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Research Article

Three new records of criconematids (Criconematidae: Criconematinae) from Iran

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Abstract: Three known species of the family Criconematidae namely *Criconema crotaloides*, *C. princeps* and *Ogma zernovi* were recovered from natural forests in Golestan province, and were characterized based upon their morphological and morphometric characters. The Iranian population of *C. crotaloides* was recovered from the rhizosphere of *Fraxinus excelsior* and is characterized by females 517-594 µm long, having 64-70 not retrorse body annuli with smooth margin without lateral differentiation and RV = 11-14. The second species, *C. princeps*, was associated with *Carpinus betulus* and is characterized by females 421-506 µm long, having 63-69 rounded to retrorse body annuli with smooth margin, marked by distinct lateral triangular arches and RV = 11-12. The main characteristics of this species and the status of some other previously reported populations as *C. princeps* were discussed. The last species, *Ogma zernovi*, was recovered from the rhizosphere of *Quercus* sp., and is characterized by females 350-653 µm long, having 58-64 retrorse body annuli, each annulus with nine rows of short smooth scales in the middle of the body, the scales uni- or bi-lobulated, RV = 9-11, tail conoid and the last three annuli without projections. New observations on its morphology were added, comparisons were made with the type populations of the species and characteristics of the species were updated with including data of males and juveniles. Compared to the type populations, no remarkable differences were observed for the three studied species. All the three species represent new records for Iran.

Keywords: *Criconema crotaloides*, *Criconema princeps*, forest, Golestan province, *Ogma zernovi*, taxonomy

Introduction

The family Criconematidae Taylor, 1936 includes five subfamilies *viz.* Criconematinae Taylor, 1936, Hemicriconemoidinae Andrássy, 1979, Macroposthoniinae Skarbilovich, 1959, Blandicephalanematinae Geraert, 2010 and Discocriconemellinae Geraert, 2010 (Geraert, 2010).

The subfamily Criconematinae is mainly characterized by having a variety of cuticular ornamentations. The annuli margin could be smooth, crenate or with ornamentation-like scales or spines, the lateral fields are rarely detectable, lip region is offset from the body with a first large disk-shaped annulus, pseudolips are present and submedian lobes are either present or absent.

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Juveniles have spines or scales in longitudinal rows. According to Geraert (2010), the subfamily currently includes eight genera namely *Criconema* Hofmänner & Menzel, 1914, *Crossonema* Mehta & Raski, 1971, *Croserinema* Khan, Chawla & Saha, 1976, *Lobocriconema* De Grisse & Loof, 1965 (De Grisse & Loof, 1965), *Neolobocriconema* Mehta & Raski, 1971, *Ogma* Southern, 1914, *Orphreyus* Siddiqi, 2000 and *Pateracephalanema* Mehta & Raski, 1971. So far, 18 species of the subfamily are recorded from Iran, including seven species of *Criconema*, four species of *Crossonema*, four species of *Ogma*, two species of *Lobocriconema* and one species of *Neolobocriconema* (Eskandari, 2018; Jahanshahi Afshar *et al.*, 2019a,b; Nikkar, *et al*., 2019; Hosseinvand, *et al*., 2020).

During our surveys in forests of north of Iran, two populations of *Criconema* and one population of *Ogma* were recovered from Afratakhteh, Naharkhoran and Olang forests in Golestan province. The recovered populations were characterized using morphological and morphometric characters.

Materials and Methods

Soil and root samples were collected from undisturbed natural forests in Golestan province, north of Iran, during 2017-2018. Two populations of the genus *Criconema* were recovered in association with *Carpinus betulus* L. in Olang forest, and *Fraxinus excelsior* L. in Afratakhteh forest and one population of *Ogma* was recovered in association with *Quercus* sp. in Naharkhoran forest. The nematodes were extracted from the soil samples using the rapid flotation-sieving and centrifugation method (Jenkins, 1964). The slow fixation method was used for mounting of the specimens in anhydrous glycerin and preparing permanent slides (Manzanilla López, 2012; Jahanshahi Afshar *et al*., 2019a). The morphological studies of the recovered nematodes were carried out using an Olympus BX51 light microscope equipped with differential interference contrast optic (DIC). The light microphotographs were taken using an Olympus DP72 digital camera attached to the microscope. Morphometrics were obtained using a drawing tube attached to a Nikon E600 light microscope.

Results

Criconema crotaloides (Cobb, 1924) Schuurmans Stekhoven & Teunissen, 1938 (Fig. 1; Table 1).

