

Nematodes of the order Dorylaimida from Romania: two interesting species of the subfamily Qudsianematinae Jairajpuri, 1965

Marcel Ciobanu^{1,2}, Iuliana Popovici¹ and Reyes Peña-Santiago²

¹Institute of Biological Research, Department of Plant and Animal Taxonomy and Ecology,
Str. Republicii 48, RO-400015 Cluj-Napoca, Romania
e-mail: icb@cluj.astral.ro

²Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Campus "Las Lagunillas" s/n,
Edificio B3, 23071, Jaén, Spain

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Summary. Two known species of the nematode family Qudsianematidae were studied on the basis of material collected from natural habitats in Romania. Two populations of *Labronema carusoi* are compared to the original description, with new observations that allow a better characterisation of this taxon, especially those referring the morphology of lip region and female genital system. The female of *Labronemella labiata* is reported and described for the first time. Descriptions, measurements, illustrations, including LM pictures for both species and SEM pictures for *L. carusoi*, are provided. Data concerning the occurrence of the species in Romania are also given.

Key words: Carpathians, Danube Delta, distribution, *Labronema carusoi*, *Labronemella labiata*, morphology, morphometrics, SEM, survey, taxonomy.

As a result of an extensive ecological survey on natural ecosystems aiming to evaluate the diversity of nematode communities throughout Romania, material including several genera belonging to the family Qudsianematidae was deposited in the nematode collection of the Institute of Biological Research in Cluj-Napoca.

According to Popovici *et al.* (2008), Romanian nematode fauna belonging to the genera *Labronema* Thorne, 1939 and *Labronemella* Andrassy, 1985 is poorly known: *Labronema plica* Ciobanu, Popovici & Decraemer, 2004, probably a Romanian endemic species, was reported from a salt-affected area at Cojocna/Cluj (Ciobanu *et al.*, 2004) and *Labronemella czernowitziensis* (Micoletzky, 1922) Andrassy, 2002 was originally described from Northern Moldova.

This paper provides information on the occurrence of two rare species, *Labronema carusoi* Vinciguerra & Orselli, 1998 and *Labronemella labiata* Andrassy, 1985 as new records in the Romanian fauna, and new data about them is provided for their characterisation.

MATERIAL AND METHODS

Nematodes were collected by the second author (I.P.) during two field sampling trips carried out in 1993 and 1995 in natural ecosystems located in the Eastern Romanian Carpathians and within the Danube Delta

Biosphere Reserve (see Table 1). Nematodes were extracted using the centrifugation method of De Grisse (1969), killed and preserved in a 4% formaldehyde solution, heated at 65°C and mounted in anhydrous glycerol according to Seinhorst (1959). Microphotographs were taken with a Nikon Eclipse 80i light microscope provided with differential interference contrast optics (DIC) and Nikon Digital Sight DS-U1 camera. For scanning electron microscopy (SEM), glycerol embedded nematodes in the permanent slides were first transferred, after measuring, into a drop of glycerol. Distilled water was then gradually added until nematodes were in almost pure distilled water and they were left so for 24 h. The nematodes were then initially dehydrated by passing through a gradual ethanol concentration series of 25, 30, 50, 70, 95 and 100% at intervals of 2 h, followed by an overnight dehydration in 100% ethanol, and subsequently putting them into 100% acetone for about 1 h. After critical point drying with CO₂, dried specimens coated with gold were examined with a JEOL (JSM-5800) microscope operating at 13 kV.

Data on the presence and distribution of the species were included in the Romanian nematode fauna database. The paper is also a contribution towards an inventory of the species belonging to the family Qudsianematidae in Romania.

Table 1. Site locations, vegetation and soil types of a nematological survey in Romania.

Site no.	Locality	Altitude (m)	Geographical position	Plant association*	Soil type**
1	Danube Delta	0.3	45°08'N-29°39'E	<i>Plantaginetum coronopi</i>	salt-affected sand dune
2	Danube Delta	1.5	44°50'N-29°37'E	<i>Acorellatum pannonicum</i>	salt-affected sand dune
3	Gurghiu Mts.	830	46°45'N-25°01'E	<i>Sympyto cordati-Fagetum</i>	acid brown

*according to Coldea (1991) and Popescu *et al.* (1980)**according to the Romanian System of Soil Classification (Conea *et al.*, 1980).**Table 2.** Measurements for *Labronema carusoi* Vinciguerra & Orselli, 1998 and *Labronemella labiata* Andrassy, 1985 from Romania. Measurements in µm (except L, in mm), and in the form: mean ± standard deviation (range).

