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Revision of the species *Psyllotylenchus pawlowskyi* (Kurochkin, 1960) Poinar & Nelson, 1973. II. Description of *Kurochkinitylenchus laevicepsi* gen. n., sp. n. and Spilotylenchidae fam. n.

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Summary. A new genus and species is proposed as the result of a revision of the species *Psyllotylenchus pawlowskyi sensu* (Kurochkin, 1960) Poinar & Nelson, 1973, which was found to represent three distinct species. A description of morphology and life-cycle of *Kurochkinitylenchus laevicepsi* gen. n., sp. n., a parasite of *Nosopsyllus laeviceps* fleas, is given. The nematode has alternation of two parasitic heterosexual generations during its life-cycle. Peculiarities in the life-cycle of the new genus correspond to the diagnosis of the subfamily Parasitylenchinae, but morphological features of the new genus do not coincide with contemporary diagnosis of this subfamily. New genus can be distinguished from other genera of Parasitylenchinae by dorsal curvature of the female body, stylet morphology (strong basal thickening but without knobs), absence of cephalic cone, well separated spermatheca and several other features. An analysis of the phylogenetic relationships of *Kurochkinitylenchus* gen. n. with other genera of the superfamily Sphaerularioidea is presented. Morphological comparison is made with the genera *Spilotylenchus* (Contortylenchinae), *Psyllotylenchus, Incurvinema* (Heterotylenchinae, Parasitylenchidae) and *Rubzovinema* (Phaenopsitylenchidae). A new family Spilotylenchidae fam. n., new subfamilies, Spilotylenchinae subfam. n., Psyllotylenchinae subfam. n., Rubzovinematinae subfam. n. and Kurochkinitylenchinae subfam. n., are proposed.

Key words: *Kurochkinitylenchus laevicepsi* gen. n., sp. n., fleas, morphology, biology, phylogenetic relationship.

Kurochkin (1960) described a tylenchid nematode, *Heterotylenchus pawlowskyi* (= *Psyllotylenchus pawlowskyi*) from the haemocoel of *Coptopsylla lamellifer* Wagn. and *Nosopsyllus laeviceps* Wagn. fleas collected from around the Astrakhan Station of Plague Control in the Kharabali and Krasnyi Yar districts of the Astrakhan region in the Lower Volga. Alternation of heterosexual and parthenogenetic generations was reported to occur in the life-cycle of the nematode.

The type locality from which *P. pawlowskyi sensu* (Kurochkin, 1960) Poinar & Nelson, 1973 had been visited during 1985 and parasitic tylenchid nematodes associated with *C. lamellifer* and *N. laeviceps* fleas were collected. Investigation of the morphology and life-cycle of the newly collected nematodes revealed that Kurochkin's description included representatives of three parasitic nematode species, none of which belonged to the heterogonic genus *Psyllotylenchus*. Furthermore, it was found that the parasitic female of the heterosexual generation described by Kurochkin (1960) represents a species which only parasitizes *C. lamellifer* fleas. This species belongs to the genus *Spilotylenchus*, in which alternation of generations does not occur during the nematode life-cycle. This species was redescribed as *S. pawlowskyi* (Kurochkin, 1960) *partim* Slobodyanyuk, 1997 in the first part of a revision of *Psyllotylenchus pawlowskyi* species (Slobodyanyuk, 1997). Further results from this revision are reported here, which include a re-evaluation of morphology, biology and taxonomic status of the second species, *Kurochkinitylenchus laevicepsi* gen. n., sp. n. which only parasitizes *N. laeviceps* fleas. These data are provided to assist with resolving the taxonomic problem associated with the original description of *Psyllotylenchus pawlowskyi sensu lato*.

Classification of the superfamily Sphaerularioidea was revised on the basis of examination of the morphology of *Kurochkinitylenchus laevicepsi* gen. n.,

sp. n. A new family, Spilotylenchidae fam. n., and four new subfamilies, Spilotylenchinae subfam. n., Kurochkinitylenchinae subfam. n., Psyllotylenchinae subfam. n. and Rubzovinematinae subfam. n. of which it is comprised, are described.

MATERIALS AND METHODS

The material and methods used in the present study were described in the first part of this investigation (Slobodyanyuk, 1997). The oesophagus, including the oesophageal glands, was measured to calculate the "b" index.

Spilotylenchidae fam. n.

Diagnosis. Sphaerularioidea. Several types of life-cycles are possible: one or two parasitic heterosexual generations, alternation of parasitic heterosexual and parasitic parthenogenetic generations, and alternation of parasitic heterosexual and free-living heterosexual generations. Also, one, two or three types of adults may be present in the host's body cavity and from two up to four types occur in the environment.

Parasitic female. Body obese, curving, with ventral side always turned outward. Center of cephalic region never elevated as a cone. Head-end dimorphism in parasitic females of primary and secondary generations present when alternation of two parasitic generations occurs. Stylet strong and large in primary female, generally basally thickened, never knobbed. Orifice of dorsal gland in vicinity of stylet base, orifices of subventral glands slightly more posterior. Oesophagus cylindrical, isthmus absent. Distinct large spermatheca present in females of both parasitic generations. Sac-like uterus occupying half or more of the body cavity. Vulva not deeply cleft. Ovoviviparous.

Partially free-living forms. Excretory pore anterior to nerve ring. In infective-stage female, three long oesophageal glands extending over intestine. Ovary still underdeveloped. In male, glands shorter; spicules tylenchoid; gubernaculum and bursa present.

Female and male of free-living generation (when alternation of parasitic and free-living generations occurs). Sexual dimorphism of anterior body end absent. Stylet fine, with minute knobs, three short oesophageal glands. Oviparous. In males, spicules tylenchoid; gubernaculum and bursa present.

Hosts. Parasites of Syphonaptera.

Differential diagnosis. The new family is related to Fergusobiidae, Phaenopsitylenchidae (except *Beddingia*, which is not considered to be a true Phaenopsitylenchidae) and Contortylenchinae (Allantonematidae) as a result of the dorsally curving parasitic female body. However, Spilotylenchidae fam. n. differs from these families and subfamily as follows:

- In Fergusobiidae the parasitic female stylet is short, generally under 10 μm long, with prominent knobs, a distinct spermatheca is absent, the uterus is tube-like and rather short, being less than half a body length long. Oviparous. In partially free-living forms, the excretory pore is posterior to the nerve ring, oesophagus is broad and fusiform with a valve-like structure situated anteriorly, a short istmus is present, the oesophageal glands are short, bulboid, and only partially extending over the intestine. In females, the ovary and oviduct are flexured, consist of numerous cells; in male, the spicules robust, angular, and a gubernaculum is absent. Parasites of Diptera.
- In Phaenopsitylenchidae (except the genus *Rubzovinema*) the parasitic female has a cephalic cone, the stylet is short, under 10 μm long, the genital tube does not exceed a body length, there is not a distinct spermatheca, and the uterus is tube-like. Oviparous. Infective-stage heterosexual female has only two short oesophageal glands. Parasites of Coleoptera.
- In Contortylenchinae (except genus *Spilotylenchus*) parasitic female cephalic cone is always present, the stylet is generally under 10 μm long with prominent knobs, a distinct spermatheca is absent, and the uterus is tube-like and less than half a body length long, and the vulva is deeply cleft, in a depression. Oviparous. In partially free-living forms, the excretory pore is posterior to the nerve ring. Parasites of Coleoptera.

Type subfamily: Spilotylenchinae subfam. n.

Other subfamilies: Kurochkinitylenchinae subfam. n.
Psyllotylenchinae subfam. n.
Rubzovinematinae subfam. n.

Key to subfamilies of the family Spilotylenchidae fam. n.

1. One type of adults (parasitic female) present in host's body cavity 2
- More than one type of adults present in host's body cavity 3
2. Two types of adults (partially free-living male and female) occur in the environment. Life-cycle without alternation of generations ..... Spilotylenchinae subfam. n.
- Four types of adults occur in the environment (males and females of free-living generation laying eggs outside of the host, and partially free-living males and females). Sexual dimorphism of head end absent in representatives of the former generation but

present in the latter one. Life-cycle with alternation of heterosexual parasitic and free-living generations... .....Rubzovinematinae subfam. n.

3. Two types of adults (parasitic females of heterosexual and parthenogenetic generations) present in host's body cavity. Head-end dimorphism of two types of females present. Life-cycle with alternation of both parasitic heterosexual and parthenogenetic generations. Copulation occurs outside the host's body cavity .....Psyllotylenchinae subfam. n.

- Three types of adults (parasitic female of primary heterosexual generation, parasitic female and male of secondary heterosexual generation) present in host's body cavity. Head-end dimorphism of two types of females present. Life-cycle with alternation of two parasitic heterosexual generations. Copulation occurs in host's body cavity..... ..... Kurochkinitylenchinae subfam. n.

Spilotylenchinae subfam. n.

Diagnosis. Spilotylenchidae. Only one heterosexual generation known. One type of adult (parasitic female) present in host's body cavity and two types (partially free-living female and male) occur in the environment.

Parasitic female. Small to large, elongate-obese, dorsally arcuate or spiral with ventral side always turned out when relaxed. Head-end without cephalic cone. Tail end short, generally directed dorsally. Typical spilotylenchid stylet, oesophagus and dorsal and subventral oesophageal gland orifice topography. Oesophageal lumen narrow, not strongly sclerotized. Distinct spermatheca filled with minute (dia. < 1 μm) spermatozoa. Sac-like uterus occupying up to 2/3 of body volume, filled with numerous juveniles and eggs. Vulva not prominent, not deeply cleft, not in a depression. Ovoviviparous.

Partially free-living female and male are typically spilotylenchid.

Type genus: *Spilotylenchus* Launay, Deunff & Bain, 1983.

No other genera.

Psyllotylenchinae subfam. n.

Diagnosis. Spilotylenchidae. Life-cycle with alternation of both parasitic heterosexual and parthenogenetic generations. Two types of adult (primary heterosexual and secondary parthenogenetic parasitic female) present in host's body cavity and two types (partially free-living female and male) occur in the environment.