Description

Female: Body straight or slightly ventrally arcuate after fixation, bluntly truncate in anterior end, slightly tapers towards posterior end. Cuticle moderately thick, annuli not retrorse, their margin smooth. Lip region offset, with two rounded, not retrorse annuli, separated from the rest of the body by a narrow constriction. The first lip annulus saucer-shaped, 19-21 µm wide, with distinct notch in ventral edge, slightly wider than the second 18-20 µm wide lip annulus. The second lip annulus close to, and narrower than the first body annulus. Labial disc slightly raised in lateral view. Submedian lobes absent. Stylet with anchor shaped knobs. Excretory pore posterior to the pharynx base. Vulva with anterior lip projecting backward, overlapping the posterior lip and forming a flap-like structure. Tail conical, regularly tapering to a single pointed or finely rounded tip.

Juvenile: Not found.

Male: Unknown.

The related plant and locality

The specimens were collected from the rhizosphere of *Fraxinus excelsior*, in Afratakhteh forest in Golestan province, north of Iran. GPS coordinates: 36°47.32856′ N, 54°58.083′ E. Altitude: 1625 m.a.s.l.

Remarks

Criconema crotaloides was described by Cobb (1924), recovered in association with Douglas fir, *Pseudotsuga taxifolia* (Lamb.) Britt. in Salt Lake City, Utah, USA. The species was later reported from California, Utah and Colorado, USA, by Raski and Golden (1966). In comparison with the type population (paralectotypes assigned by Raski and Golden, 1966) and the other populations studied by Raski

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and Golden (1966), the presently recovered population has a slightly shorter body (517-594 *vs* 590-740 µm); but there is no difference in body length ranges of the Iranian population and the populations reported by Raski and Golden (1966) (see Table 1).

