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Redescription of *Macrolaimus canadensis* Sanwal, 1960 and *M. ruehmi* Andrásy, 1966 (Nematoda, Rhabditida, Chambersiellidae), and new data on *M. crucis* Maupas, 1900

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Abstract

Three species belonging to the genus *Macrolaimus*, namely *M. canadensis*, *M. crucis* and *M. ruehmi*, have been obtained from areas of natural vegetation in Bosnia and Herzegovina, Corsica (France), the Czech Republic and Spain. These three species are characterized by the body length of males and females, stomatal proportion of the gymnostom and cheilostom, excretory pore position, postvulval uterine sac length, male and female tail length and morphology, and the length and morphology of the spicules and gubernacula. The occurrence of *M. crucis* in Spain has, as a result of this study, now also been expanded to a larger area of the southern Iberian Peninsula. Morphological and morphometrical analyses showed that *M. canadensis* and *M. ruehmi* are very similar, sharing apomorphic characters. In contrast, *M. crucis* has pleisiomorphic characters. Description, measurements and illustrations are provided for these three species.

Introduction

The genus *Macrolaimus* Maupas, 1900 represents nine valid species and also includes four *species inquirendae* (see Abolafia & Peña-Santiago, 2014). Specimens of these species are characterized by the cheilostome–gymnostome proportions, postvulval uterine sac length of females, morphology of male and female tail tips, and the morphology of the spicules and the gubernaculum. Nonetheless, some of these species have been poorly described, since the morphological description of some diagnostic characters, which could facilitate their correct identification, are not detailed enough.

In Europe, four species have been described, namely *M. canadensis* Sanwal, 1960, *M. crucis* Maupas, 1900, *M. ruehmi* Andrásy, 1966 and *M. somniorum* Andrásy, 1984. The latter species had, however, not been illustrated by Andrásy (1984). Hence, in the present study, two of these species, *M. canadensis* and *M. ruehmi*, are redescribed according to specimens obtained from areas where natural vegetation was growing in the Czech Republic, Corsica (France) and Bosnia and Herzegovina. In addition, new data for *M. crucis*, identified from natural vegetation in the southern Iberian Peninsula of Spain, are provided.

Materials and methods

Sampling and nematode extraction

Specimens of *M. canadensis* and *M. ruehmi* were extracted from bark samples that were obtained from several areas of natural vegetation in central Europe. Additionally, new specimens of *M. crucis* were extracted from coastal sandy soil that was obtained in the southern area of the Iberian Peninsula.

Nematodes obtained from all locations were extracted from either bark or soil samples using a modified Baermann funnel technique (Baermann, 1917) and fixed in a 4% formalin solution. Specimens obtained from the Czech Republic, Corsica and Bosnia and Herzegovina were transferred to pure glycerine according to the protocol of the De Grisse (1969), and those obtained from Spain using the method of Siddiqi (1964). Specimens, representative of each species, were then permanently mounted on glass microscope slides to enable species identification using light microscopy (LM), while others were prepared for scanning electron microscopy (SEM).

Light microscopy (LM)

Measurements of various structures and organs of all mounted specimens were recorded using a Leitz Laborlux S microscope (Leitz, Wetzlar, Germany), after which the De Man's indices (De Man, 1880) were calculated for each specimen. Drawings of the bodies and body structures of individual specimens were made using a drawing tube (camera lucida) attached to a Leitz Laborlux S microscope. Pictures of the bodies and structures of studied specimens were taken with a Nikon Eclipse 80i light microscope (Nikon, Tokio, Japan) furnished with differential interference contrast optics (DIC) and a Nikon Digital Sight DS-U1 camera. Micrographs were edited using Adobe® Photoshop® CS.

Scanning electron microscopy (SEM)

Specimens of each species, preserved in glycerine, were also selected for observation using SEM according to the protocol of Abolafia (2015). Nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical-point dried, coated with gold and observed with a Zeiss Merlin microscope (5 kV) (Zeiss, Oberkochen, Germany).

The terminology used for the morphology of stoma and spicules follows the proposals by De Ley *et al.* (1995) and Abolafia & Peña-Santiago (2017), respectively.

Results

Macrolaimus canadensis Sanwal, 1960

Material examined

Twenty-seven females and 17 males, originating from the Czech Republic.

Description

Measurements. See table 1.

Adults. See figs 1–3. Moderately slender nematodes of small size, 1.03–1.48 mm long. Body cylindrical, tapering towards both extremities, more towards the posterior end. Habitus curved ventrad after fixation, to form an open 'C' shape. Cuticle *c.* 1 µm thick, with very fine annuli, *c.* 1 µm wide, frequently appearing divided somewhere in its outline, hardly discernible with LM but readily visible with SEM. Lateral field covers 10–11% of body diameter, consisting of two wings (three longitudinal incisions) with transverse incomplete striations, starting at level of anterior part of corpus and ending at tail tip. Lip region convex, continuous with the adjacent body, with the oral region strongly elevated or protruding; lips fused, developing six curved, triangular liplets covering the oral aperture; anterior sensillae arranged in two circles, with six conoid labial setae *c.* two annuli long and projecting forwards and located at the margin of the oral field, and four (two subdorsal and two subventral) very small cephalic papillae situated beside the labial setae. Amphidial aperture small, oval, located at base of lateral lips. Stoma 1.3–2.2 times longer than wide and 0.6–1.0 times as long as lip-region diameter, heavily sclerotized, subdivided into cheilo-, gymno- and stegostom; cheilostom *c.* 50% of the stoma length, having cheilorhabdia strongly sclerotized, convergent (curved) and thinner anteriorly; gymnostom very short, *c.* one-third of the cheilostom length, with arched gymnorrhabdia; buccal cavity (=cheilo-gymnostom) 1.4 times longer than wide; stegostom one-half of the cheilostom length and slightly longer than the gymnostom, with funnel-shaped lumen, enveloped by the anterior end of pharynx, lacking

visible rhabdia; dorsal pharyngeal gland opening at base of stegostom. Pharynx cephaloboid; pharyngeal corpus cylindrical, 1.7–3.0 times longer than isthmus, with procorpus and metacarpus similar in width; isthmus narrower than corpus and clearly delimited from it; basal bulb nearly pyriform with strongly developed valvular apparatus located at its posterior half; pharyngeal gland nuclei obscure. Cardia short and subcylindrical. Nerve ring at 57–78% of neck length, encircling pharynx at level of anterior half of isthmus. Secretory–excretory gland cell located ventrally to isthmus; excretory pore located posterior to nerve ring, at 67–85% of neck length, at level of isthmus. Deirid located posterior to nerve ring, inside the lateral field, at 78% of neck length, five or six annuli posterior to excretory pore, not clearly visible with LM.

Female. Reproductive system monodelphic–prodelphic. Ovary reflexed near to oviduct junction, very long, surpassing the level of the vulva and reaching the posterior part of intestine; oocytes first situated in two or more rows, then in only one row. Oviduct narrow and short; no distinct narrowing is observed at ovary–oviduct junction. Uterus located on right-hand side of intestine, with two sections separated by a weak internal narrowing; the distal part is long, 5–6 times longer than the body width, tubular, with thick walls, whereas the proximal part (ovejector) is short and swollen and with thin walls. Uterine eggs were absent in the specimens examined. Postvulval uterine sac present, 1.1–1.8 times longer than the body width at its level. Vagina occupying one-third (*c.* 33–40%) of body diameter, perceptibly bent forward. Vulva a transverse slit, with distinctly protruded lips, especially the anterior one that forms a vulval cone; advulval cuticle lacking differentiations. Rectum 1.3–2.2 times longer than anal body diameter, its anterior part swollen with walls including gland-like cells; anal lips not prominent. Tail conical, slightly ventrad curved, tapering very gradually and ending in an acute dorsad-bent mucro. Phasmids located at posterior half of tail, at 57–69% of tail length from anus.

