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RECORD OF PLAGIORCHIS NEOMIDIS BRENDOW, 1970 (TREMATODA: PLAGIORCHIDAE) IN CZECHOSLOVAKIA AND STUDIES ON ITS LIFE CYCLE

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Abstract. During the studies on helminth fauna in small mammals in the vicinity of České Budějovice, the trematode *Plagiorchis neomidis* Brendow, 1970 was found in *Neomys fodiens*. This is the first record of this parasite in Czechoslovakia. The molluscs *Radix p. peregra* and *Radix auricularia* served as its first intermediate hosts. It was demonstrated experimentally that the second intermediate hosts are larvae of the mosquitoes, *Culex pipiens molestus*, *C. territans* and *Dixa* sp., and of blackflies, *Prosimulium tomosvaryi*, *Eusimulium angustipes*, *Odagmia ornata* and *Simulium noelleri*.

Brendow (1970) described *Plagiorchis neomidis* from *Neomys fodiens* from two localities near Giessen (F.R.G.). He described also the larval stages and life cycle of this trematode. Theron (1976) confirmed the validity of the species on the basis of the studies of the chaetotaxy of cercariae.

The helminth fauna of insectivores of the family Soricidae from Czechoslovakia has been studied intensively (Prokopič 1956, 1957a, b, c, 1959, Mituch 1964), but of Plagiorchidae only *Plagiorchis exasperatus* (Rudolphi, 1819), *Opisthioglyphe megastomus* Baer, 1943 and *O. anomali* Prokopič, 1957 (from *Neomys anomalus*) have been recorded.

Since the cercariae of *P. neomidis* were abundant in *Radix p. peregra* in a locality near České Budějovice, also small mammals were examined for the presence of this parasite. All developmental stages of *P. neomidis* were studied in detail. The faunistical data were supplemented with some new facts concerning the life cycle and by scanning electron microscopic studies of the cercariae.

MATERIAL AND METHODS

The molluscs were collected in a draining canal with low level of slowly flowing water. The water was drawn from 4 mutually connected small ponds situated about 6 km to the north of České Budějovice. The canal flows first through the bushes and the whole area in which the small mammals were caught is soaked with the water from ponds.

A total of 41 small mammals of the following species were dissected: *Sorex araneus* (9), *S. minutus* (1), *Neomys fodiens* (1), *Micromys minutus* (1), *Apodemus flavicollis* (3), *A. sylvaticus* (3), *Clethrionomys glareolus* (1), *Microtus arvalis* (10), and *M. agrestis* (12).

Adult specimens of trematodes recovered from the mammals were fixed in 70 % alcohol, stained with borax carmine and drawn using a projector. The morphology of larvae was studied mostly in live specimens. Cercariae releasing the snail, as well as cercariae inside the sporocysts were observed. Vital stains (neutral red, Nile Blue sulphate) were used for staining of some structures. Permanent mounts were prepared by the method of Ždárská (1963). The cercariae were measured after heat fixation (at 80 °C) after Bock (1982). The stylet of cercariae, sporocysts and cysts with metacercariae were measured in live specimens slightly pressed with the cover glass. Always 30 specimens were measured. The measurements with arithmetic mean in parentheses are given below.

Larvae of mosquitoes and blackflies bred in the laboratory and fed with micromin up to the 3rd instar were used for experimental infections. Each of them was put in 1 ml of water (using haemaglutination plates) and 15 cercariae immediately after the release from the snail were added for 3 h. Then the larvae were put into flasks containing about 100 specimens each. The water in the flasks was aerated and the larvae were examined for the presence of metacercariae. The same procedure was used for the control specimens.

For the studies in scanning electron microscope, the cercariae were washed with glucose solution, transferred to 0.1 M cacodylate buffer (pH 7.2 for 2 × 5 min) and fixed in 4 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 °C for 2 –24 h. After fixation the material was washed with 0.1 M cacodylate buffer and postfixed in 2 % osmium solution in 0.1 M cacodylate buffer. Then it was dehydrated through an alcohol series, transferred to aceton, critical point dried and coated with gold. Tesla BS-300 scanning electron microscope operating at 15 kV was used for the examination of the material.

