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Parasitic nematodes of earthworms: II. Two new species of *Dicelis* Dujardin, 1845 from Siberia and a key to the species of the genus

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Summary. Two new species of *Dicelis* are described from earthworms collected in Western and North-Eastern Siberia. *D. sibirica* sp. n. is similar to *D. nira*, but can be distinguished by the shape of the spicular apparatus, egg shell ornamentation and morphometric differences. *D. rubidi* sp. n. is similar to *D. nira* and *D. sibirica* sp. n. in the shape of the male tail and to *D. rossica* and *D. hyrcanus* in the shape of the female tail. *D. rubidi* sp. n. differs from *D. nira* by having more numerous genital papillae; from *D. sibirica* sp. n. by the shape of the amphids and different structure of the egg shell; from *D. rossica* by egg shell ornamentation and smaller suckers and amphids and from *D. hyrcanus* by the different position and size of suckers and more numerous genital papillae. The composition of the genus *Dicelis* is reviewed and only 11 species are considered valid. A dichotomous key for the identification of *Dicelis* species is provided.

Key words: *Drilonematidae*, morphology, *Dicelis*, *D. sibirica* sp. n., *D. rubidi* sp. n., earthworm host, identification key, taxonomic revision, species list.

Two new species of *Dicelis* nematodes are described from earthworms collected in Siberia. The composition of the genus *Dicelis* is discussed and dichotomous key is provided for the identification of species in the genus.

MATERIALS AND METHODS

Nematodes were recovered from the coelomic cavity of earthworms fixed in 4 % formalin. The method of Ivanova (1993) was used to prepare specimens for examination by light microscopy.

DESCRIPTIONS

Dicelis sibirica sp. n. (Figs. 1 & 2)

Holotype male: L = 3400 μm, a = 52.3, b = 17.5, c = 19.3, Oes = 194 μm, NR = 295 μm, Ex = 316 μm, Cd = 175 μm, spicule length across the chord = 75 μm, spicule length along the arc = 90 μm, gubernaculum length = 47 μm.

Paratype males (n = 5): L = 2552 (2010-3400) μm, a = 32.7 (21.6-52.3), b = 17.5 (11.5-32.7), c = 16.5 (11.9-19.4), Oes = 191 (175-203) μm, NR = 261 (215-295) μm, Ex = 276 (225-326) μm, Cd = 164 (145-178) μm, spicule length across the chord = 80 (75-84) μm, along the arc = 93 (90-95) μm, gubernaculum length = 46 (45-47) μm.

Paratype females (n = 4): L = 3550 (3000-4520) μm, a = 27.3 (22.1-38.0), b = 17.2 (14.7-21.2), c = 13.1 (11.1-15.9), Oes = 206 (192-220) μm, NR = 293 (220-342) μm, Ex = 323 (270-377) μm, V = 52 (47-58) %, Cd = 270 (237-290) μm, eggs = 55-79 x 38-52 μm.

Female. Body tapering gradually towards ends. Lateral fields 25 μm wide with 9-10 cuticular ridges. Four short submedian cephalic setae. Amphid with 5 μm dia. oval opening and prominent pouch 10 μm wide x 15 μm long. Buccal cavity 9 μm wide x 4 μm long. Oesophagus slightly expanded at base (30 μm wide at anterior, 45 μm at base). Cardia conical, embedded in intestine. Intestine two-celled in cross section. Excretory pore inconspicuous. Terminal excretory duct ill-defined, short, slightly sclerotized. Lateral

