A microsporidian infection in Otiorrhynchus equestris (Coleoptera, Curculionidae)

Article · January 1980

CITATIONS
12

READS
27

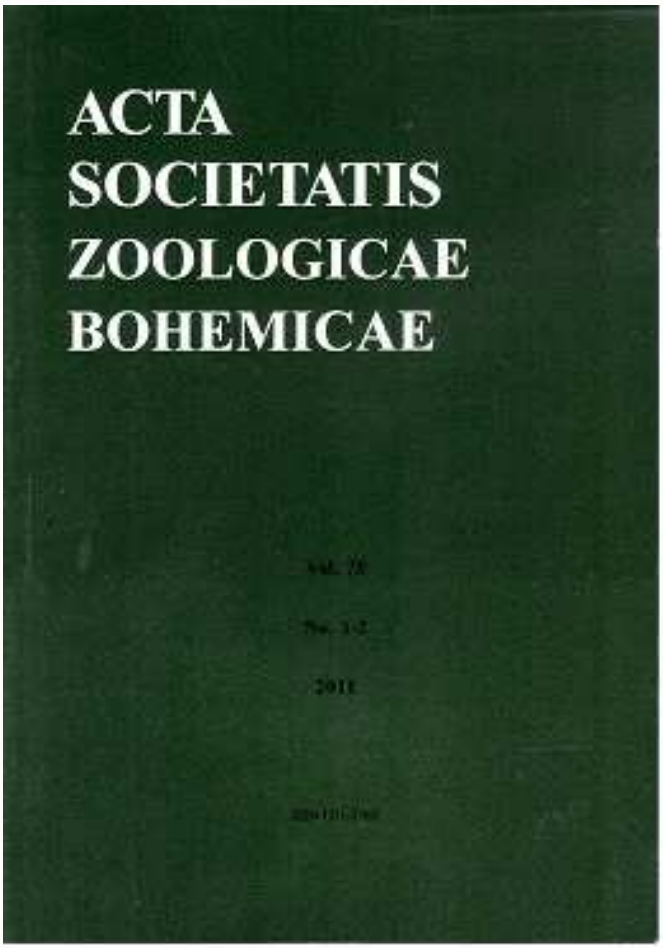
2 authors, including:

Zdeněk Hostounský
The Stentor Institute
15 PUBLICATIONS **64** CITATIONS

SEE PROFILE

VĚSTNÍK
ČESKOSLOVENSKÉ
SPOLEČNOSTI ZOOLOGICKÉ

Vol. XLIV
1980



Věst. čs. Společ. zool., **44**: 160–165, 1980

Department of Insect Pathology, Institute of Entomology, Academy of Science, Praha

A MICROSPORIDIAN INFECTION IN OTIORRHYNCHUS EQUESTRIS (COLEOPTERA, CURCULIONIDAE)

Zdeněk HOSTOUNSKÝ and Jaroslav WEISER

Received October 18, 1978

Abstract: Two microsporidian infections were found in one infected *Otiorrhynchus equestris* Richter: *Nosema adjuncta* sp. n. infects the ovaries, *Nosema equestris* sp. n. infects the connective tissue, Malphighian tubes, oenocytes and the fat body. The infection was transferred to *Gastroidea viridula* and *Leptinotarsa decemlineata* where it infected the same tissues and caused larval mortality.

In an attempt to find microsporidians capable of holding the Colorado beetle in check, we examined various chrysomelid beetles which might act as hosts of a suitable microsporidian. It was of interest that microsporidians commonly found in other chrysomelids caused in the Colorado beetle either an acute, lethal infection or an infection which was so light that it could be eliminated prior to pupation by regeneration of damaged cells of the intestinal epithelium.

A comparison of the frequency of the incidence of microsporidians in various chrysomelids (Hostounský, Weiser, 1973, 1975, 1978) and of that in the Colorado beetle provided conclusive evidence that the latter was an extrinsic element in Europe which had not become adapted as yet to local pathogens. The barrier was constituted partly by an inadaptibility of microorganisms to the insect, partly by an ecological barrier between the infection and the host. In spite of these facts we did not give up hope that one of these microsporidians might have become adapted to the Colorado beetle and thus be capable of holding the population of this pest in check.

