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Review of the genus *Neotobrilus* Tsalolikhin, 1981 (Nematoda, Enoplida: Tobrilidae)

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Summary. The composition of the genus *Neotobrilus* was revised and new synonyms were established. Descriptions of a new species *Neotobrilus sinensis* sp.n. from Taiwan and a number of species from new localities are given. Some morphological characters as taxonomic ones are proposed. A species identification key is given.

Key words: morphology, morphometric, *Neotobrilus* identification key, *Neotobrilus sinensis* sp.n., taxonomy.

The genus *Neotobrilus* (Tsalolikhin, 1981) belongs to the higher tobrilids and to the tribe Neotobrilini of the subfamily Neotobrilinae, together with the genera *Semitobrilus* and *Brevitobrilus*. (Tsalolikhin, 2001). The type species of the genus, *N. longus* (= *Anguillula longa* Leidy, 1852) was the first described specimen of tobrilids (Bastian, 1865). Currently, there are 11 (Zullini, 2006) to 19 species (Andrássy, 2007) assigned to this genus. The first complete review of the group *Tobrilus-longus* = *Neotobrilus* was performed about 40 years ago (Loof & Riemann, 1976). There has been little change in the species composition of the genus since that. The characteristic features of the genus are non-overlapping stoma pockets divided by a duct, 6 supplements of unequal size arranged in two groups, and thin spicules. By the structure of stoma *Neotobrilus* is similar to the genus *Brevitobrilus*, and by the structure of stoma and spicules, to the genus *Semitobrilus*. The structure of the supplementary apparatus is unique and occurs nowhere else in the family Tobrilidae. Given below are descriptions of some insufficiently known species from the collection of the Zoological Institute of the Russian Academy of Sciences and brief diagnoses of all valid species with relevant comments and also a key for identification of species.

Genus *Neotobrilus* Tsalolikhin, 1981

Diagnosis. Cuticle finely annulated (visually smooth), somatic setae numerous. Stoma with two pockets situated one after another along the longitudinal axis of the body; buccal cavity goblet- or cup-shaped, distinctly separated from the anterior pocket, posterior pocket separated from the anterior pocket by a duct; one relatively large tooth in each pocket. Nerve ring encircles oesophagus just in front of its middle (*ca.* in 40% from anterior). Oesophageal pericardial glands well-developed, slightly oval. Female genital system paired, amphidelphic, well-differentiated, vagina wide with strong musculature (in a number of species, vaginal musculature composed from concentric or radial layers). Vulva pre-equatorial. Male genital system paired, testes situated near the midbody; spermia either flagelloid or, in some species, clavate; *ductus ejaculatoris* strong, muscular, its length in some species corresponds with the length of the supplementary row, in another ones, exceeds it considerably.

Spicules thin. Supplementary row consists of six supplements divided into two groups: three very small rudimentary supplements are situated immediately anteriorly to cloaca, followed by distinct 0-supplement and next three very large supplements. In the majority of species, there are micropapillae between the first supplements. Subterminal seta usually present. Male tail usually shorter than female tail, spinneret present. Species of the genus can be subdivided into two groups: 1) species which males have *ductus ejaculatoris* with length remarkably exceeding that of the supplementary row length and 2) species with *ductus ejaculatoris* equal in length to the supplementary row or exceeding it slightly. Females of *Neotobrilus* species of the first group always have very strong globose vaginal musculature. This character can be achieved in two ways:

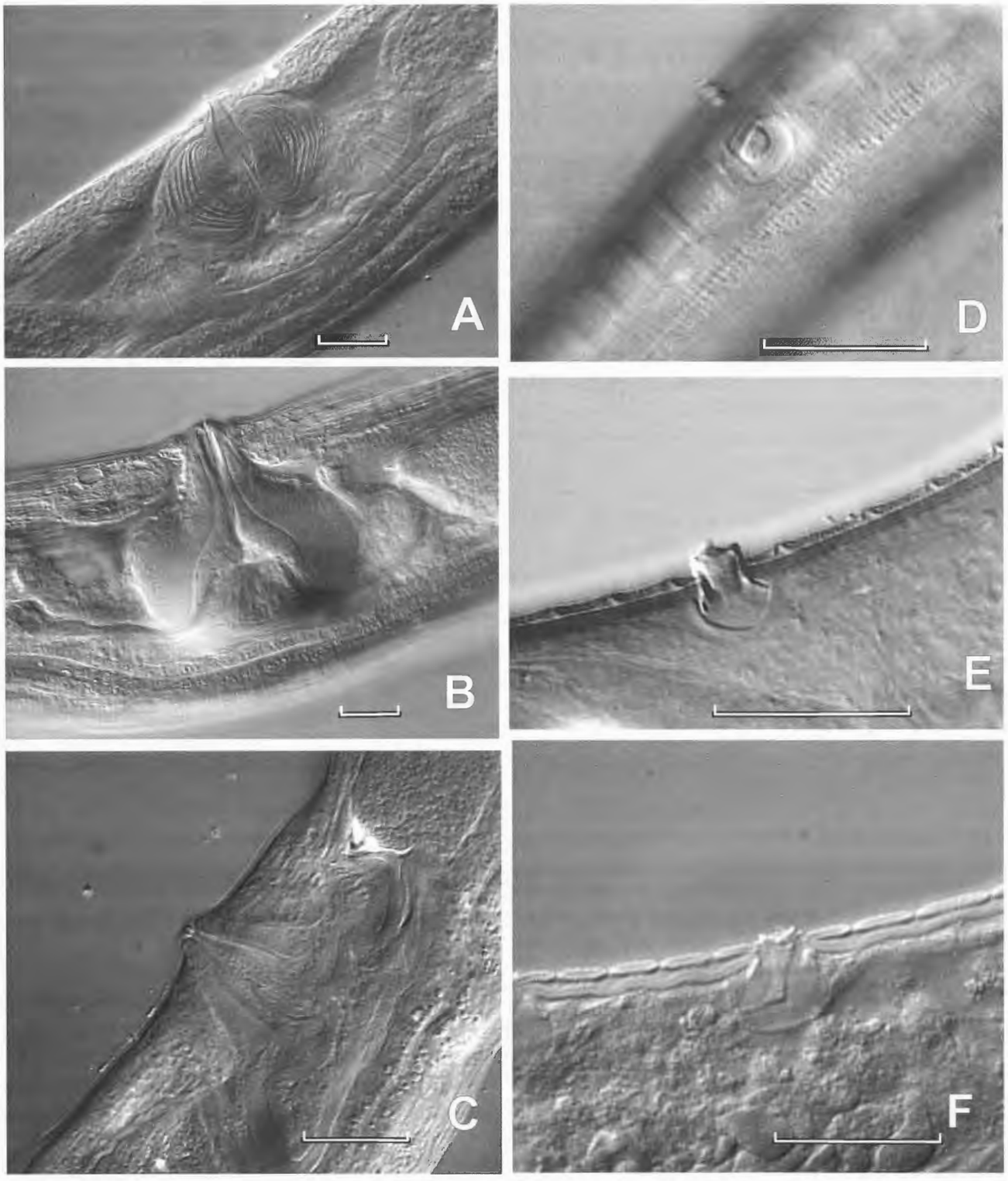

Fig. 1. *Neotobrilus filipjevi* (A, E), *Neotobrilus longior* (B), *Neotobrilus breviductus* (C, D), *Neotobrilus sp.* (F). A – globose vagina with strong development of internal longitudinal concentric muscular layers; B – globose vagina with strong development of the external radial muscular layer; C – non globose vagina; D – supplement (III) and micropapillae, ventral view; E – supplement (II) and micropapillae, lateral view; F – supplement (III) and poorly visible micropapillae (lateral view).

i) by strong development of internal longitudinal concentric muscular layers, as in *N. longus, N. filipjevi, N. hopei* (Fig. 1A);

ii) by strong development of the external radial muscular layer as in *N. diversipapillatus, N. longior, N. telekiensis* (fig. 1B).

The second group includes *N. breviductus, N. vicinus, N. nicaraguensis, N. nicasimilis, N. longiformis, N. macrospiculum, N. ampie, N. sinensis* sp.n. Females of these species have well developed vaginal musculature, which is not globose (Fig. 1C); the only exception are females of *N. macrospiculum*, possessing strong radial vaginal musculature.

Structure of the vaginal musculature is a very good diagnostic character, enabling females of the first group to be differentiated from females of the second group and enabling species to be distinguished by the developmental characteristics of vaginal layers. For example, in females of *N. longus,* the longitudinal layers of vaginal musculature are pronounced whereas in *N. filipjevi* or *N. hopei,* the layers are much thinner.

Neotobrilus longus (Leidy, 1852) (Tables 1 & 2)

This is type species of the genus, which originally was not described adequately enough (Leidy, 1852) and was subsequently re-described (Cobb, 1914; Loof & Riemann, 1976). It is characterized by following features: female body length does not exceed 1.7 mm, length of cephalic seta one half of head width, vaginal musculature very strong and multilayered, length of the *ductus ejaculatoris* exceeds the length of the supplementary row, spermia are flagelloid; subterminal seta is absent.

For a long time, all species with characteristics of the genus were regarded as *Neotobrilus longus.* The concept of the species group 'longus' (genus *Neotobrilus*) had been gradually formed but later it became evident that *N. longus* itself also was heterogenous and contained at least two subspecies/species. During the study of material from different water-bodies of Russia and Mongolia, a subspecies of *N. longus, N. longus rossicus* was discovered (Tsalolikhin, 1983), which was then regarded as a separate species (Andrássy, 2007). However, a detailed comparison of this subspecies with the Canadian species *Tobrilus filipjevi* (Ebsary, 1982) proved the validity of the Canadian species.

Neotobrilus filipjevi (Ebsary, 1982) (Syn. *Neotobrilus longus rossicus* Tsalolikhin, 1983*) (Tables 1 & 2)

***here and below only new synonymy is given**

The species is similar to *N. longus* in general morphology but differs by the following characters: larger body size (character present in different geographically remote populations); shorter cephalic setae (one third of head width long), relatively and absolutely longer spicules, subterminal seta present. The latter character, though, is not always significantly displayed and is not of much value in taxonomy of the genus *Neotobrilus. N. filipjevi* is widely spread in the Holarctic and, judging by descriptions of different authors, is distributed in Russia from the Gulf of Finland (Filipjev, 1929) up to the Far East (Alekseev & Dizendorf, 1981). In a number of

Table 1. Morphometrics of males of *Neotobrilus filipjevi* and *N. longus*

Characteristics*	*N. filipjevi*			*N. longus*
	European Russia (orig.)	West Siberia, Mongolia (orig.)	Canada (Ebsary, 1982)	U.S.A. (Loof, Riemann,1976)
	n=11	n=8	n=5	n=18
Body length, L	1700-2100 (1800)	1294-1942 (1686)	1700-2000 (1850)	1120-1430 (1275)
Oesophagus	366-406 (390)	257-367 (334)	(377)	(245)
Tail	141-186 (160)	147-154 (150)	(187)	(111)
Cephalic diameter	25-27	21-23	~30	(22)
Cephalic setae	9-10	7-9	9-10	10-11
Spicules (along axis), Sp.	101-112 (106)	91-101 (95)	100-109 (105)	67-84 (76)
Supplement row, SR	240-310 (290)	209-256 (237)	(312)	(250)
Ductus ejaculatoris, DE	406-440 (430)	440-490 (470)	(502)	(382)
SR/L, %	(14)	9-18 (14)	(17)	(20)
Sp/SR, %	(36)	35-44 (40)	(34)	(30)
Sp/L, %	(6)	4-7 (6)	(6)	(6)
SR/DE, %	(65)	(50)	(62)	(65)
a	22.3-42.4 (30.5)	22.6-36.6 (29.6)	23-28 (26)	23-34 (28.5)
b	4-5.6 (4.8)	4.3-5.5 (5)	4.7-5.1 (4.9)	4.6-5.8 (5.2)
c	8.2-15.1 (12)	8.7-12.8 (11.2)	9.8-10 (9.9)	11.5
c'	3-4	4	5	3-4

* All absolute measurements in μm

Table2. Morphometrics of females of *Neotobrilus filipjevi* and *N. longus*.

