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DEFORMATIONS OF THE ATTACHMENT ORGAN IN DIPLOZOIDAE (PALOMBI, 1949) (MONOGENEA)

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ABSTRACT. This work consisted in studying the frequency of deformations of the attachment organ in specimens representing the family Diplozoidae, occurring in roach, common bream, and white bream from five different water bodies. These water bodies differed in morphometric parameters, levels of eutrophication, and character of their pollution. The presence of three types of deformation was stated. The most common were deformations of type II (morphological changes of clamps, such as sclerite deformation, incomplete sclerite separation or sclerites missing altogether in a clamp), while less frequent were those of types I (changes in the size of clamps not associated with the structural changes) and III (missing clamps in a set). Most frequently anomalies of the attachment organ were stated in polluted Łyna River, polluted Wulpińskie Lake, and in dystrophic Warniak Lake. Less frequent were anomalies in less eutrophied lakes Dgał Wielki and Ukiel. Also the prevalence values of those parasites were the highest in the polluted water bodies. The present study supports believes of other authors, that both infection parameters of Diplozoidae affecting fishes and deformations of monogeneans' attachment apparatus can be treated as indicators of environmental pollution.

Key words: deformations, Diplozoidae, environment, lake, pollution.

INTRODUCTION

Monogeneans of the family Diplozoidae (Palombi, 1949) parasitize gills of many fish species, in particular of the family Cyprinidae. These parasites, measuring between 0.3 and 14.9 mm have a potential to provoke distinct pathological changes in their hosts, which in turn can lower the market value of the latter. The fish infected, showed an excessive mucous production, contributing to a lower respiratory efficiency of the gills, inflammations, vacuolisation and necrotic changes in the tissues surrounding the attachment organ of the parasite, and also a hypochronic anaemia which can lead to a complete cachexia of the host (Kawatsu 1978, Kagel and Taraschewski 1993).

In the course of the ontogenesis of Diplozoidae two juvenile specimens, so called diporpae, fuse permanently and therefore such "double" parasite attaches to a fish with the aid of two powerful attachment organs (Fig. 2).

The attachment organ (haptors) consists of a single pair of median (embryonic) hooks and two rectangular attachment areas. Each attachment area consists of two rows of clamps (Figs. 3, 4). The clamps are composed of the following elements: median plate; trapeze spur; joining sclerites of the proximal tip of the median plate; sclerites of the distal tip of the median plate; median sclerite of the posterior jaw; lateral sclerite of the posterior jaw; spiky spur; anterior jaw. (Fig. 1)

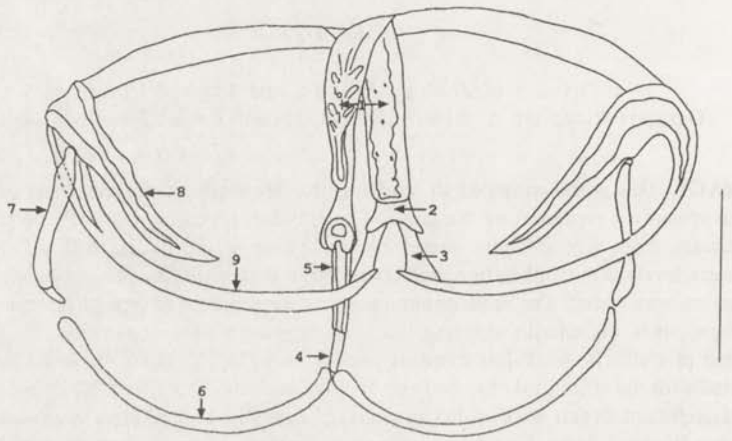

Fig. 1. Morphology of the attachment clamp of diplozoons. 1 – median plate, 2 – trapeze spur, 3 – joining sclerites of the proximal tip of the median plate, 4, 5 – sclerites of the distal tip of the median plate, 6 – median sclerite of the posterior jaw, 7 – lateral sclerite of the posterior jaw, 8 – spiky spur, 9 – anterior jaw. (scale bar = 50 µm)

The formation of the attachment organ starts at the stage of an early oncomiracidium. The median hooks develop first and are followed by clamps from the first to the fourth. Each consecutive clamp is formed after the previous one is complete (Koubkova 1999).

