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NEW DATA ON SPECIES OF ORDER MONONCHIDA (NEMATODA) FROM RILA AND THE RHODOPES MOUNTAINS, BULGARIA

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Abstract

Nematode fauna of three natural reserves in Bulgaria (in Rila and the Rhodopess) has been studied. Eleven different habitats from forest belt zone including oak, beech and spruce forests, and subalpine zone with specific plant associations, were sampled for at least 3 years.

Eight species of three families of order Mononchida were identified. Species common for two regions are *Clarkus papillatus, Mylonchulus brachyuris, Prionchulus muscorum, P. punctatus, Miconchus studeri, Mylonchulus sigmaturus.* The first one is found in all studied habitats. Specific for studied localities in Rila Mountain was *C. zschokkei* and for the Rhodopess – *C. parvus.* Distribution of each species in different habitats is discussed, morphometrics, drawings and microphotographies are provided.

Key words: Clarkus, Coomansus, Mylonchulus, Prionchulus, Miconchus, protected area

INTRODUCTION

During the studies of nematode fauna both in natural and anthropogenic habitats of Bulgaria 22 species of order Mononchida were reported (Andrássy, 1958; Gateva, 1961; Katalan-Gateva, 1965, 1966, 1982; Stoyanov, 1979; Poljakova-Krusteva, Katalan-Gateva, 1984; Baicheva, 1982; Peneva, Nedelchev, 1995; Ilieva, 1998; Peneva et al., 1999; Peneva, Nedelchev, 2000; Lazarova et al., 2000; Lazarova et al., 2004, Peneva et al., 2011). In almost all records only general information about findings with no descriptions of local populations has been provided. Poljakova-Krusteva, Katalan-Gateva (1984) studied the ultrastructure of cuticle of *Clarkus papillatus* (Bastian, 1865) Jairajpuri, 1970. Only two studies included detailed description of the species recovered (Peneva et al., 1999; Peneva, Nedelchev, 2000). Some of the records are doubtful and not actual because of changes in the systematics of the group and part of them could not be confirmed because lack of exact data on particular habitats and localities.

The aim of the present study is to reveal species composition and distribution of the mononchids in the Biosphere reserve 'Parangalitsa', Rila Mountain, as well as the Nature reserves 'Kupena' and 'Mantaritsa', the Rhodopes, to summarize information about previous distribution records and to add data on the morphology of populations recovered.

MATERIAL AND METHODS

Sampling. Each sample consisted of 10 soil cores at 20 cm depth in an area of 100 m^2 in each of the eleven studied habitats. Sampling date, number of samples per habitat and short description of the habitats is presented in Table 1.

Extraction and processing. Nematodes were extracted from soil after homogenization using a modified Bearman method with 48 h exposition (Van Bezooijen, 2006), counted alive, gently heated at 50°C and fixed in 4% formaldehyde (the Rhodopes specimens) and FAA (Rila specimens). Nematodes were mounted on temporary slides (Paramonov, 1963) for quantitative analysis. All examined specimens in the present study were transferred in glycerol (Seinhorst, 1959) and mounted on permanent slides. Measurements and photographs were taken by Olympus microscope BX60 with DIC, digital camera DP70 and specialized software Imaging System cell^B (Olympus). The classification of families and genera proposed by Zullini, Peneva (2006) was used. Old synonyms used in earlier records for Bulgaria are given for all species.

RESULTS AND DISCUSSION

Eight species belonging to three families of order Mononchida were found in 11 studied habitats.

Family Mononchidae Filipjev, 1934

Clarkus papillatus (Bastian, 1865) Jairajpuri, 1970 Syn. *Mononchus papillatus* Bastian, 1865 (Table 2, Fig. 1)

Description. Female. Body stout, small to medium size, C-form to spiral upon fixation. Cuticle very finely annulated, smooth under light microscope, 2-4 μ m wide at mid body and 6-15 μ m at hyaline part of tail. Lip region slightly setoff with prominent papillae, 5-9 μ m high. Amphidial aperture 4-5.5 μ m wide and at 7-13 μ m from the anterior edge about the level dorsal tooth apex. Bucal cavity goblet shaped 1.9 (1.7-2.3) times as long as wide. Dorsal tooth 5-6 μ m wide, its apex 78-88% of the buccal cavity base. Anterior margin of tooth straight, almost perpendicular to cavity wall. Plain ridges of subventral walls abruptly end anteriorly, opposite to dorsal tooth apex. Nerve ring 29-35% of neck lenght. Excretory pore at the level or slightly posterior to nerve ring, 30-43% of neck lenght to *pars dilatata*. Eggs oval 60-100 μ m long and 27-35 μ m wide. Vagina 1/3 to 1/2 of corresponding diameter. *Pars proximalis* frustum cone shaped, 11-18 μ m long and 12-15 μ m wide. *Pars refringens* compact rounded 3-5 μ m long and 10-14 μ m wide. *Pars distalis* 1-4 μ m long. Tail conoid ventrally arcuate, no terminal opening.

Male. Rare, with more strongly curved and slender tail. Lateral guiding pieces absent. We found males in autumn sampling but no one of the observed females contained sperms.

