

## THE MUSKRAT AS RESERVOIR IN NATURAL FOCI OF ADIASPIROMYCOSIS

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**Abstract.** A natural infection of eight muskrats (*Ondatra zibethica*) with the fungus *Emmonsia crescens* Emmons et Jellison is described. The causative agent was recovered from the lungs of seven specimens by cultivation method. A detailed description of isolated cultures is given. The muskrat is the second host species from which *E. crescens* was isolated in Czechoslovak territory. Its importance in natural foci probably consists in a possible dissemination of *E. crescens* among populations of small mammals during migrations of muskrats to greater distances.

Until recently adiaspiromycosis, caused by the fungus *Emmonsia crescens* Emmons et Jellison, 1960 has been described in 93 species of wild mammals included in five orders: Insectivora, Edentata, Lagomorpha, Rodentia and Carnivora (Dvořák, Otčenášek and Rosický 1973). The range of animals infected is clearly affected by the mode of their life. In the territory of Czechoslovakia adiaspiromycosis was microscopically detected in 11 species of rodents and insectivore (Prokopič 1971). The causative agent, however, was isolated only from voles *Microtus arvalis* (Pallas) (Otčenášek, Dvořák and Prokopič 1965). This communication contains a report on the isolation of *E. crescens* from another host, the muskrat (*Ondatra zibethica* L.) and its role as the reservoir in natural foci of adiaspiromycosis.

### OBSERVATIONS

During autopsy of eight adult muskrats, caught between April 1972 and March 1973 on the banks of the river Haná, district Vyškov, in the basin of the river Dřevnice, district Gottwaldov and of the river Jevišovka, district Třebíč, characteristic gray-white little nodules resembling fish-eggs (spawn) were found in the lung tissue of the animals. In microscopic preparations made from fragments of lung tissue and cleared by 20 % KOH these proved to be both morphologically and in size, typical adiaspores of the fungus *E. crescens*. The intensity of invasions in all animals examined was relatively low; the number of nodules did not exceed 50. The adiaspores were as large as 165 to 480  $\mu$ . It was found that their presence caused a slight tissue reaction only (Plate I, Figs. 1, 2). Individual adiaspores were separated from the unfixed tissue by preparation needles and inoculated into Mycobiotic agar (Difco)\* in Petri dishes. From about two thirds of inoculated adiaspores pure cultures of *E. crescens* were obtained. Only in one of the eight muskrats examined the cultivation failed. The morphological properties of the isolates were assessed after repeated inoculation into Sabouraud's dextrose agar containing no antibiotics.

\* Sabouraud's dextrose agar with chloramphenicol and selectively antifungal antibiotic cycloheximid.

## DESCRIPTION OF ISOLATES

On the whole, the properties of seven strains obtained were uniform. After 14 days the diameter of stabbing colonies was 45—50 mm. The colonies were flat, only in the centre they were elevated like little hills, finely haired or dusted. The central and paracentral part was white-gray of ochre shade, the peripheral part was purely white. On the surface of colonies there were irregular radial folds (Plate I, Fig. 4). In one of the isolates the radial folding was especially marked. Thick short folds surrounded the paracentral part which was ring-like and of intensive ochre colour (Plate I, Fig. 3). In some colonies there appeared sectorial dissociation in the shape of velvety haired sections of ochre or cinnamon brown colour. The reverse of colonies of all isolates was light brown.

The first subcultures intensively sporulated. Subspherical or pyriform aleuriospores 2.8—5.4  $\mu$  large originated at the tops of hyphae or in short lateral branches — conidiophores. Sometimes the longer primary conidiophores branched in right angles into short secondary branches bearing spores. Aleuriospores transferred to hyphae either directly (Plate II, Fig. 1), or sprouted in short pedicles whose distal part was mostly expanded. After repeated inoculation of cultures to Sabhi agar (Difco) with 5 % of sheep blood and following incubation at 37 °C a conversion took place of the mycelial phase of the fungus into the adiasporic phase. After 20 days the slowly growing colonies of the adiasporic phase reached 5 mm in diameter and were of cauliflower shape (Plate II, Fig. 2), crumbling, yellow-brown. They were composed of adiaspores of different size, most frequently 60—80  $\mu$ .