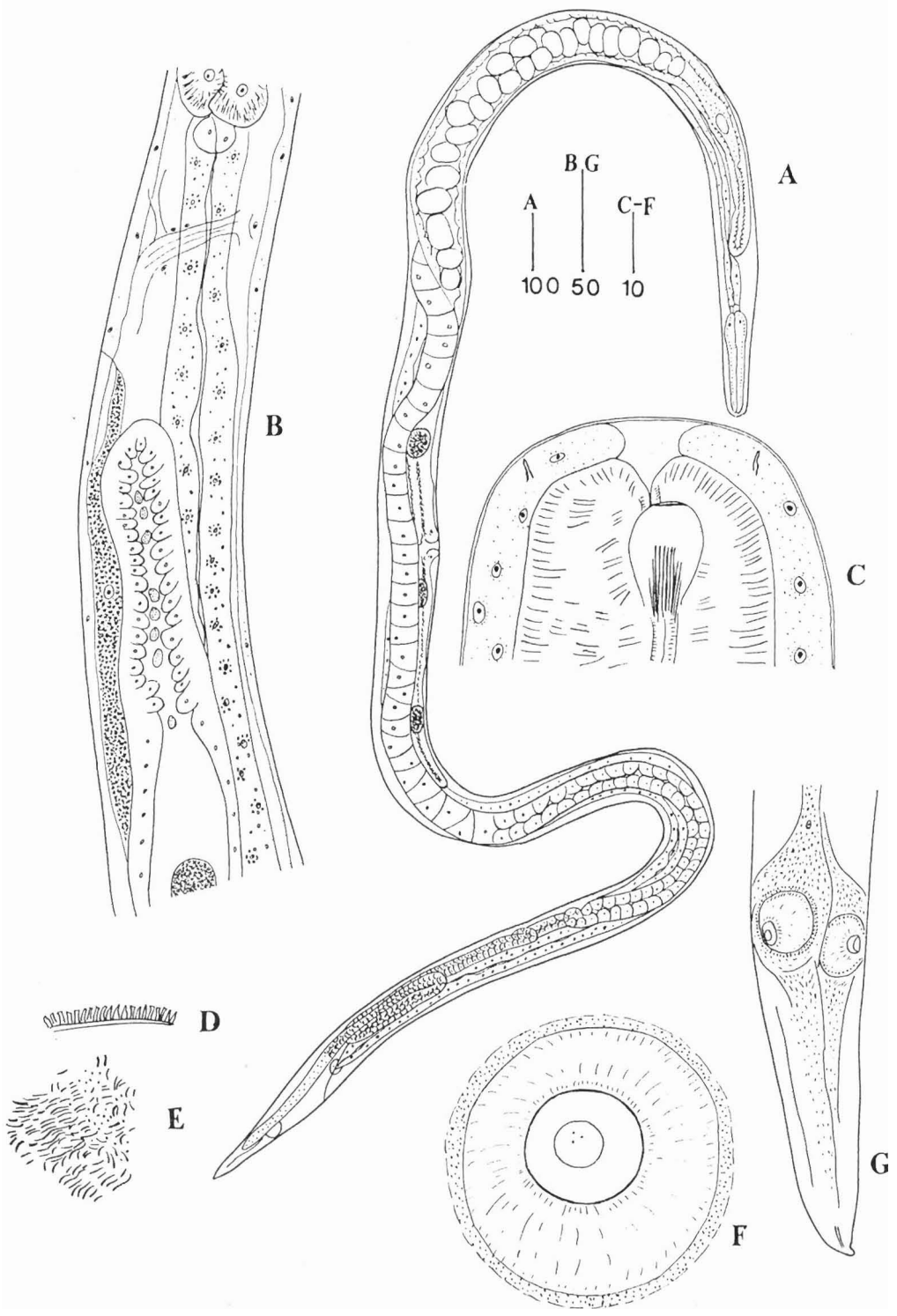

Fig. 1. *D. sibirica* female. A: Total view; B: Spermatheca region, lateral; C: Head end, lateral; D: Egg shell, lateral; E: Egg shell, surface view; F: Sucker , surface view; G: Tail, ventral. Bars in μm.