Code	Reserves	Vegetation	Longitude	Altitude	Sampling date	Number of bulk samples
-		Picea abies (L.) – Vaccinium myrtillus L. + Luzula	N42.0399		1989 – V-X	84
-		sitvatica (Huds.) 200 year forest	E23.3726	/761	1990 - V - X	(7 samples per date)
, c		Festuca valida (R.Uechtr.)	N42.0409	2001	1989 - V - X	84
1		– Juniperus sibirica L.	E23.4081	1007	1990 - V - X	(7 samples per date)
"		Pinus peuce Griseb. + P. abies –V. myrtilus	N42.0428	1975	1989 – V- X	84
<i>c</i>	es	70-80 year forest	E23.4108	<i>C 1 / T</i>	1990 – V- X	(7 samples per date)
4	ils	Alnus viridis (Chaix.) – mixtoherbosa	N42.0373	1545	1990 - X	14
+	Sue	70-80 year forest	E23.3727	(F(I	1991 – V	(7 samples per date)
v	Para	Fagus sylvestris L. +P. abies – Oxalis acetosella L.	N42.0422	1571	1990 - X	14
ſ	[100 - 120 year forest	E23.3805	1//1	1991 - V	(7 samples per date)
9		Deschambsia caespitosa (L.)	N42.0446	1885	1990 – X	14
,			E23.4105		1991 - V	(7 samples per date)
٢		Conformer a Valor	N42.0412	2031	1990 - X	14
~		JESTERIAL LUTAUSA VEICII.	E23.4087	1007	1991 - V	(7 samples per date)
o		Dinne murch time . Combrie aucordania I V mountiface	N42.0406	2005	1990 - X	14
0		1 тив тиво 1 шпа+эото из ашорити 1. — У. тутина	E23.4098	(707	1991 – V	(7 samples per date)
6	впэс	F. silvaticus	N41.9864 E24.2930	1107	2005 – IIIV, X 2006 – V, X 2007 – V, VI, IIIV, X	80 (10 samples per date)
10	Iny	F. silvaticus – Abies alba Mill. + Atropa beladona L.	N41.9317 E24.1274	1307	2005 – IIIV, X 2006 – V, X 2007 – V, VI, IIIV, X	80 (10 samples per date)
11	Mantaritsa	P. abies + L. silvatica	N41.9356 E24.0845	1560	2005 – IIIV, X 2006 – V, X 2007 – V, VI, IIIV, X	80 (10 samples per date)

Table 1. Description of studied habitats and sampling data

Mountain	Rila				the Rhodopes				
Habitat	1	2	33	8	6	10		11	
Character	5 90	3 0,0	4 90	4 99	14 \	5 90	1	17 99	1 03
L	1.0 ± 0.1 (0.9 - 1.1)	0.9 - 1.1	1.0 ± 0.1 (1.0 - 1.1)	1.1 ± 0.0 (1.0 - 1.1)	0.9 ± 0.1 (0.7 - 1.1)	1.0 ± 0.2 (0.8 - 1.2)	1.3	0.9 ± 0.2 (0.8 - 1.3)	0.9
R	24 ± 2 (22 - 29)	18 – 25	25 ± 1 (24 - 26)	21 ± 1 (20 - 22)	21 ± 4 (16 - 27)	24 ± 2 (22 - 27)	27	20 ± 2 (17 - 24)	21
þ	3.3 ± 0.1 (3.1 - 3.5)	3.2 - 3.5	3.4 ± 0.2 (3.2 - 3.6)	3.6 ± 0.0 (3.5 - 3.6)	3.2 ± 0.2 (2.8 - 3.4)	3.3 ± 0.2 (3.2 - 3.6)	3.5	3.4 ± 0.2 (3.0 - 3.7)	3.4
0	14 ± 1 (13 - 17)	13 - 15	14 ± 0.1 (13 - 14)	15 ± 1 (15 - 17)	14 ± 2 (12 - 20)	14 ± 2 (12 - 17)	17	14 ± 1 (11 - 16)	17
ົວ	2.6 ± 0.3 (2.0 - 3.0)	2.4 – 2.7	2.9 ± 0.2 (2.8 - 3.0)	2.3 ± 0.1 (2.2 - 2.4)	2.4 ± 0.4 (1.5 - 2.9)	2.6 ± 0.5 (1.9 - 3.0)	1.9	2.4 ± 0.3 (2.0 - 3.2)	1.5
V /T(%)	62 ± 1 (60 - 63)	58 - 66	62 ± 1 (61 - 63)	62 ± 1 (61 - 63)	64 ± 1 (61 - 66)	62 ± 2 (60 - 64)	48	63 ± 2 (60 - 66)	44
Buccal cavity length	23 ± 3 (15 - 25)	20 – 25	25 ± 1 (25 - 26)	25 ± 1 (24 - 25)	24 ± 2 (22 - 28)	24 ± 2 (22 - 27)	27	25 ± 2 (24 - 30)	25
Buccal cavity width	12 ± 2 (8 - 15)	10 - 13	13 ± 0.1 (11 - 14)	13 ± 0 (12 - 13)	14 ± 1 (12 - 16)	16 ± 1 (15 - 17)	17	15 ± 1 (13 - 19)	13
Neck	296 ± 22 (258 - 323)	273 - 315	302 ± 4 (298 - 305)	302 ± 13 (286 - 315)	276 ± 36 (225 - 343)	307 ± 43 (258 - 360)	359	283 ± 48 (216 - 375)	265
Rectum	22 ± 2 (19 - 25)	21 - 24	23 ± 5 (19 - 29)	23 ± 3 (20 - 26)	23 ± 2 (19 - 26)	24 ± 3 (21 - 27)		23 ± 3 (20 - 27)	
Tail	69 ± 9 (55 - 80)	67 - 70	74 ± 4 (70 - 77)	71 ± 6 (65 - 78)	62 ± 12 (39 - 88)	74 ± 4 (70 - 80)	73	69 ± 9 (53 - 88)	53
Body diameter at lip region	23 ± 1 (22 - 24)	23 – 24	24 ± 1 (24 - 25)	24 ± 2 (24 - 26)	22 ± 2 (20 - 26)	25 ± 2 (22 - 27)	27	25 ± 2 (23 - 28)	24
Body diameter at base of pharynx	39 ± 2 (37 - 41)	35 – 37	40 ± 1 (39 - 40)	49 ± 3 (46 - 53)	40 ± 7 (31 - 52)	41 ± 3 (38-46)	46	44 ± 5 (37 - 53)	44
Body diameter at mid-body	41 ± 2 (36 - 43)	35 - 53	42 ± 2 (39 - 43)	53 ± 3 (50 - 55)	43 ± 8 (32 - 56)	42 ± 3 (38 - 47)	47	47 ± 6 (39 - 59)	44
Body diameter at anus	27 ± 2 (24 - 32)	25 – 29	26 ± 1 (25 - 27)	31 ± 1 (30 - 33)	26 ± 4 (22 – 33)	27 ± 3 (24 - 31)	38	28 ± 3 (23 - 33)	36
Spiculae							67		44
Gubernaculum							16		14
Supplements							14		11

