TAXONOMY AND BIOLOGY OF DIPLOZOIDAE (MONOGENEA)

Thesis submitted in accordance with
the requirements of the University
of Liverpool for the degree of Doctor
in Philosophy

by

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Dedicated to those who made
this book possible, particularly
my parents, my brother, Mustafa
and my wife, Rajaa with gratitude
and humility.

Contents

Page

Page

Page

Abstract

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The study is divided into two parts. The first considers the taxonomy of species of Diplozoidae in the British Isles and the world. The second investigates the biology of Diplozoon homoion in field and experimental conditions.

As a result of an extensive literature survey, the distribution of species of Diplozoidae has been assessed. About 63 species were found on 78 species of fishes of the families Cyprinidae and three of Characidae. The species of parasites occurred in Asia, Europe and Africa but not in Australia or North and South America. The factors effecting this distribution were discussed.

Critical taxonomic studies on British materials revealed that there are two species, D. paradoxum from Abramis brama distinguished especially by an invagination and a few ridges (deep folds) on the posterior parts of the adult stages, and, D. homoion from various species of Cyprinidae, including A. brama, with an absence of these two characters on the adults.

A study of the morphology of all stages in the life cycle of D. homoion revealed that most of the characters currently used in systematic work on Diplozoidae showed great variation and were therefore unreliable. As a consequence, many species which have been described are synonyms. Proposals made by other authors for new genera, subgenera and subspecies of parasites are critically discussed with special

reference to phylogeny. The genera Diplozoon, Eudiplozoon and Inusiatus are thought to be valid. The genus Neodiplozoon requires further investigation. All the other genera, subgenera and subspecies are considered invalid. The Diplozoidae probably originated from both Microcotylidae and Discocotylidae, with the genera Eudiplozoon and Inusiatus ancestral to the other members of the family.

The life cycle of D. homoion from Llyn Tegid was studied experimentally under laboratory conditions. At water temperatures of 18°-21°C, the life cycle took 14-20 days from egg laying to formation of the gravid worm.

The prevalence, relative density, mean intensity and intensity of infection of Rutilus rutilus with D. homoion from Llyn Tegid were investigated from September, 1982 to December, 1983 and in June and July 1984. Infections were almost constant on all male, female and unsexed fishes throughout the year. The level of infection of fishes of different fork lengths was also investigated. Infections were minimal in fishes less than 10 cm long, but increased progressively with increasing lengths up to 20 cm but declined thereafter.

The distribution of adult D. homoion on the gills of R. rutilus from Llyn Tegid was also examined in relation to the serial number of the gill arches; the side of the fish, the inner and outer hemibranchs, the segment of the gill and the manner of attachment of the clamps to one or two consecutive primary lamellae. It was found that the adult parasites were randomly distributed in relation to the gills' structures except for a preference for attachment to the first 3 gills and to the dorsal segments of the gills. Two consecutive primary lamellae were used for attachment twice as often as attachment to a single lamella. Season, sex and fork length of the fishes had no effect on the distribution of the parasites on the gills.

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CHAPTER 1

GENERAL INTRODUCTION

I. ECONOMIC IMPORTANCE OF *DIPLOZOON* INFECTION

This group of monogenean parasites has potential economic importance by causing damage to the gill tissues and feeding on the blood of their hosts, Cyprinidae and a few Characidae. Kawatsu (1978) found that the level of the haemoglobin of *Carassius carassius* decreased with increasing numbers of *D. nipponicum* and that the relation was approximately linear. The effect on the blood of the fishes was found to be more severe in March than in October. Khaberman *et al.* (1973) also found an inverse relationship between the level of serum globulin in *Abramis brama* and the number of gill parasites including *Diplozoon* species. In contrast Wiles (1970) reported that there was no observed correlation between the level of infestation with *D. paradoxum* and the packed red cell volume or serum haemoglobin values of the blood of infected fishes, suggesting a high degree of adaptation of the parasite to the host. He thought that the worm fed at one or two sites, leaving little evidence of destruction of gill epithelium. The present results (Chapter 7, Figs. 7.1 and 7.4) show clear damage of the gill tissues not only those affected directly by the adhesive organs of the parasite but also those close to the opisthaptors. More information is needed about the effect of *Diplozoon* infections on the host.

II. THE AIM OF THE PRESENT STUDY

Many species of Diplozoidae have been recorded throughout the world, but most of the original descriptions have used characters which I shall show have no taxonomic value. Very limited data have been published on the biology and seasonality of these parasites (Chubb, 1977).

In Britain, Diplozoon occurs on most species of cyprinids but, until now, only one species has been recorded in the British Isles (Dawes, 1946; Kane, 1966; Chappell and Owen, 1969; and Kennedy, 1974).

Accordingly, my study attempted to:

A. Make a literature survey of the distribution of all species of Diplozoidae described from the world and to consider the factors which influence their distribution. This is presented in Chapter 2.

B. Make a systematic study on Diplozoon materials from Cyprinidae collected in the British Isles. Some Overseas material was also available for comparison. Observations were made on adult, egg and larval stages of these parasites. The morphological investigations of these stages were supported by experimental and field studies which are described in Chapter 3.

C. The taxonomy and phylogeny of the species of Diplozoidae of the world have been critically reassessed in Chapter 4 as a result of my studies described in Chapter 3.

D. The life cycle of D. homoion was experimentally investigated in the laboratory and supported by field observations. The longevity of each stage in the life cycle was determined and the effects of some biological factors were investigated (Chapter 5).

E. The seasonal dynamics of infections of D. homoion on the gills of Rutilus rutilus were observed for more than one year. The effect of host-sex and fork length of fish on the level of infections was also studied (Chapter 6).

F. Finally, the distribution of these parasites on the gills of their hosts in relation to the serial numbers of the gill arches, the inner and outer hemibranchs, the gills of the right and left sides of the fishes, the attachment to one or two secondary lamellae and the occupation of a particular segment of the gill. These observations were made on samples of fishes collected throughout the year. The effects of host-sex and fork length of fish on the mode of distribution of the parasites were also recorded (Chapter 7).

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CHAPTER 2

THE DISTRIBUTION OF SPECIES OF DIPLOZOIDAE

I. INTRODUCTION

In freshwater fishes and their parasites, there are many factors which govern their distribution and interrelationships, e.g. water temperature, current velocity, oxygen levels, the amount of suspension, the amount of chlorides and pollution, water structure, presence or absence of intermediate hosts, type of the bottom, fauna present in and around the habitat, the physiological and biological features of the host and the water depth (Prost, 1957; Dogiel, 1961; Shul'man, 1961). There are also many factors which influence the distribution of the parasites within the fish population, e.g. sex (Paling, 1965; Kennedy, 1968; Chappell, 1969), breeding season (Thomas, 1964a; Kennedy, 1966), age (Chappell, 1969; Thomas, 1964b; Davis, 1967; Anderson, 1974), size of fish (Anderson, 1974), behaviour of fish (Paling, 1965) and the diet of the host (Chubb, 1963; Awachie, 1966a and b; Thomas, 1958).

Diplozoon infections were first observed in Europe early in the last century (Nordmann, 1832). Since then, almost all studies on the distribution of these monogenean trematodes have been restricted to the freshwater fishes of Europe.

A parasite-host checklist of Diplozoon parasites from the freshwater fishes of the world has been assembled. The monogenean fauna of Russia was listed by Gussev in Bychowskaya-Pavlovskaya et al. (1962), of the U.S.A. by Hoffman (1967), of Canada by Margolis and Arthur (1979), of the British Isles by Kennedy (1974), of Africa by Khalil (1971) and Paperna (1979), of India by Chauhan (1953) and Tripathi (1959a). Most of the parasite collections of the world were listed by Lichtenfels and Pritchard (1982).

II. PARASITE-HOST CHECKLIST

A parasite-host checklist of Diplozoon species is provided in Table 2.1. The species are arranged according to their continents and countries of origin. The host family and type of body of water where the parasites were found are also given. It is obvious from the Table that Diplozoon populations are widely distributed in Asia, Europe and parts of Africa as illustrated in Fig. 2.1. Diplozoon infections are absent from freshwater fishes of Australia, North and South America. Their distribution from the north towards the south of the earth covers a wide range of habitats from the equator, where temperatures are high, up to Siberia in the north, where overwinter temperatures are low. The main hosts for these parasites are cyprinids and a very few of characids. Nearly 63 species of Diplozoon have been found on the gills of 78 cyprinids and 3 characids species.

In Asia alone, there are 52 Diplozoon species all found on 60 species of freshwater fish, all of them Cyprinidae. In Europe, 15 Diplozoon species are recorded from nearly 39 cyprinids and 1 characid species. The wide distribution range of Diplozoon in Asia might be attributed to the larger number of freshwater fish species available there, in contrast to west Europe. According to Wheeler (1969), there are 74 species of freshwater fish found in north west Europe against 301 species (Berg, 1949) occurring in eastern Europe and north Asia. In Africa, there are 4 species of Diplozoon found on the gills of 9 species of cyprinids and 2 of characids. Surprisingly, Diplozoon infections are absent from freshwater fishes of Australia (Beumer, personal communication, 1983 and Beumer et al., 1983) and from Canada and North America (Hoffman, personal communication, 1984, Margolis, personal communication, 1983; Margolis and Arthur, 1979). It seems that

Table 2.1. Parasite-host checklist of the Diplozoidae of the world

Country	Parasite	Host: Species	Host: Family	Locality	Author
		Continent: ASIA and Adjacent localities			
CHINA	*Diplozoon aristichthysi* Ling, 1973	*Aristichthys* species	Cyprinidae	None given	Khotenovskiĭ, 1978
	D. nipponicum Goto, 1891	*Carassius carassius* (L.)	"	None given	Yin and Sproston, 1948
	Paradiplozoon cyprini Khotenovskiĭ, 1982	*Cyprinus carpio haematopterus* Temminck, Schlegel	"	Liaohe River basin	Khotenovskiĭ, 1982
INDIA	*D. cauveryi* Tripathi, 1959	*Cirrhina cirrhosa* (Bloch)	"	Mettur Dam Reservoir	Tripathi, 1959a
	D. dayali Pandy, 1973	*Catla catla* (Hamilton)	"	District Ballia	Pandy, 1973
	D. indicum Dayal, 1941	*Barbus (Puntius) sarana* (Hamilton)	"	Gomati River, Lucknow	Dayal, 1941
	D. kashmirensis Kaw, 1950	*Schizothorax* spp.	"	Dal Lake, Kashmir	Kaw, 1950
	D. microclampi Kulkarni, 1971	*Barbus sarana* (Hamilton)	"	Hussain Sagar Lake, Hyderabad	Kulkarni, 1971

	D. soni Tripathi, 1959	*Oxygaster bacaila* (Hamilton)	Cyprinidae	River Son at Dehri-on-Son	Tripathi, 1959a
	D. thapari Gupta and Krishna, 1977	*Tor tor* (Hamilton)	"	Nanak Sagar Dam, Nainital	Gupta and Krishna, 1977
	Neodiplozoon barbi Tripathi, 1959	*Barbus chagunio* (Hamilton)	"	River Son at Dehri-on-Son	Tripathi, 1959a and b
IRAN	*D. paradoxum* Nordmann, 1832	*Rutilus frisii katum* (Kamensky)	"	South Caspian Sea	Eslami and Kohneshahri, 1978
IRAQ	*D. kasimii* Rahemo, 1980	*Cyprinion macrostomum* Heckel	"	Mosul, North of Iraq	Rahemo, 1980
ISRAEL (PALESTINE)	*D. minutum* Paperna, 1964	*Phoxinellus kervillei* Pellegrin	"	Mouth of Rubadia Stream, Western shore of Lake Tiberias	Paperna, 1964
		Tylognathus steinitziorum Kosswig	"		
JAPAN	*D. nipponicum* Goto, 1891	*Carassius vulgaris* (Nilsson)	"	None given	Goto, 1891
		C. carassius (L.)	"	One River and seven Lakes, Honshu	Kamegai, 1970 and 1974
				Lake Kitaura, Ibaraki Prefecture Lake Kasumigaura Prefecture Lake Yuno-ko, Tochigi Prefecture	Kamegai, 1972

	Carassius spp.	Cyprinidae	Tama River, Lake Biwa	Ichihara, et al., 1980 and Kamegai, 1975
	Cyprinus carpio (L.)	"	One River and seven lakes	Kamegai, 1970
			River Tamagawa, Tokyo	Kamegai, 1977
			Ponds Yabuki - and Isezaki-town Pond in Ogawa-town, Lake Kasumigaura Lake Kitaura, water system of River Tone	Kamegai, 1968
D. nipponicum Goto, 1891	Cyprinus carpio (L.)	"	River Asakawa, tributary system of River Tama, Tokyo Bay	Kawatsu, 1978
Diplozoon* (species)	Tribolodon hakonensis Ikeda	"	Hokkaido	Kamegai, 1971
	Cyprinus carpio (L.)	"	Upper water of Arakawa River, Saitama Prefecture	Kamegai, 1972

*Species not identified

RUSSIA	D. agdamicum Mikailov, 1973	Leuciscus cephalus orientalis Nordmann	Cyprinidae	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
	D. balleri Nagibina et al., 1970	Abramis ballerus (L.)	"	Rivers Dnepr, Volga and Tissa	Nagibina et al., 1970
	D. bychowskyi Nagibina, 1965	Ctenopharyngodon idella (Valenciennes)	"	Amur River	Nagibina, 1965
	D. chazarikum Mikailov, 1973	Rutilus frisii kutum (Kamensky)	"	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
	D. diplodiscus Nagibina, 1965	Elopichthys bambusa (Richardson)	"	Amur River	Nagibina, 1965
	D.(Diplozoon) mylopharyngodonis Akhmerov, 1974	Mylopharyngodon piceus (Richardson)	"	"	Akhmerov, 1974
	D. homoion Bychowsky and Nagibina, 1959	Rutilus rutilus (L.)	"	Delta of Volga and Bay of Finland	Bychowsky and Nagibina, 1959
		R. rutilus (L.)	"		
		Cyprinus carpio (L.)	"	None given	Khotenovskii, 1975
		Leuciscus idus (L.)	"	Kurshskii Bay and Lake Bol'shoi	Khotenovskii, 1977a and b

D. inustiatus Nagibina, 1965	*Hypophthalmichthys molitrix* (Valenciennes)	Cyprinidae	Amur River and Lake Bolon, far-eastern Russia	Nagibina, 1965 and Khotenovskiĭ, 1978
D. kurensis Mikailov, 1973	*Barbus lacerta cyri* Filippi	"	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
D. kuthkaschenicum Mikailov, 1973	*Alburnus filippii* Kessler	"		
	A. charusini hohenackeri Kessler	"		
D. markewitschi Bychowsky *et al.*, 1964	*Vimba vimba* (L.)	"	Dnepr River	Bychowsky *et al.*, 1964
	V. vimba vimba (Pallas)	"	Dnepr Estuary and Dnepr Delta	Komarova, 1966
	V. vimba (L.)	"	None given	Khotenovskiĭ, 1975
	Blicca bjoerkna (L.)	"		
	None given	"	Kurshskii Bay and Lake Bol'shoi	Khotenovskiĭ, 1977b
D. megan Bychowsky and Nagibina, 1959	*Leuciscus idus* (L.)	"	Delta of Volga and Bay of Finland	Bychowsky and Nagibina, 1959
	None given	"	Dnepr Estuary and Dnepr Delta	Komarova, 1966
	L. idus (L.)	"	River Neman, Delta	Khotenovskiĭ, 1977a

D. mingetschauricum Mikailov, 1973	*Barbus capito* (Güldenstadt)	Cyprinidae	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
D. nipponicum Goto, 1891	"carps"	"	The Lena River and Lake Baikal, West Siberia, Zabaikal	Lukjantzova, 1967, Naumovo, 1964 and Pronin, 1977
	None given	"	Kurshskiĭ Bay and Lake Bol'shoĭ	Khotenovskiĭ, 1977b
D.(Paradiplozoon)(species)	*Gobio* species	"	River Amur	Akhmerov, 1974
	Megalobrama terminalis (Richardson)	"		
	Phoxinus lagowskii (Dybowski)	"		
D.(P.)amurensis Akhmerov, 1974	*Pseudaspius leptocephalus* (Pallas)	"	River Amur	Akhmerov, 1974
D.(P.)erythroculteris Akhmerov, 1974	*Erythroculter mongolicus* (Basilewsky)	"	"	"
D.(P.) marinae Akhmerov, 1974	*Hypophthalmichthys molitrix* (Valenciennes)	"	"	"

*Species not identified

D.(P.) parabramidis Akhmerov, 1974	*Parabramis pekinensis* (Basilewsky)	Cyprinidae	River Amur	Akhmerov, 1974
D.(P.) skrjabini Akhmerov, 1974	*Leuciscus waleckii* (Dybowski)	"	"	"
D. paradoxum Nordmann, 1832	*Abramis brama* (L.)	"	Delta of Volga and Bay of Finland	Bychowsky and Nagibina, 1959
	A. brama (L.)	"	None given	Khotenovskiĭ, 1975
	None given	"	Kurshskiĭ Bay Lake Bol'shoi	Khotenovskiĭ, 1977b
D. paradoxum Nordmann, 1832	*Rutilus rutilus* (L.)	"	Rybinsk Reservoir	Izyumova, 1964
	Abramis brama (L.)	"		
	Blicca bjoerkna (L.)	"		
	Rutilus rutilus heckeli (Nordmann)	"	Dnepr Delta	Komarova, 1964b
	Abramis brama (L.)	"		
D. paradoxum ballerus Komarova, 1964	None given	"	"	Komarova, 1964a
D. paradoxum bliccae Reichenback-Klinke, 1961	*Blicca bjoerkna* (L.)	"	"	Komarova, 1964a and 1966
D. pavlovskii Bychowsky and Nagibina, 1959	*Aspius aspius* (L.)	"	Delta of Volga and Bay of Finland	Bychowsky and Nagibina, 1959

	None given	Cyprinidae	Dnepr Estuary and Dnepr Delta	Komarova, 1966
D. persicum Mikailov, 1973	*Vimba vimba persa* (Pallas)	"	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
D. rutili Gläser, 1967	*Rutilus rutilus* (L.)	"	None given	Khotenovskiĭ, 1975
	None given	"	Kurshskiĭ Bay and Lake Bol'shoĭ	Khotenovskiĭ, 1976 and 1977b
D. sapa Mikailov, 1973	*Abramis sapa bergi* Belyaeff	"	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
D. scardinii Komarova, 1964	*Scardinius erythrophthalmus* (L.)	"	Dnepr Estuary	Komarova, 1964a and 1966
D. schizothorazi Iksanov, 1965	*Schizothorax issykkuli* Berg	"	Kirgizian, Lake Issyk-Kul	Iksanov, 1965
D. schulmani Mikailov, 1973	*Alburnoides bipunctatus eichwaldi* (Filippi)	"	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
D. strelkowi Nagibina, 1965	*Hemibarbus labeo* (Pallas)	"	Amur River and Lake Bolon, far-eastern Russia	Nagibina, 1965
D. tadzhikistanicum Gavrilova and Dzhalilov, 1965	*Barbus capito conocephalus* Kessler	"	Kairak-kum water Reservoir and Vakhsh	Gavrilova and Dzhalilov, 1965

D. varicorhini Mikailov, 1973	*Varicorhinus capoeta sevangi* (Filippi)	Cyprinidae	Caspian Coast and Kuro River, Azerbaijan	Mikailov, 1973
Paradiplozoon alburni Khotenovskiĭ, 1982	*Rutilus rutilus* (L.)	"	Kurskig Zalir	Khotenovskiĭ, 1982
	Leuciscus idus (L.)	"		
	Alburnus alburnus (L.)	"		
	Alburnoides bipunctatus (Bloch)	"		
	Scardinius erythrophthalmus (L.)	"		
	Cyprinus carpio (L.)	"		
	Ctenopharyngodon idella (Valenciennes)	"		
P. cyprini Khotenovskiĭ, 1982	*Cyprinus carpio haematopterus* Temminck, Schlegel	"	Amur River basin	Khotenovskiĭ, 1982
P. leucisci Khotenovskiĭ, 1982	*Leuciscus leuciscus* (L.)	"	None given	"
	L. cephalus (L.)	"	None given	"
P. megalobramae Khotenovskiĭ, 1982	*Megalobrama terminalis* (Richardson)	"	Amur River basin and Lake Khanka	"
P. tisae Khotenovskiĭ, 1982	*Barbus meridionalis petenyi* Heckel	"	Tisa and Teresva River, Ukrainian	"

VIETNAM	D. doi Ha Ky, 1971	Squaliobarbus curriculus (Richardson)	Cyprinidae	Kien An Reservoirs, Hanoi and Ha Bac, North Vietnam	Ha Ky, 1971
		Hypophthalmichthys harmandi Sauvage	"		
		Cirrhina molitorella (Cuvier and Valenciennes)	"		
		Carassius auratus (L.)	"		
	P. vietnamicum Khotenovskii, 1982	Cirrhinus chinensis Gunther	"	None given	Khotenovskiĭ, 1982

EUROPE

CZECHOSLOVAKIA					
	D. homoion Bychowsky and Nagibina, 1959	Hypophthalmichthys molitrix (Valenciennes)	"	Pond Mirovy, Pohorelice, Brno	Lucký, 1981
	P. leucisci Khotenovskiĭ, 1982	Leuciscus cephalus (L.)	"	None given	Khotenovskiĭ, 1982
		L. leuciscus (L.)	"		
FRANCE	D. homoion gracile Oliver and Reichenback-Klinke 1973	Barbus meridionalis Risso	"	Herault and Pyrenees-Orientales	Oliver and Reichenbach-Klinke, 1973
		Chondrostoma toxostoma Vallot	"		
		Telestes soufia agassizi Cuvier and Valenciennes	"		
		Phoxinus phoxinus (L.)	"		
		Gobio gobio (L.)	"		

	D. nipponicum Goto, 1891	*Cyprinus carpio* (L.)	Cyprinidae	Sud-Est de La France	Lambert and Denis, 1982
	D. paradoxum Nordmann, 1832	*Gobio gobio* (L.)	"	Languedoc-Roussillon (Sud de La France)	Euzet and Lambert, 1974
		Phoxinus phoxinus (L.)	"		
		Barbus meridionalis Risso	"		
		Gobio gobio (L.)	"	Dans Le Sud-Est de La France	Euzet and Lambert, 1971
		Phoxinus phoxinus (L.)	"		
ITALY	*D. paradoxum* Nordmann, 1832	*Rutilus rubilio* Cute	"	Chiascio River, Umbria	Aisa *et al.*, 1981
		Leuciscus cephalus cabeda Cute	"		
		Barbus plebejus Cute	"		
		Phoxinus laevis (Parona)	"	None given	Palombi, 1949
		Leuciscus cephalus (L.)	"		
		Cyprinus carpio (L.)	"		
NORWAY	*D. paradoxum* Nordmann, 1832	*Abramis brama* (L.)	"	River Glomma	Halvorsen, 1969
		Rutilus rutilus (L.)	"		
		R. rutilus (L.) X *A. brama* (L.)	"		

POLAND	*D. gussevi* Gläser and Glaser, 1964	*Blicca bjoerkna* (L.)	Cyprinidae	Lake Dąbie near Szczecin, the mouth of the Odra	Wierzbicka, 1974
	D. homoion Bychowsky and Nagibina, 1959	*Alburnus alburnus* (L.)	"	Luczanski canal at Gizycko linking the Mazurian Lakes	Prost, 1972 and Pejčoch, 1968
		Scardinius erythrophthalmus (L.)	"	None given	Pejčoch, 1968
		Leuciscus cephalus (L.)	"		
		Phoxinus phoxinus (L.)	"	Mountain Stream of Wolkowyjka, Bieszczady Mountains	Prost, 1974
	D. nagibinae Gläser, 1965	*Abramis ballerus* (L.)	"	Lake Dąbie near Szczecin, the mouth of the Odra	Wierzbicka, 1974
	D. paradoxum Nordmann, 1832	*Abramis brama* (L.)	"	River Vistula, near Warszawa	Dabrowska, 1970
		Alburnus alburnus (L.)	"		
		Alburnoides bipunctatus (Bloch)	"	River Vistula	Prost, 1957
		Aspius aspius (L.)	"		
		Blicca bjoerkna (L.)	"		
		Carassius carassius (L.)	"		
		Chondrostoma nasus (L.)	"		
		Cyprinus carpio (L.)	"		

	Gobio gobio (L.)	Cyprinidae	River Vistula,	Prost, 1957
	Leuciscus cephalus (L.)	"		
	L. leuciscus (L.)	"		
	L. idus (L.)	"		
	Rutilus rutilus (L.)	"		
	Scardinius erythrophthalmus (L.)	"		
	Vimba vimba (L.)	"		
	Abramis brama (L.)	"	Drużno Lake	Kozicka, 1959
	Blicca bjoerkna (L.)	"		
	Gobio gobio (L.)	"		
	Rutilus rutilus (L.)	"		
	Scardinius erythrophthalmus (L.)	"		
	Abramis brama (L.)	"	Lake Dąbie near Szczecin, the mouth of the Odra	Wierzbicka, 1974
	Blicca bjoerkna (L.)	"		
D. rutili Gläser, 1967	Alburnus alburnus (L.)	"	Luczanski Canal at Gizycko linking the Mazurian Lakes	Prost, 1972 and Pejčoch, 1968

SWITZERLAND				
D. paradoxum Nordmann, 1832	*Rutilus rutilus* (L.)	Cyprinidae	Lake Neuchâtel	Bovet, 1959 and 1961
D.p. homoion Bovet, 1967	*R. rutilus* (L.)	"	"	Bovet, 1967
	Blicca bjoerkna (L.)	"		
D.p. paradoxum Bovet, 1967	*Abramis brama* (L.)	"		
	Blicca bjoerkna (L.)	"		
UNITED KINGDOM				
(Discussed later)				
WEST GERMANY				
D. barbi Reichenbach-Klinke, 1951	*Rasbora heteromorpha* Duncker	"	Kept in aquarium (imported from South-East Asia and North America)	Reichenbach-Klinke, 1951 and 1953
	Barbus semifasciolatus Guenther	"	Kept aquarium	
	Puntius tetrazona (Bleeker)	"		
D. gracile Reichenbach-Klinke, 1961	*Gobio gobio* (L.)	"	Main	Reichenbach-Klinke, 1951

D. gussevi Gläser and Gläser 1964	*Blicca bjoerkna* (L.)	Cyprinidae	Kieman	Gläser and Gläser, 1964
	Scardinius erythrophthalmus (L.)	"		
D. homoion Bychowsky and Nagibina, 1959	*Rutilus rutilus* (L.)	"	"	"
	Carassius carassius (L.)	"		
	Leuciscus grislagine (L.)	"		
	Alburnus lucidus Heck	"		
D. nagibinae Gläser, 1965	*Abramis ballerus* (L.)	"	O'der	Gläser, 1965
D. paradoxum bliccae Reichenbach-Klinke, 1961	*Blicca bjoerkna* (L.)	"	Donau in Bayern	Reichenbach-Klinke, 1961
D.p. sapae Reichenbach-Klinke, 1961	*Abramis sapa* (Pallas)	"	Bay Erische Donau	"
D. rutili Gläser, 1967	*Rutilus rutilus* (L.)	"	River Elbe	Gläser, 1967
D. tetragonopterini Sterba, 1957	*Ctenobrycon spilurus* (Cuvier and Valenciennes)	Characidae	From aquarium in Erfurt	Sterba, 1957
YUGOSLAVIA				
D. paradoxum Nordmann, 1832	*Leuciscus cephalus cephalus* (L.)	Cyprinidae	River Voglajna in Slovenia	Povž *et al.*, 1981

	D. homoion Bychowsky and Nagibina, 1959	*Chondrostoma nasus* (L.)	Cyprinidae	Ponds of Bosnia and Herzegovina	Kiškaroly, 1977
		AFRICA			
EGYPT	*D. aegyptensis* Fischthal and Kuntz, 1963	*Labeo forskalii* (Rüppell)	"	Giza fish market, Giza Province	Fischthal and Kuntz, 1963
GABON	*Neodiplozoon grassitrema* Price, 1967	*Barbus guilari* Thominot	"	Various bodies of water	Price, 1967
GHANA	*D. aegyptensis* Fischthal and Kuntz, 1963	*Labeo cubie* Rüppell	"	Volta Lake	Paperna, 1979
	D. ghanense Thomas, 1957	*Alestes barëmose* (Joannis)	Characidae	None given	Paperna, 1979
		A. macrolepidotus (Valenciennes)	"	Black Volta River, North region of Ghana	Paperna, 1979 and Thomas, 1957
KENYA	*D. aegyptensis* Fischthal and Kuntz, 1963	*Labeo victorianus* Boul	Cyprinidae	Nzoia River	Paperna, 1973 and 1979
		Barbus paludinosus Peters	"		
	N. polycotyleus Paperna, 1973	*Labeo victorianus* Boulenger	"		

TANZANIA	*D. aegyptensis* Fischthal and Kuntz, 1963	*Labeo cylindricus* Peters	Cyprinidae	Ruaha River	Paperna, 1973 and 1979
	N. polycotyleus Paperna, 1973	*Barbus paludinosus* Peters	"		
		B. cercops Whitehead	"		
		B. macrolepis Pfeffer	"		
UGANDA	*D. aegyptensis* Fischthal and Kuntz, 1963	*Labeo forskalii* (Rüppel)	"	Lake Albert	Paperna, 1979
		Barilius loati	"	Aswa River (White Nile system)	
		Alestes macrolepidotus (Valenciennes)	Characidae	Lake Albert, Butiaba	

NORTH AMERICA

No records — Hoffman, Personal communication, 1984, Margolis and Arthur, 1979 and Margolis, Personal communication, 1983

SOUTH AMERICA

No records

AUSTRALIA

No records

Beumer, personal communication, 1983 and Beumer *et al*., 1983

Fig. 2.1. Localizations of the Diplozoidae species in the world

A - Asia and adjacent localities

1 - China
2 - India
3 - Iran
4 - Iraq
5 - Israel
6 - Japan
7 - Russia
8 - Vietnam

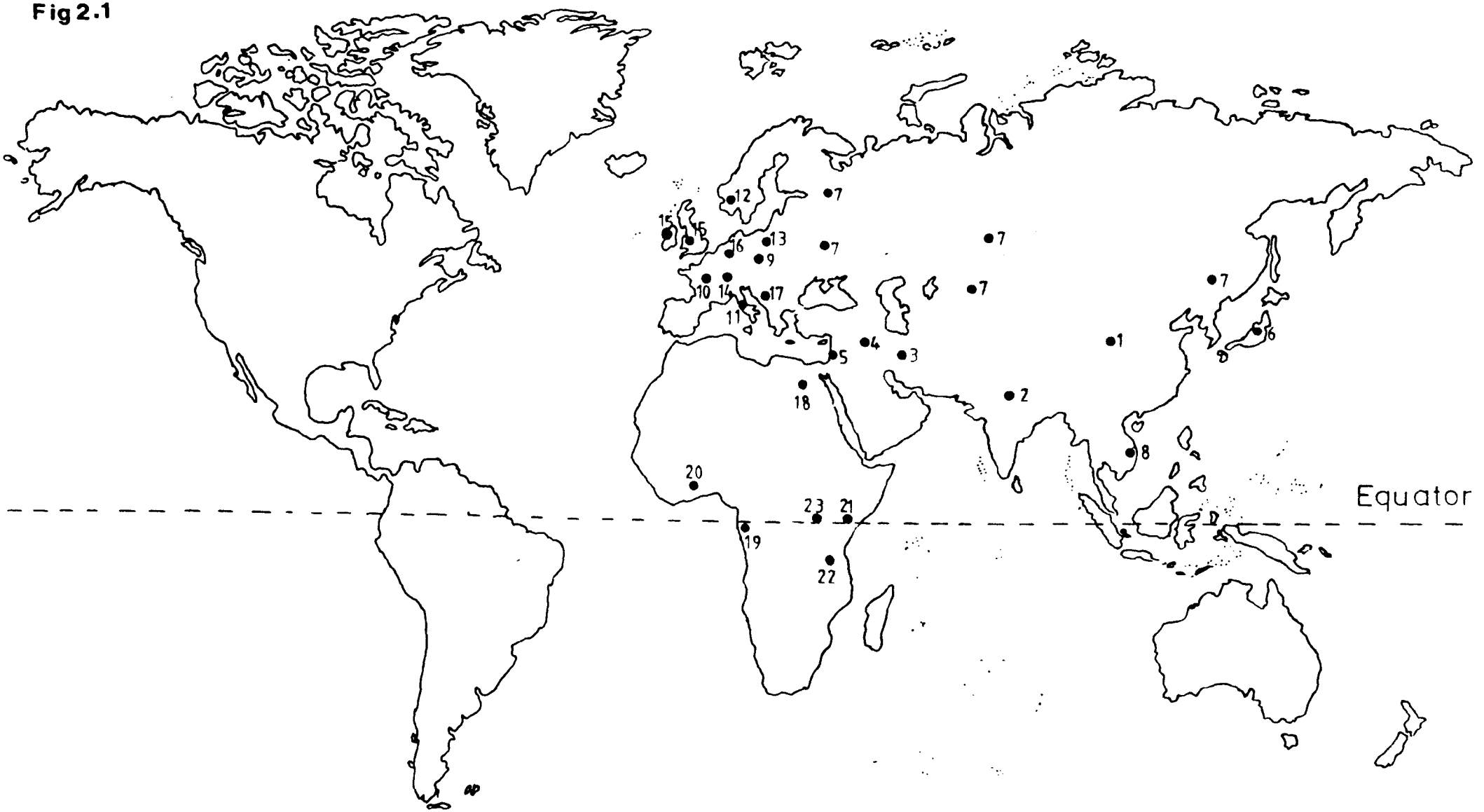
B - Europe

9 - Czechoslovakia
10 - France
11 - Italy
12 - Norway
13 - Poland
14 - Switzerland
15 - British Isles and Ireland
16 - West Germany
17 - Yugoslavia

C. Africa

18 - Egypt
19 - Gabon
10 - Ghana
21 - Kenya
22 - Tanzania
23 - Uganda

Fig 2.1



occurrence and abundance of these monogeneans decline from the north towards the south of the earth (Fig. 2.1). So most of the southern half of the earth is free from Diplozoon infection. The distribution of their potential hosts is likely to be the main reason for this. Norman (1975) clearly showed with a map of cyprinid distribution, that these fishes were completely absent from Australia, North Africa and South America. He expected that the characteristic fishes of Central and South America region might be expected to be somewhat similar to those of North America. In Australia, the absence of Diplozoon infection from freshwater fishes might be related to their origin. Beumer (personal communication, 1983) mentioned that cyprinids are exotic to Australia. According to Norman (1975), the Australian region has an almost complete absence of true freshwater fishes in that all the species available are closely allied to marine forms. Lake (1971) also mentioned 5 cyprinid species all of which have been introduced into Australia: Cyprinus carpio, Carassius auratus, Carassius carassius, Tinca tinca and Rutilus rutilus. The absence of infection from North and South America can also be attributed to the artificial introduction of their cyprinids. The parasites as shown from the Table have been recovered from fishes in different water types, e.g. rivers, streams, pools, ponds and lakes. Different species of parasites have also been found acclimatized to the fish of reservoirs, e.g. D. nagibinae, D. nipponicum (Microshnichenko and Frunze, 1983).

According to the occurrence of Diplozoon species of Asia, in Russian territory, there are at least 39 Diplozoon species found on 47 species of freshwater fishes, all of them from cyprinid fishes. Khotenovskiĭ (1977a) suggested that the presence of local fish shoals

in the Kurshskiĭ Bay influenced the distribution of Diplozoon species, because he found D. megan infected Leuciscus idus in River Neman Delta, whereas D. homoion was only found on L. idus in another area of Kurshskiĭ Bay. According to Khotenovskiĭ (1976), almost all Diplozoon species of the Soviet Union are distributed in the west and south west of the country and very few have been found in the central and eastern areas (Fig. 2.1). He revealed that vast parts of eastern Siberia and the far east of Russia remain unchecked for parasite distribution. In China,prior to 1983,only 3 Diplozoon species were recorded infecting 3 cyprinid species, but Long So (1983) reported that 18 Diplozoon species were found on Chinese freshwater fishes. The main area of distribution of these species is the South China region. In Japan, 2 species of Diplozoon are found on 4 cyprinid species. Kamegai (1974) identified them as paradoxum and nipponicum types and he found them on freshwater fishes from 10 rivers and 36 lakes in Japan. Owing to Kamegai (1974), paradoxum type represents all forms of Diplozoon which do not possess the morphological characters of D. nipponicum. D. nipponicum seems to occur on both Cyprinus carpio and Carassius carassius mainly in lakes and a few ponds (Kamegai, 1968), while paradoxum-type was discovered from Tribolodon hakonensis from area where no nipponicum-type was found (Kamegai, 1974). He confirmed that in Eurasia, paradoxum-type was distributed widely from Germany to Siberia, while nipponicum-type was reported sporadically in the mid- to western districts of the Soviet Union through to China. Owing to the theoretical origin of Japanese freshwater fish, Kamegai (1974) suggested that the paradoxum-type in Hokkaido (Northern Island) had originated in Siberia, while D. nipponicum in Honshu (Main Land) had originated on the Chinese continent independently from the former.

In India, 8 Diplozoon species were recovered from at least 7 cyprinid species. In Vietnam, 2 species of Diplozoon infected 5 species of cyprinids; in Iraq, 1 species on 1 cyprinid species; in Iran, 1 species on 1 cyprinid species; in Israel, 1 species on 2 cyprinid species.

In Europe, members of the genus are distributed in all countries (Fig. 2.1): in Germany 8 Diplozoon species have been recovered from 10 cyprinid species and 1 characid species. Sterba (1957) mentioned that infection by D. tetragonopterini was found on some imported characids from South America. Moreover, Reichenbach-Klinke (1951 and 1953) revealed that D. barbi infection was noticed on Barbus semifasciolatus imported from North America and South-East Asia. Presumably, these fishes were received the infection after importation to Germany. In Poland, at least 6 species of Diplozoon have been found on 20 cyprinid species. D. gussevi and D. nagibinae were recorded for the first time in Poland by Wiezbicka (1974). She attributed the case of a single individual of D. paradoxum on Blicca bjoerkna as an accidental infection. In Yugoslavia, 2 species have been found on 2 cyprinid species; in Czechoslovakia, 2 species on 2 cyprinid species; in Italy, 1 species on 5 cyprinid species; in Switzerland, 2 species on 3 cyprinid species; in Norway, 1 species on 3 cyprinid species; and in France, 3 species on 6 cyprinid species. D. nipponicum was first recorded in France by Lambert and Denis (1982) from Cyprinus carpio which was introduced from Hungary. In the British Isles, until the present study, all Diplozoon found infecting Cyprinidae were determined as D. paradoxum. It is only during this work that both D. homoion and D. paradoxum have been shown to occur in these islands. Thus, the checklists of Nicoll (1924), Kane (1966), Chappell and Owen (1969) and Kennedy (1974) show only D. paradoxum. Diplozoon

infections are widely spread throughout Britain and Ireland. The parasites have been found on the gills of various cyprinid species, e.g. on Rutilus rutilus from Llyn Tegid (Chubb, 1963 and Cheyne, 1977), from Shropshire Union Canal (Mishra, 1966), from Birmingham (Owen, 1963), from Northern Ireland (Kennedy, 1966 and Stranock, 1979), and from Lincolnshire (Wiles, 1968); on Abramis brama from Shropshire Union Canal (Mishra, 1966), and from Lincolnshire (Wiles, 1968); on Phoxinus phoxinus from Yorkshire (Wiles, 1968) and Northern Ireland (Halton and Jenning, 1965); on Gobio gobio from Northern Ireland (Stranock, 1979); on Scardinius erythrophthalmus from Northern Ireland (Stinson, 1954 and Kane, 1966); and on Carassius carassius from Preston (Ingersent, personal communication, 1984).

In Africa, Diplozoon species are so far recorded as follows: in Egypt, 1 species from 1 cyprinid species; in Ghana, 2 species from 2 characid and 1 cyprinid species; in Kenya, 2 species from 3 cyprinid species; in Gabon, 1 species from 1 cyprinid species; in Uganda, 1 species from 2 cyprinid and 1 characid species; and in Tanzania, 2 species from at least 5 cyprinid species.

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CHAPTER 3

STUDIES ON THE TAXONOMY OF *DIPLOZOON* SPECIES FROM BRITISH CYPRINIDAE

I. INTRODUCTION

Descriptions of most Diplozoon species, including D. paradoxum, D. homoion and D. rutili, from various parts of the world have already been given elsewhere in many reviews so that they are not repeated in detail here.

In Britain, all studies carried out on Diplozoon materials collected from various British cyprinid species have been identified as D. paradoxum (Nicoll, 1924; Chappell and Owen, 1969; Kennedy, 1974). Unfortunately, no critical taxonomic work on the identification of these materials has been made in the past.

So, the aim of the present Chapter is to examine and identify the British Diplozoon materials from a variety of cyprinid species and localities. Fortunately, some Diplozoon specimens were also available from abroad for comparison. This study was based on the following lines of systematic work: 1. Studies on the significant morphological characters of adults, eggs and oncomiracidia of the British Diplozoon materials. To achieve that purpose, scanning electron microscopic observations were used for the first time in taxonomic studies of Diplozoon; 2. The work also included preliminary observations on determining the chromosome number of D. homoion; 3. The experimental transfer of D. homoion infection in the laboratory from species to species of Cyprinidae.

II. MATERIALS AND METHODS

A. Source of Diplozoon Materials

1. Adult stage

Adult worms of Diplozoon species from a variety of cyprinid species and localities in the British Isles and Overseas were obtained or borrowed from different sources as shown in Table 3.1 A and B. The borrowed materials comprised permanent slides, adult specimens isolated from the fishes and preserved in 70% alcohol or 5% formalin solution and worms collected by checking fish samples of various British cyprinid species (Table 3.2).

In fact, only living worms of D. homoion were available during this study, while all other adult specimens of D. paradoxum and D. rutili were obtained as already preserved worms.

2. Egg stage

Few eggs of D. paradoxum and D. rutili used in the current study were found attached to the adult parasites by means of their filaments. The eggs of D. homoion were usually available during most of this study by the maintenance of the infection on Rutilus rutilus in the laboratory at a water temperature of 18^{o}-21^{o}C.

3. Oncomiracidium stage

Owing to the difficulties of obtaining other living Diplozoon species, the oncomiracidia of D. homoion were the only ones used in this study. Active larvae were obtained by collecting freshly laid eggs from adult parasites from the tanks containing infected fishes. The eggs were incubated in small dishes filled with tap water and left in the laboratory at temperatures between 18^{o}-21^{o}C. A few days later, the eggs hatched and the living larvae were collected. For further

Table 3.1. **Adult *Diplozoon* materials from various cyprinid species giving localities and sources. A. British materials. B. Overseas materials**

Host	Locality	Source
	A. British materials	
Abramis brama	River Stour, Suffolk:	Dr. C.R. Kennedy
	Moores Bakery Pond:	Dr. C. Andrews, Yorkshire Water Authority
	Vann Lake, Surrey:	Dr. W.M. Hominick
	River Thames:	Thames Water Authority
	Backford, Shropshire Union Canal	personal collection
Carassius carassius	Essex	British Museum (Natural History)
Gobio gobio	Northern Ireland	Prof. C. Arme
Leuciscus leuciscus	Sarn Bridge, Worthenbury, River Dee	personal collection
Phoxinus phoxinus	Birmingham	British Museum (Natural History)
R. rutilus	Nottingham:	
	Shropshire Union Canal:	Dr. T.N. Mishra
	Llyn Tegid	personal collection
Scardinus erythrophthalmus	Haxby Road Pond, Yorkshire	Dr. C. Andrews, Yorkshire Water Authority
	B. Overseas materials	
Europe		
A. brama	Lake Neuchâtel, Switzerland:	British Museum (Natural History)
	Unknown	
	River Glomma, Norway	Prof. O. Halvorsen
A. brama x *R. rutilus*	River Glomma, Norway	

Table 3.1 (continued)

R. frisii	Caspian Sea	British Museum (Natural History)
R. rutilus	Gulf of Bothnia and Lake Kuivas, Finland: Lake Neuchâtel, Switzerland	British Museum (Natural History)
	River Glomma, Norway	Prof. O. Halvorsen
Asia		
Carasobarbus luteus	Mousal, Iraq	British Museum (Natural History)

Table 3.2. Samples of cyprinid species from different localities of the British Isles examined for *Diplozoon* species infection.

Host	Locality	Date of sample	No. of fishes Examined	No. of fishes Infected	No. of parasites recovered
A. brama	Meols Pond	17/11/82	20 (dead)	0	-
	Hull City	20/4/83	7 (living)	0	-
	Yorkshire	20/3/83	15[a] (living)	0	-
	Backford Shropshire Union Canal	22/6/84	34[b] (living)	4	4
Ctenopharyngodon idella					
	West Peckam Fish Farm, Seven Oaks, Kent	June '1983	14[c]	0	-
Gobio gobio (fry)	Chester, River Dee	17/11/83	3 (living)	2	3
Leuciscus leuciscus (fry)	Chester, River Dee	17/11/83 Large sample	30 (living)	0	-
Phoxinus phoxinus (fry)	Atcham, River Severn	24/5/83	19 (dead)	0	-
Rutilus rutilus	Llyn Tegid	Available throughout the year	(see Chapter 6)		
	Crose Mere, Stour	23/8/83	7(dead)	0	-
R. rutilus	Crose Mere, Shropshire Lake	26/5/83	18 (dead)	0	-
R. rutilus (fry)	River Dee	November '83	2 (dead)	2	2
R. rutilus (fry)	Llyn Tegid	September '82	10 (dead)	2	2
R. rutilus	Yorkshire	20/3/83	15[a] (living)	0	-
	Backford, Shropshire Union Canal	22/6/84	14[b] (living)	4	5

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a The total number of fishes was 30, collected from a fish supplier.
b Seine net method used to catch them
c The total number of fishes was 15, all dead except one live which was used in the experiment

information about these larvae including the behaviour and type of movement during their short life span see Chapter 5 (Life cycle).

B. Preparation of Diplozoon Materials for Light and Scanning Electron Microscope Studies

1. Light microscope studies

a. Adult stage

Some adult specimens borrowed had been previously prepared as permanent slides. They were examined using dissecting and light microscopes. Other adult specimens fixed either in 5 percent formalin solution or 70% alcohol were checked for their morphological dimensions before staining using cavity slides. The examination of these specimens was carried out by putting them in a drop of their original preservative and covering the cacity slides with cover slip without putting any pressure on the specimens. Adult specimens were stained using a variety of different dyes. After trying a wide selection of these stains with adult D. homoion (because these specimens were available at all times from Llyn Tegid), it was found that haematoxylin (Ehrlich type) and Horen's trichrome stains were most useful for displaying the external and internal structures of the adult stages of Diplozoon. The technique for using these two stains was given by Chubb (1962). For achieving best results, some modifications were used either in the dilution of stains or the times of staining. Therefore these stains were used for all other specimens. The size variation of adult stages were studied using D. homoion collected from R. rutilus of various sizes and at fixed periods of time. These specimens were fixed in either 5 percent formalin or 70% alcohol and were measured using the procedure mentioned above.

b. Egg stage

Eggs of D. homoion, D. paradoxum and D. rutili were examined for their dimensions, shapes and colour by using cavity slides. Groups of eggs of D. homoion were mounted in glycerine-jelly on slides for later examination.

c. Oncomiracidium stage

Silver nitrate was only used for study of this stage. Living larvae of D. homoion, cultured by the method described earlier, were expelled from a dropper into small glass dishes of hot (40^{o}-60^{o}C) of 0.3-0.5 percent silver nitrate solution. This technique was originally described by Lynch (1933) but needed some modification both in the concentration of the solution or in the time of processing of the larvae.

2.Scanning electron microscope studies

The available materials of adult worms, eggs and oncomiracidia of D. homoion, D. paradoxum and D. rutili were prepared for electron microscope observation by washing them thoroughly several times in distilled water to remove any foreign objects attached to them. To achieve this, the washing was carried out by expelling the distilled water from a dropper rapidly onto the specimens several times. A dissecting microscope was used to facilitate the process. However, specimens preserved originally in 70% alcohol were hydrated through a series of alcohols before cleaning in distilled water. After washing, they were dehydrated again by using an alcohol series, then critically point dried in liquid CO_2, then sputter-coated with 60% gold/palladium and examined using a Philips 501B scanning electron microscope.

