

# *Drilocephalobus moldavicus* Lisetskaya, 1968 from Senegal : an odd nematode adds to its reputation under the Scanning Electron Microscope (Nematoda : Rhabditida)

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## SUMMARY

*Drilocephalobus moldavicus* Lisetskaya, 1968 is reported from marginal soil at Camberene, Senegal. The species is described as seen under light microscope and SEM; some features new for the genus are reported and illustrated : amphids appearing externally as protrusions instead of invaginations, head with 6 + 4 papillae, male tail region with variable number of papillae in cephalobid arrangement, pharynx with glandular tissue and two ventrosublateral ampullae.

## RÉSUMÉ

*Drilocephalobus moldavicus* Lisetskaya, 1968 provenant du Sénégal, un nématode singulier, ce que confirme le MEB (Nematoda : Rhabditida)

*Drilocephalobus moldavicus* Lisetskaya, 1968 a été récolté dans un sol pauvre à Cambérène, Sénégal. Cette espèce est décrite en microscopie optique et en MEB. Quelques caractéristiques nouvelles pour le genre sont rapportées et illustrées : les amphides apparaissent comme des protrusions et non des invaginations, la tête comporte 6 + 4 papilles, la région caudale du mâle avec un nombre variable de papilles disposées comme chez les Céphalobides, le pharynx un tissu glandulaire et deux ampoules ventrosublérales.

This is the third nematological paper contributing to a research project on soil improvement in Senegal's Sahel zone (Danneels *et al.*, 1987). Soil samples collected on a plot of marginal soil at Cambérène, Senegal yielded representatives of several uncommon nematode genera, which were studied with light microscope and SEM. Previous papers deal with the genera *Metacrobeles* Loof, 1962 (De Ley, Coomans & Geraert, 1989) and *Bathyodontus* Fielding, 1950 (De Ley & Coomans, 1989). This paper presents new information on *Drilocephalobus moldavicus* Lisetskaya, 1968. It is the fifth report of this species, which was first found at Kishinev, then twice more in Moldavia (Dementeva, 1968, 1973) and most recently in the Netherlands (Bongers, 1988).

The genus *Drilocephalobus* was erected by Coomans and Goodey (1965) to accommodate their new species *D. congoensis*, and considered intermediate between Drilonematidae and Cephalobidae. It was assigned to Drilonematidae by Lisetskaya (1968), when she described the species examined here, and then to a separate family Drilocephalobidae by Ali, Suryawanshi and Chisty (1973), who described two more species : *D. coomansi* and *D. goodeyi*. They pointed out that the genus differed too much from drilonematids to be considered part of even the same superfamily, and placed their new family in Cephaloboidea instead, together with a.o. Osstellidae, a family erected at the same time to accommodate *Osstella hamata* Heyns, 1962. Andrassy (1984) did not follow their reasoning, placing *Drilocephalobus* in Osstellidae, and this in turn in Drilonematoidea. Recently, Zell

(1987) described *D. humophilus*, the fifth and last species known of the genus.

## Material and methods

Soil samples were collected during the summer of 1985, on July 26 (two samples) and August 12 (twenty samples), by Mr. F. De Facq, on a small plot of marginal soil at the "Centre pour le Développement de l'Horticulture" (CDH) at Cambérène, Senegal. This research station is a division of the "Institut Sénégalais de Recherches Agricoles" (ISRA) and a co-operation centre between Senegal, Belgium and the FAO.

The soil proved very nutrient-poor (cf. Tab. 1 in Danneels *et al.*, 1987). Several combinations of agricultural treatments were tried during experiments on soil conditioning (Danneels *et al.*, 1987); since these treatments had no clear nematofaunal effects, no further details are given here.

Nematodes were fixed with hot 4% formaldehyde and mounted in dehydrated glycerine (Seinhorst, 1962). *Drilocephalobus moldavicus* Lisetskaya, 1968 was found in ten of the twenty-two samples; 32 females, 16 males and 13 juveniles were isolated. Ten females and ten males were measured (Tab. 1). One female, two males and one juvenile were treated with Spurr's low-viscosity epoxyresin (Luc, Coomans & Sarr, 1987) and examined with SEM.

All specimens are deposited in the Nematode Collec-

tion of the Instituut voor Dierkunde, Rijksuniversiteit Gent, Belgium.