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Fig. 1. Clarkus papillatus (Bastian, 1865) Jairajpuri, 1970: a., b. and c. – Lip region; d. Amphidial orifice;
e. – Neck; f. and g. – Habitus; h. – Posterior gonad; i. and j. – Vagina; k. – Spicules; l. and m. – Tail of females; n. – Posterior part of body of male (scale bar 20 μm – a, b, c, d, i, j and k; scale bar 100 μm – h, l, m and n; scale bar 200 μm – e; scale 1 mm – f and g)

Remarks. Our populations do not differ significantly from the description of the populations from oak forests in Vidin region, Bulgaria (Peneva, Nedelchev, 2000) but most of studied characters are in wider limits.

C. papillatus was found in all studied habitats. The species is very common in Bulgaria. Andrássy (1958) found the species in oak, beech and coniferous forests, moss in Balkan, Rila and the Rhodopes; in oak forest in Burgas region (Alexiev et al., 1998), in a subalpine meadow in Rila (Ilieva, 1998), and beech forests in Strandzha Mountain (Iliev, Ilieva, 2014). Species was reported in low densities from different agricultural fields in Blagoevgrad region (Baicheva, 1974; Katalan-Gateva, Budurova, 1975; Katalan-Gateva, 1982); Sofia region (Stoyanov, 1975; Katalan-Gateva, Milkova, 1979; Katalan-Gateva, et al., 1981), Pazardzhik region (Gateva, 1961); Plovdiv region (Zhivkov, Baicheva, 1973; Katalan-Gateva et al., 1981); Stara Zagora region (Katalan-Gateva, 1965, 1966).

Coomansus parvus (de Man, 1880) Jairajpuri et Khan, 1977 *Mononchus parvus* de Man, 1880 (Table 3, Fig. 2)

(Table 3, Fig. 2)

Description. Female. Body small and stout, J or C to spiral shape upon fixation. Cuticle very finely annulated, 2-4 μ m tick at mid body and 4-11 μ m at hyaline part of tail. Lip region slightly expanded with prominent papillae, 2-5 μ m high. Amphidial aperture 4-5 μ m wide, at 14-17 μ m from the anterior edge above the level dorsal tooth apex. Bucal cavity barel shaped or with more flat base, 1.7 (1.3-1.9) times as long as wide. Dorsal tooth 4-6 μ m wide, its apex at 61 (58-67%) from the buccal cavity base. Anterior margin of the tooth at acute angle to cavity wall. Thin longitudinal ridges of subventral walls gradually fading anteriorly. Nerve ring 30-41% of neck lenght. Excretory pore at the level or slightly posterior to nerve ring, 37-40% of neck lenght. Female genital system amphidelphic, with relatively short uterus, *pars dilatata* oval. Vagina 1/5 to 1/3 of corresponding diameter. *Pars proximalis* frustum cone shaped with prominent musculature, 6-10 μ m long and 10-12 μ m wide. *Pars refringens* rounded sclerotized pieces distally slightly concave, 3-4 μ m long and 6-12 μ m. *Pars distalis* 4-6 μ m long. Tail conoid ventrally arcuate, no terminal opening.

Male not found.

Remarks. Our populations do not differ significantly from the other descriptions of the species (de Bruin, Heyns, 1992; Ahmad, Jairajpuri, 2010).

C. parvus occurred only in habitat 9. It has been reported from natural habitats: oak forest in Burgas region (Alexiev et al., 1998) and beech forests in Strandzha Mountain (Iliev, Ilieva, 2014), as well as in arable lands in Sofia (Katalan-Gateva, 1968) and Kazanlak region (Katalan-Gateva et al., 1981). Present finding is a new record for the region of the Rhodopes.

Coomansus zschokkei (Menzel, 1913) Jairajpuri et Khan, 1977 (Table 3, Fig. 3, 4) Description. Female. Body long and relatively slender, adopting J- to C-form