C. Preparation of Chromosomes of D. homoion

Ten living D. homoion were obtained from freshly killed R. rutilus collected from Llyn Tegid during October and November, 1983. The parasites were fixed in 40% acetic acid for 10-15 minutes. They were then transferred to 3:1 ethanol: acetic acid (freshly made up), leaving them in this mixture for 1-3 days in deep freeze. They were then hydrolysed for 5-8 minutes in N hydrochloric acid at 60°C before staining. Thereafter, specimens were stained with leuco-basic fuchsin (Schiff's reagent) (Darlington and La Cour, 1966) for 15 minutes in the dark. The upper parts of the posterior region of each partner of the adult specimen which contains the ovaries and testes were separated in a drop of 45% acetic acid. The reproductive tissues were flattened over a flame and the chromosomes spread by a gentle fingertip pressure on the cover slip under a filter paper avoiding any lateral movement of the cover slip during this process. Then, the slides were examined under the microscope and the number of chromosomes were counted. For making permanent preparations, the dry ice method was used. After freezing the slide and cover slip, the cover slip was carefully removed from the slide, and both were transferred through the following stages (2 changes, in each for 5 minutes): 98% absolute ethanol, 1:1 absolute ethanol to xylene, finally pure xylene. The specimens were mounted in Canada Balsam.

D. Field and Laboratory Observations on the Transference of D. homoion Infections

1. In the field

Many samples of fishes (1-34 fishes) in each of various British cyprinid species and localities were examined to collect their Diplozoon species (Table 3.2). The sources of these fishes were either

from the Fishery Group of the Department of Zoology, University of Liverpool, or provided by other postgraduate students of the Zoology Department or by personal collection. Fry of various cyprinid species were caught by seine net, and other larger fishes were caught using gill nets. Some of these fish samples were obtained living and were brought back to the laboratory and their Diplozoon specimens were used for a variety of purposes. Dead specimens were used for light or scanning electron microscope studies. Living Rutilus rutilus from Llyn Tegid (many living samples were brought back to the laboratory as required), fry of Leuciscus leuciscus (30 fishes) from the River Dee, Ctenopharyngodon idella (1 fish) from Seven Oaks, Kent, Rutilus rutilus (15 fishes) and Abramis brama (15 fishes) from Yorkshire (see Table 3.2) were all used in experiments designed to demonstrate the natural transfer of D. homoion infection from one host species to another.

2. In the laboratory

D. homoion infections were maintained on Rutilus rutilus in the laboratory. Four tanks half-filled with aerated tap water were used in this experiment. 5 fishes of 15-20 cm long were placed in each tank. The water temperature was maintained between 18-21^{o}C. Thermostats and heaters were used to keep the water temperature within this range. The water of each tank was renewed every 15-30 days. About 60 uninfected fry of Leuciscus leuciscus on which no infection had been found originally were distributed through two tanks together with the infected adult Rutilus rutilus. The Leuciscus leuciscus and Ctenopharyngodon idella were examined for Diplozoon infection after one month of exposure in the tanks. The uninfected adult Abramis brama (15 fishes) and Rutilus rutilus (15 fishes) from Yorkshire were kept separately in the other two tanks containing infected R. rutilus. After one month,

5 fishes from each of the test species were examined for Diplozoon infection. Two months later (3 months of exposure to infected Rutilus rutilus) another 5 fishes from each of the test species were examined. Finally after a total period of exposure for 4 months 2 Abramis brama and all 5 Rutilus rutilus died owing to an unidentified cause. They had many haemorrhagic patches near the base of their fins. They were also examined for Diplozoon infections. The remaining three Abramis brama which survived in the tank were examined after 6 months of exposure.

The fishes were examined for Diplozoon infection after they were killed by removal of the opercular bones and the isolation of the gill arches from each side in separate dishes. The gill arches were examined carefully using a stereo microscope and any stage of the life cycle found was recorded.

III. TERMINOLOGY

The terms used throughout this study for the parts of the adult worms are defined in the following sections. Terms used for the developmental stages are defined where relevant in later sections of the thesis.

A. External Structures

1. Anterior regions (Fig. 3.1)

The anterior parts of the parasites usually have a leaf-like shape (dorso-ventral flattened). The pair of worms results from the fusion of two individuals into an X shape. Each anterior region tapers from a minimum width at its free end to reach a maximum width about its middle.

2. Prohaptor (Figs. 3.1, 3.2 and 3.3)

This is the free end of the anterior region; it comprises a cup-shaped muscular organ encircling the mouth.

3. Mouth (Fig. 3.2)

The mouth is subterminal on the ventral side.

4. Posterior regions (Figs. 3.1, 3.4 and 3.5)

These are the portions of the adult worm behind the junction area. These regions are more or less circular in cross-section although slightly flattened in a dorso-ventral plane in D. homoion. Each half has an opisthaptor.

5. Opisthaptor (Figs. 3.1, 3.4 and 3.5)

This is the disk-like out growth of the free end of the posterior region which is a muscular area. It comprises clamps and an associated muscular disc.

Fig. 3.1. Adult D. homoion showing the external morphology.

ar, anterior region; c, clamps; fc, fine constrictions; gp, genital pore; jr, junction region; md, muscular disc; oph, ophisthaptor; por, posterior region; prh, prohaptor. markers = 100 um.

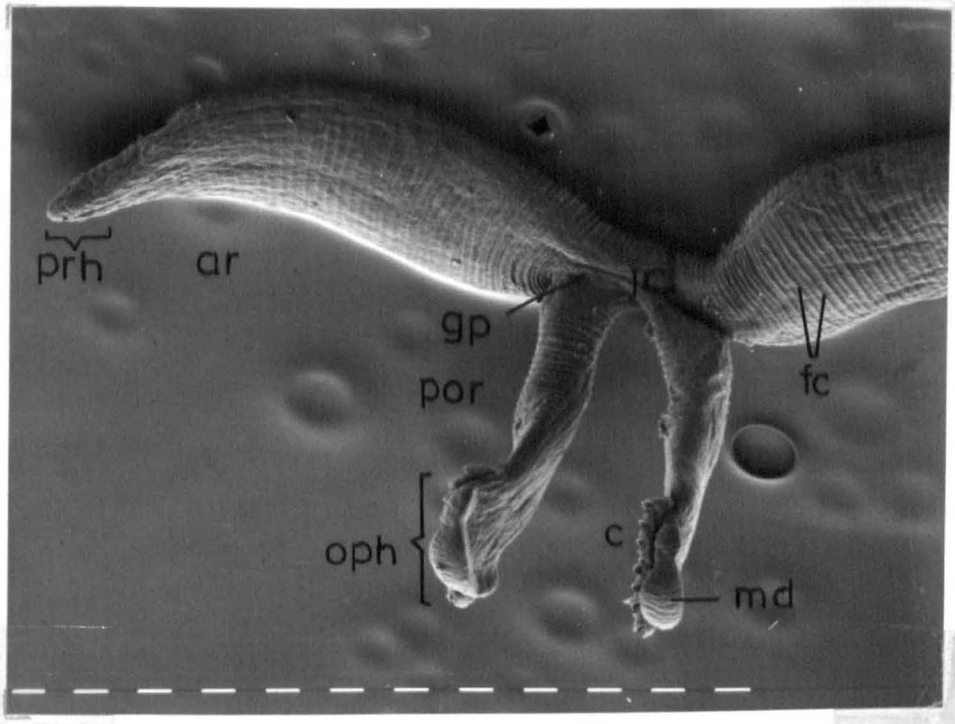


Fig. 3.2. Ventral side of anterior region of adult *D. paradoxum*. The anterior regions of *D. homoion* and *D. rutili* are similar. m, mouth; prh, prohaptor. Markers = 10 μm.

Fig. 3.3. Prohaptor of adult *D. homoion*. The prohaptors of *D. paradoxum* and *D. rutili* are similar.
os, oral sucker; ph, pharynx.

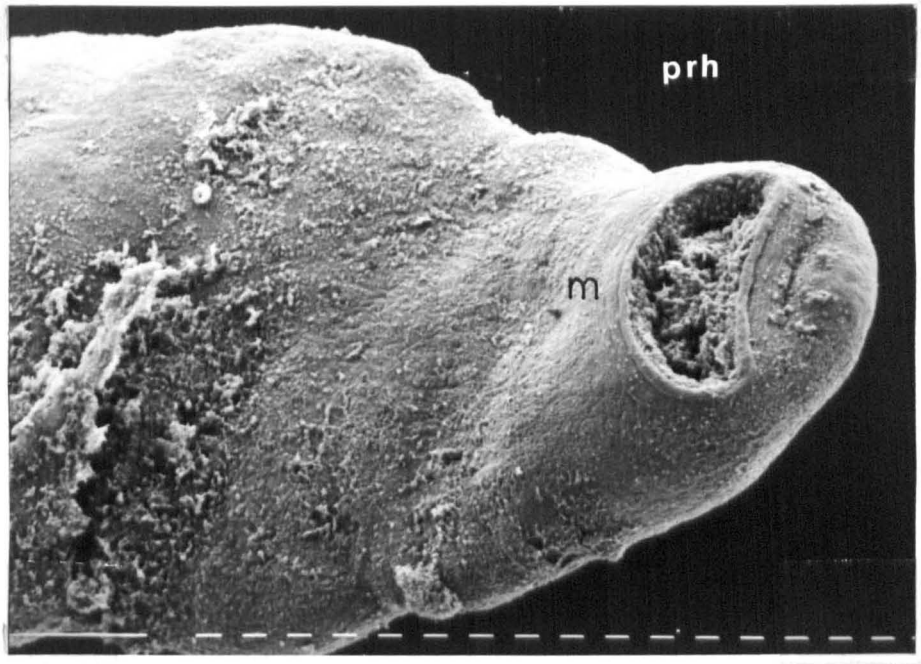


Fig.3·2

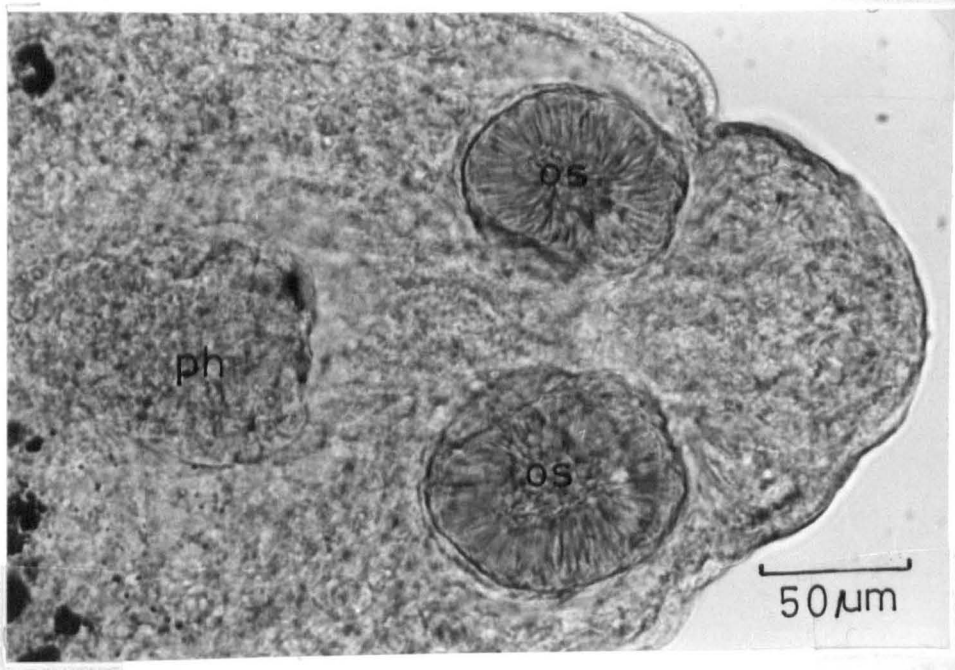


Fig.3·3

Fig. 3.4. The morphology of a small adult D. homoion (2.5mm long). ar, anterior region; c, clamps; md, muscular disc; oph, opisthaptor; os, oral sucker; ph, pharynx; por, posterior region.

Fig. 3.5. The morphology of a large adult D. homoion (5mm long). c, clamps, 1st, 2nd, 3rd and 4th; i, intestine; ov, ovary; t, testis; v, vitellaria

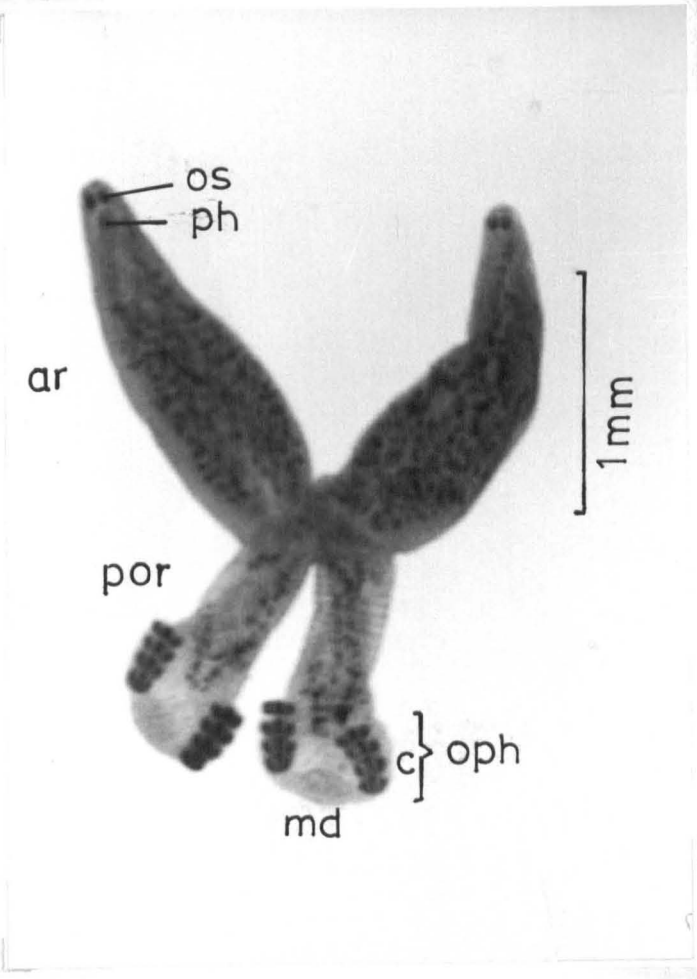


Fig. 3.4

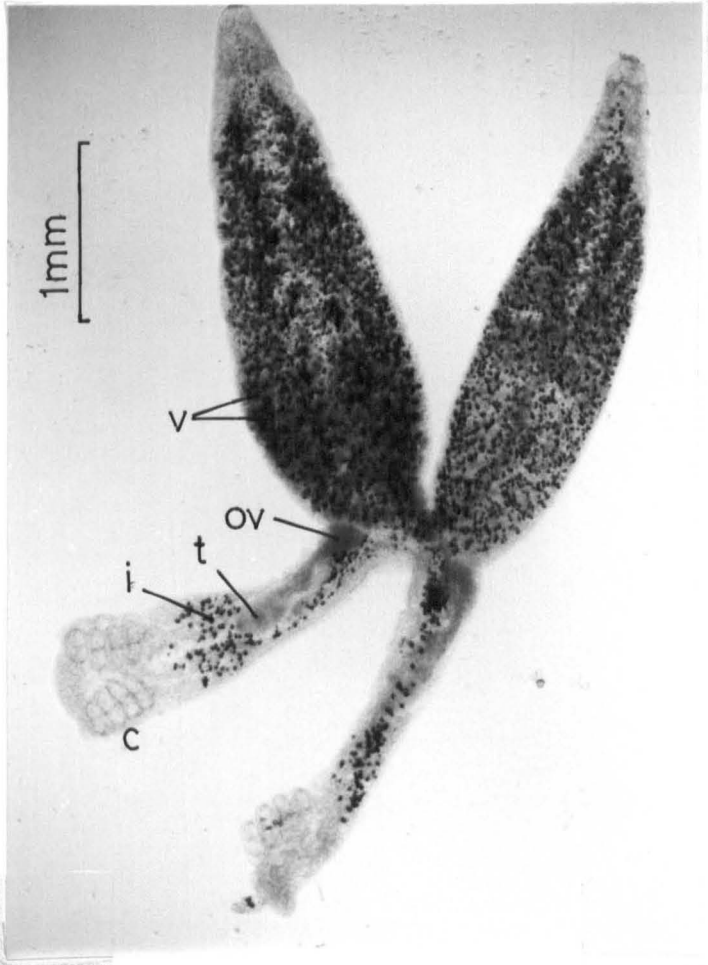


Fig. 3.5

6. Clamps (Figs. 3.1, 3.4 and 3.5)

They are four pairs of muscular attachment organs on each opisthaptor used to maintain the position of the adult parasites on the gill. They are set close together laterally on the ventral (inner) surfaces of the posterior regions. Usually the clamps have an asymmetrical arrangement on opposite sides of each opisthaptor. The pair of clamps which is placed to the posterior of the opisthaptor is the oldest as it originates in the oncomiracidium. Here this is termed the first pair of clamps. The next two pairs of clamps are formed during the diporpal stages (second and third) while the last pair (fourth) is formed in the juvenile stage.

The terminology used for parts of the sclerotized framework of the clamps follows Owen (1963b, his Fig. 2).

7. Muscular disc (Figs. 3.1, 3.4 and 3.5)

This is another structure forming part of the opisthaptor. It is a papilla-like process (thickening) at the end of the opisthaptor.

8. Larval hooks (Figs. 3.9 and 3.10)

There is a pair of crook-shaped recurved hooks, originally formed on the oncomiracidium, which persist at the ventral (inner) side situated in the area between the 1st pair of clamps and near to the posterior tip of the opisthaptor. These two hooks can be easily seen in diporpean stages with one pair of clamps.

9. Junction area (Fig. 3.1 and in many other Figs.)

The area is situated between the two anterior and two posterior regions of the adult worm.

10. Fine constrictions (Fig. 3.1 and in many other Figs.)

They are tiny thickened folds running transversely around the

body in parallel lines and entirely covering the anterior and posterior regions of the adult worm.

11. Genital pores (Fig. 3.1)

There are two pores usually situated at the junction area. Only one pore can be seen from each side of the adult worm as the other opens on the opposite face. This is illustrated in Fig. 3.1, where one genital pore appears on the left side. The other pore will be at the same level on the opposite side of the parasite.

B. Internal Structures

1. Vitellaria (Figs. 3.6 and 3.7)

They consist of numerous follicles and tend to fill the anterior region between the pharynx and the place of union of the two individuals.

2. Alimentary canal (Figs. 3.5 and 3.6)

The canal has many small branches in the anterior region just behind the pharynx and fills the anterior area. These branches first clearly appear in the juvenile stage (Fig. 3.6) at the distal end near to the pharynx. The branches at the posterior region can be seen in most adult stages as shown in Fig. 3.5.

3. Oral suckers (Fig. 3.3)

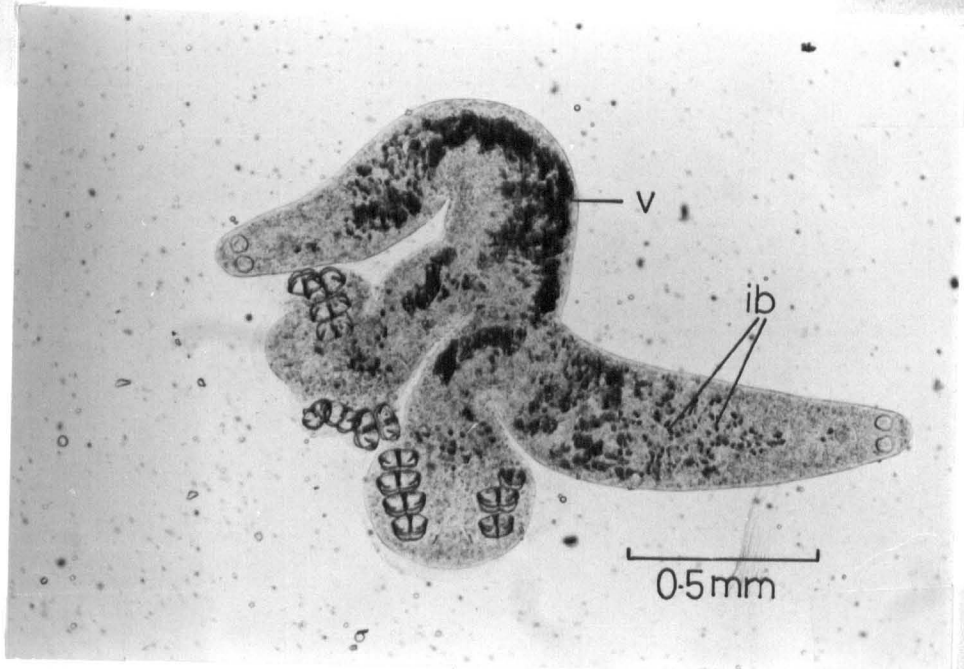
There are a pair of saucer-like muscular suckers situated some distance from the mouth at its lateral margins.

4. Pharynx (Figs.3.3 and 3.4)

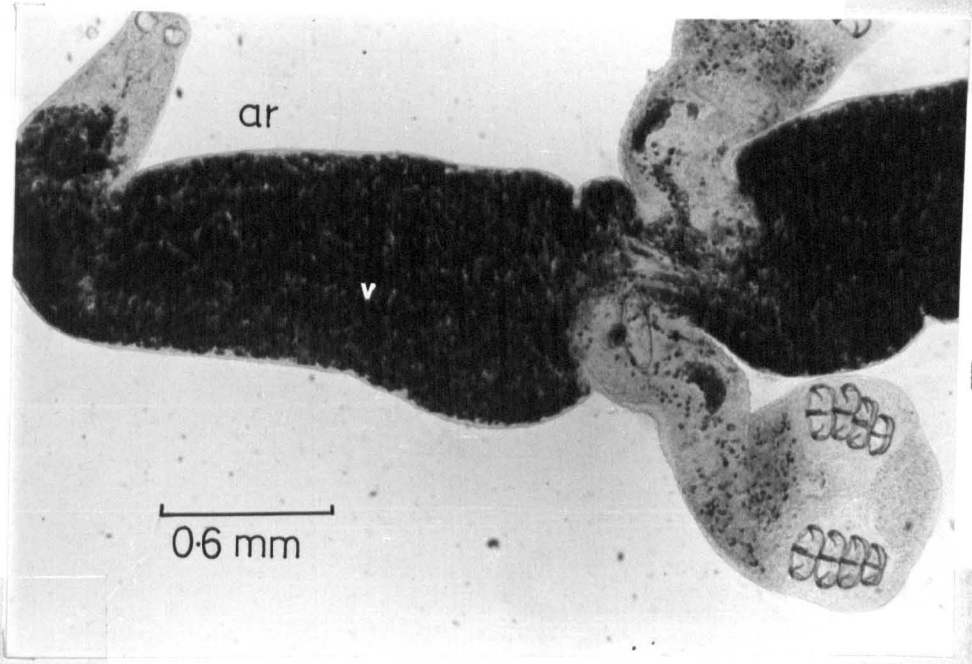
A small muscular structure, usually circular in shape as seen in mounted specimens, follows the prepharynx, and is located behind the suckers.

Fig. 3.6 A 2mm juvenile D. homoion showing formation of vitelline follicles in the anterior regions. ib, intestine branches; v, vitelline follicles.

Fig. 3.7 A 5mm adult D. homoion showing the vitelline follicles in the anterior regions which tend to fill this part of the worm. ar, anterior region; v, vitelline follicles.



Fig·3·6



Fig·3·7

5. Ovary (Fig. 3.5)

An oval-elongate organ formed as a loop which occupies the area between the junction region and the testis in the posterior part of each individual.

6. Testis (Fig. 3.5)

It usually occupies the area in the posterior region of each individual just behind the ovary and is surrounded by the intestinal caeca.

IV. RESULTS

A. Variation of the Characters of Adult Stages of Diplozoon Species

Most of the external and internal anatomical characters of adult worms of Diplozoon species were found to show marked variations within each species. These characters were studied in detail on both living and dead D. homoion from hosts of various sizes and at different periods of time.

1. The vitelline follicles

The number of vitelline follicles increases enormously during the period of maturation of each adult worm. In addition the size of the individual follicles also changes as a result of the development process (Figs. 3.6 and 3.7). In juvenile and young adult parasites (Fig. 3.6) they were relatively few in number and of smaller size. They occupied a small area of the anterior region of each partner. As the worms matured the follicles became very numerous and larger in size so that in sexually mature worms they tended to fill all the anterior part of each worm (Fig. 3.7). In gravid D. paradoxum and D. rutili a similar distribution and concentration of the vitelline follicles was seen. It is only in juvenile D. homoion that the vitelline follicles do not obscure the anterior part of the intestine which in this species was not bifurcate (Fig. 3.6).

2. The size of the adult stage of D. homoion

Samples of adult D. homoion were collected from three size ranges of fishes (less than 12 cm, 15-18 cm and 22-24 cm). The dimensions of the parasites were measured as shown in Table 3.3.

As can be seen from Table 3.3 there was a positive relationship between the mean of the total length of the D. homoion and increasing

Table 3.3. **The relationship between the mean sizes of *R. rutilus* and mean sizes of *D. homoion* on these fishes.**
The specimens of *D. homoion* were collected from fishes sampled in January, March, September and October, 1983.

	Mean length (mm) of the structures of *D. homoion* for each fork length of host		
	Fork length (cm)		
	Less than 12cm	15-18cm	22-24cm
No. hosts used	8	6	18
No. parasites examined	19	33	46
Total length of parasites	3.3	4.0	5.2
Width	0.6	0.8	0.9
Anterior region (length x width)	2.0 x 0.6	2.5 x 0.8	3.2 x 0.9
Posterior region (length x width)	1.3 x 0.3	1.5 x 0.3	2.0 x 0.4
Ratio between anterior and posterior	1.5:1	1.7:1	1.6:1
Oral sucker (length x width)	0.069 x 0.059	0.081 x 0.071	0.094 x 0.082
Pharynx (length x width)	0.086 x 0.065	0.087 x 0.072	0.096 x 0.076
Clamps width			
4th	0.190	0.199	0.226
3rd	0.188	0.197	0.222
2nd	0.170	0.180	0.208
1st	0.134	0.140	0.162
Larval hook (handle, blade)	0.056, 0.025	0.055, 0.024	0.058, 0.025

length class of R. rutilus. Mainly small and medium sized parasites were recovered from fishes less than 12 cm long, whereas D. homoion of all sizes up to 7 mm long were found on R. rutilus of lengths between 22 and 24 cm. Figs. 3.4 and 3.5 show the morphological appearance of parasites 2.5 mm in length (small) and 5mm in length (large). There was a considerable variation in the dimensions of all the external and internal structures of the parasites both in relation to size class of the hosts and during the development of adult stage. However, under experimental conditions parasites as small as 1.5 mm were capable of sexual reproduction (see for example Fig. 3.8).

The effect of the size of host on the sizes of the other morphological structures of the parasites can be seen from Table 3.3.

3. The shapes of the clamps

It was clear from this study that the shapes of the clamps of D. homoion varied during the life cycle of the parasite. A comparison between shapes of clamps of unpaired diporpae stages with one pair of clamps (Fig. 3.9), unpaired diporpae stages with 3 pairs of clamps (Fig. 3.10), juvenile stages (Fig. 3.6) and adult stages (Fig. 3.7) reveals that the shapes of clamps and their sclerites were changing continuously during the life cycle.

The shape of the clamps was almost circular in unpaired diporpa with one pair of clamps but changed to a more rectangular form in the unpaired diporpa with 3 pairs of clamps and in the juvenile stage virtually reached the normal shape of the clamps of the adult. A few days later the shape of the clamps achieved their final form. A considerable variation in the sizes and shapes of the sclerites of the clamps was seen not only between separate pairs of worms as well as on the two opisthaptors of a pair of worms but also between

Fig. 3.8. D. homoion 1.5mm in length from fry of Leuciscus leuciscus. Note the egg showing that sexual maturity had been achieved.

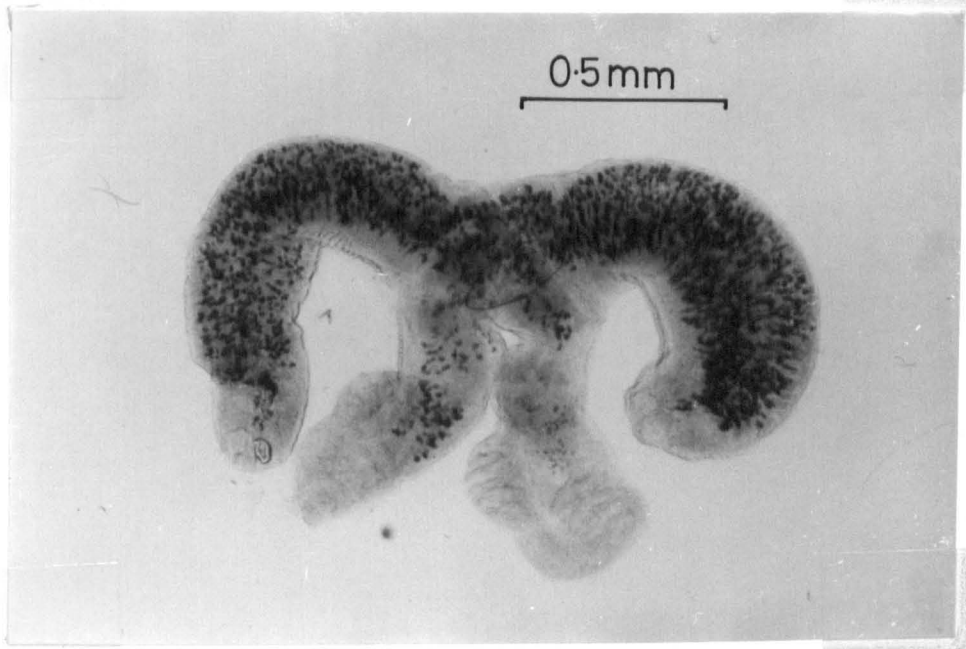


Fig. 3.9 Unpaired diporpa stage of D. homoion with a pair of circular clamps. lh, larval hook.

Fig. 3.10 Unpaired diporpa stage of D. homoion with 3 pairs of semi-rectangular clamps. lh, larval hook.

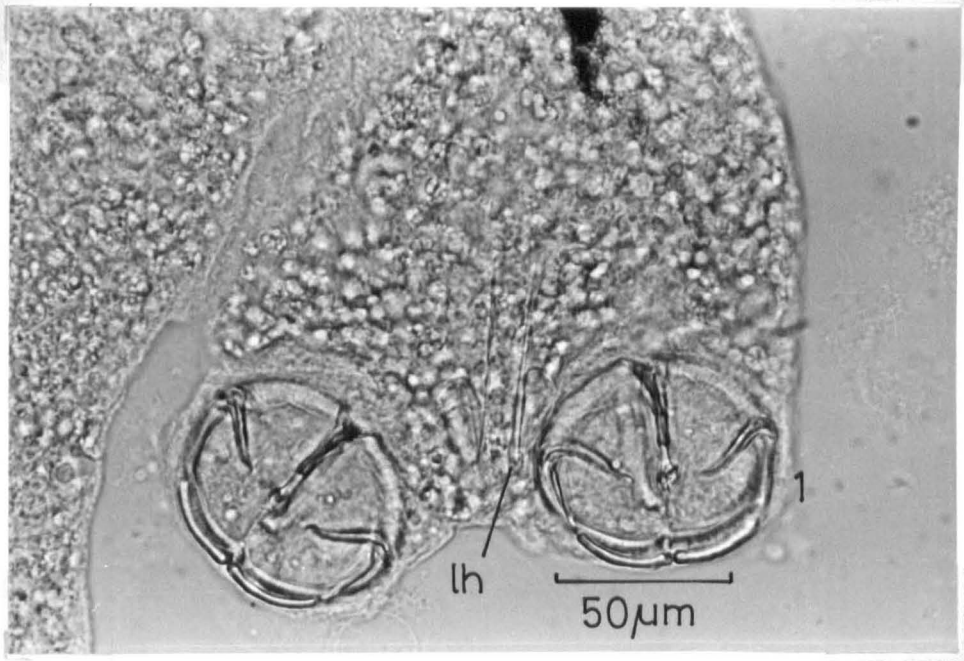


Fig.3·9

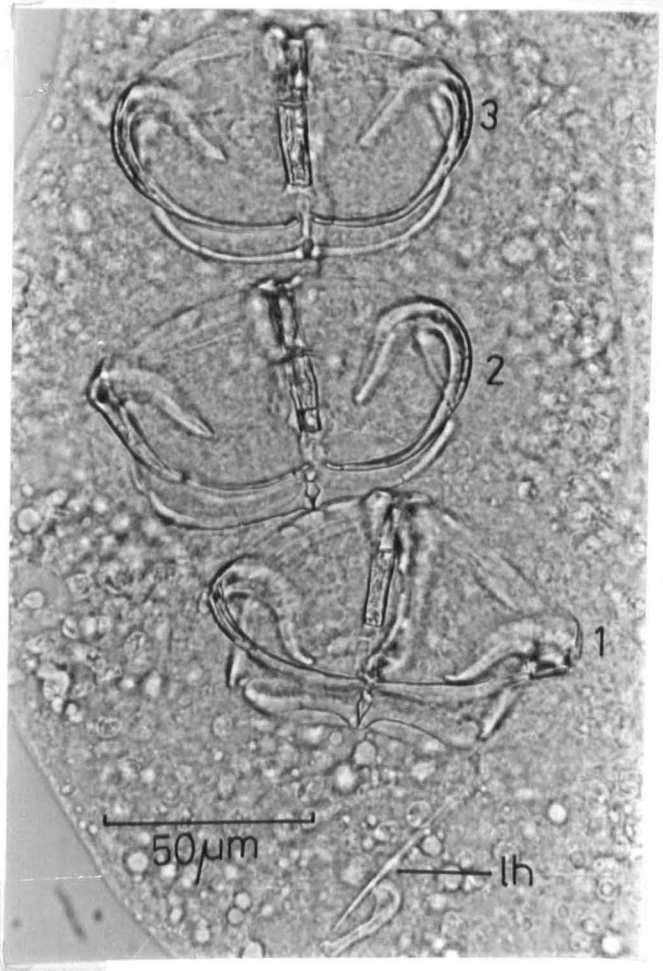


Fig.3·10

opposite members of a pair of clamps on one opisthaptor.

It was also found that these variations were considerably influenced by the process of fixation. Even the pressure of the cover slip can enormously effect the appearance of the clamps. These variations were seen on D. homoion from all cyprinid species studied.

4. The shape and size of reproductive organs

The shape and size of the ovary and the testis of D. homoion were changed with season depending on the reproductive activity. The functional ovary was oval in shape and occupied most of the upper part of the posterior region. The changes in shape will be described in Chapter 5 (life cycle). The testis also changed in shape with season but the details of these were not studied.

5. The position of genital pores

There were two genital pores opening on the ventral surface of each individual making up the compound parasite. In D. homoion two arrangements were seen, the first as shown in Fig. 3.1 where the genital pore was on the left side of the junction area as seen by the viewer, the other was on the right side of the junction area on the opposite face. The second arrangement is exactly the opposite (details in chapter 5). This variation was also seen on D. paradoxum.

6. The fine constrictions

These small thickenings covering the outer surfaces of the entire body of adult Diplozoon species were found to be highly variable on both living and dead specimens. The degree of thickening and the distance between the constrictions were recorded on living specimens examined while they were still attached to the gills. The movement of the anterior regions of the parasites enormously effected the appearance

of the constrictions. The anterior region of living Diplozoon can extend greatly so that these small folds disappear.

B. A comparison between Characters of the Adult Stages of D. homoion and D. paradoxum from British Cyprinidae with A reference to Adult Stages of Overseas Materials

My critical taxonomic studies on the adult stages of Diplozoon from cyprinid species of the British Isles have identified two species, D. homoion and D. paradoxum. The study comprised :

1. A comparison between clamp structures

The morphology of clamps of D. paradoxum and D. homoion under light and scanning electron microscopes is shown in Figs. 3.11 and 3.12 (D. paradoxum) and Figs. 3.13 and 3.14 (D. homoion). For comparison with overseas species, the morphology of the clamps of D. rutili is shown in Figs. 3.15 and 3.16. It can be seen from these Figs. that there was no significant difference between the structure of the clamps of D. paradoxum, D. homoion or D. rutili as seen by either the scanning electron or light microscopes. The clamps only differed in their sizes: in D. rutili they were larger than in the other two species and in the instance of D. paradoxum, they were smaller than in the other two, while in D. homoion, the clamp size was in between. In all 3 species studied, it was seen that the size of the 3rd pair of clamps of some specimens was slightly larger than the 4th pair. Also the 4 pairs of clamps of D. rutili seemed to occupy most of the ventral surface (clamp surface) of the opisthaptor, much more than in D. homoion and D. paradoxum. The invagination and ridges (deep folds) on the posterior region of D. paradoxum (Fig. 3.12) will be discussed later. The clamps of D. paradoxum and D. homoion from overseas had the same morphological appearance.

Fig. 3.11 The opisthaptor of adult *D. paradoxum* from British *Abramis brama*. Light microscope. c, clamps; md, muscular disc.

Fig. 3.12 The posterior region of adult *D. paradoxum* from British *Abramis brama*. S.E.M. fc, fine constrictions; inv, invagination; md, muscular disc; oph, opisthaptor; r, ridges. Markers = 10μm.

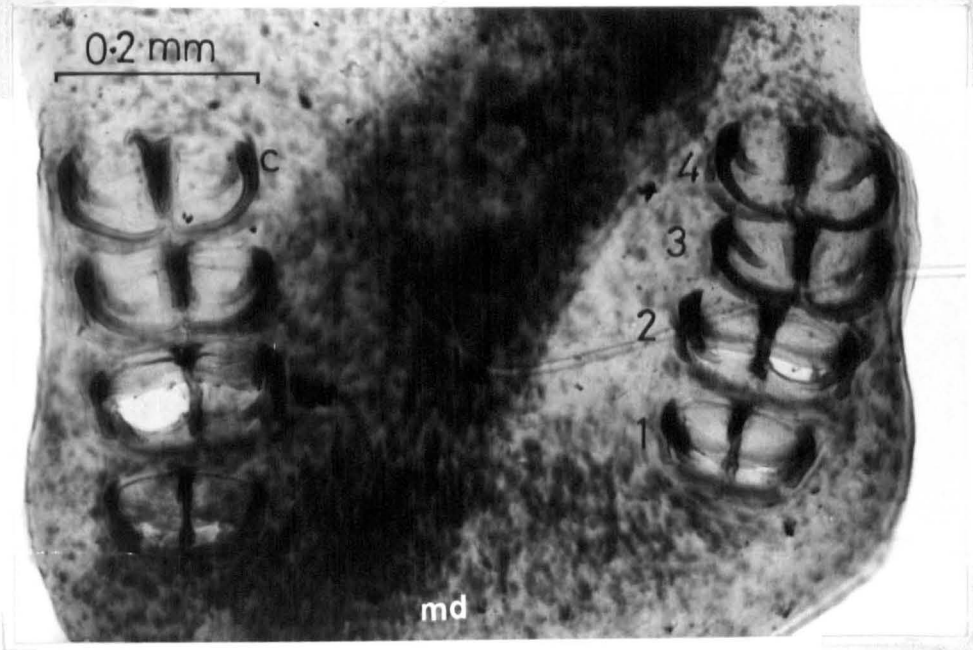


Fig.3·11

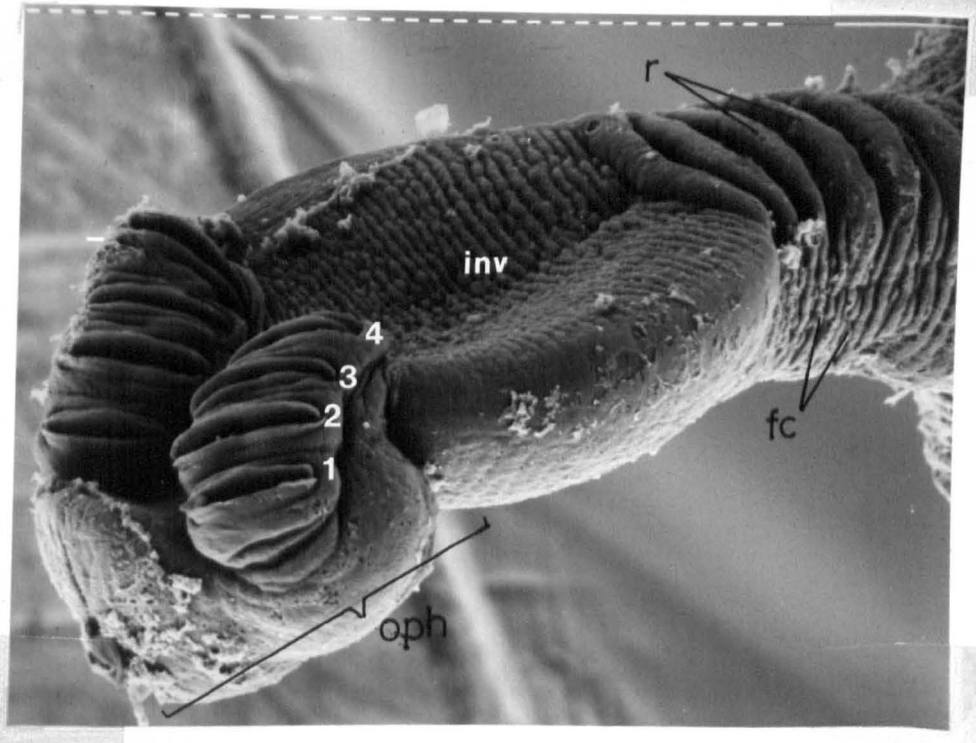


Fig.3·12

Fig. 3.13 The four clamps of one side of opisthaptor of adult *D. homoion* from British Cyprinidae. Light microscope.

Fig. 3.14 The opisthaptor of adult *D. homoion* from British Cyprinidae S.E.M. md, muscular disc. Markers = 10μm.

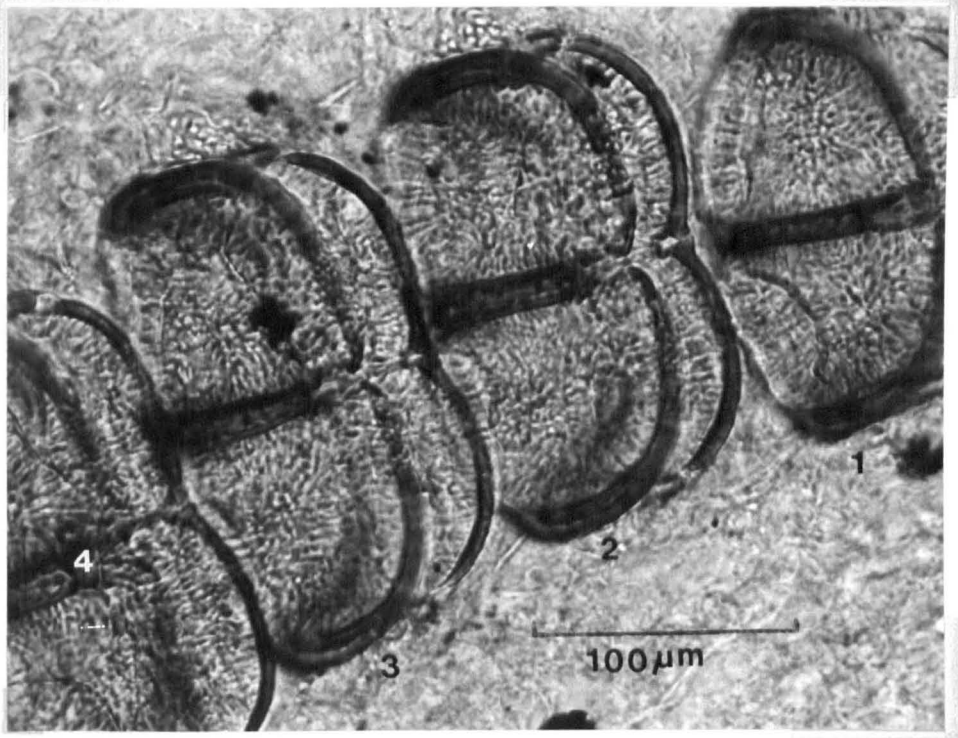


Fig.3.13

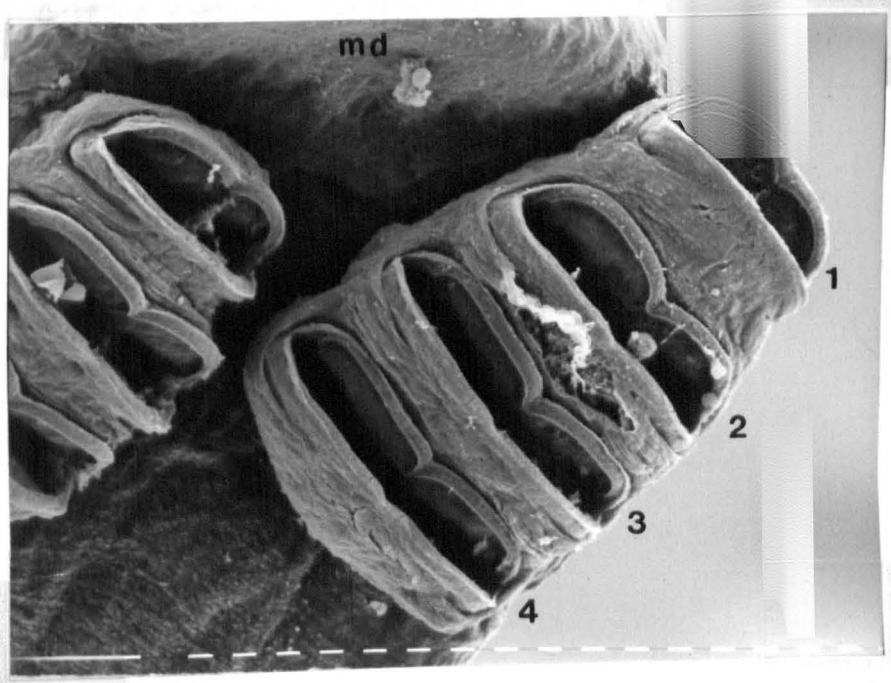


Fig.3.14

Fig. 3.15 The clamps of adult *D. rutili* from *Rutilus frisii*, Caspian Sea. Light microscope.

Fig. 3.16 The posterior region of adult *D. rutili* from *Rutilus rutilus*, Gulf of Bothnia, Finland S.E.M. fc, fine constrictions; md, muscular disc. Markers = 77μm.