RESULTS

Ten trematodes of the species *Plagiorchis neomidis* Brendow, 1970 were found in the small intestine of one of the examined *Neomys fodiens* originating from the studied locality. No other specimens of Plagiorchidae were found in other small mammals examined.

Description of the trematodes (Fig. 1): The body is oval, elongate, and measures 0.598 to 0.811 mm in length and 0.252—0.320 mm in width at the level of posterior margin of ventral sucker. The cuticle is covered with fine spines on the whole body surface. The oral sucker measures 0.135—0.172 × 0.147—0.184 mm. The ventral sucker is situated in front of midbody and measures 0.095—0.124 × 0.098—0.132 mm. The pharynx measures 0.045—0.064 × 0.042—0.070 mm. The intestinal branches are well developed and run up to the posterior end of body. The testes are situated in the posterior half of body. The anterior testis measures 0.104 — 0.124 × 0.088—0.110 mm and the posterior one 0.109—0.127 × 0.090—0.118 mm. The cirrus sac is well developed, with two distinctly separated seminal vesicles. It begins immediately below the ventral sucker, runs along its right margin and opens in the left hal fin front of the ventral sucker, together with the uterus, in genital atrium. The ovary is almost spherical, measures 0.065—0.080 × 0.068—0.094 mm and is situated immediately behind the ventral sucker, being slightly shifted to the right side. The uterus fills the space between both testes and vitelline follicles in the posterior half of body. It is separated from the posterior end of body by the vitelline follicles. In the anterior part, the uterus runs left to the ventral sucker and penetrates into genital atrium. The vitelline follicles form separated follicles of different size. In anterior part, they are united behind the posterior margin of the oral sucker, and laterally they run up to the posterior end of body. The seminal receptacle and Laurer's canal are present. The eggs measure 0.028—0.032 × 0.018—0.022 mm.

The sporocysts (Fig. 2) were found only in the hepatopancreas of spontaneously infected snails. They are elongate, saccular, 1.03—2.50 (1.82) mm long and 0.25—0.35 (0.30) mm wide, of white to light orange colour. They contain a large number of cercariae at different stages of development and 5—10 mature cercariae.

Cercaria (Fig. 3): The body of cercaria is 0.276—0.334 (0.307) mm long and 0.113 to 0.151 (0.136) mm wide. Its whole surface is covered with fine spines diminishing posteriorly. The subterminal oral sucker measures 0.050—0.065 (0.055) × 0.051—0.065 (0.059) mm and is larger than the ventral sucker, which measures 0.034—0.042 (0.038) by 0.035—0.042 (0.040) mm and is situated at the beginning of the posterior half of body. Short prepharynx and well developed pharynx (0.015—0.022 (0.019) × 0.016 to 0.022 (0.019) mm) lie behind the oral sucker. The other parts of the digestive system were not visible in the native preparation. However, in permanent preparations stained