Parasitic female body **of heterosexual generation** elongate-obese, crescent-shaped, curved on the dorsal (or ventral in *Incurvinema*) side, never curved spirally when relaxed. Tail-end short. Typical spilotylenchid stylet, oesophagus and topography of dorsal and subventral oesophageal gland orifices. Ovary and oviduct coiled in anterior region. Distinct spermatheca filled with minute spermatozoa. Sac-like uterus occupying up to 2/3 of body volume and filled with numerous eggs and juveniles. Ovoviviparous.

Parasitic female body **of parthenogenetic generation** fusiform crescent-shaped, curved on dorsal side, considerably shorter than heterosexual female. Stylet indistinct, shorter than in primary female. Reproductive tube with large sphaerical or oblong preuteral gland and sac-like uterus. Ovoviviparous.

Partially free-living forms are typically spilotylenchid.

Type genus: *Psyllotylenchus* Poinar & Nelson, 1973.

Other genus: *Incurvinema* Deunff, Launay & Beaucournu, 1985.

Rubzovinematinae subfam. n.

Diagnosis. Spilotylenchidae. Life-cycle with alternation of heterosexual parasitic and heterosexual free-living generations. One type of adult (parasitic female) present in host's body cavity and four types (partially free-living female and male and also female and male of completely free-living generation of parasitic generation) occur in the environment.

Parasitic female body elongate-obese, crescent- or spiral-shaped, dorsally curved. Tail-end short, conoid. Typical spilotylenchid stylet, oesophagus and topography of dorsal and subventral oesophageal gland orifices. Distinct spermatheca filled with minute (dia. < 1 μm) spermatozoa. Sac-like uterus occupying from 1/2 up to 2/3 of body volume. Ovoviviparous.

Female of free-living generation. Body straight when relaxed. Stylet thin and short. Three shortened oesophageal glands. Excretory pore situated anterior to nerve ring. Oviparous, with one to a few eggs in short, tube-like uterus. Spermatheca filled with minute spermatozoa.

Male of free-living generation. Head-end as in female. Spicules small, tylenchoid. Gubernaculum and narrowing peloderan bursa present.

Type genus: *Rubzovinema* Slobodyanyuk, 1991.

No other genera.

Kurochkinitylenchinae subfam. n.

Diagnosis. Spilotylenchidae. Life-cycle with alternation of two heterosexual generations in host's body cavity. Three types of adult (parasitic female of primary heterosexual generation, parasitic female and male of secondary heterosexual generation) present in host's body cavity and two types (partially

free-living female and male) occur in the environment.

Parasitic females of both generations sausage-shaped or fusiform, with ventral side always turned outward. Distinct spermatheca filled with large spermatozoa (dia. up to 7 μm). Ovoviviparous. Typical spilotylenchid primary parasitic female. Stylet dimorphism in parasitic females of primary and secondary generations present.

Parasitic and free-living male. Body slender, mobile. Stylet thin and short. Excretory pore anterior to nerve ring. Spicules small, tylenchoid. Gubernaculum and narrowing peloderan bursa present.

Type genus: *Kurochkinitylenchus* gen. n.

No other genera.

Kurochkinitylenchus gen. n.

Diagnosis. Kurochkinitylenchinae, Spilotylenchidae. Life-cycle with alternation of two parasitic heterosexual generations in host's body cavity. Three types of adult (parasitic female of primary heterosexual generation, parasitic female and male of secondary heterosexual generation) present in host's body cavity and two types (partially free-living female and male) occuring in the environment.

Parasitic female of primary generation sausage-shaped, with ventral side always turned outward. Stylet strong, thickened basaly but not knobbed, generally under 20 μm long. Excretory canal strong sclerotized, opened in stylet region or apically. Ovary and oviduct coiled in anterior region. Spermatheca filled with large spermatozoa (dia. up to 7 μm). Sac-like uterus occupying up to 1/2 of body volume and often filled with eggs and juveniles. Ovoviviparous.

Parasitic female of secondary generation sausage-shaped or fusiform, with ventral side always turned outward. Stylet thin, with minute knobs, shorter than in primary female. Excretory canal thinner, ovary and oviduct longer, distinct spermatheca much larger than in parasitic female of primary generation. Sac-like uterus occupying up to 2/3 of body volume and often filled with eggs and juveniles. Spermatheca filled with large spermatozoa (dia. up to 7 μm). Ovoviviparous.

Partially free-living female. Stylet as in parasitic female of primary generation. Excretory canal strongly sclerotized, opening anterior to nerve ring. Ovary reduced to a few cells. Spermatheca still not formed. Uterus tube-like. Vulva not far from anus.

Parasitic and partially free-living male. Body slender, mobile. Stylet thin and short. Excretory pore anterior to nerve ring. Spicules small, tylenchoid. Gubernaculum and narrowing peloderan bursa present.

Type species: *Kurochkinitylenchus laevicepsi* gen. n., sp. n.

No other species.

DESCRIPTION

Kurochkinitylenchus laevicepsi gen. n., sp. n. (Figs. 1-8).

Synonymy: *Heterotylenchus pawlowskyi* Kurochkin, 1960 *ex parte* "mature (producing juveniles) parthenogenetic generation female" (p. 1282, Fig. 1B, D), male (p.1281, Fig. 1E, K).

Psyllotylenchus pawlowskyi (Kurochkin, 1960) Poinar & Nelson, 1973 *ex parte*.

Life-cycle characterized by alternation of two parasitic heterosexual generations in host's body cavity. Three types of adults (parasitic female of primary heterosexual generation, parasitic female and male of secondary heterosexual generation) present in host's body cavity and two types of adult (partially free-living female and male) occuring in the environment.

Morphometrics of adults presented in Table 1.

Adult parasitic female of primary heterosexual generation. Short, colourless, nearly motionless, sausage-shaped or coiled spirally as an incomplete single curve, ventral side always turned outward (Fig. 1A). Sometimes body swollen in anterior third. Body walls very thick, with prominent transverse folds in most females. Cephalic end broadly rounded, not offset from body contour. Cephalic or labial papillae not present (Figs. 1B & 2A-E). Posterior end from rounded without mucro (Fig. 2H, I) or rounded with pointed mucro 5-7 μm long (Figs. 1A & 2F, G, J) to conically sharp (Fig. 2K). In some females mucro submerged in body folds (Figs. 1A & 2J). Stylet as two parts: a strongly sclerotized conical blade and a less sclerotized shaft ending with slightly thickened base (Figs. 1B & 2A-C). Total stylet length 14-17 μm, conus length 5-6 μm. Stylet lumen indistinguishable, with opening on dorsal side of stylet. Oesophagus short, almost cylindrical, without ismus. Oesophageal lumen narrow, with lightly sclerotized lining. Dorsal gland orifice 2-3 μm behind basal thickening of stylet, orifices of subventral glands not visible. Nerve ring 23-33 μm from anterior end. Excretory pore apically close to oral opening (Figs. 1A, B & 2C, E) or on ventral side at the level of the stylet (Fig. 2A, B & D). Excretory duct long, broad and winding, strongly sclerotized. Distal end of excretory duct often extending out of body surface. Excretory cell body indiscernible. Intestine narrow,

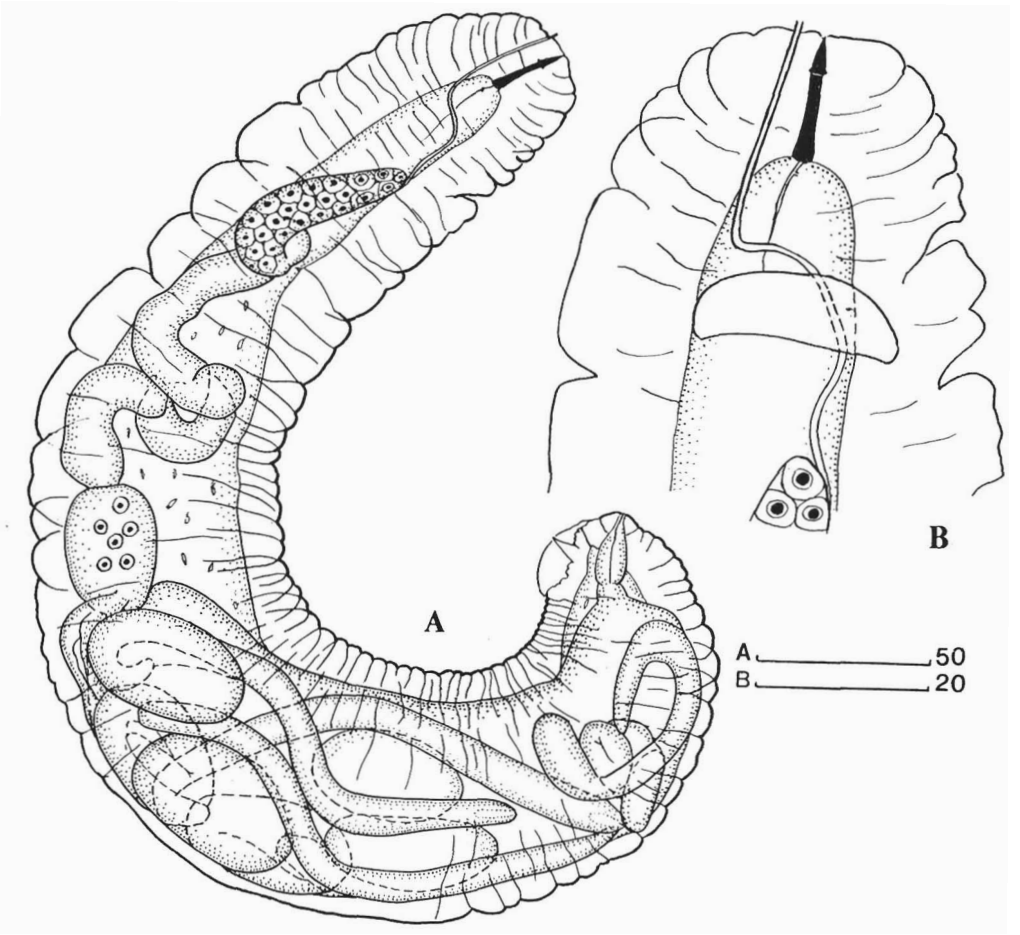

Fig. 1. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. A: Parasitic female of the primary heterosexual generation, lateral view; B: Anterior end. (Scale bars in μm).

without lumen and with microvilly outward. Rectum thin-walled, sometimes obscured in body folds. Reproductive tube relatively short, ovary and oviduct mostly forming two flexures with top always directed forward. Spermatheca rounded or oval 17-40 x 13-17 μm, considerably narrower then body width. In young parasitic females spermatheca filled with large spermatozoa (dia. 4-6 μm); in old females only a few spermatozoa present, or spermatozoa absent. Narrow and short part of oviduct behind spermatheca present. Relatively few eggs and juveniles in the sac-like uterus which occupies about half body volume. In some old females no eggs or juveniles in wrinkled uterus which is 5-6 times smaller than body. Vagina short, weakly cuticularized, vulvar lips not protruding (Figs. 1A & 2F-K). Ovoviviparous. Sometimes *endotokya matricida* occurs.