***Drilocephalobus moldavicus* Lisetskaya, 1968**  
(Figs 1 & 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

*Adult* : Body very small, straight to variously curved. Cuticle 0.5 µm thick, annulated; annuli 1.4-1.7 µm wide. Five lateral lines; annulation continuing on outer wings of the lateral field. Head helmet-shaped, 4-5 µm high and 5 µm wide, appearing under light microscope as distinctly offset by large, slit-like amphidial openings. Amphids actually covered by two pouch-like outcroppings (of the *corpus gelatum?*), as revealed by SEM. These pouches must be composed of a hyaline substance with refractory index very close to that of dehydrated glycerine : in some specimens they are also visible with light microscope, but only vaguely. Lips completely amalgamated. Four cephalic and six labial papillae present, the latter lying at the angles of a hexagonal platelet (1.5 µm wide in juvenile of Fig. 2 D) bearing the small mouth opening (0.5 µm wide in same juvenile). Papillae and platelet usually not visible with light microscope. Stoma virtually absent, mouth opening recognisable as a perforation of the head cuticle. Pharynx lumen collapsed up to head cuticle in all specimens, lumen wall not thickened (local distention of lumen in Fig. 1 B). Pharynx without distinct subsections, but widening slightly at about one third of pharynx length and more distinctly basally. Individual muscle fibers most distinct at level of both these widenings; basal "bulb" with three nuclei; ventral pharyngeal tissue granulated in between both widenings, rather glandular than muscular in appearance, with two ventrosublateral ampullae at level of anterior widening. Cardia small, rounded. Nerve ring and excretory pore usually at about two thirds of pharynx length, but rather variable in position. Hemizonid just posterior of excretory pore, two annuli wide. Deirids absent. Pharynx enveloped by a coat of small coelomocytes. One large uninucleate gland lying ventrally to lateroventrally of the anterior end of the intestine. It is usually more developed in males (34-41 µm or *ca* 2.5 neck widths long) than in females (20-29 µm or *ca* 1.5 neck widths long) and is probably associated with the excretory canal. Intestinal wall with microvilli (up to 2 µm long) and filled with globules, especially in females, thus obscuring the ventral gland more in these than in males, where it is conspicuous even at low magnification.

*Female* : Vulva at slightly less than two-thirds of body, inconspicuous. Vagina short. Postvulval uterine branch (PUB) present, 21-25 µm long, sometimes filled with sperm. Reproductive system well-developed, reflexed at level of spermatheca, with ovary straight and often reaching the rectum. Spermatheca offset, 18 µm ± 7 (11-27) long, usually pyriform : with one large globular sperm mass at base and with tapering tip of variable length sometimes containing separate, smaller sperm masses. Ovary with two rows of cells, oviduct consisting of eight or ten cells. Uterus sometimes with one egg (47-51 × 15-17 µm; n = 3). Rectum slightly longer

Table 1

Measurements (in µm) of *Drilocephalobus moldavicus* Lisetskaya, 1968 from Cambèrène, Senegal

	Females	Males
n	10	10
L	286-381 (350 ± 28)	337-377 (357 ± 15)
V/T	58-64 (62 ± 2)	42-49 (46 ± 2)
a	14.3-17.0 (15.9 ± 0.9)	18.3-25.4 (20.7 ± 2.1)
b	3.3-4.3 (4.0 ± 0.3)	3.8-4.3 (4.0 ± 0.1)
c	10.6-13.6 (12.1 ± 0.8)	10.5-12.7 (11.2 ± 0.7)
c'	2.2-2.7 (2.5 ± 0.1)	2.5-3.2 (2.7 ± 0.2)
Pharynx	79-92 (86 ± 4)	85-93 (88 ± 3)
Tail	25-31 (29 ± 2)	28-35 (32 ± 2)
Body diam.	20-26 (22 ± 2)	14-20 (17 ± 2)
Anal diam.	10-13	10-13
Excr. pore	40-73 (62 ± 9)	61-68 (65 ± 3)
Excr. pore (%)	46-81 (72 ± 11)	70-80 (75 ± 4)
Nerve ring	44-62 (56 ± 5)	54-63 (58 ± 3)
Nerve ring (%)	51-71 (65 ± 6)	61-71 (66 ± 4)
Phasmids from anus	9-13 (10 ± 2)	10-16 (12 ± 1)
Phasmids from anus (%)	31-42 (36 ± 4)	33-41 (37 ± 3)
Vagina	3-8 (5 ± 2)	—
Rectum	12-15	—
Spicules	—	14-18 (15 ± 1)
Gubernaculum	—	7-12 (9 ± 2)

