Prosolecithus danubica n. sp. (Digenea : Dicrocoeliidae) a new digenean from shrews on islands of the Danube delta

TKACH V.V.* & BRAY R.A.**

Summary :

The helminth fauna of insectivores of the Danube delta has not been studied before. Our investigation was carried out in both Ukrainian and Romanian parts of the delta. As a result, 33 helminth species from five species of insectivores were found, including a new dicrocoeliid digenean species, *Prosolecithus danubica*, from the liver of *Sorex araneus* and *Neomys anomalus*. *Prosolecithus* includes one other species, *P. pellucidus* (Pojmánska, 1957), originally described as *Dicrocoelium pellucidum*, from which *P. danubica* differs in sucker-ratio, configuration of vitelline fields and egg-size. An illustrated description of the new species and a generic diagnosis are included and comments are made on its morphological variability, site, distribution in the delta and pathogenicity.

KEY WORDS : Dicrocoeliidae. *Prosolecithus*. *Sorex*. *Neomys*. Danube delta. systematics. zoogeography. pathogenicity.

MOTS CLES : *Dicrocoeliidae.* Prosolecithus. Sorex. Neomys. *delta du Danube. systématique. zoogéographie. pathogénicité.*

Résumé : Prosolecithus danubica n. sp. (Digenea : Dicrocoeliidae), un nouveau digène de musaraignes des îles du delta du Danube

La faune helminthologique des insectivores du delta du Danube n'a jamais été étudiée avant le présent travail. Nos recherches ont porté tant sur la partie ukrainienne que roumaine du delta, et ont permis de recenser 33 espèces d'helminthes provenant de cinq espèces d'insectivores. Parmi elles, figure un nouveau Digène Dicrocoeliidae, Prosolecithus danubica, *trouvé dans le foie de* Sorex araneus *et* Neomys anomalus. *Le genre Prosolecithus comprend une autre espèce,* P. pellucidus *(Pojmánska, 1957), décrite originellement sous le nom de* Dicrocoelium pellucidum, *et dont* P. danubica *diffère par le rapport de taille des ventouses, la configuration des champs vitellins ainsi que par la taille des œufs. Une description illustrée de la nouvelle espèce, ainsi qu'une diagnose du genre sont données et nous commentons également la variabilité morphologique de* P. danubica, *sa localisation dans l'hôte, sa distribution dans le delta ainsi que sa pathogénicité.*

INTRODUCTION

The helminth fauna of shrews in most of regions of central and western Europe has been comparatively well studied. A large number of articles have been devoted to this topic during the last 150 years and a rich fauna of parasitic worms has been discovered (see e.g. Vaucher, 1971; Andreiko, 1973; Genov, 1984). Some regions, including the faunistically and biogeographically interesting Danube delta, have been almost overlooked in this respect. During expeditions into the region between 1989 and 1993 we have examined five species of insectivores from different parts of the delta. Among 33 helminths species was a new digenean species belonging to a poorly known genus of the family Dicrocoeliidae. This finding is of particular interest, as dicrocoeliids are generally rare parasites of insectivores in the Palaearctic region. Only four species, *Lyperosomum danubica* (Diesing, 1858), *L. transcarpathicus* Bykhovskaja-Pavlovskaja, Vysotzkaja & Kulakova, 1970, *Corrigia danubica* Jourdane, Théron & Gabrion, 1980 and *Prosolecithus pellucidus* (Pojmánska, 1957) have been described from Europe. Two of these were reported in the Ukraine, *L. transcarpathicus* from *Sorex araneus* in the Carpathian mountains (Bykhovskaja-Pavlovskaja *et al.* 1970, 1978) and *L. danubica* from *Crocidura suaveolens* in the Black Sea Nature Reserve, Kherson Region (Davydov 1963; our unpublished data). Other representatives of the small shrew dicrocoeliid fauna are found much further south, mostly in Africa (Yamaguti 1958; Panin 1984). All of them inhabit mainly the bile ducts and are only rarely encountered in the gall-bladder. Except for its peculiar morphology and its pathogenicity, the new species is characterised by an unusually restricted distribution.

* Dr V.V. Tkach, I.I. Shmalhausen Institute of Zoology, Ukrainian Academy of Sciences, 15 Bogdan Khmelnitsky Street, Kiev-30, 252601, Ukraine.

** Dr R.A. Bray, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK – Tel. 0171-938-9492 –Fax 0171-938-8754, e-mail r.bray@nhm.ac.uk.