Secondary heterosexual completely parasitic generation.

Fourth stage female juvenile (n=15): L = 394.7± 15.3 (306-507) μm, D=39.8±3.1 (25-50) μm. Body cylindrical, straight or slightly curved ventrally just after third molting (Fig. 3C). Cuticle smooth. Cephalic region conoid-rounded, slightly overgrown by body expansion (Fig. 3A). Tail short, conically tapering, with rounded terminus (Fig. 3B). Stylet very thin, 7-8 μm in length, with small knob-like thickening (Fig. 3A). Dorsal and subventral oesophageal glands absent. Excretory pore anterior to the nerve ring. Genital tube straight, with top reaching level of nerve ring. Spermatheca still not developed. Uterus length about a third of genital tube (Fig. 3C). Body of female juveniles gradually increasing both in length and width and curving on the dorsal side after repetitive copulations with males. Ovary and oviduct elongate and producing loop-like flexure in anterior third of the body. Initially, after copulations has occurred, the uterus is filled with large spermatozoa, 6-8 μm dia (Fig. 3D, E). Thereafter, spermatozoa move into large spermatheca formed at junction between the uterus and oviduct (Fig. 3F). Vagina not fully formed, funnel-shaped (Fig. 3D, E). Small vulvar opening ovoid 7-8 x 4-5 μm (Fig. 3F- H).

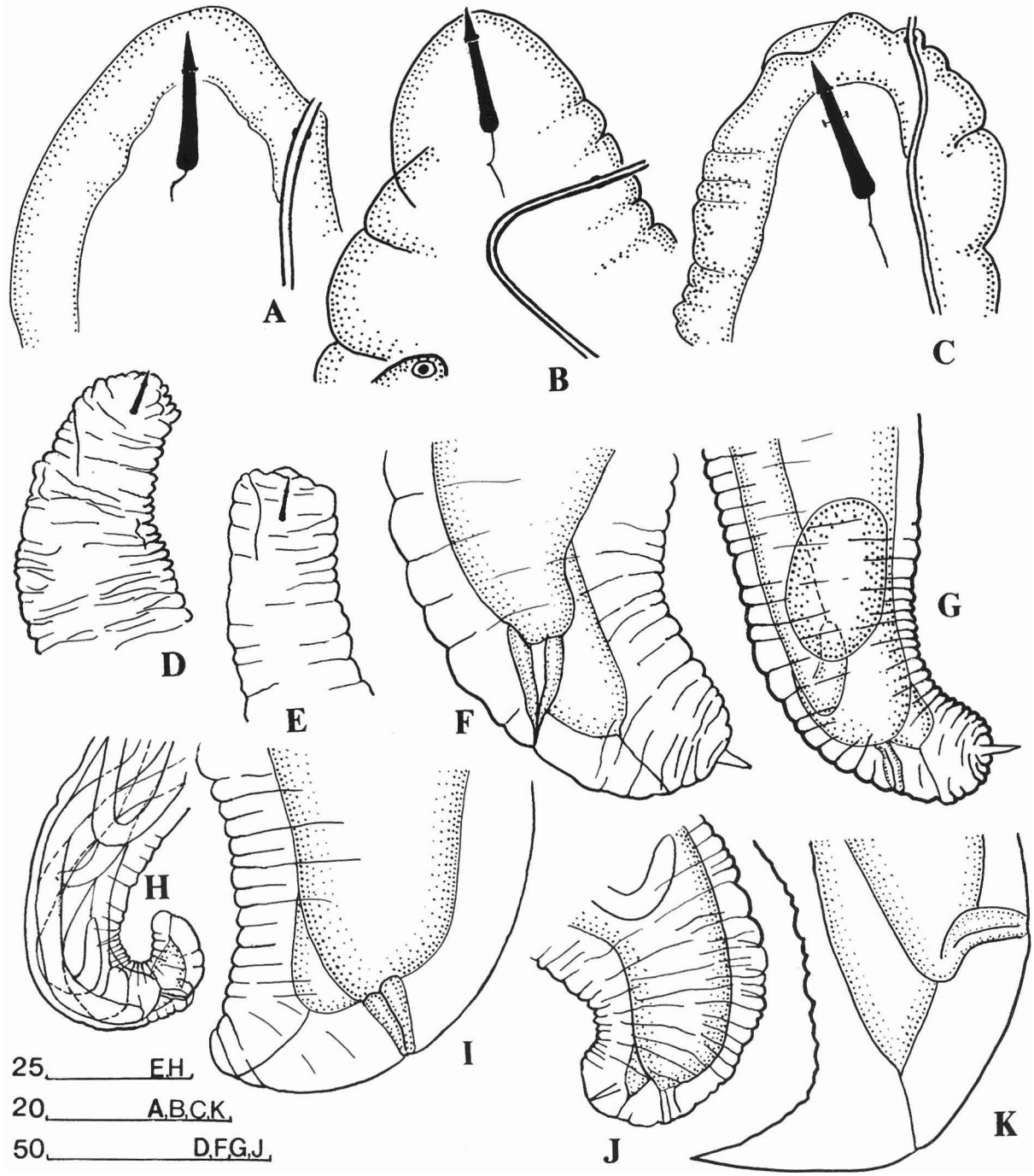

Fig. 2. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. parasitic females of the primary heterosexual generation. A - E: Anterior end; F - K: Posterior end. (Scale bars in μm).

Non-protruding vulvar lips and short vagina formed during the fourth molting. Posterior vulvar lip exceeds the anterior one. Tube-like uterus completely empty in most of female juveniles during the last molt (Fig. 5A) or contains some spermatozoa which have not yet migrated to the spermatheca. Uterus of such juveniles occasionally containing eggs (Fig. 3G). Juveniles of this stage can be distinguished from the females by the absence of vulvar lips and by having a small ovoid, not slit-like, vulvar opening and underdeveloped vagina.

Adult parasitic female. Colourless, nearly motionless, crescent-shaped with ventral side always turned outward. In young females just after last molting the body is almost cylindrical (Fig. 5A). In females with only eggs in the uterus, maximal body width is at

Table 1. Measurements and diagnostic features of *Kurochkinitylenchus laevicepsi* gen. n., sp. n. adult forms (All measurements in μm).

Character	Paratypes of primary parasitic females	Secondary parasitic females		Paratype of parasitic males	Paratypes of partially free-living nematodes	
		holotype	paratypes		females	males
n	16	1	45	22	19	6
L	457±20 (375-647)	570	550±13.6 (382-725)	364±9.7 (258-448)	446±30 (320-755)	499 (326-594)
D	63±3.4 (42-82)	110	91±4.1 (47-225)	17.0±0.6 (14-19)	18.4±0.7 (13-22)	18.8 (15-22)
Cd	14.2±2.6 (3-37)	22	17.2±1.4 (5-47)	28±0.7 (23-32)	29±1.1 (24-32)	32 (27-37)
a	7.5±0.5(5.3-12.2)	5.2	6.3±0.2 (2.4-9.7)	22±0.8 (13.5-25.3)	24±1.4 (16-38)	27 (22-38)
b	–	–	–	–	1.4±0.2 (1.2-1.9)	–
c	47±7.8 (11.2-125)	26	46±6.1 (19.2-226)	13.3±0.5 (8.3-17.3)	15.1±0.6 (12-21)	15.4 (12.1-18.3)
V%	93±0.7 (89-96)	90	91±0.2 (88-95)	–	90±0.5 (87-91)	–
Vulva-anus distance	18.0±2.1 (7-37)	38	34±1.1 (12-49)	–	21±0.9 (17-27)	–
Vulva to tail tip	32±3.5 (17-55)	60	51±1.7 (27-70)	–	49±2.2 (43-63)	–
Spicules	–	–	–	15.1±0.2 (14-17)	–	15.3 (15-17)
Gubernaculum	–	–	–	6.9±0.2 (6-9)	–	7.8 (6-9)
Anterior to excretory pore	12.0±2.3 (0-33)	18	15.6±1.5 (0-37)	26±0.8 (21-31)	30±1.4 (23-37)	34 (25-40)
Anterior to nerve ring	0-33	–	0-37	39±1.6 (29-46)	50±4.2 (35-67)	58 (56-62)
Anterior to uterus	185±26 (84-334)	230	191±5.8 (105-285)	–	–	–
Stylet	15.2±0.3 (13-17)	8	8.5±0.01 (8-10)	8.8±0.2 (8-10)	15.7±0.3 (14-18)	9.8 (9-10)
Eggs in uterus	43 (36-53)x23 (20-28)	–	49 (38-65)x27 (22-35)	–	–	–

Table 2. Infection of *Nosopsyllus laeviceps* fleas by nematodes *Kurochkinitylenchus laevicepsi* gen. n., sp.n.