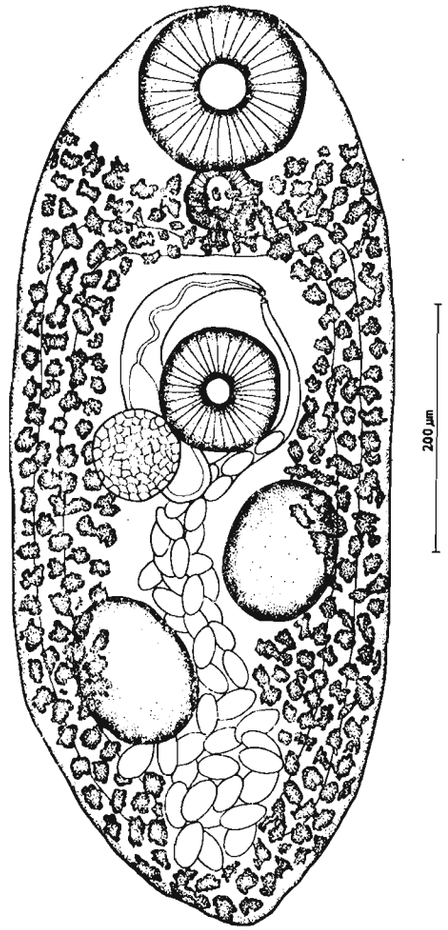

Fig. 1. *Plagiorchis neomidis* Brendow, 1970 from *Neomys fodiens*. Adult trematode (ventral view).

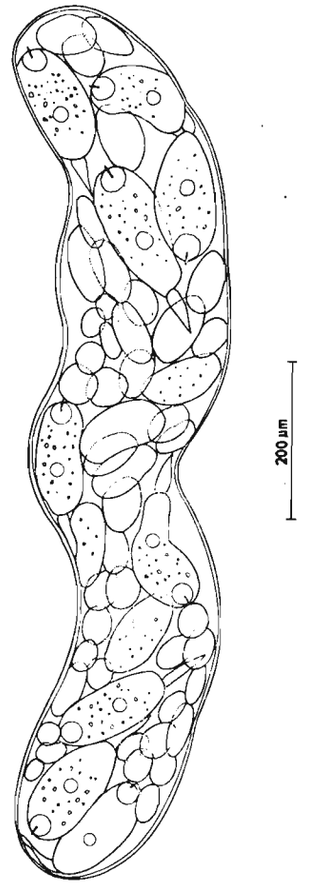

Fig. 2. Sporocyst of *Plagiorchis neomidis*.

with borax carmine, the oesophagus and intestine could be observed. The oesophagus bifurcates about half the distance between the posterior margin of oral sucker and anterior margin of ventral sucker, and the thin intestinal branches reach up to the lower margin of the excretory bladder. The thick-walled excretory bladder adheres to the caudal sheath which is provided with thick spines. The course of the excretory canal in the tail and its opening were not visible. Also the excretory canals and flame cells in the body of cercaria could be hardly observed and they cannot be described reliably. In the whole body, except for the anterior part, there is a large number of minute cystogenic gland cells (Fig. 4a), which, together with the spherical fat droplets, make the observation of inner structures very difficult. Seven pairs of large penetration glands opening near the stylet are situated between the pharynx and ventral sucker. The stylet is situated apically (Fig. 4b) and measures 0.029—0.032 (0.030) mm in lenght

and 5.5—6.0 μm in width at the base. In the middle of the stylet base there is a columella similar to that reported by Žďárská (1966) in *Plagiorchis laricola*. The horseshoe-shaped cluster of cells at the level of ventral sucker is the anlage of genital organs.

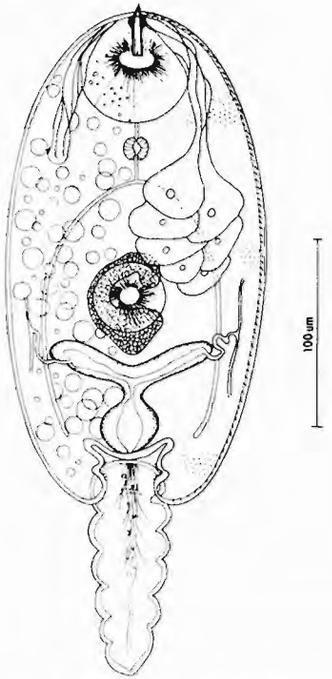

Fig. 3. Cercaria of *Plagiorchis neomidis*.

