

**A new species of the genus *Ohbayashinema*  
(Nematoda, Trichostrongylina,  
Heligmosomoidea), parasite of *Ochotona*  
*daurica* (Ochotonidae, Lagomorpha)  
from Buriatia**

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**ABSTRACT**

*Ohbayashinema erbaevae* n. sp. (Nematoda, Trichostrongylina, Heligmosomoidea) is described from *Ochotona daurica* (Rodentia, Ochotonidae) (natural host) from Buriatia and *O. rufescens* (experimental host). The new species is close to *O. ochotonae* Durette-Desset, 1974, parasitic in *Ochotona macrotis* from Nepal and *O. abei* Fukumoto, Kamyia & Ohbayashi, 1986, parasitic in *Ochotona hyperborea* from Japan, by its caudal bursal pattern (rays 6 arising from the lateral trident before the separation of rays 5 and 6). It differs from the first species by its asymmetrical synlophes in relation to the axis of orientation of the cuticular ridges. It differs from the second one by a very different ratio of the length of the spicules to the length of the body (5 to 8% in *O. erbaevae* versus 15, 5 to 16% in *O. abei*). The definition of the genus *Ohbayashinema* is emended and a dichotomous key to the species is provided.

**KEY WORDS**

Nematoda,  
Trichostrongylina,  
Heligmosomoidea,  
Heligmosomidae,  
*Ohbayashinema erbaevae* n. sp.,  
Buriatia,  
Lagomorpha,  
Ochotonidae.

## RÉSUMÉ

Une nouvelle espèce du genre *Ohbayashinema* (Nematoda, Trichostrongylina, Heligmosomoidea) parasite d'*Ochotona daurica* (Ochotonidae, Lagomorpha) en Bouriatie.

*Ohbayashinema erbaevae* n. sp. (Nematoda, Trichostrongylina, Heligmosomoidea) est décrit chez *Ochotona daurica* (Rodentia, Ochotonidae) (hôte naturel) originaire de Bouriatie et chez *O. rufescens* (hôte expérimental). La nouvelle espèce est proche d'*O. ochotoniae* Durette-Desset, 1974, parasite d'*Ochotona macrotis* originaire du Népal et d'*O. abei* Fukumoto, Kamiya & Ohbayashi, 1986, parasite d'*Ochotona hyperborea* originaire du Japon, par son type de bourse caudale (côtes 6 naissant sur le trident latéral avant la séparation des côtes 5 et 6). Elle se distingue de la première espèce par un synopse asymétrique par rapport à l'axe d'orientation des crêtes cuticulaires. Elle diffère de la seconde par un rapport longueur des spicules sur longueur du corps très différent (5 à 8 % chez *O. erbaevae* contre 15,5 à 16 % chez *O. abei*). La définition du genre *Ohbayashinema* est amendée et une clé dichotomique des espèces est fournie.

## MOTS CLÉS

Nematoda,  
Trichostrongylina,  
Heligmosomoidea,  
Heligmosomidae,  
*Ohbayashinema erbaevae* n. sp.,  
Bouriatie,  
Lagomorphes,  
Ochotonidae.

## INTRODUCTION

The trichostrongyline parasites of Ochotonidae Thomas, 1897 (Lagomorpha) are relatively rare but are represented both by the Molineoidea, with the genera *Graphidiella* Olsen, 1948, *Murielus* Dikmans, 1939, and *Rauschia* Durette-Desset, 1979 and by the Heligmosomoidea (Heligmosomidae [Travassos, 1914]) with the genus *Ohbayashinema* Durette-Desset, 1974. According to Durette-Desset 1974, the latter genus is morphologically intermediate between the genus *Citellinema* Hall, 1916, a parasite of terrestrial Scuridae and the genera *Heligmosomoides* Hall, 1916 and *Heligmosomum* Raillet & Henry, 1909, mainly parasites of arvicolid rodents. Recent field work in Buriatia (CEI) allowed one of us to identify, in *Ochotona daurica*, a new species belonging to the genus *Ohbayashinema* and to show that the posterior branch of the female genital system is atrophied and not functional. This character reinforces the intermediary systematic position of the genus *Ohbayashinema* between the didelphic genus *Citellinema* and the monodelphic genera, *Heligmosomoides* and *Heligmosomum*.

## MATERIAL AND METHODS

### MATERIAL

#### *Natural hosts*

Seven *Ochotona daurica* (Pallas, 1776) were caught between 8 and 19 August 1997, in Buriatia and necropsied between 17 and 26 August, 1997. The intestinal parasites, cestodes and nematodes (Oxyurida and Trichostrongylina) were collected from the intestinal tract. Only the Trichostrongylina are studied in this paper.