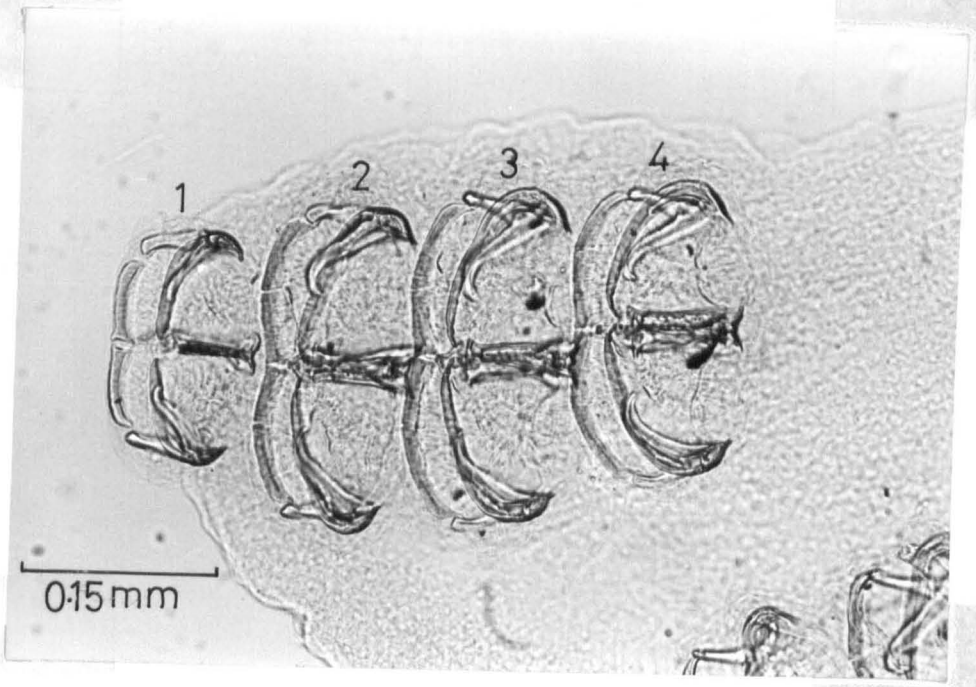


Fig.3·15

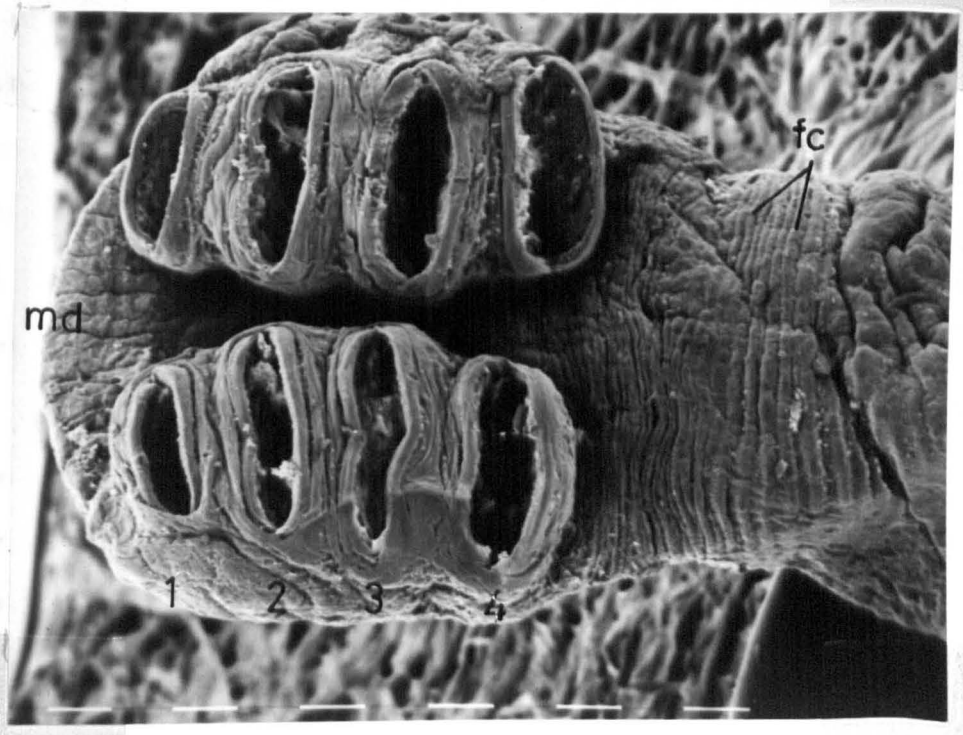


Fig.3·16

In all species studied either from Britain or abroad, the first and most posterior pair of clamps originated in the oncomiracidium stage, was the smallest and was located at the distal end of the opisthaptor. The size of the clamps increased towards the anterior 4th pair.

When it could be seen the fair-lead sclerite of the 3rd pair of clamps of D. paradoxum was triangular (Fig. 3.17). In D. homoion, by contrast, this fair-lead was thin and stalk-like (Fig. 3.18).

The current study also revealed that in these 3 Diplozoon species, the 4 clamps on each side of the opisthaptor were united by a tissue which was distinct from the general surface of the opisthaptor (Fig. 3.12 in D. paradoxum, Fig. 3.14 in D. homoion and Fig. 3.16 in D. rutili). This characteristic was also seen on dead adult specimens of D. homoion, in which the 4 clamps on one side of the opisthaptor moved together as one unit if pushed gently using a fine needle.

2. A comparison between genital pores

In this study, it was found that there were no differences between the positions of the genital pores of D. homoion and D. paradoxum either on British or overseas materials. In each species only one pore could be seen from one side and the other on the opposite. It was also noticed that in both species, the position of the genital pore seen by the viewer was located either on the left side of the parasite as in Fig. 3.1 or on the right side. In D. homoion the pore arrangements were found to depend on the position of union of the two diporpae to form the adult (See chapter 5, life cycle).
No information was collected about the position of genital pores of D. rutili owing to insufficient material.

Fig. 3.17 The 3rd clamp of an adult *D. paradoxum* from a British *Abramis brama*. s, triangular sclerite.

Fig. 3.18 The 3rd clamp of an adult *D. homoion* from a British *Rutilus rutilus*. s, thin stalk sclerite.

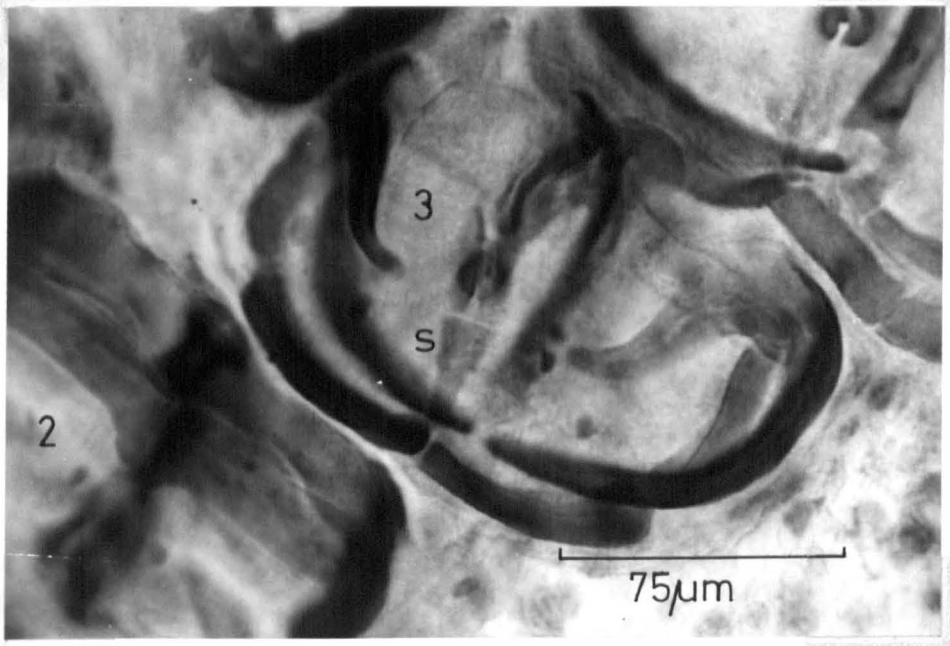


Fig.3·17

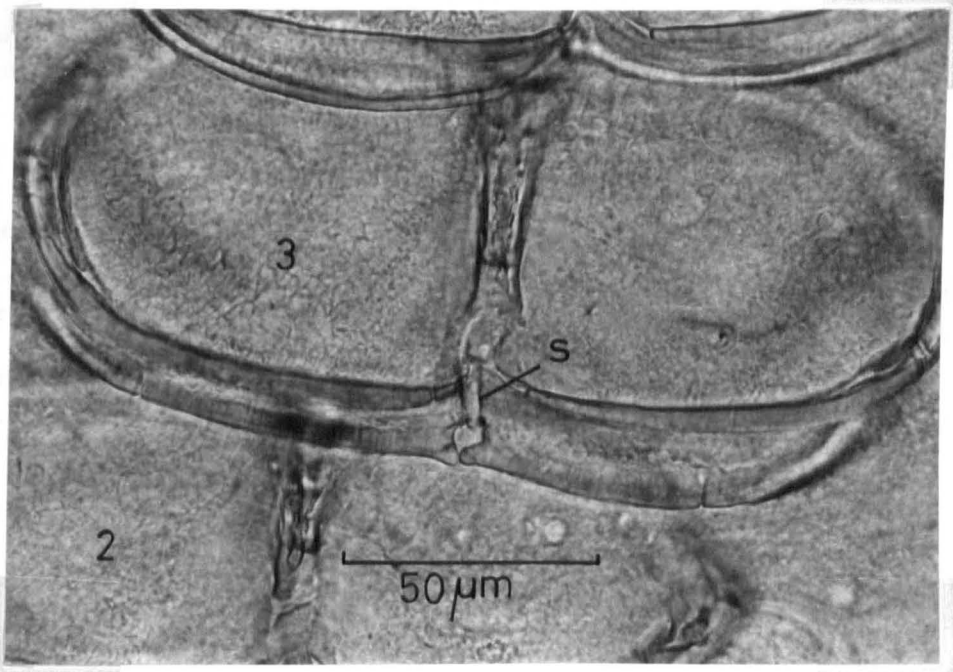


Fig.3·18

3. A comparison between larval hooks

In all specimens examined no difference was noticed between the shapes of the larval hooks of adult D. homoion and D. paradoxum. Also the hooks of D. rutili had the same morphological appearance. Fig. 3.19 illustrates a larval hook of D. homoion which is composed of a handle and a blade.

4. A comparison between muscular discs

The characteristic features of this structure were found to be very similar in D. homoion and D. paradoxum (Fig. 3.20). The outer surface of this disc appeared to be covered with numerous tiny pores. No information was obtained about the pores of the muscular disc of D. rutili.

It was also found that these pores were distributed around the genital pore in the junction area.

5. A comparison between fine constrictions

The fine constrictions normally cover the entire outer surface of the body of adult parasites of D. homoion, D. paradoxum and D. rutili from both British and overseas as shown in Fig. 3.1 for D. homoion, Fig. 3.12 for D. paradoxum and Fig. 3.16 for D. rutili. No significant differences were observed between them.

6. A comparison between intestinal branches in the posterior regions of adult parasites

The distribution of the branches of the intestine in the posterior regions was studied in D. homoion and D. paradoxum from British materials. A single unbranched intestinal caecum surrounded the ovary and testis in D. homoion (Fig. 3.5). The intestine then branched many times in the area between the testis and the 4th pair of clamps. The intestinal

Fig. 3.19 The larval hook of an adult *D. homoion*. These hooks have the same form in *D. paradoxum* and *D. rutili*. b, blade; h, handle.

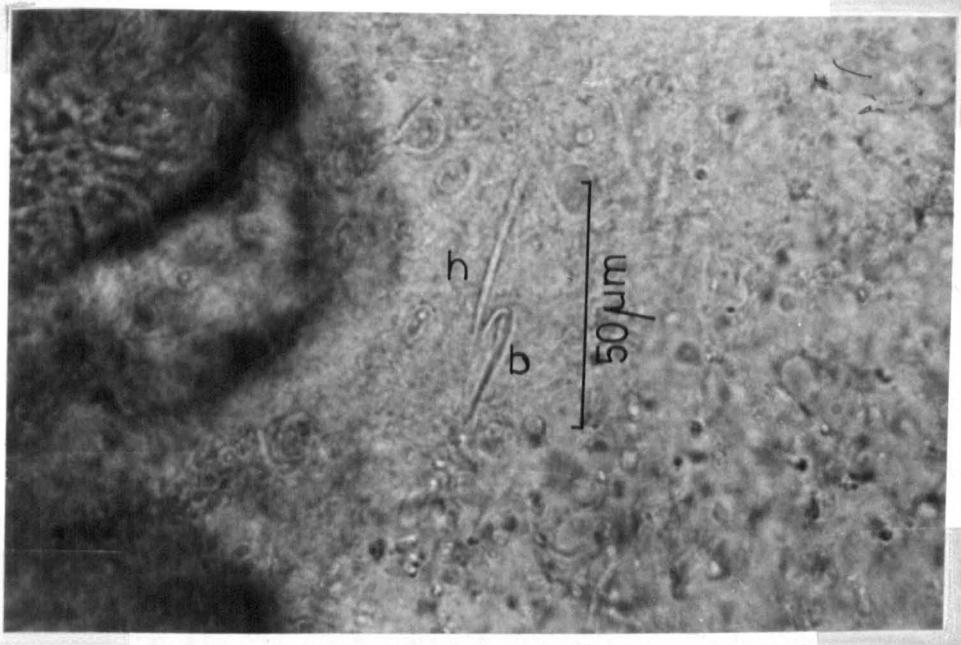
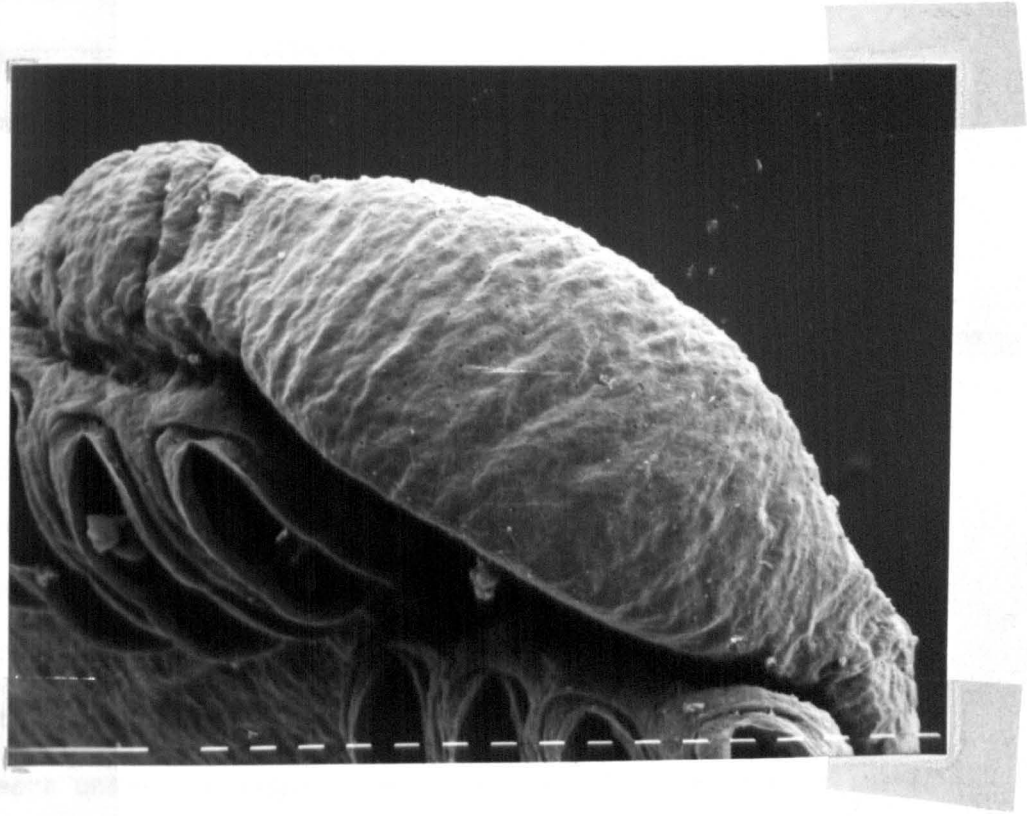


Fig. 3.20 The muscular disc of an adult *D. homoion* from British *Rutilus rutilus*. Markers = 10μm.



canal makes an incomplete circle around the reproductive organs. This can be seen in Fig. 3.5 but more clearly in Fig. 3.26. This character remained constant in all specimens of both D. homoion and D. paradoxum from Britain and overseas. There was a slight difference in the number of branches of the caeca behind the testes of D. homoion and D. paradoxum. There were fewer branches in D. homoion (Fig. 3.5) compared to D. paradoxum (Fig. 3.22). Fig 3.22 shows the distribution of the intestinal branches of D. paradoxum in the enlarged area which represents the distance between the testis and the 4th pair of clamps. Unfortunately it was not possible to examine this character on D. rutili from overseas owing to insufficient material. Fig. 3.28 shows the difficulty of distinguishing the branches of the intestine in this species.

7. A comparison between British materials of D. homoion and D. paradoxum based on characters of the adult stage of systematic value

a. D. paradoxum

i. Invagination on the posterior region of the adult stage

It was found that all adult D. paradoxum from Abramis brama collected in Britain had an enlarged area situated on each posterior region just before the 4th pair of clamps. Figs. 3.21 and 3.22 show how it appears under the light microscope. It was even more evident under the scanning electron microscope (Figs. 3.12, 3.23, 3.24). The lateral view of these enlargements on the two posterior regions of the parasite (Fig. 3.25) showed them to be cup-like invaginations. Each of them was convex towards the dorsal (outer) surface of the posterior region and concave to the ventral (clamp) surface.

ii. Large ridges on the posterior region of the adult stage

On each posterior region of the adult D. paradoxum from British

Fig. 3.21 An adult *D. paradoxum* from British *Abramis brama*. Light microscope. ar, anterior region; fc, fine constrictions; inv, invagination; por, posterior region; ri, ridges.

Fig. 3.22 The posterior region of an adult *D. paradoxum* from British *Abramis brama*. Light microscope. i, intestinal branches; inv, invagination; oph, opisthaptor; ri, ridges.

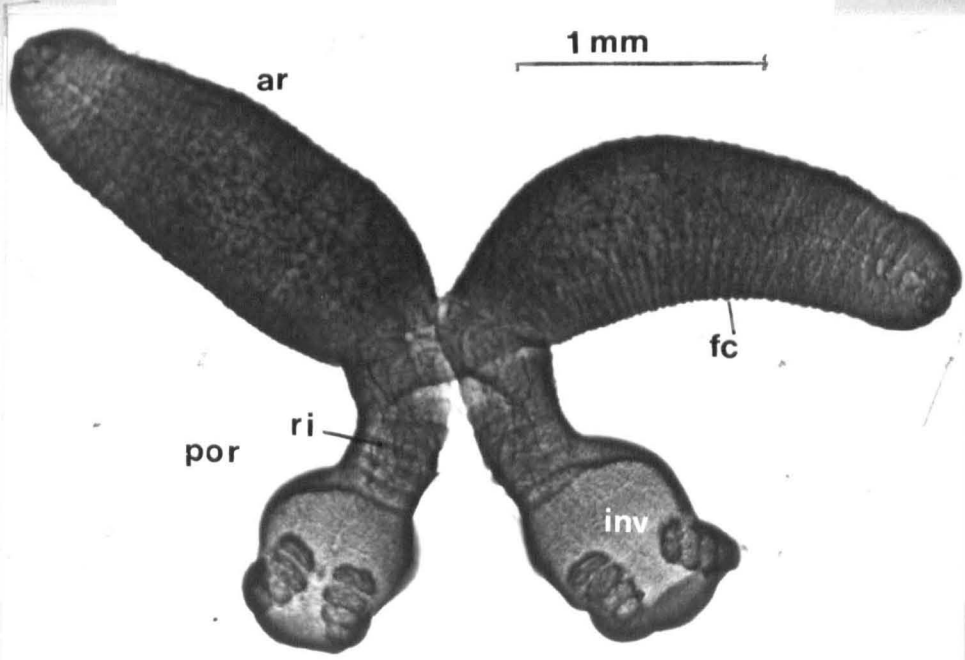


Fig.3·21

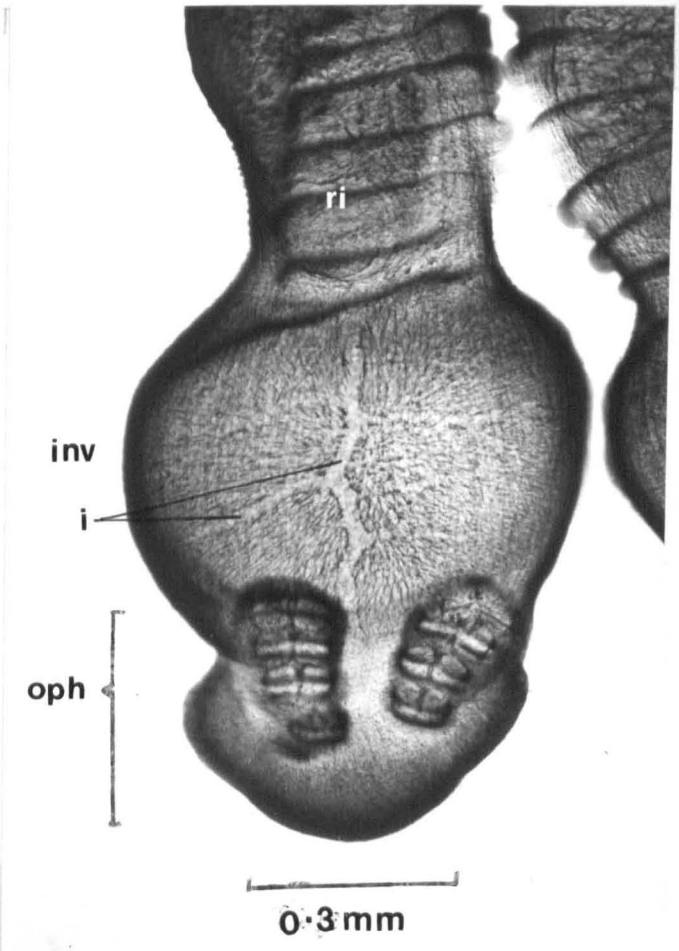
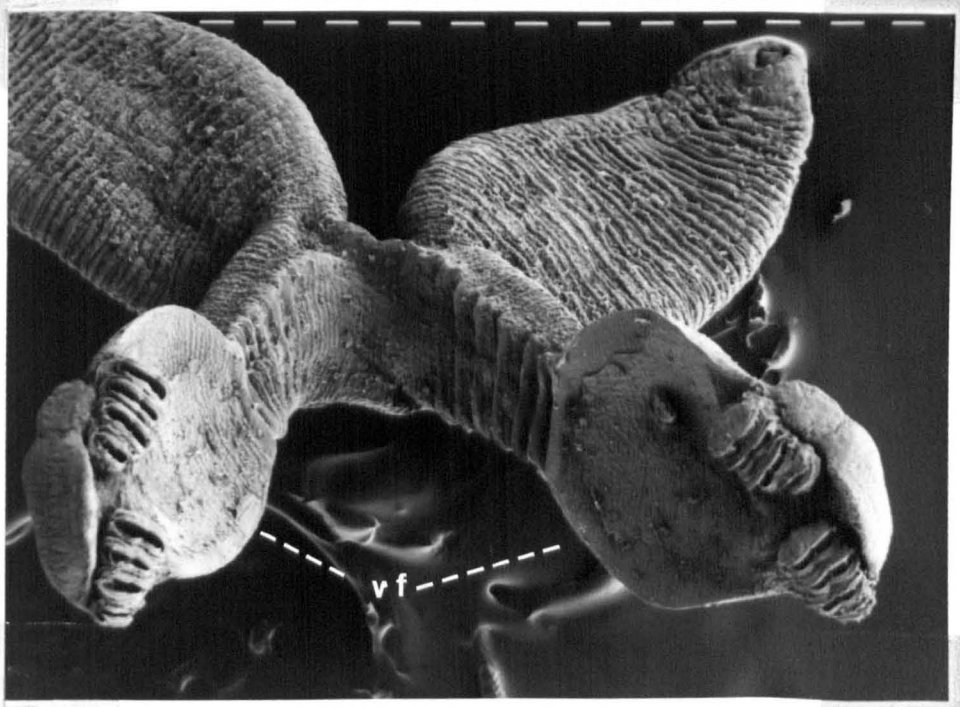


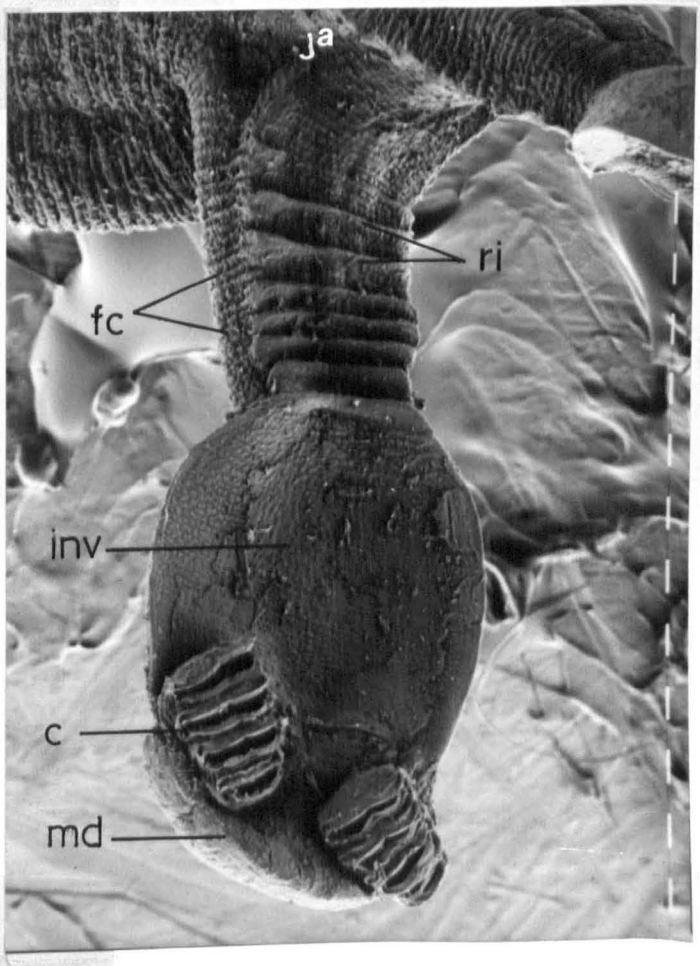
Fig.3·22

Fig. 3.23 The ventral (clamps) surfaces of the posterior regions of an adult D. paradoxum from British Abramis brama. S.E.M. vf, ventral (clamps) faces of the posterior regions. Markers = 91μm.

Fig. 3.24 The posterior region of D. paradoxum from British Abramis brama. S.E.M. c, clamps; fc, fine constrictions; inv, invagination; ja, junction area; md, muscular disc; ri, ridges. Markers = 66.7μm.

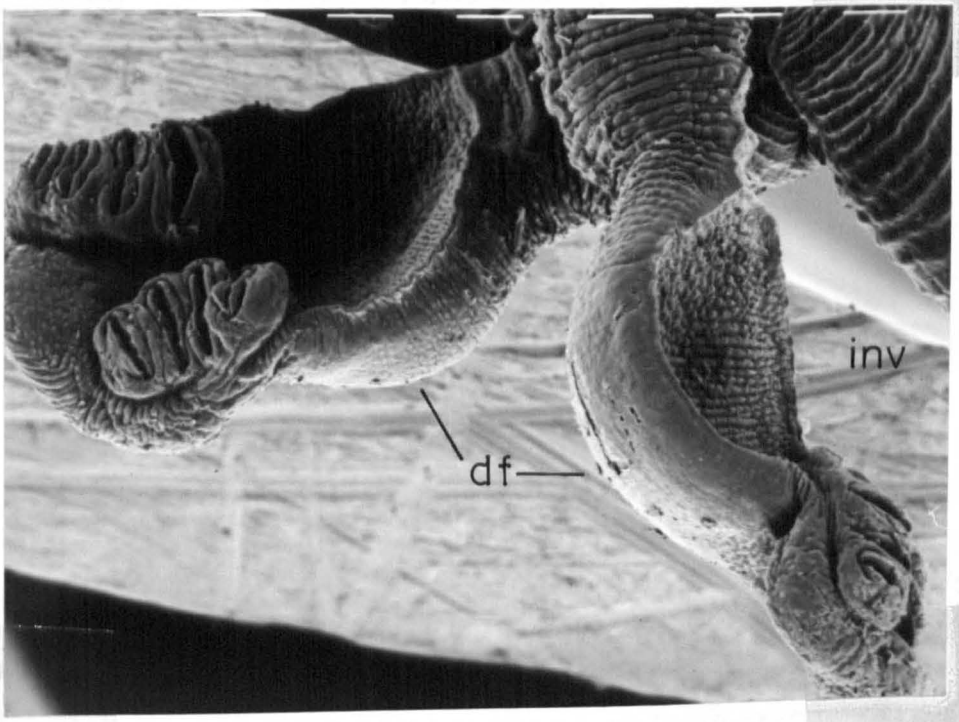


Fig·3·23



Fig·3·24

Fig. 3.25 The posterior regions of adult *D. paradoxum* from *Abramis brama* from Lake Neuchâtel, Switzerland. df, dorsal (outer) faces of posterior regions; inv, invagination. S.E.M. Markers = 100μm.



A. brama, there were 5-7 large transverse ridges (deep folds) found only on each ventral (clamps) surface in the area between the invagination (enlargement) and the junction region (Fig. 3.21 and 3.22). They ran in parallel lines transverse to the anterior-posterior axis. They were also obvious under the scanning electron microscope (Figs. 3.12, 3.23, 3.24 and 3.25). These ridges were quite different from the fine constrictions which were mentioned earlier. These characteristic features were seen on all D. paradoxum material recovered from British Abramis brama as well as on specimens from overseas.

It was noticed that there were few variations in the degree of concavity of the enlargement and its size as well as in the thickening of the ridges (deep folds) between D. paradoxum specimens from various localities (Fig. 3.19, from Moores Bakery Pond, Figs. 3.23 and 3.24 from the River Thames, England and Fig. 3.25 from Lake Neuchâtel, Switzerland). Also these two characters were seen even on small specimens of D. paradoxum (about 2 mm long) from the British Isles.

b. D. homoion

In adult specimens of D. homoion collected from a variety of British cyprinid species (including A. brama, as discussed later), no invagination was present on the posterior regions, nor were there large transverse ridges on the ventral surfaces of these parts, as has been shown in many previous figures, but can be clearly seen in Figs. 3.26 and 3.27. Only the fine constrictions appear on the outer surfaces of the entire body of adult D. homoion as described earlier. This morphological appearance was also seen on adult D. homoion from European and Asian territories. It is of interest to mention that after examining 30 permanent preparations of Diplozoon specimens provided by Prof. Halvorsen (see Table 3.1B) collected

Fig. 3.26 The posterior region of an adult *D. homoion* from British *R. rutilus*. Light microscope. Note the incomplete circle of intestinal caecum around the ovary and testis. Light microscope. fc, fine constrictions, i, intestine.

Fig. 3.27 An adult *D. homoion* from British *R. rutilus* S.E.M. fc, fine constrictions. Markers = 66.7μm.

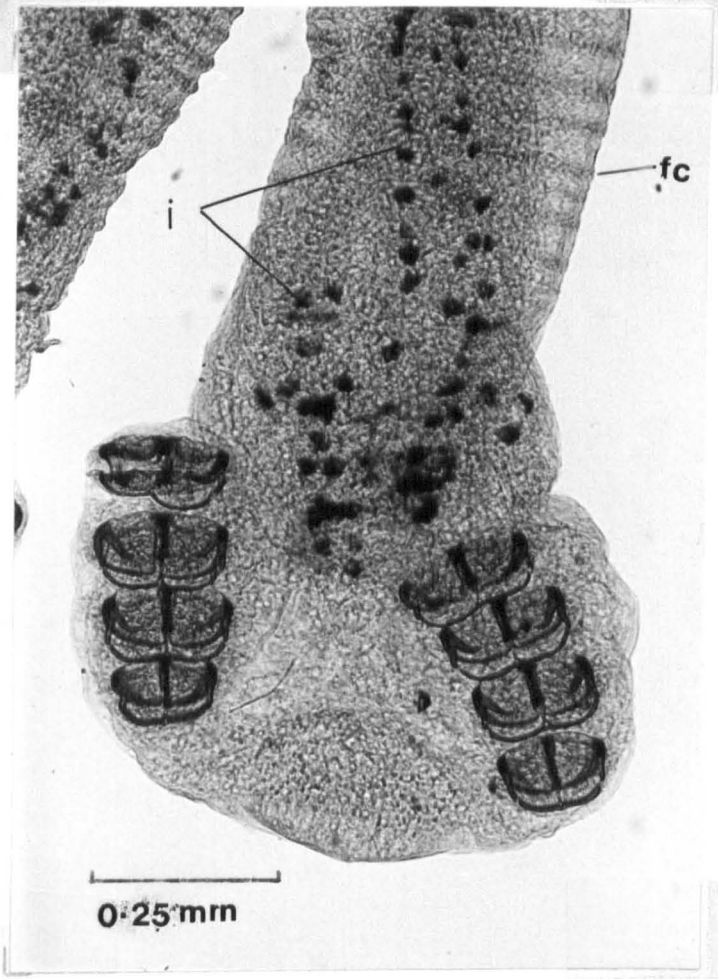


Fig.3·26

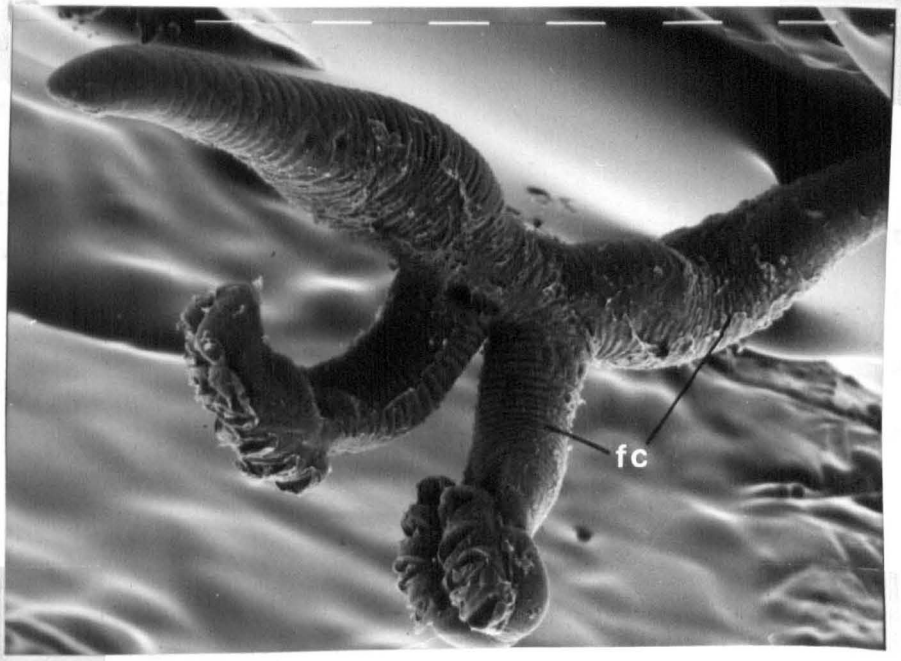


Fig.3·27

from different cyprinid species which lived together in the River Glomma, Norway, the result revealed that all the adult worms from Rutilus rutilus were D. homoion and all the specimens (8) from Abramis brama were D. paradoxum while of the 9 parasites collected from hybrid R. rutilus x A. brama 7 were D. homoion and the other 2 were D. paradoxum. There was no evidence of hybrid parasites.

c. D. rutili

D. rutili was compared with D. homoion and D. paradoxum collected in Britain. The invagination and ridges (deep folds) characteristic of D. paradoxum were not present on the posterior parts of adult D. rutili (Figs. 3.28 and 3.29).

C. A comparison between Characters of the Egg Stages of D. homoion and D. paradoxum from British Cyprinidae with A reference to the Egg Stage from Overseas Materials

The shape, size and surface texture of eggs of D. homoion and D. paradoxum from British cyprinid species were examined in detail. The study also checked the egg of D. rutili from European cyprinid species for comparison.

1. D. paradoxum

The shape of egg of D. paradoxum was an elongated oval with a long thin filament and looked obtuse rather than pointed at the end opposite to the filament. The operculum and the filament were at the same end of the egg (Fig. 3.30). The whole egg was slightly convex at one side (here termed dorsal) and concave at the other (here termed ventral). The ratio of the length of the operculum to the total length of the egg was 1:5. The egg in both Figs. 3.30 and 3.31 is hatched and part of the damaged oncomiracidium can be

Fig. 3.28 Parts of the anterior and the posterior regions of an adult *D. rutili* from the Caspian Sea *Rutilus frisii*. Light microscope. ar, anterior region; jr, junction region; por, posterior region.

Fig. 3.29 An adult *D. rutili* from *Rutilus rutilus* from the Gulf of Bothnia, Finland S.E.M. ar, anterior region; c, clamps; fc, fine constrictions; por, posterior region. Markers = 100μm.

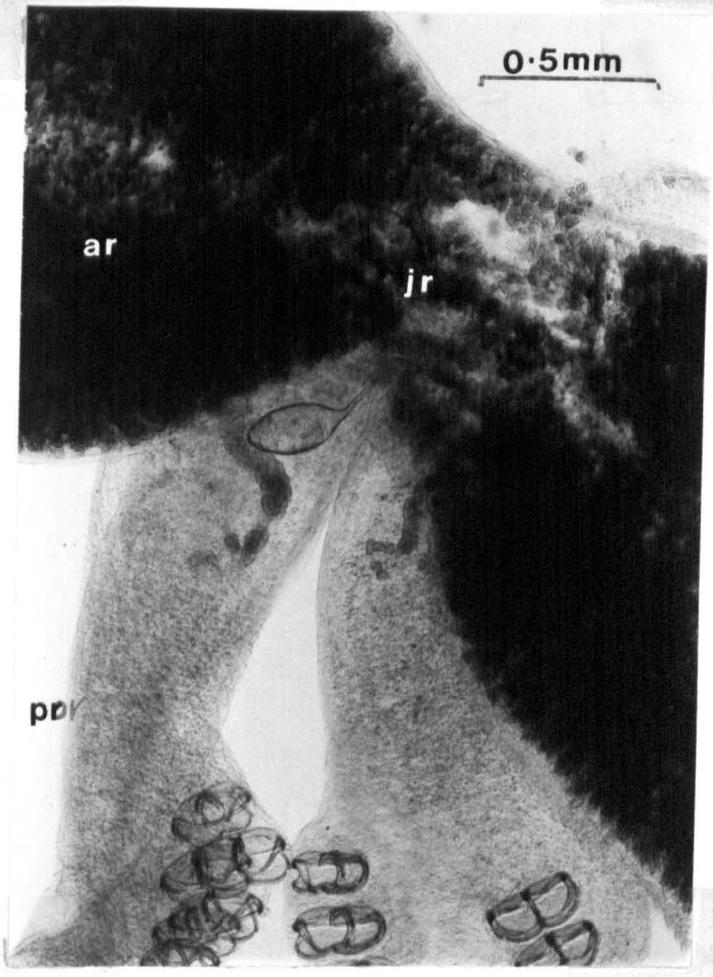


Fig.3·28

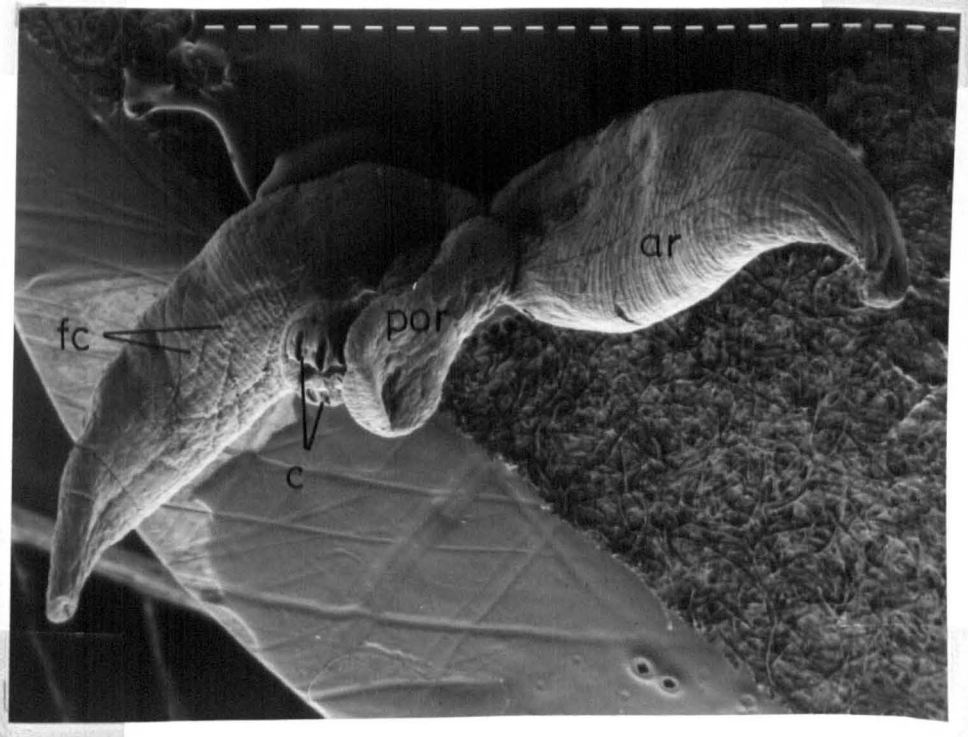


Fig.3·29

Fig. 3.30 An hatched egg of *D. paradoxum* from British *Abramis brama*. fi, filament; on, oncomiracidium; ope, operculum. Markers = 10μm.

Fig. 3.31 An opened egg capsule of *D. paradoxum* from British *Abramis brama*. ew, egg wall; on, oncomiracidium. Markers = 10μm.

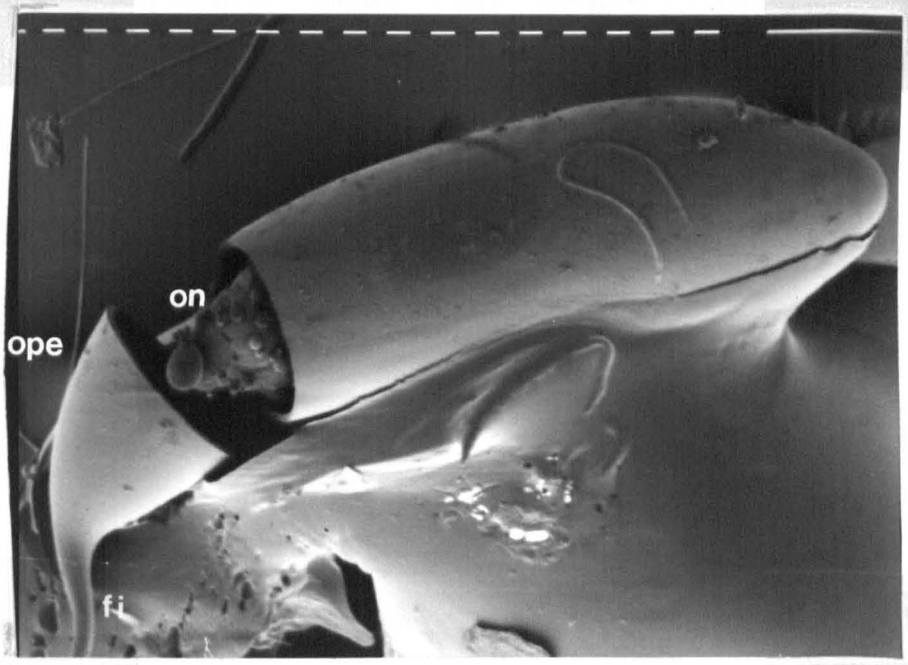


Fig.3·30

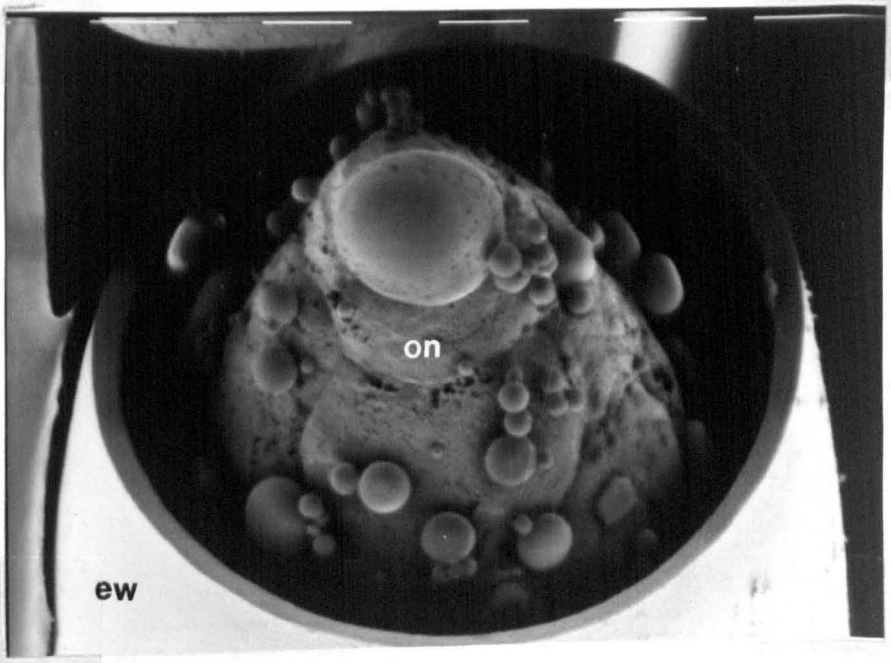


Fig.3·31

seen. The dimensions of the egg were 340 μm x 90 μm. The wall thickness (Fig. 3.31) was about 3 μm, with a smooth surface texture. The filament diameter just after the egg body was 8.3 um. The egg was yellowish-brown in colour.

2. D. homoion

The egg shape of D. homoion is shown in Fig. 3.32. The egg looked similar to that of D. paradoxum. Its shape was elongated-oval, but with a slightly pointed end at the end opposite to the filament. The whole body of egg was more convex at one side (dorsal) than in that of D. paradoxum and relatively straight at the other (ventral) side (Fig. 3.32). The line of fracture of the operculum was visible in these unhatched eggs. The ratio of the length of the operculum to the length of the egg was 1:3. The surface of the egg also had a smooth texture. One hundred and seven eggs collected from the tanks during 31/7/83 to 31/10/83 measured between 293.6 μm and 364.5 μm long and 87.8 μm-107.6 μm wide. The wall thickness was about 3 μm (Fig. 3.33) and the diameter of the egg filament was about 8.3 μm, which is similar to those of D. paradoxum. The egg also has a yellowish-brown colour.

3. D. rutili

The shape of the egg of D. rutili was considerably different from that of the other two species (Figs. 3.34 and 3.35). It looked like a rugby ball with its maximum width at the middle and tapering gradually towards the ends. At the end opposite to the filament it looked obtuse. The line of fracture of the operculum was not clear on this unhatched egg (Fig. 3.35). According to Fig. 3.34, the length of the operculum represented one fourth of the total length

Fig. 3.32 The egg of *D. homoion* from British *Rutilus rutilus*.
fi, filament; lfo, line of fracture of the operculum.
Markers = 10μm.

Fig. 3.33 The opened egg capsule of *D. homoion* from British *Rutilus rutilus*. Markers = 10μm.

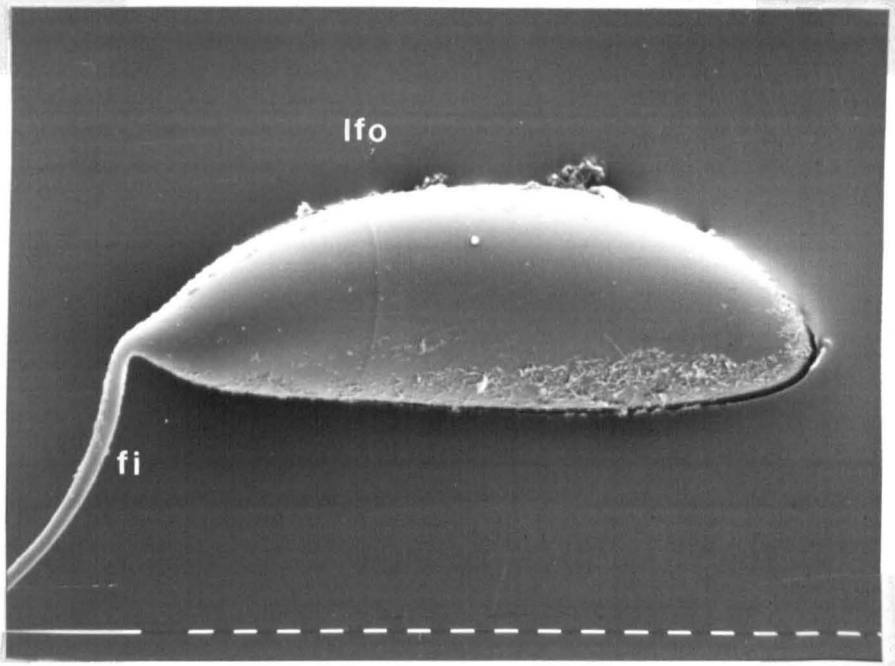


Fig.3·32

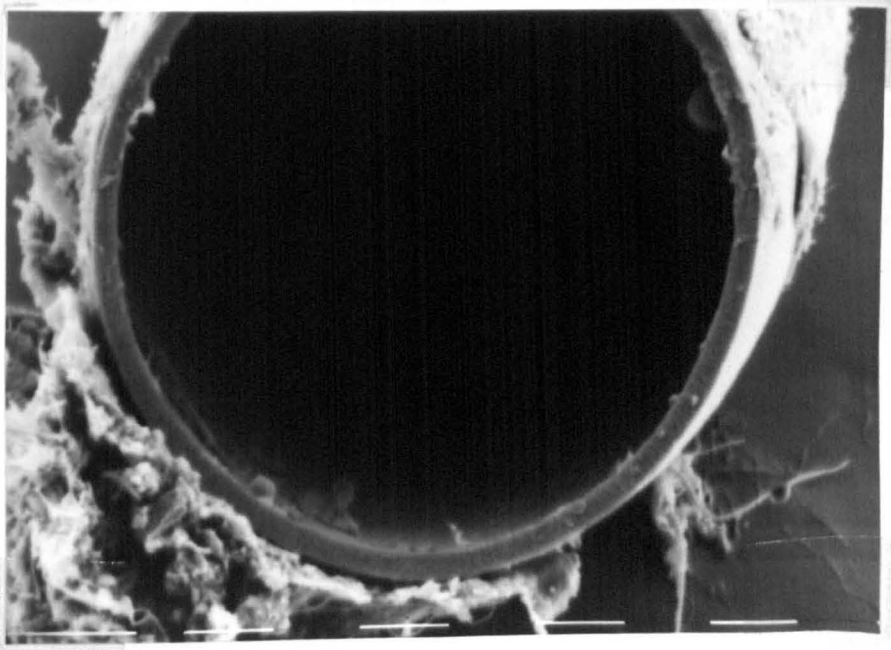


Fig.3·33

Fig. 3.34 Hatched eggs of *D. rutili* from *Rutilus frisii* from the Caspian Sea. light microscope. fi, filament; ope, operculum.