The structure of the attachment organ of Diplozoidae shows sometimes anomalies, which have been attributed, among other factors, to a mutagenic action of the environmental pollutants to parasite's ontogeny (Khan and Thulin 1991, MacKenzie et al. 1995, Dusek et al. 1998). Therefore these parasites are sometimes used as bioindicators of the state of environment (Koskivaara 1992, Kuperman 1992, Dusek et al. 1998, Zharikova 1993).

Three major types of teratological changes have been distinguished in the attachment organ of Diplozoidae (Sebelova and Koubkova 1997):

Type I. changes in the size of clamps not associated with the structural changes;

Type II. morphological changes of clamps in a form of sclerite deformation, incomplete sclerite separation or sclerites missing altogether in a clamp;

Type III. missing clamps in a set.

The aim of the present work was to determine what anomalies could occur in the attachment organ of Diplozoidae collected from fishes of five natural water bodies differing in their morphometric parameters, degrees of eutrophication, and specific character of their pollutants related to human impact.

MATERIALS AND METHODS

The study material was collected in lakes: Dgał Wielki, Warniak, Ukiel, and Wulpińskie. These lakes differ in their morphometric and trophic parameters (Table 1), and the character of their pollution. Dgał Wielki and Warniak lakes

Table 1. Morphometric parameters of lakes studied

Drainage basin	Dgał Wielki	Warniak	Ukiel Kortówka – Łyna	Wulpińskie Giłwa – Pasłęka
Longitude	21°47.6′	21°48.2′	20°24.9′	20°22.8′
Latitude	54°06.6′	54°07.4′	53°47.2′	53°42′
Elevation above sea level [m.].	120.1	120.4	104.4	105.8
Surface area [ha]	95.4	38.4	412	706.7
Maximal depth [m]	17.6	3.7	43	54.6
Average depth [m]	5.3	1.2	10.6	10.9
Water volume [in thousands m^3]	4995.9	456.7	43611.5	76990.3
Maximal length [m]	1300	1000	5360	8321
Maximal width [m]	1125	500	1715	2330
Length of coastal line [m]	5188	2625	22550	29800
Mictic type	dimictic	polymictic	dimictic	dimictic
Fisheries type	bream	tench-pike	bream	bream
Trophic type	α mesotrophic-eutrophic-	eutrophic dystrophic	α mesotrophic-eutrophic	eutrophic

are situated in the drainage basin of the Pregoła River, on the Masurian Lakes District in the mesoregion of the Great Lakes. Warniak Lake is a relatively small pond-like water body, assigned to the eutrophic-dystrophic type. Its average depth is 1.2 m. Lakes Ukiel and Wulpińskie are located in the group of Olsztyn lakes of the Masurian Lakes District. Ukiel Lake is relatively clean and eutrophic. Wulpińskie Lake is also eutrophic and it is polluted by a run-off from fields and municipal sewage. Its phytoplankton index suggests that it is a water body of advanced eutrophication and it is a subject of strong human influence (Łaźniewska 1996).

Fishes of lakes Dgał Wielki and Warniak were caught from October 1998 to November 1999, whereas those of lakes Ukiel and Wulpińskie from April 1999 to December 1999. A total of 943 roach from all lakes were studied. Their body length ranged from 12.5 to 30 cm and their weight from 17 to 370 g (Table 2). Common bream were caught at Dgał Wielki Lake in September 1998 (8 specimens) and in May 1999 (43 specimens). Totally, 51 common bream, measuring 22–33.5 cm (mean 26.7 cm) and weighing 85.76–388.8 g (mean 189.9 g) were examined. Two specimens of white bream, measuring 22.5 and 23.5 cm and weighing 124.2 to 153.5 g were caught in June 2001 in an overflow arm of the Łyna River in proximity of a sewage collector.

