INTRODUCTION

The genus Heligmosoides Hall, 1916 (Heligmosomidae) is widespread in the Holarctic region and is found mainly in the Arvicoline but also in the Murinae. Asakawa (1988) divided the species of the genus into five categories namely the “travassosi-douglasii line”, the “relic group”, the “laeviscarolinensis line”, the “longicirratum-longispiculatus line” and the “polygyrus line”. The last line parasitizes only Mus and Apodemus. Until now, three species belonging to this line have been described or recorded from China: Heligmosoides polygyrus polygyrus (Dujardin, 1845); H. neopolygyrus Asakawa and Ohbayashi, 1986 and H. asakawae Tenora and Barus, 2001, (Asakawa et al., 1990, 1992, 1993). H. neopolygyrus and H. p. bakeri Durette-Desset et al., 1972 are present in Japan (Asakawa & Ohbayashi, 1986; Hasegawa et al., 1983).

In this study we redescribe H. neopolygyrus from Sichuan Province (central China) in Apodemus peninsulae (Muridae). The use of new morphological characters on the present and previously published material allows us to examine the validity of some reports of the “polygyrus line” described as H. neopolygyrus, as well as the distribution of the genus Heligmosoides in Chinese and Japanese Muridae.

MATERIALS AND METHODS

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

The material studied here came from a single specimen of *A. peninsulae* (Thomas, 1907). The small intestine was preserved in 5% formalin and transported to the Museum national d’Histoire naturelle (MNHN) in Paris, France, one month after collection. It was then transferred to 70% ethanol. To determine the precise intestinal location of the parasites, the small intestine (SI) was divided longitudinally into four equivalent sections (SI 1 to SI 4) numbered from the pylorus to the caecum. Nematodes were collected from each section and stored in 70% ethanol. They were examined as temporary mounts in lactophenol. The synlophe was studied following the method of Durette-Desset (1985) and the axis of orientation following that of Durette-Desset & Digiani (2005). The total number of cuticular ridges reported, is followed by the number of dorsal ridges and the number of ventral ridges in parentheses. The ridges were numbered from left to right, from 1 to n on the dorsal side, and from 1’ to n’ on the ventral side. The nomenclature used for the study of the caudal bursa follows Durette-Desset & Chabaud (1981) and Durette-Desset & Digiani (2012). The nomenclature for the parasites used above the genus follows Wilson & Reeder (2005).

RESULTS

**Heligmosomoides neopolygyrus Asakawa and Ohbayashi, 1986**

Material: 88 females and 84 males, MNHN 442MQ. Material studied: 13 males and ten females

Host: *Apodemus peninsulae* (Thomas, 1907) (Rodentia: Muridae: Murinidae).

Site in host: all specimens were found in SI 1.

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

- Redescription (Figs 1-16)

Small nematode coiled along ventral side having two to four sinistral spirals in males and four to eight in females. Deirids setiform, situated at level of excretory pore (Fig. 4), observed in one male and one female. Synlophe (studied in seven males and three females): in both sexes the cuticle bears longitudinal, continuous ridges without struts. Five ridges appear posterior to cephalic vesicle (Figs 2, 3, 5, 6), other ridges appear at different levels between cephalic vesicle and excretory pore; roughly equivalent numbers on dorsal and ventral sides (Figs 3, 5). Ridges disappear at about 130 anterior to caudal bursa in male, 110 anterior to caudal extremity in females. Number of ridges: at level of esophago-intestinal junction; 23, 26 (13D/13V see Fig. 7) in two males, 25, 26 (13D/13V, see Fig. 10) in two females; at mid-body, 24-27 (13D/13V, see Fig. 8) in seven males, 24-26 (13D/11V, see Fig. 11) in three females; within distal fifth, 25-27 (12D/14V, see Fig. 9) in three males, 25-31 (13D/14V, see Fig. 12) in three females. Left ventral ridges slightly larger than the other ridges. Axis of orientation sub-frontal directed from right to left (Figs 7-12).

Male: caudal bursa dissymmetrical with right lobe larger than left lobe (Fig. 14). Prebursal papillae well developed (Fig. 14). Caudal bursa pattern of type 2-3, for both lobes. Rays 3 thicker and longer than rays 2. In both lobes, rays 6, arising first from common trunk of rays 4-6. Rays 6 parallel to rays 8 and very close to them (Fig. 14). Rays 8, of similar length, arising at base of common trunk of rays 2-6. Extremities of rays 8 curved dorsally. Dorsal ray very small divided within distal third into two branches, each branch divided into two twigs, rays 9 (external branches) slightly shorter than rays 10 (internal branches) (Fig. 13). Rays 9 and 10 arising at same level as division of dorsal ray. Spicules poorly sclerotised, subequal, ending in sharp tip (Fig. 15). Gubernaculum absent. Genital cone, bearing two long papillae 7 on dorsal lip (Fig. 14). Papilla zero not observed. Measurements of 13 males are listed in Table 1.

