SEASONAL MORPHOLOGICAL VARIATIONS IN BIRD SCHISTOSOMES

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Summary:

The present work is a contribution to the systematics of *Bilharziella* and *Dendritobilharzia*. Wildfowl was killed in hunting seasons or found dead in Champagne-Ardenne region, France, and autopsied with focus on schistosomes. Seven *Anas platyrhynchos* (mallards), one *Ardea cinerea* (grey heron) and two *Cygnus olor* (mute swans) were parasitized by *Bilharziella*. One *C. olor* was parasitized by *Dendritobilharzia*. Depending on season and hosts, various morphological forms of *Bilharziella* suggesting several species were observed. The differences in male and female worms concerned the morphology of genital apparatus, the spination on suckers, the body size and proportions. However, the comparison of DNA sequences led to a conclusion that these forms belonged to one species, *Bilharziella polonica* (Kowalewski, 1895). The morphological features and the body sizes of our samples of *Dendritobilharzia* seemed to differ from the type species of *D. pulverulenta* (Braun, 1901). Nevertheless, molecular analysis confirmed identity. We hypothesize that the differences in *Bilharziella* and *Dendritobilharzia* might be linked to internal host factors (e.g. hormonal levels), and influenced by season, host, and worm age. The definition of the genera *Bilharziella* and *Dendritobilharzia* was amended.

KEY WORDS : avian schistosome, *Bilharziella polonica*, *Dendritobilharzia pulverulenta*, morphology, systematics, molecular biology, France.

Résumé : VARIATIONS MORPHOLOGIQUES SAISONNIÈRES CHEZ LES SCHISTOSOMATIDAE AVIAIRES

Ce travail est une contribution à la systématique de Bilharziella *et* Dendritobilharzia *(Trematoda, Schistosomatidae). Des oiseaux sauvages, tués en période de chasse ou trouvés morts en région de Champagne-Ardenne (France), ont été autopsiés pour rechercher la présence de schistosomes. Sept* Anas platyrhynchos *(colvert), un* Ardea cinerea *(héron cendré) et deux* Cygnus olor *(cygne tuberculé) ont été trouvés parasités par des* Bilharziella. *Un* Cygnus olor *est parasité par* Dendritobilharzia. *Selon la saison et la nature de l'hôte, plusieurs morphes de* Bilharziella, *suggérant plusieurs espèces, ont été observés. Les différences concernaient la morphologie de l'appareil génital, la spinulation des ventouses, la taille et les proportions du corps, chez les mâles comme chez les femelles. Or la comparaison des séquences d'ADN conduit à la conclusion que ces morphes appartiennent à une seule espèce,* Bilharziella polonica *(Kowalewski, 1895). Chez nos spécimens de* Dendritobilharzia, *des caractères morphologiques et la proportion de certains organes paraissent différents de ceux de* D. pulverulenta *(Braun, 1901), espèce type. Or, l'analyse moléculaire confirme l'identité de nos spécimens avec celle de l'espèce type. Nous formulons l'hypothèse que des différences dans* Bilharziella *et* Dendritobilharzia *pourraient être liées à des facteurs internes de l'hôte, taux hormonal notamment, et influencées par la saison, l'hôte et l'âge des vers. La définition des genres* Bilharziella *et* Dendritobilharzia *est amendée en fonction de ces observations.*

MOTS CLÉS : *bilharzie aviaire,* Bilharziella polonica, Dendritobilharzia pulverulenta, *morphologie, systématique, biologie moléculaire, France.*

INTRODUCTION

Recently, occurrence of bird schistosomes and swimmer's itch became a problem in recreational areas, having important consequences, direct (health complication) and indirect (decrease of travel industry). Recent and numerous data were published for *Trichobilharzia* spp. in systematics and pathogenicity, in experimental and natural hosts: birds, mammals and humans. Conversely, data on *Bilharziella* and *Dendritobilharzia* remained less abundant. Occurrence of *Bilharziella* and/or *Dendritobilharzia* in Europe and all over the world were studied by some authors (Ejsmont, 1929; Szidat, 1929; Mehra, 1940; Bykhovskaya-Pavlovskaya & Rizhikov, 1958; Palm, 1965; Khalifa, 1972 and 1976; Sulgovstowska, 1972; Martorelli, 1981; Kolárová *et al.*, 1989 and 1997; Sepulveda *et al.*, 1994).

Furcocercariae of *Bilharziella* were reported as a causal agent of swimmer's itch in Germany by Szidat (1930). Horák & Kolárová (2000) also observed penetration of some cercariae of *B. polonica* into the skin of tails and

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legs of mice, and the schistosomulae were able to survive and migrate to the lungs in this model. On the other hand, *B. polonica* role in swimmer's itch in Poland was not confirmed by Zbikowska (2003, 2004). No data were published on a possible pathology caused by *Bilharziella* dwelling in birds.

