

HISTOLOGICAL STUDY OF COCCIDIOSIS CAUSED IN THE SILVER CARP AND THE BIGHEAD BY *EIMERIA SINENSIS* CHEN, 1956

By

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(Received June 3, 1976)

The fish coccidium *Eimeria sinensis* was originally described by CHEN (1956) in China. The naturalization of its herbivorous hosts in other countries was followed by reports on its occurrence in the USSR (MUSSELIUS, 1965) and in Hungary (MOLNÁR, 1971). The cited authors regarded *E. sinensis* as a common parasite of the silver carp (*Hypophthalmichthys molitrix*) and the bighead (*Aristichthys nobilis*). Differences in the dimensions of oocysts found in the two host species have prompted MUSSELIUS and LAPTEV (1967) as well as BAUER et al. (1969) to postulate the simultaneous presence of two coccidian species, viz., *E. sinensis* and *E. cheni* Schulman et Zaika, 1962, while MOLNÁR (1971) has suggested on the grounds of the morphological identity of the oocysts that these represent just one species. The life cycle of *Eimeria* species parasitic in fish has been studied by few authors. Most of the corresponding data have been obtained from studying the common carp's intestinal parasite, *E. carpelli* Léger et Stankovitch, 1921, which closely resembles *E. sinensis* in both morphological features and localization (LÉGER and STANKOVITCH, 1921; PLEHN, 1924; SCHÄPERCLAUS, 1943; ZMERZLAYA, 1966; LUCKY, 1965; MUSSELIUS et al., 1965). Among the other fish coccidia, *E. epithelialis* and *E. spleni* have been studied in detail (MARINCEK, 1973; LOM, 1971).

The present studies were carried out to obtain more informations on the developmental stages of *E. sinensis*, and on the nature of the gross and microscopic lesions caused by them.

Materials and methods

Silver carps and bigheads of various ages were studied in 11 pond farms to assess the frequency of occurrence of *E. sinensis* among cultured herbivorous fish in Hungary. Further to this, natural infection by the parasite was followed up over a 7-year period in the three largest fry-rearing units of Hungary by histological examination of representative groups at intervals and of individuals that had been found clinically ill.

The specimens to be examined histologically were fixed either in 4 % formalin, or in Bouin solution. Prior to fixation, the guts of smaller fish were examined under the microscope, then were coiled and sandwiched between two glass slides and so placed into the fixative. The fixed specimens were embedded in paraffin, and the paraffin sections were stained with haematoxylin and eosin.

Results

Epizootological observations. Infection of the fish stock by *E. sinensis* was demonstrable in all pond farms under study. The earliest appearance of the oocysts in the hosts was at 18 days of age. From the third week on, the infection could be regarded as general in part of the pond farms, viz., practically all silver carps and bigheads able to feed themselves, regardless of age, were passing oocysts. However, a massive infection was found only among the fry and the one-summer fish. The intensity of the infection varied by farm.

Lethal disease was frequent among the fish massively infected by *E. sinensis*. Since, however, coccidiosis was always associated with some other parasitosis, only indirect conclusions could be drawn on the pathogenic role of *E. sinensis*. Among the 4–6 weeks old fry, which harboured masses of *E. sinensis* stages in the gut, mortality sometimes reached 60–70 %, to which, however, invasion of the gills by ectoparasites (protozoans) may also have contributed.

Silver carps and bigheads showed the same symptoms of disease. The sick fish could be easily distinguished from healthy mates by their external appearance, being dark and lean. The main changes found at autopsy were oedema of the abdominal serous membranes and of the intestinal wall, absence of fat tissue and swollen intestinal mucosa. In the fry the intestinal wall is characteristically dark owing to the presence of masses of macrogametes and oocysts on the lining, and to coating of both intestinal epithelium and intestinal contents by a yellowish, viscous, mucous exudation. The granular macrogametes and, above all, the sporulated oocysts are easily visible on microscopic examination of intestinal segments sandwiched between two slides. The intestinal mucous epithelium contains many yellow bodies, either enclosing or not enclosing oocysts.

Gross morphological and histological findings. The stages recovered from the silver carp and the bighead showed in every respect identical morphological and histological features. The forthcoming description applies to stages taken from the silver carp (*Hypophthalmichthys molitrix*).

