JOHNPEARSONIA GEN. NOV. AND JOHNPEARSONIINAE SUBF. NOV. (MOLINEOIDEA, NEMATODA) FROM *BUFO MARINUS*, WITH COMMENTS ON THE PRIMITIVE TRICHOSTRONGYLE PARASITES OF AMPHIBIANS AND REPTILES.

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Summary :

A new genus is proposed in the family Amphibiophilidae based on specimens parasitic in *Bufo marinus* from Australia : *Johnpearsonia*. The species *Amphibiophilus egerniae* Johnston and Mawson, 1947, parasite of Australian lizard, is classified in this genus. The only genus that it is close to is *Batrachonema* Yuen, 1965, parasitic in malayian Ranidae and peruvian Bufonidae. The two genera were classified in a new subfamily : the Johnpearsoniinae mainly characterised with a bursa of the 1-3-1 type. *Johnpearsonia* is differentiated from *Batrachonema* in that the female is monodelphic and the dorsal lobe of the caudal bursa is not reduced. An analysis of the relationships between the other primitive genera of Trichostrongylina parasitic in poikilothermic animals is done.

KEY WORDS : Nematoda, primitive Trichostrongylina, Molineoidea, Johnpearsoniinae subf. nov. *Johnpearsonia* gen. nov. poikilothermic vertebrates Australia.

MOTS CLES : Nematodes, Trichostrongyles primitifs, Molineoidea Johnpearsoniinae nov. sub. fam. *Johnpearsonia* nov. gen. Vertébrés poikilothermes, Australie.

Résumé

JOHNPEARSONIA NOV. GEN. ET *JOHNPEARSONIINAE* NOV. SUBF. (MOLINEOIDEA, NEMATODA) PARASITE DE *BUFO MARINUS*; REMARQUES SUR LES TRICHOSTRONGYLES PRIMITIFS PARASITES D'AMPHIBIENS ET DE REPTILES.

Description d'un nouveau genre, *Johnpearsonia*, parasite de *Bufo marinus* et d'Amphibiens endémiques en Australie. *Amphibiophilus egerniae* Johnston et Mawson, 1947, parasite d'un lézard australien, est classée dans ce genre. Le seul genre proche, *Batrachonema* Yuen, 1965 est parasite de Ranidae en Malaisie et de Bufonidae au Pérou. La nouvelle sous-famille des Johnpearsoniinae appartenant à la famille des Amphibiophilidae est créée pour ranger ces deux genres. Cette sous-famille est principalement caractérisée par une bourse caudale de type 1-3-1. *Johnpearsonia* se différencie de *Batrachonema* par une femelle monodelphe et un mâle dont le lobe dorsal de la bourse caudale n'est pas réduit.

Les relations existant entre les autres genres primitifs de Trichostrongylina parasites de Vertébrés poikilothermes sont analysées.

INTRODUCTION

A new Molineoid nematode species (Trichostrongylina) was found in north-eastern Australia, in endemic amphibians and in the introduced *Bufo marinus*. The new species has similarities with *Amphibiophilus egerniae*, Johnston and Mawson, 1947, a parasite of Australian lizards. Together these two species differ from the other primitive genera known to occur in poikilothermic animals and a new genus is created for them. An analysis of the relationships between the different genera known at the present time permits a new classification which reflects the phylogeny of the group.

MATERIAL AND METHODS

Toads and native amphibians were collected from various locations throughout Queensland, Australia, and dissected within 2 days of capture. Dissection followed a fatal dose of "Lethabarb" (Euthanasia injection Pentobarbitone

solution) sprinkled onto the skin. A ventral incision was made from the pelvic to the pectoral girdle exposing the body cavity. The stomach, intestine and rectum were removed and placed in 0.85% NaCl solution, opened, and any nematodes removed. Worms were fixed in Berland's fluid (Gibson, 1979) and transferred to 70% ethanol for storage.

Morphological terms to describe the bursal rays follow Durette-Desset and Chabaud, 1981 and Durette-Desset, 1985 for the synlophe. Measurements are in micrometers.

Type specimens have been deposited in the collections of the South Australian Museum, Adelaide, Australia (SAM). Voucher specimens have been deposited in the collections of the South Australian Museum (SAM), the Museum of Paris, France (M.N.H.N) and the Queensland Museum, Brisbane, Australia (QM).

