

A detailed morphological study of *Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 (Nematoda : Cephaloboidea) from Iran and Spain

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Summary – *Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 was found in the rhizosphere of cultivated and wild plants in the North and North-East of Iran. Light microscopy and scanning electron microscopy of these specimens and of three females from Spain provided new information on the morphology of the anterior end, in particular the “guarding pieces”, and the amphids. The stoma is very different from that of Cephalobidae, and it represents a strongly derived variation of the typical cephalobid stoma. Lacking detailed information on the related and possibly synonymous genus *Kirjanovia*, *Acromoldavicus* is provisionally accepted in the subfamily Kirjanoviinae, this subfamily being nevertheless transferred to the family Elaphonematidae.

Résumé – *Étude morphologique détaillée d'Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 (Nematoda : Cephalobidae) provenant d'Iran et d'Espagne – *Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 a été récolté dans la rhizosphère de plantes sauvages et cultivées du nord et du nord-est de l'Iran. L'étude en microscopie photonique et électronique à balayage de ces spécimens et de trois femelles provenant d'Espagne a apporté des données nouvelles sur la morphologie de la partie antérieure, en particulier sur les « pièces de protection » et les amphides. Le stoma est très différent de celui des Cephalobidae et représente une variation dérivée très prononcée du stoma typique de cette famille. Manquant d'information sur le genre *Kirjanovia* – un synonyme éventuel – le genre *Acromoldavicus* est provisoirement accepté dans la sous-famille des Kirjanoviinae, elle-même toutefois transférée dans les Elaphonematidae.

Key-words : *Acromoldavicus*, Iran, morphology, nematodes, SEM, Spain, stoma, taxonomy, nématodes.

This is the first paper in a series on the soil nematodes of Iran that deals with Rhabditida. The aberrant and poorly known free-living nematode *Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 was found in four samples collected from Tajrish, in the Tehran Province, Chalus, near the Caspian sea, and Bojnurd, in the Khorasan Province, near Turkmenistan, in the north-eastern part of the country. We also studied three specimens of the same species from Spain, which were kindly provided by Dr. R. Peña Santiago.

The monotypic genus *Acromoldavicus* was erected by Nesterov (1970) based on a species that was previously described as *Acrobeloides skrjabini* Nesterov & Lisetskaya, 1965. Boström (1989) found juveniles of *Acromoldavicus* on Samos, Greece, and studied these with scanning electron microscopy (SEM). As no adults were found, he could not identify the species. Later, he found several juveniles along with a single adult female of *A. skrjabini* in Crete, Greece (Boström, 1992). It was found in Hungary as well (Andrássy, 1990). This paper contains more detailed information on the morphology of this peculiar species as studied with light microscope (LM) and SEM, with particular emphasis on the lip region and stoma structure, and a hypothesis on the

homology of buccal regions. It also adds two more localities to its known distribution.

Material and methods

Samples were taken in July 1995 from the rhizosphere of different cultivated and wild plants. Nematodes were extracted by centrifugal flotation, killed and fixed with hot FAP and transferred to anhydrous glycerine for mounting. Two specimens from Iran (one male and one female) as well as one female from Spain were selected for study with SEM. They were prepared by ultrasonic treatment for about 10 min followed by an ethanol concentration series of 25, 50, 75, 95, and 100 % at 2-h intervals, ending with an overnight dehydration in 100 % ethanol. They were then submitted to critical point drying with CO₂, coated with gold and examined with a JEOL LSM-840 at 15 kV. Unfortunately, the anterior parts of the Iranian specimens were covered with detritus, and these are therefore not shown in Fig. 1.

Stoma terminology follows De Ley *et al.* (1995). The term “labial probolae” is always written between inverted commas because we question the homology of the three perioral structures in *Acromoldavicus* with those of Cephalobidae.

Acromoldavicus skrjabini
(Nesterov & Lisetskaya, 1965) Nesterov, 1970
 (Figs 1-3)

MEASUREMENTS
 See Table 1.