Table 2. Characteristics of roach examined

Lake	No. of fish examined	Total length (cm)		Weight (g)	
		Range	Average	Range	Average
Dgał Wielki	277	14–26	20	17–180	98.5
Warniak	251	13.5–22	17.75	23–160	91.5
Ukiel	213	12.5–30	21.25	35–370	202.5
Wulpińskie	202	13–28	20.5	35–147	91

The parasites were collected directly from one side of the gills (right- and left-side alternately), while gill arches of the other side were fixed in 4% formalin. Diplozoidae specimens, freshly isolated from gills were placed on a microscopic slide. Their haptors was severed and placed in ammonium picrate (GAP) and subsequently stained with Trichrom-Gomori (Koubkova 1999). Diplozoons isolated from the gills fixed in formalin were embedded in Faure fluid.

Specific identity of the parasites was determined with the aid of a key of Khotenovskij (1985). Elements of the haptor were measured under Nikon light microscope, using phase contrast and image analysis aided by MultiScan v. 4.2. Photographs and line drawings were done under an Olympus microscope.

RESULTS

The gills of the fish studied were inhabited by the following parasite species: *Paradiplozoon homoion* (Bychovsky et Nagibina, 1959), *P. megan* (Bychovsky et Nagibina, 1959) found of roach from four lakes, *P. bliccae* (Reichenbach-Klinke, 1961) on white bream from the Łyna river, and *Diplozoon paradoxum* (von Nordmann, 1832) on common bream of Dgał Wielki Lake (Table 3). Because of certain measurement difficulties some specimens of the genus *Paradiplozoon* from roach were not identified up to the species level.

Table 3. Infection parameters and frequency of attachment apparatus deformations in the material studied

Parasite species	Fish species	Water body	No. of fish infected	Prevalence %	No. of specimens with deformation	% of specimens with deformation
P. homoion (n = 46)	Roach	Warniak	8	3.18	4	8.7
		Dgał Wielki	15	5.41	1	2.17
		Wulpińskie	9	4.4	2	4.34
		Ukiel	7	3.3	–	–
P. megan (n = 127)	Roach	Warniak	30	11.95	8	6.3
		Dgał Wielki	30	10.8	7	5.5
		Wulpińskie	29	14	6	4.72
		Ukiel	16	7.5	1	0.78
Paradiplozoon sp. (n=23)	Roach	Warniak	5	1.99	–	–
		Dgał Wielki	6	2.16	–	–
		Wulpińskie	4	1.9	3	2.36
		Ukiel	5	2.35	1	0.8
P. bliccae (n=11)	White bream	Łyna	2	100	5	45.45
D. paradoxum (n=7)	Common bream	Dgał Wielki	5	9.8	1	14.28

Table 4. Infection parameters and frequency of deformations occurring in the attachment apparatus of *Paradiplozoon* sp. sp. parasitizing roach

Lake	No. of fish examined	No. of fish infected	Prevalence %	Total no. of parasites collected	No. of specimens with deformed attachment apparatus	% of specimens with deformed attachment apparatus
Wulpińskie	202	42	20.79	54	11	20.37
Ukiel	213	28	13.14	29	2	6.86
Dgał Wielki	277	51	18.41	63	8	12.69
Warniak	251	43	17.13	50	12	24

Prevalence values of *P. homoion* and *Paradiplozoon* sp. in roach were low in all lakes. Also prevalence value of *Diplozoon paradoxum* in common bream of Dgał Wielki Lake was low. On the other hand the prevalence value of *P. megan* in roach was 2–3 times higher in all lakes. Both white bream from the Łyna River were infected by *P. bliccae* (Table 3).

Irregularities in the attachment apparatus were stated in all listed earlier parasite species. Because not all specimens of *Paradiplozoon* from roach were assigned to respective species and they also showed anomalies, additionally the data concerning the parasites of all species of this genus are summarised for each lake (Table 4).

The highest percent of *P. homoion* specimens with deformations occurred in Warniak Lake, while the lowest in Dgał Wielki Lake. No deformations were recorded in *P. homoion* specimens from Ukiel Lake. Similarly, the highest percentage of *P. megan* specimens with deformations occurred in Warniak Lake, while the lowest in Ukiel Lake. Almost 50% of the specimens of *P. bliccae* from white bream had their haptors deformed. On the other hand, only a single specimen of *Diplozoon paradoxum* from common bream exhibited deformations (Table 3). Among all parasites of the genus *Paradiplozoon* collected from roach, the highest rate of anomalies was recorded in the material from Warniak and Wulpińskie lakes, fewer anomalies in the material from Dgał Wielki Lake, and only sporadically occurring anomalies in the material from Ukiel Lake (Table 4).