An abundance of microsporidian species and small differences in developmental stages of the various species caused frequent difficulties in determination of their taxonomic position and in differential diagnosis. Therefore, various similar species of the material examined were frequently regarded as one species and, in reverse, wider groups separated into several species. The present situation calls for a better understanding of the taxonomy of microsporidia in order to complement a number of incomplete descriprions.

MATERIAL AND METHODS

A solitary female of *O. equestris* with well developed ovaries was found on the wall of a house at Holovousy, Eastern Bohemia, in July 1975. Postmortem examination disclosed infection with two different microsporidian species. A spore suspension was made of cuticular remnants and administered to other hosts. A small portion of the ovary, the Malpighian tubules and the fat body were fixed with Bouin and embedded in paraffin. Sections cut to a thickness of 4 μm were stained with Giemsa or Heidenhain's haematoxylin. Dried smears of the material were stained either with Giemsa or, for the detection of nuclei, they were hydrolyzed in 8% heated HCl (suggested by Weiser 1976) prior to staining.

Substitute hosts used in our experiments were *Gastroidea viridula* from our laboratory stock and the Colorado beetle *Leptinotarsa decemlineata*. *G. viridula* was kept on leaves of *Rumex obtusifolia* in plastic boxes. The boxes were covered with fine netting for airing. Groups of eggs were collected daily and isolated. Hatched larvae were infected with the spore suspension placed on a platinum loop. The spores had been purified by repeated centrifugation. The number of spores used for the infection ranged from 300—500/1 mm^3 in order to prevent the larvae from dying before the infection could develop. Infected larvae were kept in plastic boxes with a daily change of food. Progress in the infection was studied on individuals taken from day 5—6 p.i. for microscopic inspection.

A similar procedure was used for larvae of *Leptinotarsa decemlineata*. The development of the infection was studied on fresh material from day 5 p.i. onwards. Infected individuals were fixed with Bouin and embedded in paraffin for histological treatment in the same way as the original material.

The yield of infection was studied on a total suspension of tissues of the host, spores per 1 mm^3 were counted in Bürker's chamber. The spores were stored as a purified suspension in water at 4 °C.

RESULTS

The two microsporidian species recovered from *O. equestris* differed in the shape and size of their spores, the organisation of spores, and in the type of tissues attacked by them. Treatment of the primary material was impeded by the fact that most of it had been used up in infection experiments.

Nosema adjuncta sp. n. (Fig. 1a—g)

The infection of this species in *Otiorrhynchus equestris* Richter was concentrated in the ovary and in maturing eggs. We did not find it in other tissues. Typical of the development of this microsporidian was the small number of schizonts of which several attained a size of 5 μm; they were spherical and contained up to 8 nuclei. They produced bi-nucleate sporoblasts measuring 3×5 μm. Spores matured individually. They were elongately ovoid and measured 4—4.8×1.5—1.8 μm (average 4.2×1.8 μm); they did not stain well with Giemsa. The sporoplasm was conical with a vacuole in the anterior pole. The wall of the spore did not stain and, sometimes, a kidney-shaped deformation appeared on it during drying and fixation. In several spores, the metachromatic granule situated at the very end of the spore became stained. After hydrolysis with HCl, the two nuclei became clearly visible. They were spherical, measured 0.4 μm in diameter, and were situated in tandem position close to the wall in the second half of the spore. Hydrolysis dissolved the metachromatic granule and its former space was occupied by a posterior vacuole. Occasionally, three spherical nuclei stained in longer spores, which evidently were teratospores.

The microsporidian attacked the egg follicles, entered them and formed diffuse centres in the developing oocytes. These were present throughout the cutting plane of the egg. The groups were connected with single developmental stages and spores.

In its organisation, the spore of this microsporidian was conform to that of other typical *Nosema* species such as *N. apis* or *N. bombycis*. It was close in its size to *N. otiorrhynchi*, but differed from it in the length of spores and in its distribution in the host tissues, particularly in that it was absent from the fat body.