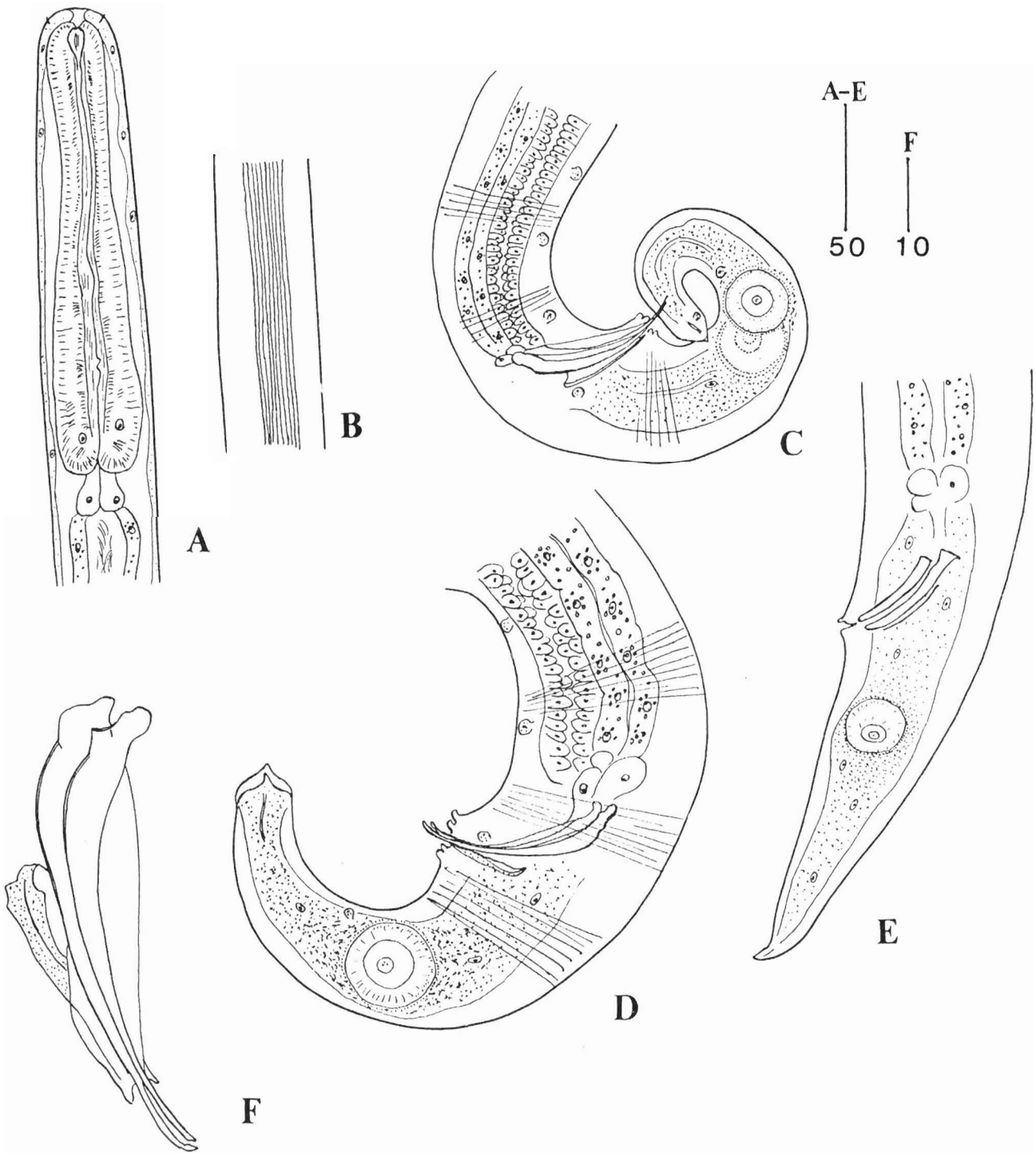

Fig. 2. *D. sibirica* male. A: Anterior part, lateral; B: lateral field; C, D: Posterior part, lateral; E: Posterior part of juvenile male (J4), lateral; F: Spicules and gubernaculum, lateral. Bars in μm.

excretory channels about 5 μm wide. Extremity of ovary in caudal region, two loops between anus and vulva. Elongated spermatheca about 150 μm long filled with 15-20 spherical spermatozoa 7 μm dia. About 20 zygotes with smooth shells in oviduct, arranged in a single or double row. Postvulvar sac longer than uterus, both narrow, thick-walled, containing 2-10 eggs. Egg shell thin (< 1 μm), covered with thin, blunt 3 μm spines. Long, conical tail with rounded terminus bearing small blunt mucron 5 μm long. Suckers on the first third of the tail, slightly asymmetrically disposed. Spherical cavity nearly 35 μm dia., aperture 8 μm dia., inner transparent rim 14 μm dia., outer slightly convex fibrous rim 35 μm dia.

Male. Spicules thin, long, slightly cephalated and curved. Body of the spicule is unmarked. Gubernaculum finely punctated. 3 pairs of subventral preanal papillae, 1 pair of adanal, 1-2 pairs of postanal (near suckers and extremity of the tail). Localization of the suckers as in female. Aperture of the sucker 8 μm dia., inner rim - 10 μm dia., outer rim - 21 μm dia. Conical tail 145-178 μm long, with rounded tip and wide, blunt 5 μm long mucron.

Fourth-stage juvenile of male. Straight, conical tail 120 μm long, with mucron. Testis flexure 140-190 μm behind the base of the oesophagus. Nerve ring on the intestine before the testis flexure. Excretory pore and duct inconspicuous. Prominent excretory channels. Spicular primordium 51 μm long. Gubernaculum and genital papillae not formed.

Type host and locality. *Eisenia nordenskioldi* (Eisen, 1879) collected from forest soil near Novosibirsk (Western Siberia) by T. S. Perel. Nematodes localized on the surface of seminal sacs in the anterior segments.

Type material. Holotype (Jc 396) deposited in the collection of Moscow State University Zoological Museum. Paratypes deposited in the collection of the Institute of Parasitology of Russian Academy of Sciences.

