

# Morphology of nematodes belonging to the genus *Rictularia* (Nematoda: Rictulariidae) collected from *Apodemus agrarius* in Shenyang, China

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**ABSTRACT.** The morphology of the rictulariid nematode species collected from *Apodemus agrarius* in Shenyang, China, and recorded as *Rictularia cristata* Froelich, 1802, was investigated. Five large gravid females, more than 40 mm long, eight small (circa 2 mm long) immature females shortly after final molt, and two males, were studied. The males showed prominent dimorphism: one had the typical male form with a ventrally-curved tail and developed precloacal fan-like crests; the other resembled a small female in its external morphology and lacked precloacal crests. Identity of the *Rictularia* species parasitic in *Apodemus* spp. of the Far East with *R. cristata* in Europe should be examined using DNA sequence analysis.

**KEY WORDS :** *Rictularia*, *Apodemus*, Shenyang, China, Far East

*Rictularia cristata* Froelich, 1802 (Nematoda: Rictulariidae) is considered to have a wide geographical range from Central Europe to the Far East [10, 15]. In Japan, *R. cristata* was first described from *Apodemus speciosus* and the morphology of both sexes was reported in 1976 [5]. Subsequently, this nematode has been recorded widely from *Apodemus* spp. in Japan [2]. In May 1989, we had the opportunity to examine helminth parasites of *A. agrarius* captured in Shenyang, China, and assigned the rictulariid nematodes we found to *R. cristata*, but without describing their morphology [3]. Recently, a new species, *Rictularia jiyeoni* Park et al., 2021, was described based on female worms collected from *A. agrarius* in South Korea [8]. Because the Korean Peninsula adjoins Shenyang, the presence of two *Rictularia* species in the same host murine species seems curious. In order to confirm the species of rictulariids from Shenyang, preserved materials were re-examined.

## MATERIALS AND METHODS

For this study of rictulariids, we examined 14 females (including one fragmented individual) and 2 males, collected from the small intestine of two out of 14 *A. agrarius* captured during 3–7 July 1989 in Shenyang [3]. Specimens were first fixed in 10% formalin, then transferred to 70% ethanol and preserved. For microscopical observation, specimens were cleared in a glycerol-ethanol solution (5% glycerol in 70% ethanol) by evaporation of ethanol, and mounted on glass slides with 50% glycerol aqueous solution. Specimens were viewed under Nikon Optiphot and Olympus BX50 microscopes equipped with Nomarski interference contrast apparatus. An en face view of the cephalic end was obtained of a severed head. Figures were drawn with the aid of drawing tubes attached to the microscopes.

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## RESULTS

The worms comprised 5 large females (more than 40 mm long), 8 small females (circa 2 mm long), and 2 males. In addition, the cephalic end of a fragmentary female was included. The males showed prominent dimorphism, tentatively named as typical male and female-like male.

## Description

*Rictularia cristata* (Froelich, 1802)

(Nematoda: Spirurida: Rictulariidae)

(Figs. 1-2)

*General:* With two ventrolateral rows of cuticular combs beginning immediately behind level of buccal capsule: combs plate-like in anterior body, becoming gradually spine-like posteriorly (Figs. 1A, 1E, 2A, 2C, 2E). Cephalic papillae arranged in two circles: outer circle of eight papillae, dorsolateral and ventrolateral quadrants, two by two; inner circle of six papillae, one on each side, two ventral and two dorsal, almost apical (Figs. 1C-E). Amphidial pores opening just posterolateral to lateral papillae of inner circle. Mouth reniform, opening dorsally, almost transversely. Buccal capsule heavily sclerotized with thicker anterior wall. Teeth on rim of mouth larger in both median parts of anterior and posterior borders than in lateral corners. One esophageal tooth present. Esophagus divided into anterior muscular and posterior glandular portions (Figs. 1A, 2A, 2C, 2E).