Characteristics	*N. filipjevi*		*N. longus*
	European Russia (orig.)	West Siberia, Mongolia (orig.)	U.S.A. (Loof & Rieman, 1976)
	n=8	n=10	n=28
Body length	1800-2200 (2000)	1750-2190 (1995±46)	1250-1690 (1470)
Oesophagus	(426)	322-435 (390±10)	(277)
Tail	(210)	194-204 (204±6)	(155)
Vulva-anus/Tail	(4)	(4)	(4)
Cephalic diameter	30-31	24-28 (26)	(22)
Cephalic setae	11-12	10	(11)
a	16.6-30.3 (23.7)	19.8-35 (25.9±1)	20-27 (23.5)
b	3.9-5.7 (4.7)	4.5-6 (5.1±0.2)	4.8-5.7 (5.3)
c	8.1-9.9 (9.5)	8.8-10.9 (9.8±0.2)	9-10 (9.5)
V%	44-52 (48)	45-51 (49±1)	45-54 (49)
c'	4-7 (5)	5	4-7 (5)

other references to *N. longus* in faunal papers it is not always possible to know with certainty which particular species is being reported. Thus, Gagarin (1993) reported *N. longus* (although most probably it was *N. filipjevi*) accompanied with illustrations of *N. diversipappilatus*. Distribution maps of *N. filipjevi* and *N. longus* in Europe need to be updated. Both species were recorded in North America, but their distribution on the continent was not detailed. References to *N. longus* in papers published before the revision of Loof & Riemann (1976) also need to be closely examined, because in the majority of cases there are other than *N. longus* species validated as a result of the above revision.

Neotobrilus diversipappilatus (Daday, 1905) (Tables 3 & 4)

The next described representatives of the genus *Neotobrilus* was for a long time synonymized with *N. longus*, until the different structure of spermia of these species was shown (Loof & Riemann, 1976; Riemann, 1983). *N. diversipapillatus* (as well as *N. longior*) has clavate spermia, instead of the 'typical' flagelloid ones. Shape of spermia is distinctly different not only in testes of males, but also in genital ducts of females. Another characteristic feature of the species is the absence of microspicules on the supplement caps (only weak tuberosity present). Subterminal seta is present. Species with clavate spermia were reduced to synonyms of *N. diversipapillatus* (Loof & Riemann, 1976); these are *Tobrilus longior* Altherr, 1963 (*sensu* Riemann, 1966A), *T. longus* (*sensu* Riemann, 1966B), *T. longus* (*sensu* Argo & Heyns, 1973), and also *T. savaryi* (Altherr, 1963A). Later on, the attention was paid to the significant differences in size of body and some organs in specimens of the species from populations of the western and eastern hemispheres, which provided a basis for division of the species into two separate subspecies, *N. diversipapillatus occidentalis* and *N. d. orientalis* (Tsalolikhin, 1983). Such division was supported by Andrássy (2007), who assigned them to two separate species. Taking into account marked difference in absolute body size and index SpIII value (see Table 9) in specimens from the eastern and western hemispheres and general similarity of all 'eastern' specimens of this species, we feel it will be reasonable to assign them to *N. longior* (Altherr, 1963), its validity thus being restored. Thus, distribution of *N. diversipapillatus* will be restricted to South America (Daday, 1905; Kreis, 1932; Tsalolikhin, 1988).

Neotobrilus longior (Altherr, 1963) (Syn. *Neotobrilus diversipapillatus orientalis* Tsalolikhin, 1983; *Tobrilus savaryi* Altherr, 1963) (Tables 3 & 4)

The type material was described from the Moselle River in eastern France (Altherr, 1963b), and redescribed when the clavate shape of spermia in type specimens was discovered. It was the reason for reducing all species with similar spermia structure and similar morphology to synonyms of *N. diversipapillatus* (Daday, 1905; Loof & Riemann, 1976). Differential characters are given below; the most valuable is a ratio of the spicule length (measured along chord) to the distance from cloaca to the third supplement. In *N. diversipapillatus,* the proximal part of spicules is situated at the level of the third supplement (SpIII $\geq$ 1), whereas in *N. longior* index SpIII is < 1. This character was used previously to differentiate closely related species of the genus *Brevitobrilus* (Tsalolikhin, 1992). Subterminal seta, as in *N. diversipapillatus,* present. The species is widespread in Europe (though in many cases, more study is needed) and occurs in Africa (Argo & Heyns, 1973 pro *Tobrilus longus*; Joubert & Heyns,

Table 3. Morphometrics of males of *Neotobrilus diversipapillatus* and *N. longior*.

Caracteristics	*N. diversipapillatus* (Daday, 1905)		*N. longior* (Altherr, 1963)			
	Paraguay (Loof & Riemann 1976)*	Paraguay (Kreis, 1932)	France (Altherr, 1963a)	Russia (Shoshin & Shoshina,2003)	Sweden (orig.)	South Africa (Joubert & Heyns, 1979)
	n=3	n=10	n=3	n=3	n=2	n=49
Body length, L	1420-1940 (1630)	1141-1685 (1436)	1800-2320 (2060)	1800-2200 (2040)	2126-2216	1910-3180 (2350)
Oesophagus	(302)	(261)	(312)	310-330 (320)	316-324	(297)
Tail	(116)	(113)	(129)	130-150 (145)	112-143	115-156 (136)
Cephalic diameter	*ca.* 20	–	25	25	24	*ca.* 22
Cephalic setae	5	3	6	8	5	6-8
Spicules (along axis), Sp	41-48 (45)	58	55-60 (57)	62	57-58	54-80 (66)
Supplement row, SR	*ca.* 200	–	*ca.* 200	300-320	242-280	*ca.* 260
Ductus ejaculatoris, DE	*ca.* 490	–	*ca.* 550	740-780 (760)	666-724	*ca.* 800
SR/L, %	(12)	–	(9)	(15)	(12)	(11)
Sp/SR, %	(22)	–	(29)	(20)	(22)	(25)
Sp/L, %	(3)	(*ca.* 4)	(~3)	(3)	(~3)	(*ca.*3)
SR/DE, %	(41)	–	(36)	(40)	(37)	(30)
a	25-35 (31)	32.9-39.8 (36)	44-51 (47)	(40)	(36)	27-40 (34)
b	4.6-6.9 (5.4)	5.2-6 (5.5)	6.4-6.7 (6.6)	(6.3)	(6.8)	5.4-8.8 (7.9)
c	13-16 (14)	11.1-14.8 (12.7)	14-18 (16)	(14.9)	(17.2)	8.3-16.1 (10.8)
c'	(4)	(3.5)	(4)	(4)	(5)	(4)

*Redescription of type material.