RESULTS

A - DESCRIPTIONS

JOHNPEARSONIA PEARSONI, SP. NOV.

Type material : holotype male, allotype female, MNHN 676 MDa, paratypes : 1 male, 1 female, 3 L4 males, 2 L4 females, MNHN 676 MDB from small

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intestine of *Bufo marinus* (Bufonidae). Coen, Queensland. Australia.

Voucher material : from small intestine of the following hosts : 26 *Bufo marinus* (Bufonidae) from Cape Weymouth, Townsville, Bentley, and Mountain View Road near Townsville (Nov. 89 to May 91) and 7 native amphibian species : 2 *Limnodynastes ornatus* (Myobatrachidae) from Coen and Bentley (Nov. and Dec 89), 2 *Cyclorana novaehollandiae* (Hylidae) from Coen and Townsville (Nov. 89 and Feb.90), 1 *Litoria alboguttata* (Hylidae) from Townsville (Dec 89), 1 *Uperoleia lithomoda* (Myobatrachidae) from Townsville (Dec 89), 1 *Notaden melanoscopus* (Leptodactylidae) from Townsville (Feb 92). Queensland. Australia.

ADULTS

Small straight nematodes or with anterior end completely coiled. Excretory pore variable, always posterior to oesophago-intestinal junction; minute triangular-shaped deirids at same level as excretory pore (figs.1,5). Excretory glands well developed.

Head (figs. 2,3,4) : Cephalic vesicle long (110 in male, 130 in female), buccal capsule well developed; dorsal oesophageal tooth present, generally smaller in male (10-13) than in female (10-15). Absence of ring at base of buccal capsule. In apical view, 6 lips separated by 6 interlabia, 6 internal labial papillae, 6 external labial papillae of which lateral labial papillae have shared peduncle with 2 amphids and 4 cephalic papillae. Mouth hexagonal in shape.

Synlophe (studied in 6 males and 5 females) (figs.6 to 9, 11 to 15) : In both sexes, longitudinal cuticular ridges present, non-interrupted, with rounded ends; slight reinforcement of ridges visible in middle part of body in some female specimens.

Ridges appear at base of cephalic vesicle and disappear, in male, at anterior ends of spicules, and in female between level of sphincter and *vagina vera*. In vulvar region, 2 mammilli form ventral alae present; 9 high in transverse section. Left ala (130 long), begins at level of vulva; right ala (150 long) begins about 10µm posterior to it. Ridges orientated perpendicular to body surface, regularly spaced; more widely spaced opposite to median fields.

Male holotype (figs. 16 to 22) : 5300 long and 100 wide in mid-body; cephalic vesicle 110 long by 50 wide; buccal capsule 11 long. Nerve ring, excretory pore and cervical papillae 190, 470, and 470 respectively from anterior end. Oesophagus 325 long.

Bursa of the type 1-3-1, very slightly asymmetric, extended transversely. Rays 5 robust, rays 6 small. Rays 8, narrow, beginning at root of dorsal ray.

Dorsal ray relatively long, divided into pairs of small terminal pointed branches. Spicules narrow, 400 long; alae start 250 from proximal end; each spicule terminates in 3 points enclosed within membrane : externolateral branch, ventral branch 70 long which appears on externolateral branch and dorsal branch. Gubernaculum 90 long by 18 wide. Genital cone rounded, 30 long by 40 wide proximally, papillae zero and papillae 7 elongated; papillae 7 bifid at tip.

Female allotype (fig. 10) : 8700 long by 120 wide in mid-body. Cephalic vesicle 130 long by 50 wide. Buccal capsule 12 long. Nerve ring, excretory pore and cervical papillae 220, 590 and 590 respectively from anterior end. Oesophagus 380 long.

Monodelphic, with vestigial posterior genital branch, reduced to a cellular mass, 80 long. Vulva 240 from posterior end. *Vagina vera* 55 long, oblique. Anterior genital branch with ovejector consisting of vestibule 195 long, sphincter 40 long and infundibulum 85 long. Uterus 2240 long containing 68 eggs in morula stage, 65 long by 35 wide. Tail 80 long by 30 wide at level of anus, with caudal spine 20 long.

