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Identification of *Diplostomum* spp. in the retina of perch *Perca fluviatilis* and the lens of roach *Rutilus rutilus* from the Baltic Sea – an experimental study

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Abstract

Metacercariae of the genus *Diplostomum* dwelling in the retina of perch *Perca fluviatilis* and the lens of roach *Rutilus rutilus* were identified on the basis of adults obtained by feeding various piscivorous birds of the families Laridae, Anatidae and Phasianidae with metacercariae on an experimental basis. Both morphological studies of the metacercariae and adults obtained from the intestine of the infected birds, as well as the suitability in these hosts, indicated that we were dealing with two different species of *Diplostomum*. The perch form was recognised as *D. baeri* Dubois, 1937. Although metacercariae of this species matured both in herring gull *Larus argentatus* and common tern *Sterna hirundo*, the principal final host seemed to be goosanders *Mergus merganser*. In contrast, the lens form in roach was identified as *D. spathaceum* (Rudolphi, 1819) and it seemed to be restricted to birds of the family Laridae. These experimental results agree with parasitological findings from birds infected in the wild.

Introduction

Members of the genus *Diplostomum* Nordmann, 1832 are strigeoid digeneans with an indirect three-host life-cycle belonging to the family Diplostomidae Poirer, 1886. The adult stage of *Diplostomum* spp. differs from that of other genera within the family by the absence of a genital cone and an asymmetrical anterior testis (Dubois, 1970; Niewiadomska, 1970). The classification and nomenclature within this genus has long been controversial. For example, many species have only been described as metacercariae inhabiting the brain, musculature or different regions of the eyes of cyclostomes, fish and amphibians (Hoffman, 1967; Sudarikov, 1971; Shigin, 1986). Others are only known as adults living in the intestine of piscivorous birds of mammals (Sudarikov, 1960; Dubois, 1970; Yamaguti, 1975; McDonald, 1981; Sonin, 1986). Thus, descriptions including both the metacercarial stage in freshwater fish and adults, verified on an experimental basis in birds were rare until comparatively recently (Williams, 1966; Dubois, 1970; Lester & Huizinga, 1977; Dick & Rosen, 1981; Niewiadomska, 1987; Shigin, 1986; Shostak *et al.*, 1987).

In the past most diplostomid metacerariae parasitic in the eyes of freshwater fish were assigned to a single species, *D. spathaceum* (Rudolphi, 1819) (see Bauer *et al.*, 1964). However, according to the keys of Sudarikov (1971) and Shigin (1976; 1986), *D. spathaceum* represents a conglomerate (complex) of several independent species inhabiting different regions within the eye, each characterised by definite biological and morphological properties. In addition, despite the fact that measurements of a range of morphological characters have been performed on groups of individuals, differences between species within the genus *Diplostomum* have seldom been tested statistically. Furthermore, variation due to different methods of preparation, killing and fixation (Hughes & Hall, 1929; Bakke, 1988), the occurrence of host induced polymorphism (Berrie, 1960; Shostak *et al.*, 1987) as well as geographical variation, age and allometry, all cause problems (Gibson *et al.*, 1985).

In this study, two species of fish were used as sources of metacercariae; namely, the retinal form found in perch *Perca fluviatilis* and the lens form found in roach *Rutilus rutilus*. The morphological similarity of the parasites was scrutinised with light and scanning electron microscopy and the data were evaluated using principal components analysis. The results are compared with earlier descriptions made of metacercariae described from, or reported to occur in, these host species and microhabitats. As the terminology previously used to describe the microhabitat of the metacercariae in perch seems to be confused, all metacercariae reported from perch, except from those in the lens, are included in the present discussion. In addition, adults were obtained by feeding various piscivorous birds with metacercariae from both sources. Thus the suitability of birds as experimental hosts and the morphology of the adult worms obtained were identified and compared with parasites obtained from birds infected in the wild. The main purpose was to identify the metacercariae, but also to assess the degree of morphological variation of adults obtained from these metacercariae in different avian host species and as introduced by different methods of preparation.

Materials and methods

Metacercariae

Collection and preparation

Perch and roach were captured with gill- and/or fyke nets in the Baltic Sea, outside the Forsmark nuclear power station (60°27′N, 18°2′E). The eyes of the fish were dissected and stored for up to 12 hours at about 4°C and metacercariae from a subsample of the fish were removed from the lens and the retinal layers of the eye. Some of the metacercariae were fixed in Berland's fluid (1 vol. 40% formaldehyde solution: 19 vols glacial acetic acid), preserved in 70% alcohol, cleared in lactophenol and mounted in glycerine jelly or polyvinyl lactophenol, whereas others were kept in tapwater for 3 hours. According to Shigin (1986) only mature metacercariae survive this treatment. Thereafter, according to the method of Shigin (1976), they were killed and stained with acetic carmine, differentiated in a mixture of 70% ethanol and 1% HCl, dehydrated in increasing concentrations of ethanol, cleared in dimethylphthalate (DMP) and mounted on slides in Canada Balsam. Specimens were examined using light microscopy (LM) and drawings were made using a drawing apparatus (Leitz Wetzler, Dialux). Others were prepared for scanning electron microscopy (SEM). Glutaraldehyde (3%) in 0.1 M phosphate buffer (pH 7.4) was used as fixative. The specimens were then rinsed in cold 0.1 M phosphate buffer, post-fixed in cold (5°C) 1% OsO_4 in the same buffer, rinsed again and dehydrated through a graded series of ethanol. The ethanol was gradually replaced with filtered Freon TF and the specimens were critical point dried using CO_2. They were mounted on stubs, coated with gold-palladium in a Jeol JCF-1000 ion sputter and examined in a JSM-35 scanning electron microscope (SEM).

Identification

Morphologically discrete body characters of metacercariae from each source (*i.e.* lens of roach, retina of perch) were measured by means of a Kontron Electronics Group image-analysis-system Mop-Videoplan (Zeiss), from drawings of 30 specimens mounted unstained in glycerine jelly and 20 specimens treated according to Shigin (1976). The following measurements were made on unstained worms: body length (TL); body width (TW); oral sucker length (OSL); oral sucker width (OSW); ventral sucker length (VSL) ; ventral sucker width (VSW); distance from anterior margin of worm to anterior margin of ventral sucker (VS); pharynx length (PHL); and distance between lappets (LAP). The product of oral sucker length and width was divided by the product of length and width of the ventral sucker (OVR), and the ratio between body length and width was calculated and expressed as a percentage (WLR). In stained specimens the width (TOL) and length of Brande's organ (TOW) and pharynx (PHW) were also measured. In addition, the product of body length and width was divided by the product of the length and width of Brande's organ (BR); and product of length and width of Brande's organ was divided by the product of the length and width of the ventral sucker (TVR), and the ratio between the distance from the centre of the ventral sucker to the anterior margin of the body and body length were calculated (VLR). Specimens were identified and compared with the keys of Shigin (1976; 1986) and the descriptions of *Diplostomum* spp. metacercariae by Sudarikov (1971) and in the papers of Hughes (1929), Williams (1966), Komiya (1938), Cichowlas (1961), Lester & Huizinga (1977), Dick & Rosen (1981) and Niewiadomska (1988).

Statistical analysis
The measurements were log transformed in order to remove effects of size. A multidimensional image of the metacercariae was produced by analysing the morphometric information using principal components analysis (PCA). Data processing was carried out at Uppsala Datacentral (UDAC). The programme used for computing the PCA was SAS procedure PRINCOMP based on the covariance matrix (SAS Institute, 1985). By plotting the first principal component against the second, bivariate scattergrams were produced.

Adults

Maintenance of birds
Eggs of the herring gull *Larus argentatus*, the common tern *Sterna hirundo* and the goosander *Mergus merganser* were taken from nets in the Forsmark archipelago. Similarly eggs of the black-headed gull *Larus ridibundus* were taken from nests in lake Barken (Dalarna, Sweden). The eggs were hatched in an egg incubator (EHRET, KMB Gr. 2 no. 11000) at 38°C. In addition, domestic chicks *Gallus gallus domesticus* were provided by the Department of Virology (Uppsala University), and hens and cocks were obtained from a local farm. The gulls and goosanders were hand-reared and fed on chopped fish that had been deep-frozen. Domestic cocks, hens and chicks were fed on commercial pellets.

Experimental infection procedures
When used for infection, the birds were at least one week old. Prior to infection, they were starved for 24 hours. The lens was separated from the rest of the fish eye. Metacercariae, either from the lens or the retinal layers of the fish eye, were thereafter administered to the birds together with a small piece of fish meat.