MATERIAL AND METHODS

The material was collected during 1989 (December), 1990 (January), 1992 (June, August, September) and 1993 (August) in the Ukrainian and Romanian parts of the Danube delta. Overall 75 specimens of insectivores (*Erinaceus concolor* – 2, *Sorex araneus* – 53, *Sorex minutus* – 4, *Neomys anomalus* – 11, *Crocidura suaveolens* – 5) from 14 localities were examined (Fig. 1), but digeneans belonging to the

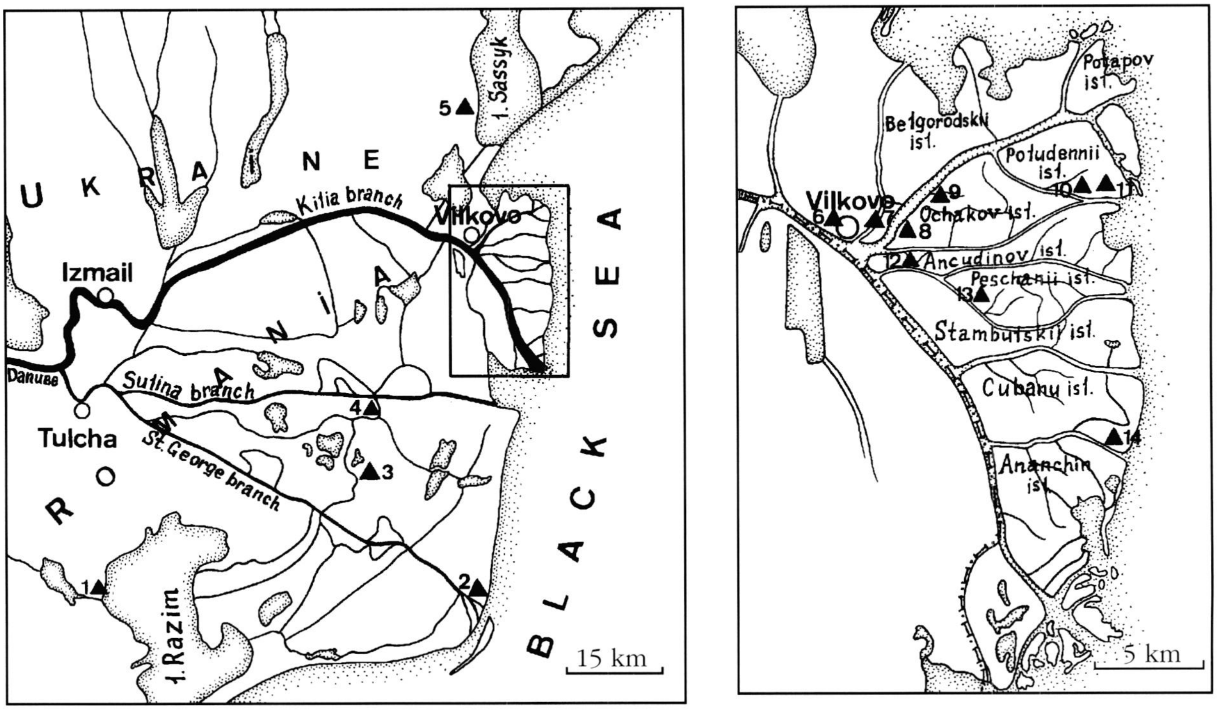

Fig.1. – Map of the Danube delta. Localities : 1. Lake Razim; 2. Sphintu George village; 3. Kara-Orman village; 4. Krishan village; 5. Lake Sassyk; 6-7. vicinities of Vilkovo town; 8-9. Ochakov Island; 10-11. Poludenii Island; 12. Ancudinov Island; 13. Peschanii Island; 14. Cubanu Island.

new species were found only in shrews captured on three islands, Ancudinov, Ochakov and Peschanii, situated in the Kilia branch of the Ukrainian part of the delta (localities 8, 9, 12 and 13 on Fig. 1). Immediately following dissection of the shrews, we examined the livers externally to check for digenean invasion, then detached the gall-bladder and carefully teased apart the lobes of the liver and the bile ducts with needles. Live worms were allowed to relax in water before being fixed in 70% ethanol, some under slight cover-glass pressure. Worms were stained with alum carmine and iron acetocarmine following Georgiev *et al.* (1986), dehydrated and mounted in Canada balsam. Several infected livers were fixed in neutral 4% formalin for histological study. 15-20 µm thick histological sections of infected livers were made to elucidate details of the morphology of the digeneans, their site and attachment to the walls of bile ducts and their pathogenic effect on the host tissues. Sections were stained with Mayer's haematoxylin and Gill's haematoxylin-eosin.

RESULTS

Family Dicrocoeliidae Odhner, 1910
Subfamily Eurytrematinae Yamaguti, 1958

Prosolecithus danubica n. sp. (Figs. 2-6)

Hosts : *Sorex araneus* L. (type-host), *Neomys anomalus* Cabrera (Insectivora, Soricidae).
Sites : gall-bladder and bile-ducts of the liver.
Localities : Ancudinov Island (type-locality), Ochakov Island and Peschanii Island, Kilia branch of the Danube, Kilia district, Odessa region, Ukraine (Fig. 1).
Material : several hundred specimens.
Holotype : Collection of the Department of Parasitology, Institute of Zoology, Kiev, Ukraine, slide number N 690-4, *Sorex araneus*, Odessa region, Danube delta, Ancudinov Island, 4.09.92, Collector V.V. Tkach.
Paratypes : Collection of the Department of Parasitology, Institute of Zoology, Kiev, Ukraine, on the same slide as holotype and also on preparations N 690-2,5,6; 684-1,2. Also Parasitic Worms Division, The Natural History Museum, London, UK, slide number N 690-1, registration number 1994.11.24.19.