Male. Reproductive system monorchic, with testis reflexed ventrad, on right-hand side of intestine. Spicules paired and symmetrical, 6.8–10.7 times longer than wide, curved ventrad with rounded manubrium, conoid calamus, ventrad curved lamina with well-developed proximal dorsal hump and thin velum at ventral side. Gubernaculum deltoid, weakly curved ventrad and acute distally. Gland-like cells are not distinguishable around the rectum. Genital papillae consist of two subventral precloacal pairs and five postcloacal pairs arranged as follows: one lateral pair located at lateral field and slightly posterior to the level of cloacal aperture; two subventral pairs, one of them bigger at the anterior third and another smaller at the posterior third to tail length; and two subdorsal pairs at the posterior half of tail, the anterior one bigger; a midventral papilla is located in front of the anterior lip of cloacal aperture. Tail conoid and tapering gradually and curved ventrad, ending in a short dorsad-bent mucro. Phasmids located at posterior half of tail, at 53–61% from anus.

Other material examined

Macrolaimus canadensis specimens were also found in Corsica (France), being similar to those examined from the Czech Republic. The Corsican specimens could only be differentiated from those of the Czech Republic by the slightly shorter length of their spicules (25 µm vs. 27–32 µm long).

Localities and habitats

Specimens of the species were collected at 16 localities (Babí u Náchoda, Bzenec Přívoz, Čechy pod Kosířem, Černá Říčka,

Table 1. Morphometrics of *M. canadensis* Sanwal, 1960. Measurements in micrometres (μm) and in the form: mean \pm standard deviation (range) where appropriate.

Reference	1				2		3		4		5	6									
Country	Czech Republic				Corsica (France)		Brasil		Austria		Quebec (Canada)		France	USA							
Locality	Sobersice (Brno)		Several		Mt. Incudine		Sao Paulo		?		Lac Tremblant		Maures	?							
Habitat	<i>Pinus sylvestris</i>		Several		<i>Pinus nigra</i>		Moss		Bark beetles		Bark beetles		Pine	Bark beetles							
<i>n</i>	12♀♀		6♂♂		15♀♀		11♂♂		6♀♀		2♂♂		♀	♂	♀	♂	10 ♀♀	9 ♂♂	?♂♂	?♀♀	?♂♂
Body length	1298.5 \pm 97.2 (1149–1481)		1309.2 \pm 102.4 (1162–1461)		1026–1422		1045–1292		1145.7 \pm 80.6 (1045–1292)		1077, 1097		1260	1170	989	714	990–1200	950–1070	1130–1170	990–1200	950–1070
<i>a</i>	46.4 \pm 5.0 (38.1–54.9)		53.5 \pm 4.3 (46.5–58.4)		5.1–7.1		43.5–52.2		42.8 \pm 4.3 (36.6–46.1)		43.9, 49.0		28–37	35.5	39.6	35.7	34.0–36.6	38.8–43.0	?	34–36.6	38.8–43
<i>b</i>	6.2 \pm 0.3 (5.4–6.5)		6.4 \pm 0.7 (5.2–7.3)		36.0–52.2		5.5–6.8		6.0 \pm 0.4 (5.6–6.4)		5.0, 5.0		4	4	5.7	4.3	4.5–4.9	4.3–4.7	5.5–5.7	4.5–4.9	4.2–4.7
<i>c</i>	19.0 \pm 2.3 (15.6–22.8)		29.2 \pm 4.1 (21.5–32.6)		16.5–23.4		22.3–31.3		18.3 \pm 2.6 (16.0–21.5)		24.4, 29.9		14	14.5	14.7	20.4	13.5–14.8	16.7–19.8	?	13.5–14.8	16.7–19.8
<i>c'</i>	4.4 \pm 0.4 (3.8–5.0)		1.9 \pm 0.3 (1.6–2.5)		3.2–4.5		1.6–2.4		4.2 \pm 0.3 (3.9–4.6)		1.6, 1.9		?	?	?	?	4.1*	?	?	4.4	1.9
<i>V</i>	54.2 \pm 2.6 (46–57)		–		48.1–59.6		–		55.4 \pm 0.6 (54.8–56.3)		–		59	–	57	–	55–57	–	–	55–57	–
Lip region width	12.8 \pm 1.1 (10–14)		11.8 \pm 0.8 (11–13)		12–14		10–12		12.0 \pm 0.9 (11–13)		11, 11		?	18	?	?	13*	?	?	16*	?
Stoma length	9.7 \pm 0.9 (8–11)		8.3 \pm 1.0 (7–10)		9–11		8–10		9.7 \pm 0.8 (8–10)		9, 10		?	?	9	8	10*	?	?	10*	?
Stoma width	5.3 \pm 0.5 (5–6)		5.0 \pm 0.0 (5–5)		5–7		5–6		5.2 \pm 0.4 (5–6)		4, 5		?	?	?	?	5*	?	?	7*	?
Pharyngeal corpus length	118.9 \pm 7.4 (108–132)		115.0 \pm 9.8 (105–130)		95–121		94–118		106.2 \pm 2.7 (102–109)		120, 123		?	?	?	?	?	?	?	135*	?
Isthmus length	60.2 \pm 7.3 (51–71)		59.5 \pm 6.3 (50–67)		40–69		50–60		59.0 \pm 4.9 (50–65)		65, 66		?	?	?	?	?	?	?	65*	?
Bulbus length	23.7 \pm 1.4 (22–26)		23.0 \pm 2.1 (20–25)		17–28		19–23		21.3 \pm 2.0 (20–25)		22, 23		?	?	?	?	?	?	?	33*	?
Pharynx length	202.5 \pm 11.5 (182–221)		197.5 \pm 14.9 (177–216)		157–218		164–200		187.0 \pm 8.0 (175–192)		208, 211		?	?	?	?	?	?	?	233*	?
Nerve ring–anterior end	150.0 \pm 10.8 (135–160)		136.8 \pm 4.3 (131–140)		120–155		130–140		133.0 \pm 7.4 (125–140)		123, 123		?	?	?	?	?	?	?	160*	?
Excretory pore–anterior end	154.3 \pm 13.2 (138–165)		160.0 \pm 7.3 (151–169)		140–173		149–152		149.8 \pm 7.9 (140–160)		143, 171		?	?	?	?	?	?	?	186*	?
Deirid–anterior end	163.5 \pm 14.8 (153–174)		?		?		?		160.5 \pm 4.9 (157–164)		?		?	?	?	?	?	?	?	?	?

(Continued)

Table 1. (Continued.)