Table 1. Measurements (in μm) of *Acromoldavicus skrjabini* (Nesterov & Lisetskaya, 1965) Nesterov, 1970 from Iran and Spain (the dimensions of Iranian specimens are in the format mean \pm standard deviation and range).

	Spain		Iran			Spain		Iran	
	Females	Females	Females	Males		Females	Females	Males	
n	3	7	5		e.p. (% neck)	72, 75	66 \pm 4.8 (60-73)	67 \pm 4.8 (64-75)	
L	611, 622, 671	616 \pm 44.5 (557-677)	624 \pm 20.6 (602-647)		Deirid (% neck)	100	84 \pm 5.6 (79-91)	85 \pm 6.6 (79-95)	
Body width (BW)	37	33 \pm 3.2 (29-37)	32 \pm 2.2 (29-35)		Rnr*	29, 31, 33	26-32	26-34	
Neck length	130, 137	142 \pm 15.8 (123-167)	145 \pm 11.2 (131-156)		Rep*	25, 32	26-35	28-31	
Tail length	32, 34, 37	38 \pm 4.5 (31-46)	42 \pm 7.9 (28-48)		Rdej*	37	33-42	36-41	
Anal body width	15.3, 15.7	17 \pm 1.7 (15-19)	25 \pm 0.9 (24-26)		Rneck*	37, 41, 45	40-53	38-52	
a	16.5, 16.8, 18.1	18.6 \pm 0.9 (17.3-19.7)	19.6 \pm 2.0 (17.2-22.3)		Rvulva*	108, 121, 126	132 \pm 5 (126-140)	-	
b	4.5, 4.8, 4.9	4.4 \pm 0.3 (3.9-4.9)	4.3 \pm 0.3 (4.0-4.7)		Ranus*	187, 199, 202	203 \pm 9 (192-219)	205 \pm 7 (196-212)	
c	16.8, 19.1, 19.7	16.4 \pm 1.6 (14.1-18.9)	15.5 \pm 3.5 (13.4-21.6)		Phasmid from anus	14.8	13.3 \pm 2.1 (10.3-15.7)	19.6 \pm 1.1 (18-21)	
c'	2.0, 2.2, 2.4	2.2 \pm 0.3 (1.9-2.8)	1.7 \pm 0.3 (1.2-1.9)		Phasmid (% tail)	40, 46	35 \pm 3.4 (32-41)	42-44	
Ant end - base of stoma	10.0, 10.3	10.2 \pm 1.5 (9.0-13.0)	11.1 \pm 1.1 (9.9-12.6)		V or T	52, 62	62 \pm 1.2 (60-64)	49-52	
Corpus	70, 72, 79	66 \pm 16.4 (48-89)	70 \pm 13.4 (55-83)		G % or flexure	36, 38, 41	30 \pm 7.1 (18-36)	68 \pm 6.1 (58-74)	
Isthmus	25, 28, 30	38 \pm 4.3 (32-45)	40 \pm 5.9 (31-45)		Vagina or spicula	8, 8.5, 10	9.1 \pm 1.1 (8-11)	29.8 \pm 3.1 (25-33)	
Bulbus	24, 25	25 \pm 2.1 (22-28)	25 \pm 1.6 (23-27)		Rectum or gubernaculum	28, 29, 31	33 \pm 4.6 (23-37)	16.4 \pm 1.7 (14.4-18)	
Nerve ring (n.r.)	103, 104, 108	95 \pm 10.4 (81-112)	98 \pm 8.6 (85-106)		PUS	36, 45	27 \pm 5.9 (20-37)	-	
Excretory pore (e.p.)	94, 103	93 \pm 6.2 (85-103)	97 \pm 6.4 (88-103)		Spermatheca	29, 36, 40	33 \pm 12.4 (9.0-45)	-	
Deirid	130	120 \pm 12.3 (109-135)	123 \pm 5.1 (117-130)		V**	56, 66	66 \pm 1.1 (64-67.5)	-	
n.r. (% neck)	76, 79	67 \pm 2.7 (64-72)	67 \pm 1.5 (65-69)		PUS/BW	1.0, 1.2	0.8 \pm 0.2 (0.6-1.0)	-	

* Number of annules from the anterior end.