Fig. 3.35 Unhatched egg of *D. rutili* from a *Rutilus rutilus* from the Gulf of Bothnia, Finland. S.E.M. Markers = 10μm.

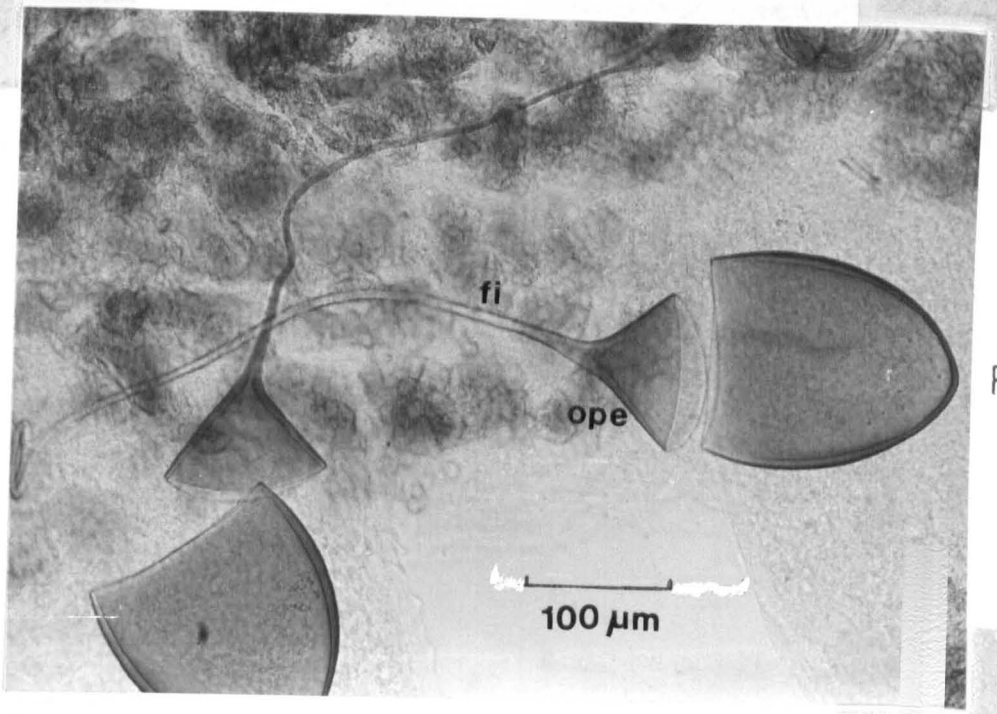


Fig.3·34

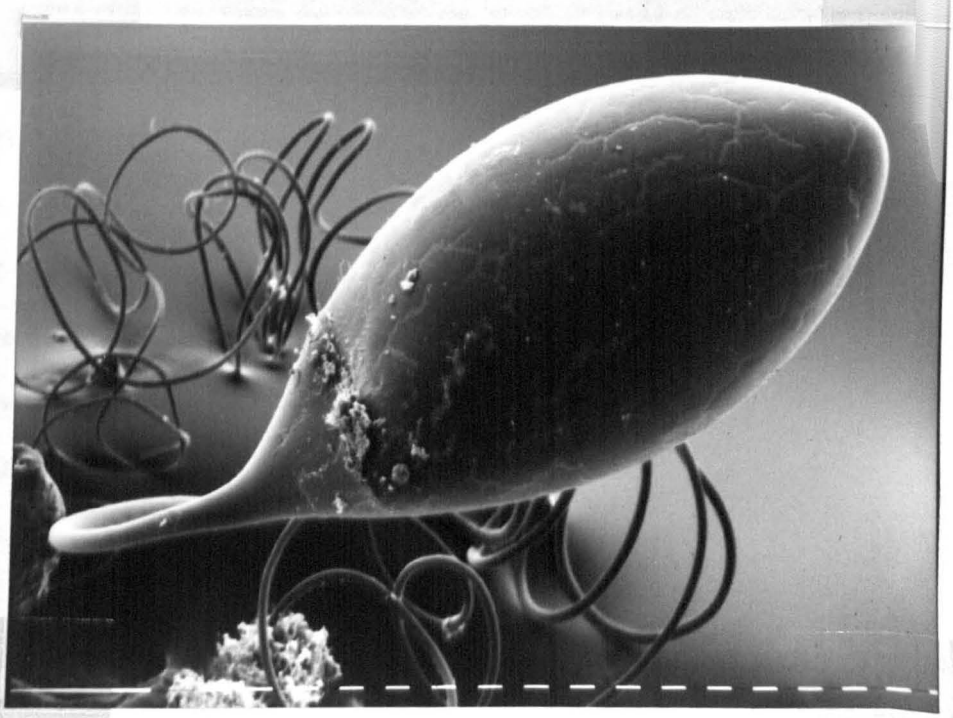


Fig.3·35

of egg. Its wall also had a smooth surface texture. The egg dimensions were 318.8 μm x 150 μm, wall thickness 5.6 μm and the diameter of the filament was about 11.7 μm. The egg also was yellowish-brown in colour.

D. Oncomiracidium of D. homoion and Morphological Variations During Its Life Span

Taxonomic studies on the oncomiracidum stage were concentrated only on D. homoion from British Cyprinidae. Unfortunately no living materials were available from D. paradoxum or D. rutili for comparison.

1. Description of oncomiracidium of D. homoion

The shape of stained larvae examined immediately after hatching was an elongated oval and the body was flattened from the dorsal and the ventral sides with a slight constriction at the anterior end (Figs. 3.36, 3.37 and in many other Figs.). There were 6 ciliated epidermal cell groups arranged on the outer wall of the larva. Most of these cells can be seen from the ventral and lateral sides. The first group was made up of 4 small ciliated cells at the tip of the frontal (cephalic) side of the larva and arranged as shown in Fig. 3.36, two lateral and two median. Their shapes and sizes will be discussed later. Two groups seen from ventral face were made up of 6 pairs of ciliated cells arranged on both antero-lateral sides in two rows each with 6 cells. These cells were relatively elongate and were placed in an oblique dorso-ventral direction (Figs. 3.36 and 3.37). Their arrangement on both sides of the larva showed a bilateral symmetry. In Fig. 3.36 one cell of this group is not visible from the left side of the picture. Another two groups, each of 6 cells, were arranged at a median-lateral position on both sides of

Fig. 3.36 Anterior region of ventral surface of oncomiracidium of D. homoion from British Rutilus rutilus. Silver treated. alc, antero-lateral ciliated cells; c, cilia; e, eyespot; fc, frontal (cephalic) ciliated cells; m, mouth; pa, papillae.

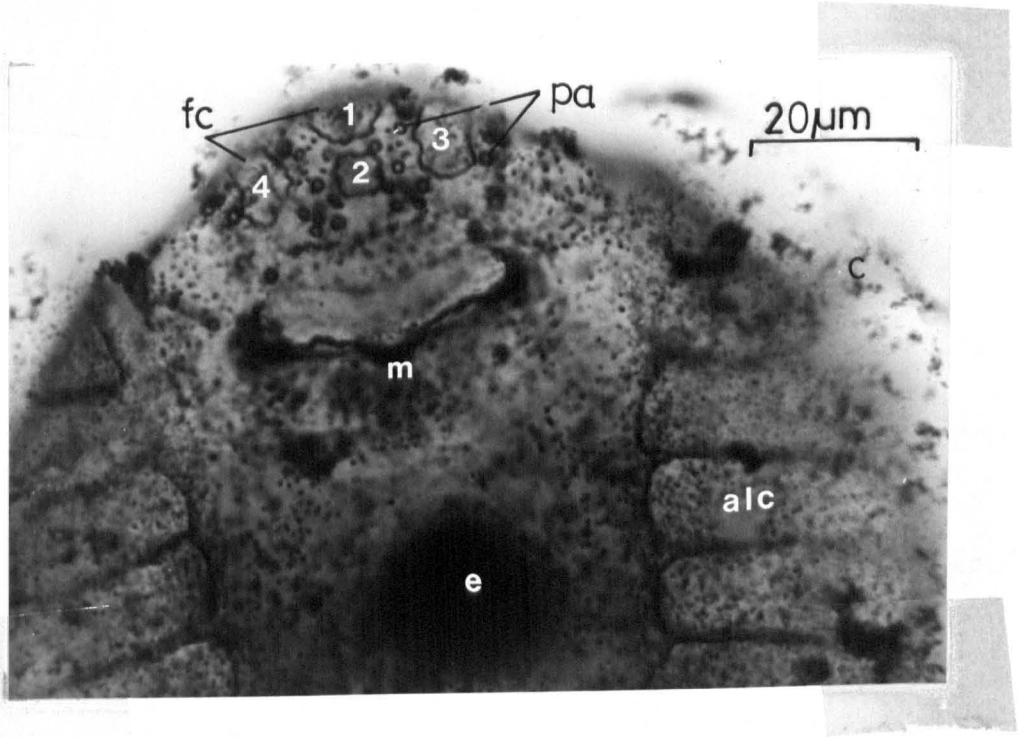


Fig.3.37 The ventral surface of an oncomiracidium of *D. homoion* from British *Rutilus rutilus*. Silver treated. alc, antero-lateral ciliated cells; cl, clamps; e, eyespot; fc, frontal ciliated cells; lc, lateral ciliated cells; lh, larval hook; m, mouth; pa, papillae.

Fig. 3.38 Posterior region of ventral surface of oncomiracidium of *D. homoion* from British *Rutilus rutilus*. Silver treated. lh, larval hook (trace); pc, posterior ciliated cells.

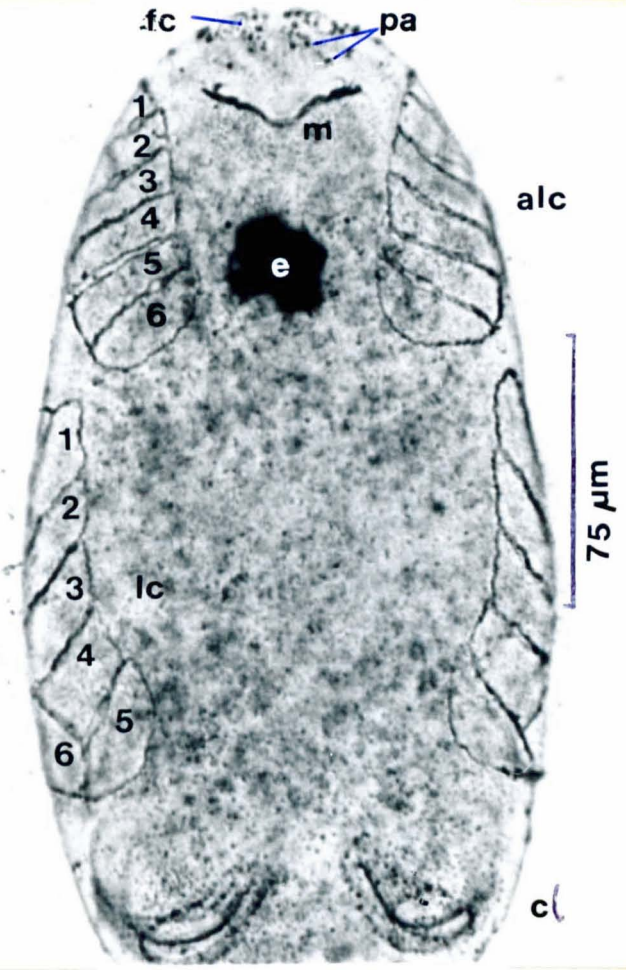


Fig.3·37

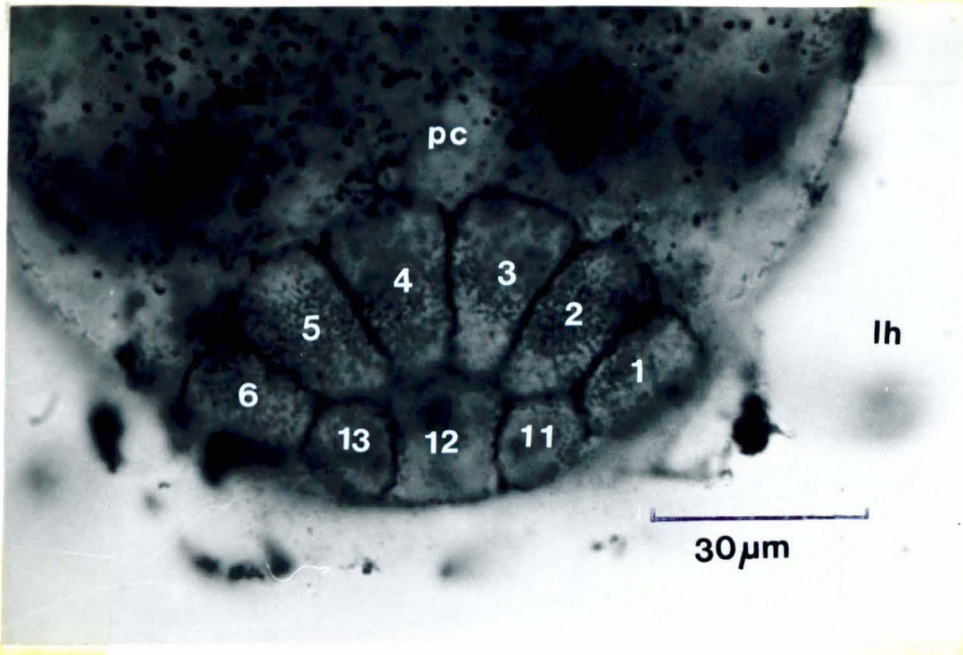


Fig.3·38

the larva. Each group was aligned in one row on each side (Fig. 3.37). Usually 5 pairs were clearly visible from the ventral surface and the sixth pair, which lay near from the fourth and fifth was obvious not only from the lateral view but also from the ventral surface as well (Fig. 3.37). The size and shape of these cells vary within each group as illustrated in Fig. 3.37. One group of ciliated cells was situated at the posterior part of the larva. It consisted of 13 cells; 10 of them were elongated oval and surrounded the posterior end. Six of them were visible from the ventral surface of the larva (Fig. 3.38), and 4 from the dorsal surface. There were 3 other cells which covered the distal end of the posterior part as shown in Fig. 3.38. All these epidermal cells had a defintive outline with a large circular nucleus. The shapes of most of these cells can also be seen from the lateral view of the larva (Fig. 3.39). It was found that using a series of silver nitrate solutions of different dilutions and exposing the larvae to these solutions for different intervals of time gave good results. Usually 0.3 percent silver nitrate solution for 2 minutes was best.

Other structures seen from the ventral side are shown in Figs. 3.36, 3.37 and 3.39. The cilia which cover all the epidermal cells are shown in Figs. 3.36, 3.37, 3.38 and 3.39. Their form as seen under the SEM from ventral, dorsal and lateral views is given in Figs. 3.40, 3.41 and 3.42 respectively. The density of cilia cover on each group of cells is especially clear from Fig. 3.42. The mouth of the larva is obvious from the ventral side (Figs. 3.37 and 3.40) and is subterminal in position. A pair of clamps can also be seen on the ventral side (Figs. 3.37 and 3.40). Neither the osmoregulatory pores nor the flame cells were visible on specimens prepared for

Fig. 3.39 Lateral view of an oncomiracidium of D. homoion from British Rutilus rutilus. Silver treated. alc, antero-lateral ciliated cells; cl, clamps; ds, dorsal surface; lc, lateral ciliated cells; pc, posterior ciliated cells; vs, ventral surface.

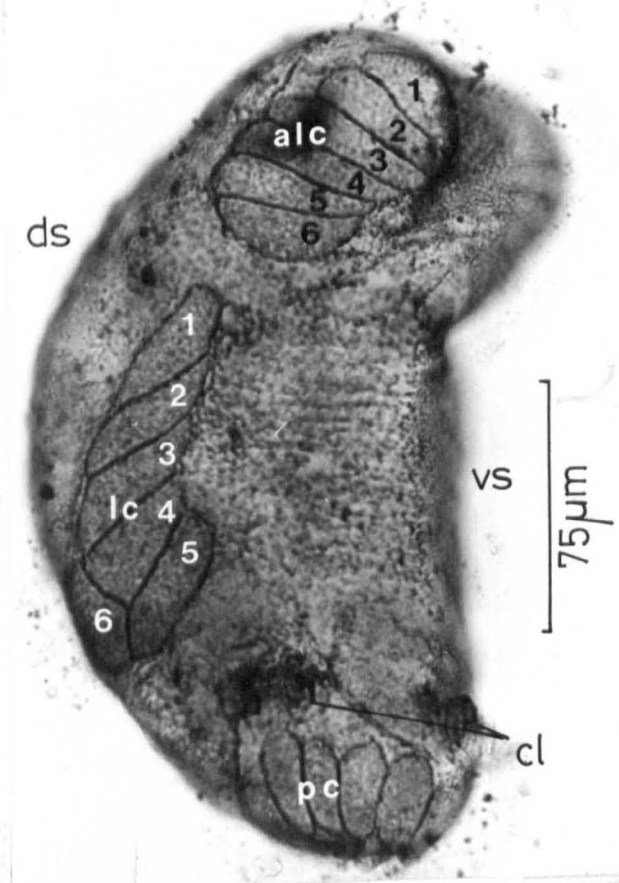


Fig. 3.40 The ventral surface of an oncomiracidium of *D. homoion* from British *Rutilus rutilus*. S.E.M. c, cilia; cl, clamps; m, mouth. Markers = 10μm.

Fig. 3.41 The dorsal surface of an oncomiracidium of *D. homoion* from British *Rutilus rutilus*. S.E.M. alc, antero-lateral cilia; fc, frontal cilia; lc, lateral cilia; pc, posterior cilia. Markers = 5.9μm.

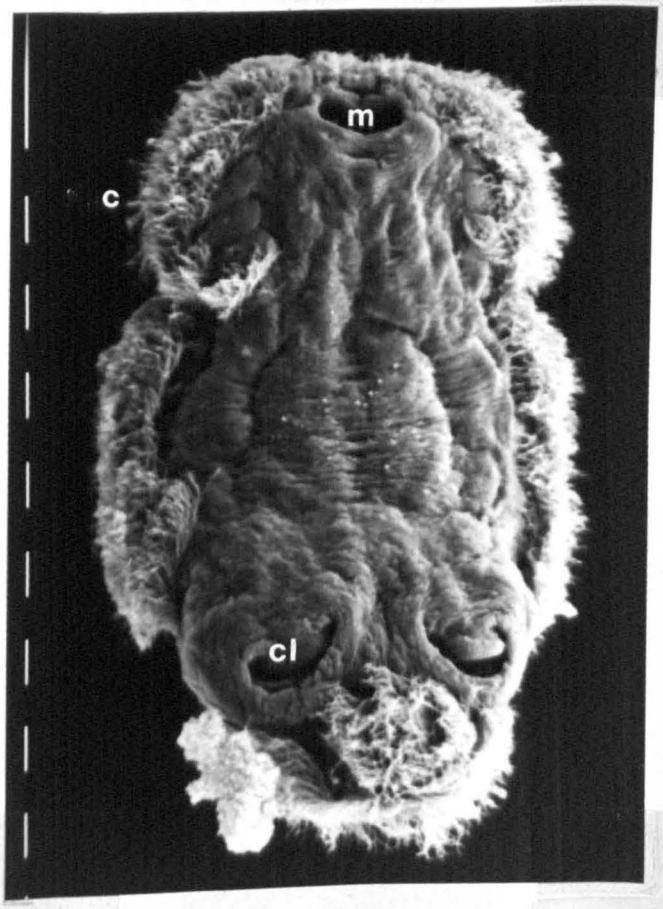


Fig.3·40

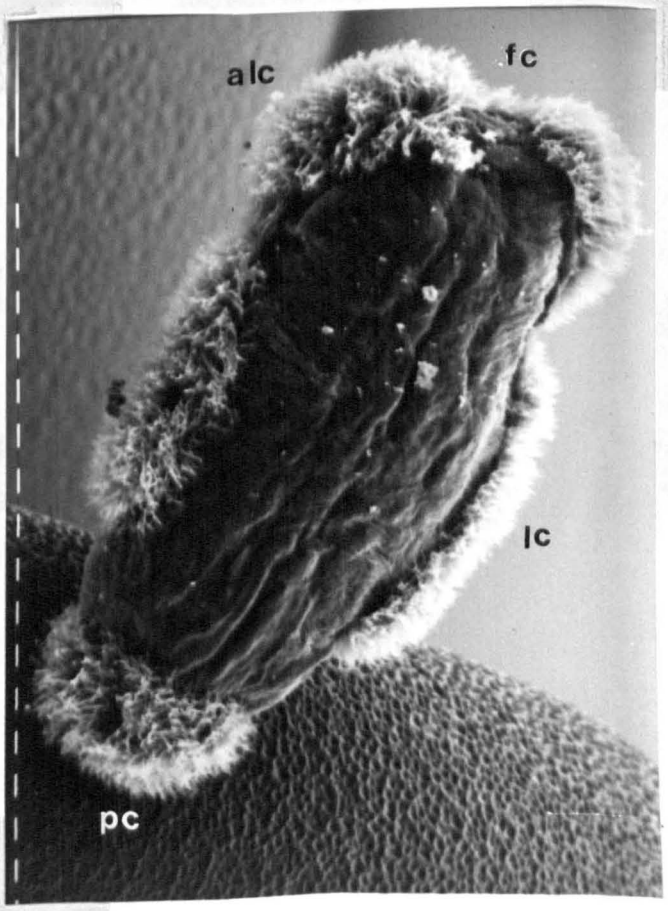
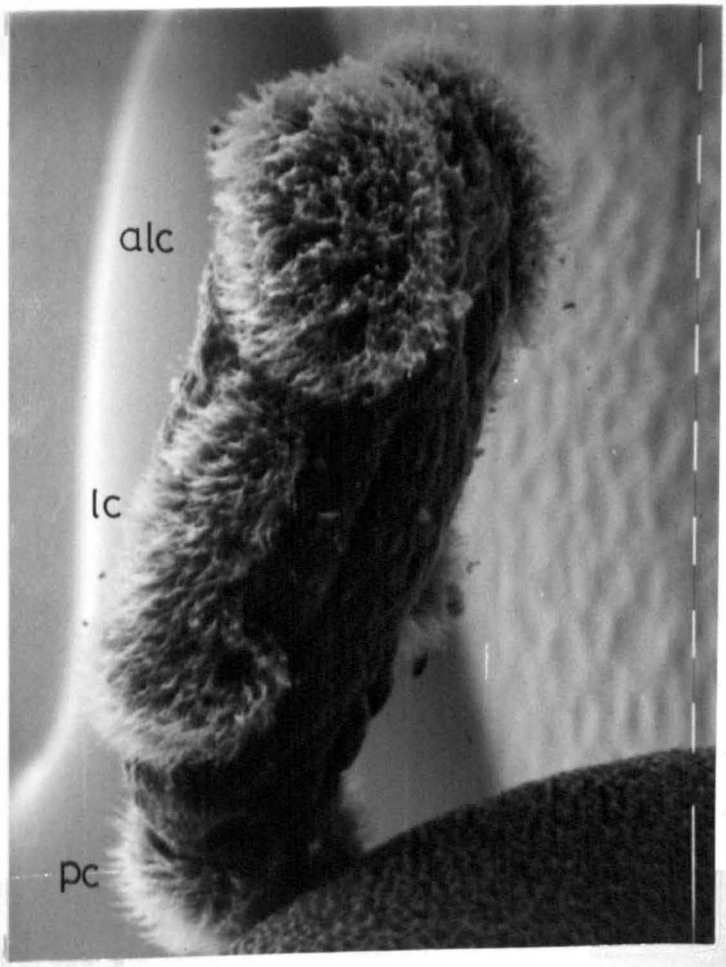


Fig.3·41

Fig. 3.42 Lateral view of an oncomiracidium of D. homoion from British Rutilus rutilus. S.E.M. alc, antero-lateral cilia; lc, lateral cilia, pc, posterior cilia. Markers = 10µm.

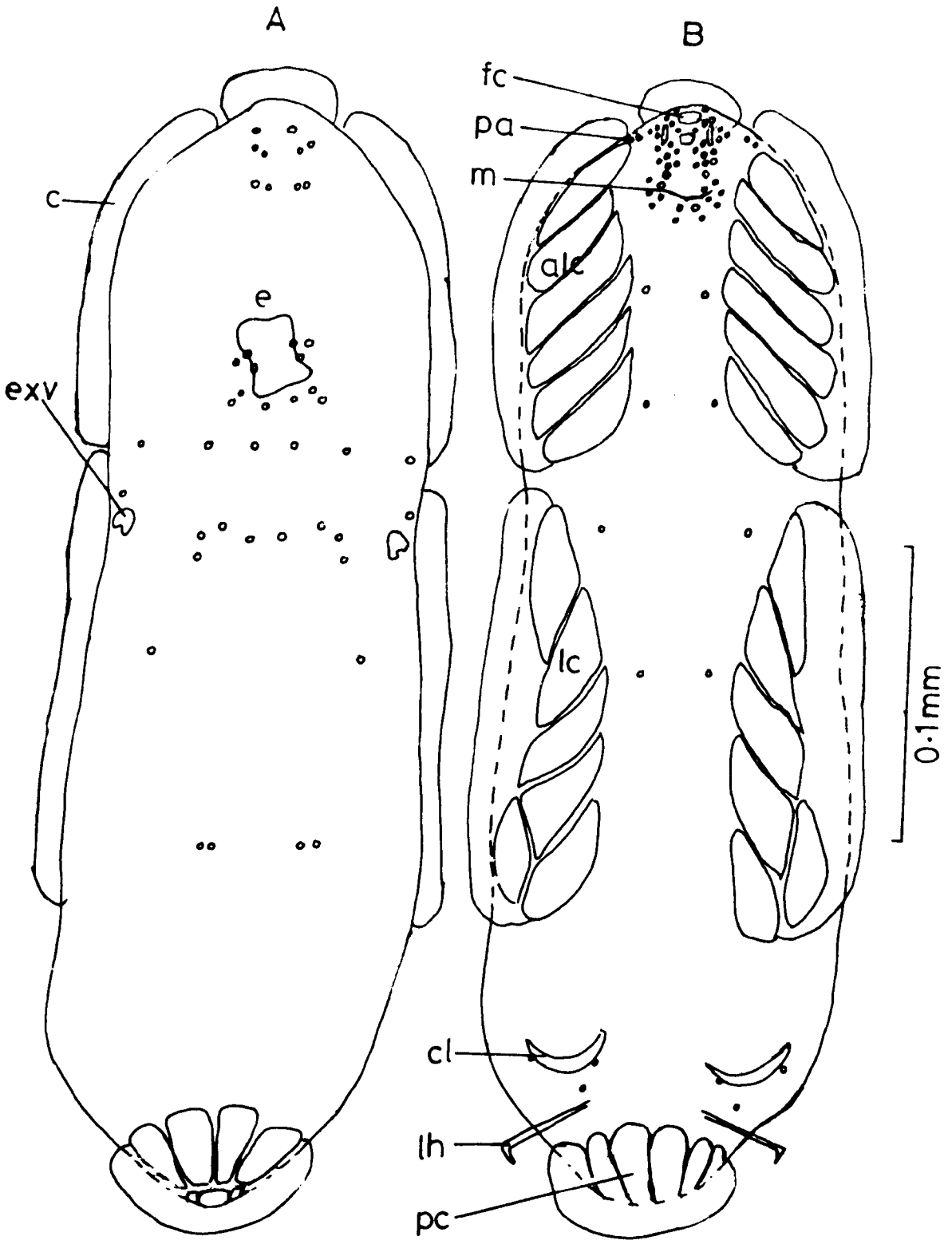


the S.E.M. and by the silver nitrate technique repectively. A pair of larval hooks were also seen near to the pair of clamps (Fig. 3.38).

The system of tiny papillae (sensillae) were also seen covering parts of the dorsal and ventral surfaces. They were concentrated in the frontal (cephalic) area around the 4 frontal ciliated cells and also around the mouth on the ventral side (Fig. 3.36) and they were also numerous in the area of the first two thirds of the dorsal surface just behind the eye spot (Fig. 3.43A). They had a bilaterally symmetrical distribution. The general distribution of papillae on both sides of the larva is illustrated in Fig. 3.43A and B. Their disposition on the dorsal side (Fig. 3.43A) was: 5 pairs near to the anterior end, 6 pairs around the eyespot, 3 pairs arranged in a straight line just behind the eyespot, 5 pairs just at the end of the first third of the larva, one pair about the middle of the larva and 2 pairs about two thirds along the dorsal surface. The distribution of the papillae on the ventral side (Fig. 3.43B) was: 17 pairs were seen around the small frontal ciliated cells and near to the anterior end (their arrangement can be seen in Fig. 3.36), 7 pairs around the mouth, 2 pairs near to the anterolateral ciliated cells, 2 pairs near to the lateral ciliated cells and 3 pairs around the clamps at the posterior end.

Near to the dorsal surface of the larva in the area between the two fronto-lateral cell groups, the eyespot was found (Fig. 3.43A) but its position can also be seen from the ventral side using the light microscope (Fig. 3.37). It was also noticed that in some stained specimens a pair of cone-like structures appeard on the dorsal side fixed laterally just at the end of the frontal one third of the larva.

Fig. 3.43 The general morphology of the oncomiracidium of D. homoion from British Rutilus rutilus. A. Dorsal surface, B. Ventral surface. alc, antero-lateral ciliated cells; c, cilia; cl, clamp; e, eyespot; exv, excretory vesicle; fc, frontal ciliated cells; lc, lateral ciliated cells; lh, larval hook; m, mouth; pa, papillae; pc, posterior ciliated cells.



It is thought they may be the excretory vesicles. The general morphology of the oncomiracidium of D. homoion is also presented in Fig. 3.43A and B.

2. Variations in the morphological characters of oncomiracidium during its life span

Examining many stained specimens of oncomiracidia of D. homoion from different times of its short life revealed that there was a wide range of variations of size and shape both in the body of the larvae as a whole and in its morphological structures on both sides. The larval dimensions, the size and shape of the ciliated cells of each group, the distance between the cells from the identical groups on both sides of larva, the distance between the papillae and their orientation were all under a process of continuous change during the life of the oncomiracidium of D. homoion (Figs. 3.36, 3.37, 3.39, Figs. 3.44 and Fig. 3.45). For example, the shape and size of the antero-lateral ciliated cells was quite different, especially as seen between Figs. 3.37, 3.44 and 3.45). The range of sizes (length and width) of the stained oncomiracidia examined from different periods of its life (data based on 34 specimens) was: lengths between 221.3 μm and 300 μm (mean 264 μm), widths between 123.8 μm and 146.3 μm (mean 124.5 μm).

E. Preliminary Observations on the Chromosome Number of D. homoion from British Cyprinidae

A cytological approach to the study of D. homoion has not been extensively used. Meiotic chromosomes were clearly observed in the cells of the gonads although some cells remained attached to each other, and the chromosomes in them were small and condensed. From Fig. 3.46, the diploid chromosome number of D. homoion in the

Fig. 3.44 The ventral surface of an oncomiracidium of D. homoion shortly after hatching. Silver nitrate treated. alc, antero-lateral ciliated cells; c, cilia; lc, lateral ciliated cells; pa, papillae; pc, posterior ciliated cells.

Fig. 3.45 The ventral surface of an oncomiracidium of D. homoion towards the end of its life span. Silver nitrate treated. alc, antero-lateral ciliated cells; lc, lateral ciliated cells.

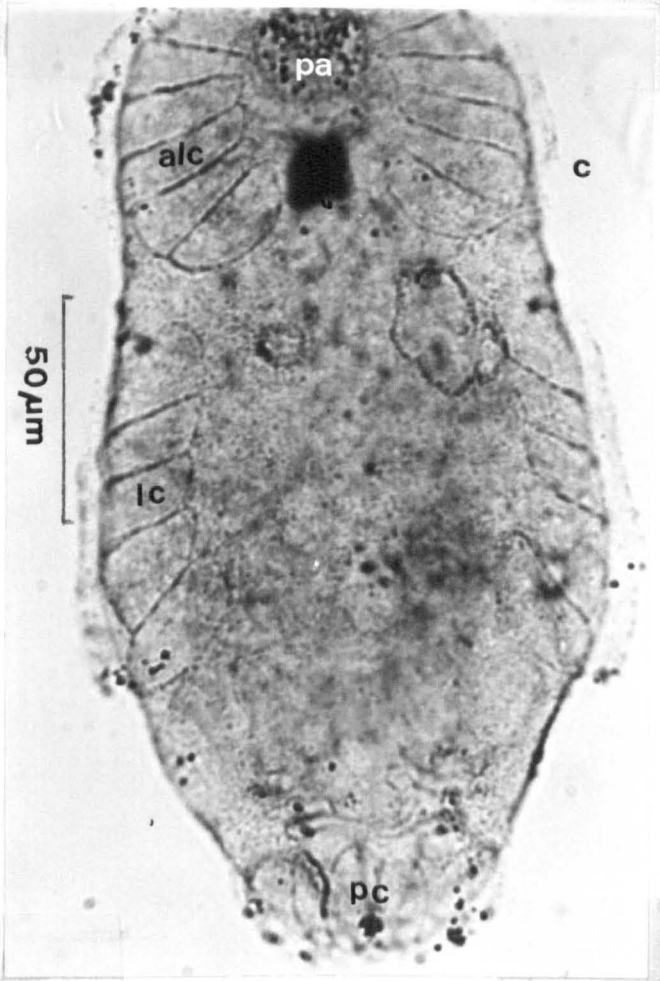


Fig.3·44

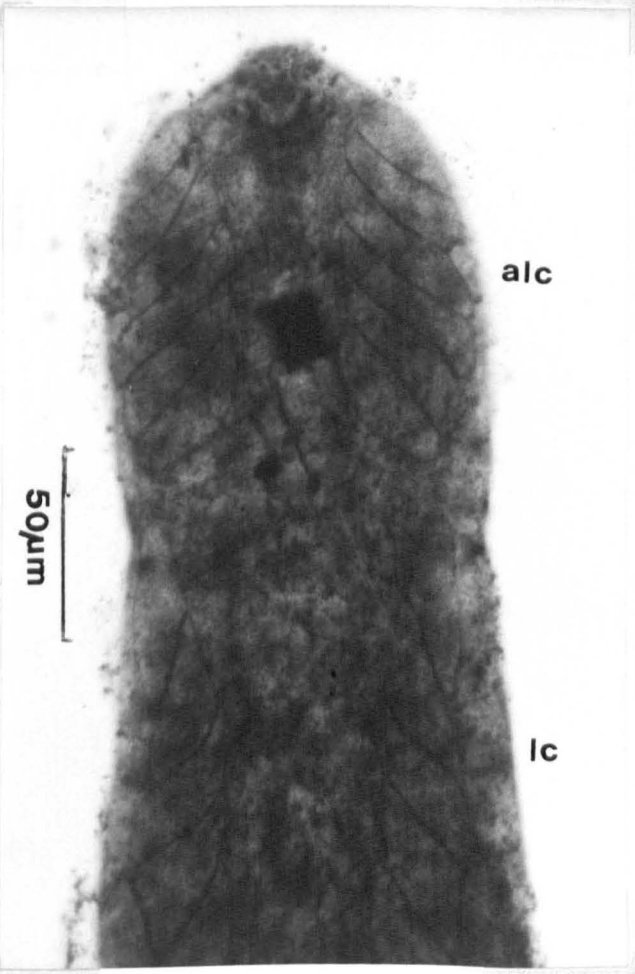
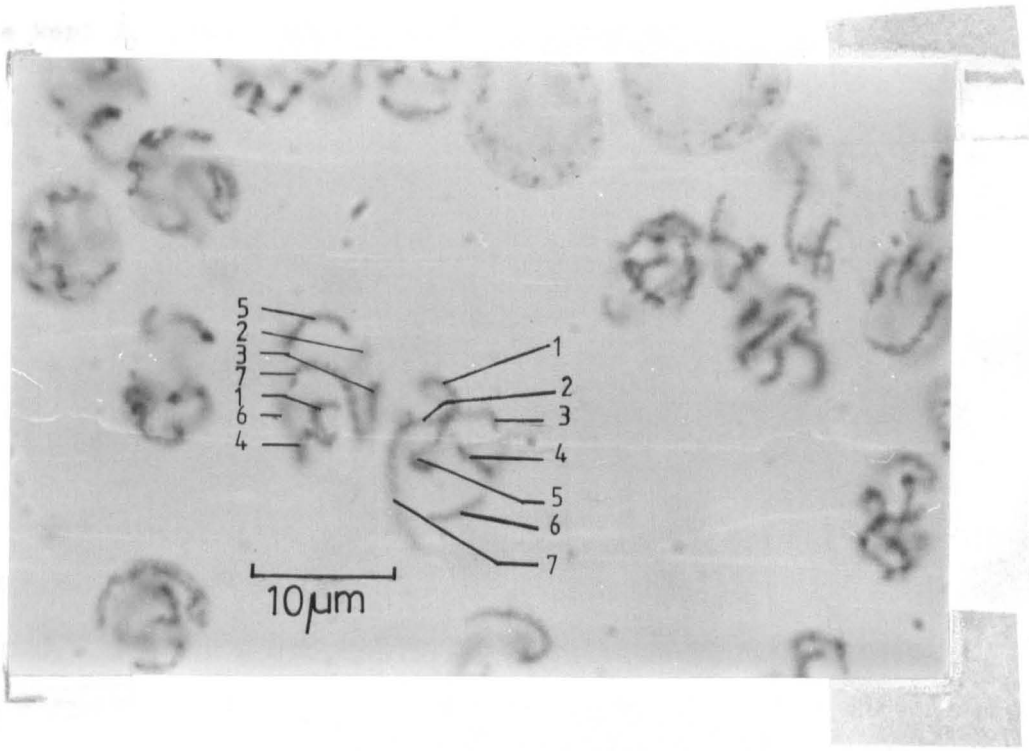


Fig.3·45

Fig. 3.46 The diploid chromosome number (2N) of D. homoion at meiotic metaphase, testis.



spermatocyte at meiotic metaphase was 14. The chromosomes were thin and the range of their lengths was between 2.8 and 9 µm.

F. Laboratory and Field Observations on the Transfer of D. homoion Infection between Cyprinid Species

1. Laboratory observations:

Laboratory observations on D. homoion infections indicate that this species was capable of transfer from Rutilus rutilus to fry of Leuciscus leuciscus and adult Ctenopharyngodon idella if uninfected fishes were kept in tanks containing either infected Rutilus rutilus with adult D. homoion or in contact with viable eggs of this parasite, at least for a month at a water temperature of 18°-21°C. All the different stages of the life cycle of D. homoion were seen on the gills of these cyprinid species mentioned above. However, in the instance of Abramis brama from Yorkshire, no infections of D. homoion became established even after 6 months of incubation, while R. rutilus from the same locality took the infection.

2. Field observations

The infections of different British cyprinid species with D. homoion collected from various localities (Table 3.4) strongly supported the laboratory observation about the ability of this parasite to infect a wide range of British Cyprinidae.

One important part of these investigations was the new record of D. homoion infection on small A. brama collected from Backford, Shropshire Union Canal during June 1984. The sample of fishes from this area consisted of 2 cyprinid species A. brama and R. rutilus (Table 3.2). Of 34 Abramis brama only 4 (11.7%) were infected with D. homoion with a mean intensity 1. R. rutilus were also infected

Table 3.4. *Diplozoon* **from various localities in the British Isles identified using the criteria described in this Chapter. *Indicates specimens from collections of British Museum (Natural History).**

Parasite	Host	Locality	British Museum (N.H.) Registration No.
1. *D. homoion*	*A. brama*	Backford, Shropshire Union Canal	
	Carassius carassius	Essex*	1976.9.23.21
	Gobio gobio	Northern Ireland	
	G. gobio fry	River Dee, Chester	
	Leuciscus leuciscus	Sarn Bridge, Worthenbury, River Dee	
	Phoxinus phoxinus	Birmingham*	1976.4.8.119-121
	Rutilus rutilus	Nottingham*: Backford, Shropshire Union Canal: Llyn Tegid	1944.11.14.181
	Rutilus rutilus fry	Llyn Tegid: River Dee	
	Scardinus erythrophthalmus	Haxby Road Pond, Yorkshire	
2. *D. paradoxum*	*A. brama*	River Stour, Suffolk: Moores Bakery Pond: River Thames: Vann Lake, Surrey	

with D. homoion. From 14 R. rutilus (Table 3.2 and 3.4) 4 (28.5%) were infected with mean intensity 1.3. All the infections on A. brama were recovered from fishes less than 10 cm long but all the adult parasites were mature. In R. rutilus, the infection was observed on fishes between 10 and 15 cm long. The D. homoion infection on A. brama from Shropshire Union Canal appears to contradict the laboratory observations. These points are discussed later. No evidence to suggest that D. paradoxum occurred at Backford on A. brama or R. rutilus was found, neither during the examination of the sample of these fishes indicated above, nor a re-examination of many specimens of Diplozoon collected by Dr. T.N. Mishra.

G. D. homoion and D. paradoxum and their British Cyprinidae Hosts.

The current taxonomic studies of British Diplozoon specimens from a variety of cyprinid species and localities so far indicate that D. paradoxum and D. homoion are found in the British Isles. This decision has been reached by studying the adult and egg stages and by field and laboratory observations on the transfer of the infection between the different host species. Table 3.4 shows the hosts and localities of the two species for the British Isles. Some permanent preparations of adult Diplozoon from the British Museum (Natural History) were reidentified using the characteristic features described in this chapter. They are indicated in Table 3.4 by an asterisk.

H. D. homoion, D. paradoxum and D. rutili from Overseas Cyprinidae used as Comparative Material During This Study

These 3 Diplozoon species from cyprinid species overseas were borrowed from Prof. O. Halvorsen and the British Museum (Natural

History) and used for comparison with British Diplozoon species. Their hosts and localities are shown in Table 3.5. It was found that some of these materials were originally incorrectly identified. Therefore, they were reidentified correctly using the characters described earlier.

Table 3.5. *D. homoion*, *D. paradoxum* and *D. rutili* from Cyprinidae collected Overseas. *Indicates specimens from collections of British Museum (Natural History).

Parasite	Host	Locality	British Museum (N.H.) Registration No.
1. *D. homoion*	*A. brama* x *Rutilus rutilus*	River Glomma, Norway	
	R. rutilus	Lake Kuivas, Finland*	1981.5.13.165-169
	R. rutilus	Lake Neuchâtel, Switzerland*	1960.8.22.1-50
	R. rutilus	River Glomma, Norway	
	Carasobarbus luteus	Mousal, Iraq*	1983.8.19.7-9
2. *D. paradoxum*	*A. brama*	Lake Neuchâtel, Switzerland*	1960.8.22.1-50
	A. brama	River Glomma, Norway	
	A. brama x *R. rutilus*	River Glomma, Norway	
3. *D. rutili*	*R. frisii*	Caspian Sea*	1983.10.22.1-2
	R. rutilus	Gulf of Bothnia, Finland*	1982.10.22.3-4

V. DISCUSSION

The original descriptions of the adult D. paradoxum, D. homoion and D. rutili were given by Nordmann (1832), Bychowsky and Nagibina (1959) and Gläser (1967) respectively. Later studies on the identification of these 3 species have included information about the stages in the life cycle.

When this study began, it was thought that the species of Diplozoon occurring on the gills of Rutilus rutilus from Llyn Tegid were not similar to the original description of D. paradoxum. Up to this time all specimens of this genus from British Cyprinidae had been referred to D. paradoxum. Therefore the critical examination of British Diplozoon materials from various Cyprinidae has revealed that there are two species, D. homoion and D. paradoxum, present in the British Isles. The overseas materials also confirmed this conclusion.

The results of this study also strongly suggest that, owing to the wide range of variations in the shapes and sizes present in the populations of Diplozoon species studied, the identification of any species of this monogenean must, if to be accurate, be carried out using many ways: by morphological studies which must be made on the different stages of the life cycle; by chromosome studies which can contribute much to an understanding of the delimitation of the species; by examination of the isozymes present in the parasite tissue, which may be constant in any particular species of parasite; and finally to test, by experimental transfer of infection of a Diplozoon from one species of cyprinid host to another, to investigate whether or not the morphological characters of the species become changed in response to the changed host species. Laboratory experiments must

be supported by field observations in each instance. All these ways are vital to allow us to achieve a better understanding of the systematics of these organisms.

The results of this study provide evidence that many characters used currently in the taxonomy of Diplozoon species are continuously variable in a population.

A. Adult Stage of Parasite

According to the present results, the overall size of the adult Diplozoon and most of the dimensions of their structures, the number and size of the vitelline follicles in the anterior regions, the shapes of the clamps, the shapes of the reproductive organs, the presence of the fine constrictions on the body, the positions of the genital apertures on the left and right sides of the parasites, and the size of the larval hooks all change between the individuals of the same species during the life cycle of the parasite as well as in response to variations in the size of the hosts. Most Russian and Indian species have been entirely based on the sizes and shapes of the structures of the adult parasites.

The present results show that the size of host as well as the stage of the life cycle play a great role in determining the size of the worm. Although a wide range of sizes of adult parasites were seen on large fishes, especially during the reproductive season of the parasite when both the old and new generations of parasites are present on the large hosts, nevertheless a comparison of the mean sizes of these parasites on large and small fishes revealed some differences between them. In small fishes, the range of sizes of the adult parasites was narrower than that in the large fishes. These observations were carried out on D. homoion from Llyn Tegid, but it seems likely that it will apply

also in other Diplozoon species. The results also indicate that the size of the parasite does not effect its sexual maturity as shown in Fig. 3.8, where a parasite of only 1.5 mm from fry maintained in the laboratory had an egg.

These results agree with the findings of some other authors. Gussev and Kulemina (1971a) found that the size of the body and some other structures in Dactylogyrus auriculatus and Diplozoon megan from young fishes compared with those of older fishes were not identical. The greatest differences were between specimens from fishes 0+ - 3+ or 0+ - 1+ which, according to them, represented the period of most intensive growth of the fishes. In other monogeneans, Paling (1965) stated that young Salmo trutta harboured only small parasites, while the large fishes were hosts to both small and large worms. He gave two reasons for that, either the size of the parasites was physically limited in some way by the size of the host or the parasites lived for more than one year and continued to increase in size over a period of several years.