Nosema equestris sp. n. (Fig. 1 h—n)

In the original host, *Otiorrhynchus equestris*, the microsporidian attacked the connective tissue, the Malpighian tubules and the oenocytes which were considerably enlarged. A small number of spores were present in the fat body. Shizonts were

tinotarsae or *N. longifilum*, but the organisation of spores and especially of nuclei differed from that in our species. The new species differs from earlier described species in the location of the metachromatic posterosome in the posterior vacuole of the spore, and in the distribution of the infection in the organs of the host.

The development of an infection with *N. equestris* in a substitute host

In *Gastroidea viridula*, the microsporidian attacked first the Malpighian tubules and infected them gradually throughout their length. At first, spores attacked and filled individual cells which increased in volume, ruptured and released spores into the lumen. The number of infected cells increased until the infection spread to almost the majority of cells in the lumen of the Malpighian tubules. Infected tubules still contained urate crystals. A gradual, sac-like swelling of the attacked parts was followed by a reduction in the number of urate crystals and finally by their complete disappearance. This damage caused to the Malpighian tubules evidently affected both their excretory and resorptive parts, and it was possible to observe the ceasing of the process of resorption and the production of crystals from the watery excreta. The absence of crystals indicated that both the process of secretion and that of water resorption had been disturbed.

In the fat body of the host, the infection was first disseminated in the form of small islets, later it affected whole lobes and finally the entire fat body. A lighter colour of the infected parts indicated the presence of microsporidia.

The infection became generalized in *Gastroidea viridula*, and the oenocytes known as the seat of infection in the primary attack in *O. equestris* disappeared in *G. viridula* as a result of their rupture under the pressure of the parasite in the first phase of infection. Later, the infection spread to the muscle fibres of the kinetic muscles, and to the intestinal muscles, but the intestinal epithelium was not attacked by the parasite.

In spite of progressing infection, the activity of larvae of *G. viridula* was not greatly affected by the presence of the parasite. Although slightly retarded in their development, they pupated and developed into normal adults which fed normally and copulated. However, distinct changes occurred in fertilized females. As the eggs developed, their abdomen did not increase in size as much as it did in normal females. Also the number of eggs laid by infected females was smaller than that of normal females. As feeding conditions worsened in the autumn, lightly infected imagoes survived while all the other infected individuals died. No mortality was recorded among healthy individuals, the females laid eggs. An appreciable number of spores was recovered from dead animals.

In the Colorado beetle, the course of the infection was acute. However, the pattern of the development of infected larvae was most ununiform. The highest rate of mortality occurred in the prepupal stage. After a normal preparation for pupation, the larvae stopped responding to touch, their body shortened and desiccated. In soil with a higher relative humidity, dead larvae began to decompose. A disseminated infection was found in the Malpighian tubules at the time of larval development of the Colorado beetle. The Malpighian tubules of infected last-instar larvae were densely packed with spores, infected lobes of the fat body contained white masses of spores, a focally disseminated infection was found in kinetic muscles of the legs and in the muscle layer of the intestine. Although an occasional individual attained the adult stage in cage experiments, it was much smaller in size than a healthy individual, and no oviposition occurred in the female.

infrequent, we recovered mainly spores and an occasional sporoblast. Sporogony was bi-nucleate. Mature spores were widely ovoid in shape and attenuated towards the anterior pole they measured 4—5×3 μm. A posteriorly located vacuole was visible in fresh spores. The sporoplasm stained fairly well and so did part of the spore surface. After HCl hydrolysis, the two nuclei stained inside the spore. They evidently continued in their development as the spore matured. Sometimes, we observed two spherical nuclei (0.5—1 μm) in tandem position similar to that of nuclei