Species Population	<i>Labronema carusoi</i>			<i>Labronemella labiata</i>
	Sulina Sand dunes	Sacalin Island Sand dunes		Gurghiu Valley Beech forest
Character				
n	7 ♀♀	15 ♀♀	8 ♂♂	1 ♀
L	1.52 ± 0.0 (1.38-1.65)	1.57 ± 0.1 (1.30-1.98)	1.66 ± 0.1 (1.46-1.85)	2.56
a	29.2 ± 0.9 (28.3-30.8)	30.8 ± 3.0 (27.4-36.2)	32.8 ± 4.7 (27.6-39.0)	33.2
b	5.0 ± 0.6 (4.3-6.0)	4.7 ± 0.4 (4.1-5.4)	4.9 ± 0.6 (3.9-5.6)	4.1
c	71.9 ± 9.3 (61.4-77.0)	70.6 ± 9.0 (56.1-83.3)	62.3 ± 7.5 (53.3-74.1)	94.8
c'	0.7 ± 0.0	0.7 ± 0.1 (0.6-0.8)	0.8 ± 0.1 (0.7-0.9)	0.6
V	54.0 ± 3.5 (52.1-55.8)	54.3 ± 1.5 (51.0-56.7)	-	50.6
Lip region diam.	20.0 ± 0.0 (20.0-20.0)	19.9 ± 0.8 (18-22)	19.8 ± 0.4 (19-20)	25
Odontostyle	23.4 ± 1.8 (20-25)	24.1 ± 0.9 (22.5-25)	24.2 ± 1.6 (21-25)	30
Odontophore	46.5 ± 4.2 (38-50)	45.1 ± 5.5 (38-58)	47.1 ± 4.0 (40-50)	53
Guiding ring from ant. end	15.0 ± 1.4 (12.5-17.5)	16.0 ± 1.5 (12.5-18)	15.6 ± 1.0 (15-17.5)	20
Neck length	307.1 ± 43.4 (245-373)	332.5 ± 25.0 (283-370)	339.6 ± 29.6 (288-373)	619
Pharyngeal expansion length	161.1 ± 22.5 (125-195)	159.2 ± 11.6 (137-175)	164.2 ± 15.3 (138-180)	300
Diam. at neck base	47.0 ± 1.5 (47-50)	47.0 ± 1.5 (47-50)	47.0 ± 1.5 (47-50)	78
at mid-body	52.1 ± 2.6 (48-56)	51.3 ± 4.5 (45-60)	51.2 ± 2.8 (47-55)	77
at anus	30.4 ± 1.5 (29-33)	33.3 ± 2.9 (30-40)	34.4 ± 2.6 (33-39)	45
Prerectum length	99.6 ± 18.8 (68-125)	79.8 ± 18.8 (60-110)	118.0 ± 45.0 (75-193)	95
Rectum length	33.3 ± 3.4 (28-38)	34.3 ± 4.8 (25-43)	33.8 ± 6.3 (30-45)	50
Tail length	21.3 ± 1.9 (20-23)	22.6 ± 2.5 (19-28)	26.9 ± 1.6 (25-29)	27
Spicule length	-	-	52.9 ± 4.3 (45-58)	-
Ventromedian supplements	-	-	19-23	-

DESCRIPTIONS

Labronema carusoi Vinciguerra & Orselli, 1998 (Figs. 1, 2)

Material examined: Twenty-two females and eight males from two localities, in general in good condition.

Measurements: See Table 2.

New observations (based on Romanian specimens):

Adult: Cuticle two-layered: outer layer thin and with very fine transverse striations, easier to distinguish under SEM; inner layer about as twice as thick as the outer one; cuticle thickness 2.0-3.5 µm

at anterior region, 2.5-3.5 µm in mid-body and 4.0-7.0 µm on tail. Cervical pores present, two or three dorsal and two or three ventral ones at level of odontostyle plus odontophore; lateral pores present along the whole body, but more developed in the caudal part. Lateral chord occupying about one-third of the corresponding body diameter at midbody, containing gland bodies which are very distinct in some specimens. Lip region 2.7-3.3 times as wide as high and about half of body diameter at neck base. SEM observations show that lips are mostly fused, each lip consisting of a few concentric striations with one papilla at the centre; lips are separated by radial striations extending across the oral field; outer and inner labial papillae appear close together at the