Species	C. parvus	C. zschokkei					
Mountain	the Rhodopes	Rila					
Habitat	9	2		3		8	
Character No of specimens	22 ♀♀	3 ♀♀	1 ð	1♀	1 ð	7 ♀♀	6 ථ්ථ්
L	0.8 ± 0.1 (0.6 - 1.0)	2.4 ± 0.2 (2.3 –2.5)	2.2	2.2	2.4	2.5 ± 0.2 (2.2 - 2.8)	2.2 ± 0.1 (2.0 - 2.3)
a	18 ± 2 (15 – 23)	32 ± 2 (31 – 33)	35	34	37	33 ± 3 (28 – 38)	32 ± 4 (25 – 36)
b	3.3 ± 0.2 (3.0 - 3.7)	4.9 ± 0.0 (4.8 - 4.9)	4.7	4.3	4.8	4.7 ± 0.3 (4.2 - 5.0)	4.4 ± 0.3 (4.1 - 4.8)
c	14 ± 1 (10 - 17)	33 ± 1 (32 - 34)	38	30	32	31 ± 4 (26 – 35)	33 ± 3 (29 – 38)
c'	2.2 ± 0.4 (1.6 - 3.6)	1.6 ± 0.3 (1.5 - 1.8)	0.9	1.7	1.2	1.6 ± 0.3 (1.2 - 2.0)	1.0 ± 0.2 (0.8 - 1.2)
V/T (%)	65 ± 2 (62 – 68)	70 ± 2 (69 – 71)		70		72 ± 3 (67 – 75)	47 ± 7 (37 – 53)
Body diameter at lip region	21 ± 2 (18 – 23)	35	38	40	40	39 ± 3 (34 – 44)	38 ± 3 (36 – 40)
Buccal cavity length	25 ± 1 (22 - 27)	47 ± 2 (45 – 48)	42	47	45	48 ± 3 (46 – 53)	44 ± 3 (40 - 48)
Buccal cavity width	15 ± 1 (13 – 17)	21 ± 2 (20 – 23)	19	22.5	22.5	25 ± 2 (23 – 29)	23 ± 2 (21 – 26)
Neck	228 ± 22 (195 – 228)	454 ± 65 (385 – 513)	466	518	493	525 ± 37 (488 – 600)	493 ± 12 (483 – 515)
Rectum	20 ± 2 (16 – 26)	21 – 22		28		32 ± 1 (30 - 34)	
Tail	55 ± 8 (45 – 73)	64 ± 14 (48 – 73)	57	75	75	81 ± 10 (65 – 93)	66 ± 9 (55 – 78)
Body diameter at lip region	21 ± 2 (18 – 23)	35	38	40	40	39 ± 3 (34 – 44)	38 ± 3 (36 – 40)
Body diameter at base of pharynx	42 ± 6 (33 – 55)	59 ± 18 (38 – 70)	60	62	63	70 ± 5 (62 – 78)	64 ± 7 (55 – 73)
Body diameter at mid-body	43 ± 6 (31 – 57)	63.± 19 (42 – 75)	63	65	65	74 ± 3 (70 – 80)	69 ± 8 (57 – 78)
Body diameter at anus	25 ± 3 (19 - 30)	39 ± 10 (28 - 48)	63	45	65	52 ± 10 (45 - 74)	67 ± 8 (55 – 75)
Spiculae			88		88		90 ± 6 (80 – 98)
Gubernaculum			30		31		26 ± 3 (24 - 28)
Lateral guiding pieces			18		20		18 ± 4 (14 - 23)
Supplements			23		23		19 – 23

Table 3. Morphometrics of specimens of genus *Coomansus* found in present study. Measurements in μ m (except L, in mm), and in the form: mean ± standard deviation (range).



Fig. 2. Coomansus parvus (De Man, 1880) Jairajpuri et Khan, 1977: a. – Neck; b. and c. – Lip region; d. – Amphidia; e. and f. – Vagina; g. – Habitus; h – Posterior gonad; i. – Tail; j – Tail tip (scale bar 20 μm – b, c, d, e, f, h, i and j; scale bar 100 μm – a; scale bar 200 μm – g)



Fig. 3. Coomansus zschokkei (Menzel 1913) Jairajpuri et Khan, 1977: a. – Neck; b., c. and d. – Lip region; e. – Amphidia; f., g. and h. – Variations of vagina shape; i. – Cardia; j. – Posterior end of body – male; k. – Anterior gonad – female; l. – Guiding pieces; m. – Spicules and gubernaculum; n., o. and p. – Tail – females; q. – Caudal pore and rectum; r. – Tail tip (scale bar 20 μm – b, c, d, e, f, g, h, i, l, m, q and r; scale bar 100 μm – n, o and p; scale bar 200 μm – a)



Fig. 4. Coomansus zschokkei (Menzel 1913) Jairajpuri et Khan, 1977: a. – Neck; b. – Lip region; c. – Variation of vagina; d. – Anterior gonad of female; e – Spicules; f. – Tail of female; g. – Posterior end of body – male

upon fixation. Cuticle very finely annulated under light microscope smooth, 3-6 μ m tick at mid body and 4-7 μ m at hyaline part of tail. Lip region expanded with prominent papillae, 6-9 μ m high. Amphidial aperture 5-6 μ m wide and at 12-19 μ m from the anterior edge above the level dorsal tooth apex. Buccal capsule barrel shaped, 1.7-2.4

times longer than wide. Dorsal tooth 5-7 μ m wide with apex at 61 (58-67%) from buccal cavity base. Apex with wide base and small prickle like tip. Anterior margin of tooth at acute angle to cavity wall. Thin longitudinal ridges of subventral walls gradually fading anteriorly. Nerve ring 22-31% of neck lenght. Excretory pore at the level or posterior to nerve ring, 27-40% of neck lenght. Small disk like structure visible at pharyngeal base. Female reproductive system with two functional gonads and a sphincter muscle between uterus and oviduct as described by Loof, Winiszewska-Slipnska (1993). Vagina about ¹/₄ to 1/3 of diameter. *Pars proximalis* 12-16 µm long and about 24-26 µm wide at distal part, *pars refringens* triangular rounded pieces 3-7 µm in length and about 7-9 µm width. *Pars distalis* about 3-4 µm. Tail conoid ventrally curved with rounded tip.