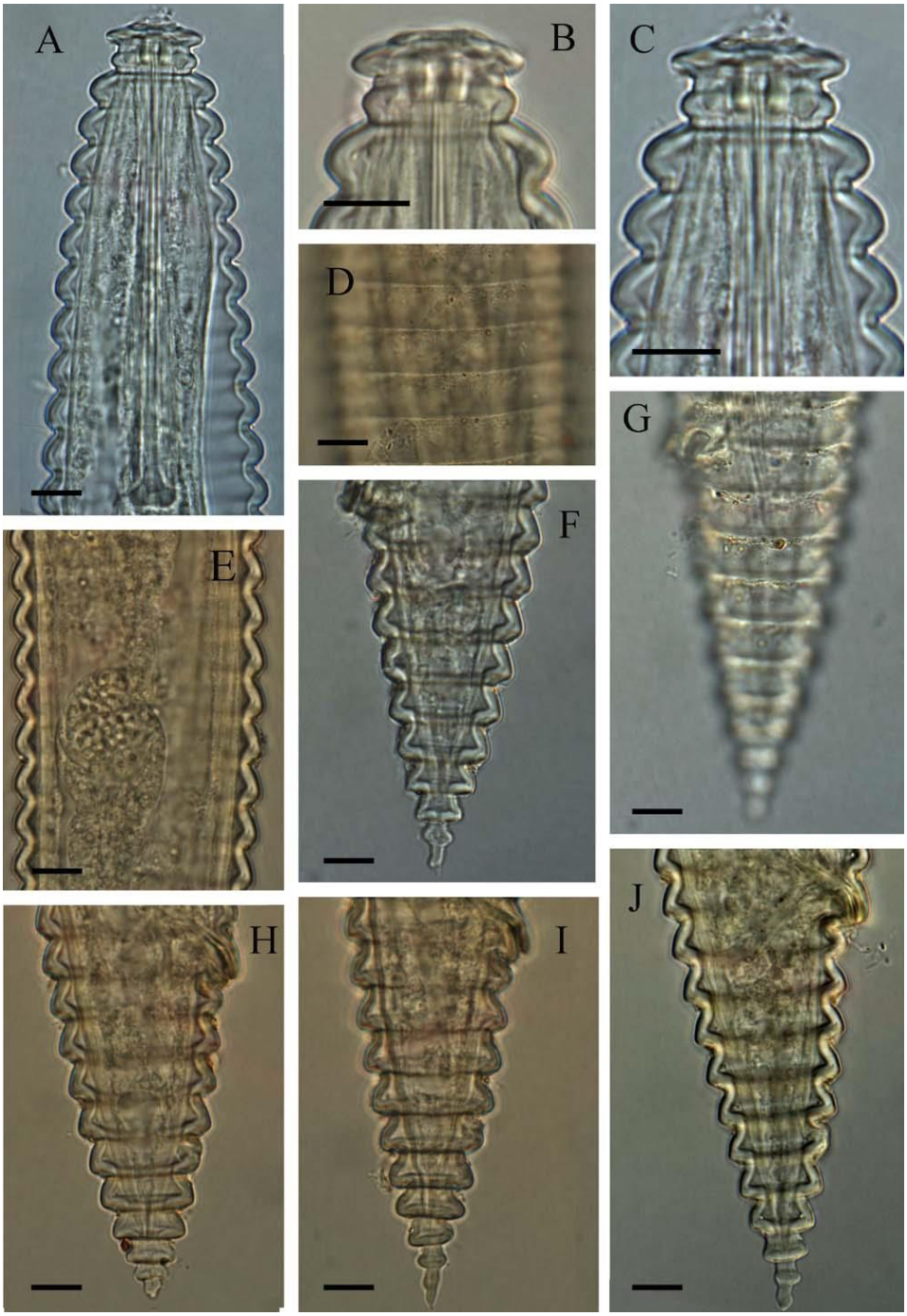

Figure 1 Light microphotographs of the Iranian population of *Criconema crotaloides*, Female. A-C: Anterior region (B & C: Lip region with two annuli); D: Body annuli margin; E: Spermatheca; F: Posterior region of body; G: Anterior vulval lip projecting backward; H-J: Variation of posterior body end morphology. (All scale bars = 10 µm).

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Table 1 Morphometrics of *Criconema crotaloides* females from Iran, the paralectotype specimens and the range of several populations from USA.

Characters	*Criconema crotaloides*		
	This study	Paralectotypes by Raski and Golden (1966)	Raski & Golden (1966)
n	**12**	**10**	**28**
L	551 ± 27.4 (517-594)	660 (590-740)	430-820
a	10.9 ± 0.4 (10.5-11.7)	-	8-13
b	3.9 ± 0.3 (3.6-4.3)	4.2 (4.0-4.6)	3.5-5.9
c	15.9 ± 1.9 (13.3-18.8)	15-16	13-30
V	86.8 ± 1.2 (84.9-88.4)	87 (84-89)	83-89
R	67 ± 2 (64-70)	72 (68-76)	62-74
RSt	13.0 ± 0.7 (12-14)	-	-
RPh	17 ± 1 (16-19)	-	-
Rex	21 ± 1 (20-23)	20-23	-
RV	12.0 ± 0.9 (11-14)	11-14	-
Ran	7 (7-8)	7-9	-
RVan	4.0 ± 0.7 (3-5)	-	-
Stylet length	98.8 ± 7.1 (92-112)	101 (95-108)	89-114
Conus length	81.4 ± 6.9 (75-94)	-	-
Shaft length	18.0 ± 1.2 (16-20)	-	-
m	81.8 ± 1.3 (80.2-83.9)	-	-
Stylet knob height	4.3 ± 0.5 (4-5)	-	-
Stylet knob width	11.0 ± 0.7 (10.5-12.0)	-	-
Pharynx length	143.4 ± 8.3 (136-159)	-	-
Excretory pore	176.4 ± 10.7 (165-193)	-	-
Head to vulva	478.7 ± 22.2 (446-518)	-	-
Max. body diam.	50.7 ± 3.0 (46-55)	-	-
Tail length	35.1 ± 4.8 (28-41)	-	-
VL/VB	1.9 ± 0.2 (1.6-2.1)	-	-
VL/ Stylet	0.7 ± 0.1 (0.6-0.9)	-	-
Stylet (% L)	17.9 ± 1.4 (16.2-20.3)	-	-
Stylet (% pharynx)	68.8 ± 2.3 (64.4-72.3)	-	-
Excretory pore (% L)	32.0 ± 0.9 (31.3-33.8)	-	-

All measurements are in µm and in the form: mean ± s. d. (range).

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Present population of *C. crotaloides* comes close to *C. annuliferum* (de Man, 1921) Micoletzky, 1925 by its morphological and morphometric characters, but can be separated by having greater RV and Ran (11-14 and 7-8 *vs* 7-12 and 3-7, respectively), anteriorly located vulva (V = 84.9-88.4 *vs* 87-92%) and lip region with two annuli (*vs* two or most often, three annuli). This is a new report of the species from Iran.

