Description of a new species of Heligmosomoides (Nematoda: Heligmosomidae) parsitic in Microtus limnophilus (Rodentia: Cricetidae) from Rangstang, Sichuan, China

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DESCRIPTION OF A NEW SPECIES OF *HELIGMOSOMOIDES* (NEMATODA: HELIGMOSOMIDAE) PARASITIC IN *MICROTUS LIMNOPHILUS* (RODENTIA: CRICETIDAE) FROM RANGTANG, SICHUAN, CHINA

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Summary:

Heligmosomoides craigi n. sp. (Nematoda: Heligmosomoidea) is described from *Microtus limnophilus* Büchner, 1889 (Rodentia: Cricetidae) from Rangtang, Sichuan, China. It is related to *H. protobullosus* Asakawa, 1987 and *H. longispiculum* Tokobaev & Erkulov, 1966 both parasites of *Microtus* spp. from Japan and USSR, respectively by the following features: a ratio of spicule length/body length of more than 45 % and rays 9 shorter than rays 10. The new species is differentiated by rays 8 being closed to rays 6 and 19-22 cuticular ridges *versus* 14 in *H. protobullosus* (synlophe not described in *H. longispiculum*). *H. longicirratus* (Schulz, 1954) also a parasite of *Microtus* sp. from the USSR is the most closely related species based on the number of cuticular ridges (20) and the ratio of spicule length/body length (48 % *versus* 50 %). There are no illustrations of this species and the female has not been described; for that reason, it is not possible to compare it accurately with our specimens.

KEY WORDS : Nematoda, Trichostrongylina, Heligmosomoidea, Heligmosomidae, *Heligmosomoides craigi* n. sp., Cricetidae, Arvicolinae, Rodents, China.

Résumé : DESCRIPTION D'UNE NOUVELLE ESPÈCE D'*HELIGMOSOMOIDES* (NEMATODA : HELIGMOSOMIDAE) PARASITE DE *MICROTUS LIMNOPHILUS* (RODENTIA : CRICETIDAE) ORIGINAIRE DE LA PROVINCE DE RANGTANG, SICHUAN, CHINE

Heligmosomoides craigi *n. sp. (Nematoda : Heligmosomoidea) est décrit chez* Microtus limnophilus *Büchner, 1889 (Rodentia : Cricetidae) originaire de la province du Rangtang, Sichuan, Chine. Cette espèce est morphologiquement proche de* H. protobullosus *Asakawa, 1987 et de* H. longispiculum *Tokobaev & Erkulov, 1966 tous deux parasites de* Microtus *spp., provenant respectivement du Japon et de l'URSS, par les caractéristiques suivantes : le rapport longueur des spicules/longueur du corps supérieur à 45 % et des côtes 9 plus courtes que les côtes 10. La nouvelle espèce se différencie par des côtes 8 proches des côtes 6 et par 19-22 crêtes cuticulaires contre 14 chez* H. protobullosus *(synlophe non décrit chez* H. longispiculum*). H. longicirratus (Schulz, 1954), également parasite de* Microtus *sp. d'URSS est l'espèce la plus proche par le nombre de crêtes cuticulaires (20) et le rapport longueur des spicules/longueur du corps (48 %* versus *50 %). Il n'y a pas d'illustration de cette espèce et la femelle n'a pas été décrite ; pour cette raison, il n'est pas possible de la comparer correctement avec nos spécimens.*

MOTS CLÉS : *Nematoda, Trichostrongylina, Heligmosomoidea, Heligmosomidae,* Heligmosomoides craigi *n. sp., Cricetidae, Arvicolinae, Rongeurs, Chine.*