Female: monodelphic. Vestibule very long. Tail rounded with caudal spine (Fig. 16). Measurements of ten females are listed in Table 1.

- Differential diagnosis

The specimens described above belong to the genus *Heligmosomoides* Hall, 1916 (Heligmosomoidea: Heligmosomidae), as redefined by Durette-Desset (1968), which is characterized mainly by longitudinal cuticular ridges, a poorly developed dorsal ray, a very long vestibule and long deirids. Asakawa (1988) divided the genus into five categories one of which was the “polygyrus line”. This line was proposed for *Heligmosomoides* parasitic only in Muridae (*Mus* and *Apodemus*) and is made up of *H. neopolygyrus* Asakawa and Ohbayashi, 1986 in *A. peninsulae* from Japan and three subspecies of *Heligmosomoides polygyrus*: *H. p. polygyrus* (Dujardin, 1845) in *Apodemus* spp. and rarely in *Mus musculus* from the Palearctic region (Eurasia,
Figs 1-16. – *Heligmosomoides neopolygyrus* Asakawa and Ohbayashi, 1986, in *Apodemus peninsulae*, from China: 1-6, male, anterior extremity, 1, right lateral view, 2-3, origin of cuticular ridges, 2, dorsal view, 3, right lateral view, 4, detail of excretory pore and deirids, ventral view, 5-6, origin of cuticular ridges, 5, left lateral view, 6, sub-ventral view; 7-12, transverse sections of body, 7-9, male, 7, at level of esophago-intestinal junction, 8, at mid-body, 9, within distal fifth, 10-12, female, 10, at level of esophago-intestinal junction, 11, at mid-body, 12, within distal fifth; 13-15, male, 13, dorsal ray with rays 9 and 10, ventral view, 14, caudal bursa, ventral view, 15, spicules, *in situ*, ventral view; 16, female, posterior extremity, right lateral view.

Scale bar: Figs 1-3, 5-6, 14, 16: 100 μm. Figs 4, 7-12, 15: 50 μm. Fig. 13: 20 μm. Abbreviations: de: deirids, r: right side, d: dorsal side, d.r.: dorsal ray, p.7: papillae 7. Transverse sections are oriented and numbered as in Fig. 7.
|                           | Asakawa and Ohbayashi, 1986 | This article |
|---------------------------|----------------------------|--------------|
|                           | Min-Max                    | Min-Max      |
| **Male**                  |                            |              |
| Number of specimens observed | 5                          | 13           |
| Total length              | 7,600-10,000               | 4,625-6,400  |
| Maximum width             | 150-200                    | 80-100       |
| Length of cephalic vesicle | 54-83                      | 40-65        |
| Width of cephalic vesicle | 37-56                      | 30-40        |
| Distance from nerve ring to anterior extremity | 188-223                   | 130-170      |
| Distance from deirids to anterior extremity | not observed | 210          |
| Distance from excretory pore to anterior extremity | 254-464                   | 195-390      |
| Length of esophagus       | 557-636                    | 465-650      |
| Ratio esophagus / total length | 6 to 7 %                   | 7 to 12 %    |
| Length of right spicule   | 510-640                    | 570-620      |
| Length of left spicule    | 510-640                    | 570-620      |
| Ratio spicule length/ Total length | 6 to 7 %                   | 7 to 12 %    |
| Gubernaculum              | no data                    | absent       |
| Number of ridges at midbody | 28-35                      | 24-27        |
|                           |                            |              |
| **Female**                |                            |              |
| Number of specimens observed | 5                          | 10           |
| Total length              | 17,500-27,300              | 9,600-11,500 |
| Maximum width             | 150-320                    | 70-150       |
| Length of cephalic vesicle | 51-76                      | 45-60        |
| Width of cephalic vesicle | 48-60                      | 35-40        |
| Distance from nerve ring to anterior extremity | 127-191                   | 115-170      |
| Distance from deirids to anterior extremity | n.o.                       | 220          |
| Distance from excretory pore to anterior extremity | 239-347                   | 210-300      |
| Length of esophagus       | 378-576                    | 530-685      |
| Ratio esophagus / Total length | 2 %                        | 3 to 7 %     |
| Length of caudal tip      | no data                    | 3-20         |
| Length of vagina vera     | no data                    | 12-25        |
| Length of tail            | 108-118                    | 70-115       |
| Distance from vulvar opening to tail | 315-410                   | 230-330      |
| Genital branch            |                            |              |
| Length of vestibule       | no data                    | 385-570      |
| Length of sphincter       | no data                    | 40-60        |
| Width of sphincter        | no data                    | 30-50        |
| Length of infundibulum    | no data                    | 130-240      |
| Length of uterine branch  | no data                    | 1,170-2,370  |
| Ratio uterus/ Total length | no data                    | 10 to 22 %   |
| Eggs                      |                            |              |
| Number of eggs            | no data                    | 8-45         |
| Length of eggs            | 76-87                      | 50-75        |
| Width of eggs             | 54-60                      | 30-50        |
| Number of ridges at midbody | 29-33                      | 25-26        |