Collection and preparation
The birds were killed and examined at minimum 3 days post-infection. The worms removed were fixed in Berland's fluid. They were either mounted unstained in glycerine jelly or stained in Mayer's paracarmine, differentiated in acid alcohol, dehydrated in ethanol, cleared in creosote and mounted on slides in Canada Balsam. Drawings were made with a drawing apparatus as described above. For preparation for SEM, see also above under collection and preparation of metacercariae.

Identification
The following measurements were made from unstained adults mounted in glycerine jelly: body length (TL); forebody length (FL); forebody width (FW); pseudosucker length (PSL); pseudosucker width (PSW); hindbody legnth at the bor-

der to the forebody (HL1); oral sucker length (OSL); oral sucker width (OSW); ventral sucker length (VSL); ventral sucker width (VSW); pharynx length (PHL); pharynx width (PHW); and Brande's organ length (TOL). The hindbody to forebody (HLFL) and oral to ventral sucker ratios (OSVS) were also calculated. In addition from stained specimens measurements were taken as: hindbody width at junction with forebody (HW2); Brande's organ width (TOW); ovary length (OVL); ovary width (OVW); anterior testis length (ATL); anterior testis width (ATW); posterior testis length (PTL); posterior testis width (PTW); distance between anterior testes and anterior end of body (TS). The oral sucker length to pharynx length ratio was also calculated (OSPH). Specimens were identified using the keys of Sudarikov (1960), Dubois (1970), McDonald (1981) and Sonin (1986), and compared with the descriptions of adult *Diplostomum* spp. by Williams (1966), Sweeting (1976), Lester & Huizinga (1977), Dick & Rosen (1981), Niewiadomska (1984) and Shostak *et al.* (1987).

Statistical analysis
As for the metacercariae, adult worms were compared using PCA.

Results

Metacercariae

Identification
Measurements of metacercariae dwelling between the retina and choroid of perch, from both the present study and the literature, are presented in Table I. Table II lists corresponding morphometric values of metacercariae reported from or living in the roach's lens. As a rule, regardless of the source of metacercariae, the degree of morphological variation found in the present study was greater than previously reported, especially in the size of the lappets and calcareous corpuscles, as indicated in the photograph in Fig. 5. Furthermore, fixatives and staining methods affected dimensions, especially in the case of the retinal form. Examination of the metacercariae with the SEM technique shows the general body surface and it confirms the external features observed with LM, namely, the oral and ventral suckers, the pseudosuckers and the tribocytic organ (Fig. 6C–D).

Judged from the information in Sudarikov (1971) and the key of Shigin (1976), the metacercariae from the retina of perch treated according to Shigin (1976) appeared morphologically most similar to *D. baeri* Dubois, 1937 and, with respect to the forms appearing in the roach lens, with *D. indistinctum* (Guberlet, 1923). Using the key of Shigin (1986), the perch form most probably must be identified with *D. volvens* Nordmann, 1832 and that from the roach with *D. helveticum* (Dubois, 1929). However, irrespective of their identity, it was possible to separate these metacercariae by overall differences in their morphology. This is especially true for the specimens fixed in Berland's fluid. Fig. 1 displays the grouping of individuals fixed in Berland's fluid when PRINC is based on the covariance matrix, whereas Fig. 2 shows specimens treated according to Shigin (1976). The associated eigen values and coefficients are listed in Table VIII.

Adults

Identification
The measurements of adult worms obtained in the present study are shown in Table IV. Corresponding figures for species reported in the literature as metacercariae from the lens of roach and from layers between the retina and choroid of perch are presented in Table V, whereas body dimensions from various descriptions of *D. spathaceum* and *D. baeri* are shown in Table VI. As in the case of the metacercariae, there is a considerable degree of variation within both groups of adults. Although there is some overlap, the adults of the retinal form can be morphologically distinguished from the lens dwelling ones by a smaller hind- to forebody ratio (Figs 6, 7). However, from the bivariate scatter diagrams showing the grouping of the specimens according to their first two principal components, it also appears distinct, but slight

Table I. Comparisons of the metric dimensions (in micrometres) of *Diplostomum* spp. metacercariae described, or reported, from between the retina and choroid in percid, with the exception of *D. baeri* (see Table III).

Reference		Lester & Huizinga	Williams	Sweeting	Hughes & Hall	Shigin	Present work	Present work
		(1977)	(1966)	(1976)	(1929)	(1986)		
Species		*D. adamsi*	*D. gasterostei*	*D. gasterostei*	*D. huronense*	*D. volvens*	Unstained	Shigin's
Source of worms		*Perca flavescens*	*Gasterosteus aculeatus*	*Gasterosteus aculeatus*	*Perca flavescens*	*Perca fluviatilis*	*Perca fluviatilis*	*Perca fluviatilis*
		Canada	Great Britain	Great Britain	USA	USSR	Sweden	Sweden
Number of specimens		5	10	10	10	20	30	20
		mean	range	range	range	range	mean (range)	mean (range)
Body	length	355	220–333	342–400	226–317	410–475	569 (510–640)	436 (297–552)
	width	180	210–220	228–325	155–219	185–230	304 (270–350)	227 (158–286)
Oral sucker	length	37	30–50	56–66	31–39	55–62	66 (50–80)	52 (38–61)
	width	38	20–40	42–52	31–39	40–50	62 (50–80)	45 (36–55)
Ventral sucker	length	35	30–40	38–64	28–39	45–50	64 (50–70)	48 (35–57)
	width	45	30–40	47–67	35–53	48–53	66 (60–80)	51 (35–63)
Brande's organ	length	100	20–60	77–117	28–77	90–115		93 (63–113)
	width	80	60–80	97–141	53–88	95–110		108 (80–135)
Pharynx	length	26	20–30	37–47	35–40		60 (40–80)	36 (28–46)
	width	24	10–10	19–27	26–28			27 (15–37)
Anterior to ventral sucker				155			305 (270–340)	240 (146–319)
Lime bodies	number							
Between lappets							139 (110–160)	
Ratios:								
AB body to AB Brande's						7.7–9.7		9.9 (8.7–11.0)
AB oral to AB ventral sucker						0.88–1.2	0.99 (0.61–1.8)	0.96 (0.76–1.2)
AB Brande's to AB ventral						3.4–5.0		4.1 (2.9–5.3)
B to A of body (%)						43.0–53.6	53.6 (48.3–64.2)	54.9 (39.1–96.4)
O to A of body (%)						50.5–56.8		54.5 (48.2–60.2)

A, length; B, width; product of AB; O, distance from centre of ventral sucker to anterior margin of body.
Tribocytic organ = Holdfast organ = Brande's organ; Ventral sucker = Acetabulum.

Table II. Comparison of the metric dimensions (in micrometres) of *Diplostomum* spp. metacercariae described, or reported, from the lens of roach *Rutilus rutilus*, with the exception of *D. spathaceum* (see Table III).

Reference		Shigin (1976)	Shigin (1986)	Shigin (1976)	Shigin (1986)	(Shigin (1986)
Species		*D. commutatum*	*D. helveticum*	*D. indistinctum*	*D. mergi*	*D. parviventosum*
Source of worms		lens	lens	lens	lens	lens
		Perca fluviatilis	*Leuciscus idus*	*Leuciscus idus*	*Abramis bramae*	*Gobio gobio*
		USSR	USSR	USSR	USSR	USSR
Number of specimens		20	20	20	20	20
		range	range	range	range	range
Body	length	350–435	352–469	352–460	382–458	405–485
	width	165–245	166–206	166–206	157–174	185–220
Oral sucker	length	45–55	48–58	48–58	33–50	37–45
	width	45–55	32–42	32–42	33–40	35–45
Ventral sucker	length	45–53	37–46	37–46	45–53	40–45
	width	50–55	44–53	44–53	45–55	40–53
Brande's organ	length	75–110	69–88	69–88	70–85	80–120
	width	80–115	78–106	78–106	70–90	80–105
Pharynx	length		28–35		25–33	25–30
	width		18–22		18–23	20–29
Lime bodies	number	122–279	304–449	304–449	702–854	496–724
Between lappets						
Anterior to ventral sucker						
Ratios:						
AB body to AB Brande's		7.24–10.5	8.0–11.7	8.0–11.7	9.43–13.38	6.95–13.4
AB oral- to AB ventral sucker		0.82–1.11	0.75–1.23	0.75–1.23	0.56–0.79	0.63–1.07
AB Brandes' to AB ventral		2.64–5.12	2.89–4.16	2.89–4.16	2.21–2.99	3.09–7.32
B to A of body (%)		45.7–59.7	43.5–53.6	43.5–53.6	34.5–43.2	39.6–49.4
O to A of body (%)		52.9–62.0	56.1–61.5	56.1–61.6	56.0–60.2	59.0–67.9

A, length; B, width; AB, product of AB; O, distance from centre of ventral sucker to anterior margin of body.
Tribocytic organ = Holdfast organ = Brande's organ; Ventral sucker = Acetabulum.