## DISCUSSION

Adiaspiromycosis of muskrats has been known from the USA (Jellison 1950), Finland (Jellison, Helminen and Vinson 1960, Jellison and Vinson 1961), Yugoslavia (Jellison and Vinson 1961), Switzerland (Hörning and Hörning—Pezenburg 1962) and from the USSR (Sharapov 1969). The degree of invasion was very variable. In some specimens only single adiaspores were found, while in others heavy affliction was observed, when elements of the causative agent, together with the surrounding granulomatose tissue, almost filled out the lung parenchyma. In previous pilot examinations of 98 muskrats collected in south Bohemia when the material was not cultivated, adiaspiromycosis was diagnosed in 9 specimens (Křivanec, unpubl. data). The invasions were mostly weak or of medium intensity. The presence of adiaspores in the lung parenchyma caused only hardly discernible tissue reactions in these animals. These findings are markedly different from our findings of adiaspores in other mammal groups. For example, in small carnivores we observed considerable tissue reactions forming round *E. crescens* elements layers which exceeded many times the diameter of adiaspore proper.

The muskrat *Ondatra zibethica* is not the original Euroasian mammal. It was introduced to many places of Eurasia (the Central-European population is the progeny of individuals introduced in 1905 in the Dobříš feudal estate) and became fully adjusted to its new environment. It is an ecological form of mammal well adapted to the life near and in water. It lives mainly in burrows, making them in the stream banks, with entrance holes under the normal water level and the nest chamber situated in the unflooded bank soil. Apart from nest burrows the muskrat also builds refuge and store burrows as well as air-shafts (air-chambers). We suppose that even this animal, although adapted to life in water, may get infected with *E. crescens* like other fossorial mammals by inhalation of aleurics from soil and nest material.

The muskrat mainly feeds on small water vegetation. In winter it digs out underground parts of plants and reeds (Kratochvíl 1973). Sharapov's supposition (1969) that muskrats, like other representatives of the genus *Arvicola*, get infected while gnawing underground parts of herbs and tree species, has not been experimentally confirmed as yet. The possible existence of saprophytic phase of the adiaspiromycosis agent in the root parts of plants and the opinion that *E. crescens* is a component of rhizospheric microflora, however, deserve more attention and should be more profoundly studied.

In Eurasia the muskrat has become a member of natural foci of tularemia and the Omsk haemorrhagic fever. In America two infections heavily afflict its populations: tularemia and haemorrhagic infection called Errington's disease by veterinarians in North America. Hence, adiaspiromycosis does not belong to infections occurring in mass epizootics among muskrat populations (Errington 1962). The weak or medium invasions ascertained by us testify to this fact. We assume that at this state the observed migrations of muskrats to greater distances between water streams and water reservoirs may be important for the long-distance dissemination of *E. crescens* among populations of small terrestrial mammals.