4 TH LARVAL STAGE

Head (figs. 23, 24, 25) : Absence of cephalic vesicle; small buccal capsule present. In apical view, 6 lips, 6 external labial papillae, 4 cephalic papillae and 2 amphids. Mouth hexagonal; oesophageal tooth absent.

Synlophe (fig. 26) : 2 small lateral ridges with an internal support, orientated perpendicular to body surface.

Male (figs. 27, 28) : Length 2170 width 50 nerve ring, excretory pore and deirids 120, 245 and 245 respectively from anterior end; parts of the genital system well differentiated : testis 460, seminal vesicle 270, deferent canal 270.

Female (fig.29) : Length 2720, width 50 nerve ring, excretory pore and deirids 145, 330 and 330 respectively from anterior end. Vulva 190 from caudal extremity. Anterior genital branch 3 times larger than posterior branch. Latter does not develop but remains visible in adult in vestigial form. Tail, 90 long.

REMARKS

The species does not belong to any of the genera already known in amphibians and reptiles. We regard it as the type species of the new genus *Johnpearsonia pearsoni* named in honour of our colleague Prof. J. C. Pearson from the University of Queensland.

Figs. 1-22. – *Johnpearsonia pearsoni* n.gen., n.sp. from *Bufo marinus*. Adults. 1, anterior part of female, left lateral view; 2, head of female, dorsal view; 3, 4, head of female, apical view, showing the 6 lips and the interno labial papillae then the oesophagal tooth and the other papillae; 5, detail of excretory pore and deirids of female, ventral view; 6-9, synopse. 6, male, at the oesophago intestinal junction; 7, male, at the middle of the body; 8, female, at the middle of the body; 9, female, at level of the ovejector; 10, ovejector and tail of female, left lateral view; 11-14, anterior part of female showing the appearance of cuticular ridges. 11, left lateral view, 12, ventral view, 13, dorsal view, 14, right lateral view; 15, tail of female showing the vulvar lateral alae; 16-18, right spicule, ventral, externo-lateral and dorsal views, 19, 20, gubernaculum, right lateral and ventral view; 21, genital cone, ventral view; 22, caudal bursa of the male, ventral view.

Cross sections of the body are orientated as figure 7

a = amphid, ilp = interno-labial papilla, elp = externo-labial papilla, c = cephalic papilla, d = dorsal, v = ventral, r = right, l = left

Scale lines : 1-10, 17-21 : 20µm; 11-15 : 100µm; 16 : 50µm; 22 : 40µm.

Figs. 23-29. – *Johnpearsonia pearsoni* n.gen., n.sp. from *Bufo marinus*. Fourth larval stages. 23, anterior part of female, right lateral view; 24, head of female, lateral view; 25, head of male, apical view; 26, synophe of male in midbody; 27, dissected genital apparat of male, showing the testicule, the seminal vesicle and the deferent canal; 28, tail of male; 29, tail and genital apparat of female, showing the developed anterior genital branch and the atrophied posterior genital branch, right lateral view.

Figs. 30-32. – *Wanaristrongylus ctenoti* from *Ctenotus grandis*. 30, 31, head of male, left lateral view, showing the dorsal oesophageal tooth and apical view, showing the 6 lips, the 6 interno labial papillae, the 6 externo labial papillae, the 2 amphids and the 4 cephalic papillae; 32, head of female, left lateral view.

Scale lines : 23, 29 : 50µm ; 24, 28 : 30µm ; 25, 26 : 10µm ; 27 : 70µm ; 30-32 : 20µm.

JOHNPEARSONIA EGERNIAE
(JOHNSTON & MAWSON, 1947), COMB. NOV.

Synonymy : *Amphibiophilus egeriae* Johnston & Mawson, 1947, p. 23, figs. 1-3

Material examined : 2 males, 2 females, (SAM n° 8667) from the small intestine of *Egernia striata* (Scincidae) ; 5 females (SAM n° 8666) from the small intestine of *Egernia kintorei* (Scincidae), Itavi Rock, Musgrave Range, South Australia.

ADULTS

Small nematodes, loosely coiled. Excretory pore and cervical papillae posterior to oesophago-intestinal junction (fig. 35).