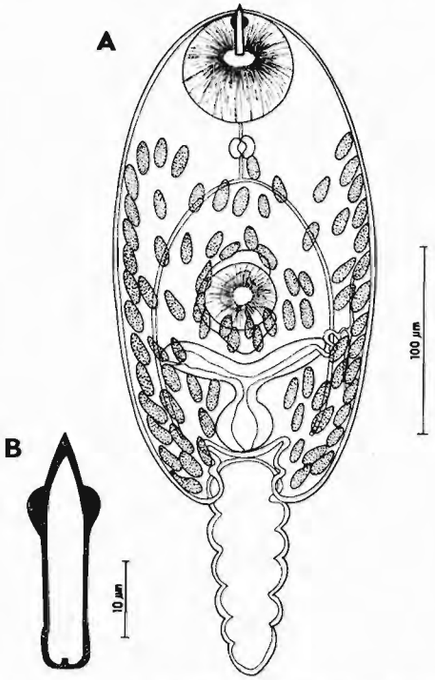

Fig. 4. A — Cercaria of *Plagiorchis neomidis* (arrangement of cystogenous cells), B — Stylet.

The observations in the light microscope were supplemented by scanning electron microscopy (SEM) (Plate I, II, Figs. 1—4). The majority of cercariae observed in SEM have strongly sunken ventral side and convex dorsal side (Plate I, Fig. 1). The tegument of cercaria body, with the exception of the apical part around the stylet, is covered with spines on the dorsal and ventral sides (Plate I, Figs. 2, 3, 4, Plate II, Figs. 2,3). The spines are orientated posteriorly and they are arranged in regular rows. The size of cuticular spines is about 1.0—1.2 μm, the smallest ones being in the posterior part of body, particularly around the tail base. The spines occur also on the overlapping tegument of both suckers; their number and size on the acetabulum (Plate II, Fig. 3) are much smaller than of those on the tegument on the remaining parts of body. Sensory papillae can be observed between the spines on the whole tegument. They are most numerous in the vicinity of the oral sucker, where they are arranged in three circles, and around the stylet. On the body of cercaria, the papillae are arranged in two ventral (Plate I, Fig. 1), two dorsal and two lateral lines. Around the oral sucker and stylet (Plate I, Figs. 2, 4, Plate II, Fig. 1), there occur papillae without cilium (with empty middle pit), papillae with a short cilium, and papillae with a long cilium. The maximum length of cilium in this part of body is about 4 μm. The cilia of papillae on the dorsal

(Plate I, Fig. 3) and lateral (Plate II, Fig. 2) sides of body, however, are much larger, up to 11 μm. The papillae with short cilium arranged in two circles occur around the ventral sucker (Plate II, Fig. 3) and the same type of two papillae, which are characteristic of the family Plagiorchidae, are situated mediodorsally even in the midlength of tail (Plate II, Fig. 4). The cilia on the tail are about 2 μm long.

Under experimental conditions, the metacercariae (Fig. 5) developed in the larvae of mosquitoes and blackflies and their development were observed for several days. The stylet releases from the oral sucker already after two days and freely moves inside the cyst. The penetration glands disappear, the fat droplets in the body decrease in number, and the number of excretory granules in the excretory bladder increases. The cysts are oval to spherical, with 0.003—0.005 mm thick envelope, and their diameter is 0.163—0.226 (0.201) mm after five days of development.

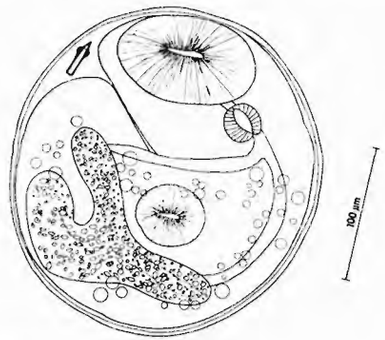

Fig. 5. Metacercaria of *Plagiorchis neomidis*.

Life cycle: In the locality under study, the cercariae of *P. neomidis* were found in molluscs of the species *Radix p. peregra* (incidence of infection 11.9 %), which is eudominant in this locality, and *R. auricularia* (3.2 %). In the examined *Lymnaea stagnalis* and *Planorbarius corneus*, the number of which was much smaller, no *P. neomidis* were found. The larvae of water insects, potential second intermediate hosts, were not examined in this locality.