Differential diagnosis. *Dicelis sibirica* sp. n. resembles *D. nira* Chitwood & Lucker, 1934 in the shape of the oesophagus and tail end and in the number of genital papillae. The new species can be distinguished from *D. nira* by differences in body length, size and shape of spicules and gubernaculum and suckers. *Dicelis sibirica* sp. n is characterised by having an ornamented spiny surface on the egg shell, whereas *D. nira* have smooth egg shells. The present species also resembles *D. rossica* Timm, 1962 in the shape of the tail end, several morphometrics, size of suckers and the number of the genital papillae. However, *D. sibirica* sp. n. differs from *D. rossica* in the shape and size of the spicular apparatus (spicules are twice the length in *D. sibirica* than in the latter species), peculiar egg shell (smooth in *D. rossica*) and by the larger size of the amphids. *Dicelis sibirica* sp. n. is similar to *D. pereliae* Ivanova, 1993 in the shape of the tail end and size and location of the suckers but differs in egg shell ornamentation (tuberculated in former), longer mucron, different arrangement of the ovary and larger amphids. The new species is similar to *D. rubidi* sp. n. in the shape of the tail end but differs by having larger amphids and in the shape of the suckers and the peculiar egg shell. From *D. eiseniae* Timm, 1967, for which similar ornamentation of the egg shell was reported, the present species can be distinguished by tail shape, larger size of the amphids, different arrangement of the ovary and more elongated eggs.

Dicelis rubidi sp. n. (Figs. 3 & 4)

Holotype male: L = 2881 μm, a = 55.1, b = 15.8, c = 10.8, Oes = 182 μm, NR = 372 μm, Ex = 426 μm, Ts = 392 μm, Cd = 266 μm, spicule length across the chord = 86 μm, gubernaculum length = 52 μm.

Paratype males (n = 7): L = 2778 (2589-3216) μm, a = 31.8 (22.7-55.1), b = 14.7 (12.5-16.9), c = 11.1 (7.5-14.4), Oes = 190 (167-211) μm, NR = 289 (220-372) μm, Ex = 349 (278-426) μm, Cd = 263 (183-431) μm, spicule length across the chord = 83 (72-88) μm, gubernaculum length = 49 (38-54) μm.

Paratype females (n = 9): L = 3575 (3064-4039)