Reference	1						2		3		4		5	6							
Country	Czech Republic				Corsica (France)		Brasil		Austria		Quebec (Canada)		France	USA							
Locality	Soberšice (Brno)		Several		Mt. Incudine		Sao Paulo		?		Lac Tremblant		Maures	?							
Habitat	<i>Pinus sylvestris</i>		Several		<i>Pinus nigra</i>		Moss		Bark beetles		Bark beetles		Pine	Bark beetles							
<i>n</i>	12♀♀		6♂♂		15♀♀		11♂♂		6♀♀		2♂♂		♀	♂	♀	♂	10 ♀♀	9 ♂♂	?♂♂	?♀♀	?♂♂
Neck length (stoma + pharynx)	212.3 ± 11.3 (193–230)		205.8 ± 15.3 (186–224)		166–229		173–209		196.5 ± 7.8 (185–202)		217, 221		?	?	173	164	?	?	?	243*	?
Body diameter at neck base	24.7 ± 2.2 (18–27)		22.7 ± 0.8 (21–23)		20–28		21–25		23.8 ± 0.8 (23–25)		21, 24		?	?	?	?	?	?	?	31*	?
Body diameter at midbody	28.5 ± 2.6 (23–32)		24.5 ± 0.8 (23–25)		22–32		22–26		26.7 ± 2.4 (25–31)		22, 25		?	?	25	20	40*	22*	?	37*	?
Lateral field width	3.0 ± 0.0 (3)		3.5 ± 0.7 (3–4)		3		3		3.5 ± 0.7 (3–4)		?		?	?	?	?	?	?	?	?	?
Anterior ovary/testis length	473.2 ± 82.7 (381–571)		131.0 ± 17.3 (111–142)		402–545		133–151		386.2 ± 36.5 (345–435)		147, 172		?	?	?	?	?	?	?	600*	?
Anterior genital branch	165.5 ± 4.9 (160–172)		–		173–190		–		170.4 ± 23.4 (145–205)		–		?	–	?	–	169*	–	–	210*	–
Postvulval sac length	42.6 ± 5.5 (37–55)		–		34–45		–		36.8 ± 4.4 (32–42)		–		?	–	?	–	31*	–	–	29*	–
Vagina length	10.3 ± 0.5 (10–11)		–		9–11		–		9.5 ± 0.5 (9–10)		–		?	–	?	–	15*	–	–	6*	–
Vulva–anterior end	701.8 ± 60.0 (623–818)		–		578–825		–		639.3 ± 50.5 (578–727)		–		?	–	?	–	?	–	–	?	–
Rectum length	29.7 ± 2.7 (23–35)		27 (n = 1)		21–30		25–28		28.0 ± 0.9 (27–29)		?		?	?	?	?	24*	?	?	31*	–
Anal body diameter	15.9 ± 1.3 (14–18)		24.2 ± 1.5 (22–26)		13–19		20–28		15.3 ± 0.5 (15–16)		22, 24		?	?	?	?	18*	?	?	19*	23*
Tail length	69.8 ± 7.1 (59–80)		45.3 ± 5.4 (40–54)		50–75		40–58		66.0 ± 5.2 (59–71)		36, 45		?	103**	67	35	73*	62*	?	85*	45*
Phasmid–anus distance	42.3 ± 2.9 (40–46)		27.7 ± 4.9 (22–31)		33–50		23–30		38.8 ± 3.6 (34–43)		24, 25		?	?	?	?	43*	?	?	?	?
Length of spicules	–		29.3 ± 2.1 (27–32)		–		27–32		–		25, 25		–	45	–	22	–	33*	?	–	29*
Gubernaculum length	–		12.0 ± 1.3 (11–14)		–		12–15		–		10, 13		–	19**	–	13	–	15*	?	–	18*

References: 1, present paper; 2, Rahm (1928, 1929); 3, Fuchs (1938); 4, Sanwal (1960); 5, Laumond & Carle (1971); 6, Massey (1974).

a = body length/body diameter; *b* = body length/pharynx length; *c* = body length/tail length; *c'* = tail length/anal body diameter; *V* = (distance from anterior region to vulva/body length) × 100.

*Measurement taken from drawings. ** Measurement taken from other measurements. – Character absent. ? Unknown measurement.

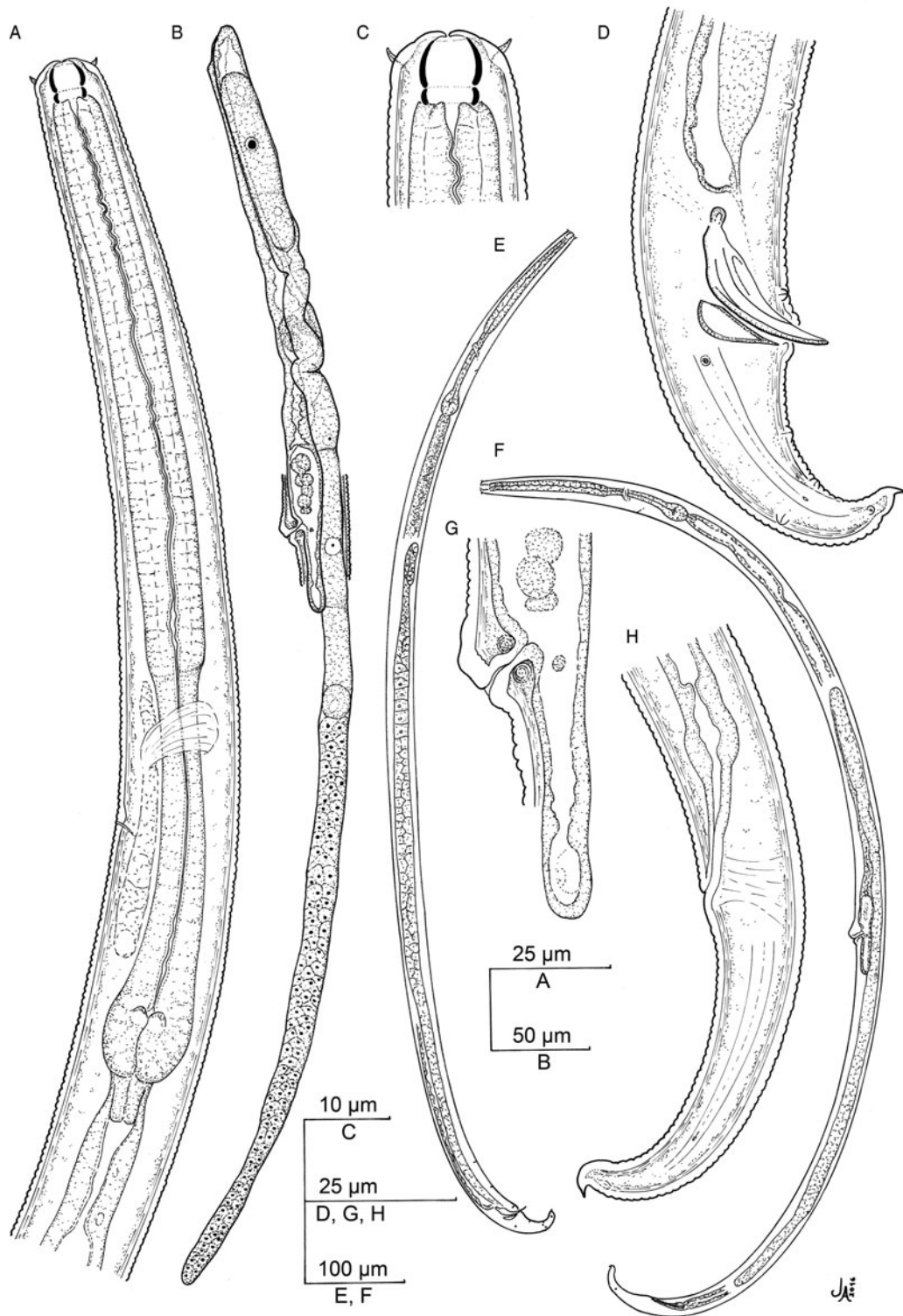


Fig. 1. *Macrolaimus canadensis* Sanwal, 1960: (A) neck; (B) female genital system; (C) stoma; (D) male posterior end; (E) entire male; (F) entire female; (G) vaginal region; (H) female posterior end.

Černá za Bory, Dívčí Hrad, Hodonín, Jevíčko, Město Albrechtice, Orlovice, Proskovice (Ostrava), Ptení, Rovensko, Salajna, Skřípov and Soběšice (Brno)) in the Czech Republic and one locality (Incudine Mount) in Corsica. The species was associated with the bark of *Larix decidua* Mill., *Pinus nigra* var. *corsicana* (Loudon) Hyl., *P. sylvestris* L. and *Pinus* sp.