#### ОНДАТРА КАК ЖИВОТНОЕ-РЕЗЕРВУАР В ПРИРОДНЫХ ОЧАГАХ АДИАСПИРОМИКОЗА

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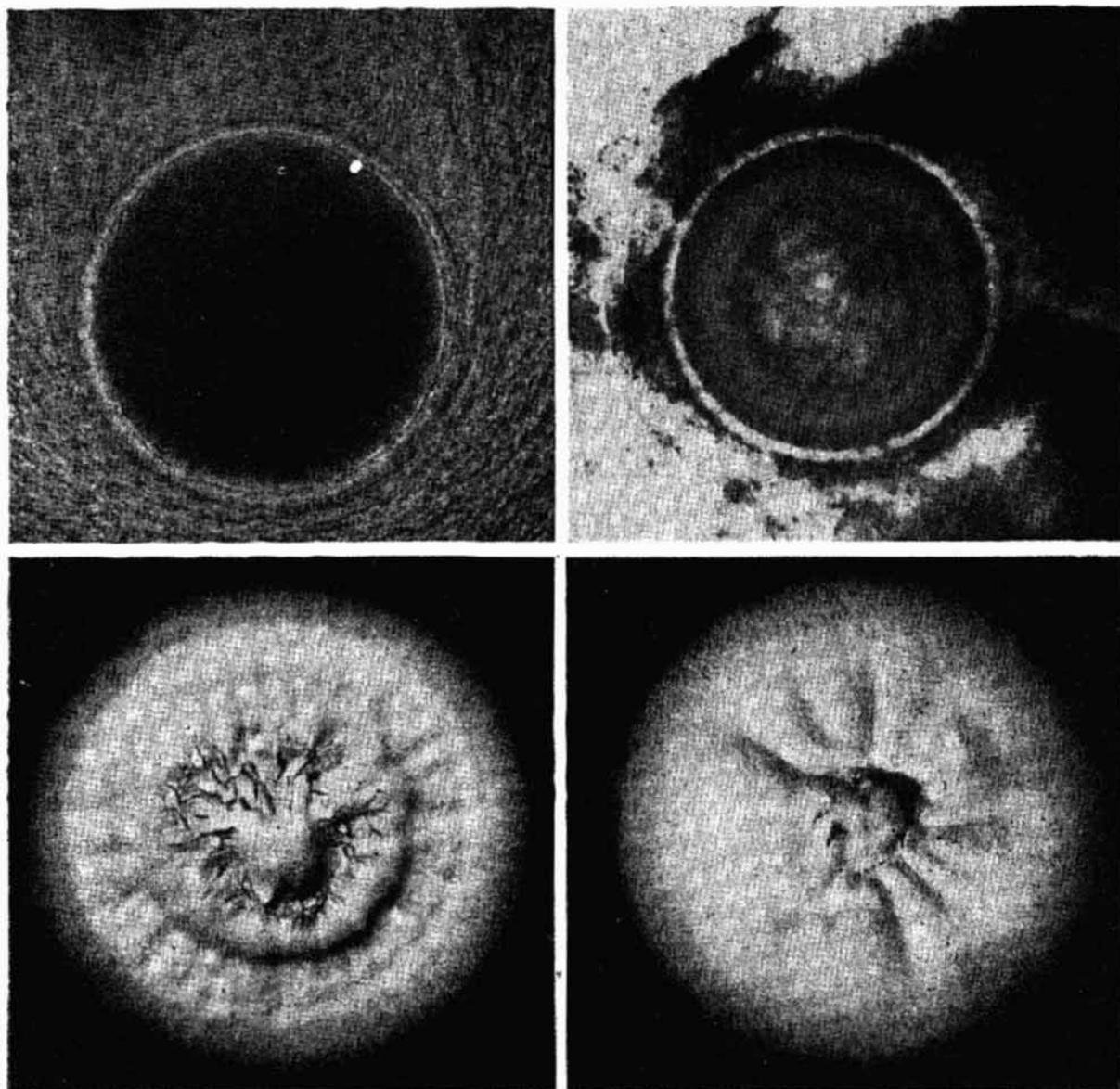
**Резюме.** Дано описание естественного заражения восьми ондатр (*Ondatra zibethica*) грибом *Emmonsia crescens* Emmons et Jellison. Возбудителя удалось обнаружить из легких семи особей путем культивации. Дано подробное описание выделенных культур. Ондатра является вторым хозяином вида, от которого выделена *E. crescens* на территории Чехословакии. Ее значение в природных очагах состоит в возможном распространении *E. crescens* на большие расстояния среди популяций мелких млекопитающих во время миграций ондатр.

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**Figs. 1, 2.** Adiaspores in lung tissue of the muskrat, 20 % KOH, mult.  $\times$  100.