Furcocercariae of *Dendritobilharzia* were reported as a causal agent of swimmer's itch in Germany by Dönges (1965), and in New Zealand by Rind (1989). Pathological changes in hosts naturally infected by *Dendritobilharzia* were studied by Levine *et al.* (1956), Wilson *et al.* (1982) and Wojcinski *et al.* (1987).

Systematics of *Bilharziella* Looss, 1899 was reviewed by Yamaguti (1971) and Khalil (2002). Systematics of *Dendritobilharzia* Skrjabin and Zakharow, 1920 was studied by several authors (Mehra, 1940; Cheatum, 1941; Macko, 1959; Baugh, 1963; Ulmer & Van de Vusse, 1970; Farley, 1971; Van de Vusse, 1979 and 1980; Leite *et al.*, 1982; Rind, 1989; Sepulveda *et al.*, 1994). The present work describes *Bilharziella* and *Dendritobilharzia* found in Champagne-Ardenne region, France. It focuses on systematics of *Bilharziella* and *Dendritobilharzia* using morphological and molecular investigations.

MATERIAL AND METHODS

Birds were either killed during the hunting period or found dead by members of ONCFS (*Office National de la Chasse et de la Faune Sauvage*) and network SAGIR. They originated mainly from Champagne region: Der-Chantecoq lake and surrounding ponds. Nine *Anas platyrhynchos* (mallards), four *A. clypeata* (shovelers), two *A. strepera* (gadwalls), four *A. crecca* (teals), one *Aythya ferina* (pochard), five *C. olor* (mute swans), two *Ardea cinerea* (grey herons), one *Gallinula chloropus* (moorhen) and three *Larus ridibundus* (black-head gulls) were examined. They were frozen before autopsy. The adult worms were found after the dissection of their intestinal, mesenteric and hepatic vessels and were fixed in alcohol or formaline, stained in chlorhydric carmine, dehydrated, mounted in Canada balsam and drawn with *camera lucida*.

Specimens of worms (*Bilharziella* and *Dendritobilharzia*) were analyzed for molecular biology. For each worm the anterior part of the body was cut for mounting, and morphological evaluation and determination. DNA extraction was done using the Qiamp DNA mini kit (Qiagen, Germany) following manufacturer's instructions. During the first step (tissue lysis), the worms were crushed one by one using a piston pellet (Treff, Switzerland), and the DNA was eluted in 50 µl of the elution buffer provided by the manufacturer. PCR was performed in a 50 µl volume using 5 µl of extracted DNA solution and 50 pmol of each of the primers C2'b (5'-GAAAAGTACTTTGRARAGAGA) and D2 (5'- TCCGTGTTTCAAGACGGG) for amplification of D2 domain of 28S rDNA. The PCR mix contained (final concentrations) 10 mM Tris HCl, pH 8.3, 1.5 mM $MgCl_2$, KCl 50 mM, Triton X 100 0.01 %, 200 µM each dNTP, and 0.25 µl (1.25 units) of *Taq* polymerase (Qiagen, Germany). Initial denaturation at 94° C for five min was followed by 40 cycles of denaturation at 94° C for 45 s, annealing at 50° C for 45 s and extension at 72° C for two min with a final elongation time of 10 min at 72° C. Amplicons were analysed by electrophoresis in 1.5 % agarose gel containing ethidium bromide. PCR products were directly sequenced in both directions by Qiagen (Hilden, Germany) using the primers used for DNA amplification. Sequence alignment was performed using the MUST software package (Philippe, 1993). The sequences are deposited in Genbank (accession Nos. DQ813437 to DQ813443).

RESULTS

Bilharziella was found in two *C. olor* (out of five examined) and *A. cinerea* found dead in autumn in Outines. *Bilharziella* was also found in seven *A. platyrhynchos* (out of nine examined), three coming from Vanault-les-Dames and killed by hunting in September and October, and four from Larzicourt, killed at the end of June (Table I). *Dendritobilharzia* was only found in one *C. olor* parasitized also by *Bilharziella*. Previously, *Bilharziella* and *Dendritobilharzia* were rarely mentioned in findings from wildfowl in Western Europe.

BILHARZIELLA (Figs 1, 2)

Specimens have been found in mesenteric or hepatic vessels. Their digestive and genital apparatuses allowed identification as *Bilharziella* sp. Besides the characters common to *Bilharziella*, the worms displayed an excretory system visible only at the end of the body. The genital apparatus in males showed a long gynaecophoric canal extending from the level of oral sucker to the hindbody. It appeared as a tubercled strip or drainpipe, opened or closed. Size and morphological features allowed to distinguish four morphological types: one from *C. olor* (71 TO), one from *A. cinerea* (72 TO, 76 TO 1, 2) and two in *A. platyrhynchos*: one observed in June (64 TO, 65 TO, 77 TO, 78 TO) and the others in September-October (63 TO, 66 TO, 73 TO, 74 TO). Only one male was observed in each *C. olor* which died in November. One of the worms put in a drop of water measured 5,830 µm in length, the diameters of oral sucker and acetabulum were respectively 150 µm and 190 µm, the distance between the two suckers was