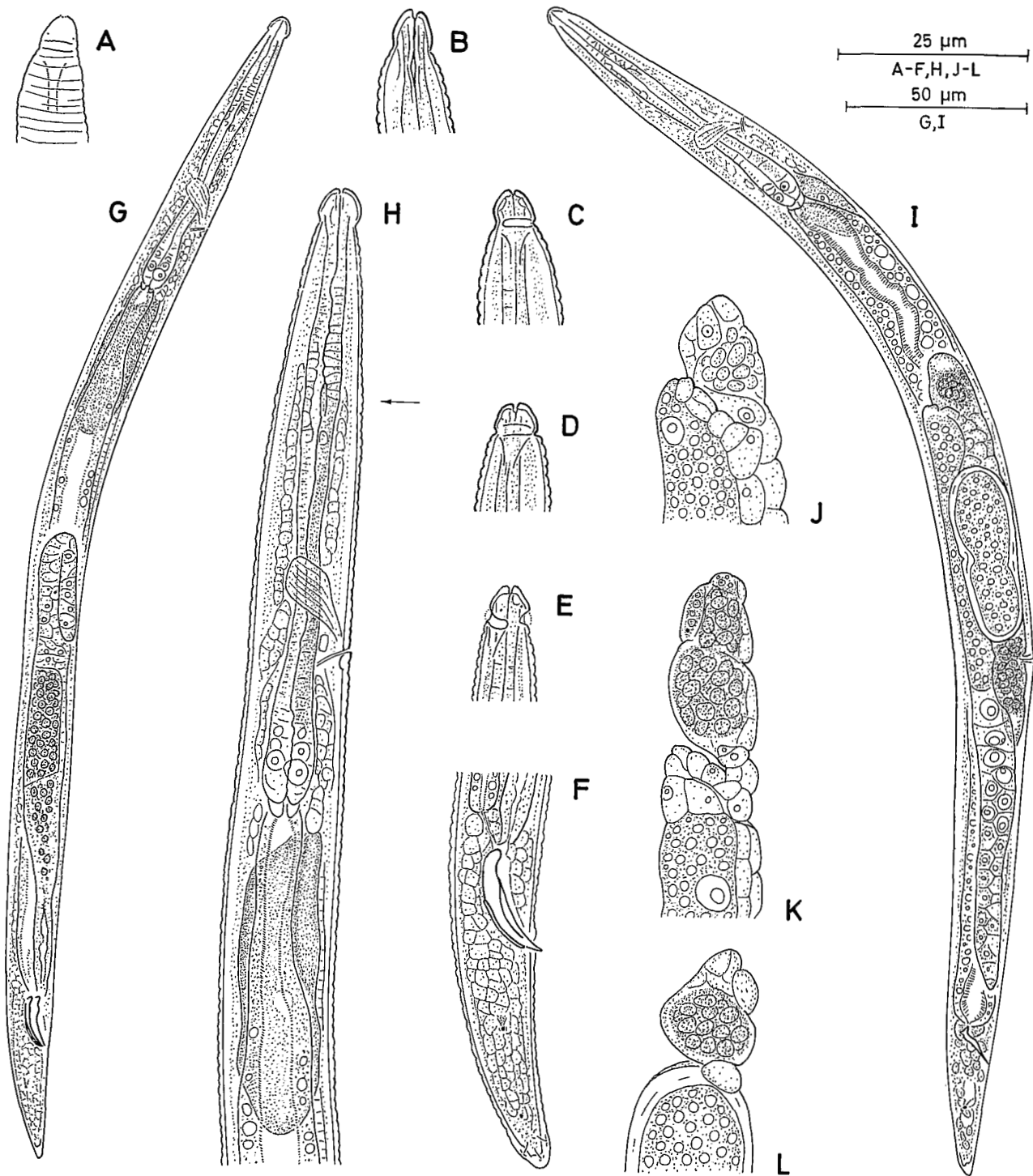


Fig. 1. *Drilocephalobus moldavicus* Lisetskaya, 1968 — A-E : Different aspects and shapes of head. A : External view; B : Internal view; C-E : Internal view with amphid superposed (E : sublateral view, with amphidial pouches faintly visible); F : Male tail; G : Whole male; H : Male, anterior half of body (arrow is at level of ampullae); I : Whole female; J-L : Different spermathecae more or less packed with sperm (L : intermediate, in dorsal view, with egg).