Description

Measurements of holotype are given in text; data on other specimens are in table 1; all measurements in mm).

Body small, length 2.58, maximum width 1.08, body length/width ratio 2.4; elongate, strongly flattened, wide in middle portion and narrowing at both ends

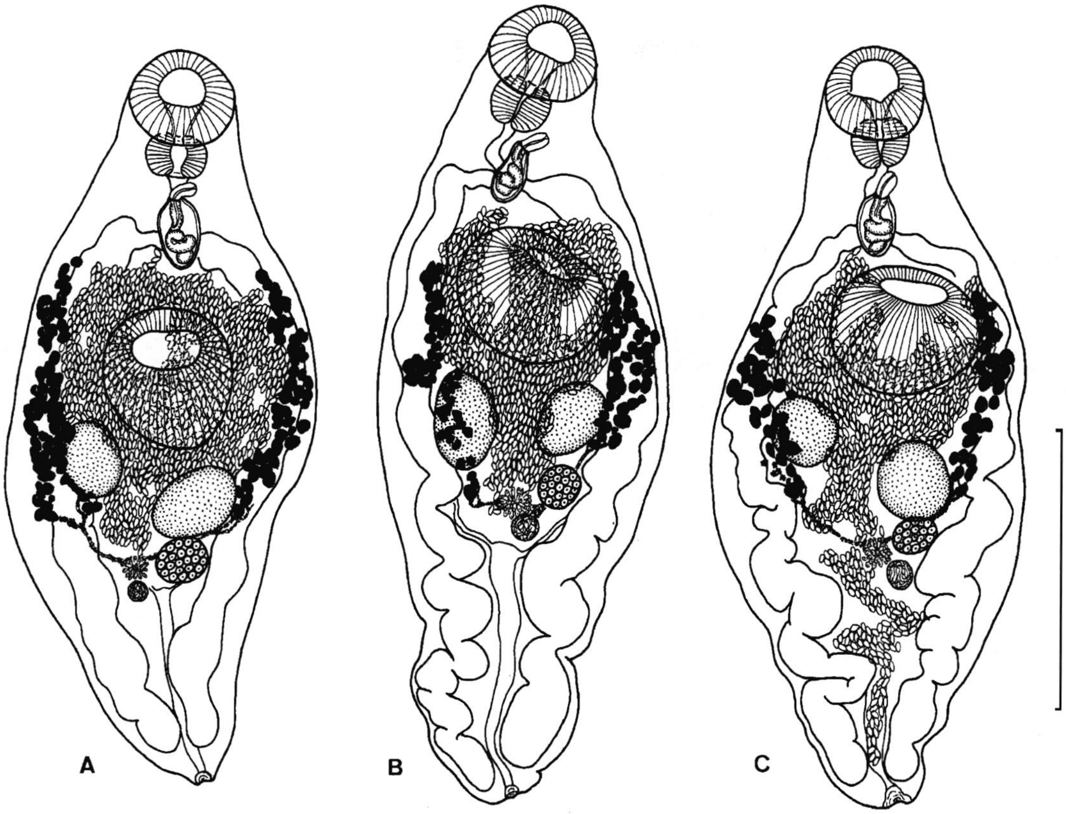

Fig. 2. – *Prosolecithus danubica* from *Sorex araneus* : A. Holotype ; B,C. Paratypes. Scale-bar : 1 mm.

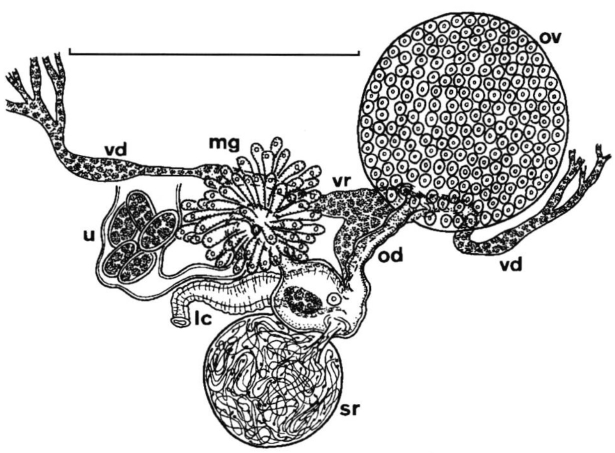

Fig. 3. – Part of female reproductive system of *Prosolecithus danubica.* Scale-bar : 0.2 mm. Abbreviations: ov, ovary; od, oviduct; o, ootype ; sr, seminal receptacle ; lc, Laurer's canal; mg - Mehlis' glands ; u, uterus ; vd, left and right common vitelline ducts; vr, vitelline reservoir.