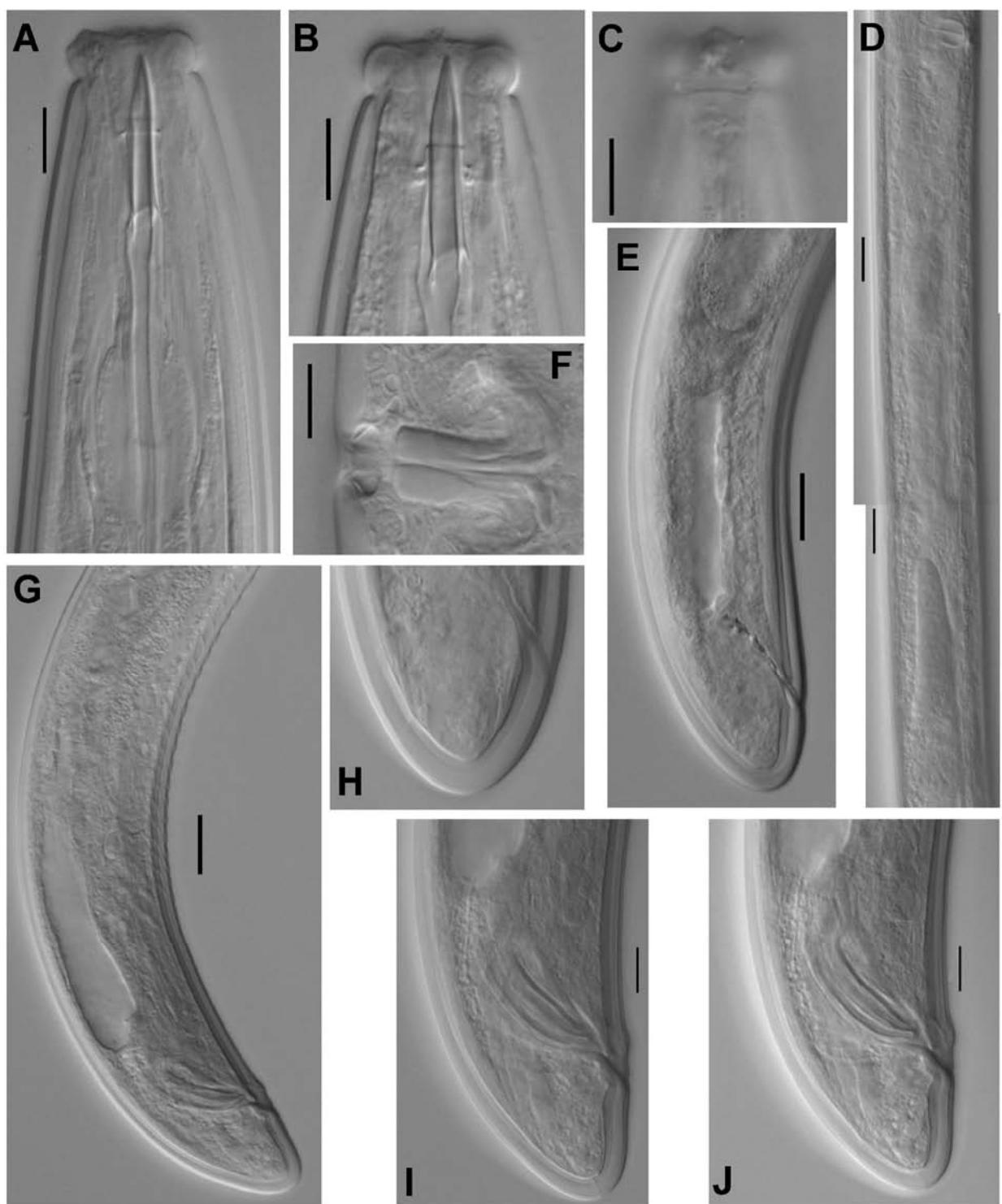


Fig. 1. *Labronema carusoi* Vinciguerra & Orselli, 1998. A, B: Anterior region in median view; C: Lip region in surface view; D: Female, posterior genital branch; E: Female, posterior body region; F: Vagina; G: Male, posterior body region; H: Female tail; I, J: Male, caudal region and spicules. (Scale bar: A-C, F, H-J = 10 μ m; D, E, G = 20 μ m).