Locality	Dessected specimens			Infected specimens		
	females	males	total	females	males	total
Astrakhan region	111	40	151	8 (7.2%)	1 (2.5%)	9 (6.0%)
Stavropol region	86	56	142	2 (2.3%)	5 (8.9%)	7 (4.9%)
Guriev region	172	90	262	5 (2.9%)	1 (1.1%)	6 (2.3%)
Uralsk region	914	376	1290	–	–	–
Azerbaijan	50	29	79	4 (8.0%)	5 (17.2%)	9 (11.4%)
Total	1333	591	1924	19 (1.4%)	12 (2.0%)	31 (1.6%)

Table 3. Measurements and diagnostic features of *Psyllotylenchus pawlowskyi* (after Kurochkin, 1960) (All measurements in μm).

Character	Parthenogenetic females	Males	Non-impregnated free-living females
L	600-820	300-550	430-500
D	56-120	–	18-20
Cd	25-30	25-30	–
Vulva to tail tip	60-78		–
Spicules	–	13-15.7	–
Gubernaculum	–	4	–
Anterior to excretory pore	18-30	–	–
Stylet	–	4-10	8-10
Eggs in uterus	47-59 x 30-38	–	–

level of gonad loops or spermatheca (Fig. 5B, C). In mature female with uterus packed with eggs and juveniles, maximal body width is at level of anterior third of uterus (Figs. 4A & 5D). Cephalic-end broadly rounded, not offset from body contour. Cephalic cone absent (Figs. 4B & 5E). Posterior end conically tapered, with top from almost pointed (Fig. 5B, D) to rounded (Figs. 4C & 5C, F-H). Stylet thin, with short distal blade and minute knobs (Figs. 4B & 5E). Oesophagus almost cylindrical, short, without sclerotized lining. Oesophageal glands not visible. Nerve ring often displaced by gonad to head end. Excretory pore often apical, or at level of stylet, never more than 37 μm from head end. Excretory duct less sclerotized than in primary female. Intestine narrow, without lumen. Rectum thin-walled, anus present. Ovary and oviduct forming several flexures in anterior third of body. Number and orientation of these flexures differ between specimens. Spermatheca large, almost rounded, 37-85 μm long and 32-92 μm wide, in both young and old females filled with large spermatozoa. Narrow tube of oviduct behind spermatheca present (Fig. 4D). Uterus short and tube-like just after fourth molting (Fig. 5B). In mature female uterus sac-like, occupying up to 2/3 body volume (Fig. 5D). Vagina short, not sclerotized. Vulvar opening a traverse slit 11-15 μm wide (Fig. 5H). Ovoviviparous.

Parasitic male. Body very mobile, short, slender, slightly narrowing towards head end (Fig. 6A). Cephalic-end not offset (Fig. 6 B). Posterior-end curved ventrally when relaxed. Tail short, rounded at the end (Fig. 6A, C, D). Cuticle with slight annulation. Lateral field smooth, 2-3 μm wide (Fig. 6E). Stylet fine, with three small knobs (Fig. 6B). Oesophagus short, cylindrical. Oesophageal glands not visible. Excretory duct sclerotized. Excretory pore opens anterior to nerve ring. Hemizonid indiscernible. Genital tube outstretched with top almost reaching nerve ring. Spicules tylenchoid, slightly cephalated, with distal ends merging together (Fig. 6C, D). Gubernaculum present. Bursa peloderan, very narrow, in lateral view almost indiscernible (Fig. 6A, C, D).

Partially free-living forms.

Female. Short and slender, cuticle sharply annulated, especially in anterior half of body (Fig. 7A, B). Lateral field smooth, 5-6 μm wide. Head continuous with body. Stylet as in primary parasitic female (Fig. 7B). Oesophagus short, cylindrical, slightly narrowing at junction with intestine, where nerve ring occurs. Oesophageal lumen slightly enlarged up to subventral gland orifices, thereafter obscure. Oesophageal glands lobe-like, with three cells extending into anterior half of body, not reaching top of ovary. Dorsal gland opening 2-3 μm behind basal thickening of stylet, openings of subventral glands about 1 stylet length posterior. Broad, strongly sclerotized excretory duct opens anteriorly to the nerve ring. Distal end of excretory duct often extruding out of body surface (Fig. 7B). Hemizonid indistinguishable. Outstretched genital tube situated in posterior third of body: represented by oval 6-10 celled ovary primordium (Fig. 7A, C, D), undifferentiated oviduct and tube-like uterus, which filled with spermatozoa after copulation in the environment. Vagina not formed completely. Vulva without protruding lips. Anus present.

Male. Body as in free-living female. Cuticle deeply annulated (Fig. 8A, B). Lateral field with 7 lines (Fig. 8E). Sexual dimorphism pronounced in anterior body part. Head continuous with body, shape similar to that of female. Stylet shorter than in female, very thin with three small knobs (Fig. 8B). Oesophagus short, cylindrical. Oesophageal lumen weaker than in female, dorsal and subventral gland orifices obscure. Oesophageal glands shorter than in female with bases not distinguishable because of outstretched genital tube (Fig. 8A). Excretory duct narrower and less sclerotized than in female, with distal end never extruding out of body surface. Excretory pore and nerve ring similar in position to those in female. Hemizonid not observed. Gonad extending anteriorly to behind nerve ring. *Vas deferens* filled with sperm, longer than half genital tube length (Fig. 8A). Spicules, gubernaculum and bursa similar to those in parasitic male (Fig. 8C, D).

Biology. From a total of 1924 dissected specimens of *Nosopsylla laeviceps*, 31 (1.6%) were found to be infected with *K. laevicepsi* gen. n., sp. n., the latter being encountered not only in type locality in the Astrakhan region, but also in the Stavropol region, (Russia), Guriev region, (Kazakhstan) and from Azerbaijan, the habitat of the host. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. specimens were not found during dissection of a large number of fleas from northernmost Uralsk region (Table 2).

The life-cycle of *K. laevicepsi* gen. n., sp. n. is characterized by obligatory alternation of two heterosexual generations in the host coelom (Fig. 9). The partially free-living stages of *Kurochkinitylenchus laevicepsi* gen. n., sp. n. occur under the nest of the flea host. Fertilized female nematodes with underdeveloped genital systems make contact with flea larvae and enter the body cavities, probably by penetrating through the cuticle, as occurs with other sphaerularioids. In the flea's hemocoel, the fertilized female develops into a primary heterosexual parasitic female. The process of sexual maturation of these females is synchronized with the host's morphogenesis. From one to seven (mean=1.9) mature ovoviviparous pri-

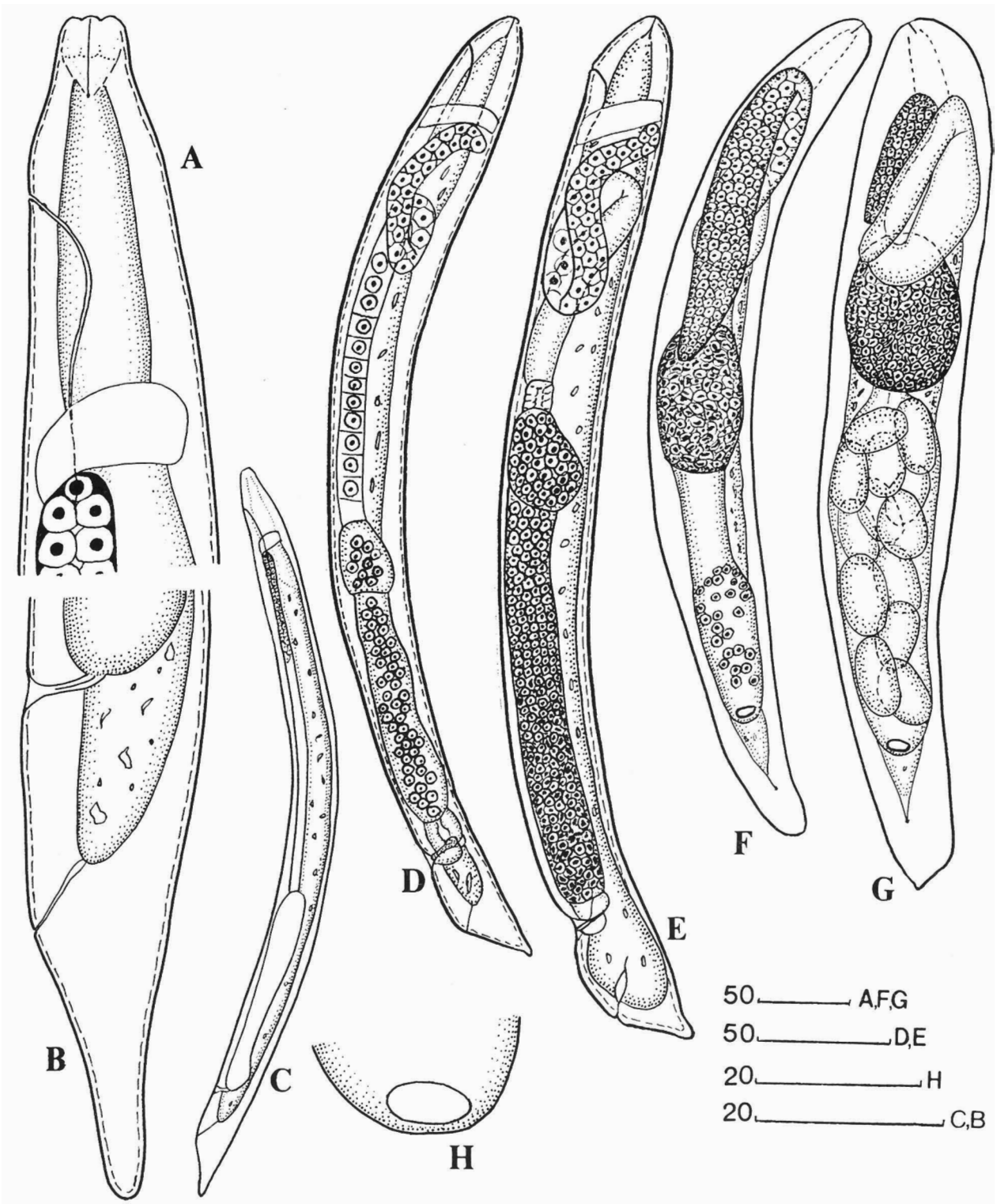

Fig. 3. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. parasitic fourth stage female juveniles of the second heterosexual generation. Juveniles before copulation: A: Anterior end; B: Posterior end; C: Lateral view. Juveniles after copulations: D & E: Lateral view; F & G: Ventral view; H: Posterior uterus end with vulvar opening, ventral view. (Scale bars in μm).