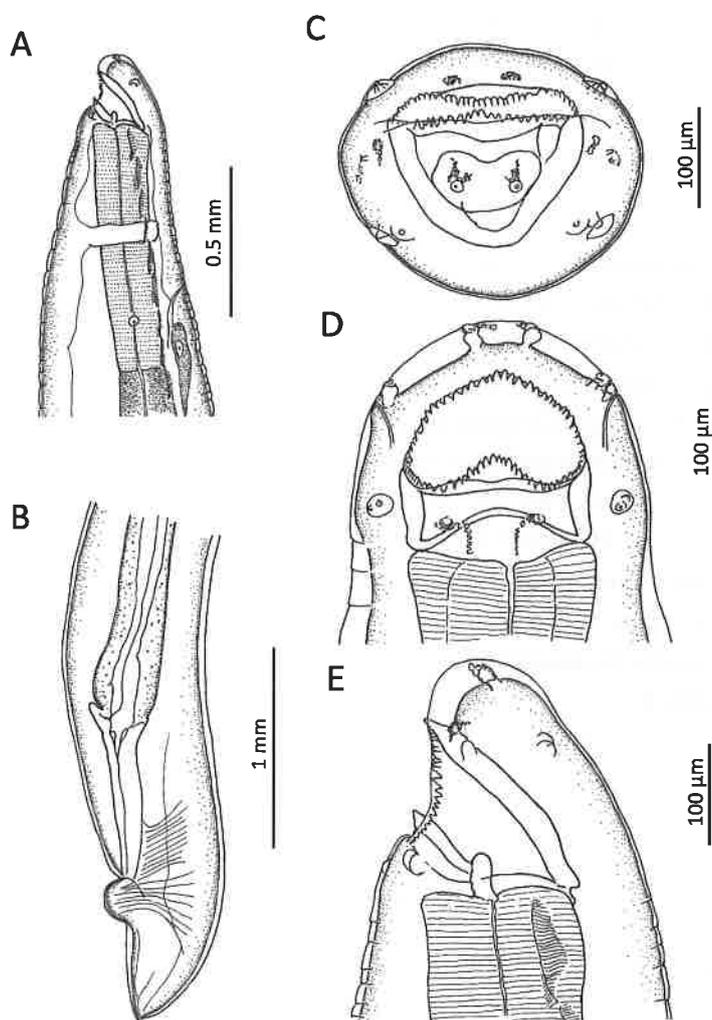


Fig. 1. Large female *Rictularia cristata* collected from *Apodemus agrarius* in Shenyang, China. A: Anterior body, right lateral view. B: Posterior end, left lateral view. C-E: Cephalic end, apical (C), dorsal (D) and right lateral (E) views.

**Table 1.** Comparison of morphometric characters of female *Rictularia* (large type) collected from *Apodemus* from the Far East.

Species	<i>R. jiyeoni</i>	<i>R. cristata</i>	<i>R. amurensis</i>	<i>R. cristata</i>
Host	<i>A. agrarius</i>	<i>A. agrarius</i>	<i>A. agrarius</i>	<i>A. speciosus</i>
Locality	Korea	Shenyang, China	Maritime Territory	Niigata, Japan
Source	Park et al. [8]	Present study	Skrjabin et al. [15]	Hasegawa [5]
No. worms measured	6	5		20
Length (mm)	26-30	44-69	51-60	14.4-67.2
Maximum width (mm)	0.11-0.13	0.93-1.25	1.1	0.45-1.94
No. denticles on oral rim	36-58	57-69 (n=4)	52	35-40
Nerve ring (mm)*		0.56-0.64		0.24-0.46
Excretory pore (mm)*		0.72-0.76	0.70-0.83	0.31-0.68
Deirids (mm)*		0.80-0.99	0.82-1.02	0.34-0.70
Vulva (mm)*	2.41-2.78	3.63-4.45	4.77	2.23-4.25
Esophagus length (mm)	1.55-2.10	Muscular: 0.80-0.98 Glandular: 3.7-3.9	1.1 4.15	0.38-0.71 1.82-4.10
Pairs of cuticular combs	37-44	43-47	50	
Prevulval pairs	32-37	33-34	34	32-34
Postvulval pairs	5-11	10-13	16	10-15
Tail length (mm)	0.040-0.049	0.48-0.66		0.20-0.57
Size of egg (µm)	38-42 × 21-24	45-48 × 29-31	48 × 33	44-52 × 26-33

\* Distance from cephalic apex.

*Large females* (5): Body stout. Vulva opening in large depression formed at distance of 6.4-10% of worm length from cephalic end. Uterus filled with eggs. Nerve ring at middle of muscular esophagus; excretory pore anterior to posterior end of muscular esophagus; deirids slightly posterior to level of excretory pore (Fig. 1A). Tail conical with pointed tip tilted dorsally (Fig. 1B). Measurements were compared with those of females parasitic in *Apodemus* spp. from the surrounding regions (see Table 1).

*Small females* (8): Vulva opening at distance of 45-60% of worm length from cephalic end (Fig. 2A). Nerve ring near posterior end of muscular esophagus; excretory pore at level of junction between muscular and glandular esophageal portions; deirids slightly posterior to level of excretory pore (Fig. 2A). Tail conical, pointed distally (Fig. 2B). Measurements were compared with those of 'fifth-stage female larvae' obtained in the experimental infection with *Rictularia amurensis* Schulz, 1927 by Morozov [7] (see Table 2).