We managed to infect experimentally the larvae of mosquitoes, *Culex pipiens molestus*, *C. territans*, *Dixa* sp. of 2nd—3rd instar, and larvae of blackflies, *Prosimulium tomosvaryi*, *Eusimulium angustipes*, *Odagmia ornata*, and *Simulium noelleri* at the same stage of development. These species of larvae are very attractive for *P. neomidis* cercariae and are attacked by them immediately after the contact. Encysted metacercariae were found already after some minutes. They are usually localized in the abdomen (65 %), less frequently in the thorax (25 %) and head (10 %). Positive results were obtained in all of the above-mentioned species of intermediate hosts.

The attempts to infect the molluscs *L. stagnalis*, *Planorbis planorbis*, *P. corneus*, and *R. auricularia* with the cercariae, as well as the control experiments, were negative.

Since the definitive hosts, insectivores, were not available, it was not possible to verify the life cycle under laboratory conditions in order to obtain adults of *P. neomidis* or to verify the infectivity of metacercariae at different stages of development.

No positive results were obtained in the intermediate hosts containing 10-day-old metacercariae were fed to laboratory mice and chickens.

DISCUSSION

In the present paper, a new distribution area of *Plagiorchis neomidis* is recorded supplementing thus the data published by Brendow (1970) and Theron (1976). These two authors found *P. neomidis* in the same host — *Neomys fodiens*. In our studies, when 6 species of rodents and 3 species of insectivores were examined, this parasite was found also in *N. fodiens* only. We consider this fact to be important since no narrow specificity to the definitive host occurs in the majority of Plagiorchidae.

The morphology and measurements of adult specimens conform to the data published by the above authors. The localization in the small intestine of the definitive host corresponds to the record by Brendow (1970), whereas Theron (1976) found the trematodes only in the rectum, in both spontaneous and experimental infections. The measurements and morphology of the cercariae also correspond to the description by the two authors. We described a different number of penetration glands (7 pairs), the course of digestive system and shape of the stylet (columella). Brendow (1970), who considered the great variability of adults of *P. neomidis* and *P. elegans*, admitted that the two species might be mistaken for one another. He gave some criteria for the larval stages which he regarded as important diagnostic characters for the differentiation of these two species. Although we do not fully agree with this author (spinulation of the body of cercaria and metacercaria of *P. elegans*) we supplement his data by the fact that the two species can be differentiated from one another by the presence of numerous and conspicuous fat droplets in *P. neomidis* cercaria.

Theron (1976) states that the metacercariae reach the maximum size after 60 days of development inside the second intermediate host at the temperature of 10 °C. He describes well differentiated anlages of genital organs (they start to differentiate on about day 12) in older metacercariae. Although the maximum size and infectivity do not seem to be reached at the same time, we assume that the metacercariae of this species become infective at the temperature of 25 °C after 7—10 days (with regard to the morphology of the metacercariae at that time and to the experience with metacercariae of other species of this family). This corresponds to the conclusions by Brendow (1970) who successfully infected 2 *Sorex* species with 10—15-day-old metacercariae (the temperature was not given).

In conclusion it should be stressed that *R. auricularia* is recorded as a new first intermediate host and larvae of Simuliidae as new second intermediate hosts of trematodes of the family Plagiorchidae.

НАХОДКА ТРЕМАТОДЫ *PLAGIORCHIS NEOMIDIS* BRENDOW, 1970 (TREMATODA: PLAGIORCHIDAE) В ЧЕХОСЛОВАКИИ И ИЗУЧЕНИЕ ЕЕ ЖИЗНЕННОГО ЦИКЛА

Я. Бушта и В. Нашинцова

Резюме. При изучении гельминтофауны мелких млекопитающих в окрестности г. Ческе Будеевице была обнаружена трематода *Plagiorchis neomidis* Brendow, 1970 у *Neomys fodiens*. Это первая находка этого паразита в Чехословакии. Молюски *Radix p. peregra* и *Radix auricularia* являлись первыми промежуточными хозяевами этой трематоды. Было показано экспериментально, что в качестве вторых промежуточных хозяев используются личинки комаров, *Culex pipiens molestus*, *C. territans* и *Dixa* sp. и мошек, *Prosimulium tomosvaryi*, *Eusimulium angustipes*, *Odagmia ornata* и *Simulium noelleri*.