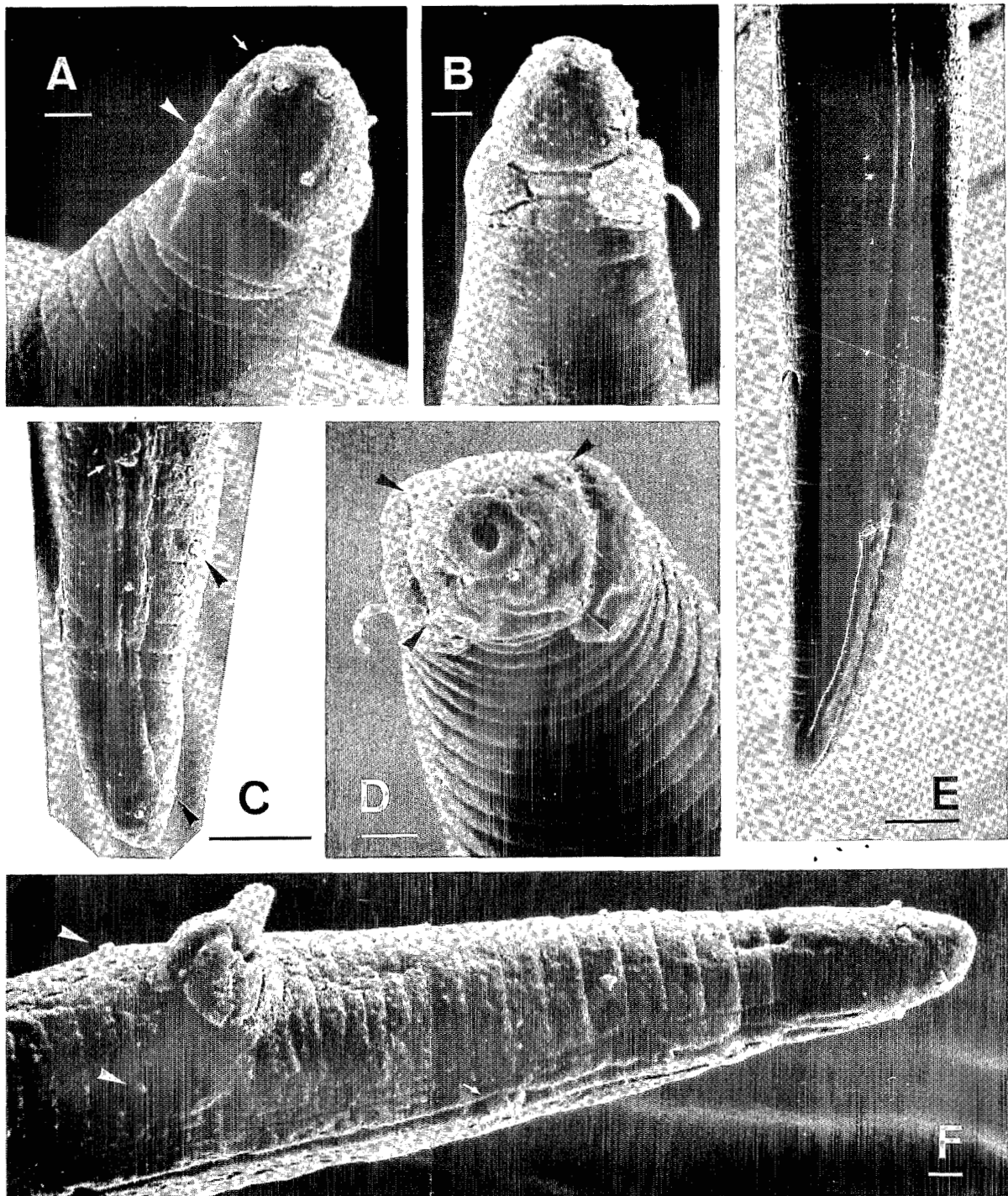


Fig. 2. *Drilocephalobus moldavicus* Lisetskaya, 1968 — A, B, D : Head of juvenile (sensory ending (?) protrudes at right side of head; arrowheads point at cephalic papillae, arrow at a labial papilla); C, F : Male tail (arrowheads indicate two subventral male papillae in C, one preanal pair in F; arrow points at phasmid); E : Tail and lateral field of juvenile (similar to female tail and lateral field). (Bar = 1  $\mu$ m in A, B, D, F; = 5  $\mu$ m in C, E.)

than one anal body width. Female tail straight, conical, blunt. Phasmids at about one third of tail length.