Characters	Min-max	Average	Std	CV*
Body length	2.16-3.30	2.72	0.281	10.3
Body width	0.84-1.29	1.06	0.109	10.3
Body length/width ratio	1.97-3.03	2.58	0.276	10.7
Oral sucker	0.30-0.39	0.36	0.019	5.3
Ventral sucker	0.43-0.58	0.50	0.040	8.0
Ventral:oral sucker ratio	1:0.58-0.90	1:0.72	0.071	9.9
Distance between suckers centres	0.85-1.15	0.97	0.089	9.2
Pharynx	0.14-0.25	0.22	0.024	10.9
Right testis	0.20-0.34	0.26	0.038	14.6
Left testis	0.20-0.37	0.29	0.039	13.4
Cirrus sac length	0.23-0.35	0.28	0.035	12.5
Diameter of ovary	0.11-0.17	0.13	0.013	10.0
Eggs	0.038-0.046	0.041	0.0019	4.6

Table 1. – Metric characters of *Prosolecithus danubica* (type series, N = 20). * CV - coefficient of variation

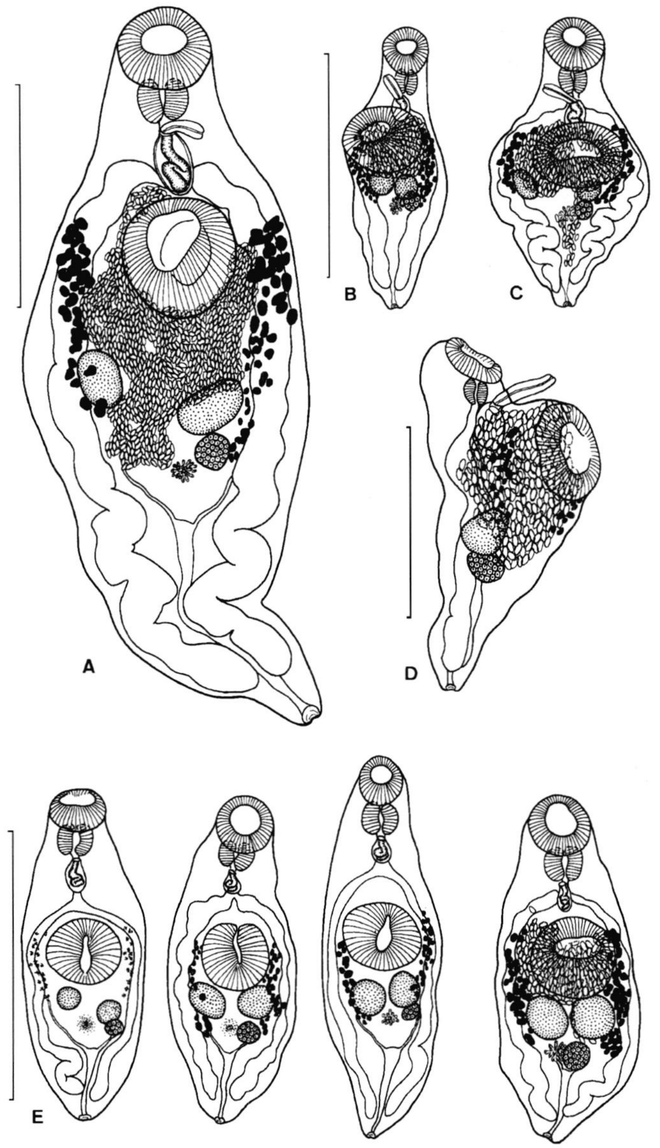

Fig. 4. – Variability of *Prosolecithus danubica* (note that A,B,C are figured to the same scale) : A. Specimen from an infection with an intensity of 30; B,C,D. Specimens from an infection with an intensity of more than 100; E. Four specimens at different stages of maturation from the same host. Scale-bars : A,B,C,E. 1 mm; D. 0.5 mm.

(Figs 2, 4). In living state posterior end usually curved ventrally. Tegument unspined, very thin. Subtegumental musculature weak, in three layers of sparsely distributed smooth muscle fibres - circular (external), diagonal (middle) and longitudinal (internal); fibres distributed more densely in anterior part of body. Weakness of subtegumental muscles precludes significant contraction in length of body.