Parasite	Host	Location in bird	Registration number (Paris)	Geographical data	Death of the bird
Bilharziella polonica large morph without spine	*Cygnus olor* (DER 1)	Mesenteric, male	71 TO 1	Outines Landres pond 4° 41' 14" E	November, 25
	Cygnus olor (DER 3)	Mesenteric, male	71 TO 2	48° 34' 33" N	*id.*
Bilharziella polonica large morph without spine	Ardea cinerea (ARD 1)	Mesenteric, male	72 TO 1	Outines	Autumn
		Mesenteric, female	76 TO 1		
		Mesenteric, female	76 TO 2		
Bilharziella polonica medium morph without spine	*Anas platyrhynchos* (KAL 5)	Mesenteric, male	63 TO 1	Vanault-Les-Dames Neuf pond 4° 47' 12" E	September, 30
		Mesenteric, female	74TO2	48° 50' 31" N	
	Anas platyrhynchos (KAL 9)	Mesenteric, female	66 TO 1	*id.*	October, 6
		Mesenteric, male	73 TO 1		
Bilharziella polonica medium morph with spines	*Anas platyrhynchos* (FRA 1)	Hepatic/mesenteric male	64 TO 1	Larzicourt Der-Chantecoq lake 4° 43' 04" E	June, 29
		Hepatic/mesenteric male	77 TO 1	48° 34' 33" N	
		Hepatic/mesenteric male	78 TO 1		
	Anas platyrhynchos (FRA 2)	Hepatic/mesenteric female	65 TO 1	*id.*	June, 29
Dendritobilharzia pulverulenta	*Cygnus olor* (DER 1)	Hepatic males and females	55 TO 1, 10	Outines Landres pond 4° 41' 14" E 48° 34' 33" N	November, 25

Table I. – Origin of samples.

1,310 µm (Fig. 1J). One male and two females occurred in *A. cinerea* found dead in autumn. The male was not measured, because it was fragmented (Fig. 1E). The two females put in a drop of water had a respective length of 2,149 and 2,161 µm. The diameter of their oral sucker was 52 µm, acetabulum 73 µm and the distance between their two suckers 401 µm (Fig. 1F). In the worms recovered from *A. cinerea* and from *C. olor*, there were no spines on the suckers and males showed a reduced seminal vesicle. In *A. platyrhynchos* hunted in Vanault-les-Dames in autumn (September-October), nine males and three females of *Bilharziella* were studied. Five males and one female were examined from *A. platyrhynchos* from Larzicourt killed in summer (June). All the worms were measured after fixation and staining. Always males were longer and wider than females. The lengths in samples from ponds of Vanault-les-Dames were: 2,977 ± 366 µm (range 2,628-3,878) for nine males and 1,664 ± 112 µm (1,556-1,780) for three females. Males and female from Larzicourt measured in lengths 3,165 ± 481 µm (2,410-3,707) and 1,811 µm, respectively. All the samples appeared to be adult. Specimens of *Bilharziella* from *A. platyrhynchos* killed in Vanault-les-Dames in autumn were named A group, and those from *A. platyrhynchos* killed in Larzicourt in summer S group.

In A group, the bodies of male and female *Bilharziella* were relaxed. The males (Fig. 1A) showed a reduced linear seminal vesicle, with prostatic glands similar to the homologous organs (Fig. 1B) in the already synonymized species *Chinbuta indica* Lal, 1937. The females had a sinuous S-shaped ovary, a uterus with one (or none) poorly developed egg devoid of long spine, and a vitelline mass in midbody and hindbody (Fig. 1C, D). The hindbody was slender. The oral sucker and the acetabulum of males and females were smooth, devoid of spines (Fig. 1H, I). In S group, the males were frequently constricted (Fig. 2A, D). They displayed a long spiraled seminal vesicle (Fig. 2G) more similar to the vesicle of *Bilharziella lali* Baugh, 1963 than that reported in *B. polonica* (Kowalewski, 1895). The female had a globular ovary and vitelline follicles of equal dimensions filling the midbody and hindbody (Fig. 2K). The uterus and the hindbody were widened. The oral sucker and the acetabulum of males and female were spiny (Fig. 2B, C, E, F, H, I).

Molecular analysis performed with specimens from *C. olor, A. cinerea* and *A. platyrhynchos* (A and S groups) showed that all isolates belonged to *Bilharziella polonica*; the sequences of samples coming from the above mentioned birds were identical. They were homologous in 100 % with the two sequences depo-