*Male* : Body more slender than that of female. Reproductive system with ventrally reflexed testis tip. Testis with two rows of spermatocytes. Globular sperm cells lying in a separate mass (*vesicula seminalis*) anterior to *vas deferens*. Spicules and gubernaculum cephalobid. Male tail similar to female tail but with papillae and packed with small cells. The two males examined with SEM differed considerably in position and number of papillae, but both exhibited the following arrangement of postanal paired papillae : two pairs posterior to phasmid, one of which on lateral field; three pairs on tail tip. One male had a single unpaired papilla preanally left-subventrally, the other one had one full preanal pair at this level, plus three more unpaired papilliform structures subventrally on its left side : two anteriorly of the preanal papillae, one postanally. It is not clear which of the two males represents a more aberrant condition, because usually only the papillae on the tail tip are distinguishable with the light microscope.

#### DISCUSSION

*Comparison with described species* : The Senegalese specimens agree best with *Drilocephalobus moldavicus* : five lateral lines and blunt tail. Lisetskaya (1968) does not mention presence of spermatheca, PUB or ventral gland, however, and gives a smaller egg size ( $39 \times 13 \mu\text{m}$ ), slightly larger spicules ( $19.5\text{--}21.2 \mu\text{m}$ ) and single-row ovary and testis. Furthermore, an illustration of the species (Fig. 2 in Lisetskaya, 1971) shows annulation continuing on all four lateral wings and spermatheca absent. Unfortunately, the reliability of this plate is severely put in question by a dorsal excretory pore (!) and a very schematic representation of the organs. Not having seen paratypes of *D. moldavicus*, it seems prudent not to assign our material to a new species.

*New features of Drilocephalobus and relationships with other genera* : The genus shows resemblances to four other nematode groups, viz. the families Cephalobidae (Filipjev, 1934) Chitwood & Chitwood, 1934 and Elaphonematidae Heyns, 1962, and the genera *Perodira* Baylis, 1943, *Daubaylia* Chitwood & Chitwood, 1934 and *Osstella* Heyns, 1962. Very striking are, firstly, the characters resembling but not exactly similar to those of Cephalobidae and Elaphonematidae :

— Pharynx : suggesting, but not actually showing clear subdivision in corpus, isthmus and bulb; basal "bulb" not valvate. The presence and position of the ventrosublateral ampullae reported here imply homology of the long, narrow part between both pharyngeal widenings in *Drilocephalobus* with the proportionally much shorter pharyngeal isthmus in Rhabditidae, Cephalobidae and Elaphonematidae. It should be noted

that the ampullae in *D. moldavicus* lie much more anteriorly in the pharynx than is known for any other secernentean nematode.

— Female reproductive system : reflexed at level of spermatheca, but without double flexure at ovary tip. Spermatheca not always offset.

— Male papillae : two mediocaudal pairs of papillae (with one pair on lateral fields), but these lying *posterior* instead of anterior to phasmids; three pairs on tail tip; only one (?) preanal pair. A similar arrangement of the postanal papillae relative to phasmid, with phasmid in anterior half of tail, has also been found in *Elaphonema messinae* van den Berg, Swart & Heyns, 1984 and in *E. karoense* van den Berg, Meyer & Heyns, 1986. The phasmid position shifts within this genus : phasmid at half the tail length and at level of both mediocaudal pairs of papillae (both of which lie ventrally of the lateral field) in males of type species *E. mirabile* Heyns, 1962 as originally described; phasmid as in most Cephalobidae at half the tail length or more, always posterior to mediocaudal pairs (one of which lies on lateral field) in *E. juanheynsi* van den Berg, 1988, *E. noca* van den Berg, 1988, *E. proteae* van den Berg, 1986 and in males of *E. mirabile* as described by van den Berg (1988). Probably, the condition in *D. moldavicus* and the two *Elaphonema*-species first mentioned is plesiomorphic : there is less difference in phasmid position between males and females (both with phasmids in anterior half of tail) than in Cephalobidae and in the other *Elaphonema*-species (phasmids in anterior half in females, in posterior half in males).

The differences between both males of *Drilocephalobus* examined with SEM, and particularly the presence of three unpaired papillae or papilliform structures on one side of the body in one of them, hints at the phenomenon of occasional reappearance of reduced organs : male Cephalobidae and Elaphonematidae have two or more preanal pairs of papillae, males of *D. moldavicus* may represent a stage in the loss of preanal papillae. More males of the genus should be studied to check this. It is worth noting that under light microscope males of *D. humophilus* are characterized by a pair of small papillae close to the tail tip and two very prominent pairs close to the anus, one preanal and one postanal.