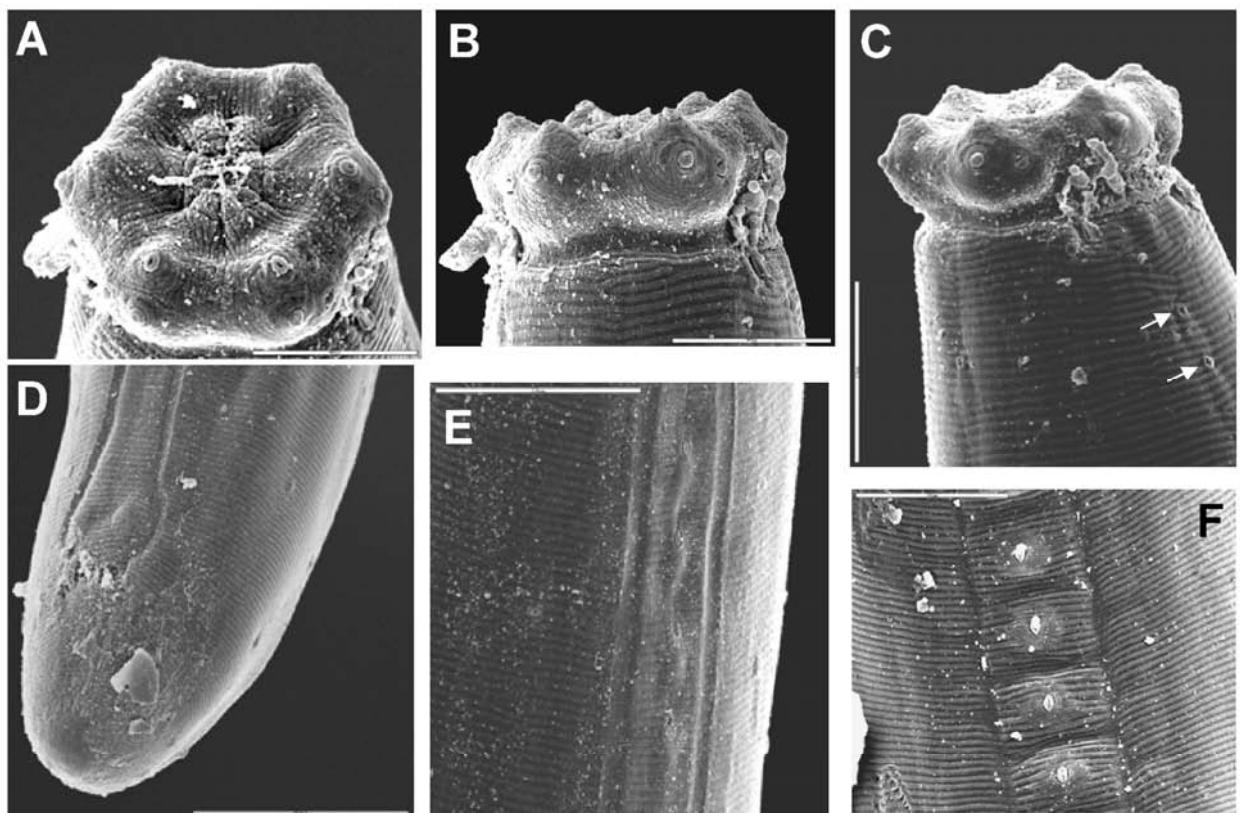


Fig. 2. *Labronema carusoi* Vinciguerra & Orselli, 1998 (SEM). A: Lip region, in frontal view; B, C: Same in dorsal or ventral view (arrow heads pointing to lateral body pores); D: Male caudal region in ventral view; E: Male posterior region showing the most anterior ventromedian supplements; F: Same showing contiguous ventromedian supplements. (Scale bar: 10 μ m).

margin of lip region; and perioral area divided into six sectors differentiated as small, low liplets. Amphid opening cup-like, occupying 8.0-12.5 μ m or about half of lip region width. Odontostyle 7-8 times as long as wide, 1.0-1.3 times longer than lip region width, and equal to or thicker than body cuticle at its level. Guiding ring double, but this condition is difficult to observe in some specimens due to fixation process. Basal pharyngeal expansion 4.1-5.3 times as long as wide or 3.0-3.4 times longer than body diameter at neck base, and occupying about half (48-52%) of total neck length. Pharyngeal gland nuclei difficult to observe in the material examined. Nerve ring located at 38-41% of the total neck length. Junction between pharyngeal base and cardia surrounded by a ring-like structure.