Oocyst. The oocysts (Fig. 1) are spherical, 8.5–10.5 μm in diameter (smaller or larger oocysts may occasionally occur). The wall is thin, colourless, and consists of a single, max. 0.1 μm thick layer. The sporocysts do not fill the entire space within the oocyst. There is no oocyst residuum, nor a micropyle, but a small polar granule is found at the periphery of the oocyst. Two sporocysts reside within the oocyst in an identical plane and direction. The sporocysts are prismatic rather than oval in shape, and end bluntly. They are 8.0–9.5 \times 3.4–4.0 μm in size and are surrounded by a unilamellar, approx. 0.2 μm thick wall. There is no Stieda body. Two worm-like sporozoites, recurved at one end, reside in each sporocyst in head-to-tail position. The sporozoites

are $8.0\text{--}9.0\ \mu\text{m} \times 1.5\ \mu\text{m}$ in size. There is a coarsely granular sporocyst residuum, elongated oval in shape, $3.5\text{--}4.5 \times 1.5\text{--}2.0\ \mu\text{m}$ in size. The sporocyst residuum falls apart to dispersed granules in older oocysts.

Oocyst sporulation is completed already in the gut of the host. The mature sporocysts become extruded to the outside world either freely or comprised in a "yellow body", either single, or by 2 or 3.

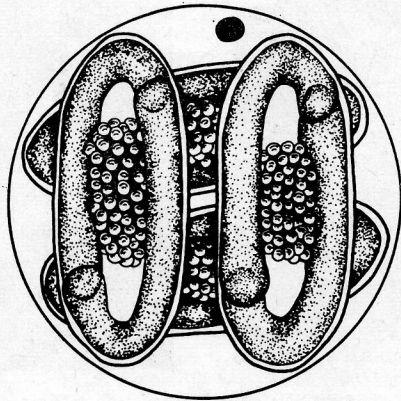


Fig. 1. Sporulated *Eimeria sinensis* oocyst

The schizogonic stages occur exclusively inside epithelial cells, always in supranuclear position, so that the nucleus of the host cell surrounds the schizont basally, in a semilunar manner.

The schizonts are round or slightly oval-shaped bodies, $8.5\text{--}12.0 \times 6.8\text{--}10.0\ \mu\text{m}$ in size. Most schizonts carry 16 merozoites (Figs 2, 3); these are banana-shaped, $5.0\text{--}6.8 \times 0.5\text{--}0.8\ \mu\text{m}$ in size, arranged in a rosette-like order inside the schizont.

Occasionally, schizonts in which only 8 merozoites were visible occurred in sections (Fig. 4), but no evidence of their existence emerged when cross-sectioned stages were examined.

Unlike the vegetative stages the sexual stages equally occur in the epithelium as well as in the mucosal propria and the submucosa. Younger stages, however, occur chiefly inside, or nearby the epithelium.

The earliest microgametocytes are mononuclear and can scarcely be differentiated from the macrogametocytes. The nucleus of the microgametocyte soon begins to divide: multinucleated, dividing microgametocytes are often found (Fig. 5). The initial phase of nuclear division takes place in the centre of the male gametocyte, but as soon as at least 16 nuclei have formed, these migrate towards the periphery, leaving behind a lighter-coloured mass of central residue. The multinucleated early microgametocytes (Fig. 6) stain dark

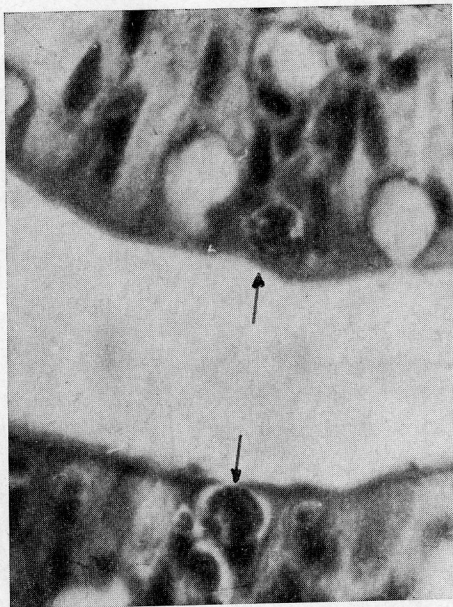
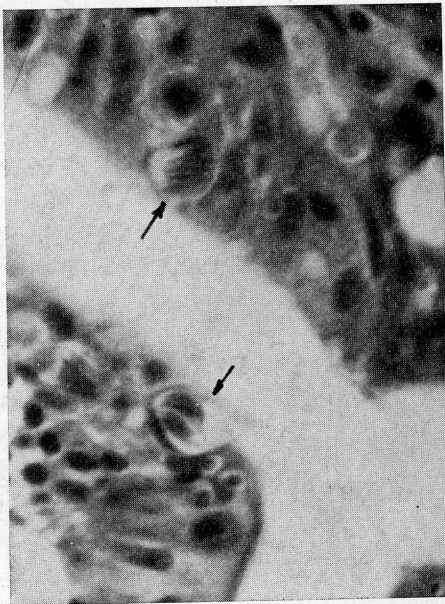