Table 4. Morphometrics of females of *N.diversipappilatus* and *N.longior*.

Characteristics	*N. diversipapillatus*	*N. longior*		
	Paraguay (Kreis, 1932)	France (Altherr, 1963a)	Sweden (orig.)	South Africa (Joubert Heyns,1979)
	n=10	n=1	n=7	n=60
Body length	1695-2241 2059)	2300	2174-2846 (2481)	1660-3180 (2560)
Oesophagus	(332)	328	331-381 (355)	(324)
Tail	(221)	209	190-285 (228)	187-266 (227)
Vulva-anus/Tail	(4.5)	–	(5.5)	(6)
Cephalic diameter	*ca.* 20	–	22-25	*ca.* 20
Cephalic setae	5	–	4-5	6-8
a	23.9-35.3 (29.8)	40	28-39 (32)	27-40 (34)
b	5.7-6.4 (6.2)	7	6.3-8.2 (7)	5.4-8.8 (7.9)
c	8.8-10 (9.3)	11	9.4-11.4 (10.9)	8.3-16.1 (10.8)
V%	35-42 (39)	39	34-41 (37)	34-44 (39)
c'	*ca.* 8	–	(6)	6-8

1979 pro *T. diversipapillatus*). Gagarin (1993) reported its absence in Russia; however, later it was found in the Saratovskoe Reservoir of the Volga River (Shoshin & Shoshina, 2003).

Neotobrilus hopei (Loof & Riemann, 1976)

The species was described (without differential diagnosis) basing on just two males from the Potomac River (Loof & Rieman, 1976). Later on, in the Ottawa River, Canada, a few specimens were found including females identified as belonging to the species (Ebsary, 1982). The species is characterized by a large size and specific structure of supplementary apparatus (Table 9). ♂: L=2720-289 μm, a=41-55, b=4.7-5.8, c=11.8-12.4, spicule length=105-120 μm; ♀: L=2800-3200 μm, a=36-58, b=4.3-5.6, c=7.7-9.1, V%=47-53%. Length of head setae *ca.* 15 μm or a half of head width. So far the species has been only discovered in North America.

Neotobrilus telekiensis (Allgen, 1951)

The largest representative of the genus. The original description was incomplete and poorly illustrated.

Table 5. Morphometrics of males of *Neotobrilus sinensis* and *N. vicinus*.

Characteristics	*Neotobrilus sinensis sp.n.*	*Neotobrilus sp.*	*N. vicinus* (Loof, 1973)
	Taiwan	Australia	Suriname
	Holotype	n=2	n=9
Body length, L	1705	1435-1752	1450-1760
Body diameter (max.)	39	39-40	(46)
Oesophagus	245	205-227	(287)
Trophico-genital part	1330	1115-1428	(1198)
Tail	130	97-115	(120)
Cephalic diameter	15	19-20	24
Cephalic setae	3	3-4	5-6
Spicules (along axis), Sp	57	53-56	50-58
Supplement row, SR	235	194-200	200
Ductus ejaculatoris, DE	280	250-255	200
SR/L, %	14	11-14	12
Sp/SR, %	24	27-29	27
Sp/L, %	3	3-4	3
SR/DE, %	84	76-80	100
a	44	37-44	29-41
b	7	7-8	5.2-6
c	13	12-18	10.4-16.3
c'	4	4-5	5

Table 6. Morphometrics of females of *Neotobrilus sinensis* and *N. vicinus*.

Characteristics	*Neotobrilus* sinensis	*Neotobrilus sp.*	*N. vicinus* (Loof, 1973)
	Taiwan	Taiwan	Suriname
	n=9	n=7	n=15
Body length	1418-2076 (1790±87)	1355-1685 (1544±53)	1490-2060 (1775)
Body diameter (max)	60-74 (67±2)	38-54 (48±2)	(54)
Oesophagus	260-290 (280±5)	211-274 (238±10)	(306)
Trophico-genital part	960-1600 (1306±85)	978-1250 (1135±46)	(1263)
Tail	214-235 (221±3)	157-196 (171±4)	(206)
Vulva-anus/tail	2.5-3.5	4	4
Q_1	301-373	218-300	–
Q_2	343-424	245-314	–
Vagina depth	23-29	22-30	27
Rectum	33-37	29-33	33
a	22.1-30.1 (26.7±1.1)	27.6-35.9 (31.9±1.1)	26-40 (33)
b	5.1-8 (6.4±0.4)	5.9-7.4 (6.5±0.2)	5.3-6.2 (5.7)
c	6.3-9.6 (8.1±0.4)	8-10.2 (9±0.3)	7.8-9.3 (8.5)
V%	37-48 (43±2)	39-47 (43±1)	38-47 (43)
c'	5-9 (6±0.3)	5-9 (6±0.3)	6-10 (8)

(Allgen, 1951). Validity of this species was confirmed by re-description of topotypes (Andrássy, 1964) and type collection material (Loof & Riemann, 1976). The major generalized characteristics of the species are as follows: ♂: L=2880-3610 μm, a=30-45, b=5-6, c=14-15, spicule length=94-117 μm; ♀: L=2850-4330 μm, a=24-38, b=4.6-6.5, c=8.7-11, V%=39-42%. Absence of micropapillae is characteristic for the supplementary apparatus of males (Table 9). This species was found in single site of Teleki Lake in Kenya only.

Neotobrilus breviductus (Loof et Riemann, 1976) (Tables 5 & 6)

Material from Brazil (Tsalolikhin, 1988): head setae length is one third of head width. Buccal cavity size is 8 x 7 μm in females and 7 x 6 μm in males; distances between teeth 8-11 μm; stoma 22-29 μm deep in females and 22-24 μm in males. NR=39-43%. Gonads of females: Q1=190-290 (245) μm, Q2=220-310 (286) μm; gonads of males: T1=160-180 μm, T2=190-195 μm. Spermia flagelloid. Average distances between supplements (μm): cloaca-I: 10, I-II: 15, II-III: 17, III-IV: 62, IV-V: 34, V-VI: 32. Shape of tail different in males and females, subterminal seta present. Distribution: South America (Loof & Riemann, 1976; Tsalolikhin, 1988) and Africa (Andrássy, 1970; sensu *Tobrilus longus*).