#### *Experimental hosts*

Males of *Ochotona rufescens rufescens* Gray, 1842, reared parasite free between two and three months-old, originating from the Laboratoire de Pharmacologie et Toxicologie fondamentales, CNRS, Toulouse, France. This strain was established from Ochotonidae originating from Afghanistan (Puget 1973).

### METHODS

In the field, faeces from the seven pikas were collected daily and mixed in equal proportion with charcoal and vermiculite (Durette-Desset &

Cassone 1987). The mixture was placed in a Petri dish with mineral water (pH7) according to Brumpt's method (1922). One hundred ensheathed infective L3 were collected and transported to Paris. Two pikas (MNHN 90 and 91 MH) were infected orally with 50 larvae each. From the eggs of the parasites collected in the faeces of these two pikas, a strain was established and maintained by successive passages on *Ochotona r. rufescens*. This strain was used to describe the new species and for the realisation of its life cycle which will be treated in another paper. The reason of this choice was to preserve the material collected from the natural hosts since the study of the morphology damaged the specimens (sections of the body, dissection of the female ovejectors, harsh clearing of the specimens...).

The small intestine of the natural and experimental hosts were cut in eight parts of equivalent length, numbered from SI 1 to SI 8, from the duodenum to the caecum. Both nematodes collected in natural and experimental hosts were fixed and stored in 70% ethanol, studied in temporary mounts in water and, when necessary, cleared in lactophenol. Apical views and sections were mounted and studied in lactophenol.

The nomenclature used above the family-group is that of Durette-Desset & Chabaud (1993). The synopse was studied following the method of Durette-Desset (1985). The morphology of the synopse being complex, the following nomenclature was used to simplify its description in the oesophageal region of both males and females: the number of dorsal ridges comes first and is followed by the number of ventral ridges. For example, if there are four dorsal ridges and three ventral ridges, in the description we write "stage 4/3".

The nomenclature used for the study of the caudal bursa is that of Durette-Desset & Chabaud (1981).

The measurements are in micrometres except where otherwise stated. Type specimens were deposited in the Helminthological collections of the Muséum national d'Histoire naturelle, Paris, France (MNHN).

## SYSTEMATICS

Genus *Ohbayashinema* Durette-Desset, 1974

*Ohbayashinema erbaevae* n. sp.

(Figs 1-3)

TYPE MATERIAL. — Holotype ♂, allotype ♀, MNHN 41 MHa; 24 ♂♂, 34 ♀♀ paratypes, MNHN 41 Mhb (7<sup>th</sup> experimental generation, collected at D15 post infestation, 24.XI.1998).

ETYMOLOGY. — After the name of Dr M. Erbaeva, specialist of the paleontology of the Ochotonidae.

GEOGRAPHICAL ORIGIN. — Kudara Somon (107°E, 51°N), Buriatia.

HOST. — *Ochotona rufescens rufescens* (Gray, 1842), (Ochotonidae) (experimental host).

SITE. — Duodenum (SI 1).

VOUCHER MATERIAL. — 16 ♂♂, 32 ♀♀, 6 L4 (MNHN 1ME, 2 ME, 12 ME, 22 ME, 26 ME, 44 ME, 52 ME) from 2 ♂♂ and 5 ♀♀ *Ochotona daurica* (Pallas, 1776), originating from Kudara Somon (107°E, 51°N), Bouriatia. The worms collected in six pikas were located just behind the pylorus at the beginning of the duodenum and entangled like a ball of wool. In the pika (26 ME) parasitised by 1 ♂ and 6 L4, the distribution in the small intestine was the following: SI 2: 1 male, 2 L4; SI 3: 2 L4; SI 4: 2 L4.

## DESCRIPTION

Nematodes with marked sexual dimorphism, females being twice to three times longer than males. Anterior part of male coiled along ventral side with two to three sinistral rings. Entire body of female coiled with 16 to 20 very tight sinistral rings. Deirids rounded at their base, with very thin thread, about 10 to 12 long (Fig. 1D). Deirids always situated at about same level of excretory pore but varying in position (above, at same level, below excretory pore), not laterally but slightly displaced to dorsal side. Ratio of oesophagus length to body length greater in female (7% on average) than in male (3% on average). Separation between muscular and glandular oesophagus with varying visibility and situated at level of nerve ring when visible (Fig. 3B).