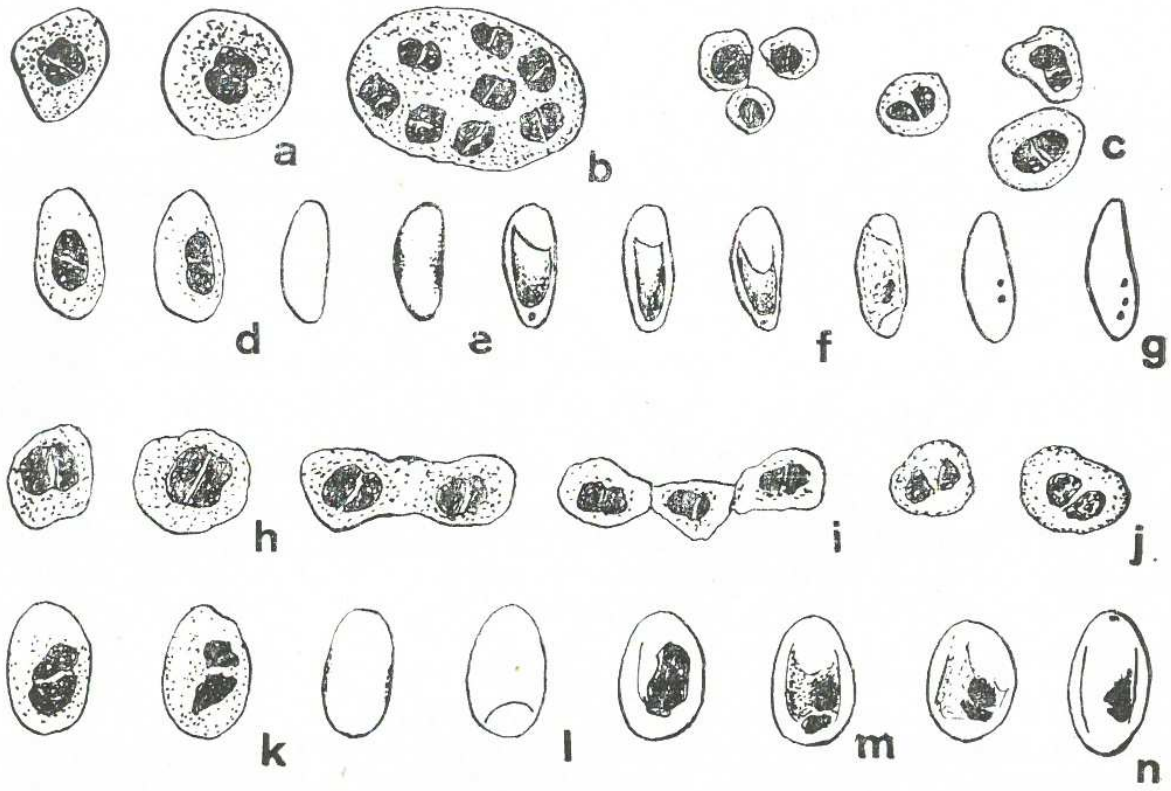


Fig. 1. *Nosema adjuncta* (a—g) and *Nosema equestris* (h—n) in the curculionid *Otiorrhynchus equestris:* a — schizonts, b — late schizont before merogony, c — development of meronts into sporonts, d — sporoblasts, e — fresh spores, f — Giemsa stained spores with the cone of the sporoplasm and the dotlike posterosome, g — spores with stained nuclei, h — schizonts, i — merozoites formed in rows, j — early sporonts, k — sporoblasts, l — fresh unstained spores, m — Giemsa stained spores with sporoplasm and the large posterosome, n — nuclei stained inside the spores.

in sporoblasts. More frequently, however, the posterior nucleus which in normally stained spores was lying close above the posterosome, was flattened or disk-shaped; the second, more anteriorly situated nucleus was extending into the first. The second nucleus stained less intensively and was often triangular, passing anteriorly into a tapering protrusion. This nuclear structure, atypical of the genus *Nosema*, will have to be studied in detail in other material in order to disclose conformities and differences among the species inside the genus *Nosema*.

Developmental stages and spores of the microsporidian were found to concentrate in the connective tissue, lymphocytes, Malpighian tubules and the fat body of the original host.

Similar spores have been described from various other coleopterans (Lípa, 1968a, b, Lípa, Steinhaus, 1959) such as *N. coccinellae*, *N. hippodamiae*, *N. lep-*

DISCUSSION

The new microsporidian species differed from other microsporidia obtained from related host species in many morphological features.