** Distance between anterior end and vulva as percentage of ant. end-anus distance.

DESCRIPTION

Female: Body stout, generally arcuate ventrad. Cuticle coarsely annulated, annules at midbody 2.4-3.1 and 2.7-3.2 μm wide in Iranian and Spanish specimens, respectively. Annules deeply longitudinally striated by incisures, not continuous near the lip region but continuous elsewhere, forming 30-31 ridges at midbody,

excluding the lateral fields. Lateral field with three incisures, beginning anterior to bulbus and continuing to near tail terminus. Lip region offset, 16-19 μm wide at base, with three pairs of well-separated, leaf-shaped lips extending into pointed cephalic probolae. Primary axils each with one long triangular guarding piece. Midventral guarding piece well developed, symmetrical and

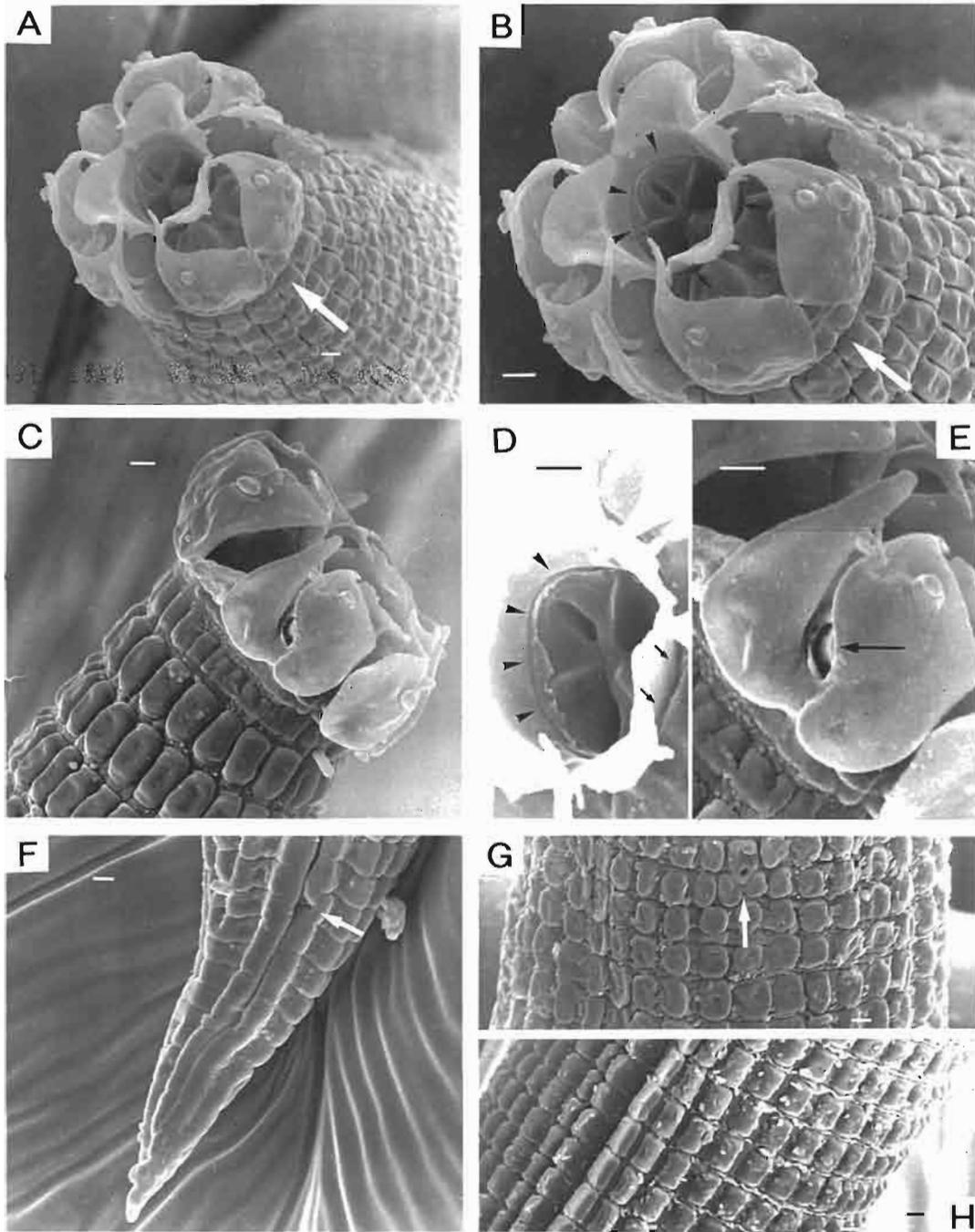


Fig. 1. *Acromoldavicus skrabini*. Females. *A, B* : Lip region seen from a dorsal angle; *C* : Lateral view of lip region; *D* : Oral aperture; *E* : Amphid, lateral lip and dorsosublateral guarding piece; *F* : Phasmid; *G* : Excretory pore; *H* : Lateral field; (White arrows in *A* and *B* show dorsal side, black arrows in *B* and *D* point at outer incisure encircling the bases of "labial probolae" externally, arrowheads point at inner incisure demarcating oral aperture, and arrows in *E, F* and *G* show amphid, phasmid and excretory pore, respectively; *A-E* from Spanish and *F-H* Iranian specimens; all scale bars equal 1 μ m).