Remarks

This species was previously found in Brazil by Rahm (1928, 1929), Germany by Fuchs (1938), Canada by Sanwal (1960), France by Laumond & Carle (1971) and the USA by Massey (1974). The specimens examined during this study, originating from the Czech Republic and Corsica, are similar to those from Brazil, Germany,

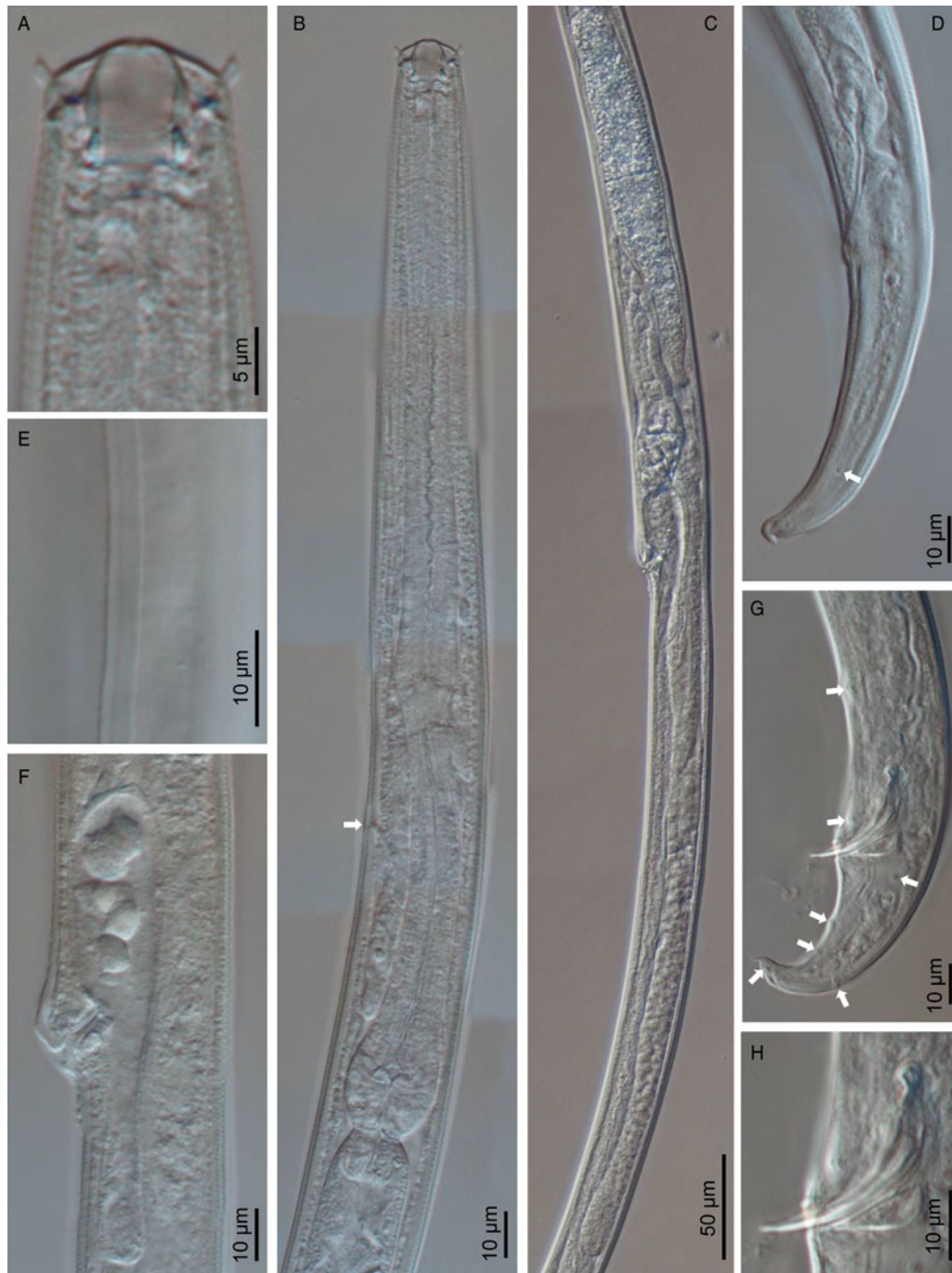


Fig. 2. *Macrolaimus canadensis* Sanwal, 1960 (light microscopy): (A) stoma; (B) neck (arrow pointing the excretory pore); (C) female reproductive system; (D) female posterior end (arrow pointing to the phasmid); (E) lateral field; (F) vaginal region; (G) male posterior end (arrows pointing to the genital papillae); (H) spicules and gubernaculum.

Canada, France and the USA. However, in general, specimens originating from the Czech Republic showed greater ranges of measurement values for the various characteristics (see table 1). This is the first record of the genus and species from the Czech Republic.

Macrolaimus ruehmi Andr ssy, 1966

Material examined

Twenty-four females and 19 males, originating from the Czech Republic.

Description

Measurements. See table 2.

Adults. See figs 4–6. Moderately slender nematodes of small size, 0.90–1.59 mm long. Body cylindrical, tapering towards both extremities, more towards the posterior end. Habitus curved ventrad after fixation, to an open ‘C’ shape or sigmoid. Cuticle c. 1 µm thick, with very fine annuli, c. 1 µm wide, sometimes appearing divided somewhere in its outline, hardly discernible with LM, but readily visible with SEM. Lateral field covers 9–15% of body diameter, consisting of two wings (three longitudinal incisures)



Fig. 3. *Macrolaimus canadensis* Sanwal, 1960 (scanning electron microscopy): (A) anterior end (black arrow pointing to the excretory pore, white arrow pointing to the right deirid); (B, C, D) lip region in subventral, lateral and frontal views, respectively (arrows pointing to the amphids); (E) lateral field; (F) deirid (white arrow) and excretory pore (black arrow); (G, H) male posterior end in ventral and lateral views, respectively (arrow pointing to the phasmid).

with transverse incomplete striations, starting at level of anterior part of corpus and ending at tail tip. Lip region convex, continuous with the adjacent body, with the oral region strongly elevated or protruding; lips fused, developing six curved, triangular liplets covering the oral aperture; anterior sensillae arranged in two circles, with six conoid labial setae *c.* as long as two annuli wide and projecting forward, and located at the margin of the oral field, and four (two subdorsal and two subventral) very small cephalic papillae situated beside the labial setae. Amphidial aperture small, oval, located at base of lateral lips. Stoma 1.7–2.5 times

longer than wide and 0.7–1.1 times as long as lip region diameter, heavily sclerotized, subdivided into cheilo-, gymno- and stegostom; cheilostom *c.* 50% of the stoma length, having cheilorhabdia strongly sclerotized, convergent (curved) and thinner anteriorly; gymnostom long, slightly shorter than the cheilostom, with slightly arched gymnorhabdia having a constriction outside at their middle length; buccal cavity (= cheilo-gymnostom) 1.8 times longer than wide; stegostom one-half of the cheilostom length, with funnel-shaped lumen, enveloped by the anterior end of pharynx, lacking visible rhabdia; dorsal pharyngeal gland

Table 2. Morphometrics of *M. ruehmi* Andrassy, 1966. Measurements in micrometres (µm) and in the form: mean ± standard deviation (range) where appropriate.

Reference	1				2		3		
	Czech Republic		Bosnia and Herzegovina		Germany		Germany		
Country	Czech Republic		Bosnia and Herzegovina		Germany		Germany		
Locality	Horka nad Moravou		Severál		Banja Luka		?		
Habitat	<i>Quercus</i> sp.		Severál		Pine		Bark beetles		
<i>n</i>	10♀♀	12♂♂	14♀♀	7♂♂	2 ♀♀	?♀♀	?♂♂	?♀♀	?♂♂
Body length	1077.7 ± 117.2 (903–1292)	1020.8 ± 65.6 (922–1116)	1136–1590	1006–1357	1180, 1190	?	?	800–1000	700
<i>a</i>	35.4 ± 4.5 (27.6–46.1)	38.4 ± 2.4 (35.1–43.9)	32.6–47.4	37.3–47.0	39.1, 38.7	?	?	22.0–36.9	32.0–35.7
<i>b</i>	5.0 ± 0.6 (4.3–6.0)	4.9 ± 0.3 (4.6–5.4)	5.5–7.0	4.8–6.7	5.1, 4.9	?	?	4.0–5.7	4.0–4.3
<i>c</i>	18.6 ± 1.0 (16.1–20.1)	20.4 ± 2.2 (16.7–24.1)	17.5–22.5	18.6–22.7	16.7, 17.4	?	?	13.7–15.0	14.5–20.4
<i>c'</i>	3.3 ± 0.2 (2.9–3.5)	2.1 ± 0.2 (1.8–2.6)	3.2–4.0	2.1–2.6	4.1, 3.8	2.1**	1.9*	2.1**	1.9**
<i>V</i>	56.7 ± 3.1 (53–65)	–	50.5–58.2	–	57, 56	?	–	55–58	–
Lip region width	11.9 ± 0.7 (11–13)	10.0 ± 0.0 (10)	10–14	10–12	15, 13	14*	?	?	?
Stoma length	10.3 ± 0.8 (9–12)	9.6 ± 0.5 (9–10)	9–11	8–10	13, 12	11*	?	?	?
Stoma width	5.0 ± 0.5 (4–6)	4.8 ± 0.4 (4–5)	4–6	4–5	4, 5	5*	?	?	?
Pharyngeal corpus length	116.1 ± 7.9 (105–128)	109.9 ± 3.6 (103–114)	102–122	105–115	132, 131	99*	?	?	?
Isthmus length	61.0 ± 3.6 (55–68)	56.9 ± 4.2 (50–65)	49–68	55–67	57, 71	48*	?	?	?
Bulbus length	30.9 ± 3.4 (26–36)	27.2 ± 2.7 (22–30)	21–35	22–30	30, 29	40*	?	?	?
Pharynx length	208.0 ± 12.4 (194–230)	194.0 ± 6.1 (185–204)	177–225	191–203	219, 231	227*	?	?	?
Nerve ring–anterior end	131.3 ± 0.6 (131–132)	131.3 ± 8.4 (119–142)	140–160	128–145	166, 159	159*	?	?	?
Excretory pore–anterior end	145.3 ± 10.4 (135–165)	145.5 ± 8.8 (136–157)	155–184	150–172	175, 171	164*	?	?	?
Deirid–anterior end	150 (<i>n</i> = 1)	?	?	?	?	?	?	?	?
Neck length (stoma + pharynx)	218.3 ± 12.3 (206–240)	203.6 ± 5.8 (195–213)	187–235	199–212	232, 243	238*	?	?	?