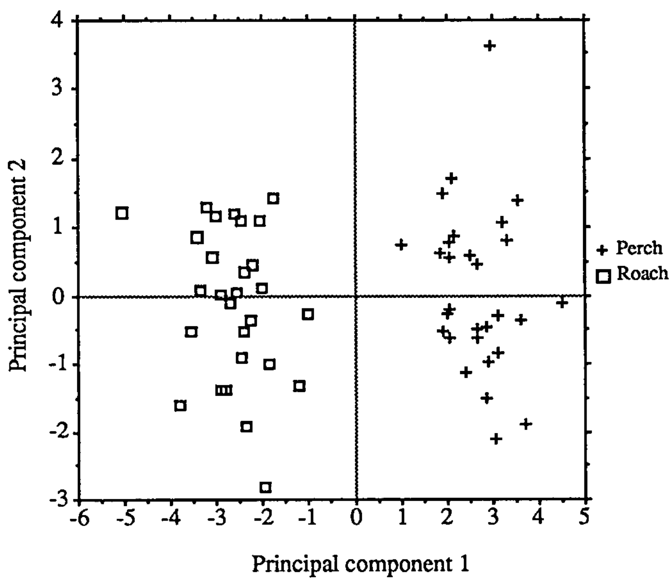

Fig. 1. Diplostomum spp. metacercariae unstained and mounted in glycerine jelly. Relative position of specimens from lens of roach and retinal layers of perch in the projection of the first two principal components based on a covariance matrix of morphometric variables shown in Table VIII.

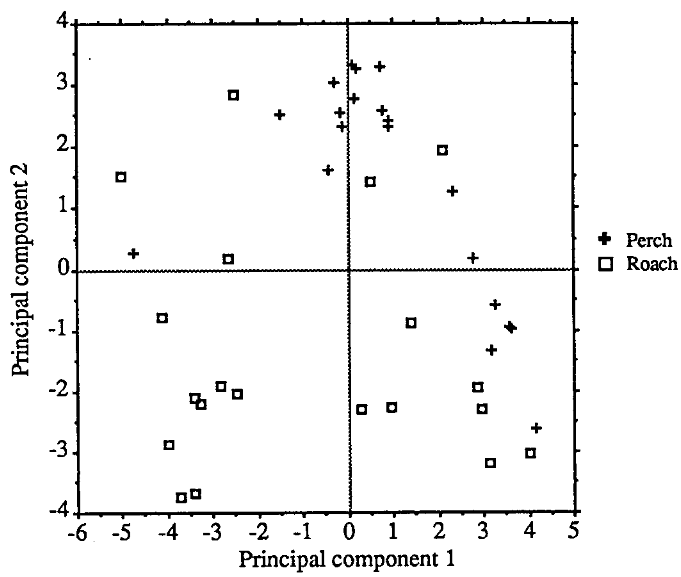

Fig. 2. Diplostomum spp. metacercariae treated according to Shigin (1976). Relative position of specimens from lens of roach and retinal layers of perch in the projection of the first two principal components based on a covariance matrix of morphometric variables shown in Table VIII.

Shigin (1976)	Shigin (1986)	Shigin (1976)	Shigin (1986)	Present work	Present work
D. paraspathaceum	*D. paraspathaceum*	*D. rutili*	*D. rutili*	Unstained	Shigin's
lens	lens	lens	lens	lens	lens
Leuciscus idus	*Salvelinus alpinus*	*R. r. lacustris*	*Rutilus rutilus*	*Rutilus rutilus*	*Rutilus rutilus*
USSR	USSR	USSR	USSR	Sweden	Sweden
20	20	20	20	30	20
range	range	range	range	mean (range)	mean (range)
395–465	370–455	370–470	355–390	342 (280–400)	331 (237–511)
155–185	170–200	200–290	195–210	253 (220–300)	231 (150–311)
45–50	45–50	47–55	49–55	51 (30–60)	47 (37–57)
35–42	43–47	36–49	40–47	51 (40–60)	42 (33–54)
35–40	38–42	47–52	40–47	42 (20–50)	40 (27–55)
40–45	40–45	52–60	47–55	51 (40–70)	48 (38–60)
65–80	70–85	70–107	80–95		67 (47–99)
60–75	60–75	88–128	90–100		98 (73–127)
	27–30	36–41	28–32	36 (30–40)	29 (19–39)
	17–22	25–30	18–22		22 (15–33)
201–271	188–258		122–279		
		105–157			
					187 (139–284)
13.0–16.7	10.1–15.8		8.09–9.70		12.0 (8.7–11.0)
0.98–1.31	1.08–1.47	0.66–0.91	0.92–1.13	1.1 (0.54–1.4)	0.96 (0.76–1.2)
2.17–3.56	2.50–3.99	2.28–4.50	3.29–4.28		4.1 (2.9–5.3)
34.7–45.4	40.2–50.0	46.0–64.0	51.3–57.7	52.3 (48.4–59.1)	54.9 (39.1–96.4)
63.8–67.2	60.5–72.5	51.5–63.2	51.4–55.8		54.5 (48.2–60.2)

differences are found within each group (Figs 3, 4). The associated eigen values and coefficients are listed in Table IX. Following the keys of Sudarikov (1960), Dubois (1970), McDonald (1981) and Sonin (1986), the average adult worms obtained from our birds experimentally infected with the lens metacercariae, as well as those found in the herring gull infected in the wild, should be identified as *D. spathaceum* (Rudolphi, 1819). On the other hand, adults of the retinal form obtained from the experimental infections and from the goosanders infected in the wild are more likely to be identified as *D. baeri* Dubois, 1937 or *D. mergi* Dubois, 1932 (depending on the host) following the keys of Sudarikov (1960) and Dubois (1970), to *D. mergi* Dubois, 1932 according to McDonald (1981) and to *D. volvens* Nordmann, 1832 or *D. mergi* Dubois, 1932 using the key of Sonin (1986). Furthermore, as in the case of the metacercariae, examination of the adults with SEM confirms the external features observed when using LM, rather than providing any additional information (Fig. 6A, B).

Recovery in the various bird hosts

The recovery of the *Diplostomum* adults in various birds infected on an experimental basis either with metacercariae from the lens of roach or with metacercariae from between the retina and choroid are given in Table VII. Compared with the

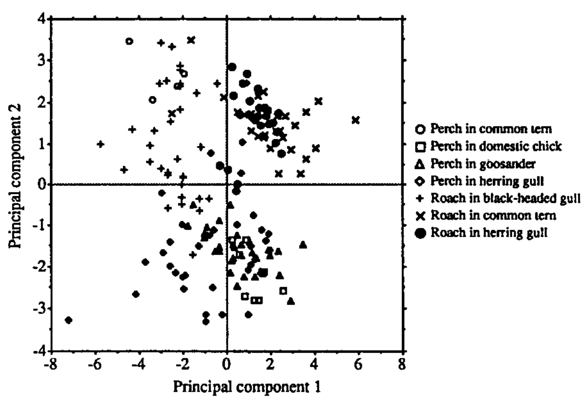

Fig. 3. Diplostomum spp. adults unstained and mounted in glycerine jelly. Relative position of specimens of seven samples groups in the projection of the first two principal components based on a covariance matrix of morphometric variables shown in Table IX.

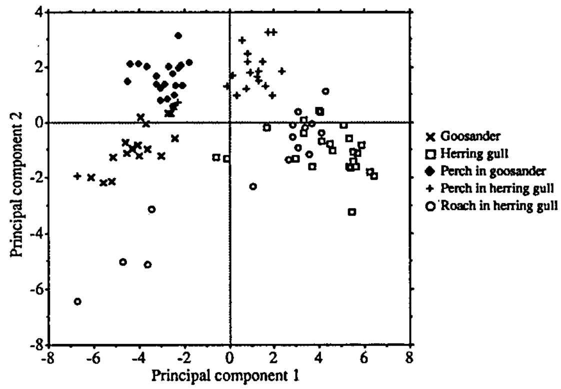

Fig. 4. Results of *Diplostomum* spp. adults stained with Mayer's Paracarmine. Relative position of specimens of five samples groups in the projection of the first two principal components based on a covariance matrix of morphometric variables shown in Table IX.

retinal form, the recovery of the lens form was better in the family Laridae. However, whereas the retinal form produced heavy infections when given to the goosander (family Anatidae), no adults were obtained when goosanders were fed with the lens form. Furthermore, in our experiments the domestic chicken was infected only with the retinal form.