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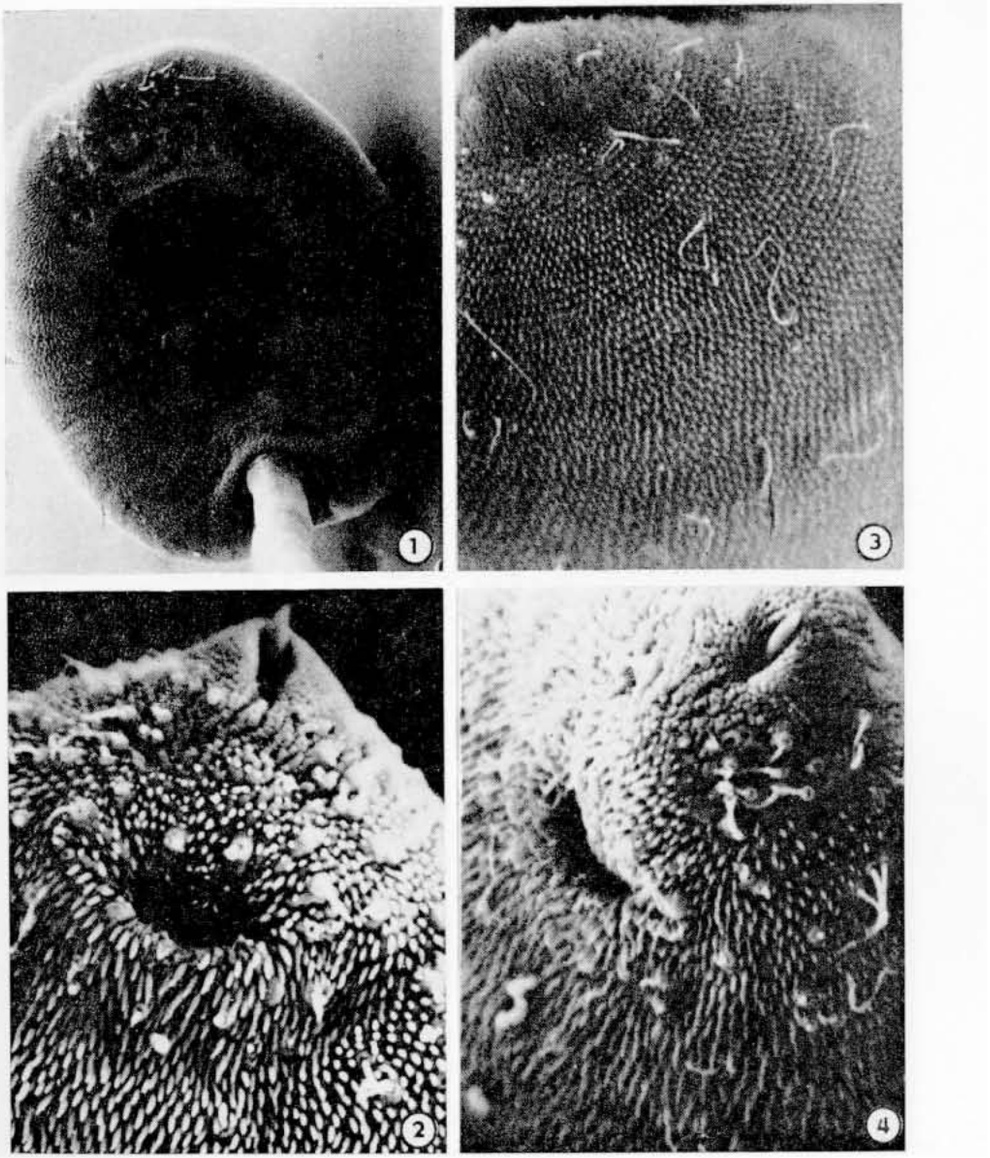
OCCURRENCE OF SOLENOPOTES CAPREOLI (ANOPLURA) IN CZECH LANDS