Also, my results show that the sizes and shapes of most internal and external structures of D. homoion change in line with the size of the whole body. For example, the size of the clamps changed between different sizes of parasites. Such changes were also seen by Gussev and Kulemina (1971b) for the lengths of the larval hooks of adults which indicated that the increase in their lengths in relation to the age of the hosts coincided with the growth of thickness of the secondary gill filaments of the host. According to them, this was a direct adaptation of the parasites to the growth in the dimensions of the gills which permits their continued survival as the fishes grow.

During this study it was seen that the arrangement and maturity of the vitelline follicles affected the appearance to the viewer of most structures of the anterior regions of the worms, especially the branches of the intestine. Accordingly it can be suggested that the branches of the intestine in the anterior region in the early stage of the adult D. homoion (Fig. 3.6) is not bifurcate. It is probable that the arrangement of these branches is a generic character.

This study showed that the shapes of the clamps and the sclerites of D. homoion changed during life cycle as well as between the adult specimens of this species. Some differences were also noticed even between the clamps on the same individual. As sometimes these variations can be caused by the pressure of the cover slip. All examinations on unstained specimens during this study were carried out using depression slides so that these slight differences between clamps probably relate to increase of the host sizes. However Owen (1963b) indicated that certain sclerites of the clamps of D. paradoxum were visible only in material sectioned in various planes.

The shape of the reproductive organs was also variable in the specimens of D. homoion (see Chapter 5, life cycle) and D. paradoxum. Despite this variation this character has been widely used in the past for identifying many Diplozoon species.

It was also found that there was great variation between specimens of D. homoion in the presence or absence and thickness of the fine constrictions. These variations were also seen on D. paradoxum. The function of these constrictions was associated with the flexing of the body of the parasite in order that it can respond to the strong water current ventilating the gill chamber. In Fig. 3.2, no fine constrictions are seen on the body of this specimen. Some authors

have used the presence of the fine constrictions as a specific character for some species (D. paradoxum, D. homoion, D. megan, D. pavlovskii and D. markewitschi) (Bychowsky and Nagibina, 1959 and Bychowsky et al., 1964).

As a result of this detailed study it was realized that all these characters of the adult stages of D. homoion are population variations and must be avoided not only in the comparison between British D. homoion and D. paradoxum but also in every other circumstance.

The light and scanning electron microscope studies of Diplozoon materials revealed that it is easy to separate the adults of D. homoion from D. paradoxum by two characters on the posterior parts of D. paradoxum but not found on D. homoion. These characters are the invagination and the 5-7 ridges (deep folds) between the invagination and junction region on the ventral sides of the posterior parts. The invagination can be seen by the naked eye. It is not possible to state the function of this structure or whether it belongs to the opisthaptor or to the whole posterior region. The two characters were seen on all specimens of D. paradoxum collected from A. brama in Britain and abroad but not on the D. homoion taken from A. brama at Backford.

On the other hand, none of the specimens of Diplozoon homoion from British Cyprinidae showed these two characters. Also D. homoion and D. rutili from abroad had the same appearance as British D. homoion.

On hybrid R. rutilus and A. brama from the River Glomma, Norway both D. homoion and D. paradoxum retained these features in a completely typical manner, exactly as described by Halvorsen (1969).

The slight variation in these two characteristic features of adult D. paradoxum from various localities (Moores Bakery Pond, River Thames, England and Lake Neuchâtel, Switzerland) may be attributed to slight

differences in the behaviour of their hosts or to environmental conditions at these localities. Mayr (1949) indicated that geographical variation between populations was the most common form of group variation encountered during taxonomic work.

Nordmann (1832) noticed the invaginations on his D. paradoxum material collected from A. brama. Later studies also reported these two characters on Diplozoon specimens from the gills of species of genus Abramis as will be discussed in Chapter 4.

This study also revealed not only variation in morphology of the clamps within the species but also some slight differences between the sclerites of the third clamps of D. homoion and D. paradoxum. This variation was also reported by Bychowsky and Nagibina (1959) Wiles (1965), Gläser and Gläser (1964). It must be stated that this difference was seen in stained permanent preparations and therefore is a possibility that it might be the result of pressure caused by the coverslip rather than a real difference between these species. It may be possible that in the future studies on the chemical analysis of the clamp components of each Diplozoon species could solve this problem, in D. homoion and D. paradoxum as well as in other species.

The variation in the number of intestinal branches between D. homoion and D. paradoxum in the area between the testis and the 4th clamp on the posterior part of the body which has also been seen by many authors seems to be a good character in some species especially in mature adult stages to distinguish between British D. homoion and D. paradoxum. However other intestinal characters are similar in both species.

Comparison of other structures in both British and overseas species, for example the positions of the genital pores, the muscular disc and

tissue surrounding each row of 4 clamps on both sides of the opisthaptor indicates that they are similar. The apparent change in the positions of the genital pores to either the right or the left side of the observed face of worm was seen in both species. This was related to the mode of attachment of the two diporpae as explained in Chapter 5. Bovet (1967) wrongly interpreted that the genital aperture of D. paradoxum lay on the right side of the observed face while in D. homoion it was on the left side of this face.

The tiny pores which covered the outer surface of the muscular disc and the area around the genital pores in both D. homoion and D. paradoxum have also been seen in other monogenean groups. Bresciani (1972) found that the epidermis of Polystoma integerrimum was thrown into ramifying folds which enclosed shallow pits, while the surface of Rajonchocotyle emarginata was smoother than that of Polystoma and was not elevated into folds but was also pitted (Lyons 1972). Lyons suggested that these pores might be sites of secretion because mucus on the general surface was most obvious around the pores. These pores on both D. homoion and D. paradoxum and perhaps also on other species including D. rutili might also cover the whole bodies of these parasites. This was mentioned in Chapter 5 where it was suggested that they might play a role in the life cycle of the parasites. The discovery of these tiny pores only on these two areas of Diplozoon specimens happened accidentally because the study at high magnifications was concentrated on the texture of the clamps and the area round them as well as the genital pores and not on the other parts of the parasite body.

The scanning electron microscope study and the laboratory observation strongly indicated that the four clamps on each side of the opisthaptor functioned as one segment so that the 4 clamps can rotate around on

their axis to a certain angle. This phenomenon was found on all British and overseas material. It has also been mentioned by many authors (Owen, 1963a, Khotenovskiĭ, 1980). This kind of adaptation would allow the parasite to arrange the clamps on the opisthaptor to conform with the direction of the gill filaments and to accommodate in position between the gill hemibranchs.

B. Egg Stage of Parasite

My results showed that the shape and size of the egg were valuable systematic characters for the three species of Diplozoidae. Although no significant differences were seen between the eggs of D. homoion and D. paradoxum in terms of general shape and size of body, colour, surface texture, thickness of the egg wall or the diameter of egg filament, but the ratios between the length of the operculum to the total length of the egg in both species were obviously different, also the degree of curvature in the egg wall of D. paradoxum was slightly more than that in the egg of D. homoion. Some of these egg characters were observed by Bovet (1959, 1967) and Khotenovskiĭ (1975). But in D. rutili, the shape of egg, the thickness of egg wall, diameter of filament, the ratio between the length of the operculum and the total length of the egg were found to be quite different from the other two species. Gläser in 1967 gave a description of the egg of D. rutili. The present observations agree with Gläser's and Khotenovskiĭ's (1975) description of the egg shape of D. rutili.

It was clear that the egg sizes of D. homoion varied during certain periods of egg laying. This difference depended on the total size of the individuals producing the eggs, in which small, newly formed sexually mature adult worms produced small eggs whilst larger, older adult worms released larger eggs. This phenomenon was seen only in the laboratory but not in the field.

C. Oncomiracidium of D. homoion

The oncomiracidia of these parasites can sometimes provide valuable taxonomic characters for the identification of certain Diplozoon species.

A comparison between the morphological characters of the oncomiracidium of D. homoion from Rutilus rutilus, Llyn Tegid given in the present study and those, illustrated in the literature, for the oncomiracidia of D. paradoxum from Gobio gobio and Phoxinus phoxinus (Euzet and Lambert, 1971), of D. paradoxum from Abramis brama (Euzet and Lambert, 1974), of D. paradoxum from A. brama (Bovet, 1959 and 1967), of D. gracile from Gobio gobio (Euzet and Lambert, 1974), of D. megan from Leuciscus idus, of D. markewitschi, D. paradoxum, D. rutili (Khotenovskiĭ, 1975), were all similar in the numbers of ciliated epidermal cells, in the numbers of papillae (sensillae) and other morphological characters. Euzet and Lambert (1971) wrongly identified only 3 ciliated cells on D. paradoxum at the tip, frontal (cephalic) group which is usually made up of 4 small cells (Fig. 3.36). This error might be related to their process of staining of the larvae.

Although many concentrations of silver nitrate solution were used for staining oncomiracidia at a variety of different temperatures (20°-60°C), I was unable to identify the osmoregulatory system. No reason can be offered for this.

It was shown in this study that many of the morphological characters of the oncomiracidium of D. homoion changed considerably during its life span, especially the shapes and sizes of the ciliated epidermal cells, the distance between each of the two identical groups of cells on the sides of the larva and the distribution of the papillae (sensory organs of many authors) and the distance between them were varied from specimen to specimen as shown in the Figs. 3.37, 3.44 and 3.45. However,

Khotenovskiĭ (1975, 1977) built up his knowledge on the identification of the oncomiracidial stages of D. markewitschi, D. homoion, D. paradoxum, D. rutili and D. megan mainly on the variation of the sizes of ciliated epidermal cells and distance between papillae (sensilla). He reported that all these larvae had a similar general structure but he did not take into account changes which might occur during the life span of the oncomiracidia.

D. Importance of Determination of the Chromosome Number of Species of Parasites

Owing to the great morphological variation which has been shown to occur in this study of D. homoion and D. paradoxum it is clear that morphological characters alone may not be sufficient to separate these species from those which are reported to occur in other parts of the world. It is strongly recommended that future systematic work on the Diplozoidae should include cytotaxonomic and chemotaxonomic methods which provide extra information to characterise each species more accurately.

Unfortunately the results for this section were very limited for a number of reasons: 1. Lack of living material of D. paradoxum and D. rutili. 2. Living adults of D. homoion were also very few when these experiments were begun. 3. Shortage in the time available after the other parts of the study had been completed.

It was shown that D. homoion from Llyn Tegid had a 2N Chromosome number of 14. Bovet (1967) also found that the 2N chromosome number in D. paradoxum from A. brama and D. homoion from Rutilus rutilus were 14. Koroleva (1968a, b; 1969) demonstrated that the 2N chromosome numbers of D. homoion, D. pavlovskii, D. gussevi, D. nipponicum, D. megan, D. nagibinae, and D. sapae were 14. But she reported that D. paradoxum had only 8 diploid chromosomes, which contradicted Bovet's (1967) result.

The range of measurements of length of the chromosomes in D. homoion from Llyn Tegid were relatively similar to those for other Diplozoon spp. given by Koroleva. According to this study, only one pair of chromosomes was 9μm and the rest were smaller. Koroleva found two large pairs.

E. Transference of D. homoion Infections

The results of this part of the research programme are very important to support the taxonomic studies as follows:

1. It was clear from the field and laboratory observations that D. homoion had a wide range of Cyprinid hosts including A. brama while D. paradoxum infections were limited to A. brama only. The results of Gläser and Gläser (1964), Prost (1972 and 1974), Kiškaroly (1977) and Lucký (1981) agree with the present finding of the wide range of specificity for D. homoion (see Chapter 2). The natural infections of D. homoion on small A. brama at the Shropshire Union Canal, Backford are believed to be the first occasion that D. homoion has been found on A. brama in the British Isles or elsewhere. It should be noted that the infection of D. homoion was only seen on A. brama less than 10 cm in fork length. Larger A. brama from this habitat were not infected by any Diplozoon species. One reason for only the small fishes having the infection may be determined by the size of the clamp aperture of the parasite in relation to the size of the secondary lamellae of the gills of the potential fish host.

2. It was also shown that after keeping A. brama from Yorkshire in tanks containing D. homoion infections for 6 months, the A. brama did not become infected while R. rutilus from the same locality did take the infection. However the discovery of D. homoion on small A. brama from Backford raises a number of interesting questions concerning the specificity of D. homoion. For example, why should this species be

able to occur on small, wild A. brama but not transfer from R. rutilus to large A. brama in the laboratory? This aspect needs further experimental study.

3. One significant result of this study was the discovery of typical D. homoion on A. brama at Backford, without the morphological features of D. paradoxum the usual parasite of this host. This indicates beyond all reasonable doubt that the differences in the characteristic features of the posterior regions of D. homoion and D. paradoxum are not adaptations to their particular hosts, but are features typical of each of the two species.

Halvorsen (1969) studied 'D. paradoxum' on A. brama, 'D. homoion' on R. rutilus and Diplozoon specimens from hybrid R. rutilus x A. brama found at the River Glomma, Norway. He concluded that there was only one species, D. paradoxum. The present results, as well as a reexamination of Halvorsen's materials clearly showed that the two species were quite constant in their morphology regardless of the species of host on which they were found. Also, the opinion of Bovet (1967) that the two species were subspecies, D. paradoxum paradoxum and D. paradoxum homoion, is untenable and unnecessary as the present chapter has shown that there are perfectly valid criteria for distinguishing the two species.

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CHAPTER 4

PROBLEMS OF THE TAXONOMY AND PHYLOGENY OF DIPLOZOIDAE SPECIES

I. INTRODUCTION

During the last few decades more than 60 species of Diplozoidae have been described from different parts of the world (see Chapter 2). Various morphological characters have been used to identify these species. In addition, these parasites have been divided into a variety of different levels of taxa. There have been 3 main trends in the taxonomy of the Diplozoidae. Firstly, particularly Russian authors have tended to add genera and subgenera by giving more attention to the host specificity and morphological characters of the adult stages of the species. Secondly, some workers have prefered to keep more than one Diplozoon species within D. paradoxum (Halvorsen, 1969 and Wiles, 1965). Thirdly, some authorities have split a few species into subspecies (Bovet, 1967; Oliver and Riechenbach-Klinke, 1973 and Komarova, 1964, 1966).

Studies on the phylogeny of Diplozoidae have been stimulated especially after the recent discovery of new, essential, systematic characters in the life cycle stages of some species. This has resulted in many authors changing their views about the accuracy of the position of these parasites amongst the Class Monogenea.

This chapter attempts to critically assess the taxonomic value of the characters used in the identification of subspecies, species, subgenera and genera of Diplozoidae. This assessment relates not only to my results presented in Chapter 3, but also to the value of the various features used by other authors in the recognition of species and in the development of understanding of the phylogeny of these parasites.

II. EVALUATION OF THE CHARACTERS USED IN THE IDENTIFICATION OF SPECIES OF DIPLOZOIDAE

A. Host Specificity

Host specificity has been widely used in fish parasitology. Ginetsinskaya (1961) believed that the ability of the parasite to infect the host depended on the morphological, physiological and biological adaptations developed by the parasitic helminths in the course of evolution. Cameron (1964) revealed that there was a tendency for parasites to be confined to a single species or group of species of hosts. He reported that even when a single parasite species was apparently limited to a single host, it might be because other hosts had not been exposed to it. However, the presence of parasite in a host could not always be attributed to specificity alone (Bychowsky, 1957), so he introduced the concept of 'occurrence' as distinct from specificity.

The Monogenea are mostly ectoparasites and include a number of very widely distributed and very pathogenic parasites of fishes. The members of this order have been thought to exhibit a strong degree of host-specificity (Hargis, 1957 and Llewellyn, 1963, 1965 and 1968). Hargis (1957) proposed the term infraspecificity for the occurrence of a single monogenean species on members of a single fish taxon. He suggested that infraspecificity of monogeneans was less strongly developed in freshwater than in marine fishes. In view of the well-developed specificity of Monogenea, Prost (1957) assumed that species of fishes which were hosts of a particular monogenean species should be closely related to one another. According to Bychowsky (1957), the majority of Monogenea (74.1%) occurred on one species of host only.

In the genus Diplozoon, Bychowsky and Nagibina (1959) recognised

a high-level of specificity in Diplozoon species, but later studies have shown that some members of this genus had a wide range of host species (see Chapter 2, Table 2.1).

According to the present field observations, D. homoion had a wide range of host specificity, including Abramis brama, whereas D. paradoxum had a narrow range and was restricted to A. brama only. A wide range of specificity has also been indicated in many other Diplozoon species, e.g. D. rutili, D. doi, etc. (see Chapter 2, Table 2.1).

Despite the problem of host-specificity mentioned above, most authors have used specificity as part of the basis for the recognition of the species they described. However, in my opinion host specificity alone is not enough for the identification of Diplozoon species unless it is supported by other valid systematic characters. The limits of host specificity for each species have not yet been fully elucidated despite the efforts of Russian workers to give more attention to this area.

B. Morphological Characters of:

1. Adult Stage

Most taxonomic papers on the identification of new Diplozoon species have mainly depended on the morphology and size of adult worms. The descriptions have most been based on a single or a few specimens, sometimes even using immature rather than mature worms, and in most instances, the worms were taken from only a single host. Mayr (1949) stated that a careful analysis of a natural population will show that there is a considerable degree of variability, grouped around a mean which is typical for the particular taxonomic category. He thought that no single individual can represent at the same time, the minimum, the maximum, and the mean

of such variation, but it is possible to represent this variation fairly accurately, if an adequate sample of the population is available. These characters are:

a. Dimensions of adult, anterior and posterior regions,

b. Size and shape of the external and internal structures which include:

1. Suckers
2. Pharynx
3. Branches of the intestine at the anterior and posterior parts of the worm.
4. Distribution of the vitelline follicles.
5. Reproductive organs and their positions.
6. Clamps and their arrangement on the opisthaptor.
   The shape and structure of some of their sclerites.
7. Invagination on the posterior region.
8. Ridges (deep folds) on the posterior region.
9. Larval hooks.

There are many other characters which have also been used occasionally for a few species but which have been dismissed by other authors.

In the present study (Chapter 3), most of these characters have been seen on adult D. homoion and they have been shown to change considerably during the year or through the life cycle of the parasite. However, some important taxonomic characters, e.g. the invagination and the ridges (deep folds) which are found on the posterior parts of the adults of a few species including D. paradoxum were not present on D. homoion.

Some authors have used particular characters, for example the invagination, to divide the genus Diplozoon into new genera or subgenera, whereas other authors have not considered that such a division was

justified on the basis of a single character.

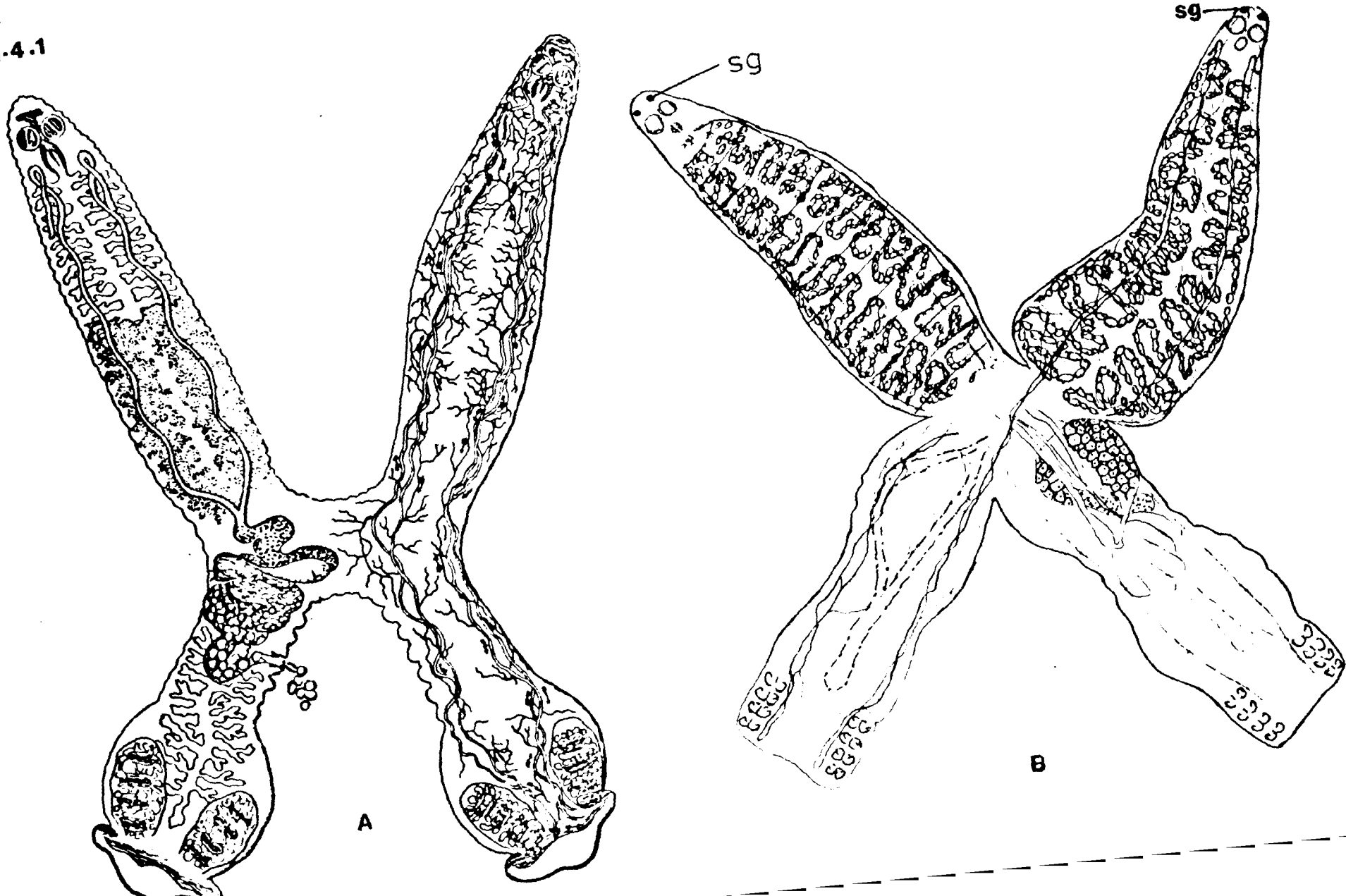
In order to clarify the discussion of the taxonomy, it was decided to reproduce illustration of all the species of adult parasites including other anatomical features from the literature. To facilitate an understanding of the taxonomic problems of Diplozoidae species, the species are arranged according to the date of their first description. The hosts of these parasite species were given earlier in Chapter 2, Table 2.1.

Fig. 4.1 (A and B) shows the first drawings of D. paradoxum (from Europe) given by Nordmann (1832) and D. nipponicum (from Japan) given by Goto (1891) respectively. Nordmann made many mistakes about the morphology and structures of adult D. paradoxum, for example in the details of the structure of the reproductive organs and the positions of the genital pores, the distribution of the intestinal branches in the anterior and posterior parts of each partner of the whole adult (Fig. 4.1A). Nevertheless, Nordmann recognised the invagination as can be seen from his figure (Fig. 4.1A). He thought that oval form of the invagination was owing to the mode of preparation of the slide. His specimens were taken from Abramis brama. The trace of the deep folds on the posterior part can also be seen.

Goto (1891) described in detail the morphology and histology of adult D. nipponicum (Fig. 4.1B). He recognized this as a new species by two main characters. Firstly, by a pair of sticky glands besides the suckers and secondly by an elliptic cylinder form of the body in the anterior portion of the posterior region which then passes posteriorly into an irregularly-edged rectangular prism (the margin is deeply crenate or even zig-zag in killed specimen). He also mentioned that the intestine of this species has no branches behind the testis and formed a single

Fig. 4.1. Adult stages of D. paradoxum (A) and D. nipponicum (B) as given by Nordmann (1832) and Goto (1891) respectively. sg, sticky gland

Fig.4.1



tube (see Fig. 4.1B). His observations were accurate and have been confirmed by many authors (Kamegai, 1968; Kamegai et al., 1966; Denis et al., 1983 and Khotenovskiĭ, 1980).

Fig. 4.2, A, B, C and D reproduces drawings of whole adults of species of Euro-Asia, India and Africa species which have been described during the last forty years. Unfortunately a few of the original descriptions of some species did not include drawings. In many of these descriptions size and other highly variable characters were used for identification.

Most authors of the European and Russian species have placed them either in the genus Diplozoon or into the subgenera D. (Diplozoon) and D. (Paradiplozoon) with a few into subspecies (Fig. 4.2 A 1-26). Most of the species of the genus Diplozoon was based on the size and shape of the internal structures of adult stage except for D. paradoxum, D. diplodiscus, D. bychowskyi, D. inustiatus and D. strelkowi (Fig. 4.2 A3, 11, 12, 13 and 14) which had an invagination on the posterior part of their bodies. Nagibina (1965) called this structure the secondary disc. She also reported that the positions of the genital pores in D. inustiatus were located on the middle portion of the anterior part of the worm (Fig. 4.2 A13) and the positions of the gonads were far from the junction area close to the invagination. It must be stated that if the presence of the genital pore on the middle of the anterior portion of this species is correct, it represents an important taxonomic step in the evolution of Diplozoidae as will be discussed later. Akhmerov (1974) divided the genus Diplozoon into two subgenera depending mainly on the presence or absence of the invagination which he called the posterior sucker. Therefore, according to his opinion, D. (Diplozoon) mylopharyngodonis and D. (D.) paradoxum have this enlargement while all others of his

Fig. 4.2 *Adult stages of species of Diplozoidae reproduced from the original descriptions*

A. European and Russian species:

1.	*D. barbi*	Reichenbach-Klinke, 1951
2.	*D. tetragonopterini*	Sterba, 1957
3.	*D. paradoxum*	As revised by Bychowsky and
	a. Internal structures	Nagibina, 1959
	b. Lateral view	
4.	*D. homoion*	Bychowsky and Nagibina, 1959
5.	*D. pavlovskii*	Bychowsky and Nagibina, 1959
6.	*D. megan*	Bychowsky and Nagibina, 1959
7.	*D. gracile*	Reichenbach-Klinke, 1961
8.	*D. paradoxum sapae*	Reichenbach-Klinke, 1961
9.	*D. p. bliccae*	Reichenbach-Klinke, 1961
10.	*D. markewitschi*	Bychowsky *et al.*, 1964
11.	*D. diplodiscus*	Nagibina, 1965
12.	*D. bychowskyi*	Nagibina, 1965
13.	*D. inustiatus*	Nagibina, 1965
14.	*D. strelkowi*	Nagibina, 1965
15.	*D. schizothorazi*	Iksanov, 1965
16.	*D. nagibinae*	Gläser, 1965
17.	*D. tadzhikistanicum*	Gavrilova and Dzhalilov, 1965
18.	*D. rutili*	Gläser, 1967
19.	*D. (Diplozoon) mylopharyngodonis*	Akhmerov, 1974
20.	*D. (Paradiplozoon) amurensis*	Akhmerov, 1974
21.	*D. (P.) marinae*	Akhmerov, 1974
22.	*D. (P.) erythroculteris*	Akhmerov, 1974

Fig. 4.2 (contd.)

23.	*D. (P.) skrjabini*	Akhmerov, 1974
24.	*D. (P.) parabramidis*	Akhmerov, 1974
25.	*D. (P.)* sp. 1 (species not identified)	Akhmerov, 1974
26.	*D. (P.)* sp. 2 (species not identified)	Akhmerov, 1974

B. Indian species

1.	*D. indicum*	Dayal, 1941
2.	*D. kashmirensis*	Kaw, 1950
3.	*D. cauveryi*	Tripathi, 1959a
4.	*D. soni*	Tripathi, 1959a
5.	*Neodiplozoon barbi*	Tripathi, 1959 a and b
6.	*D. microclampi*	Kulkarni, 1971
7.	*D. dayali*	Pandy, 1973
8.	*D. thapari*	Gupta and Krishna, 1977

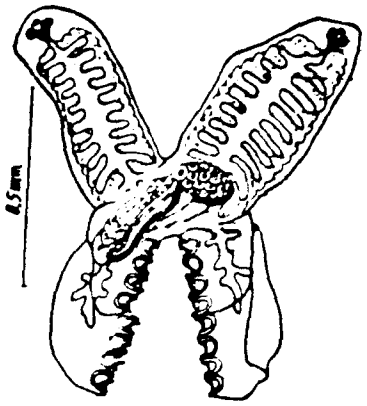
C. Other Asian species

1.	*D. minutum* (Israel)	Paperna, 1964
2.	*D. aristichthysi* (China)	Ling, 1973 (quoted from Khotenuvskiĭ, 1978)

D. African species

1.	*D. ghanense*	Thomas, 1957
2.	*D. aegyptensis*	Fischthal and Kuntz, 1963
3.	*Neodiplozoon polycotyleus*	Paperna, 1973 and 1979
	a. young	
	b. gravid	

Fig· 4·2 A



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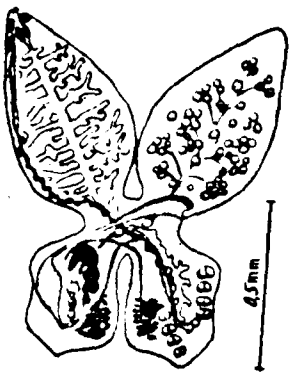

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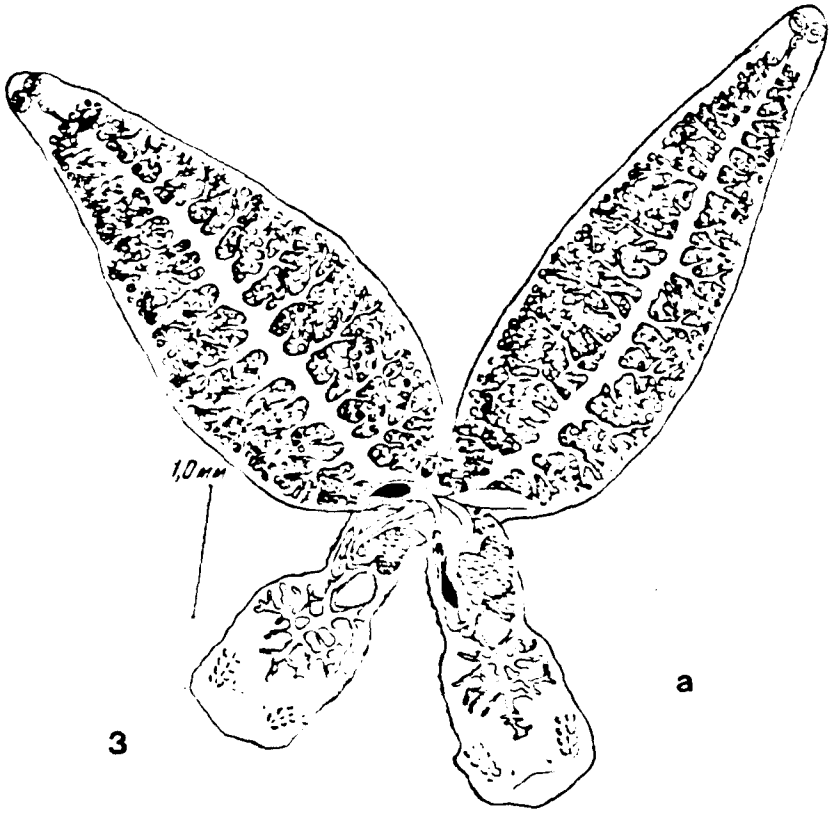
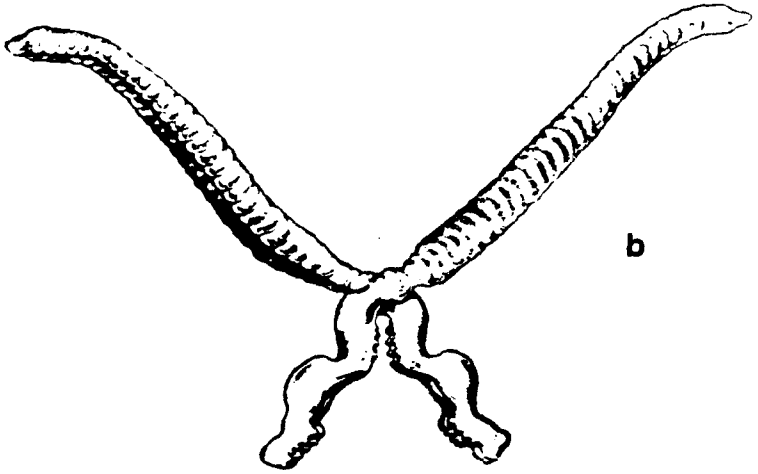


3

a



b

Fig. 4-2 A continued

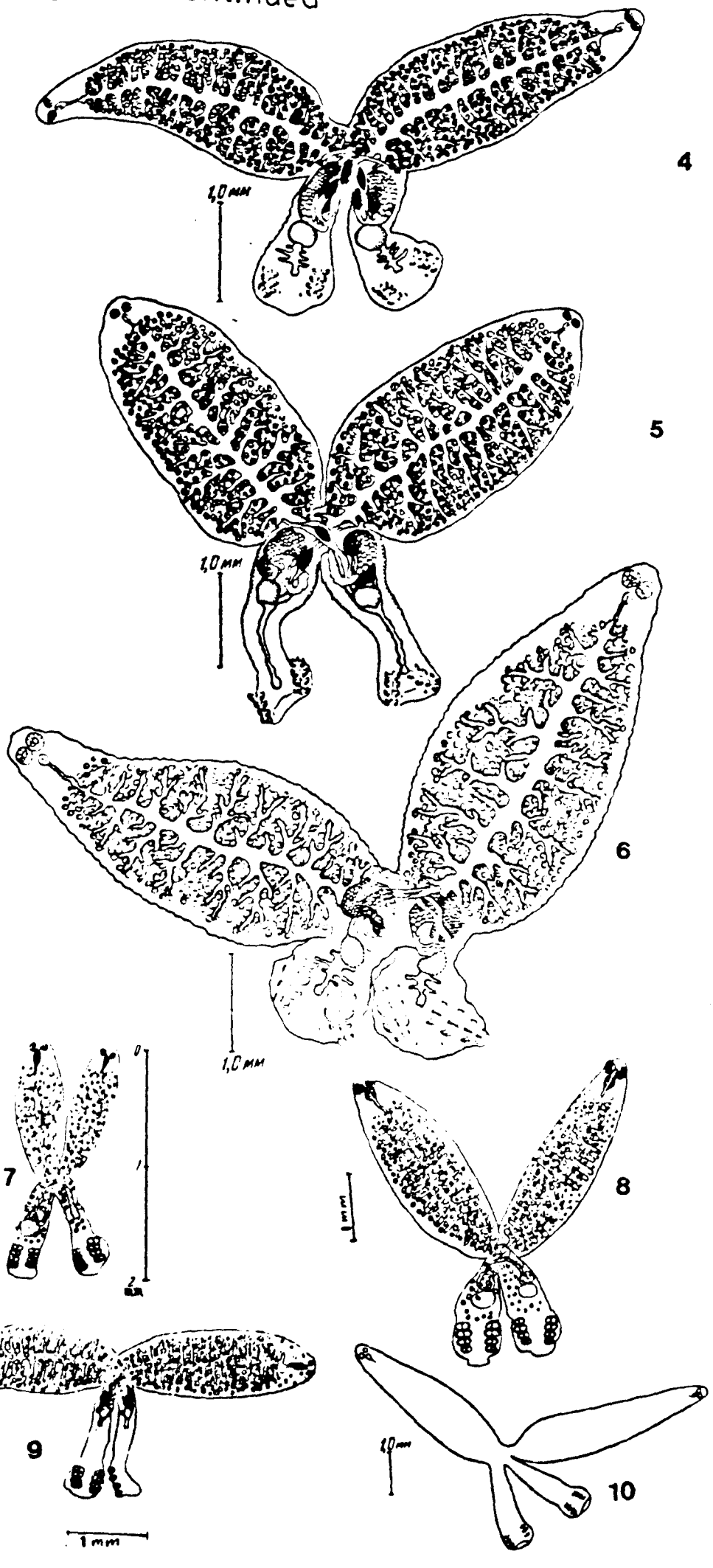


Fig.4.2A continued

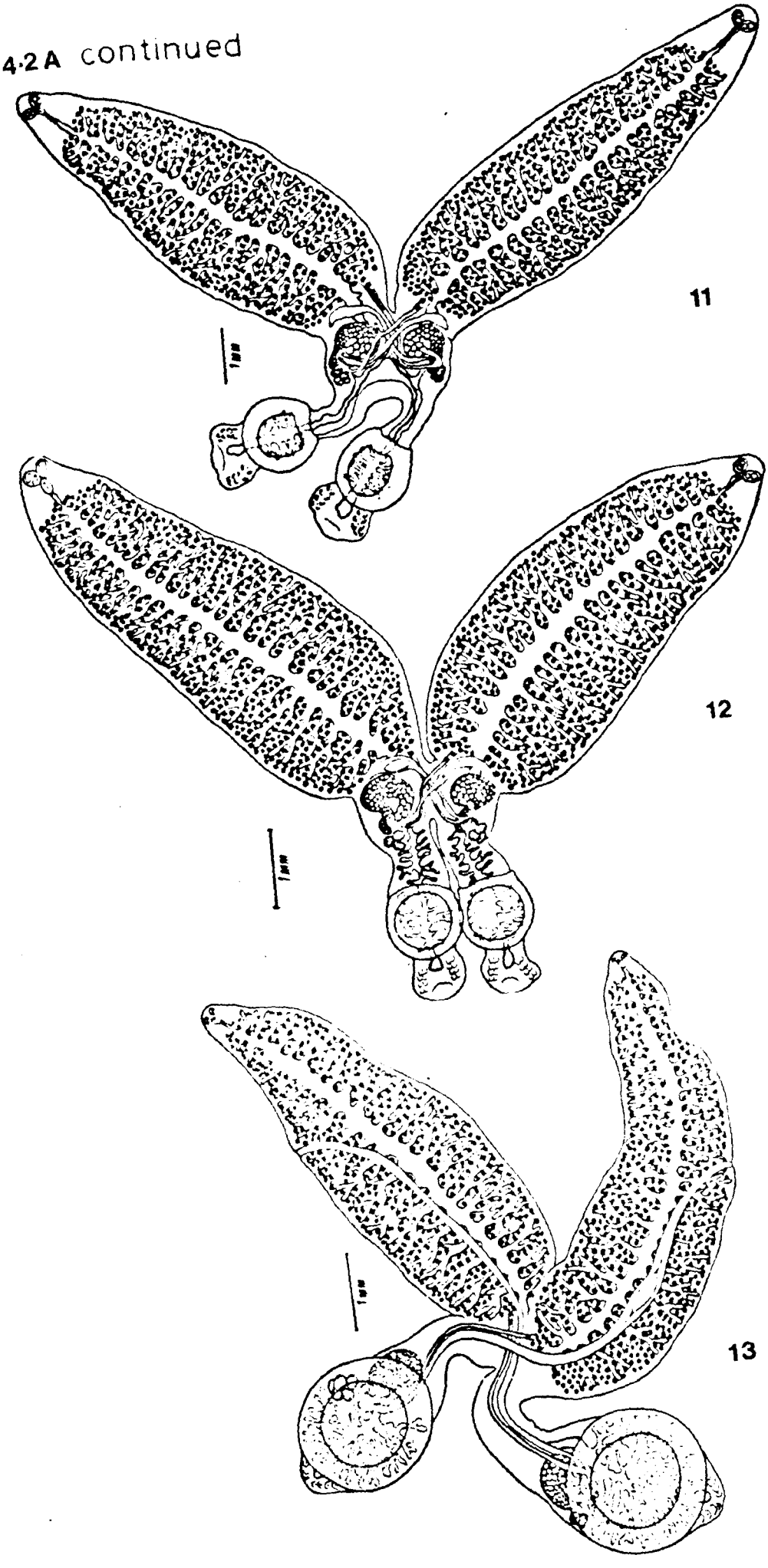
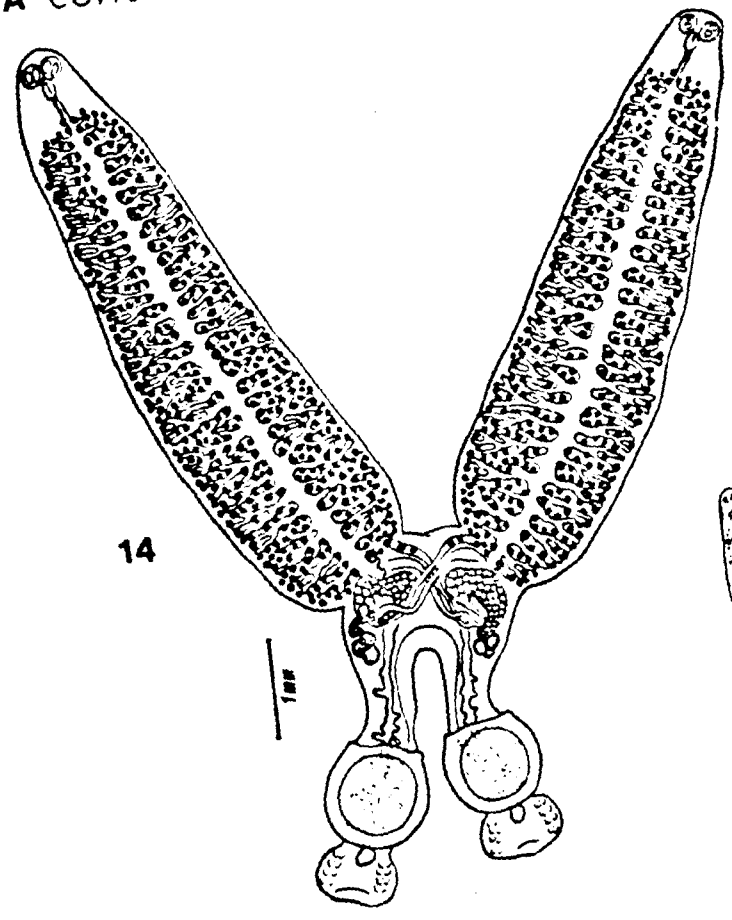
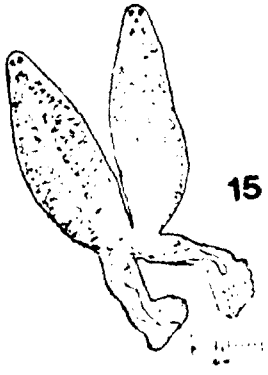
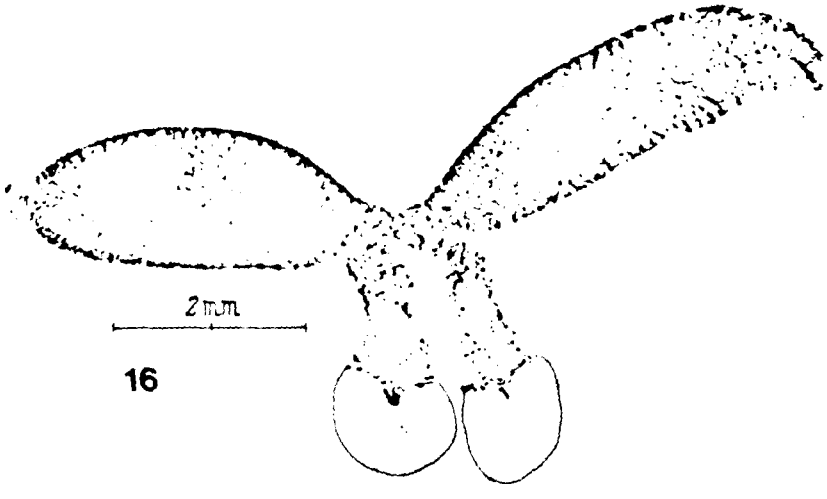


Fig. 4.2 A continued







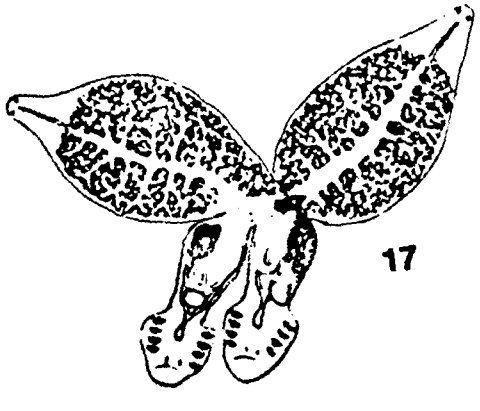
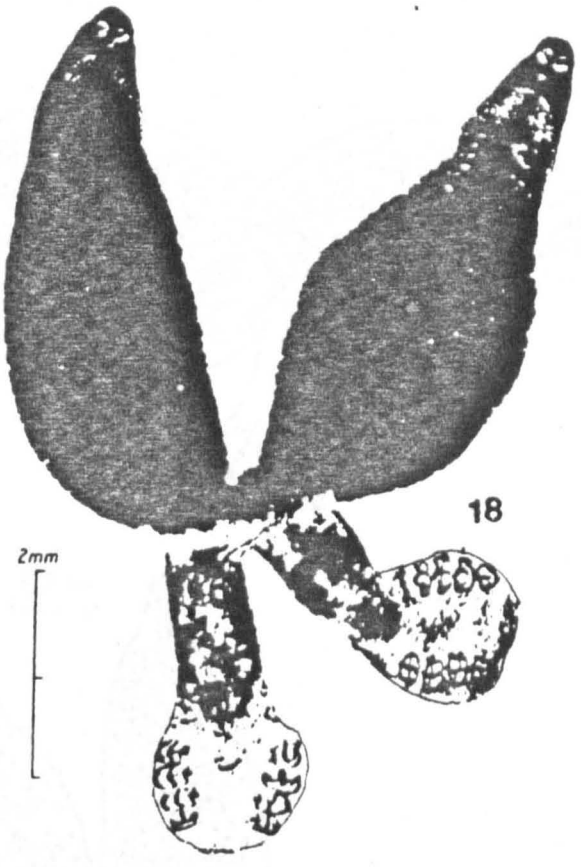
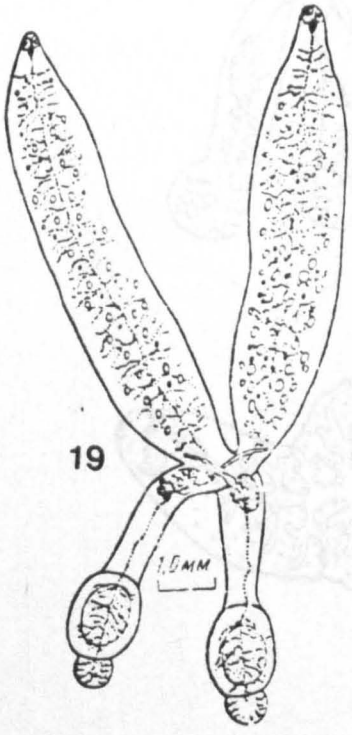