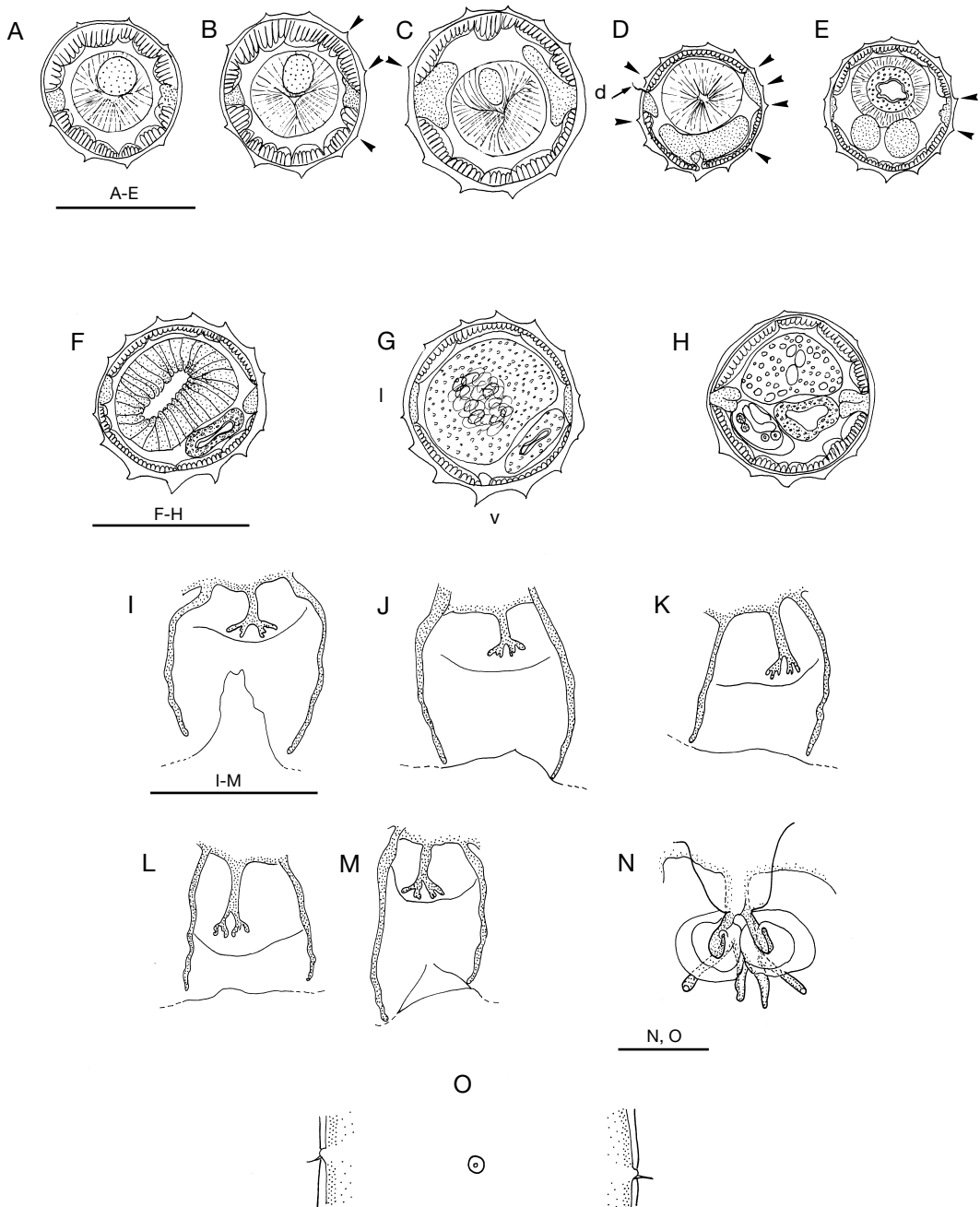


FIG. 1. — *Ohbayashinema erbaevae* n. sp. in *Ochotona r. rufescens*, male; **A-H**, male 10 mm long, transverse sections of the body; **A**, at 40  $\mu$ m from apex, stage 3/4; **B**, at 160  $\mu$ m from apex, stage 5/5; **C**, at 200  $\mu$ m from apex just before level of excretory pore, stage 6/5; **D**, at level of left deirid, stage 8/6; **E**, at oesophago-intestinal junction, stage 9/7; **F**, at mid-body (4.5 mm from apex), stage 7/7; **G**, at 3.8 mm above caudal bursa, stage 6/7; **H**, at 200  $\mu$ m from caudal bursa, stage 2/5; **I-M**, male, caudal bursae, different patterns of rays 8 and dorsal ray; **I-K**, dorsal views; **K**, holotype male; **L, M**, ventral views; **N**, genital cone and papillae 7, ventral view; **O**, detail of the excretory pore and the deirids, ventral view. All the sections of the body are orientated as G. Arrows indicate the origin of the new ridges. Abbreviations: **d**, deirid; **l**, left side; **v**, ventral side. Scale bars: A-E, 50  $\mu$ m; F-H, 100  $\mu$ m; I-M, 150  $\mu$ m; N, O, 30  $\mu$ m.