The newly described *Nosema adjuncta* differed from *Nosema otiorrhynchi* (Weiser, 1951) (host *Otiorrhynchus ligustici*) in that it developed at a considerably slower rate especially in the ovaries of the host. Also a comparison of the course of infection in the eggs showed a very different picture, e.g. in the absence of digested foci and widely ovoid stages of the parasite common to *N. otiorrhynchi*.

N. equestris differed from the related species *N. longifilum* (host *Otiorrhynchus fuscipes*) in its distribution in the host tissues and in a more widespread infection than that caused by *N. longifilum* attacking only the fat body. Spherical cysts in the fat body observed and described by Hesse (1905) for an infection on with *N. longifilum*, which were surrounded by a sheath of connective tissue inside which the spores were destroyed, were not observed with our species.

If the production of cysts is to be regarded as a reaction of the host to an "unadapted" parasite, we expected to obtain interesting results from a comparison of the reaction of two substituting hosts, *G. viridula* and *L. decemlineata* to the two newly described microsporidians. The results indicated that *Nosema adjuncta* did not develop too well in these hosts, the production of islets was sporadic and they were surrounded neither by connective tissue nor by lymphocytes of the host, and there was no indication of a further spreading. The same applied to *N. equestris* to which the substitute hosts did not react in any form resembling cysts produced as a reaction to *N. longifilum*. A substitution of hosts (*G. viridula* and *L. decemlineata*) was manifested solely in a more acute course and in a difference in the number of spores produced in an individual or in relation to the mg/weight of the host.

REFERENCES

Hesse E., 1905: Microsporidies nouvelles des insectes. *C. R. Assoc. France avanc. sci.*, 33: 917–919.

Hostounský Z., Weiser J., 1973: Nosema gastroideae sp. n. (Nosematidae, Microsporidia) infecting Gastroidea polygoni and Leptinotarsa decemlineata (Coleoptera, Chrysomelidae). *Acta entomol. bohemoslov.*, 70: 345 – 350.

Hostounský Z., Weiser J., 1975: Nosema polygrammae sp. n. and Plistophora fidelis sp. n. (Microsporidia, Noseatidae) infecting Polygramma undecimlineata (Coleoptera, Chrysomelidae) in Cuba. *Věst. Čs. spol. zool.*, 39: 2, 104–110.

Hostounský Z., Weiser J., 1978: Pleistophora grossa sp. n. (Pleistophoridae, Microsporidia), parasite of Chrysomelid beetles in Yougoslavia. *Věst. Čs. spol. zool.*, 42: 112–114.

Lipa J. J., Steinhaus E. A., 1959: Nosema hippodamie sp. n., a microsporidian parasite of Hippodamia convergens Guérin (Coleoptera, Coccinelidae). *J. Insect Pathol.*, 1: 304–305.

Lipa J. J., 1968: Nosema leptinotarsae sp. n., a microsporidian patogen of Colorado beetle, Leptinotarsa decemlineata Say. *J. Invertebr. Pathol.*, 10: 111–115.