Head (figs. 33, 34) : Cephalic vesicle elongate (80 in male, 82-90 in female), buccal capsule well developed, dorsal oesophageal tooth present, smaller in male (9) than in female (10-12). Absence of a ring at base of buccal capsule. In apical view, 6 lips separated by 6 interlabia; internal labial papillae not observed; 6 external labial papillae, 2 amphids and 4 cephalic papillae. Mouth hexagonal in shape.

Synophe (studied in one male and one female) (figs. 36, 37) : In both sexes, uninterrupted longitudinal cuticular ridges not with rounded apices. Ridges appear at base of cephalic vesicle and disappear about 400 anterior to bursa in male, and anterior to anus in female. In both sexes, 12 ridges in mid-body;

Figs. 33-43. – *Johnpearsonia egerniae* (Johnston and Mawson, 1947) n. comb. from *Egernia striata*. 33, 34, head of male, right lateral view then apical view; 35, detail of excretory pore and deirids in male, ventral view; 36, 37, synlophe at mid body of male then female; 38, ovejector and tail of female, right lateral view; 39, tail of female showing the two ventral tubercles, right lateral view; 40, dissected spicula, ventral view; 41, tip of the right spicule, dorsal view; 42, gubernaculum, ventral view; 43, caudal bursa, ventral view.

Cross sections of the body are orientated as figure 36

d = dorsal, v = ventral, r = right, l = left

Scale lines : 33, 35, 39 : 20µm; 34 : 25µm; 36, 37 : 50µm; 38 : 100µm; 40-42 : 10µm; 43 : 40µm.

4 dorsal, 6 ventral, 1 right lateral, 1 left lateral. Ridge situated opposite each lateral cord and adjacent ventral crest orientated perpendicular to body surface; other crests orientated slightly from right ventral direction towards left dorsal, axis of orientation inclined 35°-40° from frontal axis. Ridges unequal in size and irregularly spaced. Vulvar alae absent in female.

Male (figs. 40, 41, 42, 43) : Length 4200, 65 wide in mid-body; nerve ring, excretory pore and cervical papillae 175, 360 and 345 respectively from anterior end. Oesophagus 300 long.

Bursa of the 1-3-1 type, very slightly asymmetrical, spread transversely. Rays 5 and 6 robust. Rays 8 narrow, beginning at root of dorsal ray. Dorsal ray relatively long, divides into 2 small branches at distal extremity. Spicules narrow, alate, 160 long, divided into 2 branches at distal extremity, one ventral, one dorsal : dorsal branch slightly curved in internal view, bifurcated in dorsal view. Gubernaculum 30 long, 7 wide. Genital cone triangular in shape, bearing on ventral lip elongated papilla zero and on ventral lip, 2 finger-shaped papillae 7.

Female (figs. 38, 39) : Length 5900, 80 wide in mid-body; nerve ring, excretory pore and cervical papillae 185, 390 and 380 situated from anterior end respectively. Oesophagus 345 long.

Monodelphic; vestigial posterior branch not observed; vulva 135 from posterior end; *vagina vera*, well developed, 32 long; ovejector with vestibule, sphincter and infundibulum 85, 35 and 90 long, respectively. Uterus 1000 long, containing 28 embryonated eggs, 65 long by 35 wide. Tail 53 long with caudal spine 15 and two ventral tubercles 5 in height.

REMARKS

The species differs from *Amphibiophilus* in having a bursa of 1-3-1 type as opposed to 2-3 type, a reduced non-hypertrophied dorsal lobe and a monodelphic rather than didelphic female.

In all these characteristics it resembles the preceding species. The two species can be differentiated by the synlophe, the size of the rays 6 in relation to rays 5; the shape of the spicules and of the gubernaculum and the presence or absence of vulvar alae. We therefore regard the specimens from *Egernia* as a second species in the genus *Johnpearsonia*.

B – DEFINITIONS

JOHNPEARSONIINAE SUBF. NOV.

Amphibiophilidae. Bilaterally symmetrical synlophe in the larva but not always in adult; bursa of the 1-3-1 type; female didelphic or monodelphic with caudal spine. Parasitic in Gondwanian amphibians. Type-

genus : *Johnpearsonia* gen. nov. Other genus *Batrachonema* Yuen, 1965.

JOHNPEARSONIA GEN. NOV.