Oral sucker subterminal, 0.37 in diameter. Ventral sucker rounded, 0.49 in diameter, anterior to middle of body length. Distance between centres of suckers 1.00. Oral/ventral sucker-ratio 0.75:1. In young specimens sucker-ratio larger due to allometric growth – ventral sucker continues to grow after oral has completed growth. Musculature of oral sucker consists of peripheral circular muscle fibres and dorso-ventral fibres. Ventral sucker musculature generally similar, but differs in disposition of muscle fibres. Circular muscles concentrated around aperture of sucker and sparse groups of fibres occur in other parts of sucker. Most of dorso-ventral muscle fibres are comparatively short, straight, situated peripherally around sucker, connecting external and internal surfaces; few reach from centre of external surface to internal surface around central part of sucker (Fig. 5). Central part of sucker free or almost free of muscular elements and filled by thick pillow-like layer of vacuolised parenchyma (Fig. 5), which facilitates formation of vacuum effect in attachment to epithelium.

Pharynx rounded, 0.17 × 0.23. Oesophagus short, 0.20. Intestinal bifurcation anterior to ventral sucker. Caeca terminate blindly near posterior extremity of body.

Testes rounded or oval, symmetrical or slightly oblique (right testis anterior), just posterior to ventral sucker. Right testis 0.26 × 0.21, left 0.33 × 0.24. Cirrus-sac small; 0.26 × 0.17; anterior to ventral sucker. Internal seminal vesicle coiled, tubular; merges with long straight muscular ejaculatory duct. Pars prostatica not clear, but few prostatic cells surround junction of seminal vesicle and ejaculatory duct. Ejaculatory duct may be everted to form unarmed cirrus measuring 0.08-0.16 long. Genital atrium distinct. Genital pore median at level of posterior edge of pharynx.

Female reproductive system typical for dicrocoeliids (Fig. 3). Ovary rounded, 0.16 × 0.18, just posterior to left testis. Lumen of oviduct covered with ciliated epithelium; dilates just proximal to oötype, forming chamber receiving duct of seminal receptacle, common vitelline duct and Laurer's canal. Seminal receptacle small, 0.10 × 0.12; near ovary. Laurer's canal, with strong circular musculature, opens dorsally in region of seminal receptacle. Mehlis' gland distinct. Vitellarium well developed, consisted of two groups of small follicles, stretched along both sides of body from level of anterior edge of ventral sucker to level of posterior edge of testes. Right and left vitelline collecting ducts merge to form vitelline reservoir, which opens by means of short common duct into ootype.

Numerous loops of uterus generally in middle third of body length, occupy space between level of anterior edge of ventral sucker and posterior edges of testes (or bifurcation of stem of excretory bladder). In living or unflattened, fixed trematodes most of uterine loops concentrated in region of ventral sucker whereas in specimens flattened under cover-glass, some loops may be displaced posteriorly and, as result, eggs rarely seen posterior to testes and may even reach ends of caeca (Fig. 2C). Latter condition never observed in living worms. Distal end of uterus forms metraterm with weak circular muscles, which opens into genital atrium. Eggs yellow to light brown, operculated, 0.042-0.043 × 0.018-0.020.

Excretory vesicle I-shaped reaching almost to ovary (Fig. 2A, B). Excretory pore terminal, surrounded by small sphincter.

Variability

Digeneans inhabiting the limited space of such internal organs as the liver, gall-bladder and kidney are usually characterised by substantial size variability, depending on the intensity of infection, possibly a 'crowding effect' (Halvorsen, 1976). *P. danubica* n. sp. is also characterised by a high variability in the body size of mature specimens. Worms taken from the same host may differ by 1.5 times in their length. Our material contains adult worms which differ by up to three times in length (Fig. 4). The intensity was 30 specimens in the case of Fig. 4A and more than 100 specimens in the case of Fig. 4B,C,D. Of the measured characters only egg-size is stable and does not depend on body size. Therefore, it can be concluded that in this species, as in many other species of digeneans, the absolute size of the body, suckers and other measured characters cannot be a good basis for reliable differentiation. The general morphology, topography of organs and sucker-ratio are more reliable in this case.

Age variability is weakly expressed in *P. danubica*. Digeneans of the same size, but at different stages of maturation, were frequently found in the gall-bladder or bile ducts of the same host (Fig. 4E).

DISCUSSION

Differential diagnosis

The new species most closely resembles *Prosolecithus pellucidus* (Pojmánska, 1957) described from *Sorex araneus* in Western Poland (Pojmánska, 1956, 1957). We have examined two slides with 11 (9 and 2) 'syntypes' of *P. pellucidus* from the Wroclaw Natural History Museum, labelled : '288. *Sorex araneus*, male, 14. VIII. 1952. Turew k. Poznania, leg. Kwiatkowska'. These specimens are not well fixed, are deformed or folded, flattened under cover glasses and only one specimen is suitable for measurement. *P. danubica* differs from *P. pellucidus* slightly in the ventral:oral sucker-ratio (1:0.58-0.90, mean 1:0.72 vs 1:0.78-0.92, mean 1:0.85, respectively). The vitellarium in the new species is longer, often reaching just posterior to the testes, and consists of smaller follicles than in *P. pellucidus*, although the vitelline field length is difficult to estimate in the types. Egg-size in the new species is also larger (0.038-0.046, mean 0.041 vs 0.028-0.036, mean 0.034). Therefore, it can be concluded that the digeneans from the Danube delta represent a new species.