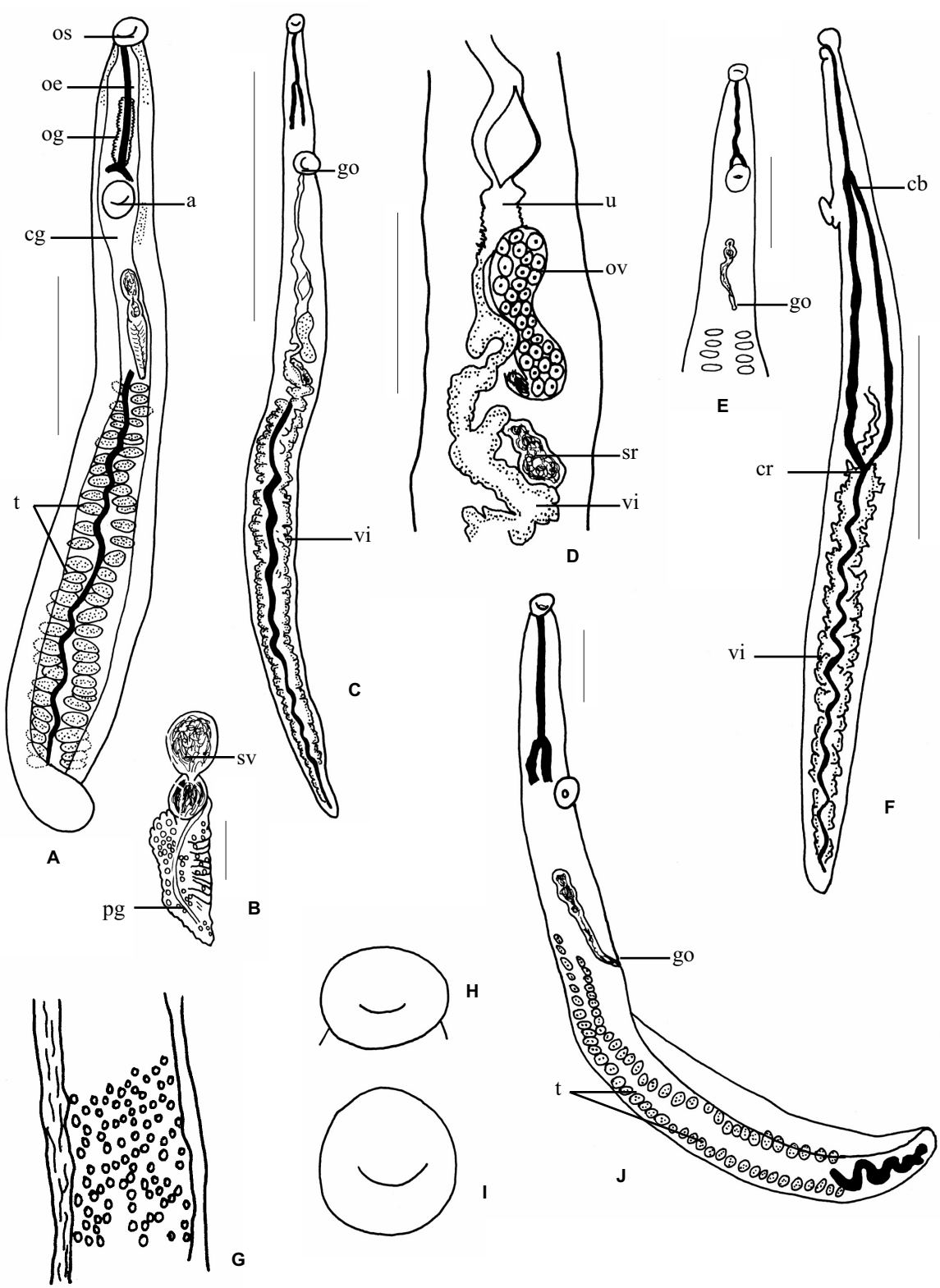

Fig. 1. – *Bilharziella polonica* autumn morph (A-D, G-I: *Anas platyrhynchos*; E, F: *Ardea cinerea*; J: *C. olor*) (male: A, B, E, G, J; female: C, D, F, H, I).

A: ventral view (63 TO). B: genital apparatus. C: ventral view (66 TO). D: genital apparatus. E: hinbody (*Ardea cinerea*). F: lateral view (*Ardea cinerea*). G: details of canalis gynaecophorus (72 TO_1). H: oral sucker (76 TO_2). I: acetabulum. J: ventro-lateral view (*Cygnus olor*) (71 TO_1).

Abbreviations. – a: acetabulum, cg: canalis gynaecophorus, cb: caeca bifurcation, cr: caecal reunion, go: genital opening, i: intestine, oe: oesophagus, og: oesophageal glands, os: oral sucker, ov: ovary, pg: prostatic gland, sr: seminal receptacle, sv: seminal vesicles, t: testes, u: uterus, vi: vitelline folicles (scale bars: A, C, E, F, J = 500 µm; B, D = 100 µm).