INTRODUCTION

The genus *Heligmosomoides* Hall, 1916 (Nematoda: Heligmosomidae) is mainly parasitic in the small intestine of Holarctic Arvicolinae. Whereas the European and Nearctic species of *Heligmosomoides* are relatively well known, only one species has been described from China, in a member of the Muridae, namely *H. asakawae* Tenora & Barus, 2001 (= *H. pokygyrus sensu* Asakawa, Tenora, Hasegawa, Jin, He, Wu, Tsuchiya, Miyashita, Moriwaki, Fukumoto & Ohbayashi, 1992) a parasite of *Apodemus uralensis* (Pallas, 1811) (= *A. microps*), from Ulumuchi, China. China is therefore a very interesting biogeographic region since it may serve as a migratory route for the Arvicolinae and their parasites to the New World. In the present paper, a new species of *Heligmosomoides* is described from *Microtus limnophilus* Büchner, 1889 (Arvicolinae) which originates from Rangtang, Sichuan, China.

M. limnophilus occurs in China and West Mongolia (Shenbrot & Krasnov, 2005; Smith & Xie, 2008). It is interesting to note that is also parasitized by the cosmopolitan family of the Heligmonellidae, which are mainly parasitic in Muridae, namely *Euzetoda ludovici* Elias & Durette-Desset, 2003, originating from South Gansu and Huang He (Elias & Durette-Desset, 2003).

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MATERIAL AND METHOD

Rodent hosts were collected during June 2004, as part of a French-British-Chinese programme, whose main target was the mass screening of human populations for alveolar echinococcosis and the study of its transmission. The study area was located in Rangtang, Sichuan, in China. The rodents were weighed and dissected in the field to determine the sex and reproductive status. Heads, tissue samples (or the whole body for a few specimens) were preserved for identification (Courant *et al.*, 1999). The nomenclature of the hosts follows Wilson & Reeder (2005).

The small intestines were preserved in 5 % formalin solution and transported to the Muséum National d'Histoire Naturelle (MNHN), Paris, one month later. They were then transferred to 70 % ethanol. To determine the precise location of the parasites, the small intestine (SI) was divided into four equivalent parts numbered, from the duodenum to the caecum, SI1 to SI4. In SI3 and SI4 some larvae (L4) were recovered. Nematodes were collected and stored in 70 % ethanol. They were examined in temporary mounts of lactophenol. The synlophe was studied following the method of Durette-Desset (1985). The total number of cuticular ridges is followed in brackets by the number of dorsal ridges then ventral ridges. The nomenclature used for the study of the caudal bursa is that of Durette-Desset & Chabaud (1981). The nomenclature for the parasites used above the family group follows Durette-Desset & Chabaud (1993). Measurements are given in micrometers, unless otherwise stated. Type specimens were deposited in the Helminthological Collection of the MNHN.

RESULTS

HELIGMOSOMOIDES CRAIGI N. SP. (Figs 1-16)

Type material (= material studied): male holotype, female allotype MNHN 438MQa. Seven male, nine female paratypes, MNHN 438MQb.

Type host: *Microtus limnophilus* Büchner, 1889 (Rodentia: Cricetidae: Arvicolinae).

Site: small intestine 1 (SI1).

Geographic origin: Rangtang, Sichuan, China, J.P. Quéré coll., June 2004.

Description

Small nematodes coiled along ventral side in three sinistral spirals in males, three to six in females. Deirids setiform, situated at level of excretory pore, observed only in one male (Fig. 1).

Synlophe: (studied in one male and one female paratypes). In both sexes, cuticle bears longitudinal, uninterrupted ridges without struts. Median ridges (about half) appear posterior to cephalic vesicle (Figs 2-3). Other ridges appear at different levels between cephalic vesicle and nerve ring; about the same number on right and on left side (Figs 2-3). Ridges disappear at about 100 anterior to caudal bursa in male, 150 anterior to caudal extremity in female. Number of ridges: 21 (11D/10V) in male (Fig. 11), 22 (12D/10V) in female (Fig. 12), at level of oesophago-intestinal junction; 19 (10D/9V) in male (Fig. 13), 22 (13D/9V) in female (Fig. 14) at mid-body; 22 (11D/11V) in male (Fig. 15), 5 (4D/1V) in female (Fig. 16) within distal fifth. Ventral ridges best developed. On ventral side, decreasing gradient of size medio-lateral. On dorsal side, ridges of similar size. Axis of orientation directed from right to left and sub-frontal (Figs 11-16).