Neotobrilus vicinus (Loof, 1973) (Tables 7 & 8)

The species was described from a ditch in Surinam (Loof, 1973) and never found elsewhere.

The supplementary apparatus of male is characterized by the absence of micropapillae. Subterminal seta present in females only.

Neotobrilus sinensis sp.n. (Fig. 2, Tables 7 & 8)

Female. Cuticle thick, on the tail up to 6 μm. Head setae short, not more than one quarter of head width. Buccal cavity wide (7-10 x 8-11 μm), duct between the pockets relatively wide, teeth large, distance between the apices of teeth 8-10 μm; total depth of stoma 20-24 μm. Amphids situated on the level of the border of the buccal cavity and anterior pocket; filaments are weakly pronounced. NR=38-45(43)%. Pericardial glands large, egg-shaped. Vulva transversal, with sclerotiums. Vaginal musculature strong but not layered. Up to five mature eggs in each uterus; egg *ca.* 50 x 50 μm in size. As the egg moves towards vulva its volume declines. Subterminal seta not observable.

Male. The only male (holotype) was similar to female in general morphology, except for a shorter tail with a very small, barely discernible subterminal seta. Supplementary apparatus typical. Distances between supplements (μm): cloaca-I: 10, I-II: 28, II-III: 27, III-IV: 80, IV-V: 45, V-VI: 45. Intersupplemental micropapillae very small, barely noticeable.

Differential diagnosis. *N. sinensis* sp.n. is closely related to *N. vicinus* (Loof, 1973), from which it differs by the presence of intersupplemental micropapillae, subterminal seta in male, long distance between supplements I and II and a longer *ductus ejaculatoris.*

Locality. Taiwan Island, San-Mung Lake; littoral, silt. 17. 03.07. Coll. V.R. Alekseev.

Holotype. ♂ № A-6840: Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia.

Addition. In material from San-Mung Lake, a few specimens of females of *Neotobrilus*, slightly different from females of *N. sinensis* sp.n., were found. It supports the assumption that there is more than one species of *Neotobrilus* in this lake. Due to the lack of males, a description of this species is not possible. Moreover, in the collection of the Zoological Institute of the Russian Academy of Sciences there are two specimens of males of *Neotobrilus* from New South Wales (Australia) which are morphologically close to the above described species. Most probably, these males belong to some other new species; however, we refrain from describing this species because of the paucity of material

Neotobrilus macrospiculum (Altherr, 1963)

The original description of the species (Altherr, 1963B) did not correspond well with the re-description of type material (Loof & Riemann, 1976). The author did not notice a presence of the supplement closest to cloaca and counted only five supplements in male. Moreover, in the original description no presence of the subterminal seta was indicated whereas it was confirmed in the re-description. In both descriptions, lack of micropapillae and considerable length of spicules were mentioned. The last characters in combination with short head setae (one quarter of head width) justify the validity of this species. ♂: L=1530 μm, a=33, b=4.6, c=10.6, spicule length=80 μm; ♀: L=1340-1650 μm, a=24-40, b=5-5.6, c=8.5-9, V%=41-48%. Distribution: South America, Argentina (Loof & Riemann, 1976).

Table 7. Morphometrics of males of *Neotobrilus breviductus.*

Characteristics	Columbia (Loof & Riemann, 1976)	Brazil (orig.)	South Africa (orig.)
	n=12	n=12	n=3
Body length, L	1200-1420 (1310)	1128-1437 (1261±24)	1203-1374 (1315)
Body diameter	(40)	30-48 (37±1)	53-61 (57)
Oesophagus	(242)	203-250 (224±4)	231-235 (233)
Tail	(112)	75-114 (104±4)	95-112 (102)
Cephalic diameter	21	16-19	18-19
Cephalic setae	7	5-6	5-6
Spicules (along axis), Sp	61-69 (65)	58-68 (63±2)	63-67 (65)
Suplement row, SR	(196)	148-205 (170±6)	159-175 (167)
Ductus ejaculatoris, DE	(196)	(200)	(200)
SR/L, %	(15)	(14)	(13)
Sp/SR, %	(33)	(36)	(39)
Sp/L, %	(5)	(5)	(5)
SR/DE,%	(100)	(90)	(85)
a	31-35 (33)	29.8-40 (34.1±0.8)	22-24 (23)
b	5-5.7 (5.4)	5.2-6.1 (5.6±0.1)	5.2-5.9 (5.6)
c	10.2-13.2 (11.7)	10.4-16.8 (12.3±0.6)	10.7-14.5 (12.9)
c'	3.5-4.3 (4)	3-5 (4)	(4)