***Criconema princeps* (Andrássy, 1962) Raski & Luc, 1985**

(Fig. 2; Table 2)

Description

Female: Body straight or slightly ventrally arcuate after fixation, bluntly truncate in anterior end, slightly tapers towards posterior end. The lateral sides with triangular markings forming arch-like lateral differentiation. Annuli rounded to retrorse, with smooth margin. Cephalic region offset, with two annuli, the first annulus not retrorse, extending sideways, wider than the second annulus.

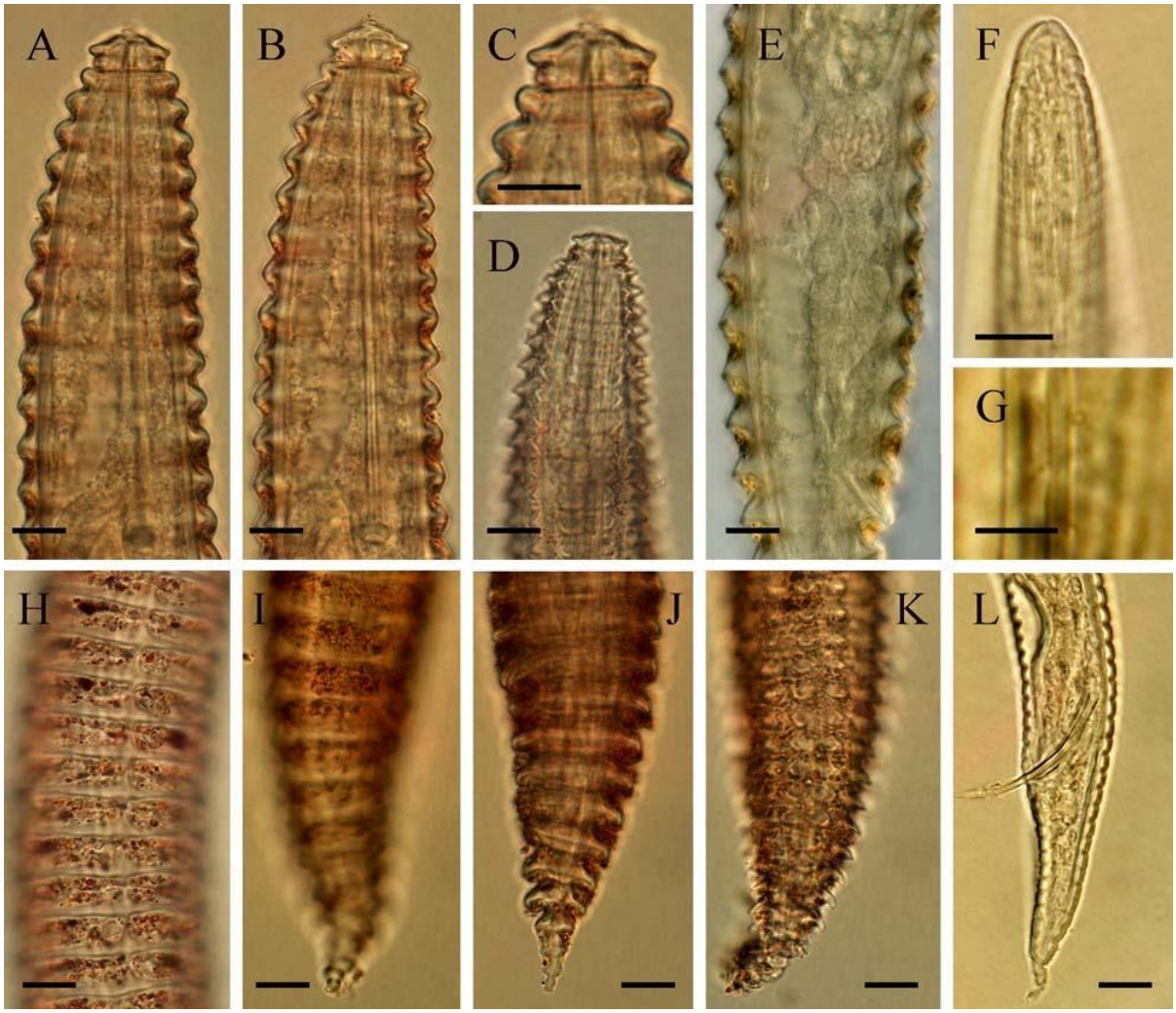

Figure 2 Light microphotographs of the Iranian population of *Criconema princeps*. A-C, E, H-J: Female, F, G, L: Male and D, K: Juvenile. A, B, D & F: Anterior region; C: Lip region with two annuli; E: Spermatheca; G: Lateral field; H: Body annuli showing arches and triangular markings; I-L: Posterior body region. (Scale bars: G = 5 µm, other scale bars = 10 µm).