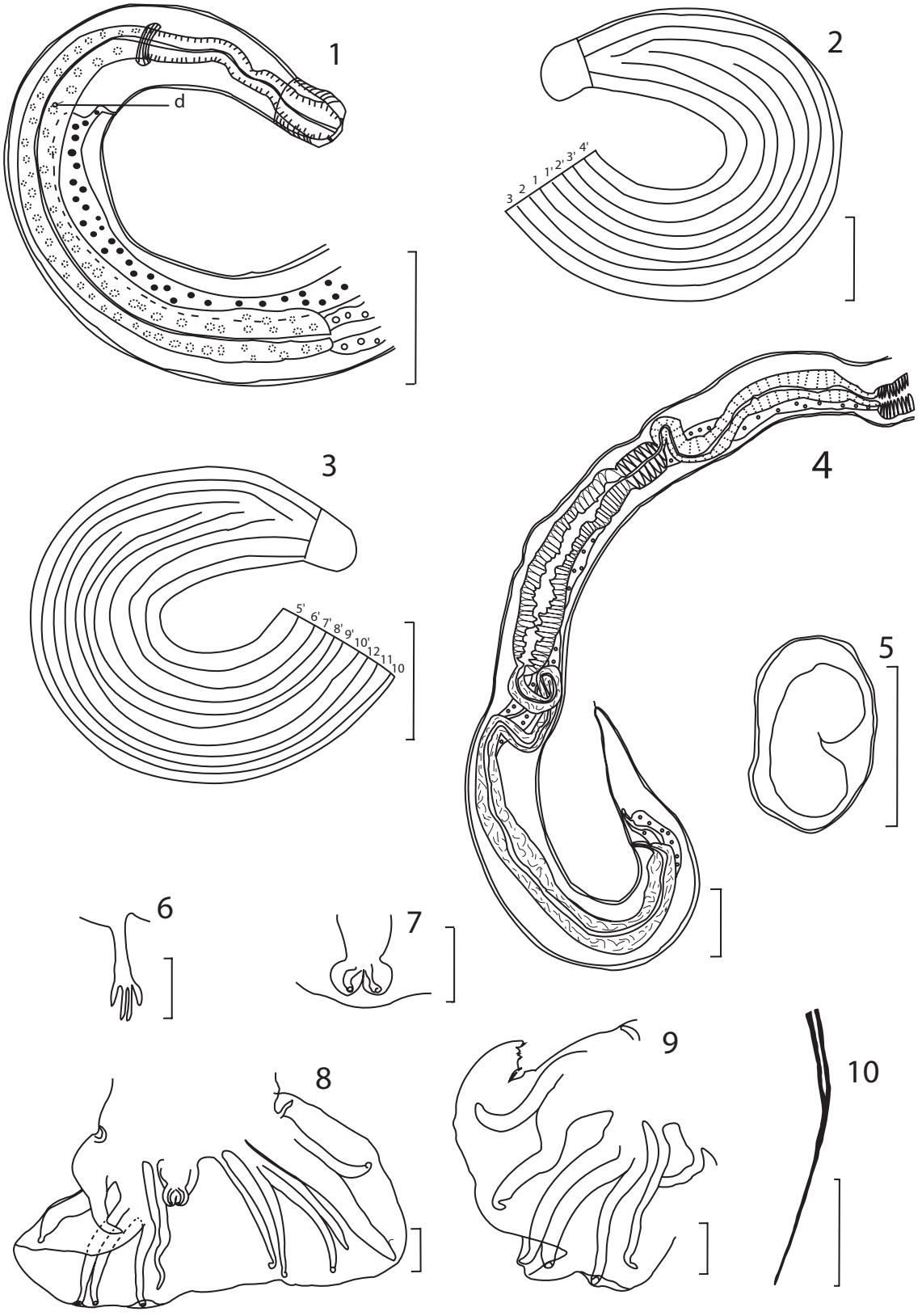
Holotype male: 5.6 mm long and 110 wide at mid-body; cephalic vesicle 50 long and 40 wide; nerve ring and excretory pore situated at 140 and 250 from apex, respectively. Oesophagus 605 long (Fig. 1).