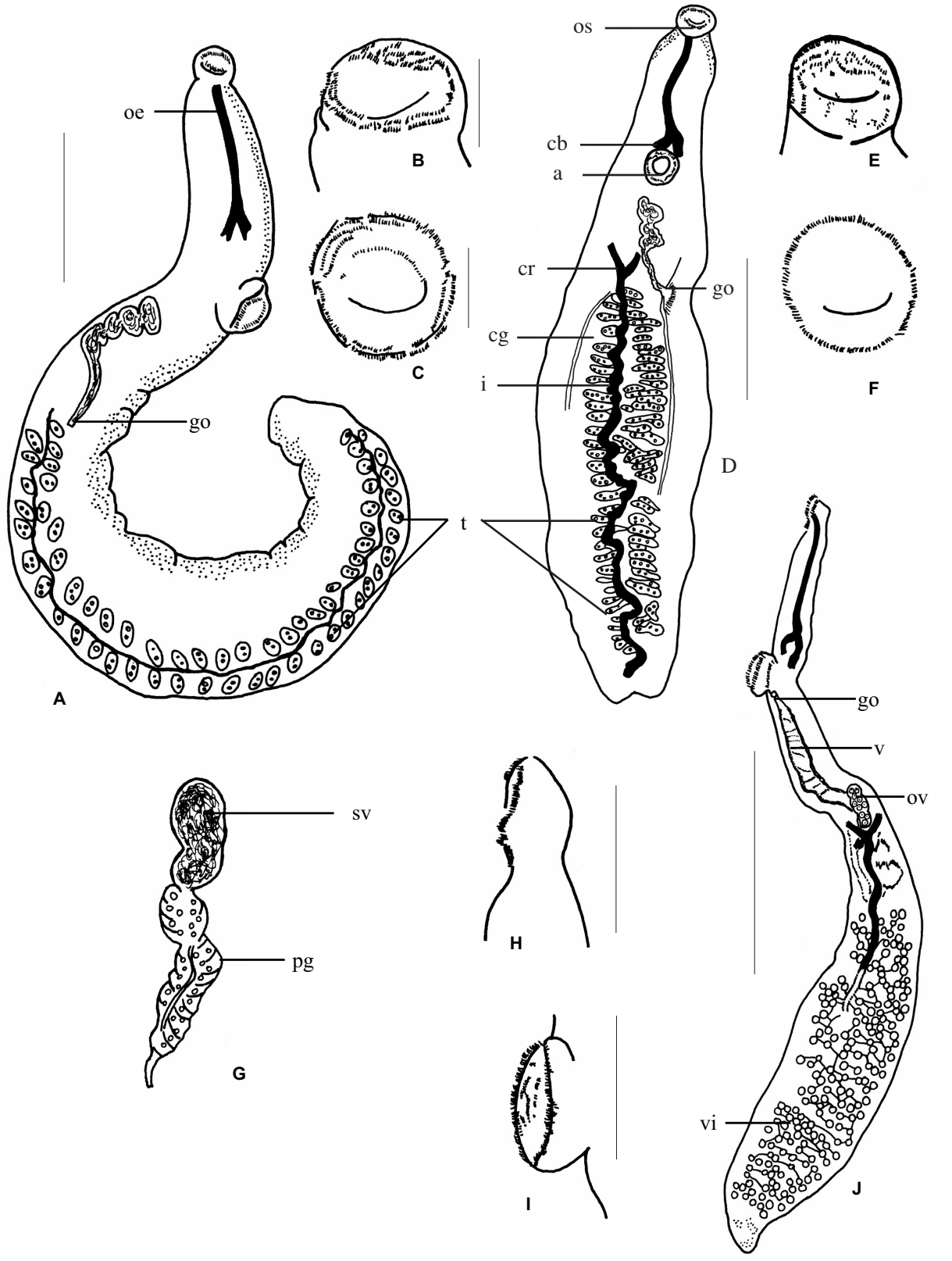

Fig. 2. – *Bilharziella polonica* summer morph (*Anas platyrhynchos*) (A-C, G-H: 77 TO, D-F, 64 TO, I-K: 65 TO) (male: A-H; female: H-J). A: lateral view. B: oral sucker. C: acetabulum. D: ventral view. E: oral sucker. F: acetabulum. G: genital apparatus. H: acetabulum. I: Female, oral sucker. J: Female, lateral view.

Abbreviations. – a: acetabulum, cg: canalis gynaecophorus, cb: caeca bifurcation, cr: caecal reunion, go: genital opening, i: intestine, oe: oesophagus, ov: ovary, os: oral sucker, pg: prostatic gland, sv: seminal vesicles, t: testes, u: uterus, vi: vitelline folicles (scale bars: A, D, K = 500 µm; B, C, I, J = 100 µm).

sited in Genbank, one obtained from a sample isolated from *Anas platyrhynchos* sacrificed in Ukraine, access number AY 157240 (Snyder & Loker, 2000) and the second one obtained from ocellate furcocercariae of *Bilharziella* emitted by *Planorbis planorbis* from the Czech Republic, access number AF 167088 (Lockyer *et al.*, 2003).