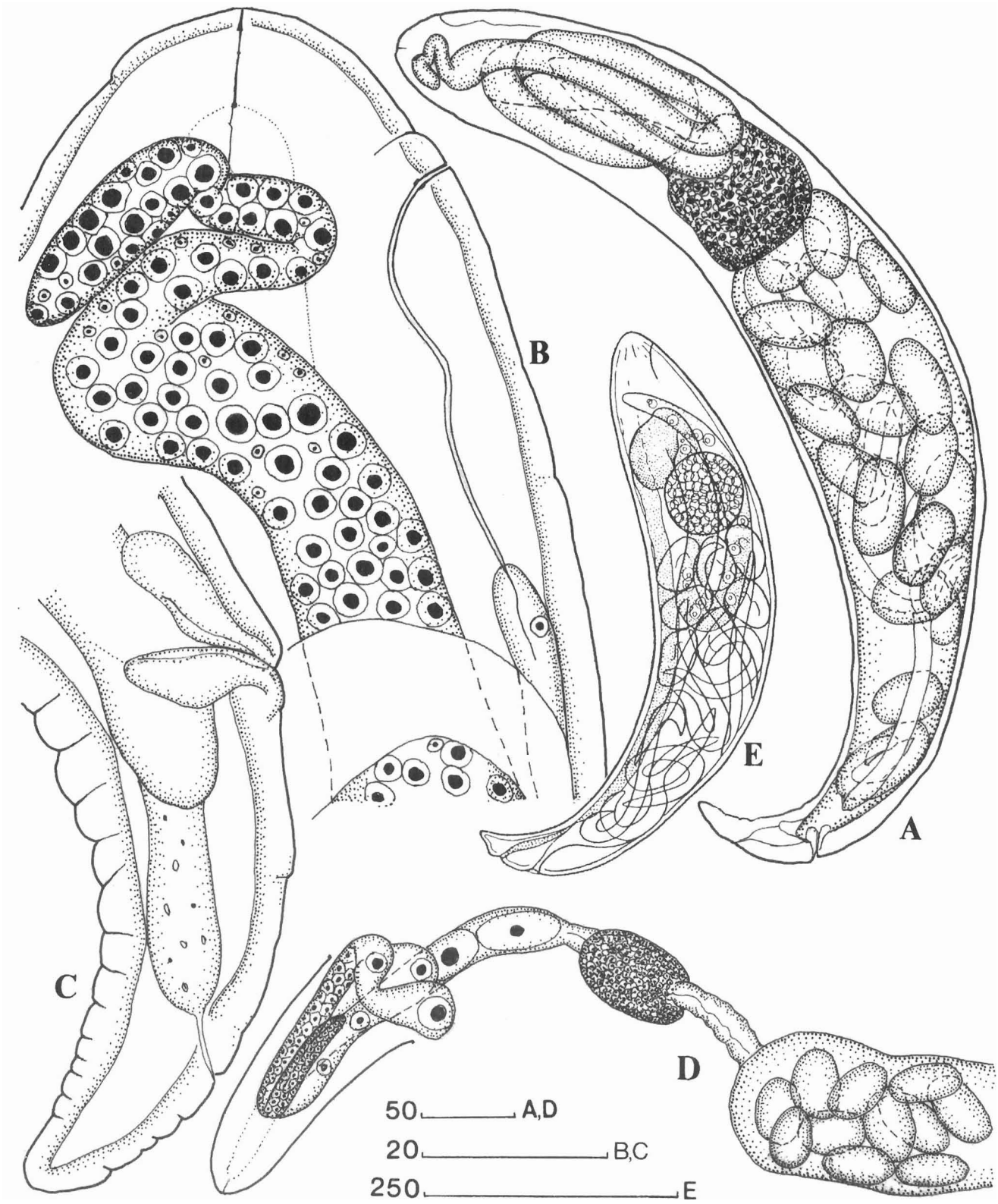

Fig. 4. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. parasitic females of the second heterosexual generation: A & E: Lateral view; B & C: Anterior and posterior ends of the same female; D: Fragment of genital tube with ovary, oviduct, well defined spermatheca and anterior part of uterus extruding from a female body. (A - D, original; E, redrawn from Kurochkin, 1960). (Scale bars in μm).

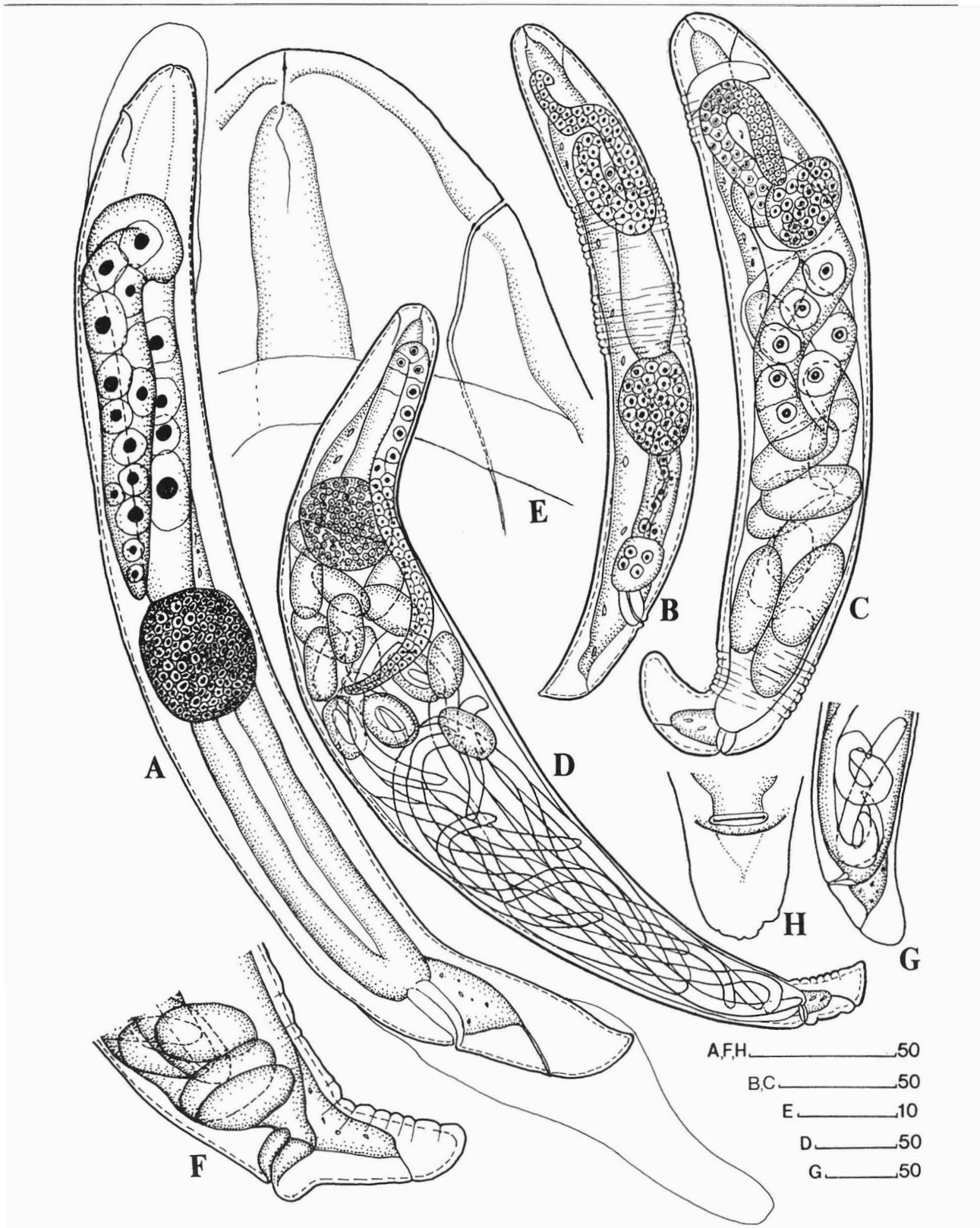

Fig. 5. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. different age parasitic females of the second heterosexual generation: A: Female during last molting, uterus still without eggs or juveniles; B: Young female just after molting with only remains of the spermatozoa and without eggs or juveniles in the uterus; C: Older female already with eggs but without juveniles in the uterus; D: Oldest female with eggs and juveniles in the uterus; E: Anterior end of the same female as C; F-H: Mature female posterior end. F & G, lateral view; H: ventral view with slit-like vulvar opening.(Scale bars in μm).