Males. Abundant. Body more curved posteriorly than female's when relaxed. Spicules massive with bifurcate tip. Gubernaculum with wider proximal part and gradually becoming thinner in its distal part. Guiding pieces bifurcate. Tail of males similar to that in females but shorter, stouter and in some specimens with more finely rounded tip that look sharp under low magnification. Cuticle in the caudal area thinner than in females.

Remarks. Wider diameter in PO region of the body all males was observed (d in mid-body is almost equal to anal body diameter). In our population, spiculae are slightly shorter than those described by Loof, Winiszewska-Slipnska (1993) (88-92 μ m *vs* 101-131 μ m). Specimens in present study slightly differ from neotype (Loof, Winiszewska-Slipnska, 1993) by wider limits of more of the characters but we do not consider it as significant.

Species is widely distributed in Europe (Fauna Europaea) and El Salvador, Mauritius, USA (Ahmad, Jairajpuri, 2010). In Bulgaria *C. zschokkei* was found only in habitats 2, 3 and 8 (Rila).

Prionchulus muscorum (Dujardin, 1845) Wu et Hoeppli, 1929 (Table 4, Fig. 5)

Description. Female. Body medium to large size, C-shaped when relaxed. Cuticle very finely annulated about 3-4 μ m tick at midbody and 4 μ m hyaline part of tail. Lip region not setoff, 5-6 μ m high. Amphidial aperture 4.5-5 μ m wide, located at 7-13 μ m from anterior end of body. Buccal cavity barel shaped. Dorsal tooth of medium size, 5 μ m wide, situated in anterior half of buccal cavity, its apex 71-81% of buccal cavity length from its base. Subventral walls with two longitudinal, denticulate ridges with 11-12 prominent sharp denticles on them. Nerve ring at 28-35% of neck lenght. Excretory pore at the level or posterior to nerve ring, or at 35-37% of neck lenght. Female genital system amphidelphic. Vagina about 1/3 of body diameter embedded in strong musculature. *Pars proximalis* concave 20-22 µm long and 20-22 µm wide at distal part, *pars refringens* triangular rounded 6-7 µm in length and about 12-15 µm wide. *Pars distalis* 0-2 µm. One egg found measuring 95/68 µm, with smooth shell. Tail conoid, arcuate ventrally, without caudal glands and spinneret.

Male not found.

Remarks. *P. muscorum* has wordwide distribution (Ahmad, Jairajpuri, 2010). In natural habitats in Bulgaria the species was found in moss and soil in Varna and the

Species	P. muscon	rum	P. punctatus
Mountain	Rila	the	Rhodopes
Habitat	1	9	10
Character	2 ♀♀	1 ♀	1 ♀
L	1.5, 2.0	2.0	1.3
a	22, 24	24	17
b	3.8, 4.1	4.0	4.0
с	17, 16	14	20
c'	2.1, 2.4	3.3	1.6
V (%)	65, 62	62	61
Body diameter at lip region (µm)	37, 40	44	33
Buccal cavity length	40, 36	48	32
Buccal cavity width	22, 16	28	21
Neck	383, 478	493	335
Rectum	33	38	28
Tail	85, 122	143	68
Body diameter at lip region (µm)	37, 40	44	33
Body diameter at base of pharynx	54, 68	68	70
Body diameter at mid-body	68, 82	83	77
Body diameter at anus	40, 51	43	41

Table 4. Morphometrics of specimens of genus *Prionchulus* found in present study. Measurements in μ m (except L, in mm), and in the form: mean ± standard deviation (range)

Balkan range, Rila, the Rhodopes by Andrássy (1958); in moss Vitosha (Andrássy, 1958), in oak forest in Burgas region (Alexiev et al., 1998) and in beech forests in Strandzha (Iliev, Ilieva, 2014). In agricultural area *P. muscorum* was identified by Katalan-Gateva and Budurova (1975) in strawberry fields in Blagoevgard region. The species is found in habitat 1 (Rila) and habitat 9 (the Rhodopes) in the present study.

Prionchulus punctatus (Cobb, 1917) Andrássy, 1958 (Table 4, Fig. 5)

Description. Female. Body of medium size, stout C-shaped when relaxed. Lip region is not setoff, 4 μ m high. Cuticle very finely annulated, about 4 μ m tick at midbody and 5 μ m at hyaline part of tail. Amphidial aperture 4 μ m wide, located at 8 μ m from anterior end of body. Buccal cavity barel shaped. Dorsal tooth of medium size, 5 μ m wide, situated in anterior half of buccal cavity, its apex 81% of buccal cavity length from its base. Subventral walls with two longitudinal, denticulate ridges each with 13 small not so prominent denticles on them. Nerve ring at 32% of neck lenght. Excretory pore posterior to nerve ring, at 38% of neck lenght. Female genital system amphidelphic. Vagina about 1/3 of body diameter embeded in strong musculature. *Pars proximalis* narrow slightly widening distally, 14 μ m long and about 12 μ m wide , *pars refringens* with triangular drop like shape 5 μ m in length and about 12 μ m width of each part. *Pars distalis* about 3 μ m. Tail stout conoid, arcuate ventrally, without caudal glands and spinneret.

Male not found.



Fig. 5. Prionchulus punctatus (Cobb, 1917) Andrássy, 1958: a. and b. – Lip region; e. – Pharyngeal cardia; g. – Vagina; i. – Posterior part of body; *P. muscorum* (Dujardin, 1845) Wu et Hoeppli, 1929: c. and d. – Lip region; f. – Pharyngeal cardia; h. – Vagina; j. – Posterior part of body (scale bar 20 μm – a, b, c, d, e, f, g and h; scale bar 100 μm – i and j)

Remarks. *P. punctatus* was reported from oak forests in Yambol and Dobrudzha regions, and in Lyulin, the Balkan range and Pirin mountains by Peneva, Nedelchev (1995), and in moss from Vitosha (Lazarova et al., 2000). Species is found in habitat 11 in the present study and it is a new record for the region of the Rhodopes.