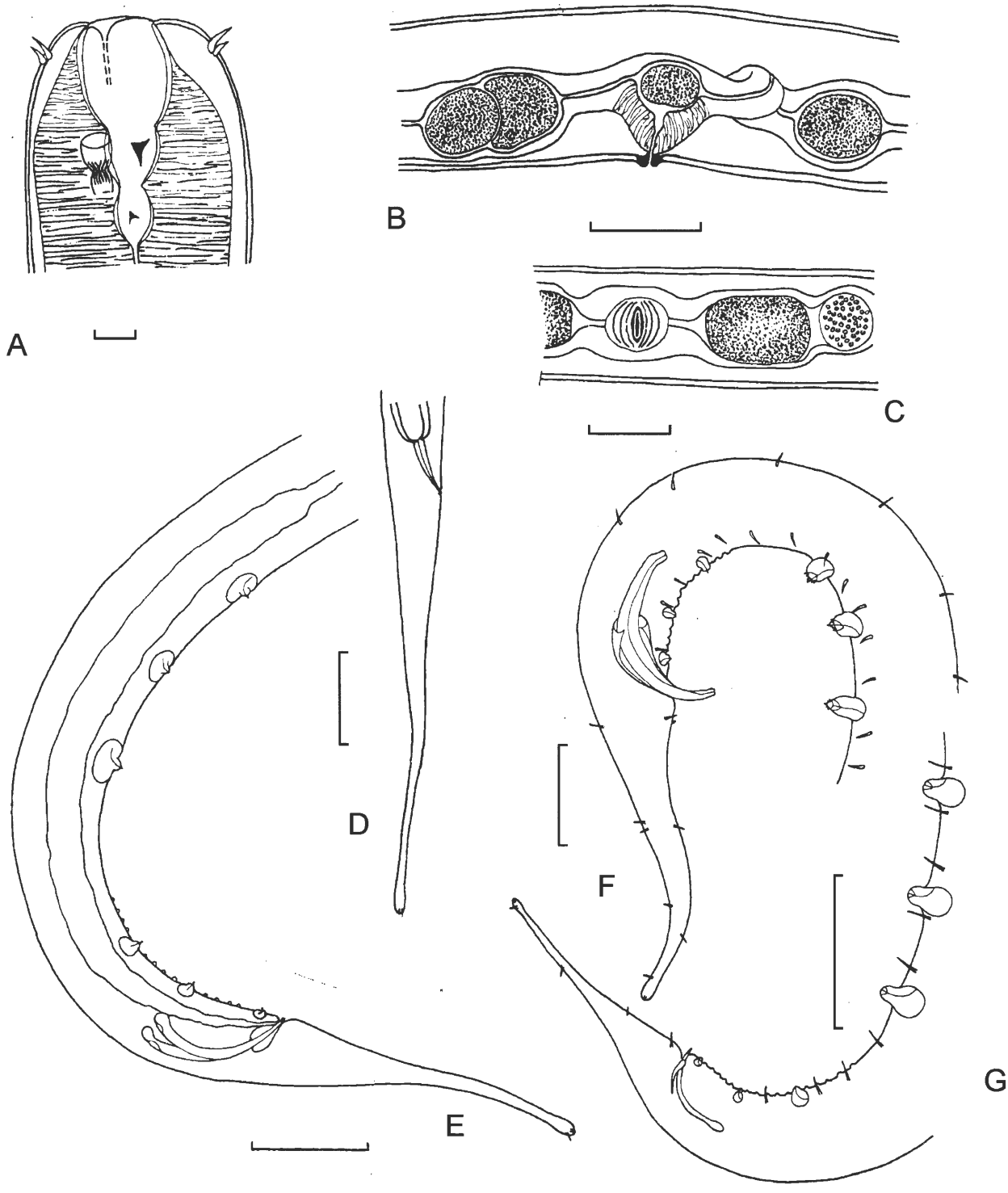

Fig. 2. *Neotobrilus sinensis* sp.n. (A-E); *N. filipjevi* (F); *N. longior* (G). A – female head, lateral view; B – region of vagina; C – vulva, ventral view; D – tail; E-G – male tail (E – holotype). Scale: A – 5 μm B-G – 50 μm.

Table 8. Morphometrics of females of *Neotobrilus breviductus*.

Characteristics	Brazil (orig.)	Columbia (Loof & Riemann, 1976)	South Africa (orig.)
	n=13	n=25	n=3
Body length	1411-1610 (1499±17)	1260-1610 (1430)	1541-1614 (1579)
Oesophagus	211-250 (232±4)	(270)	240-270 (258)
Tail	157-214 (188±4)	(161)	166-189 (176)
Vulva-anus/Tail	(~4)	(4)	(4)
Cephalic diameter	(20)	(21)	22-23
Cephalic setae	5-7	7	5-6
a	25.2-33 (29.6±0.6)	25-34 (29)	19.7-22 (20.5)
b	5.8-7.2 (6.4±0.1)	4.7-6.2 (5.3)	5.7-6.7 (6.1)
c	7-9.6 (8±0.3)	6.3-10 (8.9)	8.5-9.3 (9)
V%	34-44 (40±1)	39-46 (44)	41-43 (42)
c'	(8)	(6)	(5)

Neotobrilus longiformis (Loof, 1973)

The author of the species (Loof, 1973) assumed that this species was recorded in South and North America under the name *Trilobus longus* many times. Differentiating characters of the species are: non-layered vaginal musculature; short *ductus ejaculatoris*; characteristic shape of tail, in males, tail in the anterior third narrows abruptly and in the further two thirds it is a thin cylinder; subterminal seta absent. ♂: L= 1710-1830 μm, a=28-31, b=5.1-5.6, c=9.9-10.9, spicule length=84-96 μm; ♀: L=1500-2100 μm, a=24-32, b=4.9-6.1, c=6.7-9.3, V%=42-49%. Distribution: South America, Surinam (Loof & Riemann, 1976).

Neotobrilus nicaraguensis (Loof & Riemann, 1976)

The species is morphologically close to *N. breviductus*, from which it differs by a large number of micropapillae between supplements III and IV (12-18 *vs* 3-7), which is a good taxonomic character. ♂: L=1340-1550 μm, a=25-34, b=5-5.6, c=12-16, spicule length=48-57 μm; ♀: L=1340-1540 μm, a=23-27, b=4.9-5.5, c=11-12, V%=45-50%. Distribution: Central America, Lake Nicaragua (Loof & Riemann, 1976).

Neotobrilus nicasimilis (Loof & Riemann, 1976)

The species was reduced to a synonym of *N. nicaraguensis* on the basis of pronounced morphological similarity, including the structure of the supplementary apparatus (Tsalolikhin, 1983). The major differentiating character of these two species, in the opinion of the authors (Loof & Riemann, 1976), is the shape and length of tail, especially of females. The lack of data on intraspecific variation of these species means that their synonymization should not be justified. ♂: L=1250-1880 μm, a=28-39, b=5.8-7.1, c=12-14, spicule length=47-60 μm; ♀: L=1570-1890 μm, a=26-30, b=6-6.5, c=8.2-9.6, V%=40-44%. Distribution: Central America, Nicaragua Lake (Loof & Riemann, 1976).

Neotobrilus ampiei (Joubert & Heyns, 1979)

Morphologically close to *N. nicasimiles,* it differs from the latter by larger absolute size and head setae much longer as compared with head width (two thirds *vs* one quarter). In addition, males are characterized by closely positioned supplements IV and V; which distinguishes *N. ampiei* from all other representatives of the genus. ♂: L=1900-2190 μm, a=28-37, b=5-5.7, c=8.4-9.9, spicule length=69-80 μm; ♀: L=1960-2170 μm, a=24-33, b=4.8-5.3, c=7-8.2, V%=41-46%. Distribution: South Africa (Joubert & Heyns, 1979).