*Head*

Cephalic vesicle present, slightly assymetrical, dorsal side usually longer than ventral side. In apical view, oval-shaped buccal opening surrounded by small ring; absence of interno-labial papillae, presence of four externo-labial papillae, four cephalic papillae and two amphids. Dorsal oesophageal gland very clear in apical and lateral views (Fig. 3A, B).

*Synlophe*

Studied in two male and two female paratypes. In both sexes, body bearing continuous ridges. In transverse sections in median part of body, 14 ridges (stage 7/7) present in male (Fig. 1F), 13-14 (stages 7/6, 7/7) in female (Fig. 2E). Ridges appearing just before cephalic vesicle, stages being 3/4 in male (Fig. 1A) and 4/3 in female (Fig. 2A). Other ridges appearing at different levels between cephalic vesicle and end of oesophagus and reaching stages 9/7 in male (Fig. 1B-E) and 10/7 in female (Fig. 2B, C). New ridges appearing always on lateral sides, more numerous on right lateral side. About 100 below oesophagus, lateral ridges disappearing and becoming stage 7/7 (Fig. 1F).

Ridges disappearing just above caudal bursa in male, and progressively between level of distal posterior uterine branch and that of vulva, in female (Fig. 2F-J).

Dorsal ridges of similar size, smaller than ventral ones, difference more marked in female. Ventral ridges having double decreasing gradient of size from ventral to lateral size, therefore ventral median ridges always more developed; character very prominent in female (Fig. 2D-F).

Only lateral oesophageal ridges orientated perpendicularly to body wall, other ridges orientated from right to left side according to a frontal axis of orientation in male, sub-frontal in female. No ridges in front of lateral fields (Figs 1A-H; 2A-J).

*Holotype male*

9 mm long, width increasing gradually from 90 at level of oesophago-intestinal junction, to 130 at mid-body, then decreasing to 120 just above caudal bursa. Cephalic vesicle 75 (ventral side) 85

(dorsal side) long and 40 wide. Nerve ring, excretory pore and deirids at 190, 400 and 412 from apex, respectively. Oesophagus 580 long i.e. 6.4% of body length (Fig. 3B).

Caudal bursa assymetrical, of 2-3 type with right lobe larger (Fig. 3D). Prebursal papillae with long peduncle, situated on common trunk of rays 2 to 6 and not anterior to caudal bursa (visibility of papillae varying according to specimens). Rays 3 strongly developed. Rays 6 arising from lateral trident before separation of rays 4 and 5. Rays 8 slightly sinuous, not arising on dorsal ray but on common trunk of rays 2 to 6. Distance between extremities of dorsal ray and right ray 8 shorter than that of those between dorsal ray and left ray 8. Very short dorsal ray, divided into two branches in its distal third. Each branch divided into three branchlets (rays 9 [external], rays 10 and phasmids [internal]). In holotype (Fig. 1I) and four paratypes (Fig. 1J), rays 9 arising from dorsal ray at same level as division of latter. In five other paratypes, rays 9 arising after division of dorsal ray (Fig. 1K-M) According to specimens, phasmids (situated between rays 9 and 10) may be visible. Dorsal ray enclosed in membrane reaching rays 8. Thin, alate spicules, 530 long, i.e. 5.9% of body length. Chitinoïd axis of each spicule doubled for almost whole length of spicule. Only distal extremities single, 15 long and sharp. In dissected spicules, tips not joined (Fig. 3D'). Well-developed genital cone, bearing on dorsal lip, leaf-shaped papillae 7 with extremity ventrally curved and enclosed in membrane (Fig. 1N). Papilla 0 not observed.

Measurements of nine paratype males given in Table 1.

*Allotype female*

21.8 mm long, width increasing gradually from 120 at level of oesophago-intestinal junction to 200 at mid-body, 250 at level of median part of uterine branch, then decreasing to 210 below level of vulvar opening. Cephalic vesicle 100 (ventral side) 110 (dorsal side) long and 50 wide. Nerve ring, excretory pore and deirids at 250, 560 and 575 from apex, respectively. Oesophagus 780 long i.e. 3.5% of body length.