Discussion

Morphology has been the criterion used in studies of the characterisation of populations within the genus *Diplostomum*. So far, metacercariae of thirteen different species have been found in the eyes, excluding the lens of fish of the genus *Perca*. Some

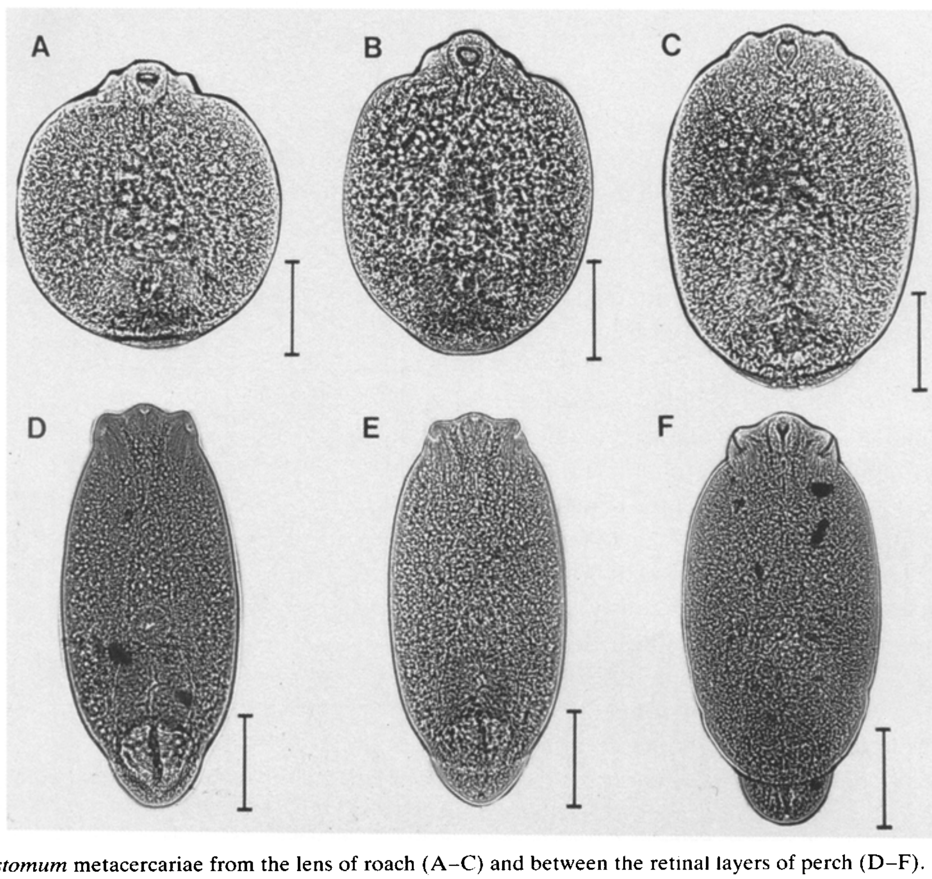

Fig. 5. Diplostomum metacercariae from the lens of roach (A–C) and between the retinal layers of perch (D–F). Fixed in Berland's fluid, cleared in lactophenol and mounted in glycerine jelly. *Scale-bars:* 0.1 mm.

Table III. Comparisons of the metric dimensions (in micrometres) of *D. spathaceum* and *D. baeri* metacercariae.

Species		*D. spathaceum*					*D. baeri*		
Reference		Komiya (1938)	Cichowlas (1961)	Sweeting (1976)	Shigin (1976)	Niewiadomska (1986)	Dick & Rosen (1981)	Shigin (1976)	Niewiadomska (1988)
Source of worms		unknown BRD	unknown Baltic	lens Cyprinidae *Great Britain*	lens *Leuciscus idus* *USSR*	lens *Cyprinus carpio* *Poland*	retina & sclera *Coregonus clupeaformis* Canada	retina & sclera *Perca fluviatilis* USSR	retina & sclera *Perca fluviatilis* Poland
Number of specimens				10	20	10	7	20	42
		range	range	range	range	range	range	range	range
Body	length	348–427	425–765	318–377	324–387	340–451	405–445	340–435 •	421–518
	width	352–264	225–391	215–260	143–163	170–296	175–255	195–220	199–259
Oral sucker	length	56–61	±51(?)	47–58	37–44	42–54	50–55	50–60	44–61
	width	47–53		40–61	35–39	42–52	50–60	35–45	40–54
Ventral sucker	length	47–54	51–54	37–47	30–35	39–56	45–53	35–47	37–54
	width	40–47		41–54	32–37	42–59	25–30	37–48	44–57
Brande's organ	length		68–105	48–73	62–74	68–93	70–115	80–1105(?)	85–122
	width		68–180	62–87	53–65	62–102	75–105	90–110	85–102
Pharynx	length	25–40		31–42		25–39	30–35		30–48
	width	18–35		18–26		12–25	25–30		23–37
Lime bodies	number				151–233	±300		443–664	500–700
Between lappets									
Anterior to ventral sucker									
Ratios									
AB body to AB Brande's					12.2–15.8			7.4–10.2	7.74–13.6
AB oral- to AB ventral sucker					1.07–1.47			0.85–1.35	0.72–1.27
AB Brande's to AB ventral					2.88–4.28			3.79–6.75	2.93–4.96
B to A of body (%)					38.6–52.7			47.7–60.3	43.53–53.38
O to A of body (%)					59.8–65.8			50.0–55.4	48.95–58.83

A, length; B, width; AB, product of AB; O, distance from centre of ventral sucker to anterior margin of body.
Tribocytic organ = Holdfast organ = Brande's organ; Ventral sucker = Acetabulum; Pseudosuckers = Lappets.