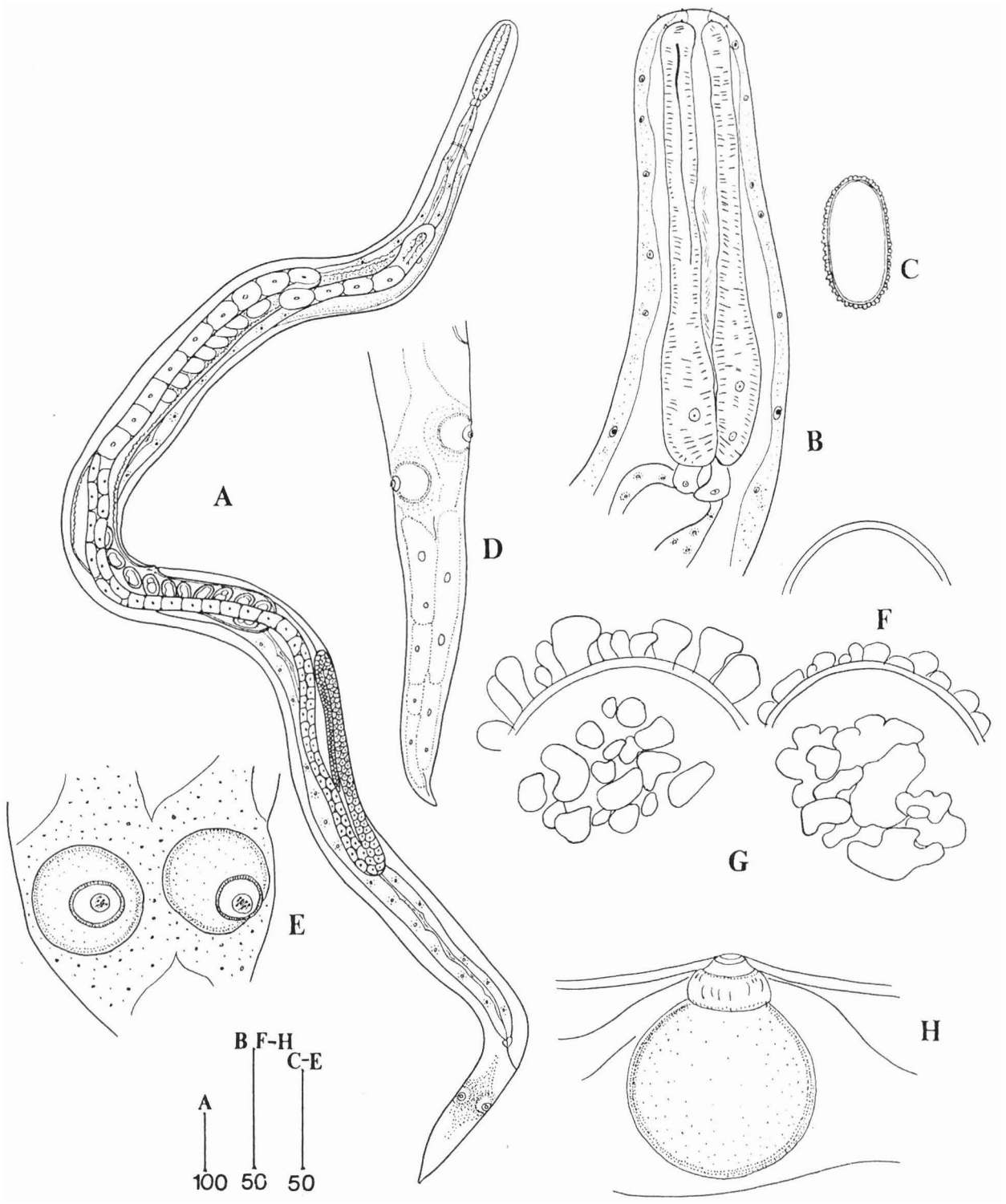

Fig. 3. *D. rubidi* female. A: Total view; B: Anterior part, lateral; C: Egg; D: Tail, lateral; E: Suckers, ventral; F: Egg with smooth shell, lateral; G: Eggs with ornamented shell, lateral and surface view; H: Sucker, lateral. Bars in μm.

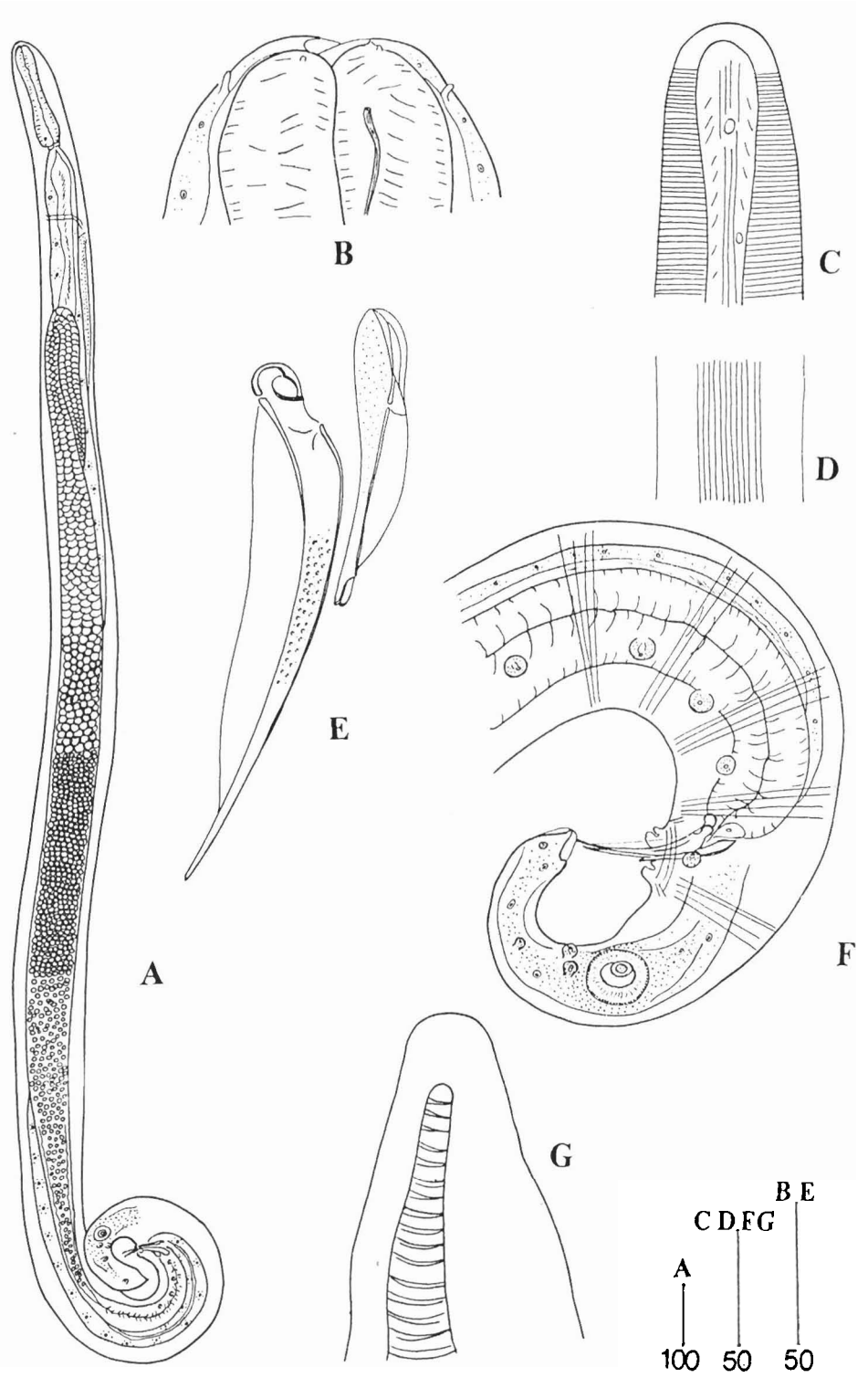

Fig. 4. *D. rubidi* male. A: Total view; B: Head end, lateral; C, D, G: Lateral fields; E: Spicule and gubernaculum, lateral; F: Posterior part, lateral. Bars in μm.