Female: Genital system didelphic-amphidelphic, with both genital branches equally and well developed, the anterior 182-415 μ m, the posterior 190-390 μ m long. Ovaries with variable development, reflexed but not always surpassing the sphincter level; oocytes first in two rows and then in a single row. Oviduct joining subterminally the ovary and consisting of a tubular part and a poorly

developed *pars dilatata*. Oviduct and uterus separated by an indistinct sphincter. Uterus 145-175 μ m or about 2.6-2.8 times the corresponding body diameter, and tripartite, i.e. consisting of three regions: proximal section, close to the vagina, a tube 80-90 μ m long with clearly visible lumen; an intermediate, shorter (50-55 μ m long), muscular section, with narrow lumen; and distal section, close to sphincter, a tube 90-100 μ m long, comparable in texture to proximal section, although slightly narrower. Spindle-shaped spermatozoa, 3.0-5.0 μ m long, often observed within the uterus. Uterine egg 92 x 35 μ m. Vagina cylindrical, extending inwards about half of the corresponding body diameter: *pars proximalis* 20-21 x 9-10 μ m with slightly sigmoid walls (i.e. proximally divergent and distally convergent) and surrounded by weak musculature; *pars refringens* with small, drop-shaped pieces separated by a less refringent area, with a combined width of 8.5-9 μ m; *pars distalis* short, 2.0-2.5 μ m long. Vulva a small longitudinal slit, usually preceded by a depression of body surface. Prerectum 2.0-4.3 times as long as anal body diameter. Rectum from slightly shorter to slightly

longer than anal body diameter. Tail broadly rounded to hemispheroid; two pairs of caudal pores, one subdorsal, another lateral, both at middle of tail.

Male: Prerectum 1.9-5.5 times the cloacal body diameter. Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair situated close to cloacal opening, there is a series of 19-23 almost contiguous ventromedian supplements, starting out the range of spicules, with the posteriormost supplement situated at 65-80 µm from ad-cloacal pair. Spicules curved ventrad, 4-5 times as long as wide and 1.2-1.8 times the anal body diameter long. Lateral guiding pieces relatively short, 7.5-10.0 µm, 3.5-4.5 times as long as wide. Tail bluntly conoid, ventrally almost straight, dorsally more convex; six pairs of caudal pores, two subdorsal, two subventral and two subterminal.

Distribution: Salt-affected sand dunes on the beach at Sulina and on Sacalin Island, both locations in the Danube Delta Biosphere Reserve - sites no. 1 and 2 in Table 1.

Remarks: This species was originally described from sand dunes in Italy on the basis of specimens collected in six different localities. The Romanian specimens perfectly fit the type material, with only minor morphometric differences. These differences include thicker cuticle (2.5-3.5 vs 1.0-2.0 µm in midbody), slightly more slender body (vs $a = 19-30$), and somewhat shorter odontostyle (vs 23-30 µm), although overlapping in the range of these measurements and ratios can be noted. Nevertheless, the above description provides many new details, especially those derived from SEM study of lip region and others concerning the morphology of female genital system, which allow a better characterisation of the taxon.

This is the first record of *L. carusoi* in Romania, extending its geographical distribution. By reporting the species from the same type of habitat as it was originally described, its preference for sand dunes is confirmed.

Labronemella labiata Andrassy, 1985 (Fig. 3)

Material examined: One female collected from the Gurghiu Valley (Gurghiu Mountains), in excellent condition, making suitable a detailed study of its morphological features.

Measurements: See Table 1.