Table I. – Comparison between measurements (μm) of Heligmosomoides neopolygyrus from Apodemus peninsulae in Japan (Asakawa & Ohbayashi, 1986) and from China (this article).

| Species                        | Asakawa & Ohbayashi (1986) | Right lobe | Left lobe | Rays 6 // to rays 8 | Direction of extremities of rays 6 | Comparative length of rays 9 and 10 |
|-------------------------------|---------------------------|------------|-----------|---------------------|-----------------------------------|-----------------------------------|
| Heligmosomoides p. polygyrus  | present                   | proximally | proximally | absent              | curved to dorsal ray               | 9 shorter 10                       |
| Heligmosomoides p. bakeri     | present                   | proximally | proximally | absent              | curved to dorsal ray               | 9 shorter 10                       |
| Heligmosomoides p. corsicus   | present                   | proximally | proximally | absent              | curved to dorsal ray               | 9 shorter 10                       |
| Heligmosomoides asakawae      | present                   | distally   | distally  | absent              | curved to rays 5                   | left 9 as similar size as 10; right 9 longer than ray 10 |
| Heligmosomoides neopolygyrus  | absent                    | proximally | proximally | present             | curved to rays 5                   | 9 shorter than 10 or as similar size as 10 |

Table II. – Morphological characters of which the four in columns 3 (a, b), 4, 5, 6 differentiate the five species of Heligmosomoides belonging to the “polygyrus line” defined by Asakawa, 1988.
The specimens described above have all the characters of *H. neopolygyrus* (Table II), but they can be differentiated by several elements from the type material described from Japan. Our specimens are about one third smaller, with smaller spicules but the spicule length/body length ratio is larger (7-12 % versus 6-7 %); the ventral cuticular ridges are slightly larger than the dorsal ones; the number of ridges at midbody in the males is 24-27 versus 28-35 and in the females is 24-26 versus 29-35 (Table I).

Durette-Desset (1968) and Durette-Desset *et al.* (1972) demonstrated that in *H. polygyrus*, a differentiation exists that is defined only by a relatively higher number of cuticular ridges in the posterior part of the body for an equivalent length of the body. These ridges are more numerous in specimens from Corsica (*H. p. corsicus*) and North America (*H. p. bakeri*) than in those from Europe (*H. p. polygyrus*). This difference is also present in *H. p. polygyrus* and *H. p. bakeri* from China and Japan.

Unfortunately, the number of Japanese specimens, in Asakawa & Ohbayashi, (1986) (five males, five females) is too small to be conclusive unlike the work by Durette-Desset *et al.* (1972) where numerous specimens were observed. Therefore, we prefer at least temporarily, to identify the Chinese specimens as *H. neopolygyrus* without considering them as a subspecies.

**DISCUSSION**

*H. neopolygyrus* was described for the first time by Asakawa & Ohbayashi (1986) in *A. peninsulae* in the Abashiri area of Hokkaido Island (Japan). Asakawa *et al.* (1990) recorded *H. neopolygyrus* in *A. agrarius* from China. In this work, only the dorsal ray and the base of rays 8 of one specimen collected in Shenyang were illustrated and showed that the swelling at the base of rays 8 was absent; a feature which is characteristic of *H. neopolygyrus*. For this reason, Asakawa *et al.* (1990) identified their specimens as *H. neopolygyrus*. In the same article, the authors reported finding *H. neopolygyrus* in *A. agrarius* from Kyonggido (Korean peninsula) but provided no description or illustrations. Asakawa (1991) confirmed the presence of *H. neopolygyrus* in *A. agrarius* from China and the Korean peninsula as well as in *A. peninsulae* from Japan.

Asakawa *et al.* (1992) reported *H. polygyrus* in *A. uralensis* (= *A. microps*) from Ulumuchi (China). However, this was, in fact, a new species later named *H. asakawae* by Tenora & Barus (2001).