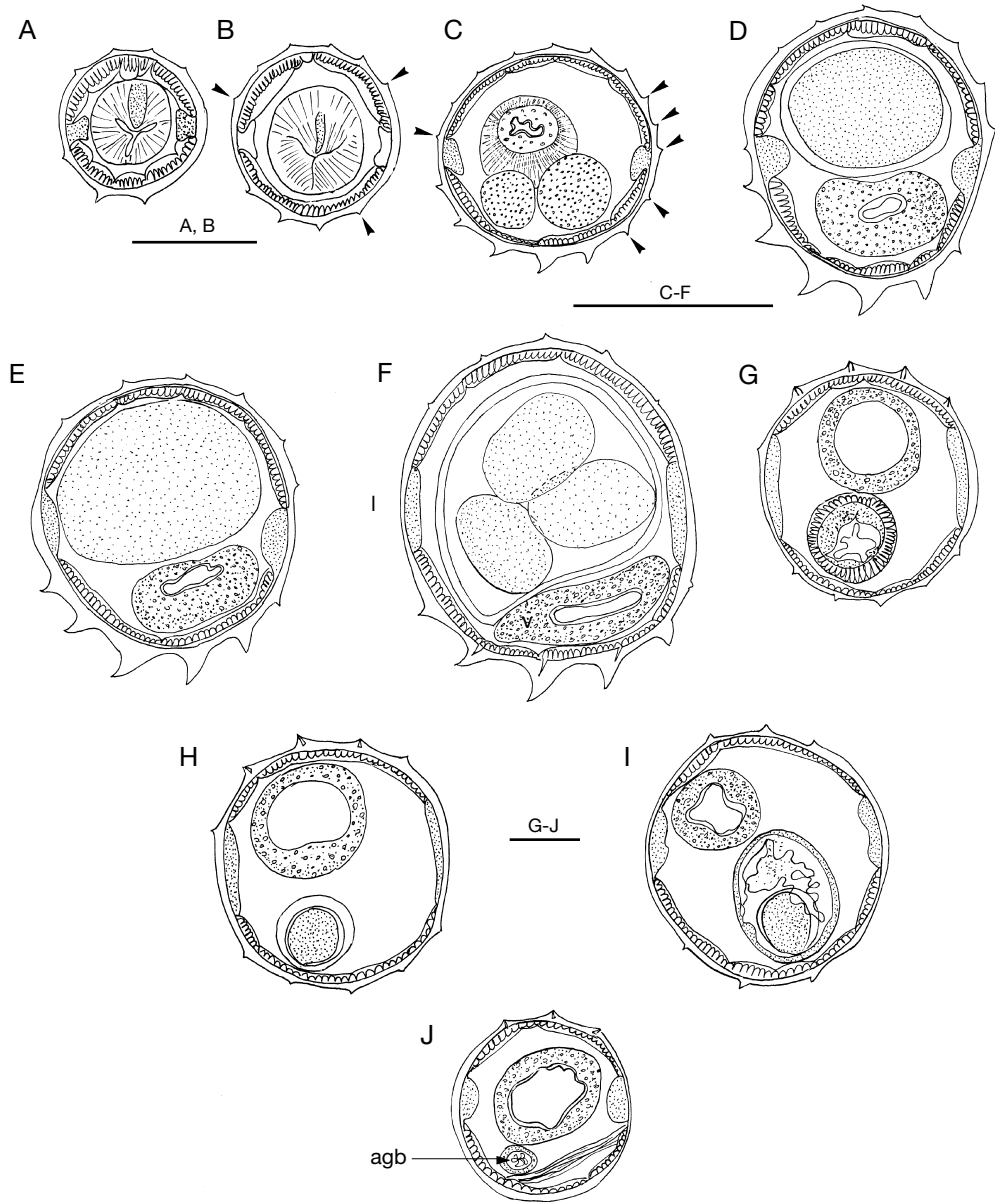


FIG. 2. — *Ohbayashinema erbaevae* n. sp. in *Ochotona r. rufescens*, female 24.9 mm long, transverse sections of the body; **A**, just behind cephalic vesicle, stage 4/3; **B**, at level of the nerve ring, stage 6/4; **C**, at level of the oesophago-intestinal junction, stage 10/7; **D**, at 5.7 mm from apex, stage 7/7; **E**, at mid-body (11.4 mm from apex), stage 7/6; **F**, at the level of the proximal uterine branch, stage 5/6; **G**, at the junction between the median and the distal parts of the vestibule, stage 5/5; **H**, at the level of the median thin part of the vestibule, stage 5/5; **I**, at the level of the proximal part of the vestibule, stage 3/3; **J**, at the level of the posterior atrophied uterine branch, stage 3/0. All the sections of the body are orientated as in **F**. Arrows indicate the origin of the new ridges. Abbreviations: **agb**, atrophied genital branch; **l**, left side; **v**, ventral side. Scale bars: A, B, 50 µm; C-F, 150 µm; G-J, 75 µm.