Prosolecithus Yamaguti, 1971 is close, both in morphological (topography of suckers and reproductive system) and biological (parasitizing the gall-bladder and bile-ducts of small mammals) features, to some representatives of the genus *Zonorchis* Travassos, 1944 and to the species *Canaania obesa* Travassos, 1944, the only representative of the genus *Canaania* Travassos, 1944. *Prosolecithus* differs from the representatives of *Zonorchis* by the much shorter vitelline fields and, more importantly, in the disposition of the uterus, which in *P. danubica* and *P. pellucidus* is situated mainly in the region of the ventral sucker and does not extend posteriorly to the ovary (apart from in strongly flattened worms; the worms figured by Pojmánska (1957) are clearly well flattened), while in *Zonorchis* it occupies all of the hinder part of the body posterior to the ventral sucker. *Prosolecithus* differs from *Canaania* in the disposition and length of the vitelline fields – in *Canaania* the fields are extracaecal and almost reach to the end of the caeca. We consider as very important the difference in host groups and geographical distribution. *C. obesa* was described from South American field mice (or grass mice) in Brazil (Travassos 1944). It should be noted that representatives of the Insectivora are absent from that region. The most southerly Neotropical records of shrews are those of species belonging to the genus *Cryptotis* from Colombia, Venezuela and Equador (Honacki *et al.* 1982; Woodman & Timm 1993). We suggest that the complete modern and historical isolation renders impossible the existence of a genus, one representative of which is living in Brazil and two are from central Europe.

Generic diagnosis : *Prosolecithus* Yamaguti, 1971. Dicrocoeliidae, Eurytrematinae. Small digeneans. Body broad, maximum width in mid-body and narrowing posteriorly. Tegument unspined. Oral sucker subterminal. Prepharynx absent. Pharynx well develo-