Asakawa *et al.* (1993) studied the distribution of *H. neopolygyrus* in the east of China in *A. agrarius* and...
Figs 17-23. – Caudal bursae of *Heligmosomoides* spp., from China, ventral views: 17, 18, *H. p. polygyrus* in *Mus musculus*, 17, from Shenyang, 18, from Changchun; 19, 20, *H. neopolygyrus* in *Apodemus agrarius*, 19, from Shenyang, 20, from Changsha; 21-23, *Heligmosomoides incertae sedis*, 21, in *A. agrarius*, from Antu, 22, in *A. peninsulae*, from Liang-Cheng, 23, in *A. peninsulae*, from Hulin. After Asakawa *et al.* (1993) and modified.

Scale bar: Figs 17-23: 100 μm. Letters in brackets referred to Fig. 1 of Asakawa *et al.* (1993). Grey bars show level of divergence of rays 4 and 5 and arising of rays 6 on the common trunk of rays 4 to 6: Figs 17, 19, 20, 23, in both lobes rays 6 arise proximally to the level of divergence of rays 4 and 5; Fig. 18, in left lobe ray 6 arising proximally to the level of divergence of rays 4 and 5, in right lobe at same level as the divergence of rays 4 and 5; Fig. 21, in both lobes rays 6 arising slightly distally to the level of divergence of rays 4 and 5; Fig. 22, in both lobes, rays 6 arise at same level as the divergence of rays 4 and 5.
in *A. peninsulæ*. Moreover, they compared the morphological characteristics of rays 8 in *H. neopolygyrus* and in *H. p. polygyrus* from China. Although this article was in Japanese, the authors presented a map of eastern China, in which they included seven illustrations of caudal bursae in ventral view: two attributed to *H. p. polygyrus* (Figs 17-18) and five attributed to *H. neopolygyrus* (Figs 19-23) from the different provinces and different hosts. No measurements were provided. A detailed analysis of these caudal bursae using the known criteria and the new criteria provided in this study, allows us to conclude the recording of *H. neopolygyrus* in *A. peninsulæ* by Asakawa et al. (1993) as erroneous. The five caudal bursae identified as *H. neopolygyrus* all clearly lacked the swelling at the base of rays 8 which is characteristic of *H. polygyrus* and differentiates it from *H. neopolygyrus*. However, these species can be distinguished from each other by the following features: (1) the relative distance between rays 6 and 8; and (2) the level at which rays 6 arise compared to the level of divergence of rays 4 and 5 on their common trunk. We consider that only the caudal bursae of the specimens parasitic in *A. agrarius* from Shenyang (Fig. 19) and Changsha (Fig. 20) may be identified as *H. neopolygyrus* due to the absence of swelling at the base of rays 8, with rays 6 arising proximally to the level of divergence of rays 4 and 5, and with rays 6 and 8 being parallel and close to each other.

In the other three specimens (Figs 21-23) rays 6 and 8 are distant from each other, which differentiates them from *H. neopolygyrus*. In addition, in the caudal bursa of the specimen from *A. agrarius* from Shenyang (Fig. 21), rays 6 arise just slightly distally to the level of divergence of rays 4 and 5 and in the one from Liang-Cheng (Fig. 22), rays 4-6 diverge at same level in both lobes. The specimen from Hulin (Fig. 23) is the only one with rays 6 arising proximally to the level of divergence of rays 4 and 5, as in *H. neopolygyrus*. In the absence of other features, particularly those of the synloph, it is not possible to attribute a specific name to these species and we consider them as *Heligmosomoides incertae sedis* belonging to the “polygyrus line”. Considering as partially inaccurate identifications of Asakawa et al. (1993), we report for the first time the identification of *H. neopolygyrus* in *A. peninsulæ* in Sichuan (central China).

Asakawa & Ohbayashi (1986) suggested that further studies may reveal the presence of *H. neopolygyrus* in *A. peninsulæ* from the Northeast Palearctic region. This work supports a widespread distribution of *H. neopolygyrus sensu lato* which seems to follow its main host *A. peninsulæ*. The presence of *H. neopolygyrus* in *A. agrarius* in China has been reported twice (Asakawa et al., 1990, 1993) (Fig. 24).

Despite the discovery of new criteria to differentiate the species of the “polygyrus” complex reported in China and Japan, their systematic position remains uncertain due to incomplete descriptions and does not allow us to use certain potentially differentiating characters such as the number of cuticular ridges in the posterior part of the body. Both molecular and morphological studies need to be undertaken to determine their systematic rank (species or subspecies).

Fig. 24. – Distribution of the species of the genus *Heligmosomoides*, belonging to the “polygyrus line” modified from Asakawa (1988) from China and Japan.
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