Body diameter at neck base	28.8 ± 2.0 (25–31)	25.7 ± 1.5 (23–28)	27–34	21–28	29, 30	?	?	?	?
Body diameter at midbody	30.7 ± 3.7 (26–39)	26.2 ± 1.2 (25–29)	30–41	25–32	30, 31	?	?	?	?
Lateral field width	3.1 ± 0.4 (3–4)	3.0 ± 0.0 (3)	3	3	4, 5	?	?	?	?
Anterior ovary/testis length	418.3 ± 56.5 (370–500)	171.8 ± 20.8 (130–185)	480–480	138–190	599, 595	?	–	?	–
Anterior genital branch	218.3 ± 23.3 (190–255)	–	260–260	–	229, 237	?	–	?	–
Postvulval sac length	40.4 ± 6.0 (30–50)	–	40–45	–	43, 50	?	–	?	–
Vagina length	10.4 ± 0.7 (10–12)	–	10–15	–	11, 12	?	–	?	–
Vulva–anterior end	608.1 ± 46.3 (558–688)	–	630–896	–	676, 666	?	–	?	–
Rectum length	29.3 ± 4.8 (22–40)	27.0 ± 2.6 (25–30)	27–33	20–26	36, 37	?	?	?	?
Anal body diameter	17.8 ± 1.6 (15–20)	23.4 ± 1.6 (22–27)	16–21	21–28	17, 18	28*	26*	?	?
Tail length	58.0 ± 5.7 (52–69)	49.6 ± 4.9 (45–60)	53–80	52–63	71, 69	59*	51*	–	?
Phasmid–anus distance	36.1 ± 4.0 (30–43)	30.5 ± 2.9 (27–34)	40–46	32–37	40, 39	?	35*	–	?
Length of spicules	–	29.8 ± 1.0 (28–31)	–	27–31	–	–	31*		
Gubernaculum length	–	12.7 ± 1.1 (11–14)	–	11–12	–	–	16*		

References: 1, present paper; 2, Rühm (1956); 3, Meyl (1960).

a, *b*, *c*, *c'*, *V*, see table 1.

*Measurement taken from drawings. **Measurement taken from other measurements. – Character absent. ? Unknown measurement.

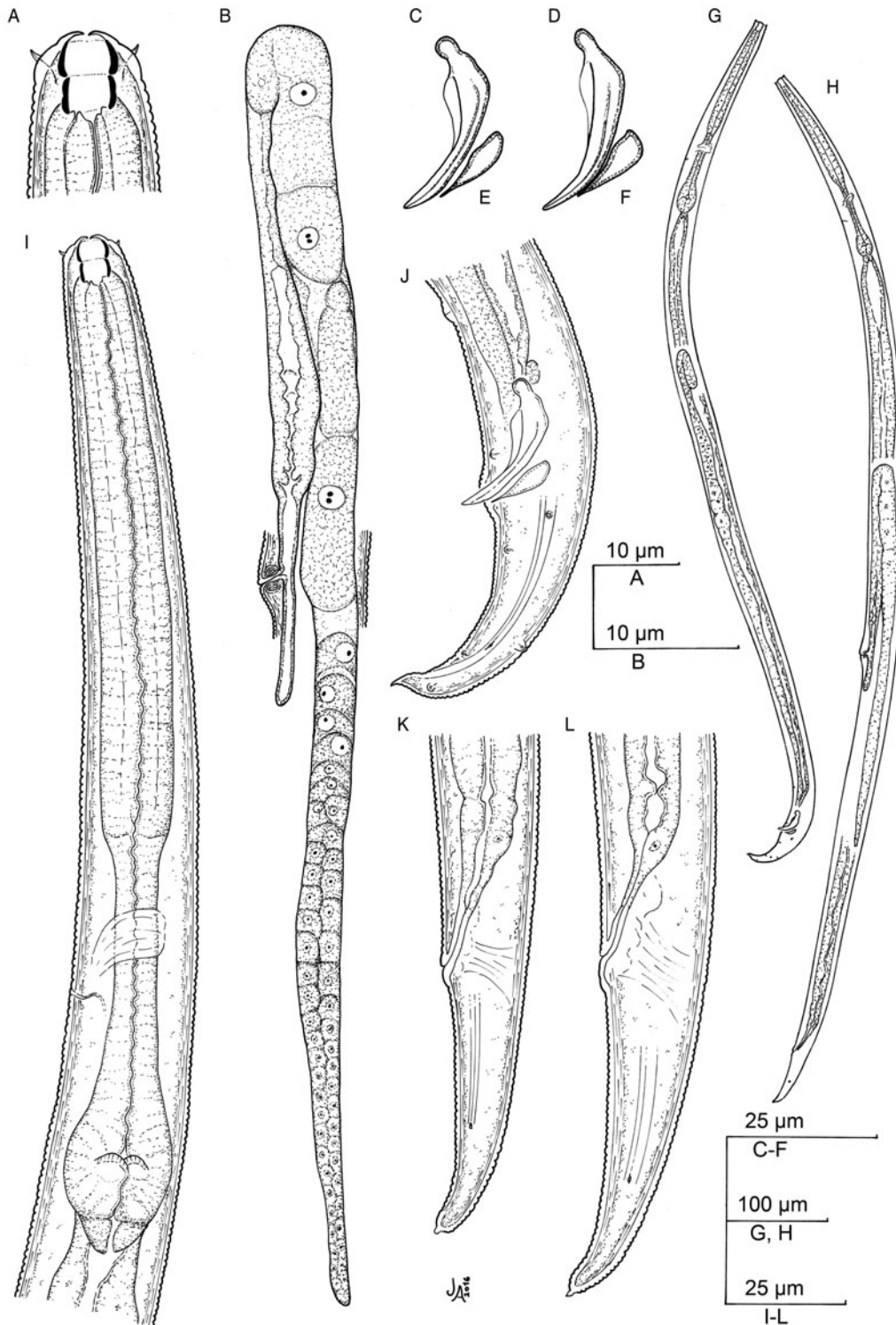


Fig. 4. *Macrolaimus ruehmi* Andr ssy, 1966: (A) stoma; (B) female genital system; (C, D) spicule; (E, F) gubernaculum; (G) entire male; (H) entire female; (I) neck; (J) male posterior end; (K, L) female posterior end.

opening at base of stegostom. Pharynx cephaloboid: pharyngeal corpus cylindrical, 1.6–2.3 times longer than isthmus, with procorpus and metacarpus similar in width; isthmus narrower and clearly delimited from corpus; basal bulb nearly pyriform, with strongly developed valvular apparatus located at its posterior half; pharyngeal gland nuclei obscure. Cardia short and conoid.