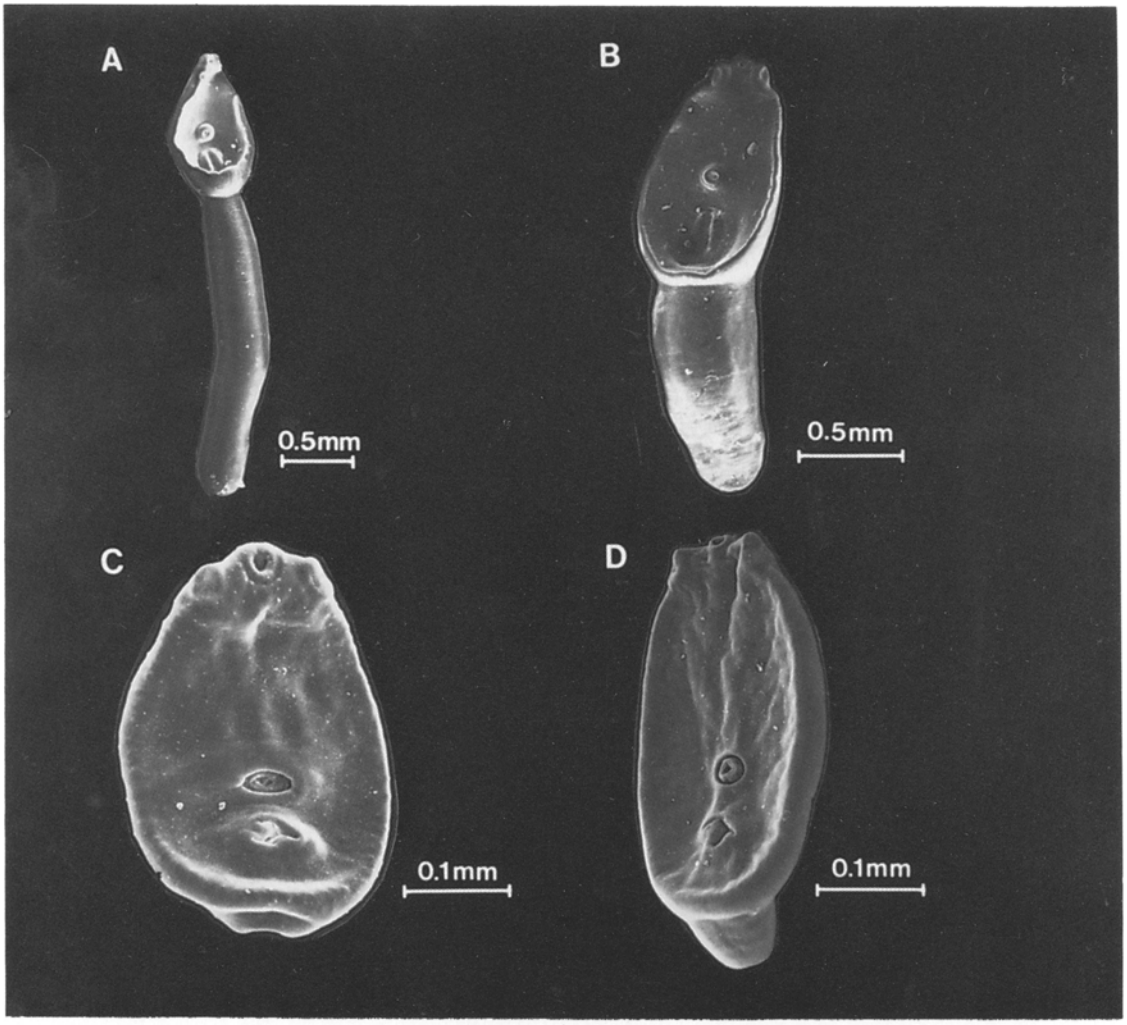

Fig. 6. SEM photographs showing adult worms from the intestine of herring gulls infected with metacercariae from the lens of roach (A) and the retinal layers of perch (B) and the metacercariae from roach (C) and perch (D).

species, like *D. clavatum* Nordmann, 1832, *D. scheuringi* Hughes, 1929 and *D. brevicaudum*, Nordmann, 1832 have been reclassified to the related genera *Tylodelphys* Diesing, 1850 and *Posthodiplostomum* Dubois, 1936. Others, like *D. gasterostei* Williams, 1966, *D. pseudobaeri* Razmaskin & Andrejuk, 1978, *D. pungitii* Shigin 1965 and *D. spathaceum* (Rudolphi, 1819), have only been reported to occur in perch without having been described (Lühe, 1909; Sudarikov, 1971; Kennedy, 1975, Shigin, 1976; Andrews, 1979). This is also the case for the American record of *D. huronense* (La Rue, 1927) from yellow perch *Perca flavescens* (Hughes & Hall, 1929; Tedla & Fernando, 1969; Sudarikov, 1971). Furthermore, as a rule the adult stages are often unknown and, of the metacercariae mentioned above, the life-cycle has only been experimentally verified for *D. gasterostei* and *D. spathaceum*. The description of *D. gasterostei* is, however, based on parasites obtained from pigeons given retinal forms of metacercariae from the three spined stickleback *Gasterosteus aculeatus* from a Scottish pond (Williams, 1966). Thus, *D. gasterostei* is considered synonymous with *D. pungitii* in the Russian keys of Sudarikov (1971) and Shigin (1986). *D. spathaceum* was found in perch by Kozicka (1958) and Wootten (1973) in Poland and the UK, re-

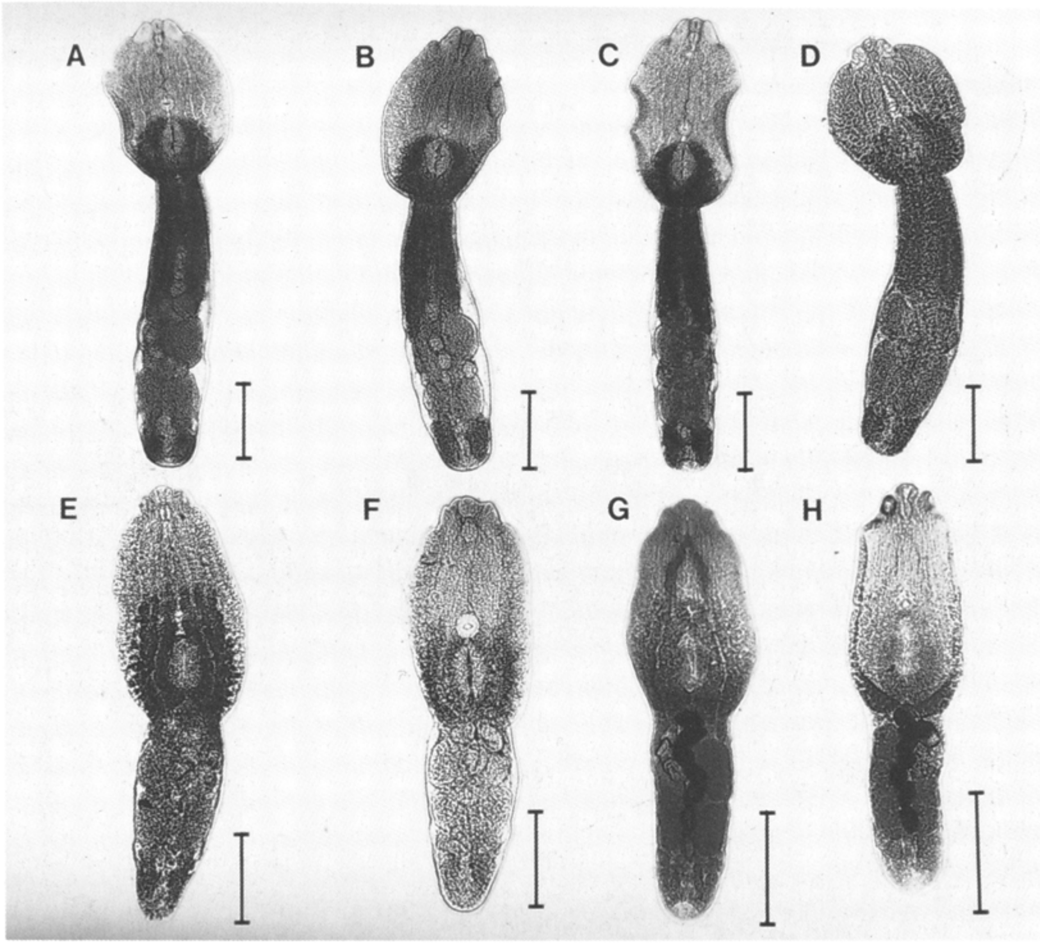

*Fig. 7. **Diplostomum*** **adults from the small intestine of herring gulls infected with metacercariae from the lens of roach (A–D) and with metacercariae from the retinal layers of perch (E–H). Fixed in Berland's fluid, cleared in lactophenol and mounted in glycerine jelly.** *Scale-bars:* 0.5 mm.

spectively. However, it is not clear whether any of these authors separated metacercariae in the lens from those in the retinal layer of the eye. The life-cycle of *D. spathaceum* has since then been verified, and subsequent experiments have clearly shown that the metacercariae live in the lens of the second intermediate fish host (Sweeting, 1976; Dick & Rosen, 1981; Niewiadomska, 1986). This is also probably true for the American records of *D. huronense* in yellow perch: feeding experiments performed by La Rue (1927) indicated that the vitreous humour form of *D. huronense* is distinct from that dwelling in the lens. *D. huronense* is considered a subspecies of *D. spathaceum* in the key of Dubois (1970).

In contrast, the descriptions of *D. baeri* Dubois, 1937 and the American species *D. adamsi* Lester & Huizinga, 1977 are both based on metacercariae from the retinal layer of perch and yellow perch, respectively (Sudarikov, 1971; Shigin, 1976; Lester & Huizinga, 1977). Also the adult stages have been described for both species. However, in the case of *D. baeri*, the adult stage was originally described from material obtained from larid birds infected in the wild (Dubois, 1970) and experiments performed to identify the retinal form of metacercariae by feeding herring gulls with metacercariae from two Canadian species of coregonid were not performed until quite recently (Dick & Rosen, 1981; Shostak *et al.*, 1987). The descrip-

Table IV. Metric dimensions (in micrometres) of *Diplostomum* spp. adults obtained from birds infected in the wild and from feeding experiments.