In the studied material all three deformation types were present (Table 5). The most frequent was type II, observed in *P. homoion*, *P. megan*, (Figs 8–12) and *Paradiplozoon* sp. collected from roach from all lakes. Type II was also recorded from *P. bliccae* of white bream. Most often this anomaly consisted in the lack of a sclerite (mainly the anterior one) in a clamp. In one case the sclerites were not separated. Type I (smaller clamps) occurred chiefly in *P. bliccae* from white bream (Fig. 7) and twice in *P. megan* from roach (Figs 5, 6, 10) and *Diplozoon paradoxum* from common bream (both cases from Dgał Wielki Lake). Type III (lack of some clamps) was rarely observed in all species of the genus *Paradiplozoon*: in *P. homoion* in the material from Dgał Wielki Lake, in *P. megan* in the material from Warniak Lake, and in material from the Łyna River (Figs 13, 15–17). Also an extra clamp was found in a single specimen of *P. bliccae* from white bream taken in the Łyna River (Fig. 14).

Besides the attachment organ, no other morphological changes were visible. Moreover, the tissue surrounding abnormal apparatus was not damaged, which is an evidence that the lack of some sclerites or entire clamps in the specimens of Diplozoidae resulted neither from inaccurate preservation nor histological processing.

Table 5. Deformations of attachment apparatus of Diplozoidae collected from fishes of particular lakes

Water body	Parasite species	Size of medial hook (in μm)	Location of clamp	Deformations
Dgał Wielki	*P. megan*	22.60	4R	Fourth clamp smaller in relation to 4L
	P. megan	23.01	4L	Lack of medial sclerite of posterior part of clamp
	P. megan	22.39	4R & 4L	Lack of anterior sclerites in clamps
	P. megan	22.45	3L	Third clamp smaller in relation to 3R
	P. megan	24.06	4R	Lack of posterior sclerite
	P. megan	22.03	4R & 4L	Not separated sclerites of posterior part of clamp
	P. megan	24.07	2L	Lack of medial sclerite
	P. homoion	21.0	L	Lack of one row of clamps in haptor
	Diplozoon paradoxum	33.90	2R	Second right clamp smaller in realtion to 2L
Ukiel	*Paradiplozoon* sp.	–	4R	Lack of anterior sclerites
	P. megan	23.21	1R	Lack of anterior sclerites
Warniak	*P. megan*	22.34	4L	Lack of posterior sclerite
	P. megan	23.06	L	Lack of 1st and 2nd clamp
	P. megan	23.56	3L	Lack of anterior sclerite
	P. megan	21.95	4R & 4L	Lack of anterior sclerites
	P. megan	22.03	2R	Lack of anterior sclerite
	P. megan	21.95	4L	Lack of anterior sclerite
	P. megan	23.45	3R & 2, 4L	Lack of anterior sclerites
	P. megan	23.02	3L	Lack of anterior sclerite
	P. homoion	20.33	1 & 2 L	Lack of 1st and 2nd clamp
	P. homoion	20.03	3 R	Lack of anterior sclerite
	P. homoion	20.61	3 R	Third clamp vestigial
	P. homoion	20.02	2 R	Lack of anterior sclerite
Wulpińskie	*P. megan*	23.41	1R	Lack of posterior sclerite
	P.megan	21.90	4R & L	Lack of anterior sclerites in clamps
	P.megan	21.60	4R	Not separated posterior sclerites
	P.megan	22.30	4R & L	Lack of anterior sclerites
	P.megan	22.63	4R	Lack of anterior sclerites
	P.megan	23.01	3L	Lack of anterior sclerite
	P. homoion	21.03	2 & 4R	Lack of anterior sclerites
	P. homoion	20.75	4L	Lack of posterior sclerites, deformations of anterior sclerites
	Paradiplozoon sp.	–	3R & 4R	Deformations of anterior sclerites
	P. sp	–	1L	Lack of anterior sclerites
	P. sp	–	4L	Lack of anterior sclerites
Łyna River	*P. bliccae*	23.45	2L	Second clamp smaller in relation to 2R
	P. bliccae	23.91	3L	Third clamp smaller in relation to 3R and deformations of anterior sclerites
	P. bliccae	24.0	L	Additional fifth clamp
	P. bliccae	23.75	2L	Both second clamps smaller on one haptor
	P. bliccae	24.01	R, L	Two haptors with a single row of clamps