*Typical male* (1, referred to as #1): Posterior body bent ventrally. Six fan-shaped projections on mid-ventral line in front of cloacal aperture (Figs. 2C, 2D). Left spicule much longer than right spicule; minute cuticular piece present ventral to spicules (Fig. 2D). With two pairs of precloacal, one pair of adcloacal and five pairs of postcloacal papillae at least. Measurements were compared with males previously reported from *A. speciosus* of Japan by Hasegawa [5] and 'fourth-stage male larvae' and 'fifth-stage male larvae' obtained in the experimental infection with *R. amurensis* Schulz, 1927 by Morozov [7] (see Table 3).

*Female-like male* (1, referred to as #2): Resembling small females described above, with conical not curling tail lacking precloacal fans (Figs. 2E, 2G). With two pairs of precloacal, one pair of adcloacal and five pairs of postcloacal papillae at least; one unpaired median papilla on anterior cloacal lip (Fig. 2G). Spicules as in typical male (Fig. 2F, 2G). Measurements are given in Table 3.

Taxonomic summary

*Host.* *Apodemus agrarius*

*Site of infection.* Small intestine.

*Locality.* Shenyang, China.

*Date of collection.* 3-7 July 1989.

*Specimens deposited.* Meguro Parasitological Museum, Tokyo, MPM Coll. No. 21822.

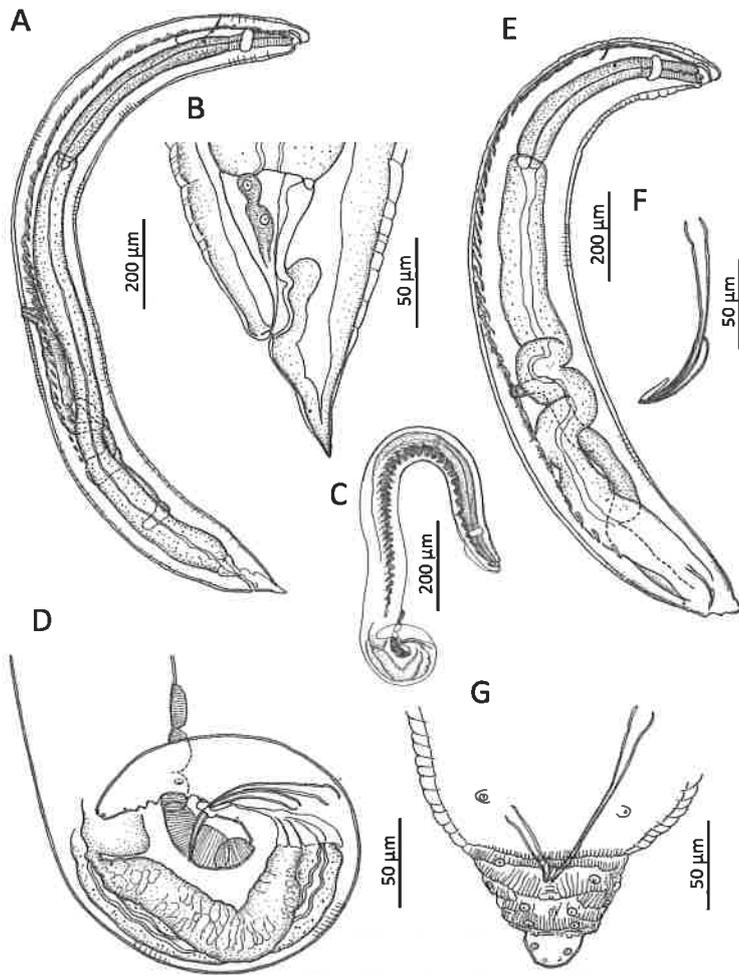


Fig. 2. Small female worm and males of *Rictularia cristata* collected from *Apodemus agrarius* in Shenyang, China. A, B: Small female, whole body (A) and posterior end (B), left lateral view. C, D: Typical male, whole body (C) and posterior end (D), right lateral view. E-G: Female-like male, whole body, left lateral view (E), spicules (F) and posterior end, ventral view (G).

Table 2. Comparison of morphometric characters of the small type of female *Rictularia* and those of premature females reported.