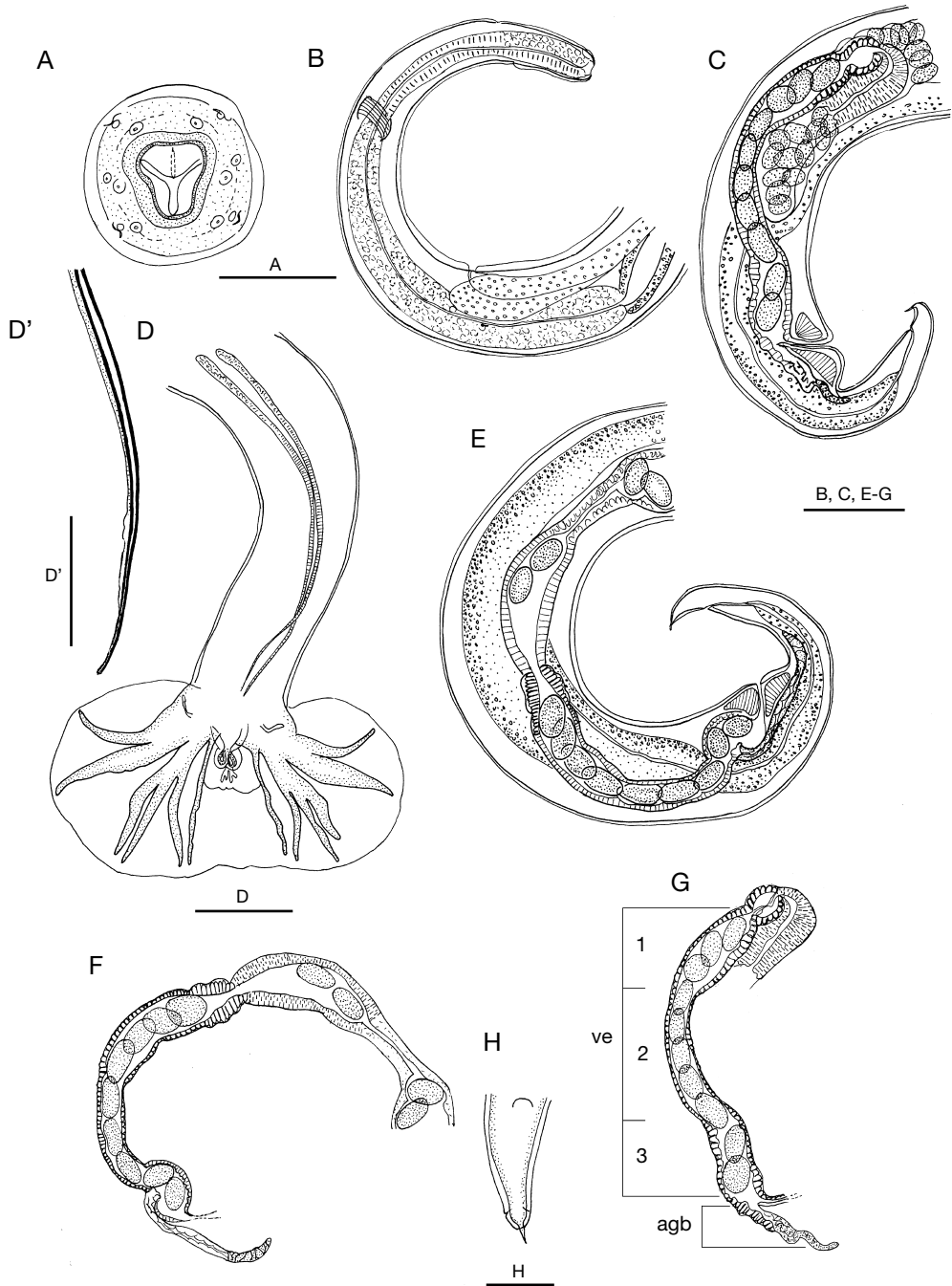


FIG. 3. — *Ohbayashinema erbaevae* n. sp. in *Ochotona r. rufescens*; **A**, female, head, apical view; **B**, male, anterior extremity, right lateral view; **C**, female, posterior extremity, right lateral view; **D**, male, caudal bursa, ventral view; **D'**, detail of the tip of a spicule; **E**, other female, posterior extremity, right lateral view; **F**, female E, detail of the ovejector with bulbous distal part of the vestibule and the atrophied posterior branch; **G**, female C, detail of the ovejector with enlarged distal part of the vestibule and the atrophied posterior branch; **H**, female caudal extremity and tip, ventral view. Abbreviations: **ve**, vestibule; **1**, enlarged proximal part; **2**, thin rectilinear median part; **3**, shorter distal part; **agb**, atrophied genital branch. Scale bars: A, 30 µm; B, C, E-G, 200 µm; D, 150 µm; D', 50 µm; H, 100 µm.