Caudal bursa sub-symmetrical (Fig. 8). Prebursal papillae developed. Caudal bursa pattern of type 2-3 with a tendency to type 2-2-1, for both lobes. Right lobe (Fig. 9): ray 3 longer than ray 2, shorter than rays 4 and 5. Left lobe (Fig. 8): ray 3 biggest. In both lobes, rays 4 and 5 joined along entire length; rays 6 arising first on common trunk of rays 4 to 6; rays 5 very distant from rays 6. Rays 8 of similar length, arising on common trunk of rays 2 to 6, slightly separated from rays 6 for entire length (Figs 8-9). Dorsal ray reduced and divided at two thirds into two branches, each branch divided into two twigs: rays 9 (external branches), rays 10 (internal branches) (Fig. 6). Rays 9 arising as same level as division of dorsal ray, shorter than rays 10. Genital cone 25.7 long and 19.7 wide, bearing two long papillae 7 on dorsal lip, papilla zero not observed (Fig. 7). Gubernaculum absent; spicules subequal, 2.85 mm long, taking up 56.4 % of body length, ending in sharp tip 40 long (Fig. 10). Each spicule divided into two closely attached of similar size parts except at both extremities. Both parts of the spicule merge in a long tip at its distal extremity (Fig. 10).

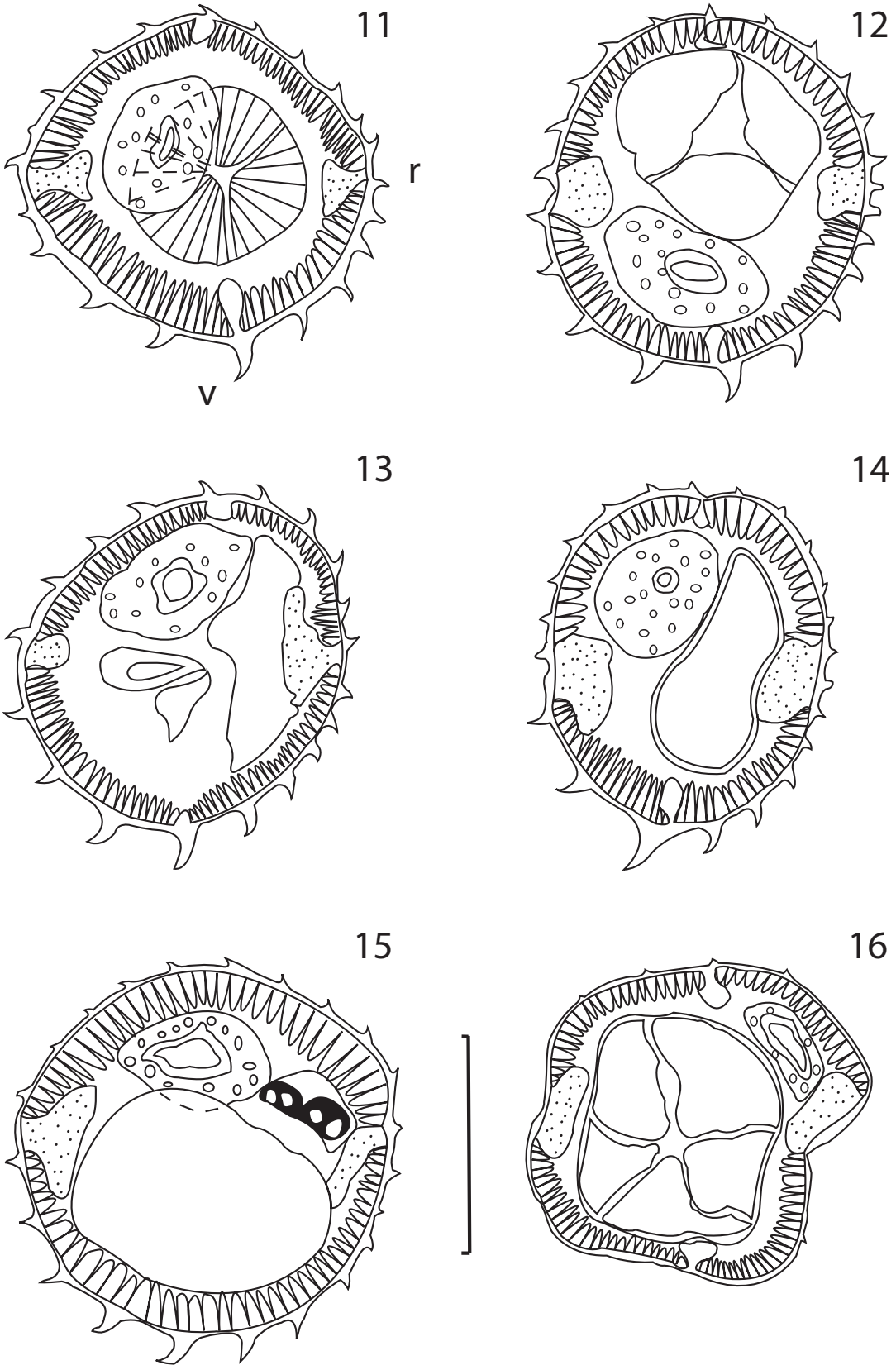
Measurements (average and range) of six male paratypes: 5.0 (4.2-5.8) mm long and 102 (85-120) wide at mid-body. Cephalic vesicle 56 (50-60) long and 41 (40-45) wide. Nerve ring and excretory pore situated at 152 (130-180) and 249 (230-290) from apex, respectively. Oesophagus 625 (540-730) long. Spicules 2.75 (2.5-2.9) mm long taking up 54 (50-60) % of body length.