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Table 2 Morphometrics of *Criconema princeps* females from Iran, the type and two other populations.

Characters	*Criconema princeps*			
	This study	Andrássy (1962)	Brzeski (1998)	Andrássy (2007)
n	**16**	**1**	-	-
L	464 ± 34.7 (421-506)	380	290-670	300-580
a	11.6 ± 0.7 (10.8-12.2)	10	7-13	7-12
b	3.4 ± 0.5 (3.1-4.1)	2.8	2.2-4.3	2.2-4.0
c	17.1 ± 4.3 (12.0-21.1)	19.1	16-37	18-35
V	87.1 ± 1.5 (85.5-88.9)	87.7	84-90	84-90
R	65.0 ± 2.9 (63-69)	63	50-71	52-68
RSt	15.0 ± 1.2 (14-16)	15	-	-
RPh	20.0 ± 0.8 (19-21)	23	14-19	-
Rex	20.0 ± 0.8 (19-21)	22	15-25	18-25
RV	11.0 ± 0.6 (11-12)	10	8-11	8-10
Ran	7.0 ± 0.8 (6-8)	-	3-7	4-6
RVan	3.0 ± 0.6 (3-4)	-	2-6	-
Stylet length	92.5 ± 2.4 (89-94)	87	86-113	88-106
Conus length	79.3 ± 2.5 (76-82)	-	-	-
Shaft length	13.3 ± 1.0 (12-14)	-	-	-
m	85.7 ± 1.1 (84.9-87.2)	-	-	-
Stylet knob height	2.8 ± 0.5 (2-3)	-	-	-
Stylet knob width	8.3 ± 1.0 (7-9)	-	9	-
1st-cephalic ann. dim.	15.0 ± 0.8 (14-16)	-	-	-
2nd-cephalic ann. dim.	13.3 ± 0.5 (13-14)	-	-	-
Pharynx length	136.0 ± 12.7 (123-151)	-	-	-
Excretory pore	133.8 ± 6.2 (127-140)	-	-	-
Head to vulva	404.0 ± 36.9 (360-450)	-	-	-
Max. body diam.	40.0 ± 1.8 (38-42)	-	-	-
Tail length	28.3 ± 5.7 (23-35)	-	17-36	-
VL/VB	2.0 ± 0.1 (1.9-2.1)	-	1.3-2.0	-
VL/Stylet	0.6 (0.6-0.7)	-	-	-
Stylet (% L)	20.0 ± 1.6 (18.6-22.1)	-	-	-
Stylet (% pharynx)	68.6 ± 7.0 (62.3-76.4)	-	-	-
Excretory pore (% L)	28.9 ± 1.2 (27.7-30.2)	-	-	-

All measurements are in µm and in the form: mean ± s.d. (range).

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Labial disk low, without submedian lobes. Stylet slender, with anchor shaped knobs, 7-9 µm wide. Excretory pore at the level with the pharynx base. Spermatheca rounded, filled with sperm. Anterior vulval lip covering the posterior one. Tail conical, tapers evenly to a pointed tip, the last 2-3 annuli not retrorse, sometimes bent.

Juvenile: Number of body annuli the same as that in female. Cuticle with about 10 rows of scales.

Male: Lateral fields with four lines. Spicules 39 µm long. Bursa narrow, apparently reaching tail tip.

The related plant and locality

The specimens were collected from the rhizosphere of *Carpinus betulus* L. in Olang forest in Golestan province, north of Iran. GPS coordinates: 36°50.7265′ N, 55°14.89199′ E. Altitude: 1676 m.a.s.l.

Remarks

Criconema princeps was originally described by Andrássy (1962) from Hungary. It is mainly characterized by triangular markings forming arch-like lateral differentiation. The species was reported from several countries (Andrássy, 2007; Geraert, 2010). The lateral differentiation is well discernible in the Iranian population as drawn for the type and Polish population (Andrássy, 1962; Brzeski, 1998). However, the lateral differentiation is not observed in the two populations reported by Gomez-Barcina *et al*., (1991) and Van den Berg and Tiedt (2001), indicating that assigning of these two populations to *C. princeps* needs further study. The recovery of males for this species revealed they have four lines in lateral field, forming three bands. The two inner lines are however hard to discern, and were originally illustrated by dot lines (Geraert, 2010). Compared with the type and the Polish populations of the species, no remarkable differences were observed for the studied population (see Table 2). This is a new report of the species from Iran.