Nerve ring at 61–75% of neck length, encircling pharynx at level of anterior half of isthmus. Secretory–excretory gland cell located ventrally to isthmus and basal bulb; excretory pore located posterior to nerve ring, at 66–86% of neck length, at level of isthmus. Hemizonid at level of excretory pore. Deirid located posterior to nerve ring, inside the lateral field, at 71% of neck length, six



Fig. 5. *Macrolaimus ruehmi* Andrassy, 1966 (light microscopy): (A) neck (arrow pointing to the excretory pore); (B) stoma; (C) female genital system; (D) male posterior end (white arrows pointing to the genital papillae, black arrow pointing to the phasmid); (E) lateral field; (F) testis; (G) female posterior end (arrow pointing to the phasmid).

or seven annuli posterior to excretory pore, inconspicuous under light microscope.

Female. Reproductive system monodelphic–prodelphic. Ovary reflexed near to oviduct junction, very long, surpassing the level of the vulva; oocytes first situated in two rows, then in only one row. Oviduct narrow and short; no distinct narrowing is observed at ovary–oviduct junction. Uterus located on right-hand side of intestine, with two sections separated by a weak internal narrowing: the distal part is long, four times longer than the body width, tubular, whereas the proximal part (ovjector) is short and with

thin walls. Uterine eggs were absent in the specimens examined. Postvulval uterine sac present, 1.0–1.8 times longer than the body width at its level. Vagina occupying a quarter (29–40%) of body diameter, slightly bent forward. Vulva a transverse slit, with distinctly protruded lips that form a vulval cone; advulval cuticle lacking differentiations. Rectum 1.3–2.2 times longer than anal body diameter, its anterior part with walls including gland-like cells; anal lips not prominent. Tail conical, almost straight but ventrad curved at posterior part, tapering very gradually and ending in a short wart-like mucro. Phasmids posterior, at 53–65% of tail length from anus.

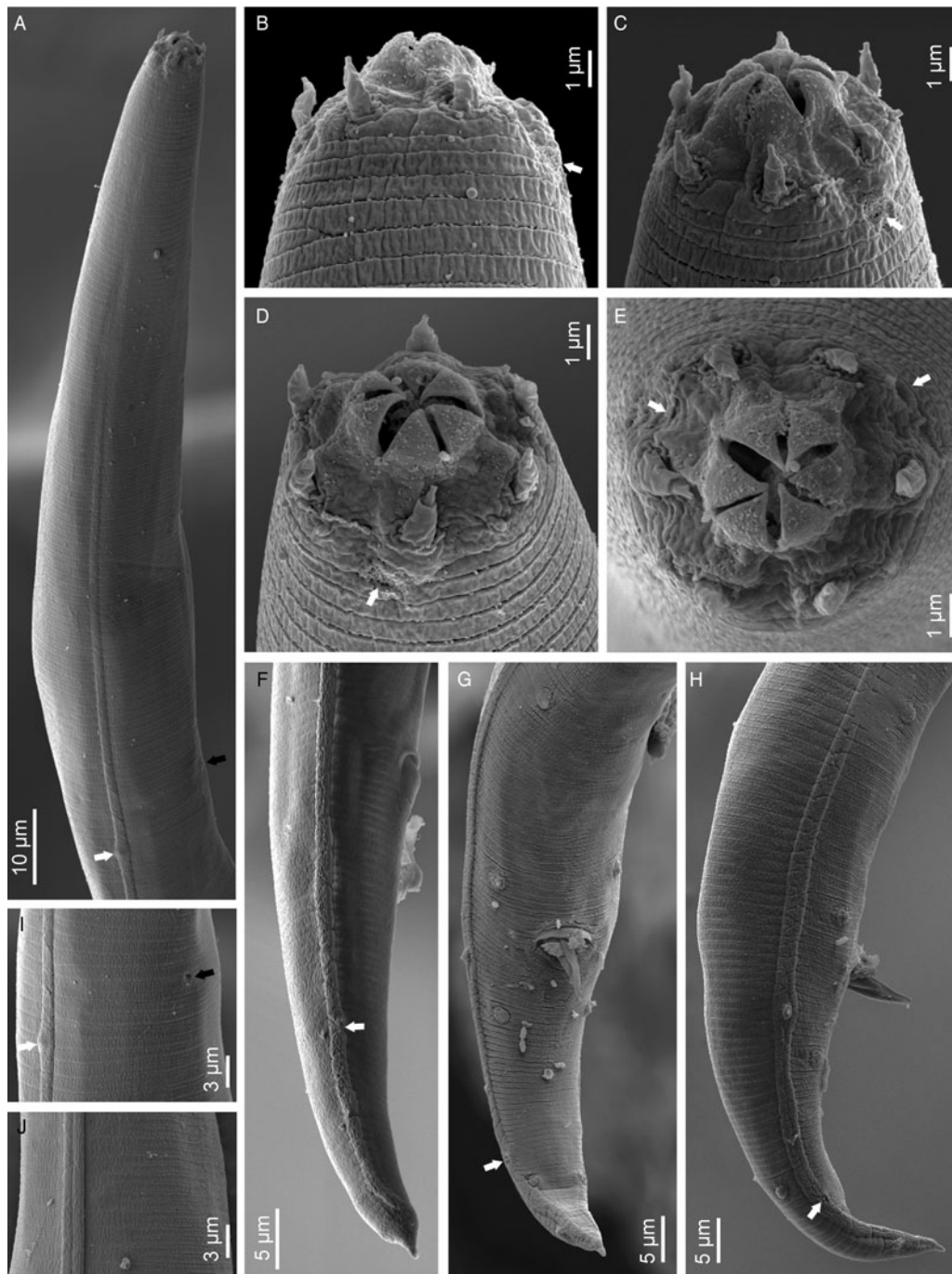


Fig. 6. *Macrolaimus ruehmi* Andr ssy, 1966 (scanning electron microscopy): (A) anterior end (black arrow pointing to the excretory pore, white arrow pointing to the right deirid); (B–E) lip region in ventral, subdorsal, lateral and frontal views, respectively (arrows pointing to the amphids); (F) female posterior end (arrow pointing to the phasmid); (G, H) male posterior end in ventral and lateral views, respectively (arrows pointing to the phasmid); (I) deirid (white arrow) and excretory pore (black arrow); (J) lateral field.

Male. Reproductive system monorchic, with testis reflexed dorsad, on right-hand side of intestine. Spicules paired and symmetrical, 5.0–7.8 times longer than wide, curved ventrad with rounded manubrium, conoid calamus, ventrad curved lamina with well-developed proximal dorsal hump and small velum at ventral side. Gubernaculum deltoid, almost straight and acute distally. Gland-like cells are poorly distinguishable around the rectum. Genital papillae consist of two subventral, precloacal pairs and five postcloacal pairs arranged as follows: one lateral

pair located at lateral field and slightly posterior to the level of cloacal aperture; two subventral pairs, one of them bigger at the anterior third and another smaller at the posterior third to tail length; and two subdorsal pairs at the posterior half of tail, the anterior one bigger. A very reduced midventral papilla is located in front of the anterior lip of cloacal aperture. Tail conoid and tapering gradually and curved ventrad, ending in a short conoid mucro. Phasmids located at posterior half of tail, at 56–76% from anus.

Table 3. Morphometrics of *M. crucis* Maupas, 1900. Measurements in micrometres (μm) and in the form: mean \pm standard deviation (range) where appropriate.