Fig. 2. Schizonts in the intestinal epithelium
 Fig. 3. Schizonts containing cross-sections of merozoites

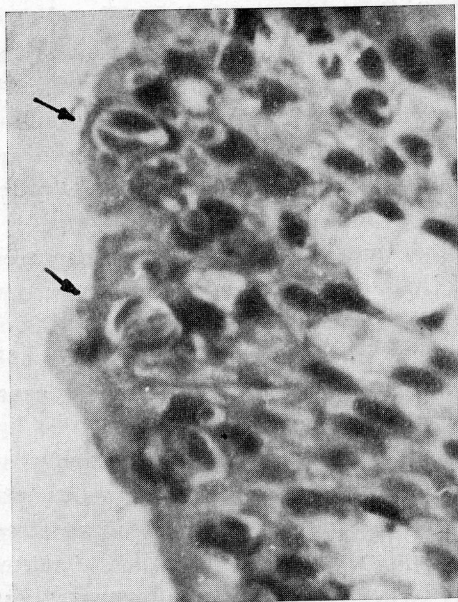


Fig. 4. Schizont with 8 merozoites
 Fig. 5. Early microgametocyte

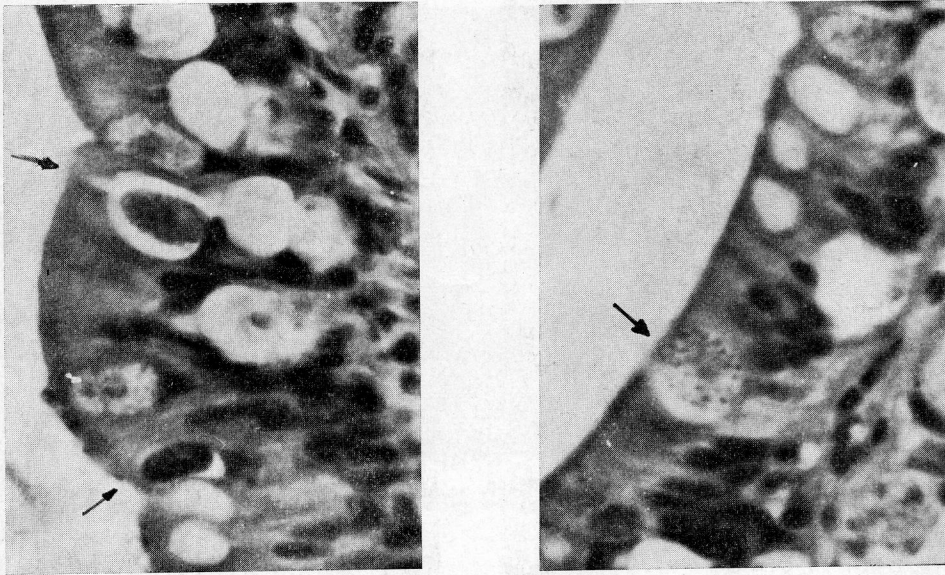


Fig. 6. Growing microgametocyte
 Fig. 7. Mature microgametocyte

and are elongated oval-shaped. Nuclear division, not followed by subdivision of the cytoplasm, is going on also in the periphery, resulting finally in the formation of 80–120 microgametes. The mature microgametocyte (Fig. 7) is an elliptic body, $10\text{--}12 \times 7.0\text{--}8.5 \mu\text{m}$ in size, with a pale-staining cytoplasm, filled by rod-shaped microgametes. These are $1.5\text{--}2.0 \mu\text{m}$ long by $0.2\text{--}0.3 \mu\text{m}$ wide.