***Ogma zernovi* Kirjanova, 1948**

(Fig. 3; Table 3)

Female: Body stout, straight or slightly ventrally arcuate, annuli retrorse. Posterior margin of annuli with nine longitudinal rows of scales in the middle of the body, the scales with smooth margin, remarkably elongated at posterior body region. Lip region with two equally wide, rounded and smooth annuli. Labial disc raised above the first annulus, submedian lobes present. Stylet thin, with anchor shaped knobs, about 9-11 µm across. Excretory pore posterior to the pharynx base. Spermatheca oval, filled with sperm. Vulva closed, anterior lip slightly overhanging, not projecting out the body contour in lateral view. Tail conoid, the last two–three annuli without scales.

Juvenile: Number of body annuli the same as that in female. Cuticular scales markedly different from those of female with 11 rows of triangular to round scales. The scales with 5-9 spines at tip.

Male: Lateral fields with three lines. Spicules 42-45 µm long. Gubernaculum 8-9 µm long. Bursa reaching near the tail tip. Anterior and posterior cloacal lips protruding.

The related plant and locality

The specimens were collected from the rhizosphere of *Quercus* sp. in Naharkhoran forest in

Golestan province, north of Iran. GPS coordinates: 36°47.2682′ N, 54°28.1103′ E. Altitude: 635 m.a.s.l.

Remarks

Ogma zernovi was originally established based on one female specimen (Kirjanova, 1948). This is a poorly known species of the genus and morphological and morphometric variations are not known for the type population. The illustrated characteristics for the type population are also of poor quality. For example, the reported 3.5 µm long scales is calculated 7 µm after the drawings. There are also no data on males and juveniles for the type population. The species was later redescribed by Escuer *et al*. (1990).

Again, some inconsistencies were observed between the redescribed Spanish population and the type population (e.g. morphology of cephalic region differs). The morphological and morphometric characters of the present Iranian population are in agreement with those of the type population, except the longitudinal rows is nine in cross section of female at mid-body (*vs* 10 in type population).

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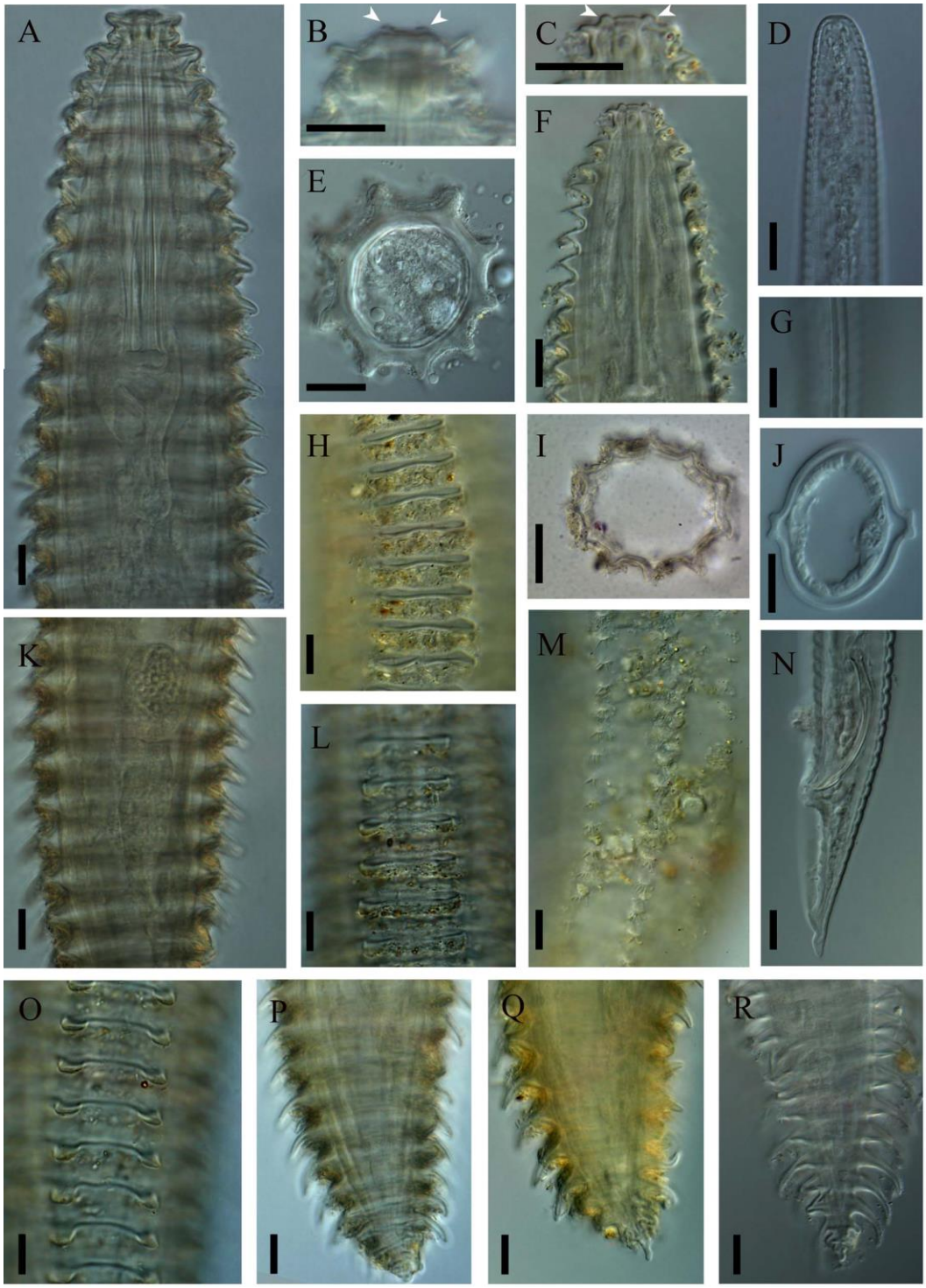