L – left side of haptor
R – right side of haptor

DISCUSSION

Anomalies in the structure of the attachment organ of Diplozoidae have been observed many times. Bovet (1967) blamed the changes in the haptor structure in *Diplozoon paradoxum* on unfavourable environmental conditions in the laboratory. Kuperman (1992) found specimens of *D. paradoxum* with structural anomalies (reduction of attachment organ, irregularities in symmetry, arrangement, and the number of clamps). Oliver (1971), who noticed various anomalies in the haptor of *D. gracile* from *Barbus meridionalis*, linked them to mutagenic effect of toxic substances in the water. Also Dzika et al. (1996) described anomalies in *Paradiplozoon homoion* from roach of a eutrophic lake in Bavaria, Germany. They consisted in the lack of a clamp row and the presence of a rudimental clamp in the armament. Comparative studies conducted by Sebelova and Koubkova (1997) in three localities (Nove Mlyny, Brodske, Bolelouc) revealed teratological changes in clamps of *P. homoion, P. megan, P. ergensi*, and *D. paradoxum.* Distinctly higher frequency of teratological changes was observed in parasites from polluted areas (Nove Mlyny and Bolelouc). Koubkova et al. (2001) stated anomalies in the attachment apparatus of *P. homoion* from *Alburnoides bipunctatus* of the River Vlara, which were associated with the highest heavy metal content in the host tissue.

The present study show that the level of eutrophication and pollutants have a distinct effect of the frequency of teratological changes occurring in four species of the family Diplozoidae. The highest percent of parasites with deformations of their attachment organ was noted in sewage-polluted Łyna River. Also a high percent of deformations occurred in polluted Wulpińskie Lake as well as in dystrophic Warniak Lake. Substantially lower percentage of specimens with deformations was recorded in eutrophic Dgał Lake, while the lowest in the least eutrophied and relatively clean Ukiel Lake (Tables 3, 4).

The polluted lakes featured also a higher prevalence of these parasites (Table 3), which is in accordance with Kuperman's (1992) observations. The latter author stated higher infection of common bream with *Diplozoon paradoxum* in a polluted water body.

The present study supports believes of other authors, that both infection parameters of Diplozoidae affecting fishes and deformations of monogeneans' attachment apparatus can be treated as indicators of environmental pollution.