DENDRITOBILHARZIA (Fig. 3)

Three females and three males out of ten adult worms, found in hepatic vessels in one *C. olor* (55 TO 1-6) and determined as *Dendritobilharzia* sp. were morphologically studied. The length and the width of the fixed specimens were 12,740 ± 770 × 1,450 ± 130 µm, without a significant difference between the males and the females. In both sexes oral and ventral suckers were not present (Fig. 3A, D). The digestive tract begun by oral opening situated subterminally. Oesophagus in its distal part was surrounded by oesophageal glands (Fig. 3B, E). Caecal bifurcation was at the level of cirrus pouch in males, or ovary, Mehlis' gland and uterus in females. Two short caeca reunited into a long dendritic intestine running zigzag to the posterior end of the body. In females, numerous eggs were present in the uterus at the morula stage; they were ovoid except for one with a terminal spine (Fig. 3F). In males the genital apparatus appeared to lack the gynecophoric canal. However, the edges of worms bore scattered spines, creases and bulges, participating probably in worm coupling. Cirrus pouch, seminal vesicle and prostatic cells were situated in the forebody. Ejaculatory duct opened in large genital bulb (Fig. 3C). A high number of testes organized in four lines were localized near the dendritic intestine. The sequence of the D2 domain of the 28S rDNA is 100 % homologous with that of *Dendritobilharzia pulverulenta* isolated from *Gallus gallus* (AF167090 or AY157241)

DISCUSSION

A species is considered as separate among closely related ones on the basis of constant differences in morphology of adult worms, specificity for first molluscan hosts, shape and size of eggs harbouring a miracidium and typical sequences in genome. Usually ITS (Internal Transcribed Spacer) sequences served as molecular markers for species identification of *Trichobilharzia* (Dvorak *et al.*, 2002). In our work, the D2 domain of 28 rDNA has been used as a relevant informative marker which is supported by use of D2 for discrimination of four species of human schistosomes (Littlewood & Johnston, 1995). Males and females of *Bilharziella* from *C. olor, A. cinerea* and *A. platyrhynchos* showed morphological differences, but molecular analysis proved they belong to one species: *B. polonica*. In addition males and females of *Dendritobilharzia* from *C. olor* seemed morphologically different from the type species, but molecular data confirmed they belong to one species: *D. pulverulenta*.

BILHARZIELLA

Khalifa (1972) obtained cercariae of *B. polonica* from *Planorbis planorbis*, *Bathyomphalus contortus* and *Planorbarius corneus*, never from *Anisus vortex*, Lymnaeidae and Physidae. He succeeded an experimental infection of young *A. platyrhynchos* with furcocercariae emitted by naturally infected Planorbidae. The ducks were sacrificed at different periods after exposure. With respect to their age, the worms presented certain variability. However, despite their differences in morphology, Farley (1971) suggested a synonymy between *B. lali* Baugh, 1963 and *B. polonica* (Kowalewski, 1895).

In the present study the two observed forms (A and S) of *Bilharziella* from *A. platyrhynchos* differed in several morphological characters according to the season (*e.g.* presence or absence of spines on the two suckers, morphology of genital apparatus). In all the birds killed in autumn male and female genital apparatuses were always reduced, independently of the size of the worms. It was impossible to characterize several structures due to immaturity of some worms. Species identification of both forms (A and S) as *B. polonica* was proved by molecular tools.

A seasonal influence of the host on blood parasites has been reported in amphibians by Stunkard (1959) and Combes (1967). Stunkard pointed out that *Polystoma* (a monogenean parasite) was strictly haematophagous and could ingest the hormone-containing blood of its hosts. Combes observed correlation between the sexual cycle of *Polystoma* and the amphibian host. In adult birds of *A. platyrhynchos*, seasonal variation in the level of hormones present in the blood is also well known (Dorst, 1956). The level increases in spring and summer and decreases in autumn and winter. The increase of hormones in the blood was linked with the lengthening of the days in spring and summer. The day light activated the process of seasonal growing of gonads, inducing nuptial parades and nesting. Conversely, the shortening of days in autumn and winter stopped the process. The hormones of birds could influence the worm morphology and, based on the above examples, *Bilharziella* worms living in the blood of birds could monitor hormonal levels, controlling in this way onset and cessation of reproduction according to the season. In agreement with this hypothesis, we recorded influence of the season on the morphology of *B. polonica*. In addition, we can also speculate on *e.g.* influences of time of infection, worm natural ageing/maturation and size of bird host.