— Spicules of cephalobid type, but cephalobid mechanism aiding spicule protrusion (copulatory muscles acting on a midventral preanal cuticular strip, cf. De Ley, Coomans & Geraert, 1989) lacking.

— 6 + 4 papillae on head, similar to but much smaller than cephalobid papillae.

— Body cavity in neck and, especially in male, tail region packed with small coelomocytes. This closely resembles the aspect of *juvenile* cephalobids.

The structure of head, amphid (under light microscope) and stoma is, however, very different from either that of Cephalobidae or that of Elaphonematidae. As

pointed out by Coomans and Goodey (1965), *Drilocephalobus* seems much closer to the drilonematid *Perodira pheretimae* Timm, 1960 in this respect, and also in presence of ventral gland and in structure of pharynx and reproductive organs. Our SEM-study confirms these similarities in more detail in two respects: similar position of cephalic and labial papillae and similar number of paired male papillae (five postanal pairs and one (?) preanal pair in one of the *Drilocephalobus*-males). There are, however, considerable differences too: *P. pheretimae* has the excretory pore well posterior of the cardia, large phasmids in asymmetrical position, females without spermatheca, males with bursa and without spicules, pronounced sexual dimorphism in tail shape and a smooth cuticle. The only other species known of the genus, *P. alata* Baylis, 1943, is less related, with both sexes lacking the distinct ventral gland and with the males even lacking genital papillae. Both species are parasites of oligochaetes.

A fourth relative of *Drilocephalobus* seems to be the genus *Daubaylia* Chitwood & Chitwood, 1934, which shows great resemblance in pharynx structure and has a rather similar female reproductive system and male copulatory apparatus. Seven species are known, some of these revealing further similarities: *D. dewiti* Schuurmans Stekhoven, 1956 has annuli and a ventral gland; females of *D. olsoni* Poinar, 1984 have a spermatheca. On the other hand, *Daubaylia* has stomatorhabdia, small amphids, six discrete lips, a single flexure in the ovary tip (sometimes), a different arrangement of the male papillae and also a pronounced sexual dimorphism in tail shape. All species are moderately specialized parasites of planorbid snails, one is also found in leeches (Poinar, 1984).

The fifth and last related form is *Ostella hamata* Heyns, 1962, of which no males are known. It is free-living and has a similar pharynx, female reproductive system, tail and cuticle, but a more developed stoma and an aberrant head (four lobes around a central projection carrying the mouth). It should be noted that *Drilocephalobus* (on an otherwise smooth head) also exhibits some differentiation around the mouth opening, the hexagonal platelet, which is apparently even visible with light microscope in *D. coomansi* (cf. Fig. 1 A-D in Ali, Suryawanshi & Chisty, 1973).

The amphidial pouches instead of openings in *Drilocephalobus* are, as far as we know, unique. SEM-studies of Ostellidae, Daubayliidae and Drilonematidae are required to shed more light on the taxonomic importance of this character, which must clearly be considered as an apomorphy. Cephalobids have small, open amphids, while amphids have not been found in *Elaphonema*, neither with SEM nor with the light microscope (Heyns, 1962; van den Berg, Swart & Heyns, 1984; van den Berg, Meyer & Heyns, 1986; van den Berg, 1988).

**Conclusion:** The genus *Drilocephalobus* seems to fit in between Cephaloboidea and Drilonematoidea. Pharynx

structure and arrangement of male papillae strongly suggest it to be derived from a common ancestor with recent cephalobids, having undergone some reductions: no stoma or valvular bulb, but still a rudimentary subdivision of the pharynx; (probably) reduction in number and size of male papillae. *Daubaylia* and *Ostella* may have evolved along this line too, as witnessed by their pharynx structure. *Drilocephalobus* exhibits some characters (head structure and ventral gland) aberrant from Cephaloboidea and reminiscent of the drilonematid genus *Perodira*, but it is not at all similar to Drilonematoidea in other respects: there is no proof of a zooparasitic habit, it lacks the strong development of the reproductive system and does not show the much more pronounced reduction or aberrant structure of other organs. At present, we agree with Cephaloboidea *sensu* Ali, Suryawanshi and Chisty (1973), assigning *Drilocephalobus* to a separate family within this superfamily.

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