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PODPISY POD RYCINY (autor E. Dzika)
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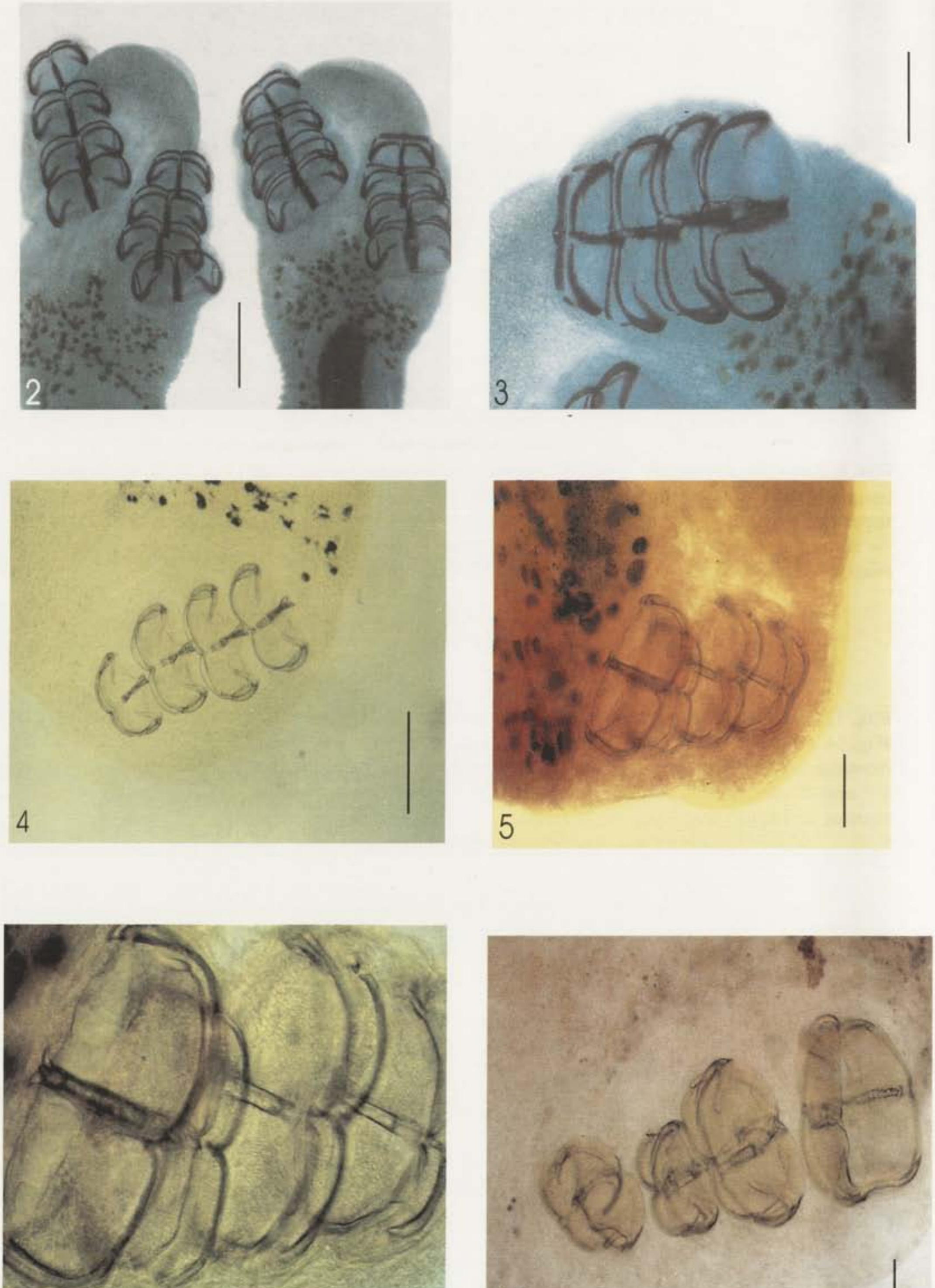
Fig. 2. *Paradiplozoon megan* – regular attachement organ (scale bar = 200 µm). Fig. 3. *Paradiplozoon megan* – regular haptor (scale bar = 100 µm). Fig. 4. *Paradiplozoon homoion* – regular haptor (scale bar = 100 µm). Fig. 5. *Paradiplozoon megan* – third clamp smaller in relation to 3R (scale bar = 100 µm). Fig. 6. *Paradiplozoon megan* – third clamp smaller in relation to 3R (scale bar = 100 µm). Fig. 7. *Paradiplozoon bliccae* – second clamp smaller in relation to 2R (scale bar = 50 µm).

Fig. 8. *Paradiplozoon megan* – lack of anterior sclerites (scale bar = 50 µm). Fig. 9. *Paradiplozoon megan* – lack of anterior sclerites (scale bar = 100 µm). Fig. 10. *Paradiplozoon megan* – not separated sclerites of posterior part of 3 clamp (scale bar = 200 µm). Fig. 11. *Paradiplozoon megan* – not separated sclerites of posterior part of 3 clamp (scale bar = 100 µm). Fig. 12. *Paradiplozoon megan* – deformations of anterior sclerites of 4 clamps (scale bar = 200 µm).

