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Redescription of *Heligmonoides vladimiri* Sadovskaja, 1952 (Nematoda: Heligmonellidae: Nippostrongylinae) parasitic in the striped field mouse, *Apodemus agrarius* (Rodentia: Muridae), of the Far East

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Abstract: *Heligmonoides vladimiri* Sadovskaja, 1952 (Nematoda: Heligmonellidae: Nippostrongylinae) is redescribed based on worms collected from striped field mouse, *Apodemus agrarius* (Rodentia: Muridae), of Northeastern China, South Korean Peninsula, Jeju Island, Uotsuri Island and Taiwan. It is closest to *Heligmonoides speciosus* Konno, 1958 parasitic in *Apodemus speciosus* and *Apodemus argenteus* of Japan, by having more than 25 ridges in midbody of which left lateral ones only slightly hypertrophied than the others, but is readily distinguished by having a dissymmetrical bursa copulatrix and rays 8 each with swollen basal portion and not extending beyond the dorsal ray. It was suggested that *A. agrarius* became to acquire the infection with *H. vladimiri* after extending its distribution to East Asia.

Key words: *Heligmonoides vladimiri*, Nematoda, redescription, *Apodemus agrarius*, East Asia, zoogeography.

Introduction

Parasitism of nematodes belonging to the genus *Heligmonoides* Baylis, 1928 (Heligmonellidae: Nippostrongylinae) in the striped field mouse, *Apodemus agrarius*, was first reported by Sadovskaja (1952) in the Maritime Territory of Russia. Although it was named as *Heligmonoides vladimiri*, no description, figures or specific remarks were given in Sadovskaja (1952). The first description appeared in Osnovi Nematodologii IV as *Heligmonella vladimiri* by Skrjabin et al. (1954). Sadovskaja (1952) also listed *Apodemus speciosus* and *Microtus michnoi* as hosts, and *Mus musculus* was subsequently added (Skrjabin et al., 1954). Because *Heligmonoides* species exhibits host genus specificity (Durette-Desset, 1970), the materials studied by Sadovskaja might contain two or more species belonging to different genera. This condition seemed to have caused confusions in the description, making the systematic position of *H. vladimiri* uncertain (Durette-Desset and Digiani, 2012). Asakawa et al. (1990) recorded *Heligmonoides* sp. from *A. agrarius* in Shenyang, Northeastern China. This species was subsequently recorded from *A. agrarius* on Uotsuri Island, Okinawa, Japan (Hasegawa et al., 1993). It has been also found from the same host species in Taiwan, Jeju Island and Korean Peninsula (Hasegawa et al., 1993; Hasegawa, 1999). Because it is highly probable that the species is actually *H. vladimiri*, a redescription is made

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herein along with a zoogeographical discussion on *Heligmonoides* in *Apodemus*.

Materials and Methods

The materials used in this study were those already referred to previously (Asakawa et al., 1990; Hasegawa et al., 1993) but not yet described in detail. The host field mice were captured in Shenyang, Northeastern China, several localities in South Korea, Jeju Island, Uotsuri Island and Taiwan (Fig. 1). The mice were captured using cage traps or snap traps during the period from 1978 to 1982 (see Shiraishi and Arai, 1980; Asakawa et al., 1990). Their viscera except those from the mice captured in Shenyang were preserved in 10% formalin at the Department of Zoology, Faculty of Agriculture, Kyushu University. The alimentary canal of each individual was cut open in a Petri dish with tap water and observed for parasites under a stereomicroscope. Those of Shenyang were examined immediately after capture and the nematodes were fixed and preserved in 10% formalin (Asakawa et al., 1990). For morphological study, the nematodes were rinsed in 70% ethanol, cleared in glycerol-ethanol mixture by evaporating ethanol, mounted on glass slides with 50% glycerol aqueous solution and observed under Olympus BX 50 microscope equipped with Nomarski differential interference contrast and a drawing tube Olympus U-DA. Nomenclature of bursal rays and papillae follows that of Durette-Desset (1983), and the numbering of body ridges follows after Beveridge and Durette-Desset (1992). Voucher specimens are deposited in the National Museum of Nature and Science, Tokyo, Japan (NSMT).