Family Anatonchidae Jairajpuri, 1969

Miconchus studeri (Steiner, 1914) Andrássy, 1958 (Table 5, Fig. 6, 7)

Description. Female. Body slightly curved to C-shaped when relaxed. Cuticle very finely annulated 2-5 µm tick at midbody and 4-6 µm at hyaline part of tail. Lips set off by a depression with amalgameted cuticle that gives in some specimens rounded shape of lip contour, 7-12 µm high. Amphidial orifice 6-8 µm wide, situated at 10-14 µm from anterior end of buccal cavity or at the first 1/4. Three equal in size teeth 6-8 µm wide at the posterior 71-80% of the buccal cavity. Smaller orifice bellow teeth looks like smaller onchs was observed as described by Khan, Coomans (1980). Pharynx envelopes base of buccal cavity at the level of teeth. Nerve ring at 29 to 40% of the pharyngeal length. Excretory pore at the middle to end of nerve ring or 35-40% of the pharyngeal length. Glandular body at the junction between pharynx and intestine 10-12 µm long. Gut is with wide lumen and often with fragments of swallowed nematodes. Vagina occupies 1/5 up to 1/3 of the relative body diameter. Pars distalis very short (1-2 µm), pars refringens triangle to drop-shaped 5-6 µm long and 7-11 µm wide, and pars proximalis 17-18 µm long and wide. Uterus usually full of sperms. Junction between uterus and oviducts muscular and distinct. Ventral cuticle pores around vulva from 0 to 3 both anteriorly and posteriorly, no clear pattern in their arrangement observed. Rectum slightly shorter to equal of the anal body diameter. Tail conoid, ventrally curved with terminal spinneret. Caudal glands obscure in most of the specimens.

Males abundant with massive spiculae and bifurcated lateral guiding pieces.

Remarks. In some of our populations (1, 9 and 11) buccal cavity is longer (53-56 μ m, 63-69 μ m *vs* 40-43 μ m, 40-49 μ m) than in earlier descriptions of the species (Mulvey, 1962; Jiménez Guirado et al, 1993; Jiménez Guirado et al, 1997). Specimens from habitat 2 and 9 are with relatively smaller spiculae than those of habitat 1 and populations described by Mulvey (1962) and Jiménez Guirado et al. (1997) and resembles populations from Jaen, Spain (Jiménez Guirado et al., 1993). Lateral guiding pieces in all studied specimens are relatively longer than described in earlier works (15-20 μ m *vs* 12-16 μ m).

The species is widely distributed in Europe (Fauna Europaea), Africa: Mauritius, and America: USA, El Salvador (Ahmad, Jairajpuri, 2010). Species was reported from subalpine meadows in Rila (Ilieva, 1998) and beech forest in Strandzha (Iliev, Ilieva, 2014). In this study the species occurred in habitats 1-3, 6, 9 and 11 and present a new record for the Rhodopes.

Family Mylonchulidae Jairajpuri, 1969

Mylonchulus brachyuris (Buetschli, 1873) Andrássy, 1958 (Table 6, Fig. 8)

Mountain	Rila					the Rhodopes		
Habitat	1		2		9	6		11
Character	5 0+0+ 2	6 dd	8 0+0+ 8	5 33	1 +0	4 0+0+	1 03	5
L	2.5 ± 0.3	2.2 ± 0.2	2.2 ± 0.2	2.1 ± 0.1	2.1	2.5 ± 0.2	2.1	2.0 ± 0.2
	32 ± 1	$\frac{(2.0-2.4)}{38+2}$	33 + 4	(1.7 - 2.2) 40 + 2		(2.3 - 2.7) 33 + 1		(1.5 - 2.2) 25 + 0
9	(31 - 33)	(34 - 40)	(27 - 40)	(37 - 42)	22	(32 - 34)	35	(25 - 26)
	4.3 ± 0.3	4.2 ± 0.1	4.4 ± 0.3	4.6 ± 0.2	67	4.8 ± 0.5	45	4.2 ± 0.2
2	(4.2 - 4.6)	(4.0 - 4.2)	(3.8 - 4.7)	(4.4 - 4.9)	7.F	(4.3 - 5.4)	<u>с.</u> ғ	(4.0 - 4.4)
U	16 ± 1.9	20 ± 2.4	16 ± 1.0	20 ± 2	15	18 ± 2	15	16 ± 0
	$(1 - \zeta I)$	(1/ - 24)	(14 - 18)	(17 - 22)		(16 - 19)		(16 - 17)
Û	2.8 ± 0.8 (1.8 - 3.3)	1.9 ± 0.3 (1.6 - 2.3)	3.1 ± 0.4 (2.5 - 3.6)	2.1 ± 0.2 (1.8 – 2.4)	3.0	3.0 ± 0.6 (2.5 - 3.4)	2.5	2.7 ± 0.6 (2.6 - 2.9)
(70) 21	66 ± 5	-	68 ± 1	~	5	74 ± 2		68 ± 2
V (%)	(60 - 70)		(66 - 70)		/0	(73 - 76)		(67 - 70)
Buccal cavity length	54 ± 2 (53 – 56)	49 ± 3 (45 - 53)	51 ± 4 (43 - 56)	40 ± 2 (37 - 42)	53	66 ± 4 (63 - 69)	48	55 ± 2 (53 - 56)
Buccal cavity width	45 ± 1 (45 - 46)	37 ± 4 (32 - 43)	35 ± 4 (31 - 44)	31 ± 5 (29 - 40)	35	48 ± 2 (47 - 50)	33	45 ± 6 (40 - 49)
Neck (µm)	587 ± 31 (552 - 610)	587 ± 31 (552 - 610)	505 ± 41 (429 - 544)	462 ± 47 (381 - 501)	500	513 ± 57 ($450 - 560$)	455	483 ± 27 (464 - 503)
Rectum (µm)	35 ± 2 (32 - 36)		35 ± 4 (32 - 37)			35 ± 4 (33 - 38)		29 ± 8 (23 - 35)
Tail (µm)	156 ± 15 (139 - 169)	111 ± 13 (100 - 131)	136 ± 18 (114 - 160)	109 ± 13 (95 - 128)	143	141 ± 12 (130 - 153)	140	129 ± 16 (118 - 140)
Body diameter at lip region	50 ± 1 (48 - 52)	49 ± 1 (48 - 50)	45 ± 3 (41 - 57)	46 ± 2 (42 - 48)	50	56 ± 7 (51 - 61)	46	50 ± 5 (46 - 53)
Body diameter at base of pharynx (μm)	75 ± 2 (72 - 77)		60 ± 7 (55 - 65)	52 ± 1 (50 - 53)		80 ± 10 (73 - 88)	59	79 ± 10 (72 - 86)
Body diameter at mid-body (μm)	78 ± 2 (77 - 80)	58 ± 5 (53 - 64)	68 ± 7 (63 - 83)	53 ± 2 (50 - 56)	98	75 ± 7 (68 - 83)	59	80 ± 7 (75 - 85)
Body diameter at anus (μm)	59 ± 14 (51 - 75)	58 ± 5 (53 - 64)	45 ± 5 (37 - 51)	53 ± 2 (50 - 56)	48	50 ± 7 (45 - 55)	55	46 ± 7 (45 - 50)
Spiculae		92 ± 5 (83 - 96)		68 ± 8 (56 - 78)			67	
Gubernaculum		18-19		19 - 22				
Lateral guiding pieces		18 ± 1 (16 - 19)		17 ± 2 (15 - 19)			20	
Supplements		9-13		9 – 11			11	