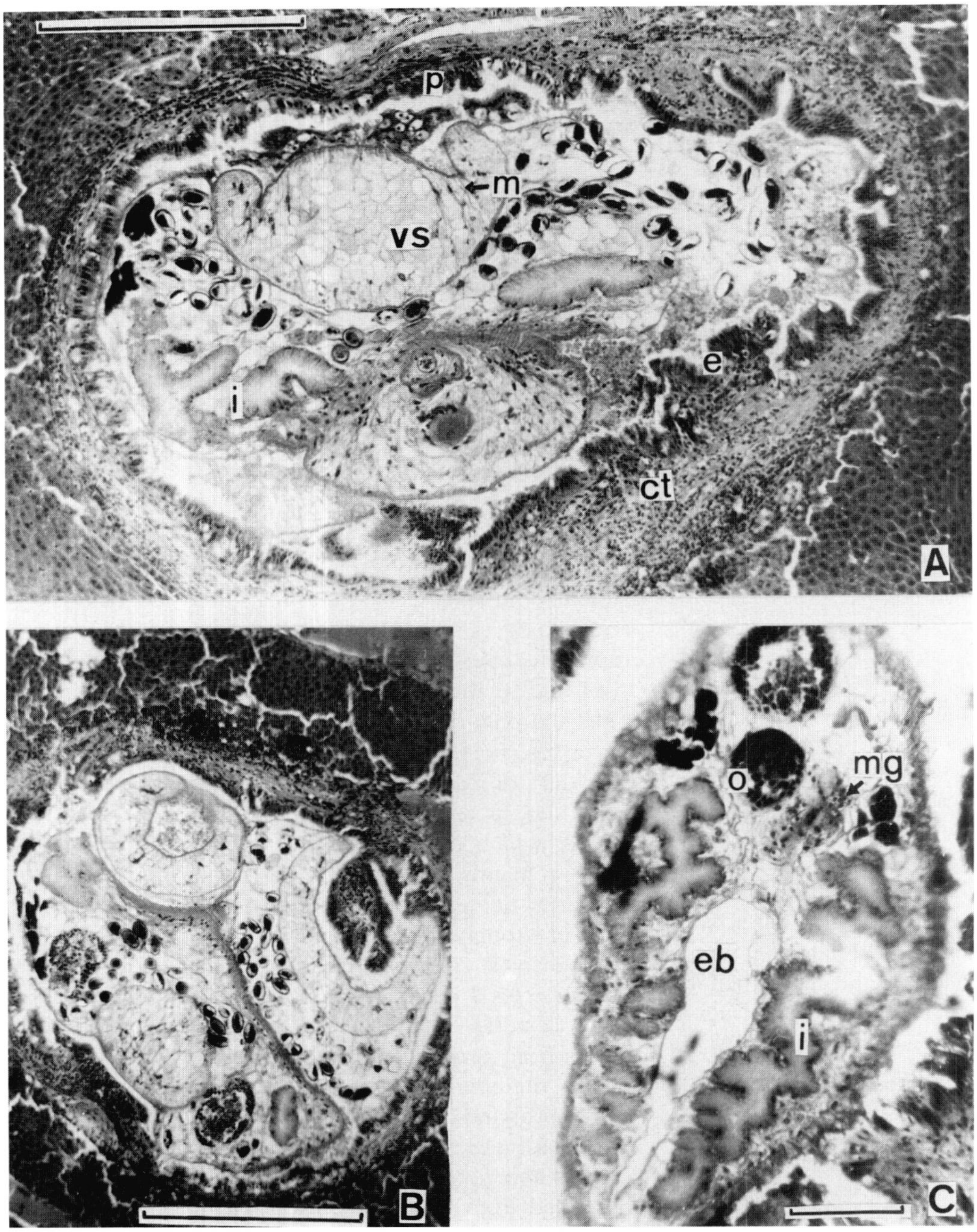

Fig. 5. – Sections of bile ducts infected with *Prosolecithus danubica*. Scale-bars : A,B. 1 mm; C. 0.2 mm. Abbreviations : e, epithelium; p, numerous pyriform cells; ct, enlarged connective tissue layer; vs, ventral sucker; m, dorso-ventral muscle fibres of the ventral sucker; i, intestine of the worm; o, ovary; mg, Mehlis' glands; eb, excretory bladder.

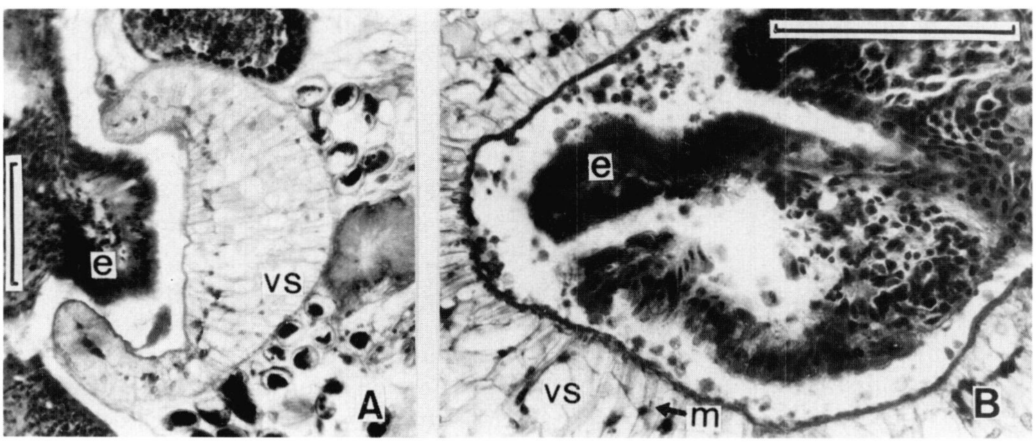

Fig. 6. – Sections of the wall of bile duct of the shrew at the site of digenean attachment. Scale-bars : 0.2 mm. Abbreviations : e, epithelium; vs, ventral sucker; m, muscle fibres.

ped. Oesophagus short. Intestinal bifurcation just anterior to ventral sucker. Caeca long, reach almost to posterior end of body. Ventral sucker pre-equatorial or nearly equatorial, usually larger in diameter than oral sucker. Testes symmetrical or slightly oblique, immediately posterior to ventral sucker. Cirrus-sac small, containing winding seminal vesicle, prostatic glands and ejaculatory duct which may form unspined cirrus. Genital pore at level of posterior edge of pharynx. Ovary sublateral, situated posterior to left testis. Seminal receptacle small, posteromedial to ovary. Laurer's canal present. Vitellarium intracaecal or overlapping caeca, reaching along middle third of body length. Uterus occupies central portion of body, its loops mainly concentrated in region of ventral sucker, only rarely extending posteriorly to ovary. Excretory bladder I-shaped. Parasitic in gall-bladder and bile ducts of Insectivora (family Soricidae).

Type-species : *Prosolecithus pellucidus* (Pojmnska, 1957) (syn. *Dicrocoelium pellucidum* Pojmánska, 1957) in *Sorex araneus*, Poland.

Other species : *Prosolecithus danubica* n. sp. in *Sorex araneus* and *Neomys anomalus*, Odessa region, Ukraine.

Yamaguti (1971) erected the subfamily Prosolecithinae for this genus, distinguishing it by its entirely pretesticular vitelline fields. Even if this minor feature were to be considered sufficient to erect a new subfamily, it is invalidated by the variation found in *P. danubica*. Therefore, we do not recognise the subfamily Prosolecithinae and consider it a synonym of Eurytrematinae. The new observations, particularly on the vitelline extension and the excretory system, warrant an emended diagnosis.