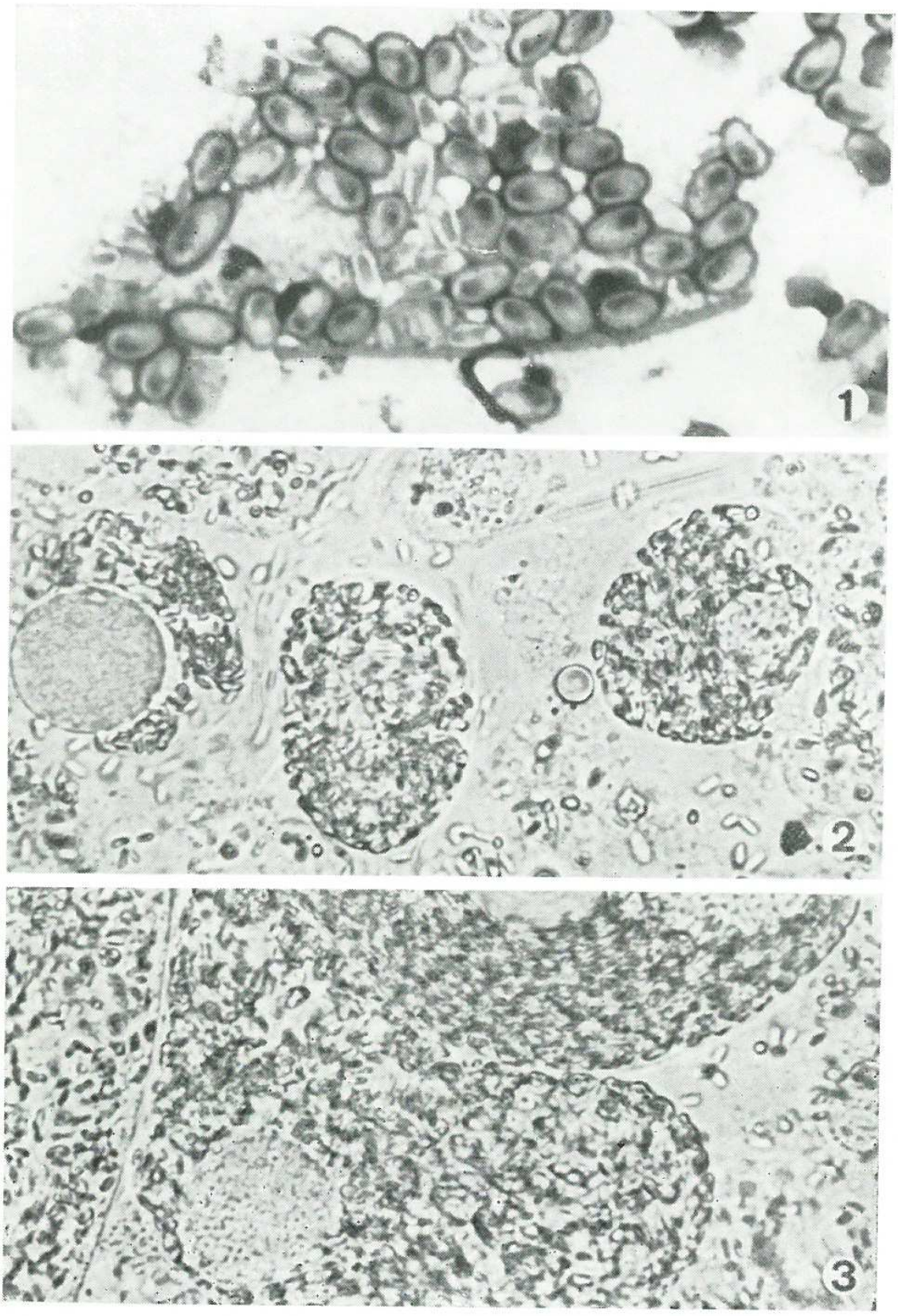
Lipa J. J. 1968: Nosema coccinellidae sp. n., a new microsporidian parasite of Coccinella septempunctata, Hippodamia tredecimpunctata and Myrrha octodecimpunctata. *Acta Protozool.*, 5: 269–272.

Weiser J., 1951: Nosematosis of Otiorrhynchus ligustici. *Věst. Čs. spol. zool.*, 15: 219–234.

Weiser J., 1976: Staining of the nuclei of microsporidian spores. *J. Invertebr. Pathol.*, 28: 147–149.