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Fig. 6. Miconchus studeri (Steiner, 1914) Andrássy, 1958: a. – Neck; b., c. and d. – Lip region; e. – Amphidia; f. – Cardia, g. – Clarkus in the gut; h. and i. – Habitus; j. and k. – Vagina; l. and m. – Tail tip; n. and o. – Tail of female; p. – Posterior end of body – male (scale bar 20 µm – b, c, d, e, f, g, j, k, l and m; scale bar 200 µm – a, n, o and p; scale bar 1 mm – h and i)



Fig. 7. Miconchus studeri (Steiner, 1914) Andrássy, 1958: a. – Neck; b. – Lip region; c. – Anterior gonad of female; d. – Posterior end of body – male; e – Spicules; f. – Tail of female

Description. Female. Body relatively small and stout for the genus, ventrally curved to C-shaped when relaxed. Cuticle very finely annulated, 1-2 μ m tick at midbody and at hyaline part of tail. Lip region slightly setoff, 3-5 μ m high. Amphidial aperture 4-6 μ m wide, located at 7-11 μ m from anterior end. Buccal cavity goblet shaped with more strongly sclerotized dorsal wall. Dorsal tooth large, 5-7 μ m wide, situated in anterior half of buccal cavity, its apex at 76-85% of buccal cavity length from its base.

Subventral wall bearing five or six transverse rows of denticles. Subventral teeth present, not always clearly visible in some specimens. Nerve ring at 24-28% of neck lenght. Excretory pore posterior to nerve ring or 31-36% of neck lenght. Female genital system amphidelphic. Vagina occupies 1/6 up to 1/4 of the relative body diameter. *Pars distalis* short or absent (0-3 μ m long), *pars refringens* triangle to drop-shaped, 2-3 μ m long and 5-8 μ m wide, and *pars proximalis* 6-10 μ m long and wide. Tail convex conoid with broadly rounded terminus. Caudal glands grouped, obcure in the most of specimens. Spinneret subterminal.

Males were often found with relatively slender spiculae, small gubernaculum and tick bifurcated lateral guiding pieces. Our population does not differ significantly by several populations in the comparative study of de Bruin, Heyns (1992).

Remarks. The species is widely distributed in Europe (Fauna Europaea) but not very often found in our country. Species was found in habitats 1, 5, 9, 10 and 11. Andrassy (1958) reported the species from soil under elm (Varna) and litter from beech forests (Vitosha and the Rhodopes); in oak forests in Vidin region (Peneva, Nedelchev, 2000) and in forests of oriental beech from Strandzha (Iliev, Ilieva, 2014). Rila is a new geographical record of *M. brahyuris.*

Mylonchulus sigmaturus (Cobb, 1917) Altherr, 1953

(Table 6, Fig. 8)

Description. Female. Body of medium size and C-shaped when relaxed. Cuticle very finely annulated, 1.5-2 μ m tick at midbody and 2-3 μ m at hyaline part of tail. Lip region not setoff, 4-6 μ m high. Amphidial aperture 6-7 μ m wide, located posteriorly at 18-19 μ m from anterior end. Buccal cavity funnel shaped with more sclerotized dorsal wall. Dorsal tooth massive, 8-9 μ m wide, situated in anterior half of buccal cavity, its apex at 76-81% of buccal cavity length from its base. Subventral wall bearing six transverse rows of denticles. Subventral teeth present at 8-10 μ m from stoma base. Nerve ring at 29-32% of neck lenght. Excretory pore at or posteriorly to nerve ring or 30-39 % of neck lenght. Both females in present study were somewhat deformed at the level of vulva and mesurments were possible only for one of them. Female genital system amphidelphic. Vagina occupies 1/6 of the relative body diameter. *Pars distalis* 3 μ m long, *pars refringens* drop-shaped about 2 μ m long and 6 μ m wide, and *pars proximalis* 12 μ m long and the 14 μ m wide. Tail convex conoid with digitate distal portion about 1/3 of tail lenght. Caudal glands grouped. Spinneret terminal.