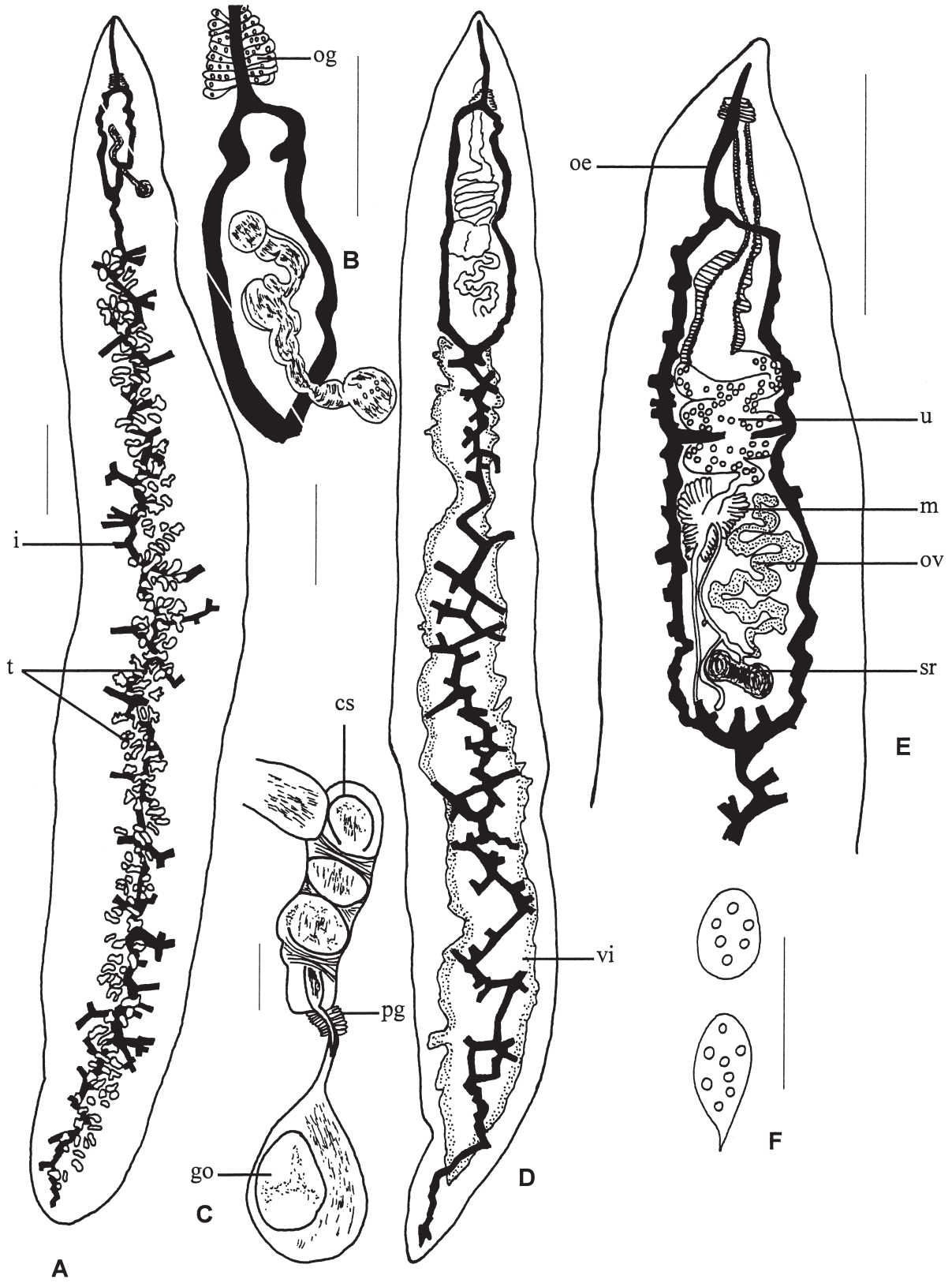

Fig. 3. – *Dendritobilharzia pulverulenta* (*Cygnus olor*) (male: A-B; female: D-F).
A: dorsal view (55 TO_1). B: genital apparatus, dorsal view (55 TO_3). C: genital apparatus, ventral view. D: ventral view (55 TO_4). E: genital apparatus (55 TO_6). F: eggs.
Abbreviations. – cs: cirrus sac, go: genital opening, i: intestine, m: Mehlis' gland, oe: oesophagus, og: oesophageal glands, ov: ovary, pg: prostatic gland, sr: seminal receptacle, t: testes, u: uterus, vi: vitelline folicles (scale bars: A, D, E = 1 mm; B = 500 µm; C, F = 100 µm).

The status of *Bilharziella* Looss, 1899 was discussed by several authors (Lal, 1937; Baugh, 1963; Yamaguti, 1971; Khalifa, 1972; Liu & Bai, 1976; Farley, 1971; Khalil, 2002). Farley (1971) synonymized *Chinhuta indica* Lal 1937 and *Bilharziella lali* Baugh 1963 *i.e.* he synonymized the genera *Chinhuta* and *Bilharziella*. For both he admitted the absence of gynaecophoric canal. Yamaguti (1971) maintained validity of the two genera. He underlined the presence of gynaecophoric canal in *Chinhuta*. Khalil (2002) accepted the Farley's view. We also agree with synonymy of *Bilharziella* and *Chinhuta*, and the presence of a gynaecophoric canal in both morphs. This canal can be opened or folded over.

In the present work, the characters of the genus *Bilharziella* can be corrected as follows: Suckers are well developed, smooth or spiny, oral sucker is smaller than acetabulum. Caecal bifurcation is situated near acetabulum, at the level or in front of it. The common caecum is without lateral branches and runs zigzag. In males, gynecophoric canal is present, clearly obvious if closed or less apparent if opened. It is marked by two longitudinal bands: one smooth and the second one covered by tubercles extending from the inferior part of the oral sucker to the hindbody (Fig. 1G).