Head with 6 lips, well developed buccal capsule and a strong dorsal oesophageal tooth. Absence of a ring at the base of the buccal capsule. Bilaterally symmetrical larval synlophe. Adult synlophe bilaterally symmetrical or with an oblique axis. Bursa of the 1-3-1 type with dorsal lobe neither hypertrophied nor reduced. Rays 8 of bursa beginning at the root of the dorsal ray. Spicules with two or three points. Gubernaculum present. Female monodelphic with caudal spine.

Type species : *J. pearsoni*, sp. nov., parasitic in Australian amphibians. Other species : *J. egerniae* (Johnston and Mawson, 1947), comb. nov. parasitic in Australian lizards.

DISCUSSION

Among Trichostrongyline which parasitize amphibians or reptiles, a group of species can be readily identified as relicts. The presence of six lips, the well developed buccal capsule and the large oesophageal tooth indicate a transitional state from the Strongyline. This exclusively Gondwanian group presents therefore a certain homogeneity, but it is important to analyse it in greater detail since it lies close to the major division within the Trichostrongyline which occurs between the Molineoidea and Heligmosomoidea (cf. Durette-Desset and Chabaud, 1993).

Seven genera occur in this group : *Herpetostrongylus*, *Vaucherius*, *Wanaristrongylus*, *Amphibiophilus*, *Batrachostongylus*, *Batrachonema* and *Johnpearsonia*.

Herpetostrongylus Baylis, 1931 is characterised by the presence of three teeth, a bursa of the 1-3-1 type with rays 2 and 3 more strongly developed than 5 and 6, a didelphic female and a synlophe of the heligmosomoidean type (a species with 2 left ventral humps, a species with an oblique axis running from a right ventral direction to a left dorsal one). The genus consists of two species from Queensland, *H. pythonis* Baylis, 1931 from *Python spilotes* and *H. varani* Baylis, 1931 from *Varanus gouldii*. As well *H. pythonis* occurs in Oceania (Schmidt and Kuntz, 1972).

Vaucherius Durette-Desset, 1980 is close to the preceding species because of the hypertrophy of rays 2 and of the conservation of the didelphic female, but the bursa has become the 2-2-1 type in place of the 2-3. The genus comprises three species, *V. vaucheri* Durette-Desset, 1980 parasitic in *Varanus rudicollis* in

Malaysia, *V. indicus* (Deshmukh, 1969), and *V. leiperi* (Sharief, 1957) parasitic in *Varanus indicus* in India. Both *Herpetostromgylus* and *Vaucherus* have an asymmetric synlophe similar to that of the Heligmosomoidea and are therefore distinct from the two species described above.

Wanaristromgylus Jones, 1987 possesses a single tooth in the buccal cavity (figs.30, 31, 32), a synlophe with ridges orientated perpendicularly to the body surface and the female is monodelphic. The bursa is of the 2-3 type in two species and has equally spaced rays in the third. The genus was placed in the Herpetostromgylidae by Jones (1987), but in this family the synlophe is never bilaterally symmetrical. Therefore the synlophe as well as the bursa, indicate that the genus is close to *Amphibiophilus* in the Molineoidea. The genus comprises three species which parasitize Australian lizards, *W. pogonae* Jones, 1987, parasitic in *Pogona minor*, *W. ctenoti* Jones, 1987, parasitic in *Ctenotus grandis* and *W. papangawurpae* Jones, 1987, parasitic in *Nephrurus laevisissimus*.

Amphibiophilus Skrjabin, 1916 has a bilaterally symmetrical synlophe with ridges perpendicular to the body surface. The bursa is of the 2-3 type with a very hypertrophied dorsal lobe. The female is didelphic. The genus comprises four species which parasitize Ranidae in eastern and southern Africa, *A. acanthocirratu*s Skrjabin, 1916, *A. natalensis* (Walton, 1935), *A. chabaudi* Puylaert, 1967 and *A. versterae* Baker, 1981.

Batrachostromgylus Yuen, 1963 appears to lack a buccal capsule and has a single, robust oesophageal tooth. It has been placed close to *Amphibiophilus* since the buccal capsule is comparable. The female is didelphic. The genus is monotypic with *B. longispiculus* Yuen, 1963, redescribed by Baker (1983), parasitic in *Megophrys nasuta* in Malaysia.