Males common. Relatively slender spiculae with small gubernaculum and bifurcated lateral guiding pieces.

Remarks. *M. sigmaturus* is distributed in Europe: Germany, Hungary, Italy, Poland, Romania, Slovenia, Sweden (Fauna Europaea), Asia: India, Iran, Pakistan, Korea, Uzbekistan; Africa: Egypt, Nigeria, Mauritus, South Africa; North America: Canada, Mexico, USA, South America: Argentina, Brazil, Columbia, Venezuela; Australia and New Zealand (Ahmad, Jairajpuri, 1992).

In Bulgaria Gateva (1961) reported *M. sigmaturus* in Pazardzhik region, and the next record of the species is from beech forests in Strandzha (Iliev, Ilieva, 2014). Species was found

Species			M. brachyu	ris				M.	. sigmaturus	
Mountain	R	ila		the	: Rhodopes			Rila	the Rhodope	(4)
Habitat	1	Ś	6		10	11		9	10	
Character	3 33	3 33	3 99	1 3	3 94	1	1	2 33	2	1 3
L	1.3, 1.5, 1.2	1.0, 1.0, 1.1	1.0 ± 0.1 (0.8 - 1.2)	1.2	0.8, 0.9, 1.2	1.1	1.3	1.8, 1.9	2.0, 2.0	1.8
ъ	36, 41, 35	30, 30, 31	22 ± 5 (18 - 33)	33	21, 21, 28	23	31	42, 43	43, 45	41
р	3.3, 3.6, 3.4	3.0, 3.1, 3.3	3.2 ± 0.2 (3.0 - 3.6)	3.5	3.1, 3.6, 3.8	3.6	3.7	3.6, 3.9	3.8	3.8
J	51, 48, 35	32, 30, 33	34 ± 6 (24 - 41)	30	31, 34, 47	39	28	45, 43	43	39
c	0.7, 0.9, 1.0	0.9, 1.0, 0.9	1.0 ± 0.2 (0.8 - 1.2)	1.2	0.9	1.0	1.2	0.9, 1.0	1.1, 1.3	1.2
V (%)			65 ± 1 ($64 - 67$)		66, 64, 65	61			70, 71	
T (%)		44, 40, 39		40			45	40, 43		37
Buccal cavity length	25, 26, 20	19	20 ± 1 (19 - 23)	20	20, 21, 21	20	19	27, 29	28, 29	28
Buccal cavity width	14, 12, 12	11, 11, 10	14 ± 1 (12 - 16)	12	15	13	16	16	16	16
Neck (µm)	390, 440, 355	340, 313, 326	294 ± 25 (263 ± 343)	343	250, 260, 305	300	355	495, 512	508, 550	472
Rectum (µm)			21 ± 2 (17 - 23)		18, 20, 21	25			21, 29	
Tail (µm)	25, 33, 35	32	29 ± 6 (22 - 40)	40	25, 28, 25	28	47.5	40,45	46, 48	46
Body diameter at lip region	24	20	22 ± 2 (18 - 25)	25	21, 22, 23	21	25	24, 28	27, 26	27
/ diameter at base of pharynx (μm)	35, 37, 35	34, 33, 34	41 ± 6 (32 - 48)	35	38, 40, 40	38	40	43, 45	46, 45	44
ody diameter at mid-body (µm)	35, 38, 35	36, 33, 36	43 ± 7 (34 - 52)	36	38, 45, 41	48	42		45, 45	
Body diameter at anus (μm)	36, 38, 35	34, 32, 35	29 ± 3 (24 - 34)	33	29, 29, 28	28	39	43, 45	41, 38	40
Spiculae	51, 55, 42	39, 37, 37		40			43	53, 59		56
Gubernaculum	13, 15, 14	13		12			19	17		17
Lateral guiding pieces	11	6		6			8	11, 13		13
Supplements	10	13, 9, 11		12			Ξ	1		11

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Fig. 8. Mylonchulus brachyuris (Büetschli, 1873) Andrássy, 1958(a, b, e, f, h, j, k, n and o); M. sigmaturus (Cobb, 1917) Altherr, 1953 (c, d, g, i, l, m, p and q) a, b, c and d – Buccal capsule; e – Habitus; f, g – Pharyngeal cardia; h, i – Vagina; j and l – Tail of female; p – Tail of juvenile; k – Spinneret on tail of male; m and n – Spicules; o and q – Guiding pieces of spicules (Scale on all pictures is 20 μm with exclusion of e – 200 μm)

in two habitats – 6 and 10. Adults were isolated in both habitats only in autumn sampling periods (October). Present record is new for the regions of Rila and the Rhodopes.

CONCLUSION

Eight species of families Mononchidae, Anatonchidae and Mylonchulidae were identified. Distribution of each species in different habitats is discussed and their morphometrics, drawings and microphotographies are provided. From Rila Mountain were described 7 species – 4 of them are new for the region (*C. zchokkei, P. punctatus, M. brachyuris, M. sygmaturus*) or in the region 8 species were reported until now. In the Rhodopes 7 species were found and 4 of them are new for the region (*C. parvus, P. punctatus, M. studeri, M. sigmaturus*). Total number of species for the region comprises 9 species.

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