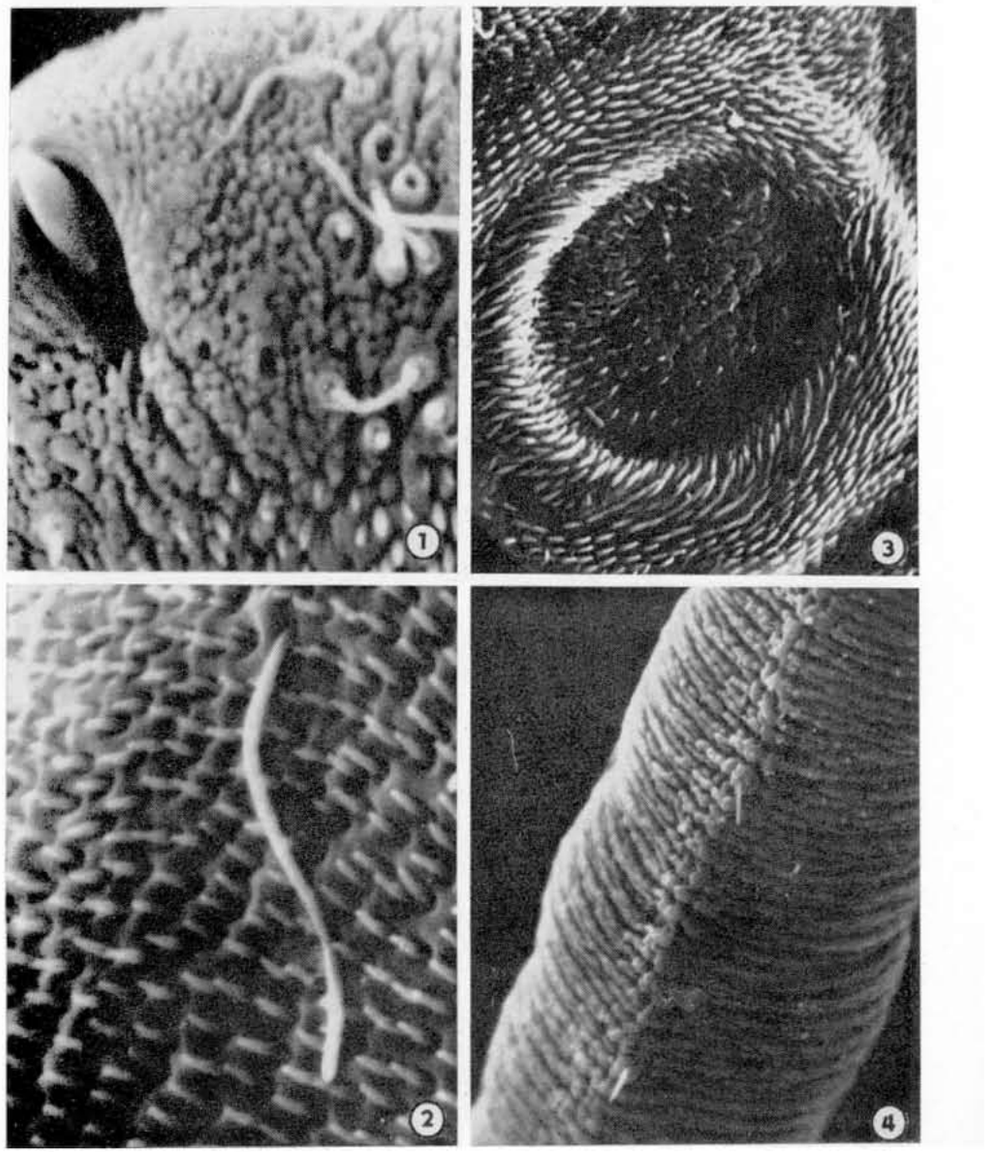
The roe deer louse, *Solenopotes capreoli* was described by Freund (Parasites, transmetteurs, animaux venimeux. Rec. Trav. dédié au 25 me Anniv. Sci. Prof. E. Pavlovsky, 1909—1934: 275—280, 1935) in a hardly available publication on the basis of two specimens from Bohemia. The locality was not given. The species was differentiated from *S. burmeisteri* (Fahrenholz, 1919) from red deer. In spite of this, *S. capreoli* was not included in the list of lice from Czechoslovakia either by Smetana (Ac. Rer. Natur. Mus. Nat. Slov., Bratislava 11: 30—38, 1965) or by Černý (Acta faun. Ent. Mus. Nat. Pragae 15, Supplem. 4: 53—54, 1977). This species, generally considered to be very rare, was not mentioned in faunistical surveys of parasites of deers from Czechoslovakia (Kotrlý A., Práce VÚLHM 44: 229—243, 1973), Switzerland (Bouvier G., Parasitol. Schr. Reihe 4: 1—18, 1956), Austria (Kutzer E., Hinaidy H. K., Z. Parasitenk. 32: 354—368, 1969), Poland (Kadulski S., Acta paras. Pol. 23: 493—535, 1975), and G. D. R. (Gräfner G., Angew. Parasitol. 21: 177—182, 1980), though several hundreds of hosts were examined in these studies.