Source of worms		Experimental, roach lens				Experimental, perch retina
Host		*L. argentatus*	*L. argentatus*	*L. ridibundus*	*Sterna hirundo*	Domestic chicken
Stain		Mayer's				
Number of specimens		15	30	33	27	9
		mean (range)	mean (range)	mean (range)	mean (range)	mean (range)
Body	length		2383 (1742–2710)	1999 (1369)–2751)	2419 (1622–3204)	1492 (1319–1681)
Forebody	length	877 (677–1116)	965 (757–1417)	1047 (726–1459)	978 (718–1292)	863 (754–948)
	width	448 (?–664)	649 (515–772)	363 (271–506)	652 (401–845)	615 (530–685)
Hindbody	length	1108 (742–1571)	1431 (908–1727)	939 (632–1353)	1410 (971–2073)	621 (566–678)
	width 1	189 (87–255)	407 (323–477)	?	446 (306–577)	455 (416–498)
	width 2	273 (160–341)				
Oral sucker	length	64 (31–82)	81 (60–98)	67 (50–86)	78 (48–129)	98 (85–108)
	width	64 (31–81)	77 (61–102)	66 (50–89)	74 (54–92)	91 (78–105)
Lateral lappet	length	82 (35–113)				
	width	53 (32–86)				
Ventral sucker	length	74 (45–95)	80 (61–98)	86 (62–105)	88 (70–117)	94 (79–114)
	width	83 (50–110)	93 (74–111)	85 (70–102)	111 (80–148)	111 (102–142)
Pharynx	length	58 (31–79)	74 (60–98)	59 (34–81)	73 (57–94)	80 (69–90)
	width	45 (27–57)	53 (43–70)	47 (35–68)	57 (40–75)	56 (47–66)
Brande's organ	length	201 (132–267)	271 (172–360)	194 (118–277)	355 (228–561)	264 (223–305)
	width	138 (107–162)				
Ovary	length	115 (77–143)				
	width	115 (81–134)				
Anterior testis	length	193 (126–279)				
	width	196 (104–279)				
Posterior testis	length	234 (168–334)				
	width	239 (138–327)				
Anterior testes to anterior end		219 (127–303)				
Oral to ventral sucker		405 (143–639)				460 (402–550)
Oral sucker to pharynx ratio		1.1 (1.0–1.4)				
Oral to ventral sucker ratio		0.9 (0.6–1.2)	1.0 (0.8–1.2)	0.8 (0.5–1.2)	0.9 (0.6–1.1)	1.1 (0.8–1.3)
Hind- to forebody ratio		1.3 (0.8–2.1)	1.5 (0.7–2.2)	0.9 (0.6–1.5)	1.5 (1.0–1.9)	0.7 (0.6–0.8)

Tribocytic organ = Brande's organ = Holdfast organ; Ventral sucker = Acetabulum; Pseudosucker = Lappets.

tion of *D. adamsi* on the other hand is based on adults from herring gulls infected experimentally with metacercariae from the yellow perch (Lester & Huizinga, 1977). Compared with *D. adamsi* the identity of *D. baeri* is much more confusing. For example, Shigin (1976) first considered *D. baeri* as synonymous with *D. yogenum* (Cort & Bracket, 1937) and then in Shigin (1986) as synonymous with *D. volvens*. This latter species was originally described by Nordmann (1832) on material from the eyes of various freshwater fish, including perch, without distinguishing metacercariae from the different microhabitats within the eye. Thus the original description of *D. volvens*, like the records of *D. spathaceum* by Kozicka (1958) and Wootten (1973), probably represents a conglomerate of species. Nevertheless, Niewiadomska (1988) found no similarity in the original description of *D. volvens* and that of *D. baeri* and the synonymy of *D. baeri* (*yogenum*) and *D. volvens* is not accepted by her. Instead metacercariae in the eye-ball of perch derived from two lakes in Poland were identified as *D. baeri*. In addition, *D. numericum* Niewiadomska, 1988 was described. However, this species is primarily a parasite of rudd *Scardinius erythrophthalmus* and ruffe *Gymnocephalus cernuus* and the infection in perch can, therefore, be considered as accidental (Niewiadomska, 1988). Thus, it is not surprising that the parasite found as a metacercariae between the retina and choroid of perch in the present study has been identified differently, apparently de-

					Infected in the wild	
L. argentatus Mayer's	*L. argentatus*	*M. merganser* Mayer's	*M. merganser*	*Sterna hirundo*	*M. merganser* Mayer's	*L. argentatus* Mayer's
19	33	20	32	4	18	24
mean (range)	mean (range)	mean (range)	mean (range)	mean (range)	mean (range)	mean (range)
	1448 (668–2090)		1554 (1293–1867)	1807 (1622–1974)		
754 (468–934)	854 (475–1202)	521 (437–623)	848 (704–1000)	799 (709–891)	707 (577–790)	986 (711–1176)
416 (315–528)	466 (262–585)	347 (286–419)	497 (399–638)	396 (380–420)	320 (232–379)	518 (411–623)
644 (405–752)	614 (198–950)	409)327–479)	710 (585–880)	1015 (974–1079)	471 (368–847)	1247 (717–1656)
273 (204–345)	336 (207–488)	241 (180–323)	358 (305–459)	391 (354–421)	209 (173–263)	231 (187–270)
259 (154–332)		204 (176–236)			176 (123–221)	331 (226–466)
72 (50–87)	84 (61–104)	66 (47–79)	82 (64–125)	61 (49–81)	61 (56–73)	82 (60–95)
69 (38–82)	81 (62–106)	69 (60–85)	91 (68–126)	51 (46–62)	52 (42–63)	73 (53–86)
78 (51–100)		75 (62–82)			66 (43–100)	119 (100–161)
55 (32–69)		51 (42–59)			45 (34–60)	68 (52–85)
89 (55–103)	89 (61–117)	73 (61–81)	912 (68–126)	66 (58–72)	77 (59–88)	82 (61–96)
84 (56–98)	94 (67–123)	75 (68–83)	103 (76–134)	76 (61–99)	69 (54–85)	81 (67–95)
70 (51–79)	75 (52–94)	62 (56–70)	83 (67–106)	57 (46–67)	52 (44–64)	67 (51–79)
50 (30–60)	55 (35–69)	51 (46–61)	66 (52–83)	37 (33–42)	35 (24–49)	47 (34–56)
195 (128–253)	231 (137–332)	156 (95–177)	245 (199–300)	236 (192–276)	175 (129–228)	237 (158–321)
112 (69–160)		106 (82–130)			115 (80–161)	182 (119–272)
104 (63–145)		74 (58–90)			115 (80–161)	182 (119–272)
108 (73–132)		81 (59–103)			61 (44–78)	117 (72–181)
172 (100–213)		105 (77–125)			94 (58–119)	224 (128–326)
142 (118–226)		97 (58–124)			84 (53–135)	270 (167–369)
185 (114–231)		107 (73–177)			108 (68–163)	279 (162–384)
230 (109–299)		177 (125–201)			149 (107–186)	260 (177–351)
217 (143–262)		167 (110–229)			172 (153–217)	214 (135–259)
422 (272–517)		304 (242–341)	415 (317–490)	439 (384–477)	410 (287–481)	551 (409–670)
1.0 (0.9–1.3)		1.1 (0.8–1.3)			1.2 (0.9–1.5)	1.2 (1.0–1.4)
0.8 (0.7–1.0)	0.9 (0.6–1.5)	0.9 (0.7–1.1)	0.9 (0.6–1.2)	0.9 (0.8–1.4)	0.8 (0.7–1.0)	1.0 (0.6–1.3)
0.9 (0.7–1.1)	0.7 (0.4–0.9)	0.8 (0.6–0.9)	0.8 (0.6–1.0)	1.3 (1.1–1.4)	0.7 (0.5–1.4)	1.3 (0.9–1.7)

pending on its stage of development and according to the key used. On the basis of its distribution and with respect both to the adult worm and the metacercariae, the species living between the retina and choroid in perch in Swedish waters most probably corresponds with *D. baeri*.

As in the case of fish belonging to the Percidae, different species of metacercariae of the genus *Diplostomum* have been reported from fish of the genus *Rutilus*. Exclusively lens forms at present are eight species, *i.e. D. commutatum* (Diesing, 1850), *D. helveticum* (Dubois, 1929), *D. indistinctum* (Guberlet, 1923), *D. parvivetosum* (Dubois, 1932), *D. mergi* Dubois, 1932, *D. paraspathaceum*, Shigin 1965, *D. rutili* Razmashkin, 1969 and *D. spathaceum* (Rudolphi, 1819) (Sudarikov, 1971; Shigin, 1976; Lee, 1977; Burrough, 1978; Shigin, 1986; Kalfa-Papaionnou & Sinis, 1985). However, some of these species at present are considered as synonymous. For example, *D. commutatum* in the keys of Sudarikov (1971) and Shigin (1976) is synonymous with *D. rutili* in the key by Shigin (1986). *D. indistinctum* in Sudarikov (1971) and Shigin (1976) is regarded by Shigin (1986) as synonymous with both *D. helveticum* (Dubois, 1929) and *D. parviventosum* Dubois, 1932. Furthermore, *D. paraspathaceum* is synonymous with *D. paracaudum* (Iles, 1959), according to Bykhovskaya & Kulakova (1987), and *D. paracaudum* is in turn synonymous with *D. spathaceum* according to Shigin (1986).