Fig. 13. *Paradiplozoon bliccae* – two haptors with a single row of clamps (scale bar = 200 µm). Fig. 14. *Paradiplozoon bliccae* – additional fifth clamp (scale bar = 50 µm). Fig. 15. *Paradiplozoon homoion* – third clamp rudimental (scale bar = 50 µm). Fig. 16. *Paradiplozoon megan* – lack of 1^{th} and 2^{th} clamp (scale bar = 200 µm). Fig. 17. *Paradiplozoon homoion* – lack of one row of clamps in haptor (scale bar = 200 µm).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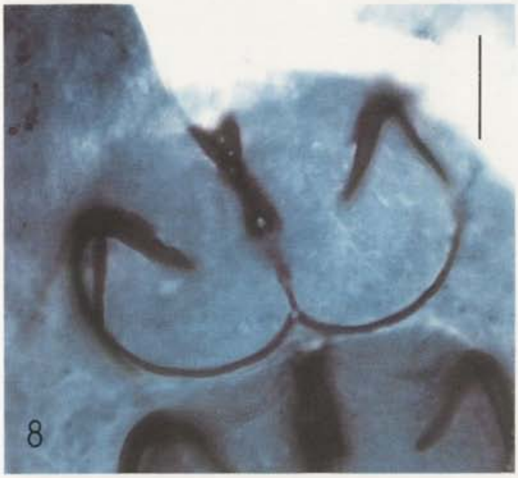
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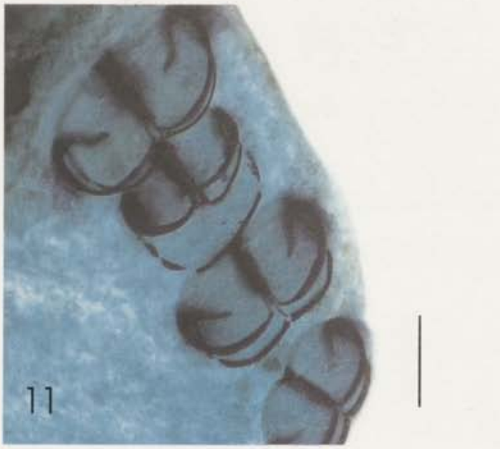
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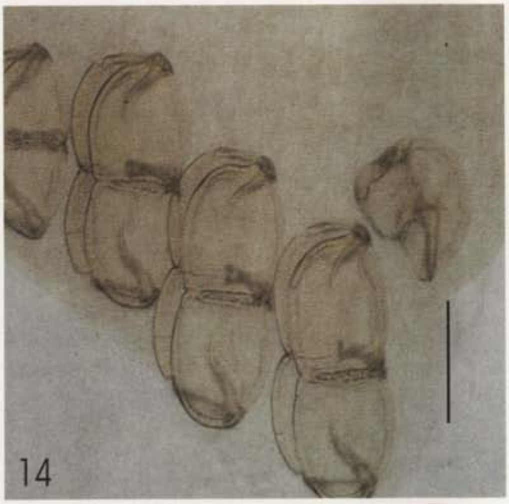

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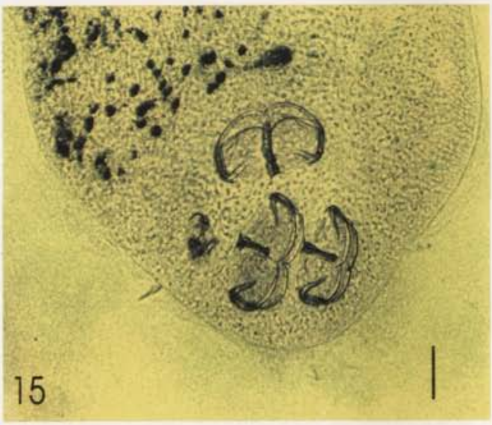

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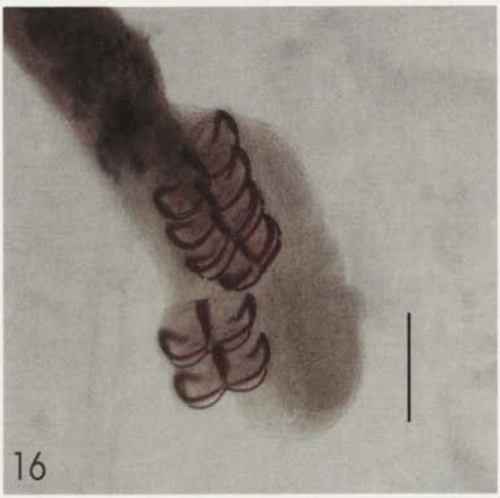

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