Batrachonema Yuen, 1965 belongs to the Molineoidea since the larval synlophe is bilaterally symmetrical. In the type-species, the bilateral symmetry is preserved in the adult. However in the adult of the second species, the axis of orientation of the ridges runs from right to left while the fourth stage larva has one ridge opposite each lateral field, orientated perpendicular to the body surface, confirming that *Batrachonema* belongs within the Molineoidea. The bursa is of the 1-3-1 type, with a marked reduction of the dorsal lobe. Rays 8 begin on the dorsal ray. The female is didelphic and a ring is present at the base of the buccal capsule. The genus comprises two species, *B. synaptospicula* Yuen, 1965, redescribed by Baker (1983), parasite of Ranidae in Malaysia

and *B. bonai* Durette-Desset, Baker and Vaucher 1984, parasitic in *Bufo typhonius* in Peru*.

Johnpearsonia gen. nov. has a bilaterally symmetrical larval synlophe whereas the adult synlophe may be bilaterally symmetrical or may have an oblique axis of orientation with oblique ridges. The bursa is of the 1-3-1 type. The only genus that it is close to therefore is *Batrachonema*. The two species studied above cannot, however, be classified in this genus since the dorsal lobe of the caudal bursa is not reduced, rays 8 begin at the base of the dorsal ray and the female is monodelphic.

We have therefore created a new genus as defined.

RELATIONSHIPS BETWEEN THE GENERA

The species belonging to the genera named above have in common a primitive cephalic structure with 6 lips, a hexagonal mouth and a buccal capsule but in reality they belong to very different groups :

Herpetostromgylus and *Vaucherus* (Herpetostromgylidae, Herpetostromgylinae) have a synlophe that is never bilaterally symmetrical either in larval or in the adult stage. They therefore belong to the Heligmosomoidea and are distinct from the other genera in the remarkable development of the ventral rays of the bursa. They are known from reptiles (varanids and more rarely snakes) in the Oriental and Australian biogeographic regions and appear to be the origin of the Nicollinidae, a family parasitic in monotremes but which has subsequently invaded Australian marsupials.

Amphibiophilus, *Batrachostromgylus* and *Wanaristromgylus* (Amphibiophilidae, Amphibiophilinae) have a bilaterally symmetrical synlophe. They therefore belong to the Molineoidea. The bursa is of the 2-3 type. The first two genera have a bursa with a very well developed dorsal lobe and have didelphic females; the third one, *Wanaristromgylus*, has a smaller dorsal lobe, and a monodelphic female. *Batrachostromgylus* has an aberrant buccal structure and ovejector. The genus *Amphibiophilus* appears the most primitive morphologically.

Batrachonema and *Johnpearsonia* (Amphibiophilidae, Johnpearsoniinae) have a bilaterally symmetrical synlophe in the larval stage that, sometimes, lose its bilateral symmetry in the adult. They therefore belong to the Molineoidea. In contrast to all the other members of this superfamily, the bursa is of the 1-3-1

* Hasegawa, 1987, reports the occurrence of *B. synaptospicula* in 3 species of Ranidae in Japan. But the axis of orientation is directed from right to left as in *B. typhonius*. According to Baker, 1983, the crests are orientated perpendicularly to body in *B. synaptospicula*. The Japan specimens cannot therefore be identified to *B. synaptospicula*.

type, a type only known up until now in the superfamilies Trichostrongyloidea and Heligmosomoidea. Due to the differences in the synopse and the type of caudal bursa, the two genera appear to be original and need to be separated from the Amphibiophilinae. These characteristics indicate a particularly archaic group. Its geographic distribution supports this view since the species are widespread, occur in amphibians only, and almost all in the Gondwanian region.

The data given above lead to the creation of a new subfamily for which we have proposed the name Johnpearsoniinae nov. subfamily as defined above.

The seven genera with primitive cephalic structures, parasitic in amphibians and reptiles are classified in the following manner :

Molineoidea : Amphibiophilidae-Amphibiophilinae : *Amphibiophilus*, *Batrachostongylus* and *Wanaristongylus*; Amphibiophilidae-Johnpearsoniinae nov. subfam. *Johnpearsonia* and *Batrachonema*. Heligmosomoidea : Herpetostongylidae-Herpetostongylinae : *Herpetostongylus* and *Vaucherus*.

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