The early macrogametes (Fig. 8) cannot be easily differentiated from the microgametocytes. They are round bodies with a centrally placed nucleus and a dark-staining cytoplasm. During the development of the gamete many granules, not taking up the stain, appear in the cytoplasm, imparting thereby a net-like structure to the macrogamete. At this stage, a few microgamete often occur nearby the surface of the female gamete. Large, eosinophilic clumps (plastin) (Fig. 9) appear inside the macrogamete before this reaches its final size. The plastin clumps gradually migrate towards the periphery of the female cell, to form the future oocyst wall. At this stage of development the zygote has a pale colour and a foamy structure, it is $8.5\text{--}10.2 \mu\text{m}$ in diameter, and has a central nucleus, $2.0\text{--}2.5 \mu\text{m}$ in diameter. Sporogony is being continued in the finished oocyst ($9.0\text{--}11.0 \mu\text{m}$ in diameter in histological sections), and after division of the nucleus into four parts, corresponding compartmentalization of the cytoplasm gives rise to the sporocysts (Fig. 10). The early sporocysts are oval bodies, tapering at one end, rounded at the

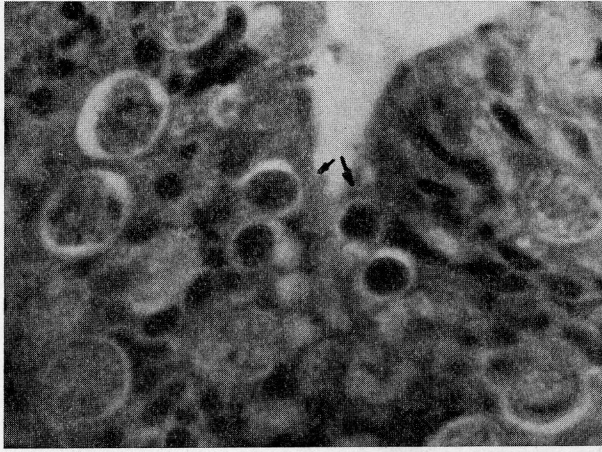


Fig. 8. Early macrogamete

other, with the nuclear substance residing at the tapering pole. Later the sporocysts gradually assume an elliptic shape, and each gives rise to two sporozoites.

Apart from the above stages, small, dark-staining trophozoites (Fig. 11), surrounded by a light halo of parasitophorous vacuole, are often encountered in supranuclear localization inside intestinal epithelial cells. The trophozoites, which probably give rise to second generation schizonts, cling close to the nucleus of the host cell, being thus surrounded by the nucleus in a semilunar manner.