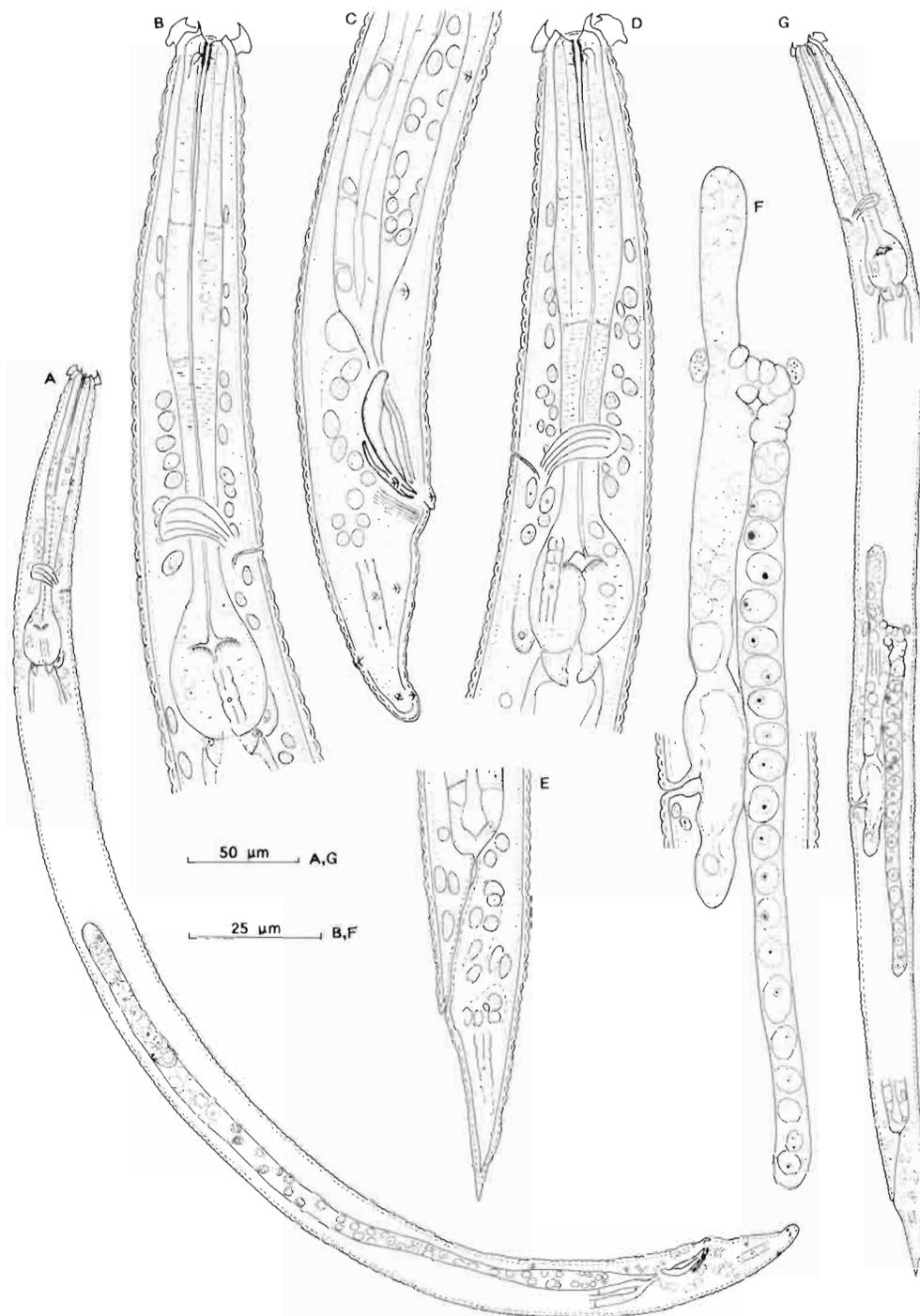


Fig. 2. *Acromoldavicus skrjabini*. Male. A : Total view; B : Pharynx; C : Tail; Female. D : Pharynx; E : Tail; F : Reproductive system; G : Total view.