LIFE-CYCLE

The infection patterns exhibited by *Prosolecithus danubica* n. sp. may be taken to indicate that the species has a shortened, two-host life-cycle, an unusual phenomenon in the Dicrocoeliidae (see Panin 1984; Korol 1993). If this is the case, shrews become infected by feeding directly on snails. Evidence for this is the fact that the prevalence of *P. danubica* in *Sorex araneus* and *Neomys anomalus* on Ancudinov Island was 100%, while several *Sorex minutus* examined there were not infected with the species. Similarly, on Peschanii Island all five *N. anomalus* and 21 *S. araneus* were heavily infected, but the three *S. minutus* were uninfected. It may be that, due to its small size, *S. minutus* rarely or never feeds on snails. An investigation of the life-cycle of the species is planned.

PREVALENCE AND INTENSITY

We have collected material in different seasons and from shrews of different ages, although the data are insufficient as yet for detailed statistical analysis. The prevalence in *S. araneus* and *N. anomalus* from Ancudinov Island was 100%, but the intensity varied from seven specimens to several hundred (more precise calculation was impossible due to their very close disposition in the bile-ducts). Old shrews were always heavily infected, while the lowest intensity was observed in young shrews collected at the beginning of August. The intensity sharply increased up to the beginning of September and from September to December all shrews were heavily infected. Digeneans recovered from young shrews at the beginning of August were mostly represented by young immature specimens. Due to the mild climate infection continues to the beginning of winter. Young specimens of *P. danubica* were observed in shrews even in the middle of December. From the host's intestine, young digeneans invade the gall-bladder through the bile duct. As our observations show, digeneans first occupy the gall-bladder and only if the intensity increases to several dozen specimens do newcomers start to invade the extra- and intra-liver bile-ducts, including interlobular ducts. At an intensity of more than 100 specimens, nearly all of the large ducts are inhabited by digeneans.

PATHOGENICITY

The heavily infected liver has a yellowish appearance, with pale spots at the sites of necrosis. At high levels of intensity *P. danubica* causes serious pathological changes in the gall-bladder, bile ducts and liver of the shrews. The gall-bladder in such a case is usually enlarged and its colour changes from yellowish to rose and its walls became thickened and opaque. Histological sections indicate the pathological changes in the structure of the wall of the bile ducts invaded by *P. danubica*. The wall becomes much thicker and its connective tissue membrane partly sclerotises (Fig. 5). This is probably a reaction to the mechanical and chemical influences of the parasite. The surface of epithelium becomes rough and friable (Fig. 5A, 6), while in uninfected ducts the epithelium is smooth. Among the epithelial cells the number of pyriform mucous cells increases (Fig. 5A). More detailed histological studies are in progress.

FEEDING

Prosolecithus danubica probably does not feed on blood, but causes intensified proliferation of the epithelium and haemorrhagy, and feeds on dying cells and the contents of the ducts. This suggestion is based on the fact that erythrocytes were never observed in the caeca, either in live worms or on the histological sections. Often the ventral sucker was seen to be attached to the wall of the gall-bladder or bile ducts (Fig. 6). Perhaps these worms can, also, absorb nutrients through their tegument.

Distribution

Finally, a short comment should be made concerning the narrow distribution of *P. danubica*, which is surprising, considering the huge range of *Sorex araneus* (from the Atlantic to the Pacific Ocean) and the low host-specificity of digeneans to the different genera of the Soricinae. Though four species of shrew were examined from 14 localities in the delta, worms were found on only three islands. It should be stressed that the natural conditions (vegetation, fauna of shrews and snails) are very similar on the range of islands throughout the delta. River branches and artificial canals cannot be considered barriers because in winter the infected shrews may easily move between the islands, crossing the frozen canals. Possibly the study of the life-cycle of *P. danubica* will provide an answer. At present it can only be supposed that the modern ranges of the species of *Prosolecithus* are remnants of former wider ranges.

ACKNOWLEDGEMENTS

Authors are grateful to Alexander Fedorchenko, Danube delta Natural Reserve, Vilkov (Ukraine) for invaluable assistance in field collection of material and for sending fixed specimens of shrews, Dr.Vasile Otsel, Institute of Danube delta, Tulcea, (Romania) for help in organizing expeditions in the Romanian part of the delta and Dr. Alexander Grabovoj, State Medical University, Kiev (Ukraine) for help in histological study of our material. Dr Teresa Pojmánska, Institute of Parasitology, Warsaw, Poland, helped VVT to obtain type material of *P. pellucidus*, for which we are most grateful. The investigation and preparation of the manuscript for publication was supported for one of the authors (VVT) by a British Council travel grant and grant N 5/189 from the State Commitee for Science and Technology of the Ukraine. Dr David Gibson, NHM, London, critically reviewed the manuscript. Dr Jean Mariaux, Université de Neuchâtel, Switzerland, kindly translated the summary.

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Accepté le 3 janvier 1995