Species inquirendae

N. brzeskii (Altherr, 1963). The species was described by single specimen, a female, from Meurthe River in France (Altherr, 1963a). The only character of the species which corresponds with generic diagnosis of *Neotobrilus* is the structure of stoma: two pockets divided by a duct. However, such stoma is characteristic also of the genus *Brevitobrilus*, which includes the smallest tobrilids, whereas the body length of 'brzeskii' exceeds not only *Brevitobrilus*, but also the largest representatives of *Neotobrilus*. Such size of the body and some organs are found in some representatives of genera *Eutobrilus* and *Epitobrilus*, e.g. *Eutobrilus andrassy*, described in the same paper as 'brzeskii' (Altherr, 1963a). It can be assumed that the author has found something different (an artifact?) instead of the second pocket of stoma, because the distance between the apices of onchs, 30 μm, is twice as a long as that of representatives of the genus *Neotobrilus*.

N. floridensis (Joubert et Heyns, 1979). The species was described from females from Florida Lake in South Africa

Table 9. Comparison of supplement structures for *Neotobrilus* species

Species	Cl-I*	I-II	II-III	III-IV	IV-V	V-VI	SpIII**	SR/DE,%	SR/L,%
N. longus (U.S.A.)	4	8 (3)	14 (5)	34 (1)	18	21	0.9	65	20
N. filipjevi (Canada)	4	11 (4)	12 (5)	33 (1)	17	22	1.3	62	17
N. filipjevi (European Russia)	5	10 (3)	13 (4)	33 (2)	19	20	1.1	65	14
N. filipjevi (Siberia)	5	11 (4)	13 (5)	37 (1)	15	18	1.3	50	14
N. hopei (U.S.A.)	4	10 (4-5)	12 (6)	35 (14-19)	18	20	1.1	*ca.* 50	*ca.* 13
N. diversipapillatus (Paraguay)	5	9 (4)	10 (4-5)	36 (3)	18	22	1.1	40	12
N. longior (France)	7	9 (4)	11 (4)	27 (6)	19	27	0.9	33	10
N. longior (Sweden)	4	11 (5)	11 (5)	30 (5)	22	22	0.8	40	12
N. telekinesis (Kenya)	4	9 (-)	12 (-)	36 (-)	16	23	0.95	54	13
N. breviductus (Columbia)	7	9 (3-4)	9 (2-3)	33 (4-7)	21	21	1.2	100	15
N. breviductus (Brazil)	5	10 (3-4)	11 (2-3)	35 (4-7)	19	19	1.2	90	14
N. breviductus (South Africa)	5	11 (3-4)	9 (3)	40 (5-6)	21	22	1.2	85	13
N. vicinus (Suriname)	5	2 (-)	13 (-)	40 (-)	19	21	1	100	13
N. sinensis (Taiwan)	4	12 (4)	12 (4)	29 (3)	19	23	0.75	75	13
N. sp.(Australia)	6	7 (1-2)	9 (2-4)	38 (5)	20	20	1	77	13
N. macrospiculum (Argentina)	4	8 (-)	15 (-)	30 (-)	23	20	1.1	100	*ca.* 20
N. longiformis (Suriname)	2	15 (3-4)	15 (4-5)	32 (3-5)	16	20	0.95	100	*ca.* 18
N. nicaraguensis (Nicaragua)	7	11 (3-5)	11 (4-5)	41 (13-14)	20	19	1.2	100	10
N. nicasimilis (Nicaragua)	7	9 (3-5)	11 (4-5)	38 (12-18)	18	16	1	100	12
N. ampiei (South Africa)	7	14 (4-5)	12 (4-5)	36 (7-12)	10	21	0.9	100	14

* Average ratio (%) of intersupplement distance / supplementary row length, number of micropapillae on the intersupplement space in parentheses; SR – supplementary row length; DE – *ductus ejaculatoris* length, L – body length.

** Spicule length (by chord) / Cl-III distance ratio (average value)

(Joubert & Heyns, 1979). Structure of stoma is not clear. Absence of males means that the structure of the supplementary apparatus cannot be described, which is needed for reliable identification of species.

N. tantloyi (Sukul, 1971). The species was described from females from the thermal spring in state Bihar in India (Sukul, 1971). The structure of the supplementary apparatus is not known due to the lack of males. Body size corresponds better to representatives of the genus *Brevitobrilus*, so this species should be probably placed to this genus after additional data about its structure were obtained.

N. hohnelensis (Allgen, 1951). This species was described in the same paper as *N. telekiensis*, (Allgen, 1951), one page earlier, under the name of *Trilobus telekinesis*. Most probably both represent a single species, which reduces one of them to a synonym of the other. In accordance to the International Code of Zoological Nomenclature, priority should be given to species name '*hohnelensis*'. However, in description of *T. hohnelensis,* only five supplements of male are mentioned, which is confirmed by the illustration, and this contradicts to the diagnosis of the genus. It is possible that the author did not see a very small supplement closest to the cloaca; thus, the validity of this species remains doubtful.

Addition. Andrássy (2007) included species *N. consimiloides* (Altherr, 1965) in the genus *Neotobrilus*: "This species is placed only tentatively to *Neotobrilus*" (p. 408). There is no reason in doing it, because the structure of spicules and supplementary apparatus of this species, judging by the original description, (Altherr, 1965), does not conform to those in the genus *Neotobrilus*. Earlier, species 'consimiloides' was placed in the genus *Brevitobrilus* (Tsalolikhin, 1983), but presently it is considered as *species inquirendae* (Tsalolikhin, 2001; Zullini, 2006).

Key for the identification of species (by males)

1(12) Length of *ductus ejaculatoris* exceeds length of supplementary row, (SR/DE <70).

2(5). Spermia clavate.

3(4). Body length usually less than 2 mm; head of spicules reaches level of 3rd supplement..... .....*N. diversipapillatus.*

4(3). Body length usually more than 2 mm; head of spicules reaches level of 2nd supplement (or extends beyond this point).....*N. longior*

5(2). Spermia flagelloid.

6(7). Micropapillae between supplement absent. .....*N. telekiensis*

7(6). Micropapillae between supplements present

8(9). 13 or fewer micropapillae between supplements III and IV.....*N. hopei*

9(8). 3 or fewer micropapillae between supplements III and IV.