μm, a = 25.6 (19.7-36.1), b = 17.9 (14.2-21.8), c = 10.2 (7.3-12.9), Oes = 202 (151-224) μm, NR = 276 (230-312) μm, Ex = 340 (264-405) μm, V = 48 (40.5-53.0) %, Cd = 358 (277-431) μm, eggs = 63-98 x 32-50 μm.

Female. Body tapering gradually towards ends. Lateral fields 50-60 μm wide with 11-12 cuticular ridges. Four prominent 2-3 μm long and four (six?) very small submedian cephalic papillae. Shallow 2 μm long slightly cuticularized stoma. Pore-like amphid with opening 1.5-2 μm dia. and pouch 2-3 μm wide. Oesophagus cylindrical, very slightly expanded at base (31 μm wide at anterior, 35 μm at base). Cardia of 4-6 cells. Excretory pore ill-defined, excretory duct distinct, short, slightly cuticularized. Termination of the ovary between vulva and anus. The ovary forms two equal loops in the middle of the postvulvar region. Long elliptical spermatheca containing 25-40 spermatozoa, 4-6 μm dia. Postvulvar sac longer than uterus, containing about 10 eggs in a single row. Two types of eggs in uterus: one thick-walled (2 μm wide), covered with abundant rounded tubercles 2-7 μm in height and 3-10 μm wide and another with finely punctate, almost smooth shell 1 μm thick. Eggs of the former type almost spherical in shape. Tail narrow, conical, with acute 4-7 μm long mucron. Suckers situated symmetrically at mid-tail. Spherical cavity beneath the surface 21-32 μm dia. (one third - one half of the tail diameter), protruding outer rim about 14 μm dia., almost flat inner rim about 10 μm dia. and openings 8 μm dia., with sensilla.

Male. Tail end conical, slightly swollen near the termination, with blunt, short mucron, covered by a thin cuticular membrane. Large genital papillae, rounded in shape, with prominent ampullae. Four pairs preanal, one pair adanal and two pairs of postanal papillae situated just before suckers subventrally, a pair behind suckers laterally and a pair near tail terminus. Suckers on the first third of the tail end, larger in size than in the females. Spicula with the long velum, round manubrium and thin, punctate body. Gubernaculum with rounded proximal portion and two distal processes and a punctated inner surface.

Type host and locality. *Dendrodrilus rubidus* (Savigny, 1826) in Magadan region, Ten'kin district, «Aborigen» station. From 111 dissected earthworms 23 specimens were infected with 2-26 *D. rubidi* sp. n. Only mature nematodes were present in the coelom cavity in the seminal sacs. It is possible that this earthworm species had been introduced to the Magadan region associated with planting material. The earthworms were collected and dissected by D. I. Berman and O. M. Orlovskaja in September, 1984.

Type material. Holotype (Jc 395) deposited in the collection of Moscow State University Zoological Museum. Paratypes deposited in the collection of the Institute of Parasitology of Russian Academy of Sciences.

Differential diagnosis. The new species is similar to *D. nira* Chitwood & Lucker, 1934 and *D. sibirica* sp. n. in the shape of the male tail. It differs from *D. nira* by having more numerous and larger genital papillae and by the shape of the spicules and gubernaculum. From *D. sibirica* sp. n. it differs in the shape of the amphids, symmetrical position of the suckers and their different structure and having tuberculate egg shells. It is similar to *D. rossica* and *D. hyrcanus* Belostotzkaya, Kozodoi & Spiridonov, 1987 in the shape of the female tail. However, it can be distinguished from *D. rossica* by having a different arrangement of the ovary, tuberculate egg shells and shorter mucron and smaller suckers and amphids. From *D. hyrcanus* it can be distinguished by having more numerous genital papillae, the more anterior position of the suckers and the shape of the male tail.

Etymology. Specific name refers to its host name.

DISCUSSION

All species of *Dicelis* have been reported from earthworms belonging to the family Lumbricidae and are known from Europe, the northern part of Asia i.e. from Polar Ural to Magadan and from North America (Table 1).

The type species of the genus, *D. filaria*, was described by Dujardin, 1845 from the seminal vesicles

Table 1. Species of the genus Dicelis isolated from earthworms.