The more surprising were the results obtained by the senior author during the examinations of 231 cadavers and 224 heads of roe deers sent to the laboratory for the examination for rabies. *S. capreoli* was found on 57 specimens (prevalence 12.5 %) and a total of 1509 females, 529 males and 366 larvae were collected (mean intensity of infestation 42.2, abundance 5.3). The highest number of lice (388) were collected from a roe caught on March 2, 1985 at Nová Ves nad Nisou (district Jablonec nad Nisou).

The species was found in Czech lands in 17 districts of 7 regions. The numbers in the following list indicate the number of specimens examined and the numbers in parentheses the number of positive specimens.
North Bohemia 290 (38): Česká Lípa 45 (3), Chomutov 21 (4), Děčín 20, Jablonec n. N. 35 (15), Liberec 44 (5), Litoměřice 63 (6), Louny 9 (1), Most 12, Teplice 16 (2), Ústí n. L. 25 (2). East Bohemia 70 (11): Chrudim 2, Havlíčkův Brod 1, Hradec Králové 1, Jičín 4, Náchod 10 (3), Pardubice 2, Rychnov n. K. 6, Semily 7 (1), Svitavy 20 (2), Trutnov 11 (3), Ústí n. O. 6 (2). South Bohemia 57 (5): České Budějovice 13, Český Krumlov 10, Jindřichův Hradec 10 (3),



Figs. 1—4. Scanning electron micrographs of *Plagiorchis neomidis* Brendow, 1970 cercariae. **Fig. 1.** Body of cercariae (ventral view) (×360). **Fig. 2.** Anterior part; stylet, oral sucker and papillae (ventral view) (×3 100). **Fig. 3.** Anterior part (dorsal view) (×2 500). **Fig. 4.** Anterior part; stylet, oral sucker and papillae (ventrolateral view) (×2 700).



Figs. 1—4. Scanning electron micrographs of *Plagiorchis neomidis* Brendow, 1970 cercariae. **Fig. 1.** Detail of the papillae without cilium, papillae with a short cilium and papillae with a long cilium around the stylet (ventral view) (×4 500). **Fig. 2.** Detail of the lateral papilla with a long cilium (at the end of the first third of anterior part of body) (×4 900). **Fig. 3.** Ventral sucker (×2 500). **Fig. 4.** Detail of the tail papillae (dorsal view) (×3 150).