Reference	1				2		3		
Country	Spain				Algeria		Spain		
Locality	Salinas		Cartagena		Aguilas	Salobreña	Wadi Zoubia	Salinas	
Habitat	Coastal dune		Coastal dune		Coastal dune	Rocky coast	Salty soil	Salty soil	
<i>n</i>	5 ♀♀	5 ♂♂	2 ♀♀	♂	♀	♂	♀	15 ♀♀	6 ♂♂
Body length	781.6 \pm 41.6 (721–831)	754.2 \pm 20.4 (720–772)	851, 857	987	857	912	958	654–863	654–767
<i>a</i>	28.4 \pm 2.1 (26.8–32.0)	31.4 \pm 3.2 (29.0–36.8)	28.4, 29.6	38.0	33.0	39.7	22.8**	23.4–30.0	29.7–33.3
<i>b</i>	4.9 \pm 0.3 (4.4–5.2)	5.0 \pm 0.2 (4.7–5.2)	5.1, 5.3	6.1	5.3	5.5	4.8**	4.0–5.8	4.1–4.8
<i>c</i>	14.9 \pm 0.5 (14.3–15.5)	15.5 \pm 0.5 (15.1–16.2)	15.2, 15.9	19.7	17.1	15.2	16.0**	13.1–4.0	14.6–17.2
<i>c'</i>	3.2 \pm 0.3 (2.9–3.6)	2.5 \pm 0.1 (2.5–2.6)	3.3, 3.4	2.5	3.3	3.0	3.3*	3.1–4.0	2.0–2.6
<i>V</i>	58.0 \pm 0.6 (57–59)	–	57, 59	–	55	–	58**	55–61	–
Lip region width	14.2 \pm 0.4 (14–15)	12.2 \pm 1.3 (11–14)	15, 14	14	13	13	10	13–18	12–14
Stoma length	10.0 \pm 0.7 (9–11)	7.8 \pm 1.3 (6–9)	9, 10	10	10	11	?	9–13	10–11
Stoma width	8.0 \pm 0.7 (7–9)	5.2 \pm 0.4 (5–6)	6, 6	6	5	5	?	6–11	6–11
Pharyngeal corpus length	84.6 \pm 1.8 (82–87)	78.4 \pm 2.6 (76–82)	86, 84	82	87	80	?	77–93	72–82
Isthmus length	38.2 \pm 3.4 (35–44)	39.8 \pm 2.3 (36–42)	44, 42	41	41	47	?	33–50	37–50
Bulbus length	26.8 \pm 1.8 (26–30)	25.0 \pm 1.2 (24–27)	28, 27	28	12	28	?	24–30	20–25
Pharynx length	149.6 \pm 3.8 (146–155)	143.2 \pm 2.2 (140–146)	158, 153	151	151	155	?	134–173	133–156
Nerve ring–anterior end	105.8 \pm 3.0 (102–110)	104.4 \pm 4.6 (100–112)	111, 110	114	120	119	?	104–139	104–124
Excretory pore–anterior end	113.8 \pm 7.4 (101–120)	112.6 \pm 5.5 (107–121)	115, 115	124	115	130	?	109–139	108–126
Deirid–anterior end	?	127.5 \pm 3.5 (125–130)	?, ?	?	?	?	?	116–140	120–128
Neck length (stoma + pharynx)	159.6 \pm 4.2 (155–165)	151.0 \pm 3.2 (146–154)	167, 163	161	161	166	200	140–188	144–171
Body diameter at neck base	26.0 \pm 1.7 (25–29)	23.6 \pm 1.1 (22–25)	28, 28	26	25	22	?	22–26	20–22
Body diameter at midbody	27.6 \pm 1.8 (26–30)	24.2 \pm 2.2 (21–26)	30, 29	26	26	23	42	23–29	21–23
Lateral field width	?	?	6, ?	?	3	?	?	2.0–3.0	2.5
Anterior ovary/testis length	305.0 \pm 15.0 (290–320)	160.0 \pm 21.2 (145–175)	283, ?	116	350	?	?	213–376	148–180
Anterior genital branch	140.0 \pm 18.0 (125–160)	–	128, ?	392	145	–	?	96–165	–
Postvulval sac length	2.5 \pm 3.5 (0–5)	–	0, 0	–	0	–	?	0–6	–
Vagina length	10.0 \pm 0.7 (9–11)	–	10, 10	–	10	–	?	9–10	–
Vulva–anterior end	453.0 \pm 24.9 (422–487)	–	486, 503	–	474	–	557	370–497	–
Rectum length	22.4 \pm 2.4 (20–25)	?	19, 24	7	25	?	?	21–26	5–9

(Continued)

Table 3. (Continued.)

Reference	1		2		3	
	Spain		Algeria		Spain	
Country	Spain		Algeria		Spain	
Locality	Salinas	Cartagena	Aguilas	Salobreña	Wadi Zoubia	Salinas
Habitat	Coastal dune	Coastal dune	Coastal dune	Rocky coast	Salty soil	Salty soil
<i>n</i>	5 ♀♀	2 ♀♀	♀	♂	♀	6 ♂♂
Anal body diameter	16.4 ± 0.9 (15–17)	17, 16	15	20	?	13–18
Tail length	52.6 ± 3.3 (50–58)	56, 54	50	60	60	38–48
Phasmid–anus distance	38.6 ± 1.8 (37–41)	35, 35	33	38	?	15–18 ¹
Length of spicules	–	–	–	30	–	26–38
Gubernaculum length	–	–	–	14	–	10–13

References: 1, present paper; 2, Maupas (1900); 3, Abolafia & Peña-Santiago (2014).

¹ SEM observations in the present study indicate that this measurement is a mistake.

a, b, c, c', V, see table 1.

*Measurement taken from drawings. **Measurement taken from other measurements. – Character absent. ? Unknown measurement.

Other material examined

The material, representing two females that were obtained from Bosnia and Herzegovina, was similar to the specimens from the Czech Republic. However, these specimens had slightly bigger stoma (1.7–2.5 vs. 2.6–3.0 times longer than wide) than those from the Czech Republic.

Localities and habitats

The species was collected at six localities (Benkov u Střelice, Horka nad Moravou, Hrušky, Ladín, Olomouc and Znojmo) in the Czech Republic and one locality (Banja Luka) in Bosnia and Herzegovina. The specimens were associated with the bark of *Betula pendula* L., *Fraxinus* sp., *Prunus armeniaca* L., *Pinus nigra* L., *P. silvestris* L., *Quercus* sp. and *Tilia* sp.

Remarks

Specimens of *M. ruehmi* from the Czech Republic are similar to *M. crucis* specimens of German populations previously studied by Rühm (1956) and Meyl (1960), which have been renamed as *M. ruehmi* by Andrassy (1966). This is the first record of the species from the Czech Republic and Bosnia and Herzegovina.

Macrolaimus crucis Maupas, 1900

Material examined

Eight females and seven males, originating from Spain.

Description

Measurements. See table 3.

Diagnostic description (additional data from Abolafia & Peña-Santiago (2014)). The Spanish specimens of *M. crucis* (fig. 7) examined during this study are characterized by the body length (0.72–0.86 mm in females and 0.72–0.99 mm in males); lip region 11–15 µm wide and bearing six short, seta-like papillae; stoma 6–11 × 5–9 µm with gymnostom slightly shorter than cheilostom; neck 146–167 µm long; pharyngeal corpus 1.7–2.4 times isthmus length; excretory pore located at isthmus level; female reproductive system monodelphic–prodelphic; postvulval uterine sac absent; female tail conical (50–58 µm, $c = 14.3–17.1$, $c' = 2.9–3.6$) with acute tip bent dorsad; phasmids located at 63–76% of tail length from anus; male tail conical and curved ventrad (47–60 µm, $c = 15.1–19.7$, $c' = 2.5–3.0$) with phasmids located at 57–64% of tail length; spicules 27–34 µm long and gubernaculum 11–13 µm long with slightly fusiform corpus.

Localities and habitats

Macrolaimus crucis has been collected from four littoral localities in the southern Iberian Peninsula: 1) sandy soil, close to the beach near Salinas de Cabo de Gata (Almería Province) where *Lycium intricatum* Boiss., *Lygeum spartum* (L.) Kunth, *Ononis talaverae* Devesa & G. López and *Thymelaea hirsuta* (L.) Endl. grew; (2) dry soil from a rocky area close to the beach near Salobreña (Granada Province) where *L. intricatum* grew; (3) sandy soil from coastal dunes near Águilas (Murcia Province) and (4) near Cartagena (Murcia Province) where *Ammophila arenaria* (L.) Link. grew.