Fig. 4·2A continued





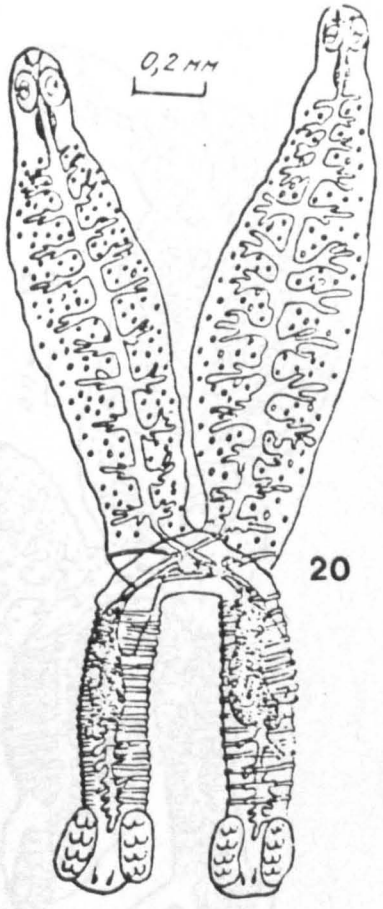


Fig. 4.2 A continued

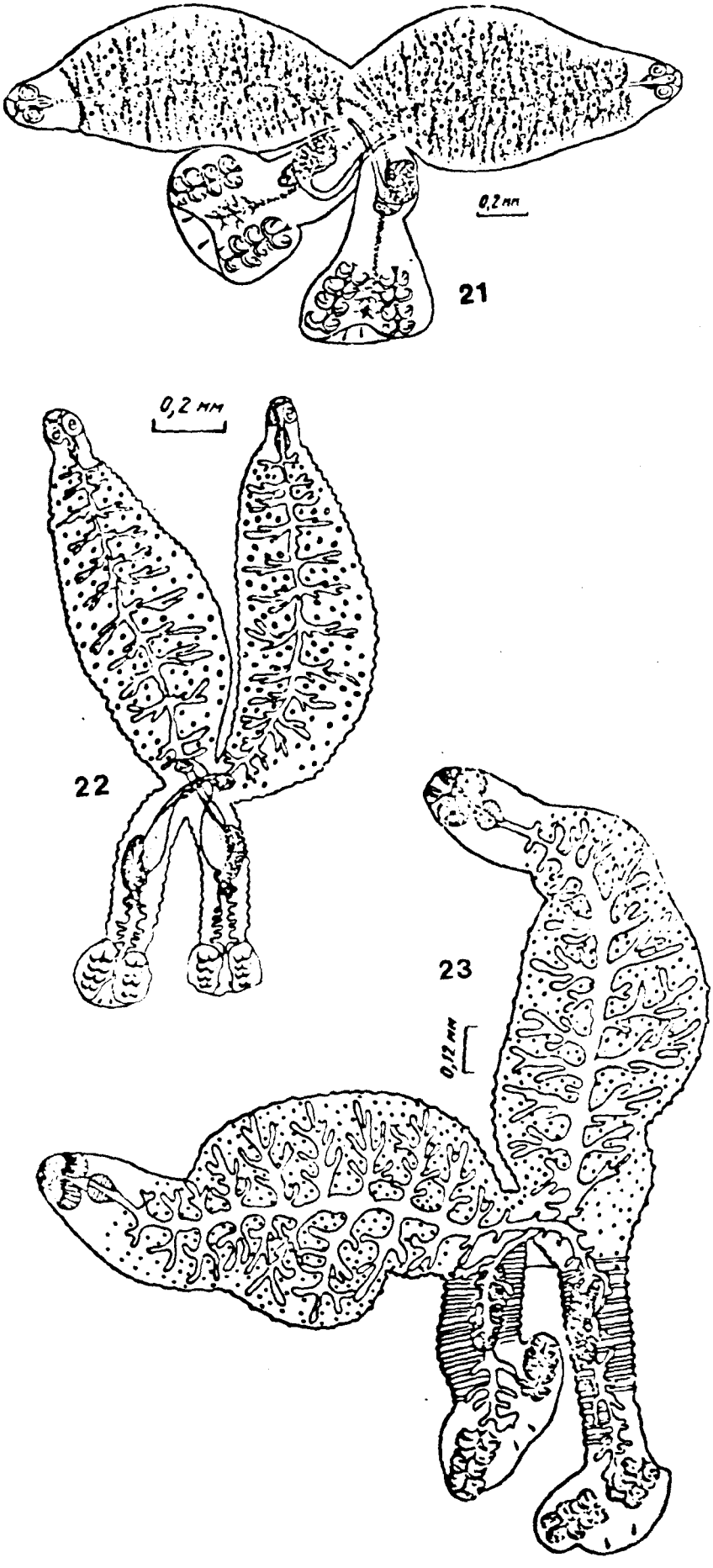
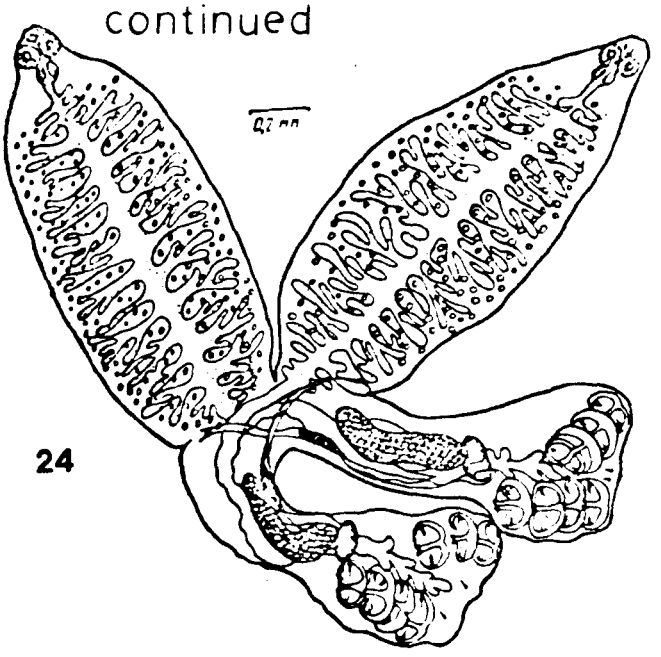


Fig.4·2A continued



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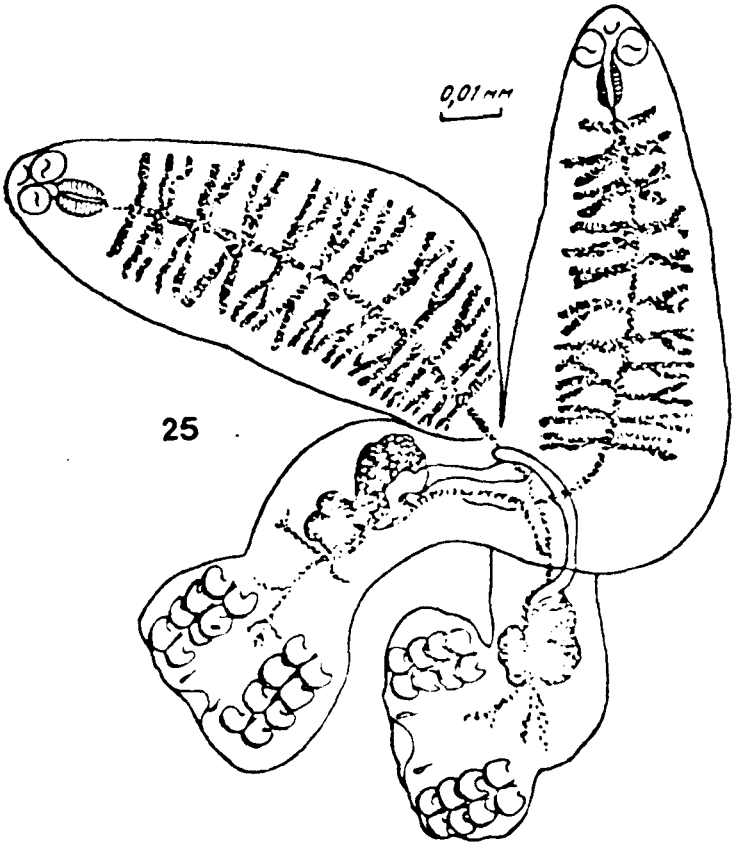

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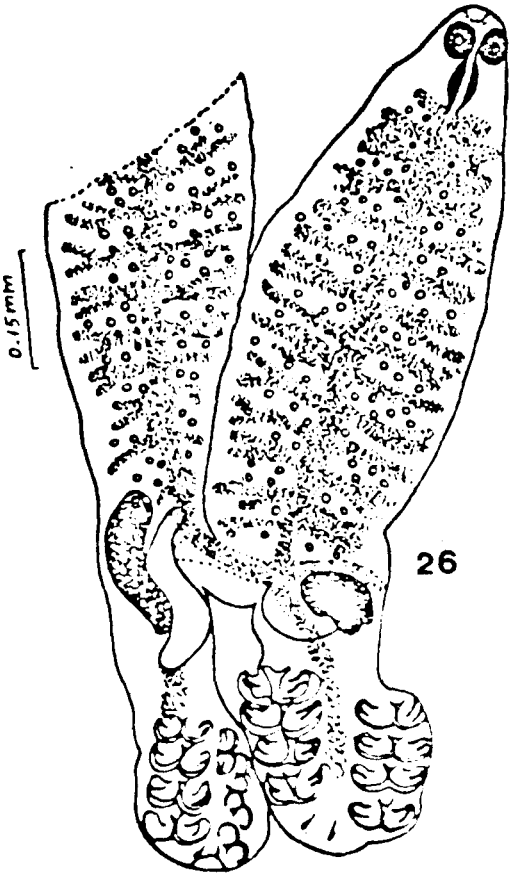


26

Fig.4.2 B

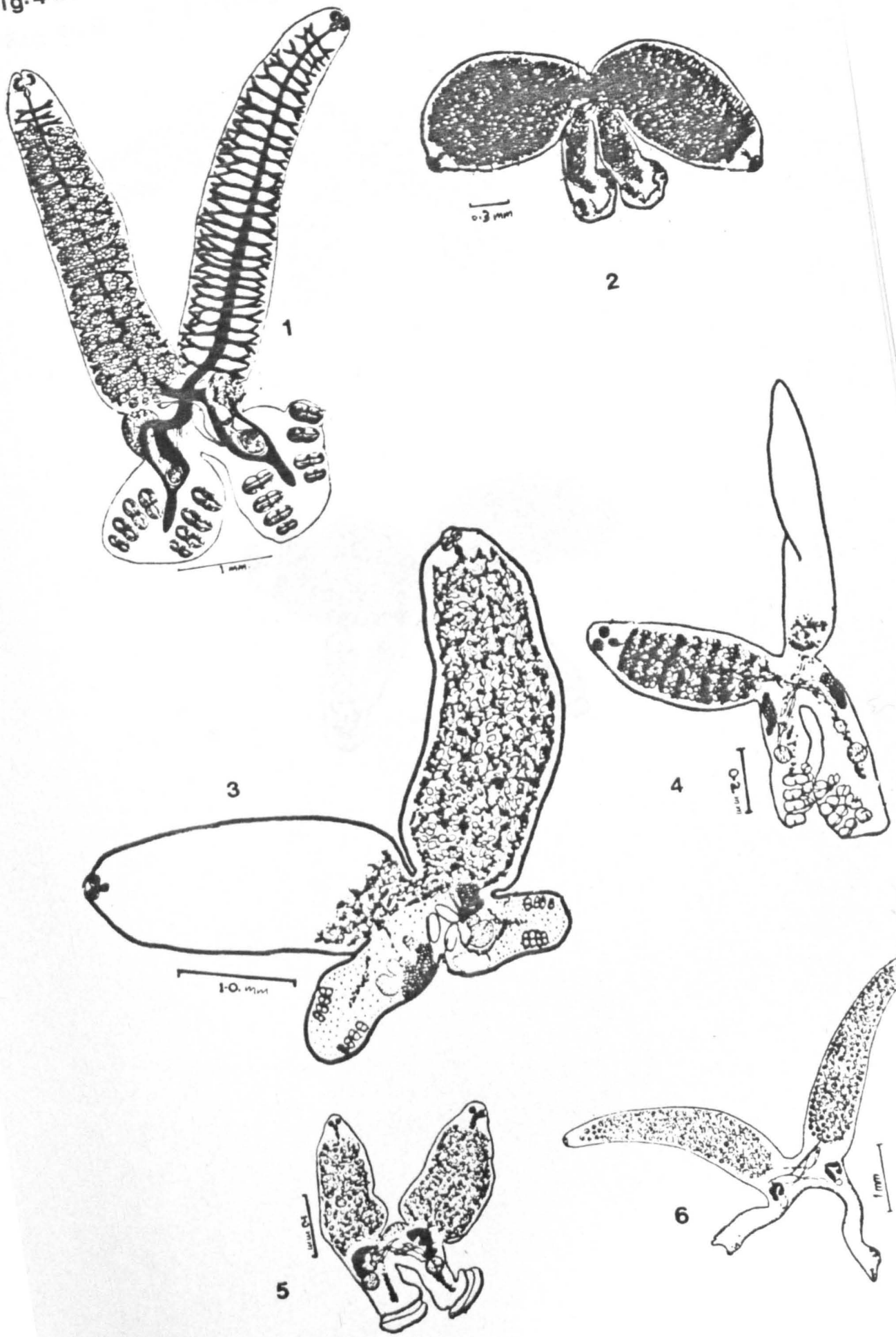
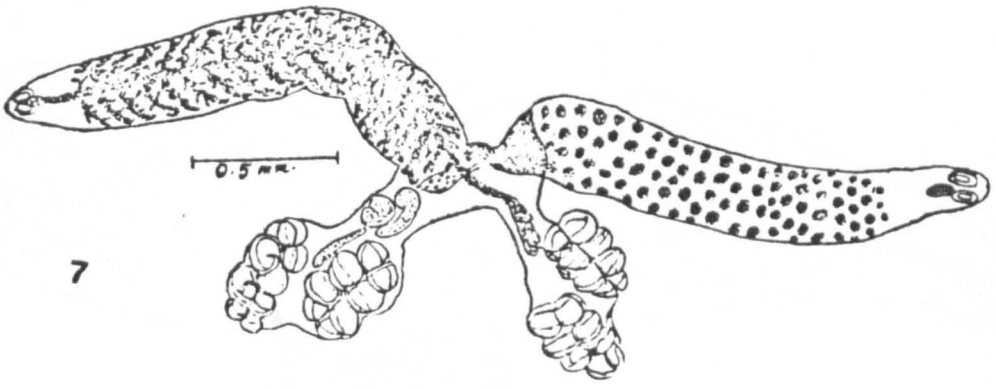


Fig. 4·2B continued



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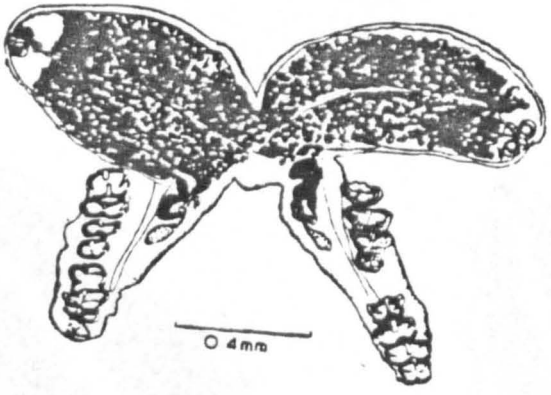
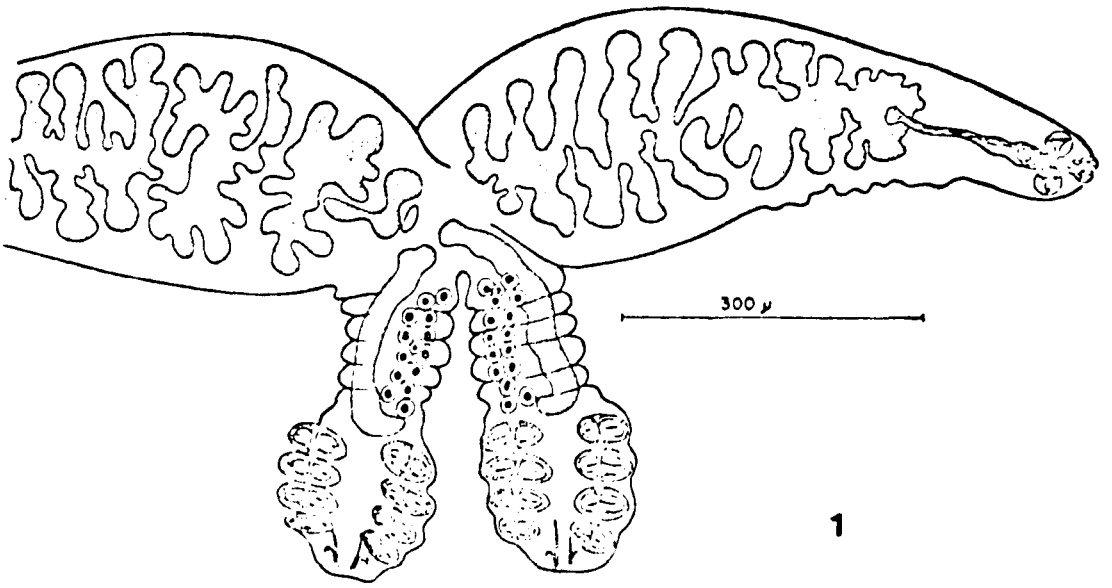
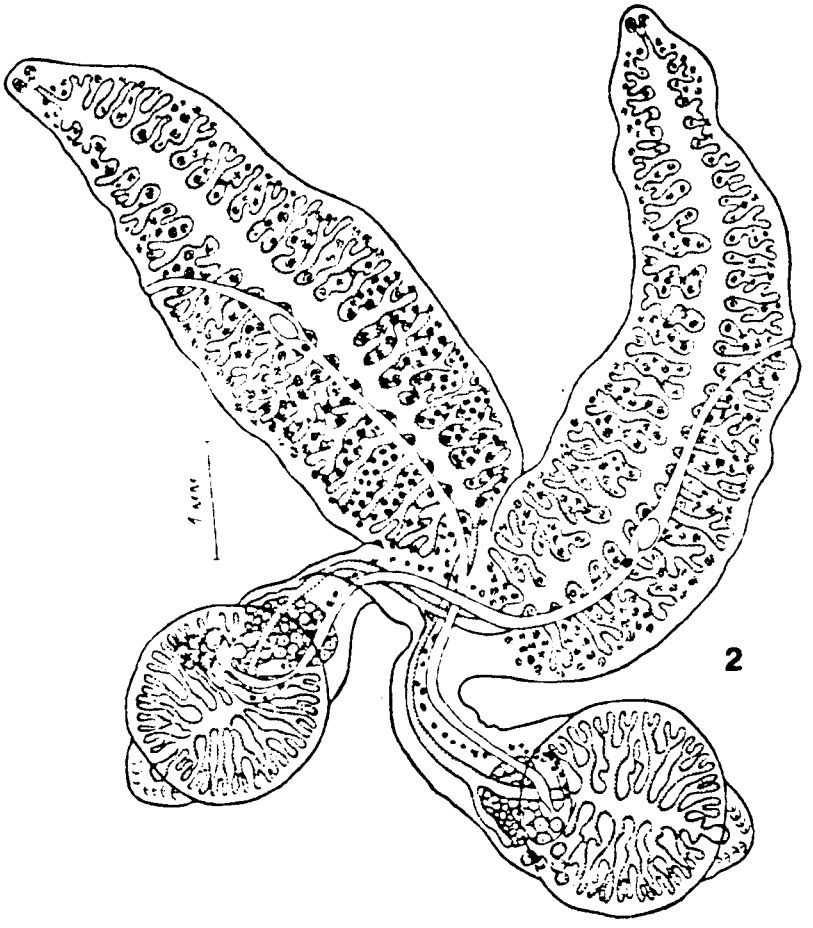


8

Fig·4·2C

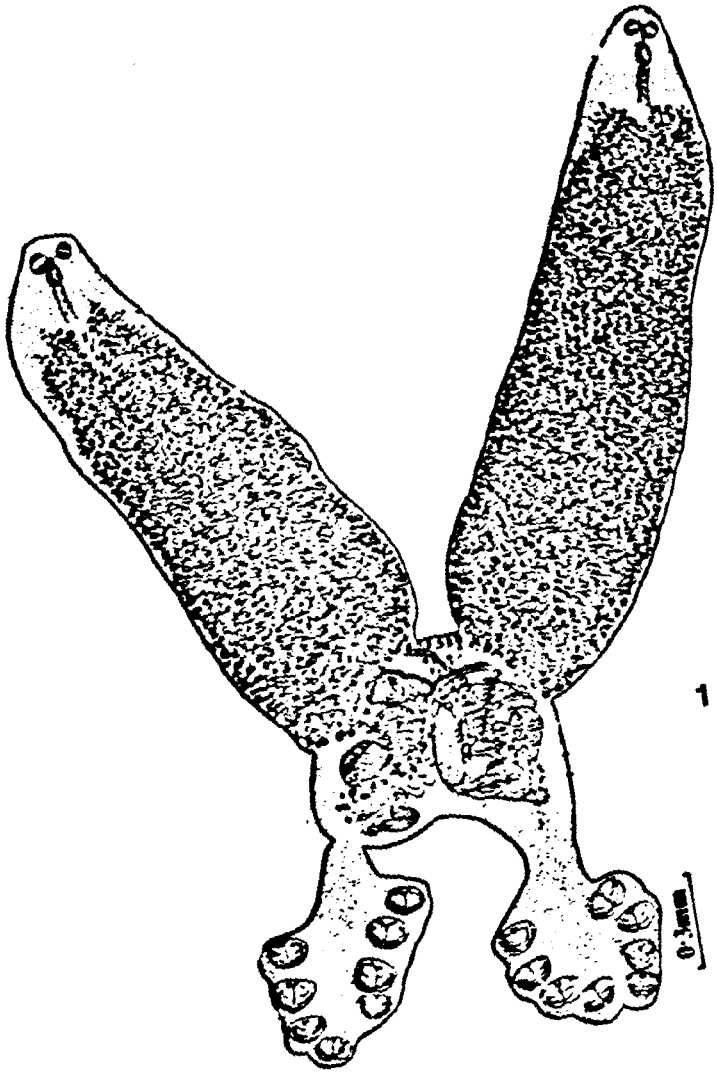


1



2

Fig.4.2 D



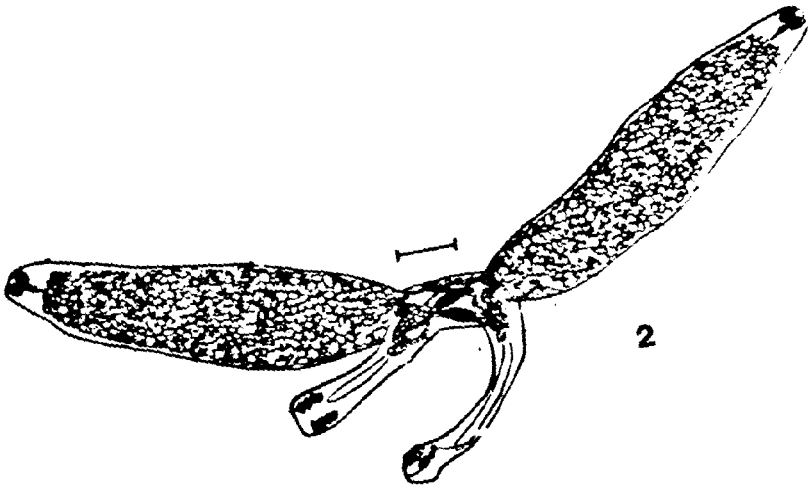
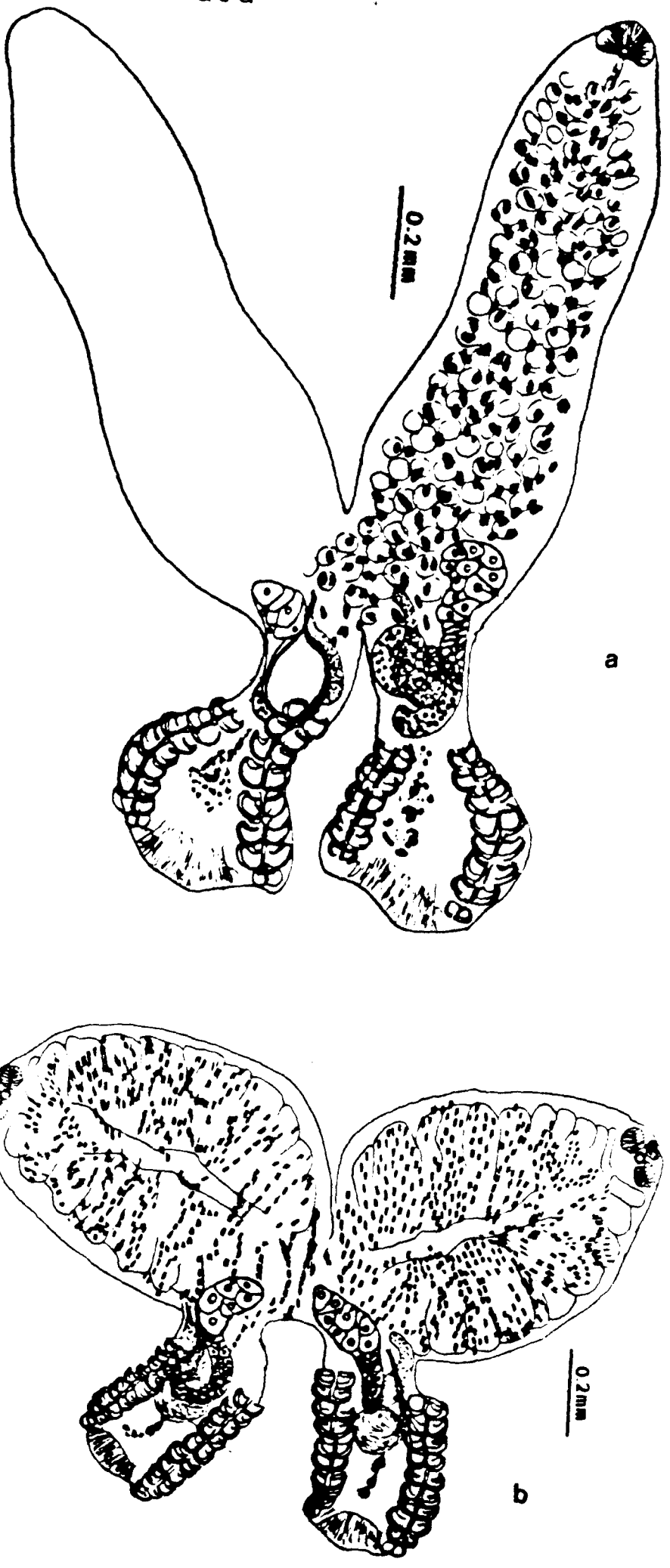


Fig.4.2 D continued



materials without this structure were ranked into the subgenus D. (Paradiplozoon). However, up to 1959 of all the European and other Russian species, the deep folds on the posterior part of the adult worms have only been reported on D. paradoxum (Bychowsky and Nagibina, 1959). Reichenbach-Klinke (1951) incorrectly used the assymetrical arrangement of the clamps on the posterior parts of adult worms as a main character to identify D. barbi (Fig. 4.2 A1). This point is discussed in detail in Chapters 3 and 7.

The adult worms of Indian species (Fig. 4.2B 1-8) have been placed into two genera. All the adult forms with 4 pairs of clamps were kept within the genus Diplozoon and only the one species where the opisthaptor was bilobed with 18-28 pairs of clamps was put into a new genus Neodiplozoon barbi (Fig. 4.2 B5). The fine constrictions on all parts of the bodies of adult Diplozoon have not been stated in any of the Indian species.

Of the other Asian species, the description of D. minutum given by Paperna, 1964 (Fig. 4.2 C1) also depends only on the size and shape of adult structures, except a few ridges on the posterior parts. D. aristichthysi (Fig. 4.2 C2 as given by Ling, 1973) recovered from China is closely similar to D. inustiatus (quoted from Khotenovskiĭ, 1978). This point will be discussed in detail later.

In the African species (Fig. 4.2D 1-3), 2 species were put in the genus Diplozoon, with 4 pairs of clamps, and one species was added to the genus Neodiplozoon, but differed from the Indian one by having 8 pairs of clamps in the newly coupled worms, and 10 in large gravid specimens (Fig. 4.2D 3a and b) (Paperna, 1973 and 1979). The number of the clamps may be different in each of the worms of the couple so Paperna (1979)

gave it the name N. polycotyleus.

2. Posterior region of adult stage

Fig. 4.3A, B, C and D illustrates for most species of Diplozoidae the distribution of the intestine branches, the arrangement of clamps on the opisthaptor and the presence or absence of the invagination and the deep folds on the posterior parts.

In European and Russian species (Fig. 4.3 A1-24) most of the posterior parts look like D. homoion, except for D. paradoxum, D. nagibina and D. balleri (Fig. 4.3 A2, 8 and 10 respectively) which have the deep folds and the invaginations (Nagibina et al., 1970). Gläser (1965) called these folds on the posterior part of D. nagibinae cuticular wrinkles, while Nagibina et al., (1970) called them cuticular folds on D. balleri and said that they looked like those of D. nagibina. In my opinion the posterior parts of these two species appear to be very similar. All 3 species were recovered from a member of genus Abramis (see Chapter 2, Table 2.1). D. (Diplozoon) mylopharyngodonis (Figs. 4.2 A19 and 4.3 A20) and D. diplodiscus, D. bychowskyi, D. inustiatus and D. strelkowi (Fig. 4.2 A11, 12, 13 and 14 respectively) have only an expansion and not the deep folds. The intestinal branches in the posterior parts show a considerable variation between the different species. The results of Chapter 3 confirm that there was a difference between D. paradoxum and D. homoion. Bychowsky and Nagibina (1959), Bovet (1967) and Gläser and Gläser (1964) have already reported this difference. However, later on, the new genus Paradiplozoon was proposed by Khotenovskiĭ (1982) following its use as a subgenus by Akhmerov (1974). This new genus covered all species which were without invaginations and ridges (deep folds) on the posterior parts. Khotenovskiĭ used the

Fig. 4.3 Posterior parts of species of Diplozoidae reproduced from the original descriptions.

A. European and Russian species

1.	*D. tetragonopterini*	Sterba, 1957
2.	*D. paradoxum*	As revised by Bychowsky and Nagibina, 1959 and Khotenovskiĭ, 1980
3.	*D. homoion*	Bychowsky and Nagibina, 1959
4.	*D. pavlovskii*	Bychowsky and Nagibina, 1959
5.	*D. megan*	Bychowsky and Nagibina, 1959
6.	*D. gussevi*	Gläser and Gläser, 1964
7.	*D. markewitschi*	Bychowsky *et al.*, 1964
8.	*D. nagibinae*	Gläser, 1965
9.	*D. rutili*	Gläser, 1967
10.	*D. balleri*	Nagibina *et al.*, 1970
11.	*D. kurensis*	Mikailov, 1973
12.	*D. mingetschauricum*	Mikailov, 1973
13.	*D. varicorhini*	Mikailov, 1973
14.	*D. sapa*	Mikailov, 1973
15.	*D. schulmani*	Mikailov, 1973
16.	*D. chazarikum*	Mikailov, 1973
17.	*D. kuthkaschenicum*	Mikailov, 1973
18.	*D. agdamicum*	Mikailov, 1973
19.	*D. persicum*	Mikailov, 1973
20.	*D. (Diplozoon) mylopharyngodonis*	Akhmerov, 1974
21.	*Paradiplozoon megalobramae*	Khotenovskiĭ, 1982
22.	*P. tisae*	Khotenovskiĭ, 1982

Fig. 4.3 (contd.)

23. *P. leucisci*	Khotenovskiĭ, 1982
24. *P. alburni*	Khotenovskiĭ, 1982

B. Indian species

1. *D. indicum*	Dayal, 1941
2. *D. kashmirensis*	Kaw, 1950
3. *Neodiplozoon barbi*	Tripathi, 1959 a and b
4. *D. microclampi*	Kulkarni, 1971
5. *D. thapari*	Gupta and Krishna, 1977

C. Other Asian species

1. *Paradiplozoon cyprini*	Khotenovskiĭ, 1982
2. *P. vietnamicum*	Khotenovskiĭ, 1982

Fig. 4-3 A

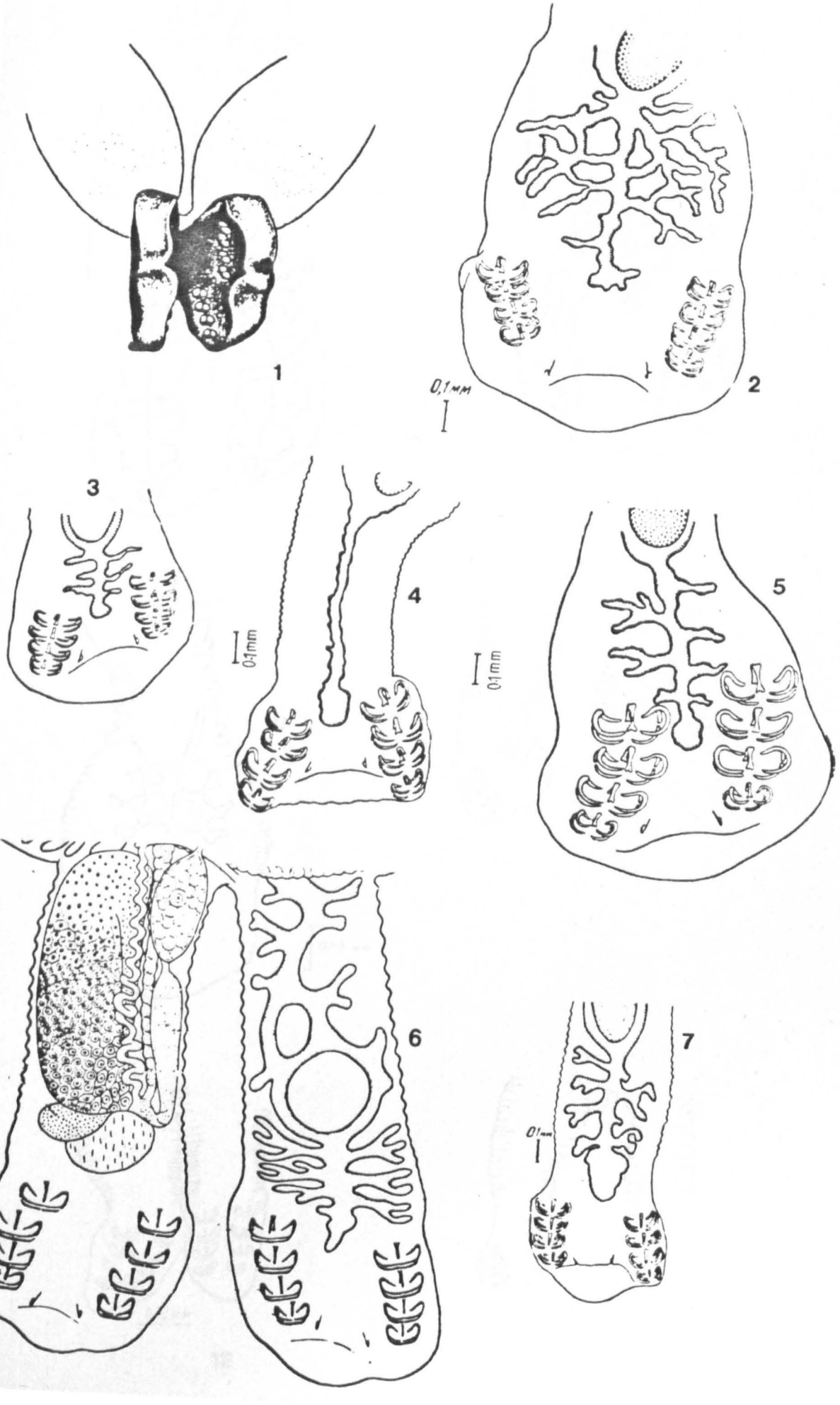
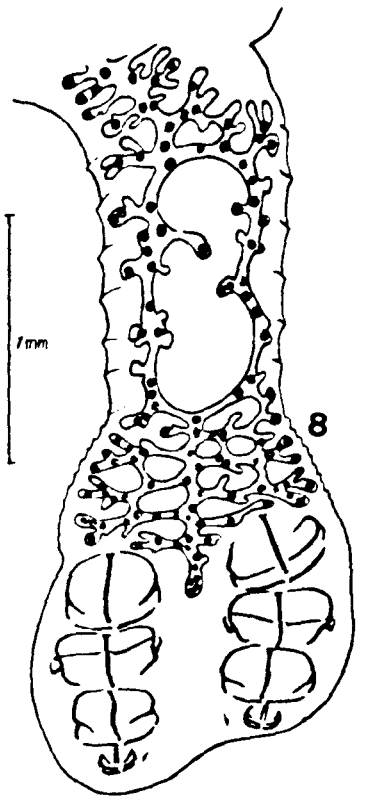
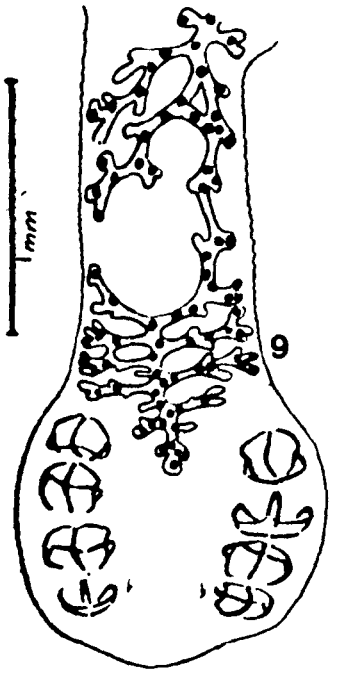
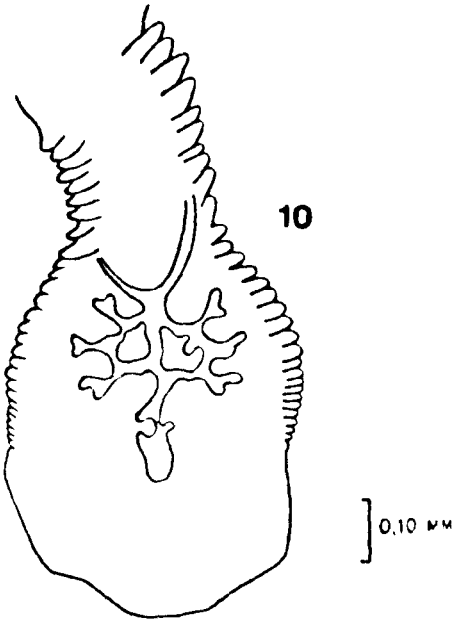
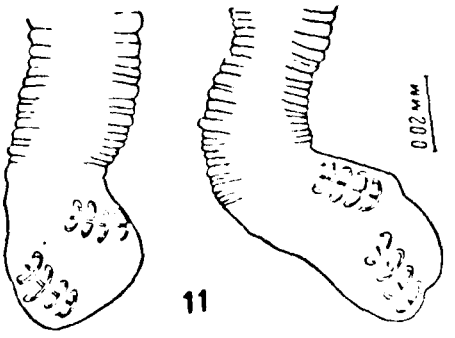


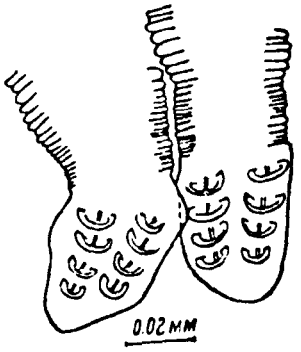
Fig. 4-3 A continued

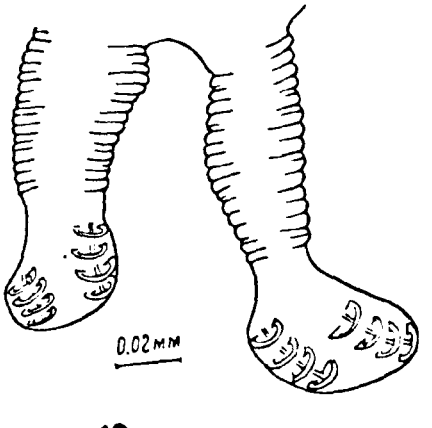




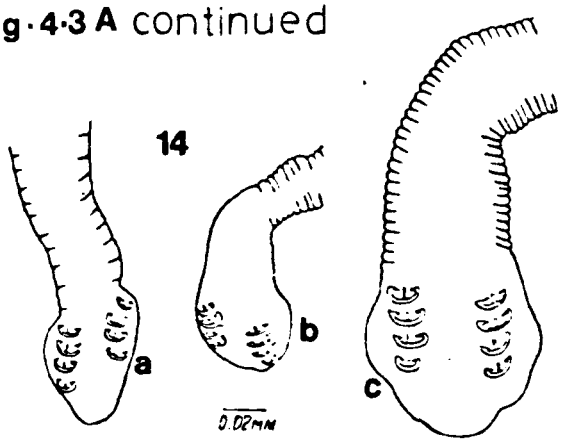


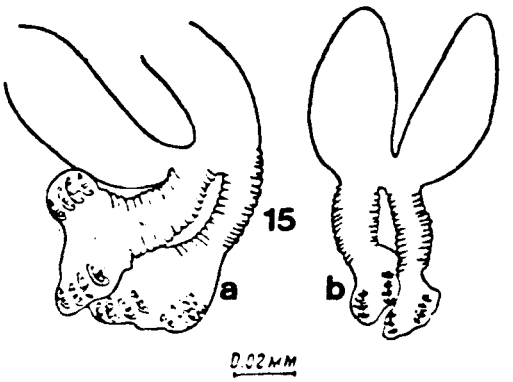


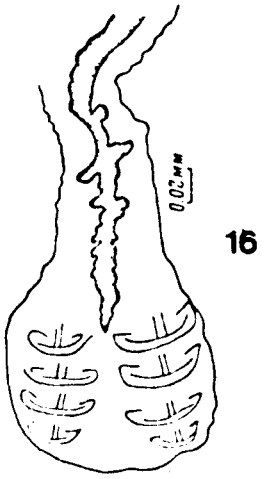


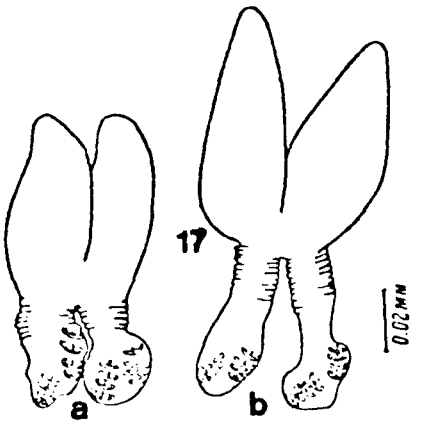


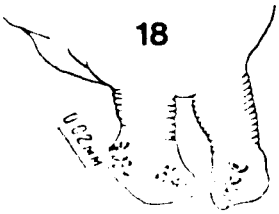
Fig·4·3 A continued

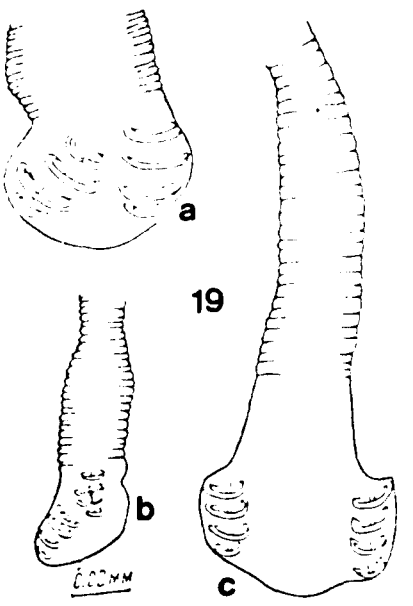












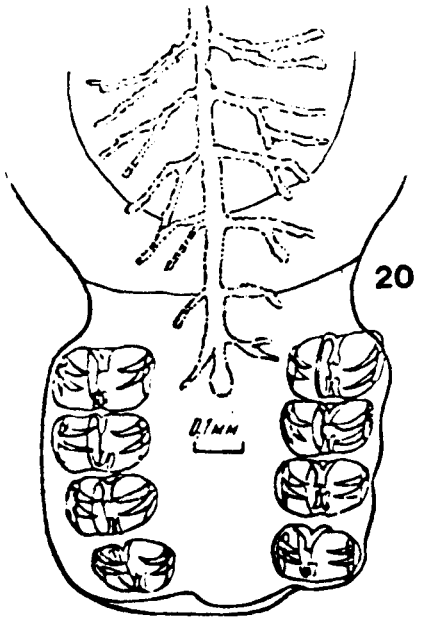


Fig. 4·3A continued

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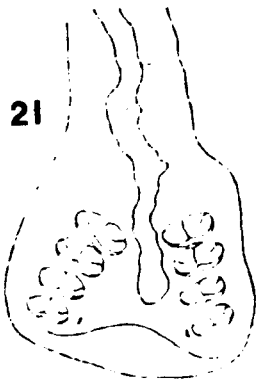

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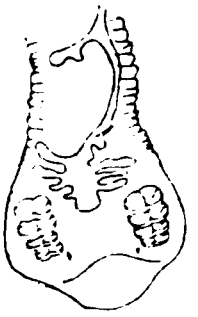

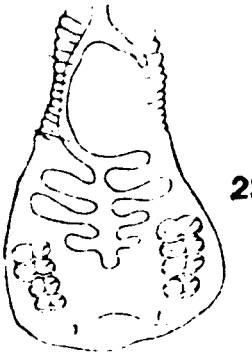

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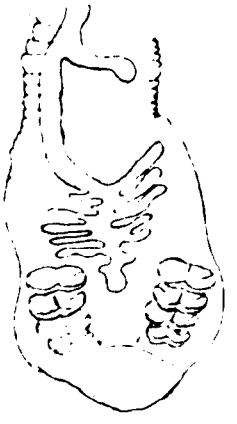
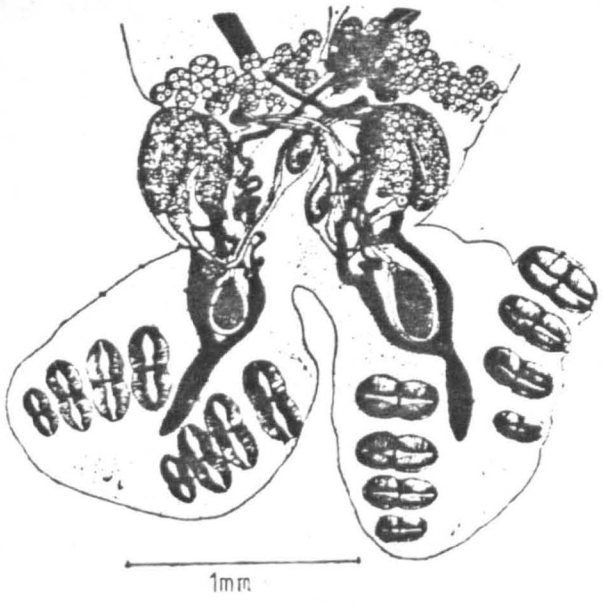


Fig. 4.3B



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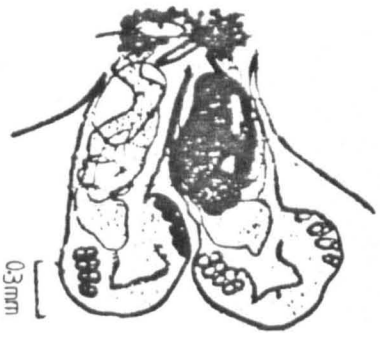

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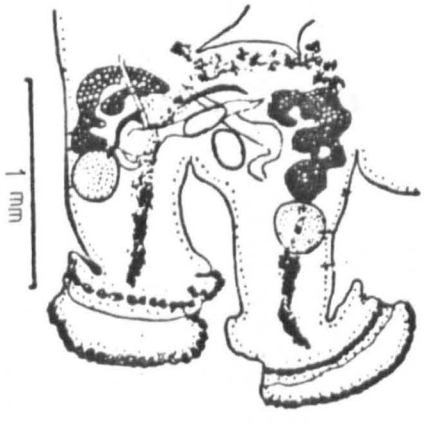

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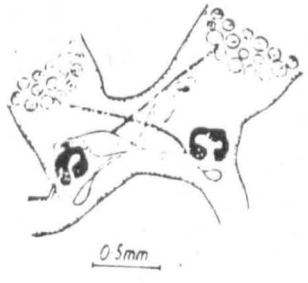

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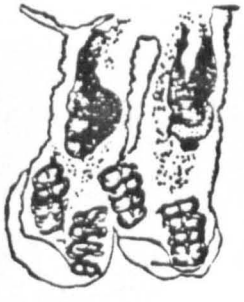
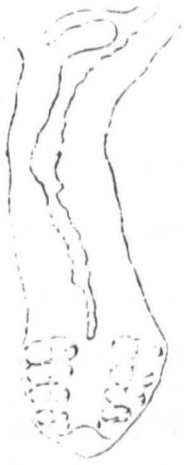


5

Fig. 4.3 C

1



2

branches of intestine in the posterior parts and the side of the opening of the intestinal loop around the gonad as shown in Paradiplozoon megalobramae, P. tisae, P. leucisci and P. alburni (Fig. 4.3 A21, 22, 23 and 24 respectively) as characters to distinguish between the species.

In the Indian species (Fig. 4.3 B 1-5), the intestinal branches in the posterior parts of all species have a similar distribution in which the intestine forms one loop behind the testis. Kulkarni (1971) found that the position of reproductive organs of D. microclampi (Fig. 4.3 B4) was at the crossing area of two individuals. According to Tripathi (1959a) there was no distinct separation of the opisthaptor on the hind body of D. cauveryi nor a muscular disc. Gupta and Krishna (1977) indicated that there was an adhesive gland at the posterior end of the opisthaptor of D. thapari, but these data were not confirmed later by any author. In Neodiplozoon barbi (Fig. 4.3 B3), the bilobed opisthaptor seen by Tripathi (1959a and b) can be clearly recognised on the posterior part of this species. The arrangement of the clamps round these two lobes was also very distinct.