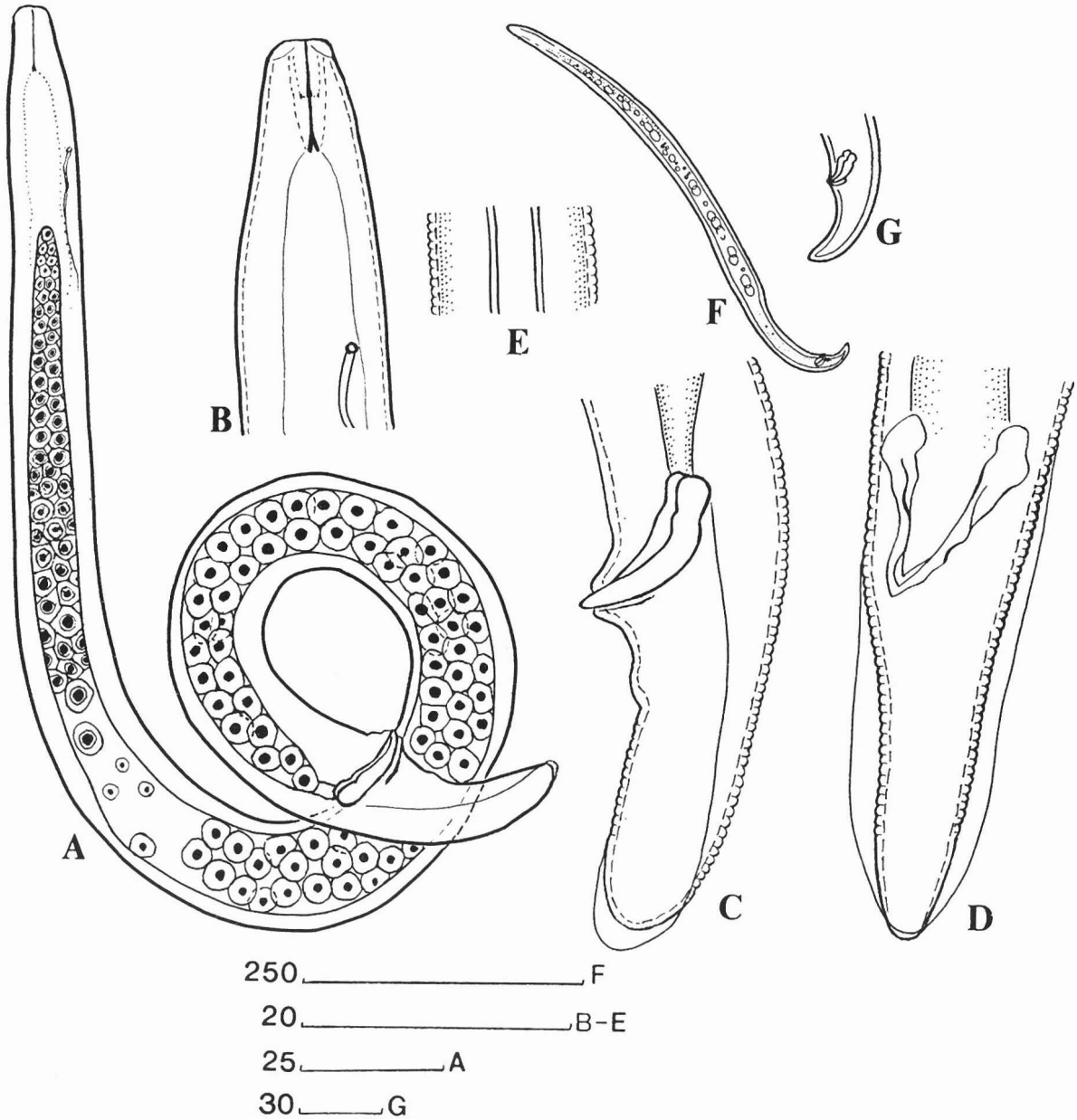

Fig. 6. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. parasitic males of the second heterosexual generation: A & F: Lateral view; B: Anterior end; C, D & G: Posterior end (C & G, lateral view; D, subventral view); E: Lateral field. (A - E, original; F & G, redrawn from Kurochkin, 1960). (Scale bars in μm).

mary parasitic females of *K. laevicepsi* gen. n., sp. n. were observed in the host's coelom during the young flea's exhatchment from pupa envelopes. The juveniles produced by these females develop into the bisexual form in the host's body cavity. Very thin male juveniles appear in the host's haemocoel, thereafter thick female juveniles appear. The former develop very quickly and after four molts turn into very mobile parasitic males. The males never exit the host haemocoel into the environment but instead wait for the female juveniles to reach the fourth stage. The final molt of juveniles and their development to secondary heterosexual parasitic females occur only after copulation. One female of the first generation can produce up to 40 males and 70 females of the second generation. These females in turn produce heterosexual juveniles that are subsequently released into the flea's hemocoel. Up to 1000 juveniles, of different size, were extracted from the coelom of individual infected fleas. These juveniles develop to the third stage before leaving the fleas via the host's intestine.

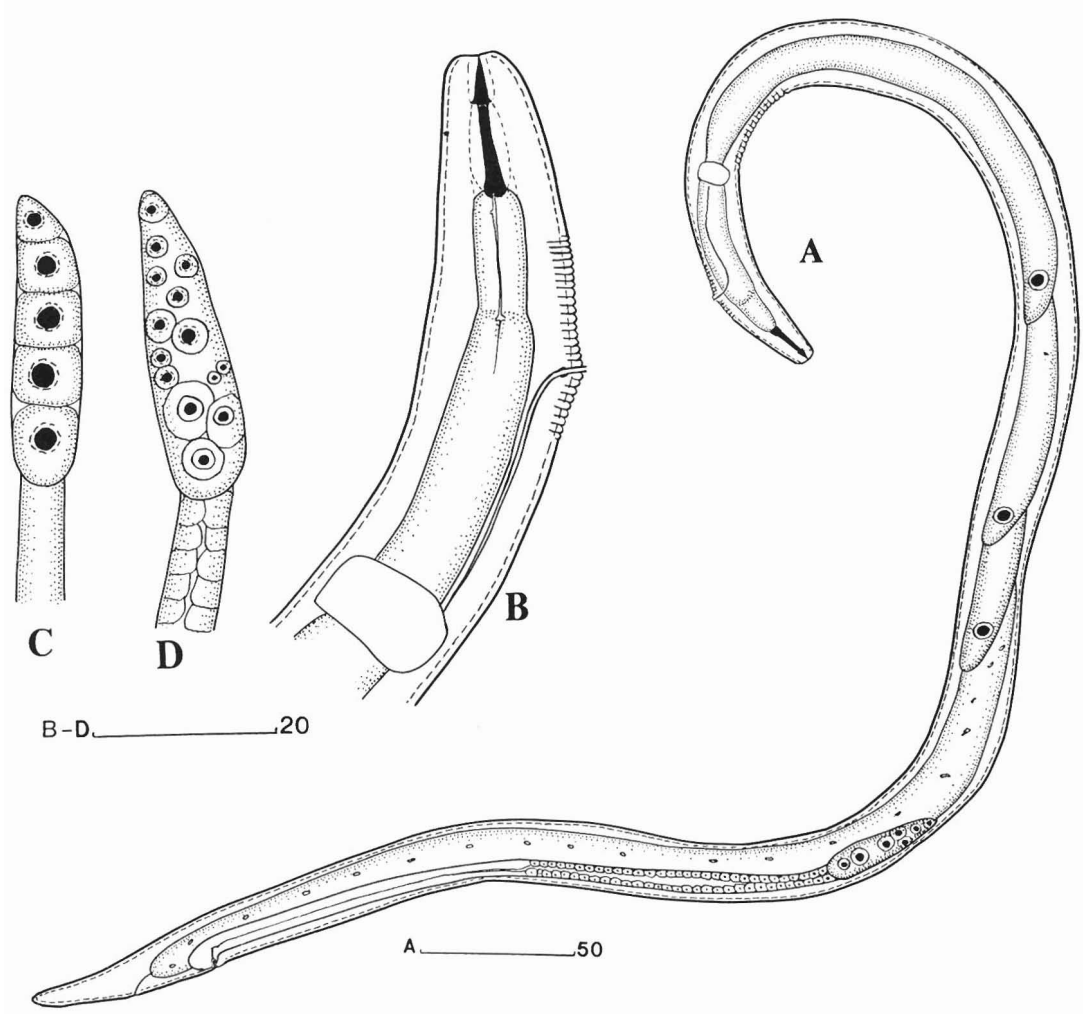

Fig. 7. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. free-living females: A: Lateral view; B: Anterior end; C & D: Ovary primordium. (Scale bars in μm).

The body length of the first stage juveniles, produced by second generation females, was 236-356 μm long and 14-18 μm wide. These specimens had a thin 6-7 μm long stylet. The first molt occurs in the host's body cavity and was observed in 302-348 μm long juveniles with the second molt occuring with 406-443 μm long juveniles. The third stage juveniles in the host's haemocoel were 426-497 μm long, with 22-25 μm body diameter and a 9-10 μm long stylet. Unlike *Spilotylenchus pawlowskyi,* penetration of juveniles into the hosts's femur muscles was not observed. In the environment, under the jird's nest, juveniles develop into partially free-living adult nematodes after two molts. Under laboratory conditions molting was observed in hanging drops after 5-7 days. The exuvia of two consecutive stages were shed simultaneously, both by male and female juveniles (Fig. 8D), although these two molts were found to be separate in male and female juveniles of the previous generation in the host's body cavity. After mating, the free-living male nematodes die without entering a host and the free-living females seek out a new host.

Type material. Holotype parasitic secondary heterosexul female, paratypes of primary and secondary heterosexual females, paratypes of parasitic and free-living males, paratypes of free-living females are deposited in the Collection of parasites in the Institute of Parasitology, Russian Academy of Sciences, Moscow, Russia.