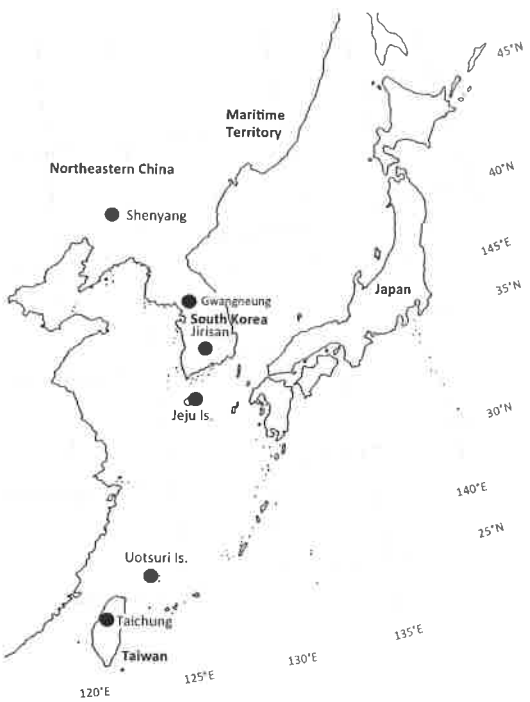


Fig. 1. Localities where the striped field mice, *Apodemus agrarius*, were captured for the present study.

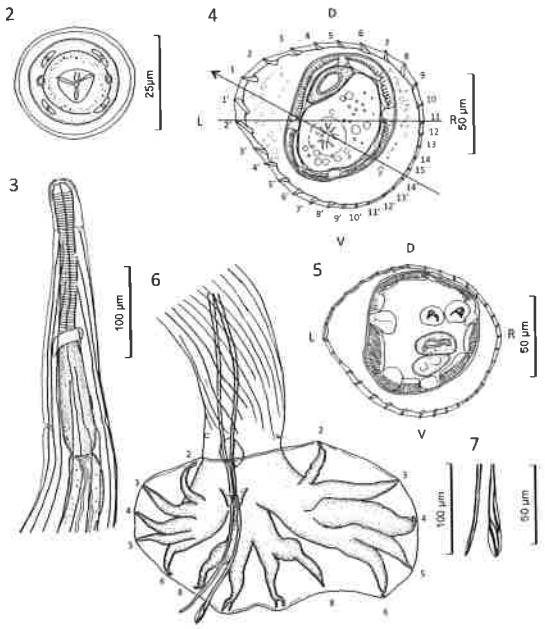
Results

Heligmonoides vladimiri was collected from *A. agrarius* captured in the following localities: Shenyang, Northeastern China (No. infected: 8/ No. examined: 12), Gwangneung, Namyangju, Gyeonggi Province, South Korea (4/4); Jirisan, Sicheon-myeon, Gyeongsangnam Province, South Korea (4/4); Jeju Island, Jeju Province, South Korea (17/18); Uotsuri Island, Okinawa, Japan (2/2); Taichung, Taiwan (16/18) (Fig. 1).

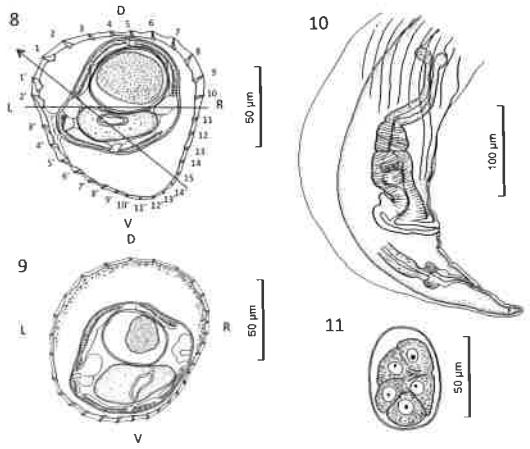
Redescription

Heligmonoides vladimiri Sadovskaja, 1952
(Syn. ***Heligmonella vladimri*** (Sadovskaja, 1952) Skrjabin et al., 1954)
(Figs. 2-11)

General: Nematoda: Trichostrongyloidea: Heligmonellidae: Nippostrongylinae. Minute nematodes forming sinistral coils. Cephalic vesicle presnet (Figs. 2, 3). Mouth triangular with rounded corners, encircled by 4 cephalic papillae and 2 amphidial pores (Fig. 2). Cuticle with fine transverse striations. Synlophe well developed with ridges commencing immediately posterior to cephalic vesicle and termi-



Figs. 2-7. Male of *Heligmonoides vladimiri* collected from *Apodemus agrarius*. 2. Cephalic end, apical view. 3. Anterior body, left lateral view. 4 Cross section through midbody. 5. Cross section through prebursal level. 6. Bursa copulatrix, ventral view. 7. Distal ends of spicules. Abbreviations: D. dorsal; L. left; R. right; V. ventral.