Nematode	Host	Locality	Reference
D. eiseniae	*Eisenia carolinensis* Michaelsen, 1910	Louisiana, USA	Timm, 1967
D. filaria	*Lumbricus terrestris* Linnaeus, 1758	Central Europe	Dujardin, 1845
	E. foetida (Savigny, 1826)		Poinar, 1978
	L. rubellus Hoffmeister, 1843		Wulker, 1926
	Dendrodrilus rubidus f. subrubicunda (Eisen, 1874)		Timm, 1962b
	Eisenia nordenskioldi (Eisen, 1879)	Polar Ural, Russia	Timm, 1962b
D. hyrcanus	*E. foetida* (Savigny, 1826)	South Azerbaijan	Belostotskaya et al., 1987
D. kimmeriensis	*Dendrobaena veneta* (Rosa, 1886)	Crimea, Ukraine	Ivanova, 1993
D. lovatiana	*L. rubellus* Hoffmeister, 1843	North-West Russia	Ivanova, 1993
D. lumbricicola	*L. rubellus* Hoffmeister, 1843	Moscow region, Russia	Ivanova, 1993
	L. terrestris Linnaeus, 1758		
D. nira	*Nicodrilus caliginosus* (Savigny, 1826)	Maryland, USA	Chitwood & Lucker, 1934
D. pereliae	*E. nordenskioldi* (Eisen, 1879)	Voronezh region, Russia	Ivanova, 1993
D. rossica	*E. kusenkoi* Michaelsen, 1903	Polar Ural, Russia	Timm, 1962b
D. rubidi sp. n.	*E. nordenskioldi* (Eisen, 1879)	Western Siberia, Russia	Ivanova, 1994
D. sibirica sp. n.	*Dendrodrilus rubidus* (Savigny, 1826)	North-Eastern region of Russia	Ivanova, 1994

of earthworms collected from garden-soil near Paris. It was characterized by its fusiform body, round mouth, muscular clavate oesophagus, expanded adjacent to the anterior part of the intestine; presence of large circular «suckers» at mid-tail, surrounded by radial fibers; spicules and gubernaculum in males and oval eggs in females. Timm (1962a,b) reviewed the composition of the genus *Dicelis* and concluded that *D. pleurochaeta* Beddard, 1883, which is characterised by the presence of a single overhanging papilla or «tooth» close to the oral opening, should be excluded from the Drilonematidae.

Belostotskaya et al. (1987) revised the genus *Dicelis* and considered that *D. guatemalana* Timm, 1962, *D. ivericus* Kvavadze & Eliava, 1975 and *D. kurashvilii* Kakulia & Kvavadze, 1974 were species *insertae sedis*. In the description of *D. guatemalana* Timm (1962b) mentioned the possible need to establish a new genus in which accommodate it, but deferred because of the absence of males (i.e. the species was conditionally referred to genus *Dicelis*). The posteriorly displaced vulva and large circular amphids reported for *D. guatemalana* are not characteristic for *Dicelis*. *Dicelis ivericus*, described only from females, also has large circular amphids and nerve ring situated around the oesophagus. *Dicelis kurashvilii* was not included in *Dicelis* by Belostotskaya et al., (1987) as the nerve ring was situated in the oesophageal region and not on intestine, only a few genital papillae were present and a terminal duct unique for drilonematids was present connecting the suckers with the tail tip. Also, the anteriorly displaced vulva (ratio V of only 14-18%) in this species differs from that in all other Drilonematidae. Therefore, Belostotskaya et al. (1987) considered as members of *Dicelis* only *D. filaria, D. eiseniae, D. nira, D. rossica* and the then newly described *D. hyrcanus*. All other species were regarded as *insertae sedis* in Drilonematidae. However, the taxonomic position of *D. dendrobaenae* Timm, 1962 was not discussed. Apart from the description of *D. dendrobaenae* Timm (1962) presented additional data of these nematodes and was certain of their identification as species of *Dicelis*. Nevertheless, *D. dendrobaenae* has a single, important feature which distinguishes it from all other *Dicelis viz.* polar caps (operculums) on the egg shells. This feature is peculiar for the genus *Adieronema* which is closest to *Dicelis* and *D. dendrobaenae* can be

easily distinguished by its much smaller size, absence of a distinct excretory pore and duct, more anterior vulva position and lesser number of eggs (hundreds in *Adieronema,* two dozen in *D. dendrobaenae*). Therefore, *D. dendrobaenae* should correctly be regarded as a *species inquirenda.*

Dicelis ghiliarovi (Kvavadze & Kakulia, 1986), which was described from the same host and locality as *D. pereliae*, was reported to be amphidelphic and had fused spicules. *Dicelis abshazicus*, described by the same authors, differs from the other *Dicelis* species by having a posteriorly displaced vulva (V=71%). The nerve ring is situated in the basal part of the oesophagus in both of these species. *D. ghiliarovi* and *D. abshazicus* obviously do not belong to *Dicelis.* Moreover, it seems impossible to assign these two species to any known family in the superfamily Drilonematoidea. As it was not possible to reexamine specimens of these two forms comments can not be made about their taxonomic position in the Drilonematoidea.