10(11). Length of head seta half of head width; spicules not longer than 80 μm .....*N. longus*

11(10). Length of head setae one third of head width; spicule length not less than 90 μm.....*N. filipjevi.*

12(1). Length of *ductus ejaculatoris* corresponds to length of supplementary row or slightly exceeds it (SR/DE >70).

13(16). Length of supplementary row *ca.* 20% of total body length (SR/L *ca.* 20).

14(15). Head setae length less than one quarter of head width; tail conical..... .....*N. macrospiculum.*

15(14). Head setae length is more than one third of head width; tail conical proximally in one third of its length, distally in the shape of thin cylinder.....*N. longiformis*

16(13). Length of supplementary row is less than 15% of total body length (SR/L<15).

17(18). Length of head setae half of head width. .....*N. ampiei*

18(17). Length of head setae *ca.* one third-one quarter of head width.

19(22). More than 10 micropapillae between supplements III and IV.

20(21). Tail blunt, index c′ not more than 3.5 .....*N. nicaraguensis*

21(20). Tail slender, index c′ more than 3.7 .... *N. nicasimilis*

22(19). 8 or fewer micropapillae or micropapillae absent between supplements III and IV.

23(2). Sp/SR ratio not less than 30% .....*N. breviductus*

24(23). Coefficient Sp/SR not more than 28%.

25(26). Length of *ductus ejaculatoris* corresponds with the length of supplementary row (SR/DE=100) .....*N. vicinus*

26(25) Length of *ductus ejaculatoris* slightly exceeds length of supplementary row (SR/DE=75). ..... *N. sinensis* sp. n.

Comments to tables and key for the identification of species

1) The largest number of species of the genus *Neotobrilus*, characterized by a 'short' *ductus ejaculatoris* was described from South America, which suggests that Gondwana was the territory of origin of the genus. It is supported additionally by the fact that some South American species and species closely related to them are present in Africa and Australia and have invaded South-Eastern Asia, demonstrating characteristic Hologondwana distribution (Tsalolikhin, 1983). These are *N. breviductus, N. vicinus, N. macrospiculum, N. longiformis, N. nicaraguensis, N. nicasimilis, N. ampie* and *N. sinensis.* Another group of species, with 'long' *ductus ejaculatoris*, has a holarctic distribution. It includes *N. longus, N. hopei, N. filipjevi, N. diversipapillatus* and *N. longior. N. telekiensis* occupies a special position being morphologically related to the first group but inhabiting Ethiopian zoogeographic region. Probably ancestors of this species have spread to Africa from the Palearctic.

The genus *Neotobrilus* was not reported for several water-bodies of Africa where nematode surveys were performed, e.g. in Nyasa Lake (Daday, 1910), Tanganyika Lake (Meyl, 1957; Tsalolikhin, 1989), in many lakes of the Rift Valley (Filipjev, 1931; Tsalolikhin, 1992; 1996).

Note: the reference to *N. floridensis* in the latter paper is erroneous (see above in *species inquirenda*). The only exception are high mountain water-bodies of Kenya, where *N. telekinesis* is present, and South Africa, where specimens of the genus of both the first and the second morphological groups were discovered in a number of lakes and rivers. The presence of *N. longior* there seems doubtful. It is quite probable that this is a separate species.

2) Another enigma is associated with the discovery of *'N. diversipapillatus'* male in Brazil and of the similar one in the Antarctic (Tsalolikhin, 1988). It would be more correct to consider these finds as unidentified *Neotobrilus* sp., because they have a combination of characters of different species, e.g. clavate spermia, relatively short spicules (SpIII=0.6), short *ductus ejaculatoris* and a large number of micropapillae between supplements III and IV (see comment 6). In a number of other cases, an insufficient material prevents a reliable identification of species,

because intraspecific variation in tobrilids is relatively high.

3) The record of *N. diversipapillatus* from Australia (Arthington et all, 1986) also seems doubtful. The authors give no morphological data, but tentatively, it can be assumed that it is related to *N. sinensis* sp.n. and to specimens of males from the collection of the Zoological Institute of the Russian Academy of Sciences (see description of *N. sinensis* sp.n.).

4) In many early papers length of spicules was measured along the chord; recently, length of spicules is commonly measured along the axis of spicule. Because both variants of measurements are used in diagnostics of *Neotobrilus* we propose a coefficient of calculation of the values equal to 0.85 which obtained from examining of extensive material of the whole range of species. Deviations in individual cases from this average figure are very low and can be ignored.

5) Table 9 demonstrates a striking similarity of the majority of structural parameters of the supplementary apparatus of *Neotobrilus* spp., which suggests the generic character of the features of this morpho-functional system. The differences in the details of its structure acquire greater significance and, therefore, can be regarded as species features. The principle of the structure and evolution of tobrilids' supplementary apparatus was considered in detail in the separate paper (Tsalolikhin, 2006).

6) In the systematics of *Neotobrilus,* such a character as presence or absence of micropapillae between supplements is of great importance. Re-examination of material from the collection of the Zoological Institute of the Russian Academy of Sciences has shown that this formation is not true papillae, i.e. shortened somatic setae or openings of excretory pores. 'Micropapillae' of *Neotobrilus* represent a row of transversal ventral folds of cuticle *ca.* 15 μm long, which probably facilitates curling of posterior part of the male body (Fig. 1D). These folds are formed in the areas of thinning of the exocuticle, where the exocuticle bends outwards like 'bellows'. The loose endocuticle, in contrast, may bend inwards (Fig. 1E, F). No setae or cuticle pore-like ruptures were observed on the folds, i.e. they most probably do not perform sensory or adhesive function.

In all probability, the number of areas of modified cuticle, and, therefore, also of ventral folds (micropapillae) is a stable character, although not of great value: sometimes, they are not found even in well-known species, which 'must' have papillae. This depends on the condition of specimens and quality of the fixation, and micropapillae may be very poorly pronounced (Fig. 1F). It is possible, that in those species, where papillae have not been described they are simply difficult to discern. An additional ultrastructural study of the structure of the cuticle of the precloacal area of males is needed.

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Резюме. Пересмотрен состав рода *Neotobrilus* с установлением новых синонимов; приводится описание нового вида *Neotobrilus sinensis* sp. n. с о. Тайвань и ряда видов из новых мест обнаружения; анализируются некоторые морфологические признаки, предлагаемые в качестве таксономических. Приводится ключ для определения видов.