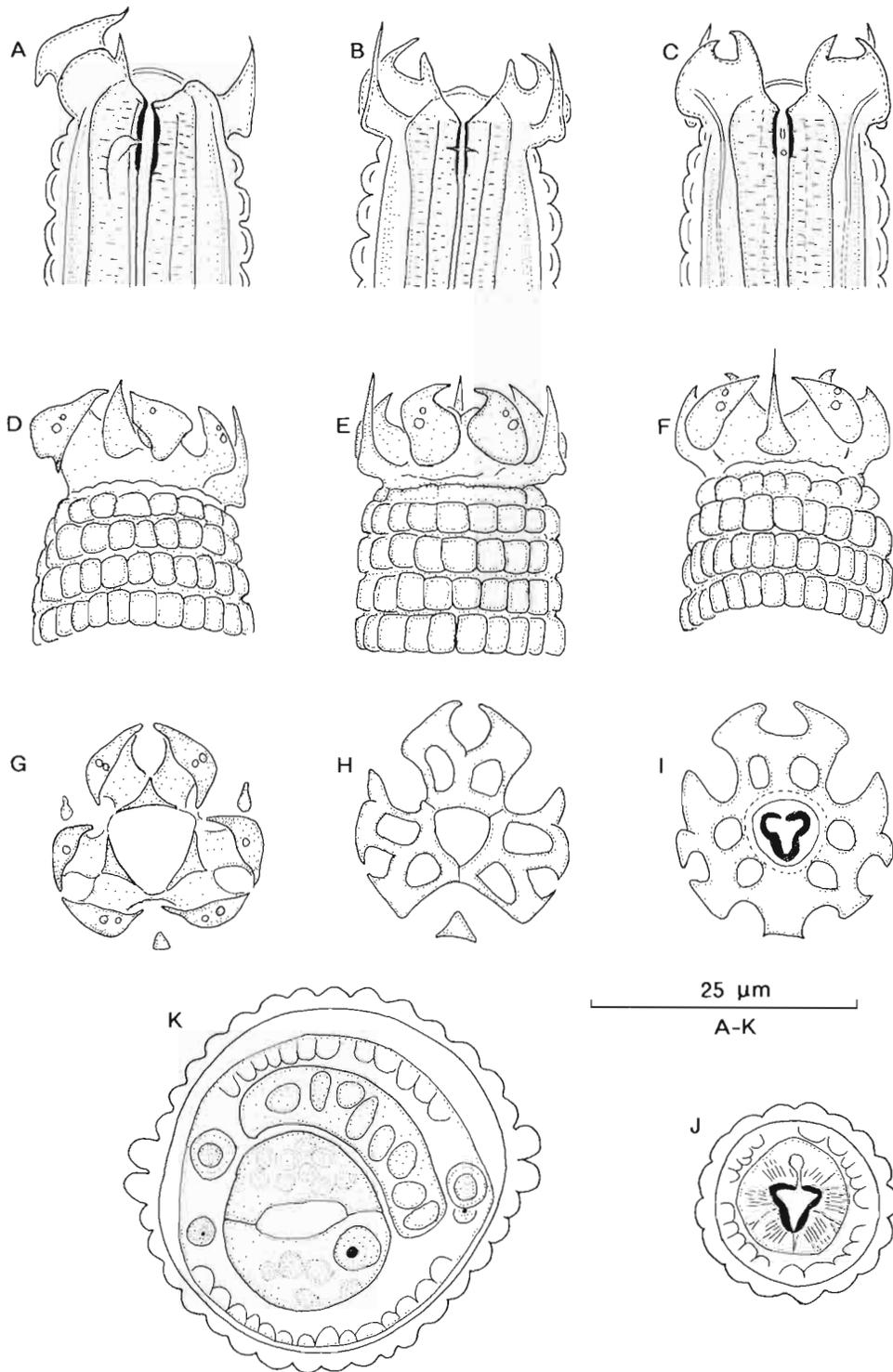


Fig. 3. *Acromoldavicus skrjabini*. Female. A-J : Anterior part; A, D : Lateral view; B, E : Dorsal view; C, F : Ventral view; G-J : Optical cross section at different levels from anterior end to the base of stoma; K : Cross section at level of spermatheca.

clearly separate from the subventral lips, while the two dorsosublateral guarding pieces being asymmetrical, actually arising from the bases of lateral lips and extending obliquely into the primary axil. Amphids slit-shaped, weakly curved, obliquely positioned in between lateral lips and dorsosublateral guarding pieces. Three thin and broad "labial probolae" present, triangular-rounded, with three small projections along their rim, bending outwards, connected at bases, and separated from the surrounding labial cuticle by an incisure encircling their bases externally. Oral aperture rounded-triangular, demarcated by another incisure, this one following the insides of the bases of the "labial probolae". Stoma short, having a triradiate lumen continuous with the pharyngeal lumen, and a cuticular lining with two faint sets of sclerotizations. Pharyngeal collar reaching the oral aperture, with two sets of muscle cells corresponding in position with the faint sclerotizations. Dorsal gland ampulla clearly visible in most specimens, located opposite posterior stoma region. Dorsal gland opening