Species	<i>R. cristata</i>	<i>R. amurensis</i>	
Host	<i>A. agrarius</i>	<i>Mus musculus</i>	
Locality	Shenyang, China	Experimental	
Source	Present study	Morozov [7]	
Stage	Adult	'Fourth-stage larva'	'Fifth-stage larva'
No. worms measured	8		
Length (mm)	1.62-2.05	1.54-1.80	4.21-4.7
Maximum width (µm)	147-189	80-86	140-200
Nerve ring (µm)*	115-139	92	182-211
Excretory pore (µm)*	163-199	172	328-340
Deirids (µm)*	195-233		
Vulva (mm)*	0.82-1.10		2.133-2.21
Vulva (mm)**	0.65-1.04	0.736-0.745	
Muscular esophagus length (µm)	111-195	118	291-313
Glandular esophagus length (µm)	397-515	361	1040-1152
Pairs of cuticular combs	46-48	56	56
Prevulval pairs	32-35	31	30-31
Postvulval pairs	13-15	25	25-26
Tail length (µm)	60-91	73-76	102-115

\* Distance from cephalic apex.

\*\* Distance from caudal apex.

**Table 3.** Comparison of morphometric characters of males of *Rictularia* (in micrometers unless otherwise indicated).

Host	<i>A. agrarius</i>		<i>A. speciosus</i>	<i>Apodemus</i> ?	<i>Mus musculus</i>	
Locality	Shenyang, China		Niigata, Japan	Maritime Territory	Experimental	
Source	Present study		Hasegawa [5]	Sadovskaya [12]	Morozov [7]	
Stage	Adult #1	Adult #2	Adult n=2*	Adult	'Fourth-stage larva'	'Fifth-stage larva'
Length (mm)	1.15	1.67	4.40 (3.55)*	—	1.552	2.218
Maximum width	85	210	329	—	86	112
Nerve ring**	103	130	237			147
Excretory pore**	143	184	326			
Deirids**	161	206	370			
Total esophageal length	295	450	920	1328		726
Muscular esophagus length	92	110	260			200
Glandular esophagus length	203	340	660			526
Left spicule length	106	114	110 (111)	99	86	76
Right spicule length	35	56	44 (52)	49	44	41
Pairs of cuticular combs	40	43	40 (36)	42	42	40
No. preanal fans	6	0	5 (6)		3	3
Caudal papillae arrangement***	2-1-5	2-1-5	2-1-5		2-1-4	2-1-4
Tail length	67	64	107 (107)		70	80

\*One worm was incomplete and its data are shown in parentheses.

\*\*Distance from cephalic apex.

\*\*\*Preanal - adanal - postanal pairs

## DISCUSSION

Although ranging widely in body size, the morphology of the large female rictulariids from Shenyang closely resembles those reported from the Maritime Territory of the Russian Far East and Niigata, Japan [5, 12, 14, 15] (Table 1). However, the number of teeth on the oral rim tends to be greater in Shenyang worms than elsewhere. The reported measurements of the body width and the tail length of *R. jiyeoni* were much smaller than those of our specimens [8]. Judging from the photomicrographs [8], we surmise that values were miscalculated by one digit. If the given body width and tail length were one digit more, namely 1.1-1.3 mm and 0.40-0.49 mm, respectively, *R. jiyeoni* would be considered conspecific with rictulariids from Shenyang and the Maritime Territory. The egg sizes reported for *R. jiyeoni*, were somewhat smaller than in our specimens, but reconfirmation may be necessary.

The small Shenyang females were intermediate in size between 'fourth-stage larvae' and 'fifth-stage larvae' of *R. amurensis* obtained in experimental infection by Morozov [7], suggesting that they were taken only a few days after infection. The small Shenyang females had many fewer comb pairs (by nearly half) in the postvulval body, than the females reported by Morozov [7].

Male #1 from Shenyang showed the typical morphology of *Rictularia* males, but was much smaller than males recovered from Niigata [5], and even smaller than 'fourth-stage male larvae' described by Morozov [7], suggesting that it was young, only several days after infection. The morphology of male #2 was unusual for a *Rictularia* male. Further careful consideration may be necessary to judge whether it was aberrant or representative of polymorphism. It is of special interest to know whether the female-like male has any reproductive significance. Similar variation has been described in a closely related genus, in which *Pterygodermatites tani* (Hoeppli, 1929) and *P. whartoni* (Tubangui, 1931) are easily distinguished by male caudal morphology but indistinguishable from females [6, 13].