Allotype female: 6.4 mm long and 120 wide at mid-body. Cephalic vesicle 55 long and 40 wide. Nerve ring and excretory pore situated at 180 and 290 from apex, respectively. Oesophagus 640 long.

Monodelphic. Vulva situated 210 from caudal extremity. Vagina vera 10 long, vestibule 1.450 long divided into two parts (with a muscular proximal part shorter than



Figs. 1-10. – *Heligmosomoides craigi* n. sp. from *Microtus limnophilus*: 1, male, anterior extremity, right lateral view; 2-5, female, 2, 3, arising of the cuticular ridges, 2, left lateral view 3, right lateral view, 4, posterior extremity, right lateral view, 5, embryonated egg, 6-10 male, 6, dorsal ray, dorsal view, 7, genital cone, ventral view, 8, 9, caudal bursa, 8, with right lobe folded, ventral view, 9, with right lateral lobe open, ventral view, 10, distal tip of one spicule.
Scale bars: Figs 1-4: 100 µm; Fig. 5: 50 µm; Figs 6-9: 30 µm; Fig. 10: 20 µm. Figs 8 and 9 from two males. Abbreviation: d: deirid.



Figs 11-16. – *Heligmosomoides craigi* n. sp. *in Microtus limnophilus*, Transverse sections of body: 11, 12, at level of the oesophago-intestinal junction, 11, male, 12, female, 13, 14, at mid-body, 13, male, 14, female, 15 at about 1.2 mm anterior to caudal bursa, male; 16, at about 1 mm anterior to vulva, female. Scale bar: Figs 11-16: 50 µm.
Abbreviations: r: right side, v: ventral side. Transverse sections orientated as 11.

the distal), sphincter 100 long and 70 wide, infundibulum 260 long (Fig. 4). Uterus 1.9 mm long taking up 21 % of body length, without eggs. Tail 85 long with caudal spine 10 long (Fig. 4).