faintly visible in three specimens, situated in between the two stoma regions. Procorpus cylindrical; metacarpus slightly fusiform, the entire corpus quite variable in length: equal to 1.2-2.5 and 2.4-3.2 times isthmus length in Iranian and Spanish specimens, respectively. Bulbus with strongly developed valves; cardia well developed, enveloped by intestinal cells. Excretory pore at the level of nerve ring. Deirid at level of bulbus. Vulva posterior to midbody. Post-vulvar uterine sac (PUS) up to one corresponding body width long. Tail conoid, pointed, without distinct annulation in the posterior part. Phasmids anterior to middle of tail.

Male: Body generally arcuate ventrad, especially towards tail. Annuli 2.6-3.0 μm wide. Testis reflexed in anterior part. Spicules strong, arcuate ventrad, 1.0-1.3 times as long as anal body width. Gubernaculum weakly curved, boat-shaped, its length 40-60% of spicule length. Tail conoid with rounded terminus. Papillae distributed as follows: three subventral preanal pairs, the first pair situated at about two times tail length anterior to anus, the second pair one tail length anterior to anus and the third pair near the tip of spicules; one single midventral papilla on anterior cloacal lip; one lateral pair, one ventrosublateral pair anterior, and one subdorsal pair posterior to phasmid; two pairs (one lateral and one subventral) near tail tip.