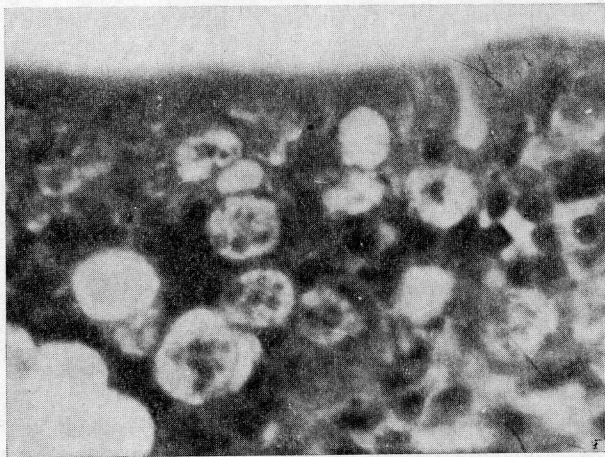


Fig. 9. Growing macrogametes with cytoplasmic plastin granules

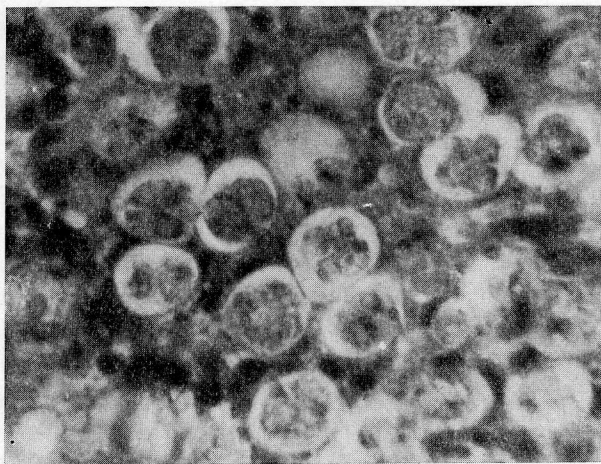


Fig. 10. Early and sporulating oocysts in the mucosal propria

The different stages of the cycle were apparently not limited to given intestinal segments. Both oocysts and schizonts have been found in all parts of the gut. Young fish, infected for the first time, chiefly harboured the parasites in the foregut, while in older hosts, the bulk of stages occurred in the midgut.

Severely infected hosts had oocysts, gametes, and a few schizonts in the first segment of the gut. Most massively invaded was the second quarter of the gut, in which macro- and microgametes filled the entire epithelial lining.

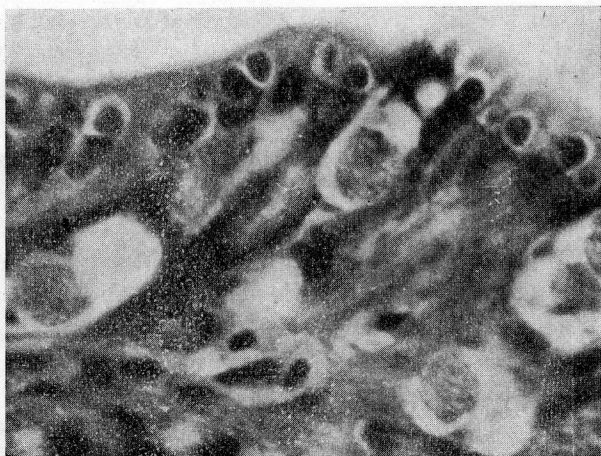


Fig. 11. Trophozoites in the intestinal epithelium. Growing macrogametes in the propria

More posteriorly oocysts or, less often, macrogametes were found in decreasing numbers. In this segment, above all in the midgut region, the epithelial layer above the oocyst-packed propria was filled by many trophozoites, indicating multiple invasions.

Although the intestinal tissues become considerably injured by the parasites, the histopathological changes are usually milder than would be expected from the intensity of the parasitosis. While sometimes a half of the epithelial cells and most cells of the lamina propria are invaded by parasites, neither inflammatory nor haemorrhagic lesions develop in the intestinal wall. Injury of the intestinal epithelium is due chiefly to schizont development and release of merozoites inside the cells, but microgametocytes, often establishing themselves in epithelial cells and propria, may also be responsible for epithelial necrosis. Macrogametes rarely occur in epithelial cells, invading above all the top row of propria cells immediately beneath the epithelium but, with more intensive infection, they may penetrate the deeper-seated layers, too, down to the submucosa, thereby giving rise to deformation of the entire propria. The nucleus of the host cell is visible for some time at one pole of the early macrogametes, but it gradually vanishes and then disappears as the gamete grows, and the developing oocyst(s) become surrounded by necrotic, coagulated cell residues. The intact propria cells form islets between oocysts. Since the oocysts, localizing either singly or in groups, are considerably larger than the cells, they block the supply of nutriment to the superposed epithelium, which thus becomes necrotic. Across the gaps so arisen, the oocysts become extruded into the intestinal lumen, embedded in the so-called "yellow bodies" arisen from cell debris. Regeneration, progressing from inside to the outward (from the submucosa towards the epithelium) also helps to push the oocysts towards the intestinal lumen. Regeneration of the intestinal mucosa may also start from the intact cell islets wedged between the oocysts. The empty space left by the extruded oocyst becomes filled first by exudation, the shrinking of which may later on be followed by primary healing.

Oocysts localizing in the submucosa were found either in intestinal segments free from folds, or in intensely infected folds. Since no stages were found to develop in submucosal cells, the oocysts found in this layer had in all probability been pushed thereto from the propria.

Although *E. sinensis* coccidiosis is characterized by the presence of viscous, mucous exudation, coating both the lining and the contents of the intestine in a tube-like manner, no quantitative increase of goblet cells was demonstrable in the specimens studied. The exudation presumably gained access to the lumen from injured epithelial cells and across the discontinuities of lining arisen where oocysts had been extruded into the lumen. Not even the massively infected hosts developed haemorrhagic or inflammatory changes in the gut.