Dendritobilharzia

Van de Vusse (1979) noted locations of *Dendritobilharzia* in several arteries of birds (aorta, femoral and renal arteries, etc.) and admitted that these locations were different in surface ducks and diving ducks. The corresponding eggs were scattered in different tissues: intestine, liver, kidney of the bird hosts, and appeared to be non-developed. Our specimens of adult worms were found in hepatic vessels and no egg was found in the surrouding tissues. Morphology of our samples of males and females of *Dendritobilharzia* from *C. olor* seemed to be different from *D. pulverulenta* (Braun, 1901) described in Africa, and specimens found by Ulmer & Van de Vusse (1970) in North America, and Rind (1989) in New Zealand. *D. pulverulenta* was reported also in Germany by Palm (1965), Poland by Sulgostowska (1972) and Khalifa (1972), the Czech Republic by Kolarova *et al.* (1989, 1997), Yakutiya by Bykhovskaja-Pavlovskaja & Rizhikov (1958), Texas by Canaris *et al.* (1981) and India by Chauhan *et al.* (1973).

Body sizes of worms in our study were different from those mentioned by Macko (1959) and Van de Vusse (1980). Our female worms from *C. olor* morphologically resembled those of *D. asiatica* Mehra, 1940, with one spiny egg among numerous ovoid eggs. Males in our findings had a large genital bulb as *D. anatinarum* Cheatum, 1941; unfortunately, the male of *D. asiatica* was never described. Although Freitas & Costa (1972) mentioned *D. anatinarum* in Brazil and Leite *et al.* (1982) also recognized this species, Macko (1959) and Ulmer *et al.* (1970) consider it as a synonym of *D. pulverulenta* (Braun, 1901). Van de Vusse (1980) considered *D. asiatica* to be a *species inquirenda*, whereas Martorelli (1981) considered it as a valid species differing from *D. pulverulenta* and *D. rionegrensis* n. sp. Evaluating their specificities towards first intermediate hosts, it was difficult to confirm their synonymy. In Poland, Khalifa (1976) reported *Anisus vortex* and *Planorbis planorbis* as the first naturally infected hosts of *D. pulverulenta*, Leite *et al.* (1982) succeeded in the experimental infection of *Biomphalaria straminea* by miracidia identified as *D. anatinarum*. These miracidia came from eggs found in naturally infected *Cairina moschata*. The same authors failed in attempts to infect *Aplexa rivalis*, *Biomphalaria glabrata* and *Lymnaea columella* by miracidia of *D. anatinarum*. In New Zealand, Rind (1989) failed to establish *D. pulverulenta* infection in *Potamopyrgus antipodarum, Gyraulus corinna* and *Lymnaea tomentosa*.

Leite *et al.* (1982) and Rind (1989) observed that the maturation of *D. anatinarum* and *D. pulverulenta* eggs represents a long process. The intra-uterine eggs, as well as the deposited eggs are at *morula* stage. They grow and become mature outside the body of the female worms, in the intestinal mucosa of the bird host. The presence of numerous intra-uterine eggs, branching intestine and the absence of oral and acetabular suckers are the main differences between *Dendritobilharzia* and *Bilharziella*.

Our morphological and molecular data lead to a conclusion that *D. pulverulenta* is a species that could morphologically differ with respect to the physiological status of and location within the bird host. It means that it is extremely difficult to determine this species only by morphology of the adult worms. We think that the shape and size of genital bulb is not a species-specific feature and it could reflect physiological status of the male worm and its bird host.

An improved species diagnosis is proposed for *Dendritobilharzia pulverulenta*: Mouth devoid of oral sucker. Body devoid of acetabulum. Oesophageal glands near the caecal bifurcation. Caecal reunion in males at the level of the genital pore, in females at the level of ovary and seminal receptaculum. Common caecum long, zigzag, with lateral diverticula. In males, numerous testes lie posterior to caecal reunion on each side of common caecum; they occur in two, three or four rows reaching the posterior body end. The cirrus pouch contains a bipartite seminal vesicle, prostate and small or dilated genital bulb with a pore. The female genital system consists of a long and sinuous ovary, intercaecal seminal receptaculum lying behind ovary, Mehlis gland, sinuous uterus full of eggs, and metraterm ending in egg-laying pore near the oesophagus. Vitellaria spread from the level of ovary or seminal receptaculum until

the hindbody. Excretory apparatus runs into a terminal pore. In male and female, crenated and brawny edges of the body with scattered spines and bulges are probably used during mating. The gynaecophoric canal is absent in males. The egg maturation continues after release into the intestine of bird host.

In the future it would be of interest to characterize the seasonality of the above mentioned morphological forms in experimental infections of birds. This might stimulate further work of taxonomists (intraspecific morphological variability) and physiologists (hormonal influence on parasite development). In addition, the naturally infected snails in the ponds and lakes where aquatic birds come from should be investigated in order to identify the intermediate hosts of *B. polonica* and *D. pulverulenta*. The intermediate host specificity should be verified experimentally by exposure of different planorbid snails to the above miracidia. The ability of cercariae to penetrate the skin of and migrate in vertebrates (birds, mammals) should also be tested.

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