Ley, P., Van de Velde, M. C., Mounport, D., Baujard, P., and Coomans, A. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). *Nematologica* 41:153–182.
- Dougherty, E. C. 1953. The genera of the subfamily Rhabditinae Micoletzky, 1922 (Nematoda). *Thaper Commemoration Volume*:69–76.
- Dougherty, E. C. 1955. The genera and species of the subfamily Rhabditinae Micoletzky, 1922 (Nematoda). A nomenclatorial analysis—including an addendum on the composition of the family Rhabditidae Örley, 1880. *Journal of Helminthology* 29:105–152.
- Eyualem, A., Karegar, A., Nabil, H. and De Ley, P. 1998. A redescription and ultrastructural study of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 from Ethiopia and Iran. *Russian Journal of Nematology* 6:17–22.
- Fitch, D. H. A. 2000. Evolution of “Rhabditidae” and the male tail. *Journal of Nematology* 32:235–244.
- Flegg, J. J. M. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb’s decanting and sieving technique. *Annals of applied Biology* 60:429–437.
- Goodey, T. 1963. Soil and freshwater nematodes. 2nd ed. rewritten by J. B. Goodey. London: Methuen & Co.
- Hirschmann, H. 1952. Die Nematoden der Wassergrenze mittelfränkischer Gewässer. *Zoologische Jahrbücher - Abteilung für Systematik, Ökologie und Geographie der Tiere* 81:313–364.
- Khera, S. 1971. Nematodes from the banks of still and running waters. XI. Subfamily Rhabditinae. *Indian Journal of Nematology* 1: 237–243.
- Kreis, H. A. 1929. Freilebende terrestrische Nematoden aus der Umgebung von Peking (China). I. *Zoologischer Anzeiger* 84:283–294.
- Kreis, H. A. 1930. Freilebende terrestrische Nematoden aus der Umgebung von Peking (China). II. *Zoologischer Anzeiger* 87:67–87.
- Linstow, O. F. B. von 1876. Helminthologische Beobachtungen. *Archiv für Naturgeschichte* 42:1–18.
- Loof, P. A. A. 1964. Free-living and plant-parasitic nematodes from Venezuela. *Nematologica* 10:201–300.
- Maupas, E. F. 1899. La mue et l’enkystement chez les nématodes. *Archive de Zoologie Expérimental and Génétique* 7:563–628.
- Micoletzky, H. 1922. Die freilebenden Erd-Nematoden mit besonderer Berücksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision sämtlicher nicht mariner, freilebender Nematoden in Form von Genus-Beschreibungen und Bestimmungsschlüsseln. *Archiv für Naturgeschichte* 87:1–650.
- Örley, L. 1880. Az Anguillulidák magánrajza. (Monographie der Anguilluliden). *Természetrázi Füzetek* 4:16–150.
- Osche, G. 1952. Systematik und Phylogenie der Gattung *Rhabditis* (Nematoda). *Zoologische Jahrbücher (Systematik)* 81:190–280.
- Paetzold, D. 1958. Beiträge zur Nematodenfauna mitteldeutscher Salzstellen im raum von Halle. *Wissenschaftlichen Zeitschrift der Martin-Luther-Universität* 8:17–48.
- Stefański, W. 1922. Excrétion chez les nématodes libres. *Archiwum Nauk Biologicznych Towarzystwa Naukowego Warszawskiego* 1:1–33.

Siddiqi, M. R. 1964. Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 2:174–184.

Sudhaus, W. 1974. Zur Systematik, Verbreitung, Ökologie und Biologie neuer und wenig bekannter Rhabditiden (Nematoda). I. Teil. *Zoologische Jahrbücher (Systematik)* 101:173–212.

Sudhaus, W. 1976. Nomenklatorische Bemerkungen über Arten und Gattungen der Unterfamilie Rhabditinae *sensu lato* (Rhabditidae, Nematoda). *Nematologica* 22:49–61.

Sudhaus, W. 1991. Check list of species of *Rhabditis sensu lato*

(Nematoda: Rhabditidae) discovered between 1976 and 1986. *Nematologica* 37:229–236.

Sudhaus, W., and Fitch, D. 2001. Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). *Journal of Nematology* 33:1–70.

Tahseen, Q., Siddiqi, M. R., and Rowe, J. 2002. Study on species of *Diploscapter* Cobb (Nematoda: Rhabditida) from India including *D. indicus* sp. n. *International Journal of Nematology* 12:183–188.

Zimmermann, A. W. P. 1898. De nematoden der koffiewortels. Deel I. *Mededelingen's Lands Plantentuin Buitenzorg* 27:1–64.