In the Asian species, Khotenovskiĭ (1982) ranked two new species from Vietnam in the genus Paradiplozoon because of the absence of the invagination, the deep folds on the posterior parts and the arrangement of the branches of the intestine in the posterior part (Fig. 4.3 C1 and 2) which were similar to the Russian species (Fig. 4.3 A21-24).

3. Clamp structure

Earlier, Price (1934) proposed the term haptor for the adhesive organ of the monogenetic trematodes including the gyrodactylids, polystomes and microcotylids. This term was to replace the other terms such as holdfast organs, adhesive organs, cotylophore etc. Later different

names for the adhesive organs of Diplozoidae appeared in the literature e.g. the opisthaptor of Dawes (1946) which represented the distal portion of the posterior part including the clamps, the pair of larval hooks and the muscular disc (see Chapter 3). Kaw (1950) and Chauhan (1953) used the term posterior sucker instead of clamp.

The structure of the clamps of the different species of Diplozoidae are shown in Fig. 4.4A, B, C and D. A wide range of variation can be seen between the clamps from different species. However, a similar range of variation is evident between the clamps of the one specimen of D. nagibina (Fig. 4.4 A10a, b and c) and in one specimen of D. rutili (Fig. 4.4 A16a, b and c). It is difficult to decide whether the differences between the clamps are of taxonomic significance or are population variations.

In European and Russian D. paradoxum and D. homoion there was a slight difference between the clamps (Fig. 4.4 A 3 and 6 respectively). This was confirmed by the results of chapter 3 . This difference was restricted to a particular sclerite called the fair-lead by Owen (1963) and Bychowsky and Nagibina (1959). This was described in detail in Chapter 3. Otherwise, in my opinion the morphology of the clamps of the remaining species are closely similar.

In the Indian species (Fig. 4.4 B1-5) including Neodipozoon barbi, other Asian species (Fig. 4.4 C1-4) and African species (Fig. 4.4 D1 and 2) including N. polycotyleus, the clamp structures are similar to those of European and Russian species. Sproston (1945) stated that form of the skeleton of the clamp must be regarded as of primary importance in the classification of the super family. She also reported that although the detail of clamps was important in systematic work but they were highly complex owing to the sclerites often being twisted bars, curving through three dimensions with the primary bar often jointed or fused.

Fig. 4.4. The structure of the clamps of species of Diplozoidae reproduced from the original description.

A. European and Russian species

1. *D. nipponicum* — Goto, 1891
2. *D. barbi* — Reichenbach-Klinke, 1951
3. *D. paradoxum* — As revised by Bychowsky and Nagibina, 1959
4. *D. pavlovskii* — Bychowsky and Nagibina, 1959
5. *D. megan* — Bychowsky and Nagibina, 1959
6. *D. homoion* — Bychowsky and Nagibina, 1959
7. *D. markewitschi* — Bychowsky *et al.*, 1964
8. *D. gussevi* — Gläser and Gläser, 1964
9. *D. schizothorazi* — Iksanov, 1965
10. *D. nagibina* — Gläser, 1965
    a. 1st clamp
    b. 2nd clamp
    c. 3rd clamp
11. *D. diplodiscus* — Nagibina, 1965
12. *D. bychowskyi* — Nagibina, 1965
13. *D. inustiatus* — Nagibina, 1965
14. *D. strelkowi* — Nagibina, 1965
15. *D. tadzhikistanicum* — Gavrilova and Dzhalilov, 1965
16. *D. rutili* — Gläser, 1967
    a. 3rd clamp
    b. 2nd clamp
    c. 1st clamp
17. *D. balleri* — Nagibina *et al.*, 1970

Fig. 4.4 (contd.)

18.	*D. kurensis*	Mikailov, 1973
19.	*D. varicorhini*	Mikailov, 1973
20.	*D. mingetschauricum*	Mikailov, 1973
21.	*D. schulmani*	Mikailov, 1973
22.	*D. sapa*	Mikailov, 1973
23.	a, b and c *D. chazarikum*	Mikailov, 1973
24.	*D. kuthkaschenicum*	Mikailov, 1973
25.	*D. persicum*	Mikailov, 1973
26.	*D. agdamicum*	Mikailov, 1973
27.	*D. (Diplozoon) mylopharyngodonis*	Akhmerov, 1974
28.	*D. (Paradiplozoon) erythroculteris*	Akhmerov, 1974
29.	*D. (P.) skrjabini*	Akhmerov, 1974
30.	*D. (P.) marinae*	Akhmerov, 1974
31.	*D. (P.) parabramidis*	Akhmerov, 1974
32.	*D. (P.)* sp.1 (species not identified)	Akhmerov, 1974
33.	*D. (P.)* sp. 2 (species not identified)	Akhmerov, 1974
34.	*D. (P.)* sp. 3 (species not identified)	Akhmerov, 1974
35.	*Paradiplozoon megalobramae*	Khotenovskiĭ, 1982
36.	*P. tisae*	Khotenovskiĭ, 1982
37.	*P. leucisci*	Khotenovskiĭ, 1982
38.	*P. alburni*	Khotenovskiĭ, 1982

Fig. 4.4 (contd.)

B. Indian species

1.	*D. kashmirensis*	Kaw, 1950
2.	*D. cauveryi*	Tripathi, 1959a
3.	*D. soni*	Tripathi, 1959a
4.	*Neodiplozoon barbi*	Tripathi, 1959a and b
5.	*D. microclampi*	Kulkarni, 1971

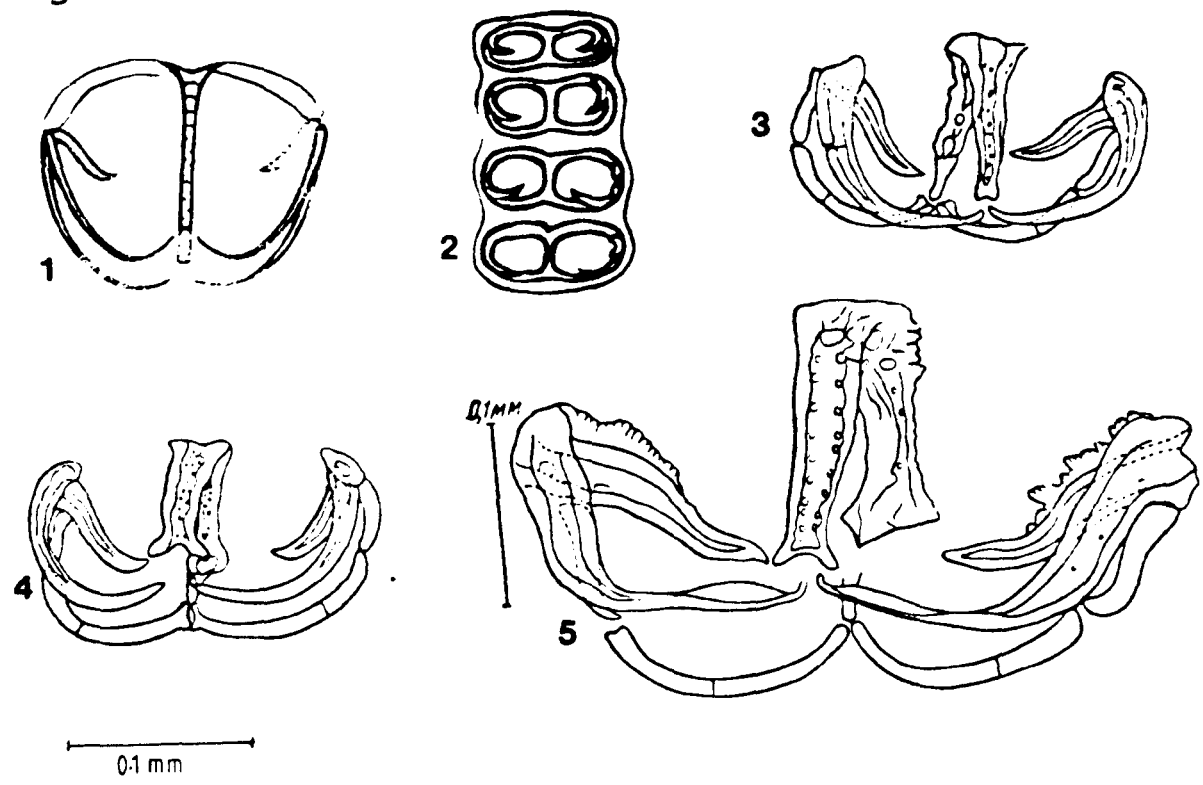
C. Other Asian species

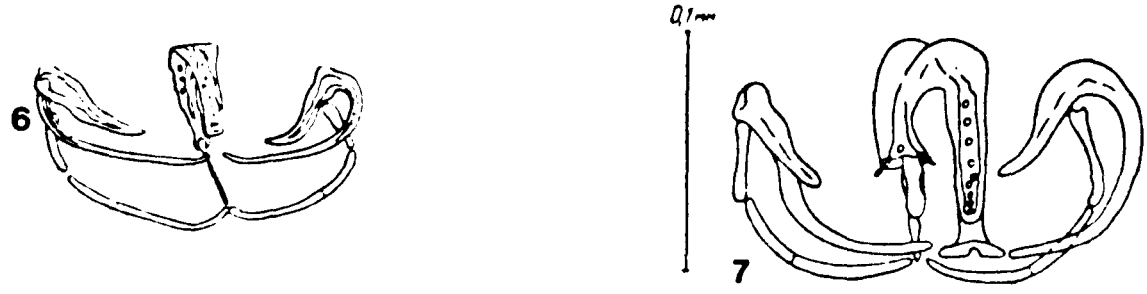
1.	*D. minutum*	Paperna, 1964
2.	*D. doi*	Ha Ky, 1971
3.	*Paradiplozoon cyprini*	Khotenovskiĭ, 1982
4.	*P. vietnamicum*	Khotenovskiĭ, 1982

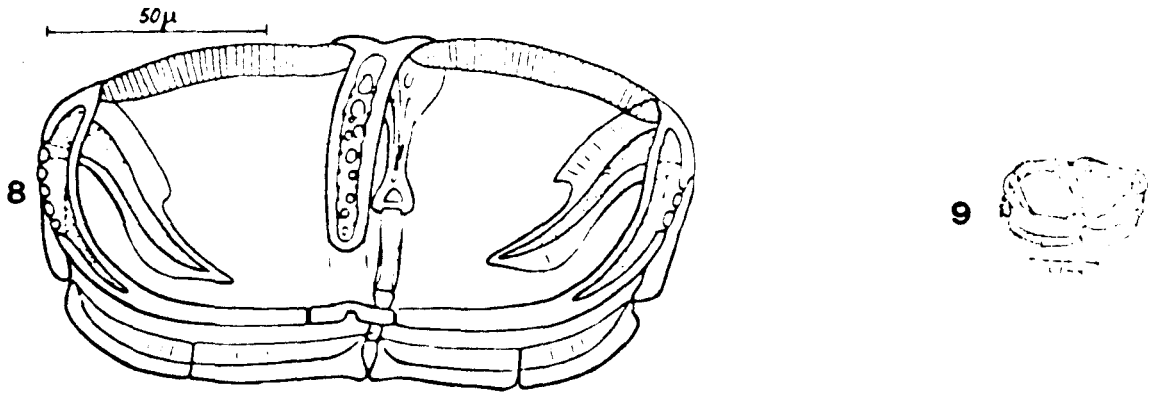
D. African species

1.	*D. ghanense*	Thomas, 1957
2.	*Neodiplozoon polycotyleus*	Paperna, 1973 and 1979

Fig. 4.4 A







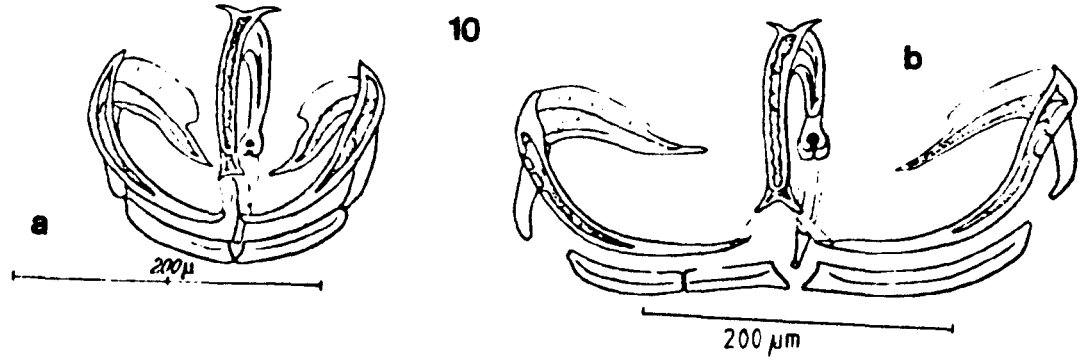
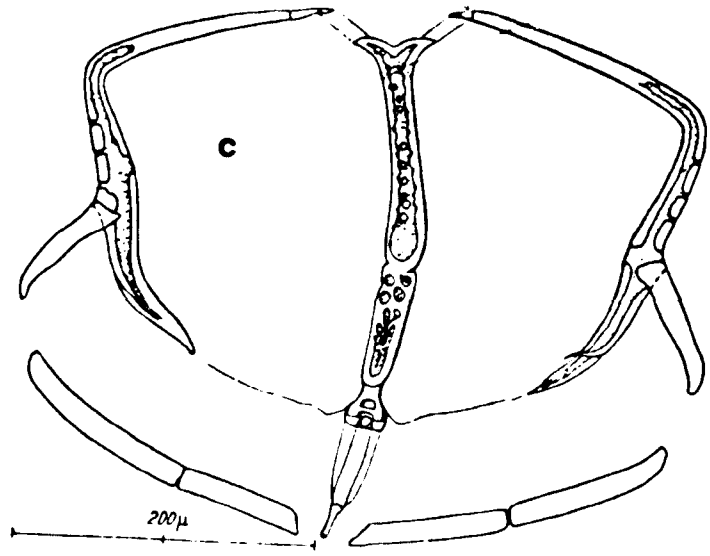
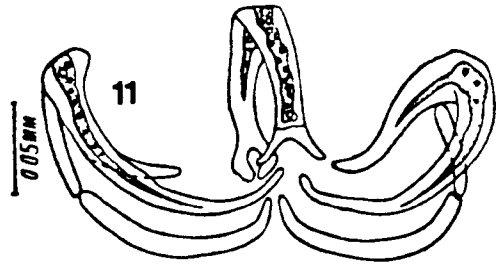
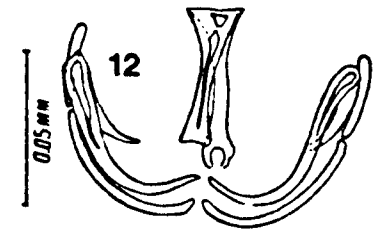
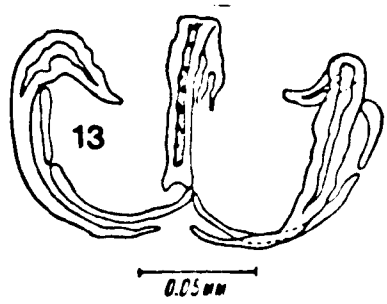


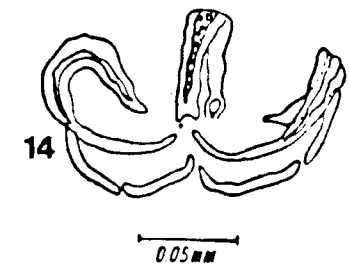
Fig. 4.4A
continued

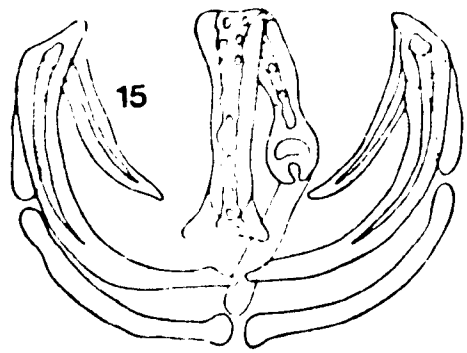


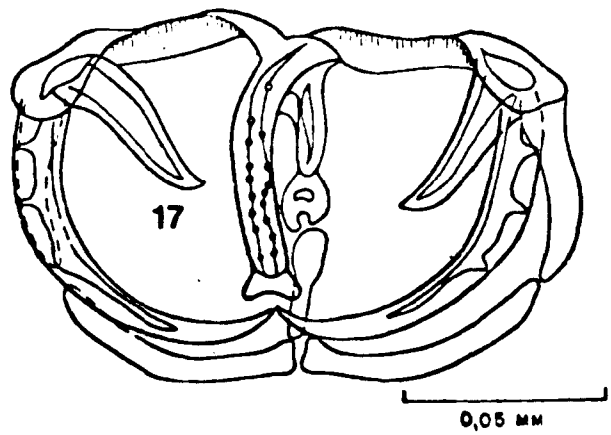












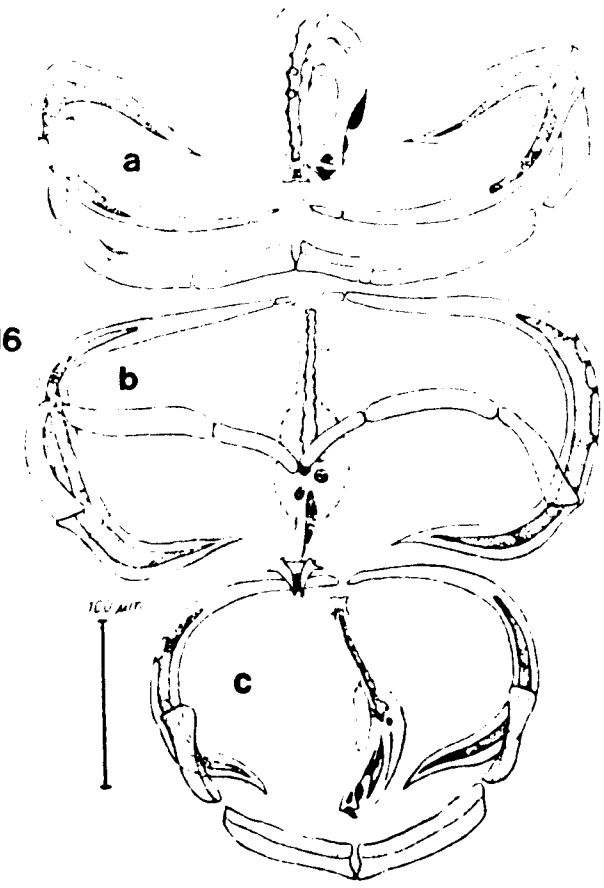
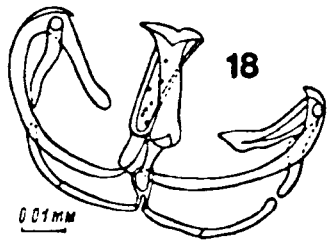
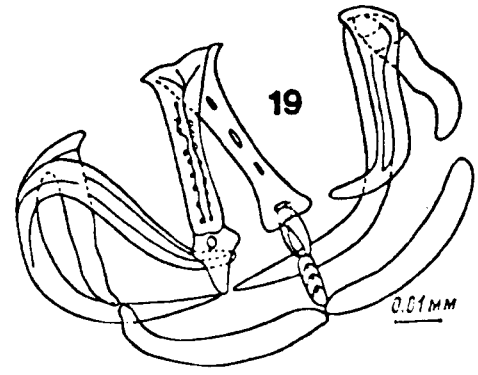
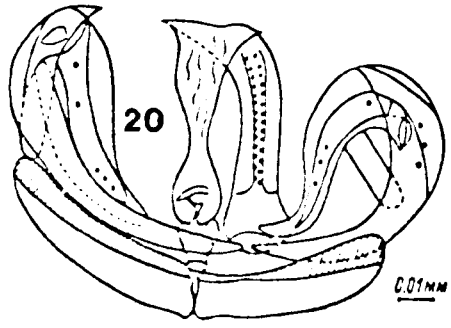
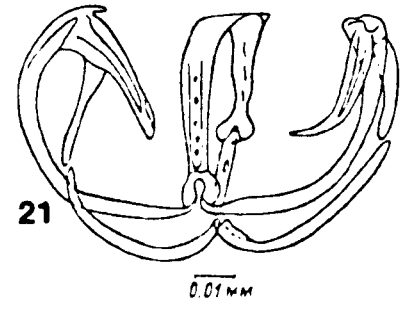


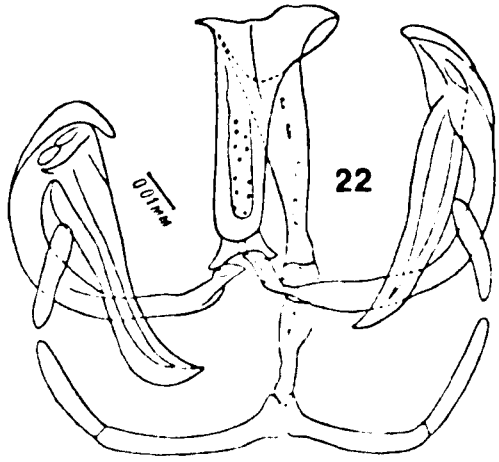
Fig. 4.4A continued

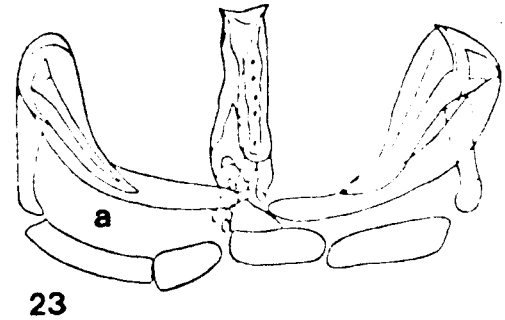












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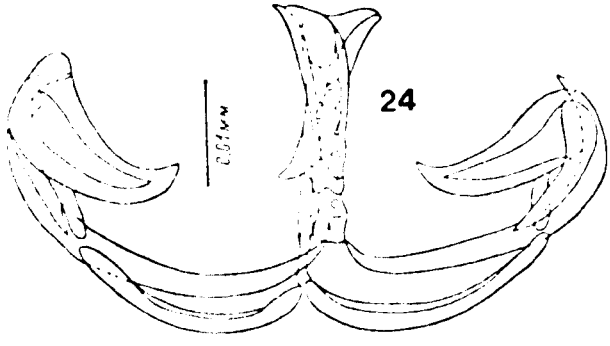
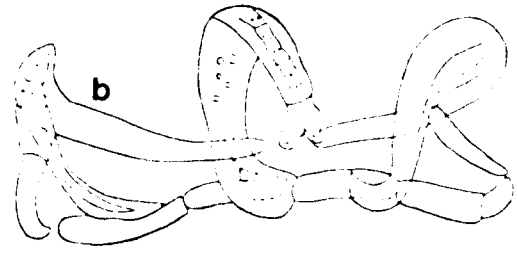
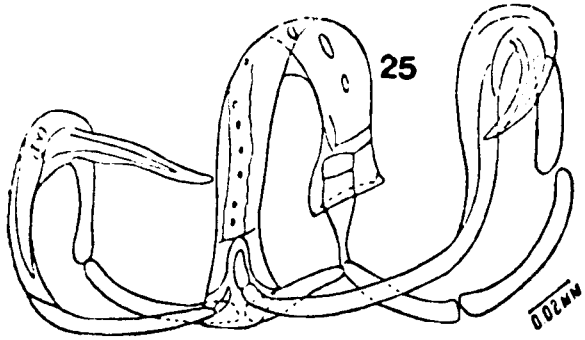
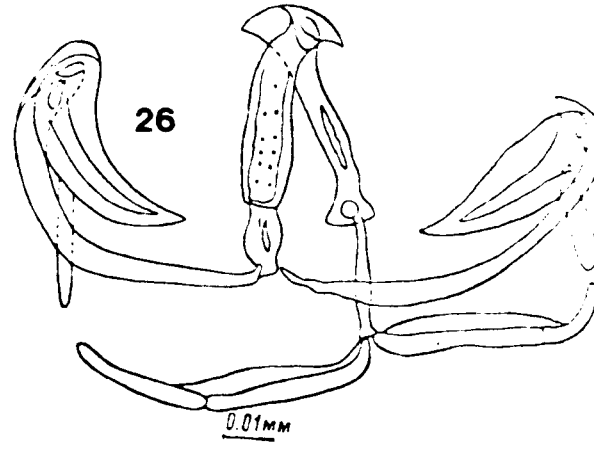
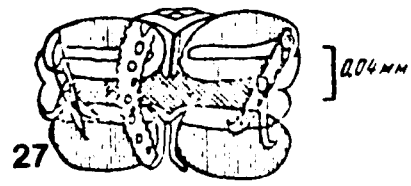








Fig·4·4A continued



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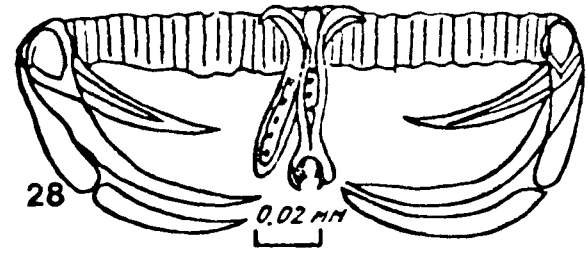

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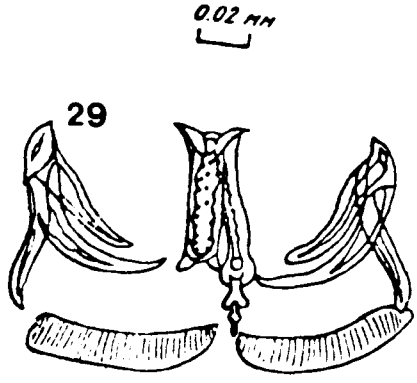

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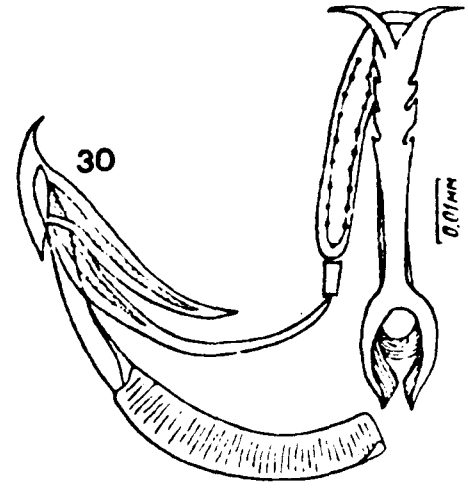

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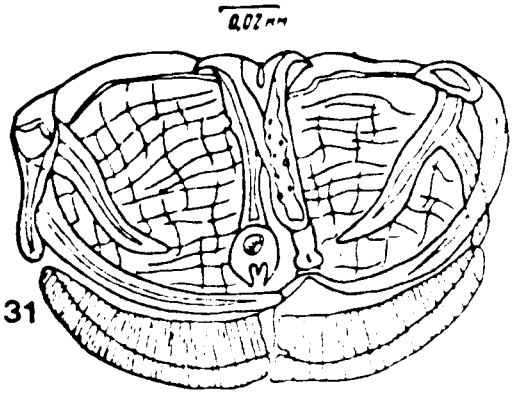

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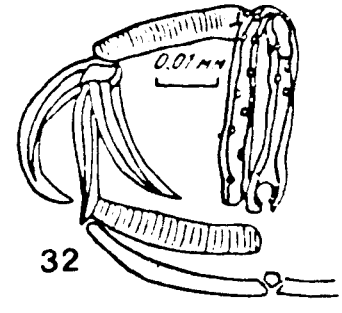

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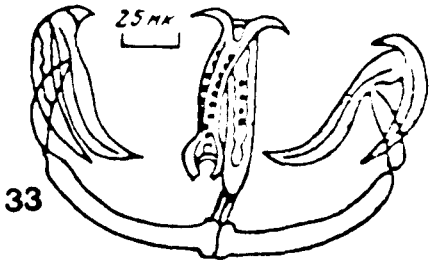

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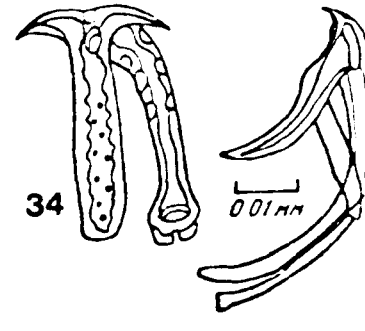

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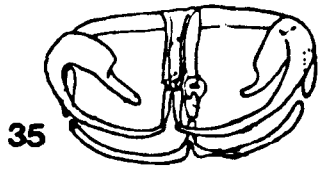

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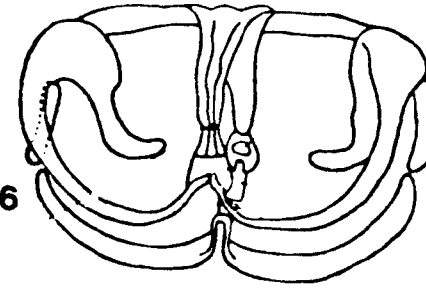

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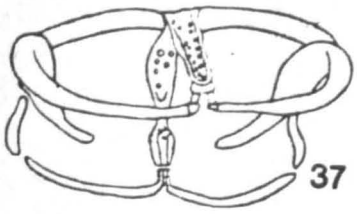
Fig.4.4A continued



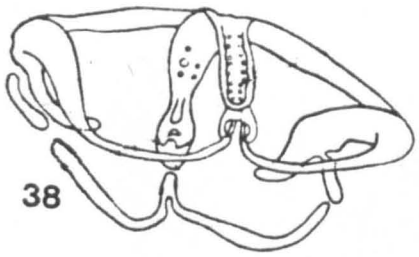


Fig 4.4 B

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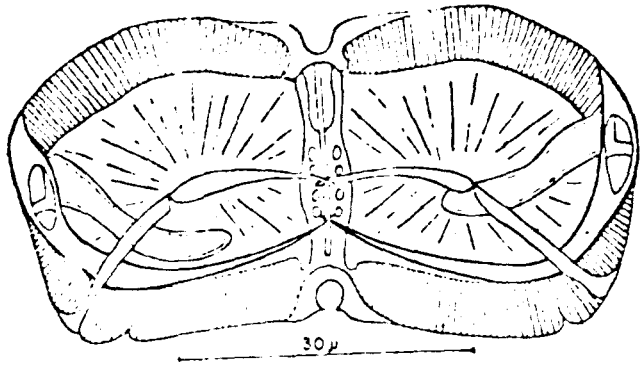
Fig.4.4 C



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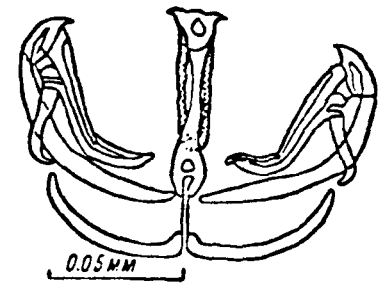

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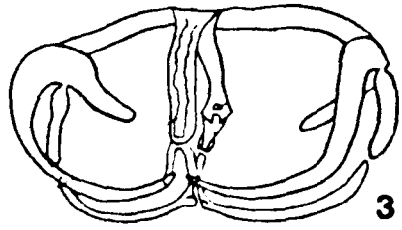

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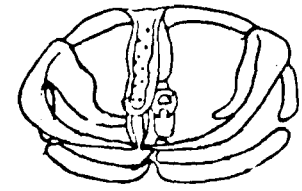

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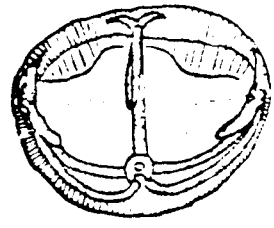
Fig.4.4 D



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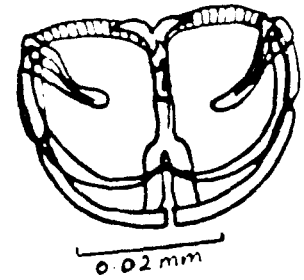

2

The difficulty of interpreting them was increased by their being semi-transparent, and appearing of different shapes when viewed from various angles. Lyons (1966) drew attention to the importance of the chemical analysis of the clamps of many monogenean groups. A chemical analysis of the clamps of different species of Diplozoidae might perhaps clarify the significance of the variations in these clamps.

4. Larval hook shapes

The shapes of the larval hooks of most species are reproduced in Fig. 4.5 A and B. I can see no significant difference between the shapes of the larval hooks of the various species of Diplozoidae from Europe, Russia, India or even Africa. The shapes of the larval hooks are also closely similar in species from the different genera which have been proposed e.g. Paradiplozoon, Diplozoon and Neodiplozoon, or from subgenera or even from subspecies. The variations which occurred between them are only in the size of the blade and shaft and in the degree of curvature of the blade. My results given in Chapter 3 showed a wide range of variation of larval hooks, on the population of D. homoion related to development during the life cycle, host sizes, seasons, or, even to the effect of pressure on the cover-slip during the permanent preparation of the specimens. However, some authors indicated in their original descriptions of some species, especially Indian ones that the larval hooks were absent from some species such as Neodiplozoon barbi (Tripathi, 1959a and b), D. dayali (Pandey, 1973), D. soni (Tripathi, 1959a) and D. thapari (Gupta and Krishna, 1977). Tripathi (1959a and b) suggested that the absence of larval hooks in Neodiplozoon barbi was of generic significance but this idea was refuted by Paperna (1973) as this character appears on other Neodiplozoon species from Africa (N. polycotyleus).

Fig. 4.5 The shapes of the larval hooks of some species of Diplozoidae reproduced from the original descriptions.

A. European and Russian species

1.	*D. nipponicum*	Goto, 1891
2.	*D. paradoxum*	As revised by Bychowsky and Nagibina, 1959
3.	*D. pavlovskii*	Bychowsky and Nagibina, 1959
4.	*D. homoion*	Bychowsky and Nagibina, 1959
5.	*D. megan*	Bychowsky and Nagibina, 1959
6.	*D. markewitschi*	Bychowsky *et al.*, 1964
7.	*D. gussevi*	Gläser and Gläser, 1964
8.	*D. diplodiscus*	Nagibina, 1965
9.	*D. strelkowi*	Nagibina, 1965
10.	*D. bychowskyi*	Nagibina, 1965
11.	*D. inustiatus*	Nagibina, 1965
12.	*D. tadzhikistanicum*	Gavrilova and Dzhalilov, 1965
13.	*D. rutili*	Gläser, 1967
14.	*D. balleri*	Nagibina *et al.*, 1970
15.	*D. kurensis*	Mikailov, 1973
16.	*D. varicorhini*	Mikailov, 1973
17.	*D. mingetschauricum*	Mikailov, 1973
18.	*D. schulmani*	Mikailov, 1973
19.	*D. sapa*	Mikailov, 1973
20.	*D. chazarikum*	Mikailov, 1973
21.	*D. kuthkaschenicum*	Mikailov, 1973
22.	*D. agdamicum*	Mikailov, 1973

Fig. 4.5 (contd.)

23.	*D. persicum*	Mikailov, 1973
24.	*D. (Diplozoon) mylopharyngodonis*	Akhmerov, 1974
25.	*D. (Paradiplozoon) amurensis*	Akhmerov, 1974
26.	*D. (P.) erythroculteris*	Akhmerov, 1974
27.	*D. (P.) skrjabini*	Akhmerov, 1974
28.	*D. (P.) marinae*	Akhmerov, 1974
29.	*D. (P.) parabramidis*	Akhmerov, 1974
30.	*D. (P.)* sp. 1 (species not identified)	Akhmerov, 1974
31.	*D. (P.)* sp. 2 (species not identified)	Akhmerov, 1974
32.	*D. (P.)* sp. 3 (species not identified)	Akhmerov, 1974

B. Other Asian and African species

1.	*D. cauveryi*	Tripathi, 1959a
2.	*D. doi*	Ha Ky, 1971
3.	*Neodiplozoon polycotyleus*	Paperna, 1973 and 1979

Fig. 4.5 A

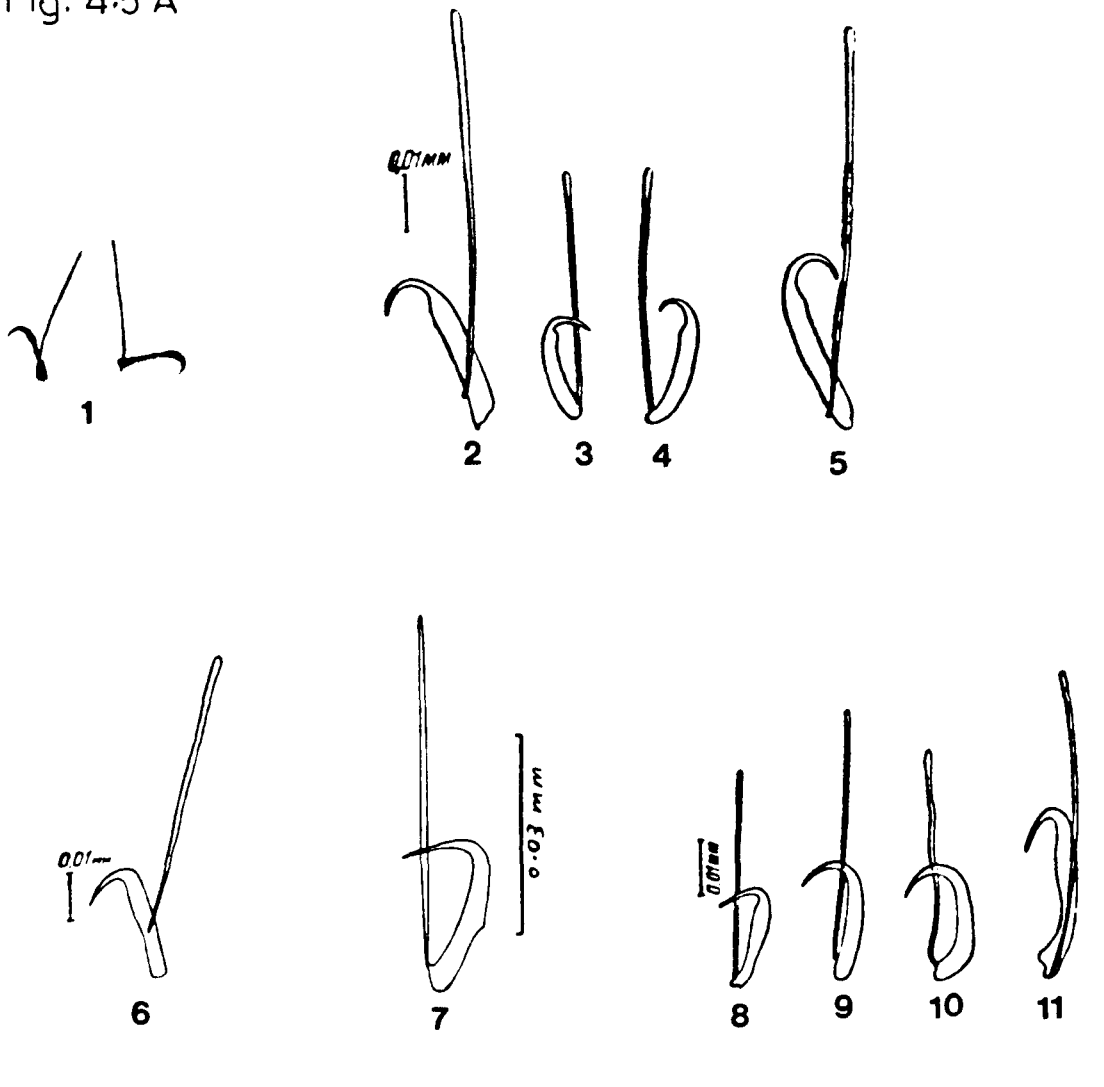
0,01mm
1
2
3
4
5
0.01mm
6
0.03 mm
7
0.01mm
8
9
10
11
30 µm
12
13
0,01 mm
14
0.01mm
15
0.01mm
16

Fig.4·5A continued

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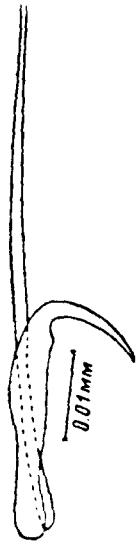

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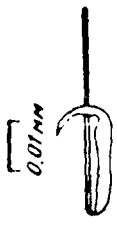

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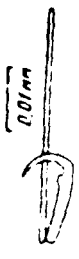

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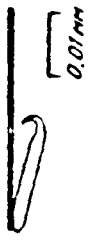

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Fig.4·5B

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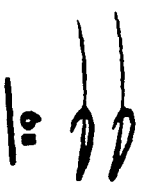


3

It is likely that the disappearance of these hooks might be related to the destroy them during the process of examination as that happened occasionally with D. homoion material used in this study. Gussev (1967) and Gussev and Kulemina (1971a and b) indicated that the change in the curvature along the length of the anchor and at the point coincided with that of growth of thickness of the secondary gill filaments of the host. They suggested that the most constant characters of Diplozoon species were the shape and size of the anchors which represented the most "ancient" organ.

5. Other morphological characters of the adult stage

The fine constrictions (Fig. 4.6A 1-4) have been used by few authors. The shape and thickness of these constrictions as shown in D. homoion (Chapter 3) were quite variable. Bychowsky and Nagibina (1959) thought that the wide ridges on D. paradoxum were similar to the fine ones. The sclerites of the clamps were used by Bychowsky et al. (1964) to distinguish D. markewitschi from other species (Figs. 4.6 B4 and 4.6 C4). I believe that the fair-leads of D. homoion and D. paradoxum are distinct (Figs. 4.6 B1 and 2), but that the differences between the sclerites, including the fair-lead, of the other species are not of taxonomic value. The variations in the shapes and sizes of the structures of the anterior parts e.g. suckers and pharynx (Fig. 4.6 D1-3) do not show any systematic importance as has been discussed earlier in Chapter 3.

6. Egg stage

The dimensions and shapes of the eggs of most species of Diplozoidae together with their characteristic features are given in Table 4.1 and Fig. 4.7A, B and C. It is clear that there was a considerable variation

Fig. 4.6 The fine constrictions, certain sclerites of the lower and upper parts of the clamps and the structures of the suckers and pharynx of some species of Diplozoidae reproduced from the original descriptions.

A. Fine constrictions

1. *D. paradoxum*	As revised by Bychowsky and Nagibina, 1959
2. *D. parlovskii*	Bychowsky and Nagibina, 1959
3. *D. homoion*	Bychowsky and Nagibina, 1959
4. *D. megan*	Bychowsky and Nagibina, 1959

B. Fair-lead sclerite on the posterior jaw of the clamp

1. *D. paradoxum*	As revised by Bychowsky, *et al.*, 1964
2. *D. homoion*	As revised by Bychowsky, *et al.*, 1964
3. *D. pavlovskii*	As revised by Bychowsky, *et al.*, 1964
4. *D. markewitschi*	Bychowsky *et al.*, 1964

C. Median sclerites on the anterior jaw of the clamps

1. *D. paradoxum*	As revised by Bychowsky, *et al.*, 1964

Fig. 4.6 (contd..)

2.	D. homoion	As revised by Bychowsky, et al., 1964
3.	D. pavlovski	As revised by Bychowsky, et al., 1964
4.	D. markewitschi	et al., 1964

D. Structure of the sucker and pharynx in the anterior region of the adult

1.	D. (Paradiplozoon) skrjabini	Akhmerov, 1974
2.	D. (P.) marinae	Akhmerov, 1974
3.	D. (P.) parabramidis	Akhmerov, 1974

Fig.4.6 A

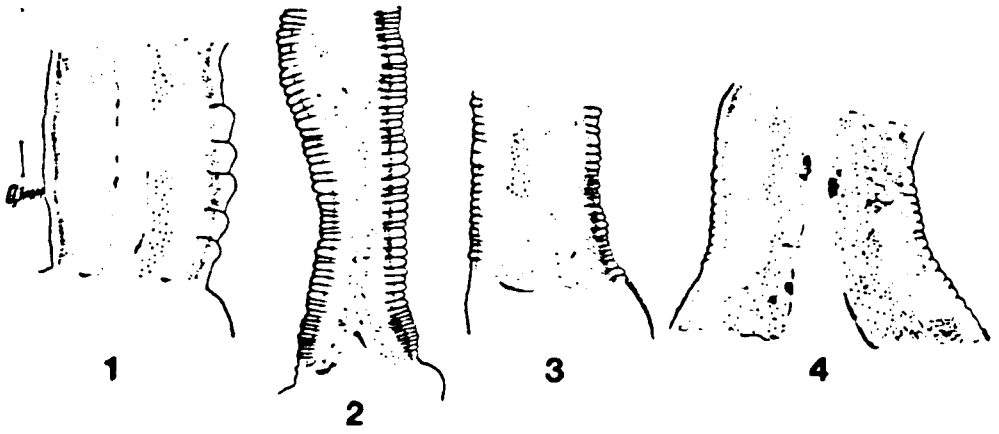


Fig.4.6B

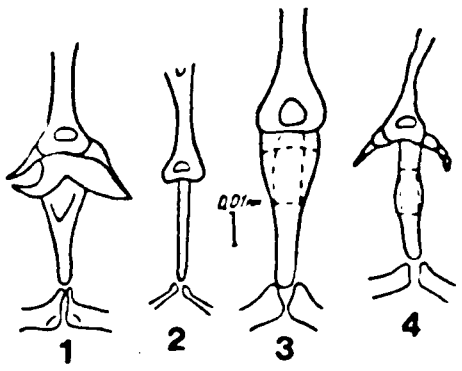
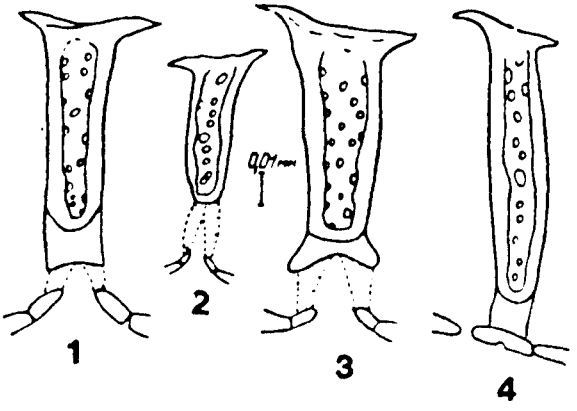
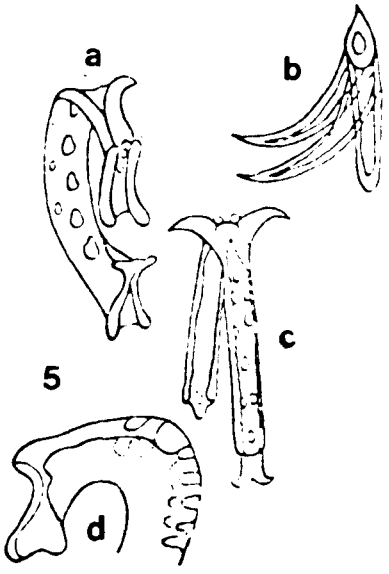


Fig.4.6 C





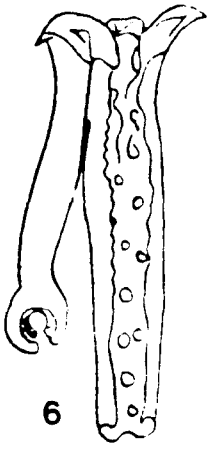


Fig.4.6 D

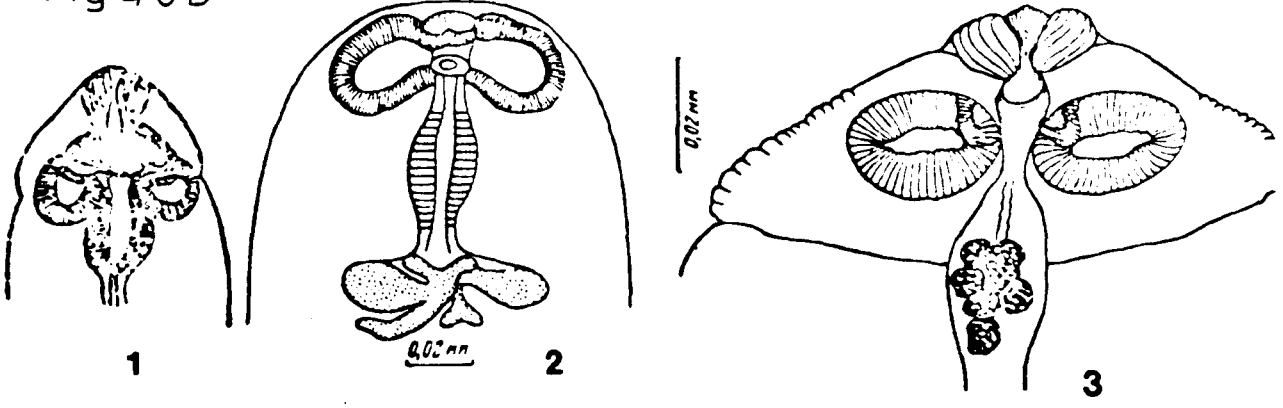


Table 4.1 The size and shape of eggs of species of Diplozoidae from the literature

Species	Dimensions (mm) Length x width	Shape and other characteristic features	Authors
		EUROPEAN AND RUSSIAN SPECIES	
D. nipponicum	0.25 -0.285 x 0.113 - 0.120	Oval, operculum at one pole and filament at the other	Kamegai et al., 1966; Kamegai, 1968 and 1974; Denis et al., 1983 and Khotenovskiĭ, 1975
D. barbi	0.200 x 0.080	Oval, bluntly pointed tip	Reichenbach-Klinke, 1951 and 1961
D. tetragonopterini	0.100 - 0.200 x 0.040 - 0.080	Oval, bluntly pointed tip	Sterba, 1957
D. paradoxum	0.317 - 0.520 x 0.084 - 0.140	Oval, thickness of wall was between 3-4 μm	Bychowsky and Nagibina, 1959; Bovet 1959 and Khotenovskiĭ, 1975
D. homoion	0.142 - 0.301 x 0.060 - 0.150	Oval, thickness of wall was 2-5 μm	Bychowsky and Nagibina, 1959 and Khotenovskiĭ, 1975
D. pavlovskii	0.250 - 0.280 x 0.110 - 0.140	Oval, thickness of wall was 3μm	Bychowsky and Nagibina, 1959
D. megan	0.250 - 0.336 x 0.106 - 0.159	Oval, rugby ball shape	Bychowsky and Nagibina, 1959 and Khotenovskiĭ, 1977a

Table 4.1 (contd..)