Monodelphic with atrophied, non functional, posterior genital branch, 180 long. Vulvar opening 410 from posterior extremity. *Vagina vera* 60 long, directed anteriorly obliquely. Ovejector with vestibule 560 long, sphincter 60 long and 65 wide, infundibulum 200 long. Vestibule divided into three distinct parts: enlarged proximal part, thin rectilinear median part of equivalent length to proximal part, shorter distal part either strongly swollen, bulbous (allotype and seven paratypes) (Fig. 3E, F) or only enlarged like proximal part (two paratypes) (Fig. 3C, G). Infundibulum usually straight, directed anteriorly in continuity with sphincter and vestibule

(Fig. 3E), or directed posteriorly in one paratype (Fig. 3C). Uterus 4.5 long, i.e. 20.6% length of body, with numerous eggs (over 200), morula stage, 80 long and 50 wide. In three paratype females, presence of a vulvar "plug". Tail 100 long with caudal spine 10 long (Fig. 3H). Main measurements of nine paratype females given in Table 1.

## DISCUSSION

### DIAGNOSIS

The genus *Ohbayashinema* (Heligmosomidae) was created by Durette-Desset (1974) in order to classify certain parasites of Ochotonidae, morphologically close to the genera *Heligmosomoides* Hall, 1916 and *Heligmosomum* Railliet & Henry, 1909. The three known species of *Ohbayashinema* are all parasites of *Ochotona* spp. from the Old World. *O. dubinini* (Gvodev, 1966) parasitic in *Ochotona alpina* from Russia (Altai); *O. ochotonae* Durette-Desset, 1974, parasitic in *Ochotona macrotis* from Nepal and *O. abei* Fukumoto, Kamiya & Ohbayashi, 1986, parasitic in *Ochotona hyperborea* from Japan. A fourth species, *Heligmosomum mongolicum* (Danzan, 1976), parasitic in *Ochotona daurica* from Mongolia, possesses a caudal bursa morphologically identical with that of *O. dubinini*, with rays 4 arising from the lateral trident before the separation of rays 5 and 6. It could be a synonym of *O. dubinini*. However, its synlophe, one of the most important differential characters, is unknown. Therefore the systematic position of *H. mongolicum* remains unclear until a redescription including the characteristics of the synlophe is available. We propose that *H. mongolicum* is actually a member of *Ohbayashinema*. However, the species is not included in the key. The specimens from Buriatia share the following characteristics with the four species cited below: – synlophe with number of ridges at mid-body less than 15; axis of orientation frontal or sub-frontal with ventral ridges having a medio-lateral double gradient of size; absence of ridges in front of the lateral fields;

TABLE 1. — Measurements ( $\mu\text{m}$ ) of adults of *Ohbayashinema erbaevae* n. sp. from experimentally infected *Ochotona r. rufescens*. Mean of 9 paratypes  $\pm$  SD.

Male worms	
Total length	8811 $\pm$ 1603
Width at mid-body	124 $\pm$ 16
Cephalic vesicle: length $\times$ width	77 $\pm$ 9 $\times$ 40 $\pm$ 0
Distance from nervous ring to apex	206 $\pm$ 30
Distance from deirids to apex	417 $\pm$ 64
Distance from excretory pore to apex	431 $\pm$ 70
Length of oesophagus	620 $\pm$ 70
Length of oesophagus / total length	7% $\pm$ 2%
Length of spicules	541 $\pm$ 30
Female worms	
Total length	24200 $\pm$ 1582
Width at mid-body	162 $\pm$ 32
Cephalic vesicle: length $\times$ width	89 $\pm$ 11 $\times$ 53 $\pm$ 4
Distance from nervous ring to apex	246 $\pm$ 22
Distance from deirids to apex	544 $\pm$ 73
Distance from excretory pore to apex	513 $\pm$ 50
Length of oesophagus	735 $\pm$ 51
Length of oesophagus / total length	3% $\pm$ 0%
Length of <i>vagina vera</i>	72 $\pm$ 14
Distance from vulvar opening to posterior extremity	429 $\pm$ 33
Length of tail	95 $\pm$ 11
Anterior branch of genital apparatus	
Length of vestibule	581 $\pm$ 63
Sphincter: length $\times$ width	72 $\pm$ 5 $\times$ 64 $\pm$ 5
Length of infundibulum	263 $\pm$ 43
Length of uterus	4300 $\pm$ 699
Eggs number	125 $\pm$ 26
Eggs: length $\times$ width	76 $\pm$ 5 $\times$ 45 $\pm$ 4
Posterior atrophied branch	204 $\pm$ 27



- caudal bursa with very long papillae 1, arising at base of rays 2;
- monodelphic female with an atrophied posterior genital branch (see Remarks below);
- ovejector divided into three distinct parts, an enlarged proximal part, strongly muscular only in the part adjacent to the sphincter, a cylindrical narrow median part with only one row of eggs and a very swollen distal part, very often bulbous.