Figure 3 Light microphotographs of the Iranian population of *Ogma zernovi*. A, B, E, H, K, L & O-R: Female, D, G, J & N: Male and C, F, I & M: Juvenile. A, F & D: Anterior body region; B & C: Lip region showing submedian lobes (arrows); E, I & J: Cross section at mid-body; G: Lateral field; H & L: Scales at anterior body region; K: Spermatheca; M & O: Scales at mid-body; N & P-R: Posterior body region. (Scale bars: E & I = 20 µm, other scale bars = 10 µm).

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Table 3 Morphometrics of *Ogma zernovi* females from Iran, the type and a population assigned to the species.

Characters	This study	Kirjanova (1948)	Escuer *et al*. (1990)
n	**10**	**1**	**11**
L	545 ± 99.7 (350-653)	390	480 (390-570)
a	7.6 ± 0.9 (7.5-8.7)	9.8	11 (9.5-14.2)
b	4.1 ± 0.7 (2.7-5.0)	3.4	3.7 (3.0-4.7)
c	36.6 ± 12.7 (20.6-59.4)	11.9	14 (13-16)
V	89.4 ± 4.2 (84.3-91.1)	85	88 (85-91)
R	61 ± 2 (58-64)	66	65 (62-69)
RSt	11.0 ± 0.7 (9-11)	15	12 (11-14)
RPh	16 ± 2 (14-20)	-	17 (16-19)
Rex	18.0 ± 0.9 (17-20)	-	20
RV	10.0 ± 0.7 (9-11)	10	10 (9-11)
Ran	5.0 ± 0.6 (4-6)	7	5-7
RVan	4.0 ± 0.8 (3-5)	-	2-4
Stylet length	85.5 ± 5.8 (79-97)	92	88.9 (82-93)
Conus length	67.0 ± 5.4 (60-77)	-	-
Shaft length	18.8 ± 0.7 (18-20)	-	-
m	78.3 ± 1.3 (75.9-80.0)	-	-
Stylet knob height	3.4 ± 0.5 (3-4)	-	-
Stylet knob width	10.0 ± 0.8 (9-11)	-	-
1st-cephalic ann. dim.	19.1 ± 1.2 (17-21)	-	-
2nd-cephalic ann. dim.	19.1 ± 1.2 (17-21)	-	-
Pharynx length	134 ± 9 (125-151)	-	-
Excretory pore	159.3 ± 27.4 (109-198)	-	-
Head to vulva	489 ± 96.2 (295-595)	-	-
Max. body diam.	71.8 ± 9.0 (60-90)	-	-
Tail length	15.9 ± 4.1 (11-22)	-	-
VL/VB	1.2 ± 0.1 (1.1-1.3)	-	1.5 (1.1-1.6)
VL/ Stylet	0.7 ± 0.1 (0.6-0.8)	-	0.6 (0.53-0.7)
Stylet (% L)	16.3 ± 4.1 (12.7-25.7)	-	19 (18-22)
Stylet (% pharynx)	63.8 ± 4.3 (55.2-69.2)	-	-
Excretory pore (% L)	29.3 ± 1.2 (27.8-31.1)	-	-