Similarly to metacercariae in perch, most meta-

Table V. Comparisons of the metric dimensions (in micrometres) of *Diplostomum* spp. adults reported as metacercariae from the lens of roach and retina of perch, with the exceptions of *D. spathaceum* and *D. baeri* (see Table VI).

		Roach lens				Perch retina		
Reference		Dubois (1970)	Dubois (1970)	Dubois (1970)	Sonin (1986)	Lester & Huizinga (1977)	Williams (1966)	Sonin (1986)
Species		*D. commutatum*	*D. mergi*	*D. parviventosum*	*D. rutili*	*D. adamsi*	*D. gasterostei*	*D. volvens*
Source of worms		Experimental in *Chlidonias* spp. Source lens *Scardinius* *erythrophthalmus* *Hydroprogne* sp. *Larus* spp. *Sterna* spp. Europe, USSR	*Mergus* spp. *S. mollissima* *Tringa* spp. (?) Eurasia	*L. argentatus* (?) *Mergus* spp. Europe, USSR	Experimental in *Larus* spp. Source unknown USSR *Chlidonias hybrida* *Larus* spp. *Sterna* spp.	Experimental in *L. argentatus* Source retina & sclera *Perca flavescens* Canada	Experimental in pigeon Source retina & sclera *Gasterosteus* *aculeatus* Scotland	*Chlidonias* spp. *Larus* spp. *Stercocarius* spp. *Sterna hirundo*
Number of specimens						9	10	
		mean (range)	mean (range)	mean (range)	range	range	mean	range
Body	length	3200	1600	1500	1370–2170	1500–2100		2380
Forebody	length	760–1220	520–1080	660–810	780–1140	660–1080	480	500–1190
	width	460–840	230–560	340–500	450–(580	370–570	500	260–600
Hindbody	length	950–2160	250–700	540–810	680–1180	840–1080	490	310–1900(?)
	width	400–900	190–540	360–490	290–420	230–380	340	300–520
Oral sucker	length	69–109	36–76	40–50	66–88	56–94	60	50–95
	width	72–116	36–83		69–91	62–94	50	70–100
Lateral lappet	length					67–109		75–125
Ventral sucker	length	75–150	40–114	72–97	83–110	62Z–109	50	60–100
	width	82–185	50–136		88–110	72–109	70	40–70
Pharynx	length	60–91	540–74	54–60	60–77	44–72	50	60–100
	width	45–85	21–50	43–45	55–63	34–62	20	40–70
Brande's organ	length	157–640	100–280	135–250	275–350	181–275	120	145–312
	width	147–640	100–300		163–280	102–237	90	130–325
Ovary	length	75–160	58–110	117–135	72–110	87–105	60	90–225
	width	96–270	63–140	100–117	97–162	87–103	100	75–155
Anterior testis	length	185–575	90–170	115–200	124–234	156–281	130	110–390
	width	198–710	150–290	200–240	228–384	141–243	190	250–360
Posterior testis	length	185–840	70–225	288–432	179–349	190–281	130	115–400
	width	219–765	150–300	135–190	212–350	187–296	300	280–460
Hind- to forebody	ratio	1.06–2.62	0.41–0.94	0.70–0.92	(0.95)	(1.13)	(1.02)	(1.10)

Tribocytic organ = Brande's organ = Holdfast organ; Ventral sucker = Acetabulum; Pseudosucker = Lappets.
Values within the brackets is the mean ratio based on the maximum and minimum values.

cercariae in the lens of roach have only been reported and the only species previously described is *D. rutili* (see Shigin, 1986). However, unlike the situation with regard to *Diplostomum* metacercariae in perch, seven out of eight species reported from the lens of roach are also known as adults. Although these descriptions have sometimes been based on adults obtained from experimental infections, the source of metacercariae, as in the case of *D. rutili*, was either unknown (Sonin, 1986) or, as in the case of *D. commutatum*, derived from the lens of the rudd *Scardinius erythrophthalmus* (Yamaguti, 1975). Thus most descriptions of adult forms with metacercarial stages in the lens of roach are based on material from birds infected in the wild and they have yet not been verified on an experimental basis. According to the taxonomic keys of Sudarikov (1960, 1971), Dubois (1970), McDonald (1981) and Sonin (1986), the adults can be divided into two groups. One frequents goosanders. It has a conical hindbody and hind- to forebody ratio varying between 0.5–1.0, namely *D. mergi* and *D. parviventosum*. The second group is harboured by birds of the family Laridae. These worms have a more club-shaped hindbody and a hind- to forebody ratio above 0.7. They are described as *D. commutatum*, *D. indistinctum*, *D. paraspathaceum*, *D. rutili* and *D. spathaceum*. In the present study metacercariae from the lens of roach and recovered as adults from birds of the family Laridae were identified as *D. spathaceum*. This species was also found in one herring gull infected in the wild. Consequently, with respect both to their habitat and morphology we suggest that both *D. mergi* and *D. parviventosum* may prove to be the adult stage of the retinal form of metacercaria.

In conclusion, as stated by Thompson & Lymbery (1988), a clear view of what constitutes a species is essential for constructing formal taxonomic groupings and understanding modes of speciation. As species concepts channel our thinking the continuing controversy over the taxonomic status of variant populations within the genus *Diplostomum* is probably partly due to disagreement over appropriate criteria for distinguishing species. However, it is clearly demonstrated in the present study that we are dealing with species characterised by different morphology as well as suitability to different final hosts. One species lives in the retina of perch with adults mainly in goosanders, but it also becomes sexually mature in birds of the family Laridae. The second species is found as a metacercaria in the lens of roach and with adults exclusively in various larid birds. Furthermore, in agreement with Shostak *et al.* (1987), we found that the degree of morphological variation was greater than previously described. Thus, on that account several of the species described in the past ought to be regarded as synonymous. As regards forms of *Diplostomum* parasitic in perch and roach, the present investigation leads to the opinion that among earlier descriptions of *Diplostomum* spp. two main forms really exist, at least in Baltic coastal areas. These are here considered to agree with *D. baeri* Dubois, 1937 in the retinal layers of perch and *D. spathaceum* (Rudolphi, 1819) in the lens of roach. The reason for this judgment is the appearance of the corresponding morphologically distinct adults in different bird hosts, although they do exhibit a certain degree of overlap.

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Table VI. Comparisons of the metric dimensions (in micrometres) of *D. spathaceum* and *D. baeri* adults.

Species		*D. spathaceum*						
Reference		Krause (1914)*	Dubois (1938)	Cichowlas (1961)	Dubois (1970)	Dubois (1970)	Dubois (1970)	Sweeting (1976)
Subspecies					*huronense*	*indistinctum*	*spathaceum*	
Source of worms		*L. argentatus* *L. fuscus* *L. canus* BRD	*L. argentatus* ?	*L. ridibundus* Poland (Baltic)	*Larus* spp. USA	*Larus* spp. USA	*Larus* spp. *Sterna* spp. *Rissa tridactyla* *Alca torda*	Experimental in *L. argentatus* Source Cyprinidae
Number of specimens								10
		range	range	range	mean (range)	mean (range)	range	range
Body	length	1500–3370	1250–4450		3250	3400	1250–4450	737–2277
Forebody	length	600–1360	640–1800	731–1003	520–1620	820–1500	600–180	737–862
	width	360–690	270–960	510–935	450–920	270–850	270–960	
Hindbody	length	770–2100	520–3220	850–1105	520–1800	880–2280	520–3220	550–1416
	width	230–710	210–750	306–442	400–710	250–810	210–750	
Oral sucker	length	54–94	40–100	68–75	65–112	55–110	40–100	70–91
	width	54–99	48–104	±51	51–105	49–97	46–104	
Lateral lappet	length				80–135		75–180	
Ventral sucker	length	54–110	48–101	±60	60–120	60–110	48–110	98–102
	width	59–140	48–130	±96	73–138	60–135	48–140	
Pharynx	length	41–81	48–91		51–98	50–94	39–91	
	width	36–72	26–75		36–64	30–79	25–75	
Brande's organ	length	220–410	125–450	119–165	180–360	110–310	125–450	200–234
	width	240–390	90–432	±119	150–340	120–320	90–390	
Ovary	length	77–130	50–205	±102	69–170	80–190	50–205	136–197
	width	100–170	70–235	±170	123–225	90–200	70–235	
Anterior testis	length	170–340	95–460	±323	200–435	170–420	95–460	494–273
	width	280–520	130–560	±459	330–630	220–490	130–560	
Posterior testis	length	210–400	90–485	±306	225–500	220–560	90–485	385–519
	width	320–590	190–650	±435	370–610	240–560	190–650	
Hind- to forebody	ratio	(1.41)	(1.30)	(1.14)	0.90–1.29	0.73–2.00	0.66–2.62	(1.19)

Tribocytic organ = Brande's organ = Holdfast organ; Ventral sucker = Acetabulum; Pseudosucker = Lappets.
Values within the brackets is the mean ratio based on the maximum and minimum values.
*As cited in Dubois (1938).