LOCALITY AND HABITAT

Iranian specimens were found in the rhizosphere of chick-pea (*Cicer arietinum* L.) and sunflowers (*Helianthus annuus* L.) in Bojnurd, Khorasan Province, near Turkmenistan in the north-eastern part of the country; silk tree (*Albizia julibrissin* Durazz.) in Tajrish, Tehran Province; and wild plants in Chalus, near the Caspian sea.

Discussion

The measurements of these populations agree fairly well with earlier descriptions (Nesterov & Lisetskaya, 1965; Nesterov, 1970; Boström, 1989, 1992), but there are some differences in the observed structure of lip region and stoma. Thus, Fig. 1 in Nesterov (1970) shows lips fused in pairs; each primary axil with one pointed, separate guarding piece extending anterior to the cephalic probolae; "labial probolae" shaped as three small, pointed, conoid thorns surround the mouth opening. Also, the stoma was described as weakly developed by Nesterov (1970) or "... without differentiations or sclerotizations, enveloped by a somewhat expanded pharyngeal collar" by Boström (1992). We assume that these reported differences with our material are primarily due to differences in preservation and observation, rather than to any genuinely distinct characters.

Our light-microscopical observations of the stoma region show that *Acromoldavicus skryabinii* does have some interesting features, allowing some comparison with the typical cephalobid stoma (cf. Van de Velde *et al.*, 1994; De Ley *et al.*, 1995): the two subdivisions of the pharyngeal collar and stoma lining resemble the meta- and telostegostom of Cephalobidae, particularly in the presence and location of dorsal gland ampulla and opening. The anterior part of the stoma of *A. skryabinii* is very short, however, and differs fundamentally from the cephalobid arrangement in *i*) the absence of a distinct cheilostom and *ii*) the fact that the pharyngeal collar reaches the oral aperture. In fact, the stoma of *Acromoldavicus* would appear to consist nearly entirely of the region homologous to the posterior stegostom of Cephalobidae.

Transmission electron microscopy (TEM) is required to resolve the homology of this peculiar condition with the cephalobid stoma, but our SEM observations do reveal one interesting clue: the external incisure encircling the "labial probolae" has never been observed in any other cephalobid genus, and it actually coincides with the expected location of the membrane wedge ring seen with TEM in typical Cephalobidae. Therefore, we hypothesize that the "labial probolae" of *A. skryabinii* are actually homologous to the three interradiial "denticles" found at the anterior rim of the buccal cuticle in typical Cephalobidae (cf. De Ley *et al.*, 1995), and that the mouth opening of *A. skryabinii* is homologous to the anterior end of the stegostom in Cephalobidae.

All this remains to be proven on fresh material with TEM, but it does illustrate that the stoma of *Acromoldavicus* diverges strongly from the cephalobid standard organisation, and yet retains some resemblances. Since we consider the stoma structure of Cephalobidae to be the main defining character of the family, it also implies that *A. skryabinii* does not belong to it. Andrassy (1976) proposed a subfamily Kirjanoviinae within Cephalobidae for *Acromoldavicus* and the possibly closely related

(or even synonymous?) genus *Kirjanovia* Ivanova, 1969. However, on the basis of our observations, we consider it more appropriate to transfer *Kirjanoviinae* to the family *Elaphonematidae* Heyns, 1962. This family currently contains only one genus, *Elaphonema* Heyns, 1962, characterized by having a strongly ventrally inclined lip region with one dorsal process and two wide, outspread ventosublateral flaps, a stoma with membranous walls, and apparently a pharyngeal collar reaching the oral aperture (cf. Figs 1, 2, 6 in Van den Berg *et al.*, 1984; Figs 1, 2, 14, 15 in Van den Berg *et al.*, 1986). The morphology of *Acromoldavicus* resembles that of *Elaphonema* in all four respects: *i*) the lip region is weakly ventrally inclined; *ii*) the pairs of leaf-shaped lips might be an intermediate state on an evolutionary line towards the strongly modified processes of *Elaphonema*; *iii*) the stoma has relatively few and weak sclerotizations; and *iv*) the pharyngeal collar also extends to the oral aperture.

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