Type host and locality. All type specimens were collected from *Nosopsyllus laeviceps* fleas in the Kharabali district of the Astrakhan region. This insect was indicated by Kurochkin (1960) as one of two type hosts and the Astrakhan region as the type locality of *P. pawlowskyi* (*sensu lato*). The *K. laevicepsi* gen. n., sp. n. parasitic female of the secondary generation designated here as the holotype was collected from the coelomic cavity of a male *Nosopsyllus laeviceps* flea, collected from a tamarisk gerbil,

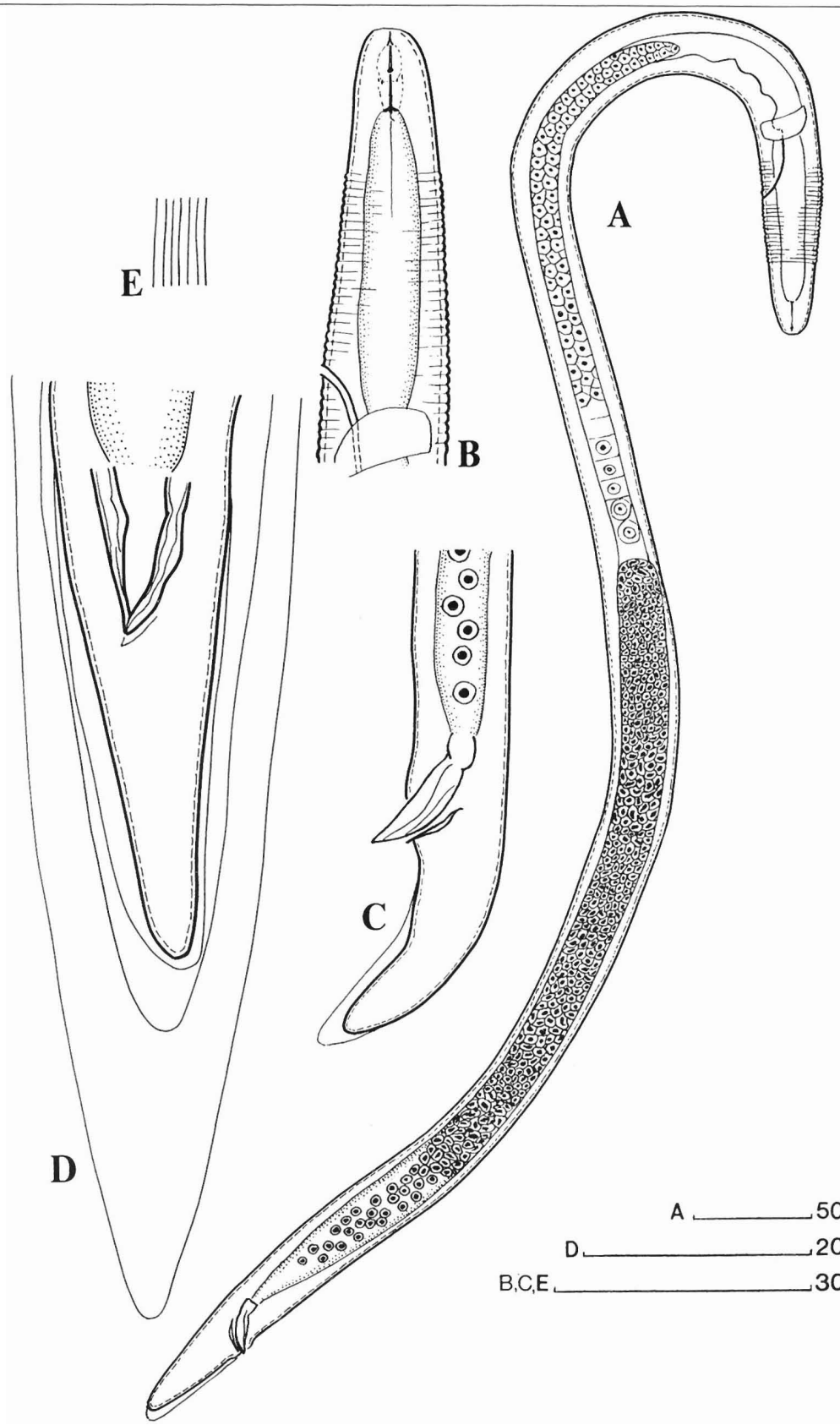

Fig. 8. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. free-living males: A: Lateral view; B: Anterior end; C & D: Posterior end. C: End of mature male in lateral view; D: End of molting male with two shed cuticles, in ventral view. (Scale bars in μm).

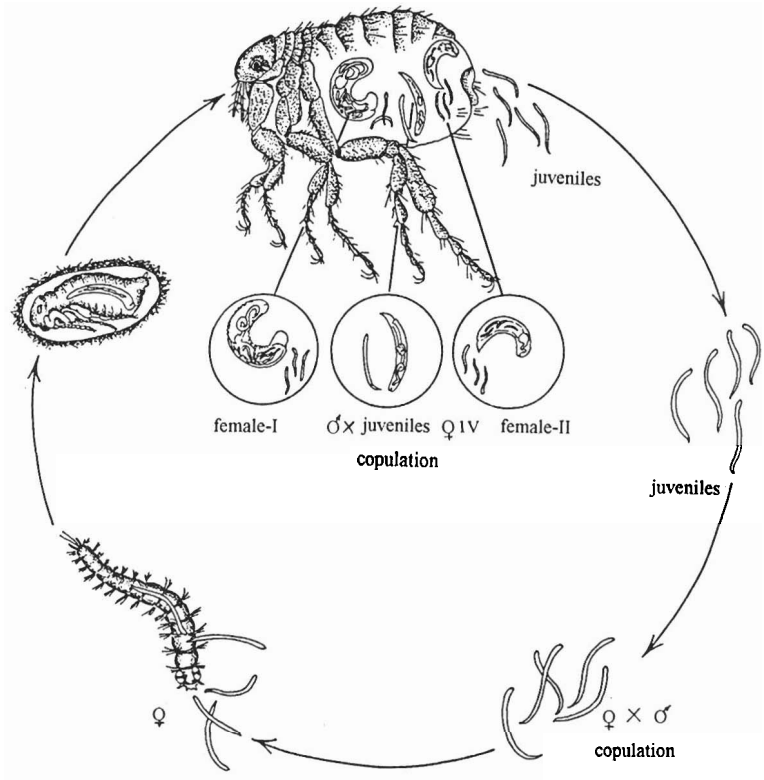

Fig. 9. *Kurochkinitylenchus laevicepsi* gen. n., sp. n. life-cycle.

Meriones tamariscinus, on 11th November, 1985, captured in bushes at the village of Tchapchachi (leg. O.V. Slobodyanyuk). The paratype parasitic females of the primary and secondary generations and parasitic males of *K. laevicepsi* gen. n., sp. n. were collected from the coelomic cavities of 5 females and 1 male of *N. laeviceps* collected from a tamarisk gerbil, *Meriones tamariscinus*, on 11th November, 1985, captured in bushes at the village of Tchapchachi. The paratype partially free-living females and males were reared from juveniles in hanging drops of physiological saline.

Diagnosis and relationships. The morphology and mophometrics of secondary, completely parasitic generation females of *K. laevicepsi* gen. n., sp. n. (Figs. 4A, B & 5D; Table 1) and males (Fig. 6A, C; Table 1) agree with those published by Kurochkin (1960) for parasitic females of parthenogenetic generation and free-living males of *P. pawlowskyi*, respectively (Figs. 4E & 6F, G; Table 3). The only exception is in the presence of bursa in males, which Kurochkin originally stated were absent. Narrow bursal alae were observed only in lateral view and were almost inconspicuous. The posterior end of the male can be positioned for ventral observation only if removed from the body. Kurochkin observed intact males, thus he was unable to examine bursa. Measurements presented by Kurochkin for the "unfertilized female of the sexual generation" of *Psyllotylenchus pawlowskyi* (Table 3) correspond closely to those of the third stage juveniles of *K. laevicepsi* gen. n., sp. n. shortly before they exit the host into the environment. Therefore, it can be presumed that the nematodes under consideration which were collected in the Kharabali district are representatives of the species described by Kurochkin (1960) from females of the parasitic parthenogenetic generation, males and unfertilized females of the "sexual generation". Kurochkin observed the development of second generation females in host coelom similar to that observed here with *K. laevicepsi* gen. n., sp. n. However, he erroneously identified these females as being parthenogenetic, and referred to the large spherical spermatheca as a "preuteral gland". He was of the opinion that males exited the host without copulation with secondary females.

In accordance with the Code of Zoological Nomenclature the nematodes reported here must be described as representing a new species. The name "*pawlowskyi*" was given earlier to the species *Spilotylenchus pawlowskyi* which was separated from the artificial species *Psyllotylenchus pawlowskyi sensu* Kurochkin (Slobodyanyuk, 1997). As the new species has alternation of two heterosexual generations and copulation occurs inside the host body cavity it can be placed in the existing genus, *Parasitylenchus* Micoletzky, 1922 (Parasitylenchinae, Parasitylenchi-

dae). However, there are sufficient morphological differences between the new species parasitizing fleas and parasitylenchs from beetles and flies for it to be placed in a new genus, *Kurochkinitylenchus* gen. n.

The new genus differs from *Parasitylenchus* by having dimorphism of body and stylet shape in parasitic females of both generations, absence of a cephalic cone in parasitic females, presence of a stronger and larger stylet without visible lumen, which is basally thickened, but not knobbed in the primary parasitic female. The genus *Kurochkinitylenchus* differs from *Parasitylenchus* and from other parasitylenchid genera in having the body of primary and secondary parasitic females curved dorsally, a genital tube with large distinct spermatheca. All members of the family Parasitylenchudae are much less specialized parasites (except the genera *Psyllotylenchus* and *Incurvinema*), the parasitic females do not have a dorsally curved body, have a weaker penetration apparatus which is short, generally < 10 μm long, with wide lumen, distinctly knobbed stylet, lightly cuticularized excretory duct, and the reproductive system is without a distinct spermatheca, as the posterior part of the oviduct performs this function. Therefore, the new genus can not be placed in the family Parasitylenchidae.

As a result of the characteristic position of the parasitic female body with the ventral surface turned outward, in the organization of the penetration apparatus and female reproductive system, a typical large and strongly thickened basally but non-knobbed stylet, distinct spermatheca, absence of a cephalic conus in parasitic females, in topography of the excretory pore, and in ovoviviparity, the new genus is related to the genera *Spilotylenchus* (Contortylenchinae, Allantonematidae), *Rubzovinema* (Phenopsitylenchidae), *Psyllotylenchus* and *Incurvinema* (Heterotylenchinae, Parasitylenchidae), each of which has been described from fleas. Despite the obvious morphological similarity of these genera they are distributed in contemporary classifications of Sphaerularioidea between three different families as a result of their various types of life-cycles.

The genus *Spilotylenchus* was placed in the family Allantonematidae by Launay *et al.* (1983), but Siddiqi (1986), in accordance with his new system of entomoparasitic tylenchids, placed *Spilotylenchus* in the subfamily Contortylenchinae Ruhm, 1956 because of its simple life-cycle, without alternation of generations. Nevertheless, Siddiqi (1986) regarded the genus as "doubtfully placed in this subfamily".