Table VII. Recovery of experimental *Diplostomum* infections in different avian hosts.

Bird hosts, family and species		Number of infected hosts	Source of metacercariae in fish	Recovery as adults in birds
Family Laridae				
Black-headed gull	*Larus ridibundus*	1	roach lens	+++
Common tern	*Sterna hirundo*	1	roach lens	+++
		1	perch retina	+
Herring gull	*Larus argentatus*	3	roach lens	+++
		2	perch retina	++
Family Anatidae				
Goosander	*Mergus merganser*	2	roach lens	–
		2	perch retina	+++
Family Phasianidae				
Domestic chicken (juvenile)	*Gallus gallus*	1	roach lens	–
		1	perch retina	+
Domestic chicken (male)	*Gallus gallus*	1	roach lens	–
		2	perch retina	–
Domestic chicken (female)	*Gallus gallus*	1	perch retina	–

– missing, + present, ++ frequent, +++ very frequent.

Dick & Rosen (1981)	Niewiadomska (1984)	Sonin (1986)	*D. baeri* Dubois (1970)	Dubois (1970)	Dick & Rosen (1981)	Shostak *et al.* (1987)
indistinctum			*baeri*	*bucculentum*	*bucculentum*	*bucculentum*
Experimental in *L. argentatus*	*L. ridibundus*	*Larus* spp.	*Stercocarius* spp.	*Larus* spp.	Experimental in *L. argentatus*	Experimental in *Larus* spp.
Source *Coregonus clupeaformis*	Poland (Lake Memry)	Eurasia	Switzerland	USA (Alaska)	Source *Coregonus clupeaformis*	Source *Coregonus sardinella*
8	20				30	3–259
range	range	mean (range)	mean (range)	mean (range)	range	range
2730–3484		3830	1800	1700	1625–2106	930–2952
1326–1651	1110–1480	850–1630	500–930	460–1060	858–1235	460–1504
585–884	590–850	400–1090	260–600	380–590	507–774	235–543
1482–1872	1560–2920	1090–280	310–850	430–730	520–1027	366–1450
355–637	560–660	325–770	300–520	330–510	325–494	282–686
65–105	57–95	55–100	50–85	73–122	100–125	64–120
65–110	74–102	60–111	72–100	73–113	105–135	71–120
90–105	102–153	70–130	75–95	52–130	65–105	41–92
75–115	78–95	65–110	60–103	76–113	100–145	60–122
100–125	89–102	65–130	63–108	89–115	110–150	75–135
65–95	59–74	50–85	60–96	57–87	68–93	53–94
50–70	51–74	45–70	40–67	46–62	60–80	37–79
225–415	238–374	150–460	145–270	125–220	215–300	112–307
	259–399	150–440	120–225			
90–170	138–222	90–180	90–105	70–105	75–155	71–209
105–195	163–236	70–270	105–155	95–113	100–200	78–172
325–400	185–540	190–430	110–235	130–220	160–315	188–216
285–500	421–629	220–500	250–360	170–300	180–390	
320–425	348–592	220–550	115–250	150–250	190–355	188–357
355–510	466–658	280–550	280–435	300–460	290–435	291–630
1.05–1.33	(1.69)	(1.62)	0.52–1.00	0.53–0.88	0.51–1.04	0.53–1.53

Table VIII. Principal components analysis of 11–17 morphological measurements of *Diplostomum* metacercariae (values are for first four components as given below).

Treatment	Unstained in glycerine jelly, n = 40				Prepared according to Shigin (1976), n = 60			
Component	1	2	3	4	1	2	3	4
Eigen value	7.61	1.27	0.71	0.48	7.42	5.39	1.47	0.81
Cumulative proportion	0.69	0.80	0.87	0.92	0.43	0.75	0.83	0.89
Eigen vectors for character:								
TL	0.35	0.03	0.15	−0.03	0.06	0.40	0.20	−0.01
TW	0.28	0.11	−0.64	0.03	0.27	−0.22	0.21	0.07
VS	0.34	0.00	0.22	−0.10	−0.05	0.40	0.18	−0.14
OSL	0.26	0.43	0.08	0.65	0.21	0.19	0.44	0.00
OSW	0.26	0.42	−0.17	−0.56	0.28	−0.09	0.24	−0.21
PHL	0.32	−0.18	0.05	−0.06	0.33	0.13	0.02	−0.16
PHW								
LAP	0.34	0.09	−0.03	−0.23	0.35	−0.04	0.01	−0.03
VSL	0.33	−0.18	−0.12	−0.04	0.33	0.13	0.02	−0.16
VSW	0.31	−0.07	−0.15	0.44	0.35	−0.04	0.01	−0.03
TOL					0.13	0.37	0.11	0.29
TOW					0.34	0.03	−0.20	−0.02
HL					0.04	−0.28	0.33	0.59
BR					−0.19	−0.18	0.48	−0.30
OVR	−0.18	0.73	0.16	−0.02	−0.25	−0.03	0.49	0.03
TVR					−0.11	0.34	−0.08	0.46
WLR					0.09	−0.41	−0.04	0.05
VLR	0.30	−0.05	0.64	−0.06	−0.30	0.11	−0.01	−0.36

For abbreviations see *Materials and methods*.

Table IX. Principal components analysis of 19–28 morphological measurements on 166-96 adults of *Diplostomum* spp. (values are for first four components).

Treatment	Unstained in glycerine jelly, n = 166				Mayer's Paracarmine, n = 96			
Component	1	2	3	4	1	2	3	4
Eigen value	4.78	3.37	1.39	0.75	13.6	3.3	2.1	1.7
Cumulative proportion	0.36	0.62	0.73	0.79	0.52	0.65	0.73	0.79
Eigen values for character:								
LEN	0.26	−0.41	−0.10	0.17				
FL	0.15	−0.24	−0.35	0.57	0.21	−0.21	−0.21	0.18
FW	0.39	−0.03	0.18	−0.20	0.19	0.05	0.17	−0.02
VS					0.15	−0.12	−0.24	0.40
OSL	0.26	0.26	0.23	0.47	0.21	0.18	0.19	0.32
OSW	0.21	0.34	0.10	0.18	0.17	0.31	0.15	0.09
PHL	0.24	0.32	−0.02	−23	0.18	0.34	0.06	−0.06
PHW	0.26	0.28	−0.08	−0.19	0.13	0.36	0.11	−0.15
PSL					0.23	0.04	0.20	0.10
PSW					0.20	0.12	0.22	−0.01
VSL	0.16	0.22	−0.52	0.12	0.13	0.26	−0.36	0.26
VSW	0.32	0.19	−0.21	−0.11	0.18	0.26	−0.06	−0.08
TOL	0.38	−0.09	−0.01	−0.03	0.22	−0.07	−0.18	0.10
TOW					0.21	−0.17	0.05	0.13
HL	0.27	−0.42	0.00	−0.01	0.23	−0.23	0.07	−0.13
HW1	0.29	0.02	−0.02	−0.15	0.08	0.41	−0.06	0.07
HW2					0.24	−0.40	−0.06	−0.02
OVL					0.24	−0.04	−0.16	−0.14
OVW					0.23	−0.02	−0.13	−0.25
OVW					0.23	−0.02	−0.13	−0.25
ATL					0.25	−0.10	−0.04	−0.14
ATW					0.23	−0.20	0.02	−0.04
PTL					0.24	−0.16	−0.05	−0.10
PTW					0.23	0.00	−0.15	−0.07
TS					0.20	0.04	−0.19	−0.08
HLFL	0.24	−0.36	0.21	−0.31	0.18	−0.19	0.25	−0.33
OSPH					0.07	−0.20	0.21	0.54
OSVS					0.11	−0.06	0.56	0.10

For abbreviations see *Materials and methods.*

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