All measurements are in µm and in the form: mean ± s. d. (range).

The length of the scales was however in accordance with the original drawings. The presently studied population has similarities with *O. fagini* Escuer & Bello, 1996, especially by having nine rows of scales (8-9 in *O. fagini*). Compared to *O. fagini*, present population has males with bursa (*vs* males of *O. fagini* lack bursa) and 11 longitudinal rows in juveniles (*vs* 10 in *O. fagini*). The other morphometric differences with *O. fagini* are as follow:

[DOR: 20.1001.1.22519041.2020.9.3.12.3]

difference in R (58-64 *vs* 51-58), RV (9-11 *vs* 6-8), cephalic region with two equally wide annuli (*vs* the second annulus wider than the first one) and shorter scales (4-8 *vs* 8-15 µm). In conclusion, the recovered population fits better to *O. zernovi*; and a number of nine rows of longitudinal scales as well as the characteristics of males and juveniles were added to the diagnostics of the species herein. This is a new report of the species from Iran.

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سه گزارش جدید از کریکونماتیدها (Criconematidae: Criconematinae) از ایران

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چکیـــده: سـه گونـه شـناخته شـده *Criconema crotaloides*، *C. princeps* و *Ogma zernovi* از خـانواده Criconematidae از جنگل‌های طبیعی استان گلسـتان جمـع‌آوری و براسـاس داده‌هـای ریخـت‌شناسـی و ریخت‌سنجی شناسایی شدند. جمعیت ایرانی گونه *C. crotaloides* به‌دست آمده از فراریشـه درخـت زبـان گنجشک (*Fraxinus excelsior*) دارای ماده‌هایی به طول ۵۱۷-۵۹۴ میکرومتـر، ۶۴-۷۰ حلقـه بـا حاشـیه صاف، غیرمتمایل به سمت عقب بدن، بدون تمایز جـانبی و RV = 11-14 مـی‌باشـد. گونـه *C. princeps* از فراریشه درخت ممرز (*Carpinus betulus*) جمع‌آوری شد. این گونه دارای ماده‌هـایی بـه طـول ۴۲۱-۵۰۶ میکرومتر، ۶۳-۶۹ حلقه گرد تا متمایـل به‌سمت عقب بدن، با حاشیه صاف و دارای فرورفتگی‌هـای قوسـی یا مثلثـی شـکل و RV = 11-12 می‌باشد. ویژگی‌های اصلی این‌گونه و وضعیت برخی از جمعیت‌های دیگـر که قبلاً با این نام گزارش شده بودند، مورد بحث قرار گرفت. گونه *O. zernovi* از فراریشه یـک گونـه بلـوط (*Quercus* sp.) جمع‌آوری شد. ماده‌هـای ایـن گونـه بـه‌طـول ۵۲۶-۵۸۹ میکرومتـر، دارای ۵۸-۶۴ حلقـه متمایل به‌سمت عقب بدن، هر حلقه در وسط بدن دارای نه ردیف فلس صاف کوتاه یک یا دو لـوبی، RV = 9-11، دم مخروطی و سه حلقه آخر بدون زائده هستند. با مقایسه جمعیت ایرانی این‌گونه با جمعیت تیـپ، اطلاعات جدیدی در مورد ریخت‌شناسی آن به‌دست آمد و خصوصیات گونه با داده‌هـای مربـوط بـه نرهـا و لاروها به‌روز شد. در مقایسه با جمعیت تیپ سه گونه گزارش شده، تفاوت‌های قابل‌تـوجهی بـرای ایـن سـه جمعیت ایرانی مشاهده نشد. این سه گونه برای اولین بار از ایران گزارش می‌شوند.

واژگان کلیدی: استان گلستان، جنگل، طبقه‌بندی، *Criconema crotaloides* ، *Criconema princeps*، *Ogma zernovi*

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