Figs. 8-11. Female of *Heligmonoides vladimiri* collected from *Apodemus agrarius*. 8 Cross section through midbody. 9. Cross section through infundibulum level. 10. Posterior end, right lateral view. 11. Egg. Abbreviations: D. dorsal; L. left; R. right; V. ventral.

nating at just prebursal level in male and at prevulval level in female (Figs. 3-6, 8-10). Orientation of ridges inclined ca. 50° to 60° from sagittal axis; left lateral 4 ridges slightly larger, forming weakly developed carene of type B; dorsal right ridges slightly developed; right subventral ridges minute. Esophagus club-shaped (Fig. 3). Nerve ring near middle of esophagus; excretory pore and deirids slightly anterior to esophago-intestinal junction (Fig. 3).

Male: Cuticular ridges 28 or 29 in number at midbody, increasing in number but becoming minute, lacking clear intracuticle supports in left dorsal field at prebursal level (Figs. 4, 5). Bursa dissymmetrical with larger left lobe; rays relatively stout, reaching bursal rim except rays 8; arrangement of bursal rays type 2-2-1 on right lobe and 2-3 on left lobe: rays 2 and 3 widely divergent, rays 4 to 6 with stout trunk, diverged widely; rays 8 with basal swelling, arising from thick common trunk with dorsal ray at same level of division of dorsal ray, directing latero-caudally; dorsal ray divided basal to midlength in form of inverted "V"; each branch with basal swelling, redivided distally into two offshoots: inner one thin and short and outer one thick, long and curved (Fig. 6). Spicules similar in length, thin, alate, distal ends slightly flattened, not fused (Figs. 6, 7). Gubernaculum small, round (Fig. 6).

Female: Cuticular ridges 29 or 30 in number at midbody, becoming minute and lacking clear intracuticle supports in left dorsal field at prevulval level (Figs. 8, 9). Vulva opening slightly anterior to anus; vagina forming small diverticulum dorsally; vestibule muscular, divided into anterior thick and posterior curved portions; sphincter muscular, narrowed anteriorly; infundibulum slender, often winding (Fig. 10). Tail conical, lacking terminal spine (Fig. 10). Eggs ellipsoidal, thin-shelled, containing early cleavage-stage embryo at deposition (Fig. 11).

Measurements are compared among the five localities and the original description in Table 1.

Taxonomic summary

Host: Apodemus agrarius (Pallas, 1771); striped field mouse (Rodentia: Muridae: Murinae).

Site in host: Small intestine.

Localities: Shenyang, Noetheastern China; Gwangneung, Namyangju, Gyeonggi Province, South Korea; Jirisan, Sicheon-myeon, Gyeongsangnam Province, South Korea; Jeju Island, Jeju Province, South Korea; Uotsuri Island, Okinawa, Japan; Taichung, Taiwan.

Specimens deposited: NSMT-As 4301 (Shenyang, Northeastern China); NSMT-As 4302 (Gwangneung, Namyangju, Gyeonggi Province, South Korea); NSMT-As 4303 (Jirisan, Sicheon-myeon, Gyeongsangnam Province, South Korea); NSMT-As 4304 (Jeju Island, Jeju Province, South Korea); NSMT-As 2187 (Uotsuri Island, Okinawa, Japan); NSMT-As 4305 (Taichung, Taiwan).

Remarks

Although the worms collected from Jeju Island had generally smaller body size than those from other localities (Table 1), principal morphology was identical. It is considered that all of the present worms belonged to one species. By having well developed synlophe, oblique axis of orientation of ridges, and by lacking tail spine in female, this nematode belongs to Heligmonellidae; by having an axis of orientation of ridges inclined between 45° and 67° from sagittal axis and having a carene, it is classified as Nippostrongylinae; by having a carene of type B and a dissymmetrical bursa copulatrix with larger left lobe, it is assigned to the genus *Heligmonoides* Baylis, 1928 (Durette-Desset, 1983; Hasegawa and Syafruddin, 1997; Gibbons, 2010; Durette-Desset and Digiani, 2012).