As previously mentioned, it is highly probable that the *Rictularia* worms parasitic in *Apodemus* spp. of Japan, the Korean Peninsula, Northeastern China and the Maritime Territory of Russia belong to the same species. But, if so, to which taxon should they be assigned? The primary candidate is *R. cristata* (described in

1802) as *R. amurensis* (described in 1927) was synonymized in 1969 with *R. cristata* by Quentin [9] and *R. jiyeoni* is a junior synonym having been described only in 2021. Hasegawa [5] at first supposed that worms collected from *A. speciosus* in Niigata, Japan, were close to *R. amurensis*. Although Hasegawa [5] considered the basis for synonymy was insufficient, he followed Quentin [9] and adopted *R. cristata*. Thereafter, all rictulariids found from *Apodemus* spp. in Japan have been identified as *R. cristata* [2].

*Rictularia cristata* is the type species of the genus *Rictularia*, first described from a dormouse, *Muscardius avellanarius*, in Germany at the beginning of the 19th century [4]. The original description was rather vague and the type host was misidentified as a wood mouse, *A. sylvaticus*, causing confusion later (see [9, 15] for the history). *Rictularia amurensis* was described by Schulz [14] in the early 20th century from *A. agrarius*, *A. peninsulae* (recorded as *A. speciosus*), *Clethrionomys rutilus* and *Microtus fortis* of the Maritime Territory of the Russian Far East. Later, Morozov [7] studied the life history of *R. amurensis*. He used millipedes as experimental intermediate hosts and gave the third-stage larvae from the millipedes to the experimental final hosts (mice and dormice), to raise adults. However, Morozov's [7] terminology for developmental stages was not congruent with the usual definitions; for example he used the term 'fifth-stage larva' whereas in typical nematode development, the fifth stage is the adult stage [e. g. 1]. Curiously, Morozov's [7] 'fourth-stage larva' showed comb plate pairs, which are not formed in usual fourth-stage larvae of *Rictularia* or *Pterygodermatites* [e. g. 10, 11]. Moreover, the spicules and caudal papillae of males and the vulva of females were drawn and measured for 'fourth-stage larvae' [7]. Thus, it is apparent that Morozov's [7] 'fourth-stage larvae' and 'fifth-stage larvae' were adults. The worms described by Morozov [7] are certainly different from the worms from Shenyang and Niigata as they have nearly twice as many postvulval comb plates (see Table 2), suggesting that they should be assigned to another species.

When Quentin [9] synonymized *R. amurensis* with *R. cristata*, no actual specimen of *R. cristata* was available and the morphology of males had not been described. Quentin [9] considered that three elements were essential for identification as *R. cristata*: (1) fully dorsal and transverse position of the mouth opening, (2) parasitic in Gliridae, and (3) geographic distribution in continental Europe. The only specimen of *R. amurensis* observed by Quentin [9] was a female collected from a garden dormouse, *Eliomys quercinus*, of South Slovakia and identified by A. G. Chabaud. Quentin [9] cited measurements of males from Sadvovskaya [12], whose specimens were collected in eastern Russia, not continental Europe. Without comparison of male worms from Europe, it may be premature to accept synonymy between *R. amurensis* and *R. cristata*.

It is more than 45 years since *R. cristata* was first reported from Japan, and subsequently, DNA sequencing techniques have been developed making strict identification and phylogenetic reconstruction of helminth parasites much easier than in the past. However, no DNA sequence data for either *R. cristata* or *R. amurensis* have been registered in the GenBank. It is expected that a comparison of the DNA sequences of the *Rictularia* worms of the Far East and Europe will elucidate the phylogenetic relationship of *R. cristata* and *R. amurensis* in the near future. DNA sequencing will also provide insights into apparent male dimorphism.

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\* Data reproduced in Skrjabin et al. (1967) were utilized.

## 中国瀋陽産セスジネズミ *Apodemus agrarius* から得られた *Rictularia* 属線虫 (リクチュラリア科) の形態

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**要約** : 中国瀋陽産セスジネズミ *Apodemus agrarius* から得られ、*Rictularia cristata* Froelich, 1802 として報告されたリクチュラリア科線虫の形態を記載した。体長 40mm 以上の大型の成熟雌成虫 5 個体、体長約 2 mm の最終脱皮直後の未熟小型雌成虫 8 個体および雄成虫 2 個体について検鏡、計測を行った。雄虫は顕著な二型を示した：一方は腹側に曲がった尾部と発達した総排泄腔前の扇状突起を有する典型的な雄型、他方は総排泄腔前の突起を欠き、外見的に小型の雌に類似していた。極東地域のアカネズミ属に寄生する *Rictularia* がヨーロッパの *R. cristata* と同一種かどうかは、DNA 塩基配列の解析によって検討されるべきである。

**キーワード** : *Rictularia*, *Apodemus*, 瀋陽, 中国, 極東