**Figs. 3, 4.** Colonies of *E. crescens* (Fig. 3 strain No. 5355, Fig. 4 strain No. 5278) after 15 days of incubation on Sabouraud's dextrose agar at 27 °C. Actual diameters of colonies 50 mm.

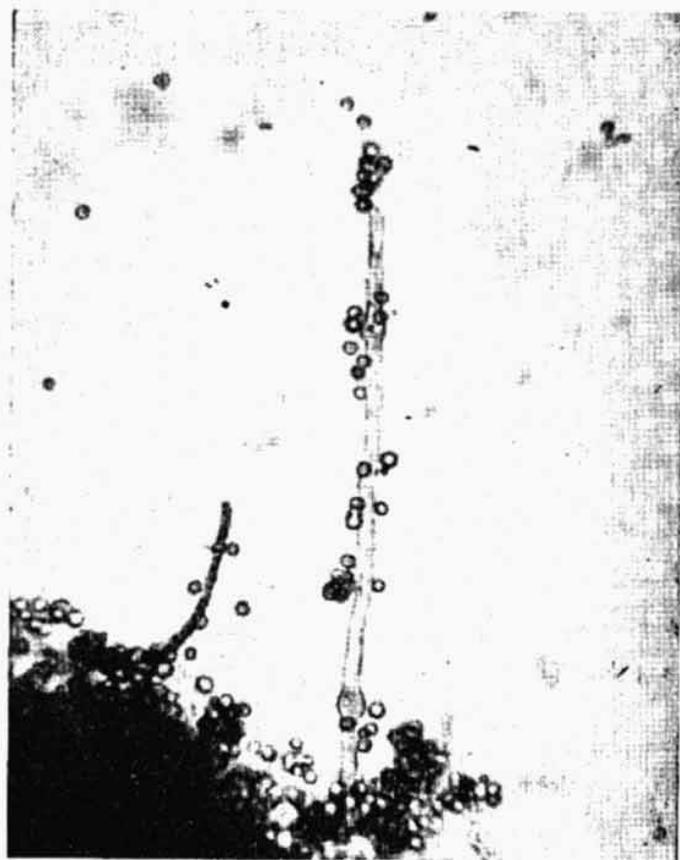


Fig. 1. *B. crescens*, strain No. 5278. Aleuriospores formed on the sides of the hypha. Lugol's solution  $\times 400$ .

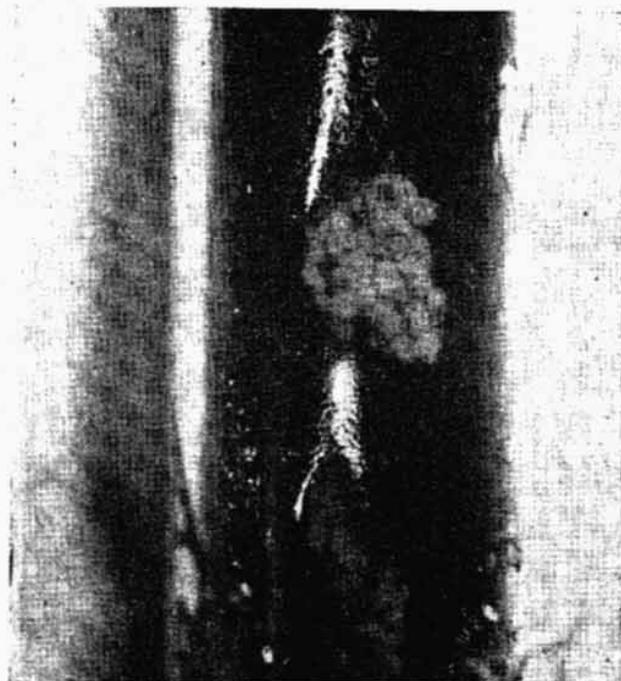


Fig. 2. Adiasporic stage of the strain No. 5278 on Sabhi agar (Difco) after 21 days of cultivation at 37 °C.