In conclusion we consider that the genus *Dicelis* Dujardin, 1845 contains the following species:

Type species: *D. filaria* Dujardin, 1845

Other species: *D. eiseniae* Timm, 1967, *D. hyrcanus* Belostotzkaya, Kozodoi & Spiridonov, 1987, *D. kimmeriensis* Ivanova, 1993, *D. lovatiana* Ivanova, 1993, *D. lumbricicola* Ivanova, 1993, *D. nira* Chitwood & Lucker, 1934, *D. pereliae* Ivanova, 1993, *D. rossica* Timm, 1962, *D. rubidi* sp. n., *D. sibirica* sp. n.

Species inquirenda: *D. dendrobaenae* Timm, 1962.

Status insertae sedis: *D. pleurochaeta* Beddard, 1883, *D. guatemalana* Timm, 1962, *D. kurashvilii* Kakulia & Kvavadze, 1974, *D. ivericus* Kvavadze & Eliava, 1975, *D. ghiliarovi* Kvavadze, Kakulia & Gorgadze, 1986, *D. abshazicus* Kvavadze, Kakulia & Gorgadze, 1986.

Key to the species of *Dicelis*

1. Egg shell unmarked 2
- Egg shell ornamented 3

2. Suckers large (1/2 - 1/3 body diameter), amphids pore-like, eggs elliptical, 54 x 38 μm..... .....*D. rossica.*

- Suckers not large (1/4 - 1/3 body diameter), amphids oval, eggs long, narrow, 68 x 30 μm..... ..... *D. nira.*

3. Egg shell bearing spines 4
- Egg shell bearing tubercles 5

4. Tail end bluntly rounded, amphids pore-like, eggs nearly round, 42 x 32 μm, shell covered with abundant fine pointed spines.....*D. eiseniae.*

- Tail with conoid tip 5 μm long, amphids oval with large pouch , eggs elliptical 67 x 45 μm, shell with blunt spines ..... *D. sibirica* sp.n.

5. Poorly visible small suckers (less than 1/4 body diameter) at the last third of tailumbricus 6
- Distinct suckers at mid-tail 7

6. Extremity of the ovary near the tail tip, no loops from anus to spermatheca, tail scarcely tapering..... ..... *D. kimmeriensis.*

- Extremity of the ovary in front of the anus, two loops at distance from the anus to spermatheca, tail conoid .....*D. hyrcanus.*

7. Outer rim of sucker nearly 15 μm in diameter, spherical cavity diameter two times more than outer rim, tail conical, pointed ..... *D. rubidi* sp. n.

- Outer rim of sucker more than 15 μm, cavity diameter nit more than former diameter 8

8. Tail broadly conical with small tip 1-2 μm long, suckers slightly asymmetrical disposed..... ..... *D. pereliae.*

- Tail scarcely tapering, rounded at tip.....9

9. Oesophagus uniformly thickened, eggs 40 μm long..... *D. filaria.*

- Oesophagus slightly expanded at base, eggs more than 65 μm long 10

10. 13-15 cuticular ridges, prominent cephalic papillae, punctate spicule body, 9 pairs preanal papillae..... *D. lovatiana.*

- 5-7 cuticular ridges, short setose cephalic papillae, smooth spicule body, 7 pairs preanal papillae.. ..... *D. lumbricicola.*

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Иванова Е.С. Паразитические нематоды дождевых червей: II. Два новых вида *Dicelis* Dujardin, 1845 из Сибири и ключ к видам рода.

Резюме. Два новых вида *Dicelis* описаны от дождевых червей, собранных в Западной и Северо-Восточной Сибири. *D. sibirica* sp. n. близок к *D. nira*, но отличается по форме спикулярного аппарата, структуре оболочки яиц и по морфометрии. *D. rubidi* sp. n. близок к *D. nira* и *D. sibirica* sp. n. по строению хвостового конца самцов, сходен с *D. rossica* и *D. hyrcanus* по форме хвостового конца самок. *D. rubidi* sp. n. отличается от *D. sibirica* sp. n. формой амфид и строением оболочки яиц, от *D. nira* - большим числом генитальных папилл, от *D. rossica* структурой оболочки яйца, меньшими по размеру хвостовыми органами и амфидами, а от *D. hyrcanus* - расположением и размером хвостовых органов и большим числом генитальных папилл. Проведена ревизия рода *Dicelis*. Признаны валидными 11 видов и предложен ключ для их определения.