The original description of *H. vladimiri* was rather vague and the bursa copulatrix figured showed larger right lobe (Skrjabin et al., 1954). Nevertheless,

Table 1. Comparison of measurements (range and mean in parenthesis) of *Heligmonoides vladimiri* collected from *Apodemus agrarius* among localities in the Far East. (In micrometers unless otherwise stated)

Locality	Maritime Territory, Russia*1	Shenyan, Northeastern China	Gwangneung, Korea	Jeju Island, Korea	Uotsuri Island, Okinawa, Japan	Taichung, Taiwan
Male (No. measured)		(6)	(10)	(5)	(4)	(5)
Length (mm)	3.95–5.00	3.15–3.45 (3.28)	2.17–3.61 (2.82)	2.25–3.21 (2.63)	3.30–3.95 (3.58)	3.42–4.31 (3.84)
Width	104–187	112–185 (137)	105–133 (119)	95–125 (108)	117–133 (123)	117–156 (128)
Cephalic vesicle length	59–64	53–65 (60)	40–56 (51)	45–53 (50)	36–56 (46)	54–60 (57)
Cephalic vesicle width	38	33–48 (38)	34–39 (36)	28–30 (29)	32–36 (34)	32–36 (35)
Nerve ring*2		165–220 (188)	110–172 (153)	117–150 (138)	124–205 (160)	175–210 (192)
Excretory pore*2		258–345 (287)[n=5]	160–289 (238)	158–228 (214)	215–299 (257) [n=2]	290–325 (303)
Esophagus length	328–353	306–348 (323)[n=5]	270–318 (297)	267–303 (282)	293–375 (333)	330–365 (345)
Spicule length	449–520	378–462 (410)	361–440 (397)	337–360 (352)	400–455 (424)	405–460 (438)
Gubernaculum length	33	33–38 (36)	32–38 (34)	30–35 (31)	34–38 (35)	31–38 (34)
Female (No. measured)		(2)	(10)	(5)	(1)	(2)
Length (mm)	3.8–5.2	4.08–4.61 (4.35)	3.60–4.51 (3.98)	2.64–3.37 (2.90)	4.54	4.28–4.47 (4.38)
Width	116	110–138 (124)	117–133 (124)	78–105 (94)	117	113–117 (115)
Cephalic vesicle length	66	58–63 (61)	56–60 (59)	38–50 (46)	40	46–60 (53)
Cephalic vesicle width	35	35–40 (38)	36–44 (42)	28–33 (29)	32	32 [n=1]
Nerve ring*2		130–167 (149)	137–177 (162)	115–170 (136)	165	155–175 (165)
Excretory pore*2		173–230 (202)	223–304 (267)	153–247 (194) [n=4]		290 [n=1]
Esophagus length	402	298–305 (302)	286–349 (318)	281–305 (296)	320	330–340 (335)
Vulva*3	116	114–148 (131)	103–150 (128)	100–113 (106)	130	130–140 (135)
Anus*3	49	45–47 (46)	36–60 (47)	41–48 (44)	50	58 (58)
Vagina vera length		63–91 (77)	48–72 (60)	50–64 (57)	60	58–60 (59)
Vestibule length		91–98 (95)	60–86 (74)	59–78 (67)	86	78–110 (94)
Sphincter length		31–38 (35)	29–44 (35)	20–28 (25)	36	33–36 (35)
Infundibulum length		69–133 (101)	64–101 (85)	63–120 (84)		90–104 (97)
Egg size	56 x 23	68–70 x 38–40	66–78 x 38–44	63–70 x 35–43	56–60 x 39–40	64–70 x 36–40

*1 Source: Skrjabin et al. (1954).
*2 Distance from cephalic apex.
*3 Distance from caudal apex.

the bursal ray arrangement is similar to that of the present male when the figure is flipped horizontally, suggesting that the original figure was drawn from dorsal side. It was stated to possess only 8 to 12 longitudinal ridges and narrow lateral alae. However, no figure of cross section was presented. Because the host list contained *Microtus fortis* (as *M. michnoi*), it is very probable that the type material contained some additional species with fewer ridges such as *Carolinensis minutus*, a common nematode of *Microtus* of the Palearctic region (Durette-Desset, 1968, 1971, 1983). Sadovskaja (1952) did not make type host and type specimen designations. However, she put *A. agrarius* at the top on the host list, suggesting that this mouse was the type host. Hence, it may be valid to consider the *Heligmonoides* in *A. agrarius* as *H. vladimiri*. In the original description, the spicules were fused in distal 1/3, differing from the present males. This discrepancy may be due to the only slightly protruded condition of the spicule or mixture of two or more species in the type material.