Female: Slender nematode of medium size, 2.56 mm long. Habitus strongly curved, G-shaped upon fixation. Cuticle with two layers: outer layer smooth and thin, the inner layer is very thick (3-4 times the outer one) and bears distinct radial striations along entire body; cuticle 3 µm thick at anterior region, 5

µm in mid-body and 7.5 µm on tail. Lateral chord occupying about one-fourth of the corresponding body diameter at midbody. Lip region offset by constriction, sucker-like, and 3.5 times as wide as high or about one-third of body diameter at neck base; lips amalgamated in their most part; oral field deeply sunk in head contour, with six small rounded liplets surrounding the oral aperture. Amphidial fovea funnel-like, its aperture 9 µm or about two-fifths (41%) of lip region width. Odontostyle straight, slender (about 12 times as long as wide), with slender walls and distinct lumen; it is 1.2 times longer than lip region width and 1.17% of body length; aperture 37% of the total length. Odontophore rod-like, 1.8 times the odontostyle. Guiding ring double. Pharynx consisting of a slender but distinctly muscular anterior part, enlarging gradually; basal expansion 8.7 times as long as wide or 4.1 times longer than body diameter at neck base, and occupying 49% of total neck length. Pharyngeal gland nuclei and outlets situated as follows: DN = 58, DO = 56, S₁N₁ = 69, S₁N₂ = 78, S₂N = 88. Nerve ring located at 38% of neck length. Cardia conoid, 23 µm long, 1.5 times as long as wide, surrounded by intestinal tissue that forms a conical extension measuring 40 µm including the cardia. Genital system didelphic-amphidelphic, with both genital branches equally and well developed, the anterior 437 µm, the posterior 525 µm long. Ovaries very large, extending beyond the sphincter level; oocytes numerous, arranged first in two or more rows and then in one single row. Oviduct joining the ovary subterminally and consisting of a tubular part and a well developed, elongate *pars dilatata* containing spindle-shaped spermatozoa, 4.0-5.0 µm long. Sphincter separating oviduct from uterus, but not very marked. Posterior uterus 549 µm long, the anterior one convoluted; it is tripartite, that is consisting of three specialized regions: proximal portion, close to the vagina, a tube 110-154 µm long with wide lumen; an intermediate dilated portion, 46-56 µm long, containing spermatozoa; and distal portion, close to the sphincter, a narrower tube 73-110 µm long with practically no distinct lumen. Vagina extending inwards to more than half (55%) of the corresponding body diameter: *pars proximalis* 23 x 6.5 µm, with slightly sigmoid walls (*i.e.* proximally divergent and distally convergent) and surrounded by moderately developed musculature; *pars refringens* with two trapezoidal, adjacent pieces measuring 9 x 6-6.5 µm and with a combined width of 12.5 µm, almost reaching the body surface; *pars distalis* very short, measuring about 1.5 µm. Vulva a transverse slit. Prerectum relatively short, about twice the anal body diameter long. Rectum slightly longer than