#### REMARKS

Amongst the species already described, only the female of *O. abei* is known and the authors of this species do not mention the presence of an atrophied posterior genital branch. This is perhaps due to the fact that this character is very difficult to detect since the specimen needs to be very well-cleared. Thus we believe that this character is present in all the species and is characteristic for the genus *Ohbayashinema* as is the case in some other genera of Trichostrongylina. For example, the 19 species of the genus *Moennigia* Travassos, 1935, parasites of marsupials in South America, have an atrophied genital branch varying in degree of reduction according to the specimens (Durette-Desset 1970).

The caudal bursa of the different species are morphologically closely related and the study of males in the material from Buriatia has revealed the great variability (in shape and length) of rays 8 and 9 (Fig. 11-M). Therefore, only one character remains constant and allows us to divide the species into two groups. In the first group, rays 4 arise from the lateral trident before the separation of rays 5 and 6. *O. dubinini* belongs to this group. In the second group, rays 6 arise from the lateral trident before the separation of rays 4 and 5. *O. ochotonae*, *O. abei* and the specimens described below belong to this group.

*O. ochotonae* is differentiated from the Buryat specimens by 10 ridges at mid-body *versus* 14; by the equivalent size of dorsal and ventral ridges and by the presence of a double gradient of size of the ridges also on dorsal side.

*O. abei* is differentiated by having spicules about three times longer and a very different ratio of the length of the spicules to the length of the body:

15.5, 16% in *O. abei* *versus* 5-4 to 8% in the Buryat specimens.

We consider that the specimens from Buriatia belong to a new species which we have named *Ohbayashinema erbaevae* n. sp.

#### REDEFINITION OF THE GENUS *OHBAYASHINEMA*

The erection of the genus *Ohbayashinema* was mainly based on the presence of a symmetrical axis of orientation of the ridges in relation to the frontal axis, passing through the lateral fields. This character is not present either in *O. abei* or *O. erbaevae*, whose synlophe is described. In addition, since the morphology of the female is now well-known, it has become necessary to amend the definition of the genus *Ohbayashinema*.

#### Genus *Ohbayashinema* Durette-Desset, 1974

TYPE SPECIES. — *O. ochotonae* Durette-Desset, 1974, by original designation.

OTHER SPECIES. — *O. abei* Fukumoto, Kamiya & Ohbayashi, 1986; *O. dubinini* (Gvozdev, 1966) Durette-Desset, 1974 = *Heligmosomum dubinini* Gvozdev, 1966; *O. erbaevae* n. sp.

#### DESCRIPTION

Synlophe with less than 15 ridges and axis of orientation of the ridges sub-frontal or frontal; presence of a medio-lateral gradient of ridges at least on ventral side; absence of ridges in front of lateral fields; caudal bursa with papillae 1 arising at the base of rays 2; chitinous axis of spicules doubled in their median part; monodelphy with atrophied posterior genital branch; setiform deirids. Parasites of Ochotonidae in the Old World.

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DICHOTOMOUS KEY OF THE SPECIES

- 1 (2). Rays 4 arising from the lateral trident before the separation of rays 5 and 6. Parasite of *Ochotona alpina* from Russia (Altai) ..... *O. dubinini* (Gvozdev, 1966)
- 2 (1). Rays 6 arising from the lateral trident before the separation of rays 4 and 5.
- 3 (4). Synlophe symmetrical in relation to the axis of orientation. Parasite of *Ochotona macrotis* from Nepal ..... *O. ochotonae* Durette-Desset, 1974
- 4 (3). Synlophe asymmetrical in relation to the axis of orientation with some ventral ridges hypertrophied.
- 5 (6). Ratio of the length of the spicules to the length of the body: 15, 5-16%. Parasite of *Ochotona hyperborea* from Japan ..... *Ochotona abei* Fukumoto, Kamiya & Ohbayashi, 1986
- 6 (5). Ratio of the length of the spicules to the length of the body: 5.5-8%. Parasite of *Ochotona daurica* from Buriatia ..... *Ochotona erbaevae* n. sp.

Pharmacologie et Toxicologie fondamentales, CNRS, Toulouse) who provided the experimental hosts. We also thank Mr Pierre Desset who helped us in the field.

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