Measurements (range and average) of eight female paratypes: 7.8 (6.0-10) mm long and 106 (90-150) wide at mid-body. Cephalic vesicle 58 (50-70) long and 43 (40-45) wide. Nerve ring and excretory pore situated at 185 (155-200) and 329 (270-350) from apex, respectively. Oesophagus 669 (610-730) long. Vulva situated 208 (170-250) from caudal extremity. Vagina vera 19 (10-30) long, vestibule 1050 (820-1450) long, sphincter 82 (60-110) long and 55 (40-95) wide, infundibulum 426 (260-550) long. Uterus 1.63 (1.08-2.7) mm long taking up 20 (15-38) % of body length. Only two females having 12 and 53 embryonated eggs respectively. No eggs in other females. Eggs 70 long and 45 wide (Fig. 5). Tail 104 (60-170) long with spine 10 (10) long.

DIFFERENTIAL DIAGNOSIS

The specimens described above belong to the genus *Heligmosomoides* Hall, 1916 (Heligmosomoidea: Heligmosomidae), as redefined by Durette-Desset (1968) mainly characterised by longitudinal cuticular ridges and a poorly developed dorsal ray.

Eight species, five Nearctic and three Palearctic, share, along with the Chinese specimens, a ratio spicule length/body length of more than 45 %.

In the Nearctic zone are: *H. carolinensis* (Dikmans, 1940), *H. longispiculatus* (Dikmans, 1940), *H. montanus* Durette-Desset, 1967 and *H. tenorai* Durette-Desset, 1967, are all parasites of Arvicolinae and *H. thomomyos* Gardner & Jasmer, 1983 is a parasite of Heteromyidae. These species are easily distinguished from the Chinese specimens since the distance anus-vulva is longer than the length of the tail. In addition the ratio of spicule length/body length is greater than 60 % or less than 50 % (*H. carolinensis*).

In the Palearctic zone are: *Heligmosomoides protobullosus* Asakawa & Ohbayashi, 1987 a parasite of *Microtus montebelli* (Milne-Edwards, 1872) from Japan; *H. longispiculum* Tokobaev & Erkulov, 1966, a parasite of *Microtus gregalis* (Pallas, 1779) from Central Asia (Kyrgyzstan) and *H. longicirratus* (Schulz, 1954) in Skjabin *et al.* (1954), a parasite of *Microtus* sp. from the USSR. In addition, *H. protobullosus* and *H. longispiculum* have, like the Chinese specimens, rays 9 shorter than rays 10. They are differentiated by rays 8 being very distant from rays 6.

H. protobullosus is also distinguished from the Chinese specimens by the following features: the synlophe has 14 cuticular ridges. In the male, the caudal bursa is markedly assymmetrical with right ray 4 arising first on the common trunk of rays 4 to 6 and left ray 4 at the same level as ray 6; in both lobes, rays 4 and 5 are distant from each other. In the female, the vestibule is very long in relation to the other *Heligmosomoides* species and the distance anus-vulva is equivalent to the length of the tail. In the Chinese specimens, the synlophe has 20 cuticular ridges, the caudal bursa is sub-symmetrical, with right ray 6 arising first on the common trunk of rays 4 to 6 and left ray 4 posteriorly to ray 6; in both lobes, rays 4 and 5 are close from each other.

H. longicirratus may be the most closely related species by the number of cuticular ridges (20 *versus* 19-22) and the ratio of spicule length/body length (48 % for a male of 6 mm *versus* 50 % for a male of 5.8 mm long). Unfortunately the species has not been illustrated and the female not described. It is not possible to compare it accurately with our specimens. If the male and the female of *H. longicirratus* turn out to be the same as those of the Chinese specimens, the latter would automatically become a synonym of *H. longicirratus*. We consider, at least provisionally, that the specimens from *Microtus limnophilus* belong to a new species which we have named *Heligmosomoides craigi* n. sp in honour of Professor Philip Simon Craig, Salford University, UK. He has devoted his professional life to parasitology and has promoted parasite eco-epidemiological studies in China.

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