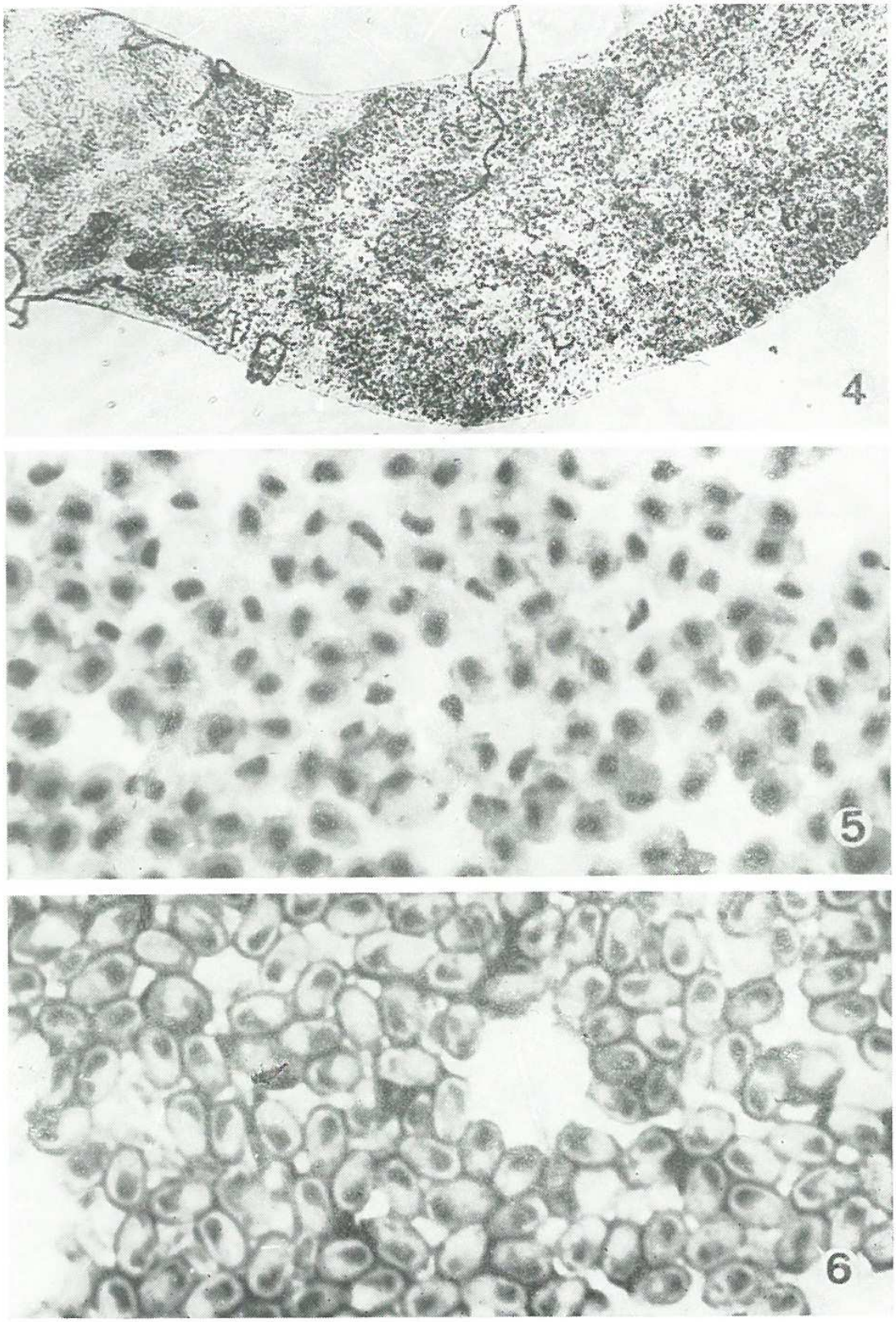
The plates (Tab. 1, 2) will be found at the end of this issue.

Authors' address: Ing. Z. Hostounský, Dr. J. Weiser, Entomologický ústav ČSAV, Flemmingovo nám. 2, 160 00 Praha 6-Dejvice, ČSSR.



Tab. 1. *Nosema adjuncta*. 1. Smear with Giemsa stained spores of *N. adjuncta* (minor, less stained ones) and *N. equestris*. 2. Oenocytes filled with spores of *N. adjuncta*. 3. Spores of *N. adjuncta* in gonads. Magn. ×1,200, (1) and ×500 (2, 3).

Original image width = 119 mm



Tab. 2. *Nosema equestris*. 4. Malpighian tube with spores of *N. equestris* spread over the wall. 5. Giemsa stained spores with dark posterosome prominent in the sporoplasm. 6. Spores of *N. equestris* with visible nuclei, the posterior flat, the anterior protruding in a flare. Magn. ×200 (4) and ×1,200 (5, 6).

Original image width = 119 mm