Remarks

The specimens examined are similar to those studied by Maupas (1900) and Abolafia & Peña-Santiago (2014). Since the latter authors provided a detailed description of the species from the southern Iberian Peninsula, there is no need to repeat the

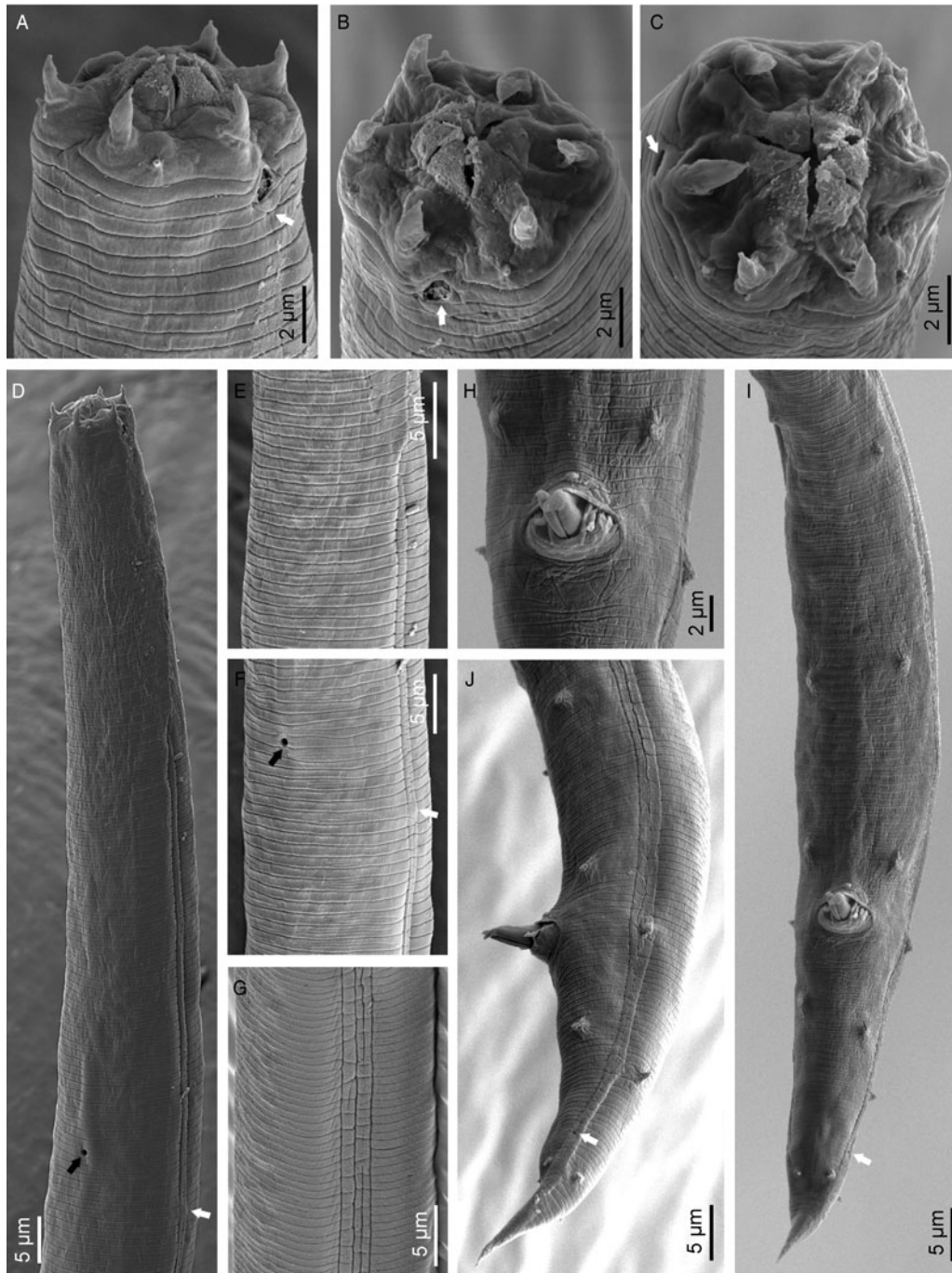


Fig. 7. *Macrolaimus crucis* Maupas, 1900 (male, scanning electron microscopy): (A–C) lip region in subventral, lateral and frontal views, respectively; (D) anterior end (black arrow pointing to the excretory pore, white arrow pointing to the right deirid); (E–G) lateral field at anterior end, deirid level and midbody level, respectively (black arrow pointing to the excretory pore, white arrow pointing to the right deirid); (H) cloacal opening region; (I, J) male posterior end in ventral and lateral views, respectively (arrows pointing to the phasmid).

description here. The SEM study revealed that the phasmid position on the male tail is posterior, not anterior, as was reported previously by the latter Spanish authors, using LM. As a result of the present study, the distribution area of this species in the southern Iberian Peninsula of Spain has been expanded.

Discussion

The study of these three species provides new and useful information on the morphology of the genus. Regarding the lip region,

the morphology of these species agrees well with the results of the studies conducted for *M. arboreus* Truskova & Eroshenko, 1977 by Shokoochi *et al.* (2018), *M. crucis* by Abolafia & Peña-Santiago (2014) and *M. richteri* Swart & Heyns, 1992 (Swart & Heyns, 1992). However, some structures of the three species examined during this study are slightly different. For example, the morphology of the postvulval sac is slightly longer than the body diameter of *M. canadensis* and *M. ruehmi* vs. very reduced for *M. crucis*; the number of preloacal papillae, of which two pairs were discernible for *M. canadensis* and *M.*

ruehmi vs. three pairs for *M. crucis*; spicules with a dorsal hump being present in *M. canadensis* and *M. ruehmi* males; and the gubernacula being deltoid in males of *M. canadensis* and *M. ruehmi* vs. slightly fusiform for *M. crucis* males. Such data confirm that more similarities occur among specimens of *M. canadensis* and *M. ruehmi* in terms of their morphology.

Regarding the number of preloacal papillae, specimens of all species of the genus *Macrolaimus* have four pairs, except those of *M. natator* Timm, 1960. Specimens of the latter species have three pairs of preloacal papillae (Timm, 1960), which coincides with the description of *M. crucis* that originated from the southern Iberian Peninsula of Spain, as noted in the present study. The number of genital papillae is reduced in some lineages (Osche, 1958; Chabaud & Petter, 1961). Within the family Chambersiellidae, specimens of the genus *Diastolaimus* Rahm, 1928, which is the closest to *Macrolaimus* (Nadler et al., 2006; Boström et al., 2011), have different numbers of preloacal papillae: three pairs are present in *D. croca* (Massey, 1963) Andrassy, 1984 and *D. damalis* (Massey, 1966) Andrassy, 1984 and four pairs in *D. mexicanus* Cid del Prado, 2012 (see Massey, 1963, 1966 and Cid del Prado, 2012, respectively). Furthermore, the only species of the chambersiellid genus *Geraldus* Sanwal, 1971, *G. bakeri* (Sanwal, 1957) Sanwal, 1971, has seven pairs of preloacal papillae (see Sanwal, 1957, 1971; Holovachov et al., 2003). In addition, the genus *Steinernema* Travassos, 1927, described by Travassos (1927a) and renamed later by the same author (Travassos, 1927b) and probable ancestor of Chambersiellidae (Bert et al., 2008), exhibits 7–10 pairs of preloacal genital papillae. Thus, according to this, the reduction of the number of genital papillae in *Macrolaimus* species could be ascribed to an apomorphic condition.

On the other hand, the number of female genital branches becomes reduced, from two (didelphic) to one (monodelphic) in nematode species (Chitwood & Chitwood, 1950; Lorenzen, 1978, 1981). In Chambersiellidae, females of the genera *Geraldus* and *Diastolaimus* are didelphic (plesiomorphic condition), while those of *Macrolaimus* are monodelphic (apomorphic condition). Females of the genus *Steinernema* also have didelphic genital branches.

According to the above-mentioned criteria for the genus *Macrolaimus*, *M. crucis* could be an ancestral species while *M. canadensis* and *M. ruehmi* could be derived species. Unfortunately, molecular analyses for these species could not be performed to support this interpretation.

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Conflict of interest. None.

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