The genera *Psyllotylenchus* and *Incurvinema* were originally described in the family Allantonematidae by Poinar & Nelson (1973) and Launay, Deunff & Bain (1983), respectively. However, Siddiqi (1986) and Remillet & Laumond (1991) placed these genera in a new subfamily Heterotylenchinae Siddiqi, 1986 (Parasitylenchidae), because of alternation of heterosexual and parthenogenetic generations in the host body cavity. These genera differ from Heterotylenchinae in possessing ovoviviparity and a different structure of the genital system, *viz.*, a large ovary, oviduct tube coiling in the anterior region, distinct spermatheca in primary heterosexual females, sphaerical preuteral gland in secondary parthenogenetic females, and sac-like uterus, occupying half or more of the body volume, filled with numerous eggs and juveniles in both types of parasitic females. Also, the shape and characteristic position with the ventral body surface turned outward of both heterosexual and parthenogenetic females, stylet in heterosexual females without knobbed base, and the excretory pore positioned anterior to the nerve ring in partially free-living forms serve to distinguish these genera. The presence of a bursa and gubermaculum in males also separate these genera from Heterotylenchinae.

The genus *Rubzovinema* was placed in the family Phaenopsitylenchidae Blinova & Korenchenko, 1986 by Slobodyanyuk (1991) because of the alternation of parasitic and free-living generations in the life-cycle. The author noted the morphological similarity between parasitic females of the genera *Rubzovinema* and *Spilotylenchus* and suggested more accurate taxonomic definition the former genus was required. The genus differs from other phaenopsitylenchids by possessing a typically spilotylenchid parasitic female, *viz.*, body curved dorsally, ovoviviparous, large ovary and oviduct tube coiling in anterior region, distinct spermatheca and sac-like uterus occupying half or more of the body volume and filled with numerous eggs and juveniles and large stylet without basal knobs. The presence of three, and not two oesophageal glands in infective stage heterosexual females also separates the genus from Phaenopsitylenchidae.

Spilotylenchidae fam. n. is here proposed in order to resolve the contradictions in the contemporary classification of these nematodes. Four subfamilies with different types of nematode life- cycles and all genera with morphological similarities can be placed in this family.

In the original description by Kurochkin (1960) males and two morphological forms of females were described for the nematodes recovered from the coelom of *Coptopsylla lamellifer* and *Nosopsyllus laeviceps* fleas. These two female forms differed substantially in their respective body shapes and the size of gravid females and young individuals. Kurochkin (1960) considered parasitic females characterized by having 2-3.1 mm long cylindrical bodies as repre-

senting the heterosexual generation, whereas females with 600-820 μm long, swollen mid-bodies represented the parthenogenetic generation. The author reported the length of juveniles hatched from heterosexual females as being 300-430 μm, and those from parthenogenetic females as being 300-800 μm, and the length of males produced from the later as being 300-550 μm. He also reported that all drawings and measurements were made from living material. Professor Kurochkin, shortly before his death, confirmed to the present author that type specimens and slides were not designated or deposited in any collection and that the temporary slides used for the original description were destroyed or lost.

A new investigation of nematodes parasitizing *C. lamellifer* and *N. leaviceps* was made on the basis of material collected at the type locality of Kurochkin's species. Three species of tylenchid nematodes were found associated with the two flea species. One of these species, a specific parasite of *C. lamellifer* fleas, belongs to the genus *Spilotylenchus* Launay, Deunff & Bain, 1983, and was redescribed as *S. pawlowskyi* (Kurochkin, 1960) *partim* Slobodyanyuk, 1997 in the first part of the revision of Kurochkin's *Psyllotylenchus pawlowskyi* species (Slobodianyuk, 1997). Parasitic females of this species resemble those of the heterosexual generation described by Kurochkin. The maximum length of juveniles from the host coelom produced by these females did not exceed 500 μm. The two other tylenchid species are specific parasites of the species *N. laeviceps*, with one of these belonging to the genus *Spilotylenchus* and which can be distinguished from the species parasitic in *C. lamellifer* by the smaller size of parasitic females (925-1880 μm) and by several morphological differences. Also, the maximum length of the juveniles from host coelom is about 900 μm. The second tylenchid species associated with *N. laeviceps* fleas belongs to the new genus *Kurochkinitylenchus* gen. n., which is characterized by an alternation of two heterosexual generations in the host coelom. Parasitic males and females of the second generation of this species resemble the free-living males and parasitic females of the parthenogenetic generation described by Kurochkin (compare Figs. 6F, G with 6A, C, and 4E with 5D). The maximum length of juveniles of this species from the host coelom is < 500 μm.

It may be concluded that data for the parasitic females of two separate species and parasitic males of one of these, were included in Kurochkin's description of *P. pawlowskyi*. Also, the morphometrics obtained from juveniles of three separate species probably were reported in the original description.

In the present material *Kurochkinitylenchus laevicepsi* gen. n., sp. n. was one of two tylenchid species found associated with *Nosopsyllus laeviceps* fleas in the type locality (Astrakhan region) and also in the Stavropol and Guriev regions. Also, it was the only species found in samples from Azerbaijan. Another species belonging to the genus *Spilotylenchus* was a specific parasite of *N. laeviceps* fleas recovered in all investigated regions, with the exception of Azerbaijan. The same two species were the only nematodes recovered by the present author during an investigation of Rubtsov's collection of slides containing nematodes obtained from *N. laeviceps* specimens from the south of the Uralsk region and identified by Rubtsov as *Psyllotylenchus pawlowskyi* (Rubtsov, 1981). These slides are deposited in the Zoological Institute of the Russian Academy of Sciences, St. Peterburg. Thus, it can be concluded that these two species are probably the only tylenchid nematodes which parasitizes *N. laeviceps* fleas.

To resolve the taxonomic uncertainly concerning the original description we propose the new genus, *Kurochkinitylenchus* gen. n. for one of two specific parasites of *N. laeviceps* fleas, and redescribe this species here as *K. laevicepsi* gen. n., sp. n. As a result of the absence of type specimens the holotype and paratypes were designated for this species from new material collected from the type location. The other parasitic species from *N. laeviceps* fleas will be described as a new taxon in the subsequent paper.

Etymology. *Kurochkinitylenchus* is derived from the name of late Prof. Yu.V. Kurochkin who first found and described some morphological forms of these nematodes in a complex species, *Psyllotylenchus pawlowskyi,* and '*tylenchus*', being a stem in a name of many soil, phyto- and entomoparasitic nematodes. The species name *laevicepsi* refers to the flea host name, *Nosopsyllus laeviceps.*

ACKNOWLEDGEMENTS

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REFERENCES

Kurochkin, Y.V. 1960. [The nematode *Heterotylenchus pawlowskyi* sp. n., castrating flea-vectors of plague]. *Doklady Akademii Nauk SSSR* 135: 1281-1284.

Launay, H., Deunff, J. & Bain, O. 1983. *Spilotylenchus arthuri* gen. n., sp. n. (Nematodea, Tylenchida: Allantonematidae), parasite de *Spilopsyllus cuniculi* (Da-

le,1878) (Syphonaptera: Pulicidae). *Annales de Parasitologie Humaine et Comparée* 58: 141-150.

Poinar, G.O.Jr. & Nelson, B.C. 1973. *Psyllotylenchus viviparus* n. gen., n. sp. (Nematodea: Tylenchida: Allantonematidae) parasitizing fleas (Siphonaptera) in California. *Journal of Medical Entomology* 10: 349-354.

Remillet, M. & Laumond, C. 1991. Sphaerularioid nematodes of importance in agriculture. In: *Manual of Agricultural Nematology.* (W. R. Nickle. Eds.). pp. 967-1024. New York, Dekker & Co.

Rubtsov, I.A. 1981. [*Parasites and Enemies of Fleas*]. Nauka, Leningrad. 104 pp.

Siddiqi, M.R. 1986. *Tylenchida: Parasites of Plants and Insects.* Commonwealth Agricultural Bureaux, UK. 645 pp.

Slobodyanyuk, O.V. 1991. [Validation of the genus *Rubzovinema* gen. n. (Sphaerularioidea) and revision of *Rubzovinema ceratophylla* comb. n.]. *Zoologichesky Zhurnal* 70, 9: 33-43.

Slobodyanyuk, O.V. 1997. Revision of the species *Psyllotylenchus pawlowskyi* (Kurochkin, 1960) Poinar & Nelson, 1973. I. Redescription of *Spilotylenchus pawlowskyi* (*sensu stricto*) comb. n. (Tylenchida: Allantonematidae). *Russian Journal of Nematology* 5: 103-112.

Слободянюк О.В. Ревизия вида *Psyllotylenchus pawlowskyi* (Kurochkin, 1960) Poinar & Nelson, 1973. II. Описание *Kurochkinitylenchus laevicepsi* gen. n., sp. n. и Spilotylenchidae fam. n.

Резюме. Новый вид и новый род *Kurochkinitylenchus laevicepsi* gen. n., sp. n. выделен из сборного вида *Psyllotylenchus pawlowskyi* (Kurochkin, 1960) Poinar & Nelson, 1973. Дано описание морфологии и жизненного цикла этой нематоды, паразита блохи *Nosopsyllus laeviceps*, развивающейся с чередованием двух паразитических гетеросексуальных генераций. Новый род отличается от близкого рода *Parasitylenchus* (Parasitylenchinae), куда по типу жизненного цикла он мог бы быть отнесен, дорсально изогнутым телом паразитических самок, наличием мощного стилета со слабым базальным утолщением, но без головок, отсутствием головного конуса, наличием крупной обособленной сперматеки и другими признаками. Дан анализ филетических отношений нового рода с другими родами в надсемействе Sphaerularioidea и выявлено морфологическое сходство с родами *Spilotylenchus* (Contortylenchidae), *Psyllotylenchus*, *Incurvinema* (Heterotylenchinae, Parasitylenchidae), *Rubzovinema* (Phaenopsitylenchidae), которые вместе с новым родом объединены в новом семействе Spilotylenchidae fam. n. Даны диагнозы четырех новых подсемейств: Spilotylenchinae subfam. n., Psyllotylenchinae subfam. n., Rubzovinematinae subfam. n. и Kurochkinitylenchinae subfam. n., а также ключ для их определения в семействе.