Discussion

The present findings suggest a high incidence of *E. sinensis* coccidiosis in silver carp and bighead populations reared in the pond farms of Hungary. The infection may occasionally be severe. Despite the diversity of measurements, I have regarded the oocysts found in the examined hosts as one species, because the morphology of the smallest to largest oocysts has been similar in every respect. The original description (CHEN, 1956) is in need of a certain revision because, unlike his written statement and drawing, the oocyst wall is not thick, but distinctly thin. MUSSELIUS and LAPTEV (1967) have referred to the occurrence of *E. cheni* Schulman and Zaika, 1962, in mixed infection with *E. sinensis*. This is, however, quite improbable because in view of the strict host specificity of the *Eimeria* species, it can be hardly imagined that the oocysts originally described from a phylogenetically distant host, (*Mylopharyngodon piceus*), could occur in *Aristichthys* and *Hypophthalmichthys* species.

Since no literary data have been available on the morphological features of *E. sinensis* schizonts and on the tissular localization of these and other developmental stages, the present findings can only be compared with related data on the better-known fish coccidium *E. carpelli*. Already LÉGER and STANKOVITCH (1921) mentioned that the schizogonic development of *E. carpelli* takes place in the epithelium, and somewhat later PLEHN (1924) reported massive infection of the epithelium by growing schizonts. SCHÄPERCLAUS (1943) found sporogonic stages in both epithelium and subepithelial cell layer, while MUSSELIUS and LAPTEV (1965) found oocysts exclusively in the epithelium. According to LUCKY (1965), the *E. carpelli* oocysts occur chiefly in the mucosal propria, frequently in the epithelium and quite rarely in the submucosa.

The schizogonic development of *E. sinensis* takes place in the epithelium. Gametogonic and sporogonic stages occur, apart from the epithelium also in the propria, and a few oocysts are even demonstrable in the submucosa. Involvement of the deeper-seated tissues does not, however, exclude the probability that gametogony, too, begins in the epithelial cells, and the stages penetrate the deeper layers only later, just as shown by MARINCEK (1973) for *E. subepithelialis*, and also postulated to occur in the coccidioses of warm-blooded animals.

Summary

Coccidiosis due to *Eimeria sinensis* has been shown to occur frequently in silver carp and bighead populations cultured in the pond farms of Hungary.

The schizogonic development of *E. sinensis* takes place in the intestinal epithelium, but gametogonic and schizogonic stages frequently occur also in the propria and submucosa.

All developmental stages of *E. sinensis* can occur in any segment of the gut, but intensive infection is chiefly found in the fore- and midgut. The oocysts are passed in a sporulated state.

The main gross lesions found in *E. sinensis* coccidiosis are swelling of the mucosa, and coating of the intestinal epithelium by a viscous mucus, in which many "yellow bodies" enclosing oocysts are encountered. Haemorrhagic and inflammatory lesions do not even occur with very massive infections.

References

- Бауер, О. Н., Мусселиус, В. А., Стрелков, Ю. А.: Болезни прудовых рыб. Москва, 1969. SHEN CHIH-LEU: Acta Hydrobiol. Sinica 2 (1956), 279—299. LÉGER, L. and STANKOVITCH, S.: Compt. Rend. Acad. Sci. 85 (1921), 742—744. LOM, J.: Folia Parasit. 18 (1971), 289—293. LUCKÝ, Z.: Sborník Vysoké Školy Zem. v Brně 13 (1965), 393—426. MARINEK, M.: Acta Protozool. 12 (1973), 195—215. MOLNÁR, K.: Acta vet. hung. XXI (1971), 1—14. Мусселиус, В. А. and Лаптев, В. И.: Паразитология 1 (1967), 333—338. Мусселиус, В. А., Лаптев, В. И. and Иванова, Н. С.: Тр. ВНИИПРХ 13 (1965), 68—77. ПЛЕНН, М.: Praktikum der Fischkrankheiten. Stuttgart, 1924. SCHÄPERCLAUS, W.: Z. f. Fisch. 41 (1943), 283—295. Змерзлая, Е. И.: Зоол. Журн. 45 (1966), 305—308.

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