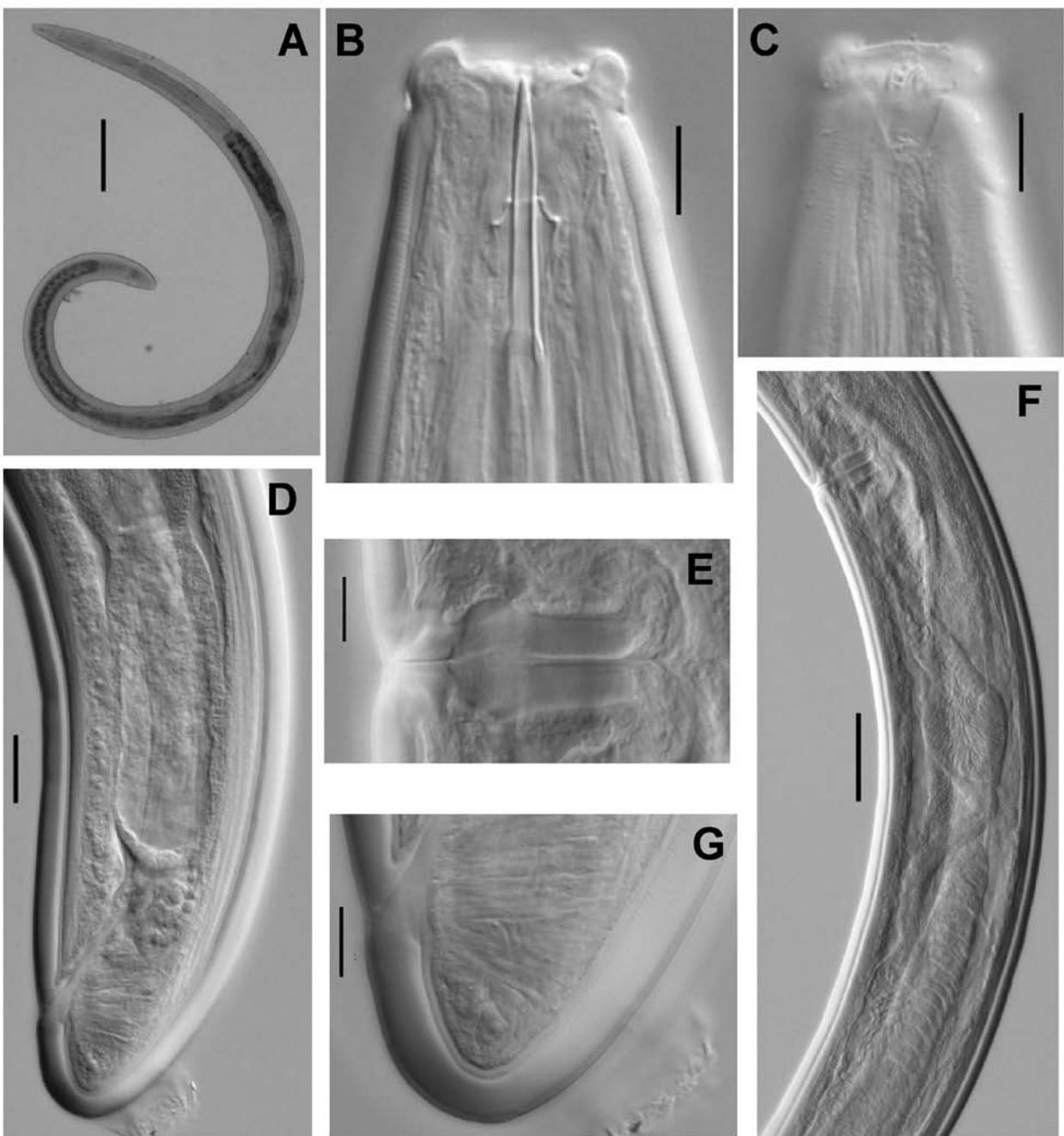


Fig. 3. *Labronemella labiata* Andrassy, 1985 (female). A: Entire female; B: Anterior region in lateral median view; C: Same in lateral surface view; D: Posterior body region; E: Vagina; F: Posterior genital branch; G: Tail. (Scale bar: A = 200 μ m; B, C, E, G = 10 μ m; D = 20 μ m; F = 50 μ m).

anal body diameter. Tail broadly rounded; three pairs of caudal pores at the posterior half of tail, two subdorsal and the other lateral.

Male: Not found.

Distribution: Natural beech forest (*Fagus sylvatica*) on acid soil located in the Gurghiu Valley (Gurghiu Mountains, Eastern Romanian Carpathians), site no. 3 (Table 1.)

Remarks: Although Andrassy (1985, see also 2009) described this species on the basis of only one male from Hungary, the Romanian female specimen herein studied is considered to be conspecific with the Hungarian male because there are many morphometric similarities between them, namely body length (2.56 vs 2.54 mm in the Hungarian male), inner cuticle layer with radial striation (vs

"cuticle not annulated but finely radially striated"), lip region width (25 vs 23 µm), odontostyle length (30 vs 31 µm or 1.2 vs 1.3 times the lip region width), odontostyle aperture (37% vs one-third of total length), etc.; moreover, both specimens were collected from the same biogeographical area. There are, nevertheless, some morphometric differences, such as body slenderness ($a = 33$ vs $a = 48$) or neck length (619 vs 410 µm) but, taking into consideration that only two specimens of different sex are compared, they are provisionally interpreted as intraspecific variations. Then, with due caution, the female of this species is reported and described for the first time.

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M. Ciobanu, I. Popovici, R. Peña-Santiago. Нематоды отряда Dorylaimida из Румынии: два вида Qudsianematinae Jairajpuri, 1965.

Резюме. По материалу, собранному в естественных экосистемах Румынии, исследованы два известных вида семейства Qudsianematidae. Проведено сравнение двух обнаруженных популяций *Labronema carusoi* с первоначальным описанием. Новые морфологические наблюдения позволяют дополнить первоописание деталями строения губ и женской половой системы. Впервые приводится описание самки *Labronemella labiata*. Даны описания, измерения и иллюстрации для обоих видов, включая фотографии, сделанные в световом и сканирующем электронном микроскопе (для *L. carusoi*). Приводятся сведения о встречаемости этих видов в Румынии.