Among the congeners, *H. vladimiri* is closest to *Heligmonoides speciosus* (Konno, 1958) (syn. *Longistriata speciosa* Konno, 1958; *Longistriata hokkaidensis* Chabaud et al., 1963; *Tenorastrongylus hokkaidensis* (Chabaud et al., 1963) Durette-Desset, 1970) parasitic in *A. speciosus* and *Apodemus argenteus* in Japan by having more than 25 ridges in midbody of which left lateral ones are only slightly hypertrophied than the others (Konno, 1958; Chabaud et al., 1963; Durette-Desset, 1970). However, the bursa copulatrix in *H. speciosus* is nearly symmetrical and rays 8 are slender, lacking basal swellings, and more extended caudally than dorsal ray, differing from *H. vladimiri* (Konno, 1958; Chabaud et al., 1963).

Discussion

Because *H. vladimiri* has been collected from *A. agrarius* of Korean Peninsula, Jeju Island, Uotsuri Island, Taiwan and northeastern China besides the Maritime Territory of Russia, it is surmised that this nematode has high affinity with this host murid. *Apodemus agrarius* is distributed widely in Eurasian continent but the distribution is interrupted forming two major areas: one from Europe to Lake Baikal and the other in the Far East, including northern Myanmar, China, the Maritime Territory of Russia, the Korean Peninsula, Taiwan, and Uotsuri Island (Musser and Carleton, 2005). *Heligmonoides* has not been recorded from the European populations of any *Apodemus* though helminthological studies have been made rather extensively (e.g. Asakawa and Tenora, 1996; Ondrikova et al., 2010; Gubanyi et al., 2015).

Sharply contrasting with the absence of records of *Heligmonoides* in Europe, *Apodemus* species other than *A. agrarius* in East Asia also harbor other *Heligmonoides* species. The two sympatric species, *A. speciosus* and *A. argenteus*, of Japan harbor *H. speciosus* (Konno, 1958; Chabaud et al., 1963; Durette-Desset, 1970; Hasegawa, 1990; Asakawa and Tenora, 1996). In Hokkaido, Japan, *Apodemus peninsulae* was also known to be parasitized by *H. speciosus* but only in limited localities, where *A. speciosus* inhabits sympatrically (Asakawa, unpublished data). In Taiwan highland, *Apodemus draco* harbors *Heligmonoides taiwanensis* Hasegawa, 1990 (Hasegawa, 1990). *Heligmonoides* sp. was also recorded from *Apodemus gurkha* of Nepal (Asakawa et al., 1997). It is surmised that *A. agrarius* acquired *Heligmonoides* after spreading its distribution to East Asia. A preliminary cladistic analysis of the *Apodemus*-parasitic species of *Heligmonoides* based on morphology suggested their close phylogenetic relationship (Hasegawa, 1999). It is thus surmised that *Heligmonoides* experienced co-speciation with *Apodemus*. Further studies including DNA sequence analysis are necessary for elucidation of evolutionary relationship of *Apodemus-Heligmonoides* in East Asia.

Because heligmonellid species are usually minute, forming complex coils and resembling each other

in appearance, identification is often difficult. Thus, the records of *H. vladimiri* from *Microtus* and *Mus* (Sadovskaja, 1952; Skrjabin et al., 1954) should be re-examined critically. In Korea, Seo et al. (1968) examined many individuals of *A. agrarius* and recorded *Heligmosomum* sp. and *Heligmosomoides* sp. They considered that these species are common ones between *A. agrarius, Rattus norvegicus* and *M. fortis*, and the former is also shared by *M. musculus* and *Crocidula russula*. More recently, Sohn et al. (2014) also surveyed helminth parasites of *A. agrarius* in southern regions of Korea, and recorded heligmosomids with high prevalence and intensity. However, it remains unclear whether *Heligmonoides* was included in their 'heligmosomids' because they withheld generic and species identification.

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