Species	Dimensions (mm) Length x width	Shape and other characteristic features	Authors
D. gussevi	0.170 - 0.329 x 0.120 - 0.155	Oval	Gläser and Gläser, 1964
D. paradoxum sapae	0.180 x 0.130	Oval	Reichenbach-Klinke, 1961
D. markewitschi	0.200 - 0.351 x 0.088 - 0.130	Oval	Bychowsky et al., 1964 and Khotenovskiĭ, 1975
D. inustiatus as Inustiatus inustiatus (discussed later)	0.270 - 0.310 x 0.110 - 0.130	Oval	Khotenovskiĭ, 1978
D. diplodiscus	0.370 x 0.090	Oval	Nagibina, 1965
D. bychowskyi	0.320 - 0.370 x 0.110 - 0.180	Oval	Nagibina, 1965
D. strelkowi	0.300 - 0.330 x 0.110 - 0.180	Oval	Nagibina, 1965
D. schizothorazi	0.130 - 0.330 x 0.150 - 0.240	Oval	Iksanov, 1965
D. tadzhikistanicum	0.310 - 0.450 x 0.130 - 0.200	Oval	Gavrilova and Dzhalilov, 1965
D. nagibinae	0.255 - 0.338 x 0.137 - 0.152	Oval	Gläser, 1965
D. rutili	0.251 - 0.367 x 0.142 - 0.184	Oval, rugby ball shape, thickness of wall was 6-11 μm	Gläser, 1967 and Khotenovskiĭ, 1975

Table 4.1 (contd.)

Species	Dimensions (mm) Length x width	Shape and other characteristic features	Authors
D. balleri	0.200 - 0.250 x 0.080 - 0.090	Oval	Nagibina et al., 1970
D. homoion homoion	0.250 - 0.300 x 0.100 - 0.150	Oval	Oliver and Reichenbach-Klinke, 197?
D. h. gracile	0.220 - 0.240 x 0.080 - 0.100	Oval	Oliver and Reichenbach-Klinke, 197?
D. (Diplozoon) mylopharyngodonis	0.297 - 0.300 x 0.118 - 0.120	Oval	Akhmerov, 1974
D. (Paradiplozoon) amurensis	0.240 - 0.320 x 0.060 - 0.130	Oval	Akhmerov, 1974
D. (P.) erythroculteris	0.190 - 0.320 x 0.085 - 0.100	Oval	Akhmerov, 1974
D. (P.) skrjabini	0.265 - 0.275 x 0.082 - 0.085	Oval	Akhmerov, 1974
	INDIAN SPECIES		
D. indicum	0.220 - 0.240 x 0.080 - 0.100	Oval	Dayal, 1941
D. kashmirensis	0.270 - 0.290 x 0.070 - 0.090	Oval	Kaw, 1950
D. cauveryi	0.260 - 0.270 x 0.100 - 0.116	Oval, fusiform, filament not present	Tripathi, 1959a

Table 4.1 (contd.)

Species	Dimensions (mm) Length x width	Shape and other characteristic features	Authors
D. soni	0.064 x 0.034	Oval	Tripathi, 1959a
Neodiplozoon barbi	0.152 x 0.091	Oval	Tripathi, 1959a and b
D. microclampi	0.225 - 0.231 x 0.115 - 0.119	Oval, thickness of wall was 4-5 µm.	Kulkarni, 1971
D. dayali	0.180 x 0.070	Oval, filament not present	Pandey, 1973
	OTHER ASIAN SPECIES		
D. minutum	0.030 x 0.012	Oval	Paperna, 1964
D. doi	0.126 - 0.168 x 0.070 - 0.103	Oval	Ha Ky, 1971
	AFRICAN SPECIES		
D. ghanense	0.260 x 0.115	Oval, filament not present	Thomas, 1957
D. aegyptensis	0.254 - 0.313 x 0.081 - 0.132	Oval	Fischthal and Kuntz, 1963
Neodiplozoon polycotyleus	0.180 - 0.220 x 0.140 - 0.160	Oval	Paperna, 1973 and 1979

Fig. 4.7 The eggs of species of Diplozoidae reproduced from the original descriptions

A. European and Russian species

1.	*D. nipponicum*	Kamegai, 1968 and 1974; Khotenovskiĭ, 1975 and Denis *et al.*, 1983
2.	*D. barbi*	Reichenbach-Klinke, 1954 and 1961
3.	*D. tetragonopterini*	Sterba, 1957
4.	*D. paradoxum*	Bovet 1959 and Khotenovskiĭ, 1975
5.	*D. homoion* a and b	Bovet 1967 and Khotenovskiĭ, 1975
6.	*D. megan*	Khotenovskii, 1977a
7.	*D. paradoxum sapae*	Reichenbach-Klinke, 1961
8.	*D. markewitschi*	Khotenovskiĭ, 1975
9.	*D. nagibinae*	Gläser, 1965
10.	*D. schizothorazi*	Iksanov, 1965
11.	*D. rutili* a and b	Gläser, 1967
12.	*D. (P.) skrjabini*	Akhmerov, 1974

B. Indian species

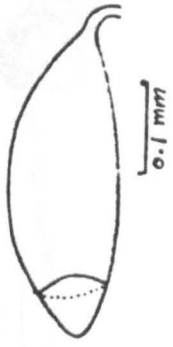
1.	*D. indicum*	Dayal, 1941
2.	*D. kaskmirensis*	Kaw, 1950
3.	*D. cauveryi*	Tripathi, 1959a
4.	*D. soni*	Tripathi, 1959a
5.	*Neodiplozoon barbi*	Tripathi, 1959 a and b
6.	*D. microclampi*	Kulkarni, 1971

Fig. 4.7 (contd.)

C. Other Asian and African species

1.	*D. minutum*	Paperna, 1964
2.	*D. doi*	Ha Ky, 1971
3.	*D. aristichthysi*	Ling, 1973 (quoted from Khotenovskiĭ, 1972)
4.	*Neodiplozoon polycotyleus*	Paperna, 1973 and 1979

Fig. 4·7 A



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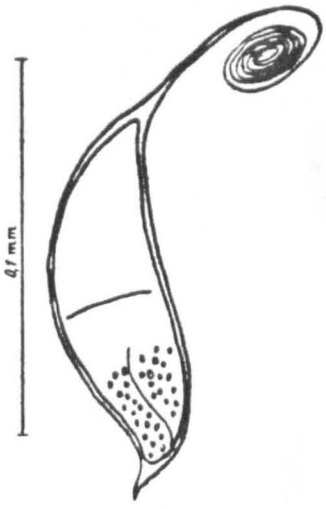

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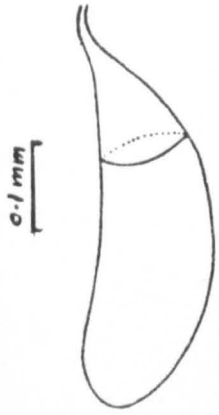

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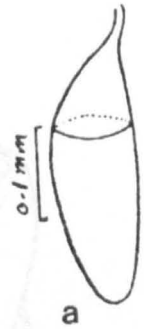

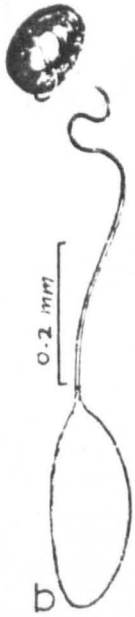

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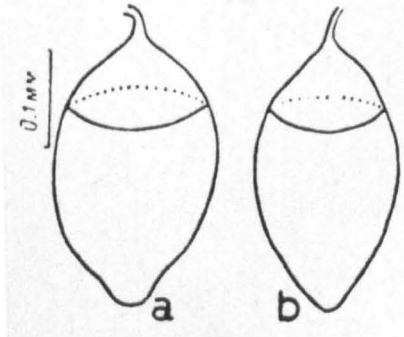

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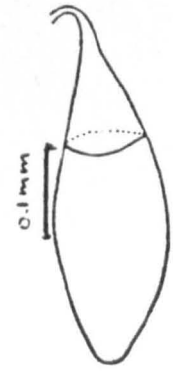

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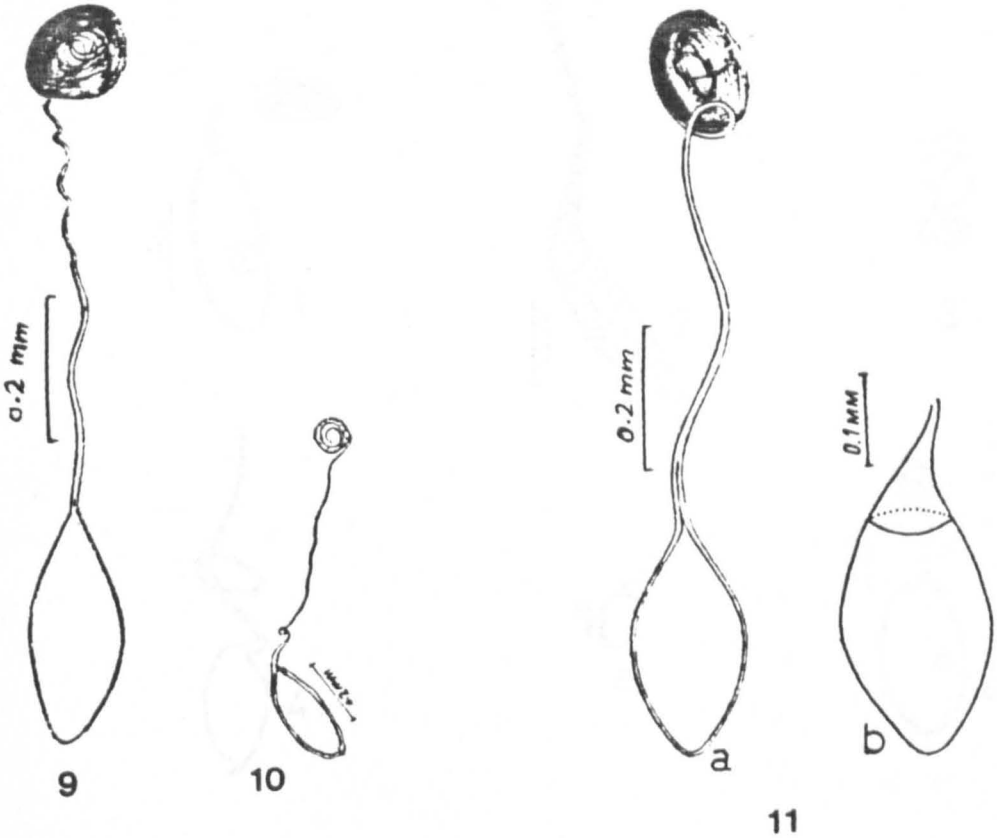
Fig.4.7 A continued



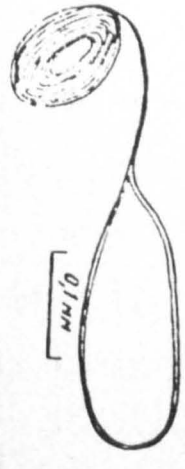


Fig.4·7B

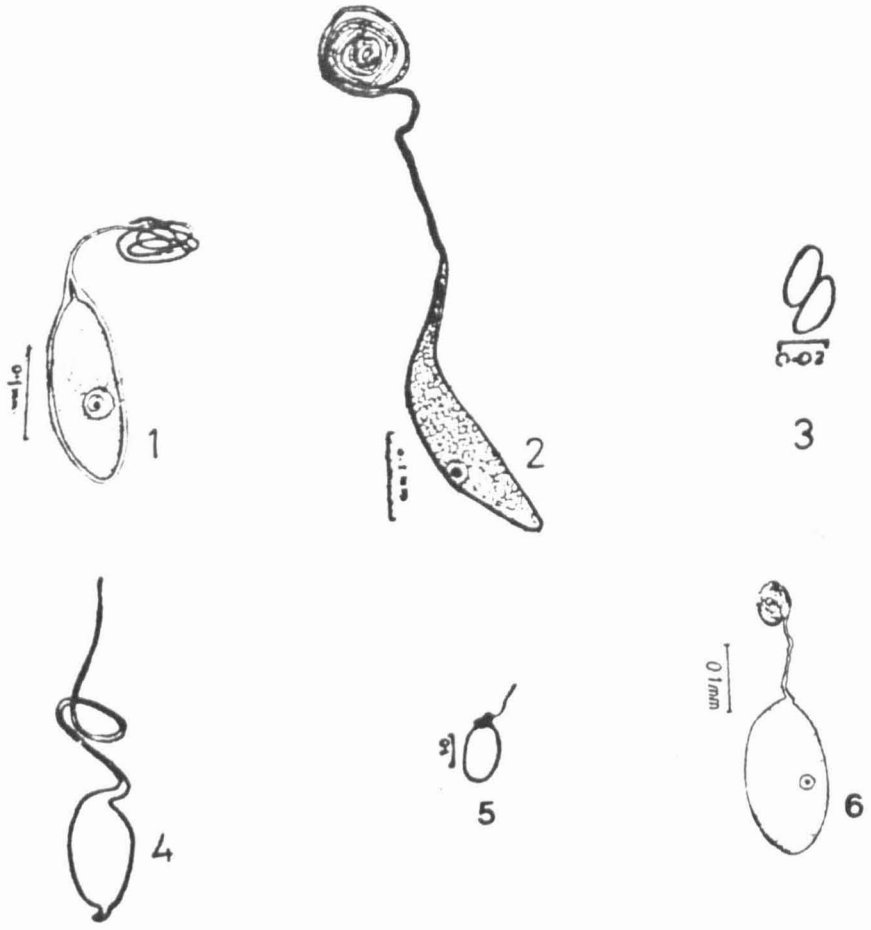
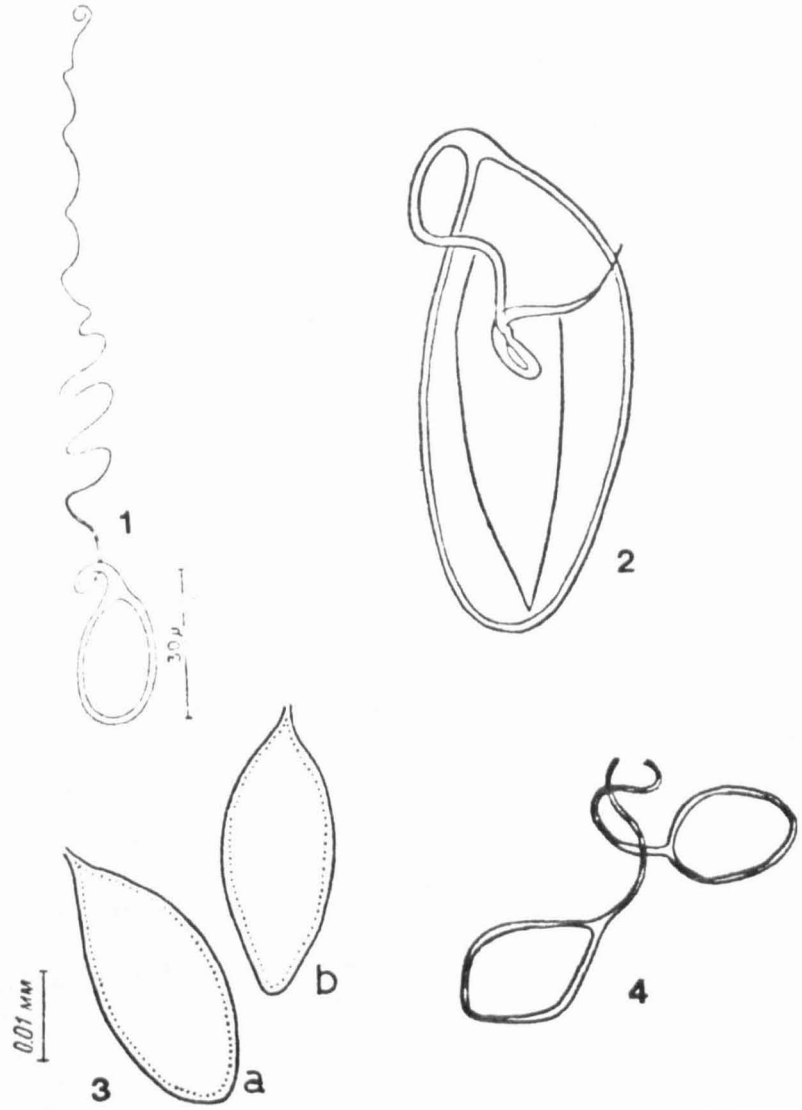


Fig.4·7 C



in the size of eggs of species of Diplozoidae from different parts of the world. The range of size of eggs of European and Russian species was 0.100 mm - 0.520 mm x 0.040 mm - 0.240 mm which was slightly higher than the egg sizes of Indian and African species. The range of egg sizes of Indian species was 0.064 mm - 0.270 mm x 0.034 mm - 0.115 mm and African species 0.180 mm - 0.292 mm x 0.107 mm - 0.160 mm. This difference may be attributed to the effect of water temperatures. The eggs of D. minutum and D. soni have a very small size (0.030 mm x 0.012 mm and 0.064 mm x 0.034 mm respectively) by comparison with other species.

In the European and Russian species (Table 4.1 and Fig. 4.7 A1-12) the difference between the size and shape of the eggs of D. paradoxum and D. homoion (Fig. 4.7 A4 and 5) is confirmed by my results given in Chapter 3. Bovet (1967) ranked D. paradoxum and D. homoion as two subspecies D. paradoxum paradoxum and D. p. homoion dependent mainly on the differences between the sizes of eggs of the two. The egg of D. rutili (Fig. 4.7 A11) has a rugby-ball shape and its size was slightly bigger than the previous ones. There was no clear information about the number and the source of the eggs used for identification purposes for most species. It was not stated whether or not these eggs had been measured within the gravid worm or after shedding. During the present observations on the eggs of D. homoion they were smaller than the normal while they were still inside the worm and some were without filaments especially in the early stages of development. Little variation was seen between the sizes of eggs of D. homoion laid by old, overwintering worms compared with young, summer adults. The measurements of the dimensions of the egg of any undescribed species should be carried out on a group of eggs which have been shed naturally to avoid any mistake in the

estimations of size. Oliver and Reichenbach-Klinke (1973) used these small variations in the eggs dimensions of D. homoion to divide the species into the subspecies D. homoion homoion and D. h. gracile. In D. barbi described by Reichenbach-Klinke (1951) and D. tetragonopterini described by Sterba (1957) (Fig. 4.7 A2 and 3), I suspect that the blunt point at the tip of the eggs may have originated either by observation of the eggs while still inside the gravid worm or by pressure of the coverslip during observation of the eggs after release.

The shape and size of the eggs of D. paradoxum sapae, D. schizothorazi and D. (P.) skrjabini (Fig. 4.7 A7, 10 and 12) resembled that of D. homoion (Fig. 4.7 A5), while those of D. megan, D. markewitschi and D. nagibinae (Fig. 4.7 A6, 8 and 9) were closely similar to those of D. rutili (Fig. 4.7 A11). Only the egg of D. nipponicum shows a significant difference from the rest. Its operculum was located at one pole and the filament on the opposite one (Fig. 4.7 A1) whereas all the other eggs of species of Diplozoidae have the filament and the operculum at the same pole of the egg.

In the Indian species (Table 4.1 and Fig. 4.7 B1-6), the shape of the eggs was generally oval. The eggs of D. cauveryi, and D. dayali were reported without filaments (Table 4.1 and Fig. 4.7 B3). My results (Chapter 5, life cycle), suggest that the egg filament of most species of Diplozoidae is important for successful completion of the life cycle of parasites. If the lack of this structure from the eggs of the Indian species is true, it will add another factor relevant to the evolution of these parasites. This will be discussed later. The egg of D. soni was very small (Table 4.1) and with a pointed tip at one pole (Fig. 4.7 B4) similar to the eggs of the European species D. tetragonopterini and D. barbi.

In other Asian species, the size of the eggs of D. minutum was remarkably small (Table 4.1 and Fig. 4.7 C1) while its shape like the others was approximately oval.

In the African species, again the eggs of D. ghanense were also without filaments similar to the eggs of Indian speices and the shape was also oval (Table 4.1).

7. Oncomiracidium stage

Few studies apart from those in Europe and Russia have used the characters of the larval stages for the taxonomy of the species. The morphology and the sizes of the oncomiracidia of D. paradoxum, D. gracile, D. markewitschi, D. rutili, D. megan and D. nipponicum are given in Fig. 4.8A, B, C, D, E, F, G and H respectively. Most of these larvae are illustrated from both dorsal and ventral surfaces. From these Figs. and the original descriptions it can be seen that they were all very similar. They have been distinguished from each other mainly by the size and shape of the ciliated epidermal cell, the distribution and distances between the papillae and on the distances between the epidermal cells apart from the larva of D. nipponicum which was quite different. From my study (Chapter 3), the variation in the size of the epidermal cells, the distance between the papillae, the presence or absence of some of these papillae from one larva to another were all variations which occurred on D. homoion. In the oncomiracidium of D. nipponicum (Fig. 4.8 H). Kamegai (1968) reported the presence of a pair of sticky glands just near the anterior border of the sucker. Later this character could not be seen by either Khotenovskiĭ (1976) or Denis et al. (1983). The pair of sticky glands by the oval sucker on the adult stage of this species is quite obvious as has been shown by many authors. They

Fig. 4.8 The oncomiracidia of some European and Russian species of Diplozoidae reproduced from the literature

A. D. paradoxum, ventral surface — Euzet and Lambert, 1971

B. D. gracile, ventral surface — Buzet and Lambert, 1971

C. D. homoion, — Khotenovskiĭ, 1975

1. ventral surface
2. dorsal surface

D. D. paradoxum — Khotenovskiĭ, 1975

1. ventral surface
2. dorsal surface

E. D. markewitschi — Khotenovskiĭ, 1975

1. ventral surface
2. dorsal surface

F. D. rutili — Khotenovskiĭ, 1975

1. ventral surface
2. dorsal surface

G. D. megan — Khotenovskiĭ, 1977a

1. ventral surface
2. dorsal surface

H. D. nipponicum — Denis et al., 1983

1. ventral surface
2. dorsal surface
3. posterior part of larva from ventral side showing the position of the 2 pairs of additional small hooks (hooklets)

Fig. 4.8 (contd.)

4. clamp structure

5. larval hook

6. lateral hooklet

Fig. 4·8

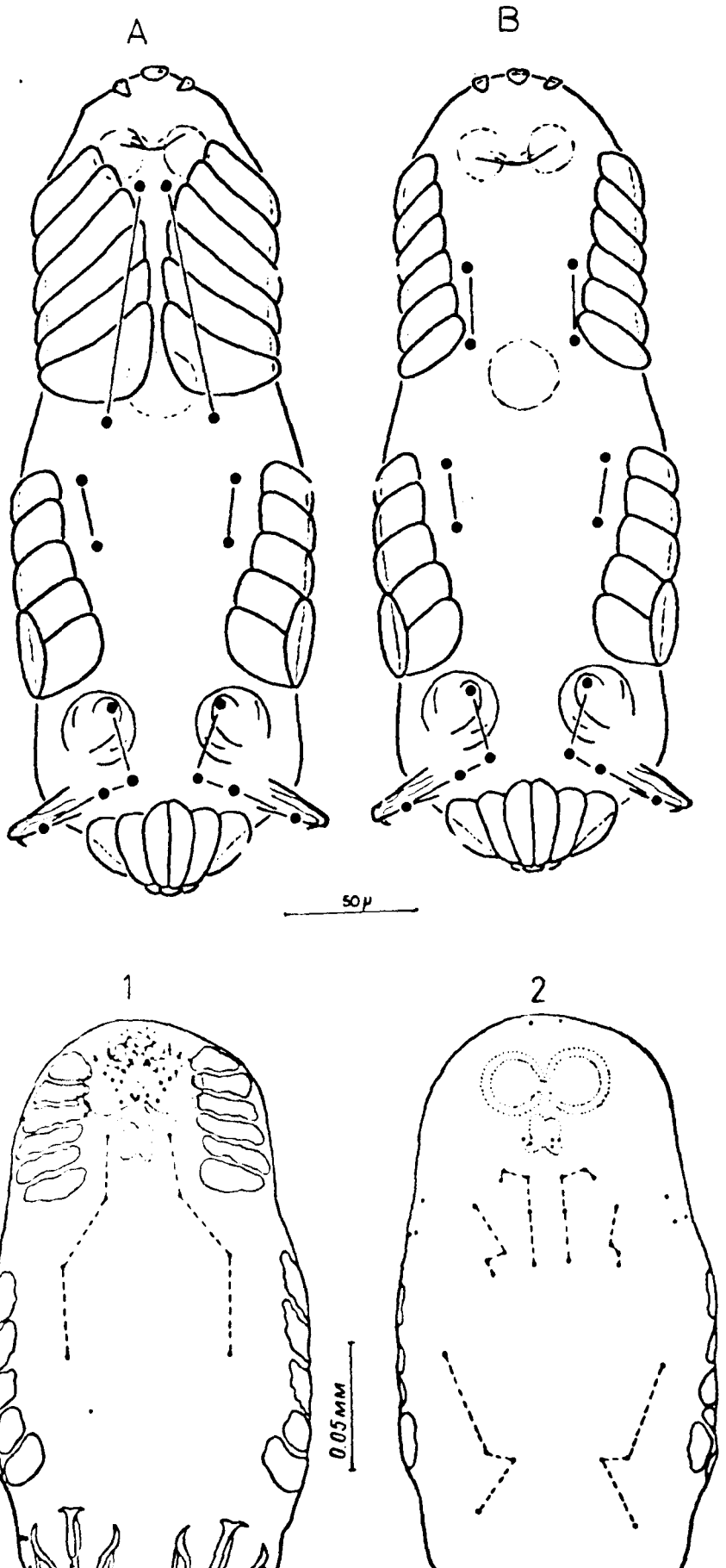
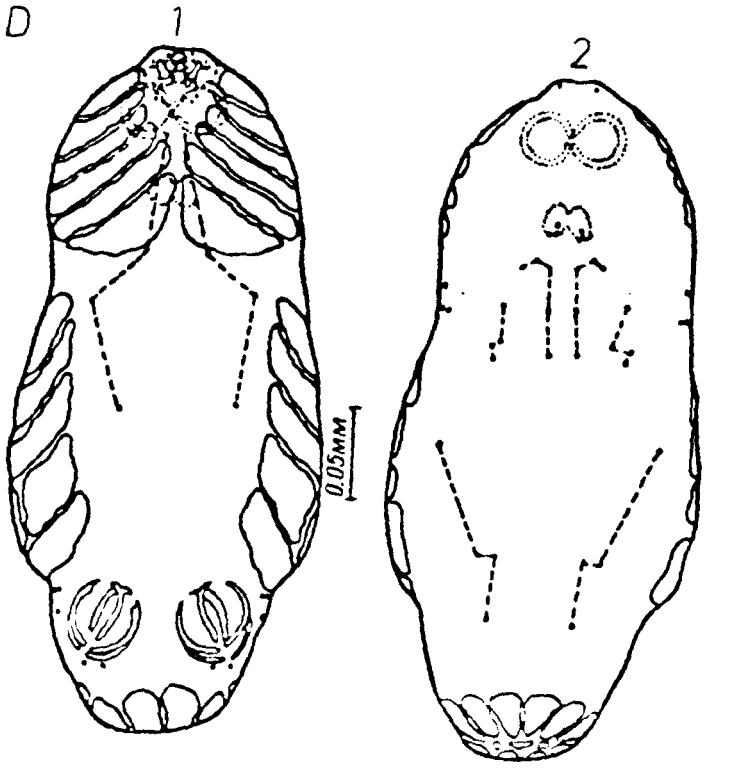


Fig.4.8 continued



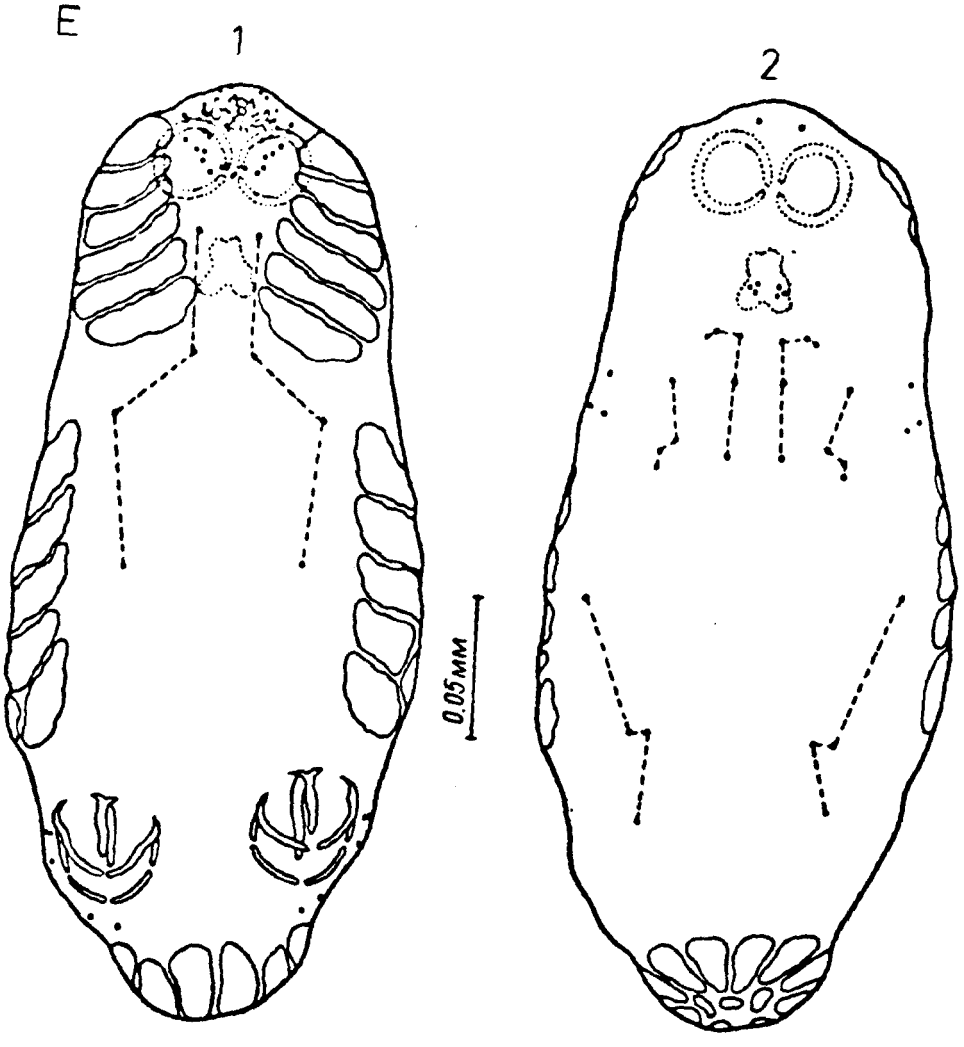


Fig.4.8
continued

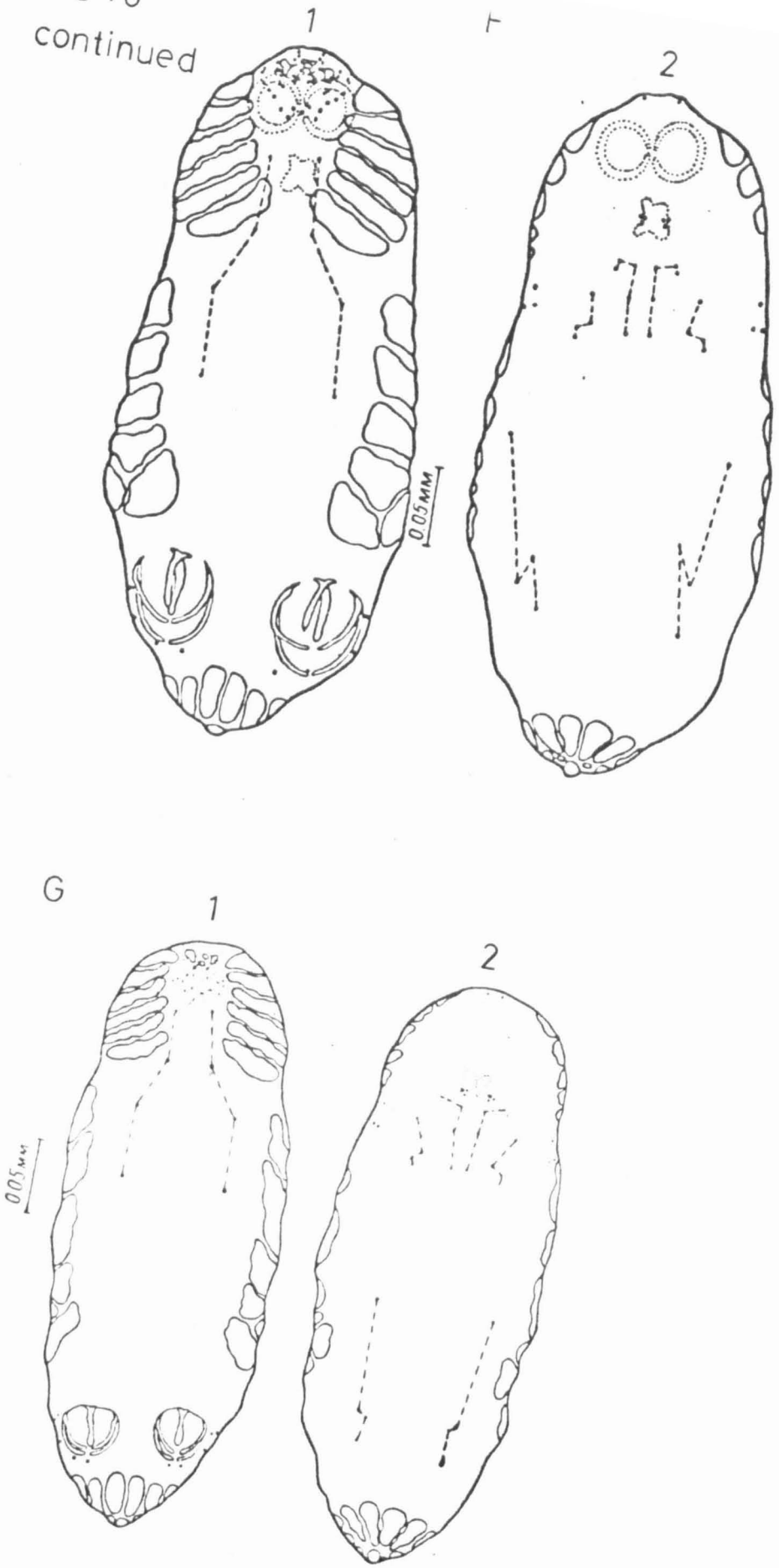
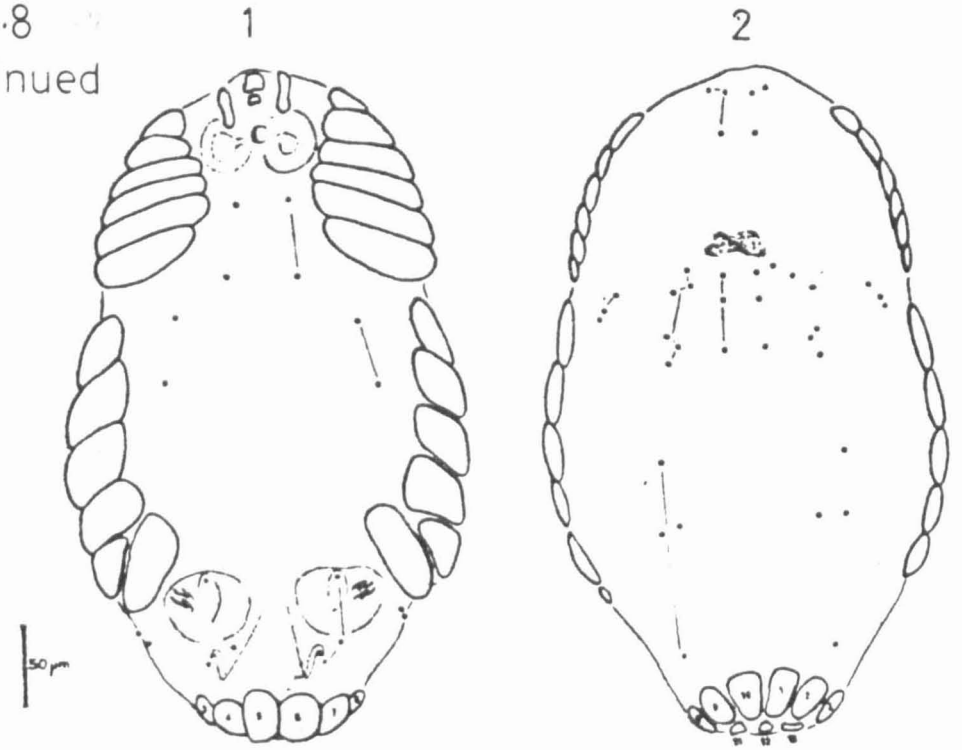
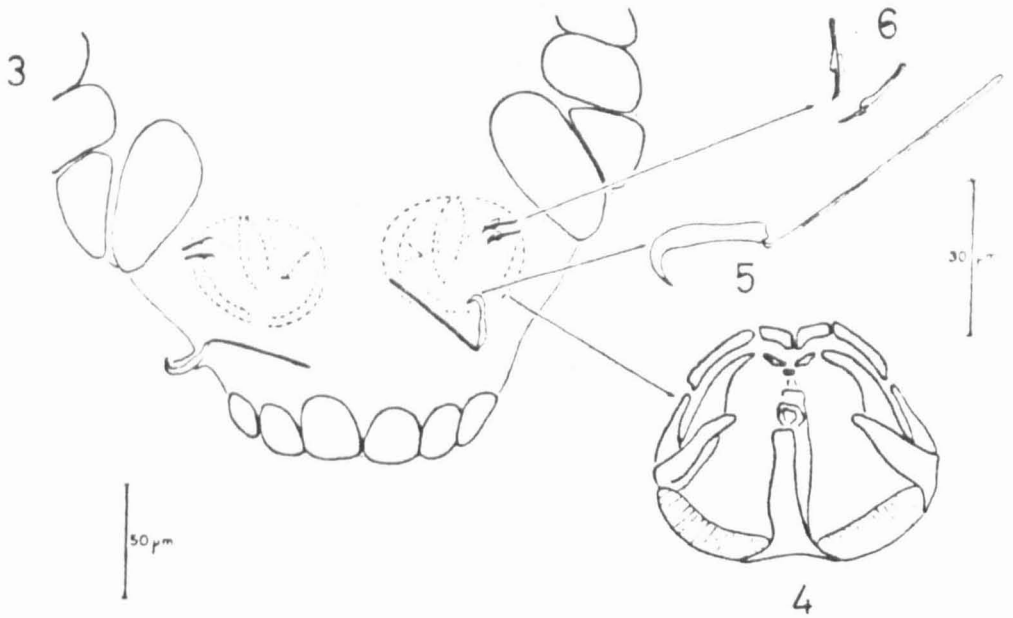


Fig.4.8 continued





are located at the same position as those described for the oncomiracidium. The second characteristic feature of this larvae is the arrangement of the 6th ciliated cell in the lateral ciliated group of cells (Fig. 4.8H 1 and 3). This will be discussed later. The third character is the discovery of two additional pairs of hooklets fixed laterally just above the pair of clamps (Fig. 4.8H 3 and 6) (Denis et al., 1983).

III. KEYS FOR IDENTIFICATION OF SPECIES OF DIPLOZOIDAE

There have been many keys devised for the identification of species of Diplozoidae. Most of them use the characters of the adult stage only. As can be seen from the example of Keys which follow, the same Diplozoon species is often distinguished by different characters.

The Key of Kaw (1950)

1. Intestine bifurcates into two branches behind the place of the union of two individuals and the branches unite posterior to testis 2

   Intestine runs as a single tube without branching behind the place of the union of two individuals and gives out lateral branches posterior to testis .....D. paradoxum

2. Testis lobed and lies midway between the crossing of individuals and the posterior margin of the body 3

   Testis smooth and lies more near the crossing of individuals than the posterior margin of the body .....D. indicum

3. A pair of sticky glands present at the entrance of the mouth .....D. nipponicum

   No sticky glands present at the entrance of the mouth .....D. kashmirensis n. sp.

The Key of Chauhan (1953)

1. Intestine bifurcates into two branches behind the place of the union of two individuals and the branches unite posterior to testis 2

   Intestine runs as a single tube without branching behind the place of the union of two individuals and gives out lateral branches posterior to testis .....D. paradoxum

2. Testis lobed and lies midway between the crossing of individuals and the posterior margin of the body 3

   Testis smooth and lies more near the crossing of individuals than the posterior margin of the body .....D. indicum

3. A pair of sticky gland present at the entrance of the mouth .....D. nipponicum

   No sticky glands present at the entrance of the mouth .....D. kashmirensis

The Key of Thomas (1957)

1. The clamps in the opisthaptors of the two individuals in permanent copula in four rows, each with four clamps 2

1' The clamps in the opisthaptors of the two individuals in permanent copula in two linear rows each with eight clamps .....D. barbi

2. The intestine with bifurcation in the area of fusion, the two branches reuniting behind testis 3

2' The intestine without bifurcation.

(a) Testes compact, occurring in region of fusion .....D. ghanense

(b) Testes lobed, occurring in posterior region behind point of fusion .....D. paradoxum

3. Testes lobed, occurring midway in the hind body behind the point of fusion, some distance in front of the cotylophore 4

3' Testes compact, occurring close to cotylophore .....D. indicum

4. A pair of sticky glands present near the mouth .....D. nipponicum

4' No sticky glands present .....D. kashmirensis

The Key of Tripathi (1959a)

1. All clamps on one side in a single series ..... *D. barbi*

   Clamps in two series of four each 2

2. Sticky glands present at the anterior end ..... *D. nipponicum*

   Sticky glands absent 3

3. Eggs without filament, intestine reticulate ..... *D. cauveryi*, sp. nov.

   Eggs with filament 4

4. Intestine ends in a single crus in the posterior part of body 5

   Intestine divided into two crura in hind part of body which again unite posterior to testis 6

5. Testis lobed ..... *D. paradoxum*

   Testis spherical ..... *D. soni* sp. nov.

6. Testis lobed, first and fourth clamp nearly equal in size ..... *D. kashmirensis*

   Testis entire, first clamp nearly twice the size of fourth clamp ..... *D. indicum*

The Key of Gussev (given in Bychowskaya-Pavlovskaya et al., 1962)

1(6). Anterior part of body of worm at least twice as large as posterior. Integument posteriorly with conspicuous folds.

2(5). Central portion of posterior part of body expanded in form of rounded cup, concave ventrally; distal part of intestinal trunk with or without lateral branches.

3(4). Distal part of intestinal trunk with lateral branches .....D. paradoxum

4(3). Distal part of intestinal trunk lacking lateral branches .....D. nipponicum

5(2). Central portion of posterior part of body without cuplike expansion.
Distal portion of intestinal trunk always without lateral branches .....D. pavlovskii

6(1). Anterior part of body of worm over twice as large as posterior. Posterior cuticle with inconspicuous folds.

7(8). Holdfast clamps of almost equal size, with delicate chitinoid components .....D. homoion

8(7). First pair of holdfast clamps hardly more than half as large as other three, clamps with thick chitinoid components .....D. megan

The Key of Fischthal and Kuntz (1963)

1. All clamps on one side in a single series ..... D. barbi

   Clamps in two series of four each 2

2. Intestine with bifurcation in area of fusion, reuniting behind testis..... 3

   Intestine without bifurcation 6

3. Egg without filament ..... D. cauveryi

   Egg with filament 4

4. Testis entire ..... D. indicum

   Testis lobed 5

5. Pair of sticky glands near mouth ..... D. nipponicum

   Sticky glands absent ..... D. kashmirensis

6. Testis lobed ..... D. paradoxum

   Testis entire 7

7. Testis occurring in region of fusion ..... D. ghanense

   Testis occurring in opisthohaptoral region 8

8. Egg very small, 0.064 x 0.034 mm ..... D. soni

   Egg large, 0.254 - 0.313 x 0.081 - 0.132 mm ..... D. aegyptensis

The Key of Diplozoon species of Europe (Reichenbach-Klinke, 1980)

1(14). Posterior part of the body enlarging smoothly to the end.

2(13). Gut in the posterior body with caeca (diverticles).

3(10). Gut in the hind body with more than 5 diverticles.

4(9). Gut in the hind body poorly anastomosing, clamps with different measures.

5(8). Last clamp smaller than the first ones.

6(7). Last clamps two-thirds as broad as the last but one, shaft of the larval hamulus more than 60 µm.....D. megan

7(6). Last clamp maximally half as broad as the last but one, shaft of the larval hamulus not more than 57 µm .....D. nagibinae

8(5). Last clamp slightly smaller than the first ones, large larval hamuli (shaft up to 66 µm, hooks up to 30 µm.....D. rutili

9(4). Gut in the hind body anastomosing like a net, clamps nearly equal in size .....D. paradoxum

10(3). Gut in the hind body with 5 or less diverticles, last clamps slightly smaller than the first ones.

11(12). Handle-ends of the clamps melt together, central buckle united with the front handle by a ribbon .....D. markewitschi (syn gussevi)

12(11). Handle-ends of the clamps not united, central buckle smoothly bent and grooved in the centre .....*D. homoion*

13(2). Gut in the posterior body without or with short diverticles .....*D. pavlovskii*

14(1). Posterior part of the body in the centre enlarged belly-like on both sides, gut in the hind body without diverticles .....*D. nipponicum*

(perhaps introduced)

IV. SPLITTING OF THE GENUS DIPLOZOON INTO SUBGENERA AND NEW GENERA

Akhmerov (1974) suggested that there was a need to divide the genus Diplozoon into two subgenera. He proposed D. (Diplozoon) and D. (Paradiplozoon) separated by the presence or absence of the invagination on the posterior part of the adult worms. He recognized only the presence of the invagination, but not the ridges (deep folds).

Some authors considered that certain morphological characters of the Diplozoidae were sufficiently distinct to justify the division of the species into new genera. In the Indian species, Tripathi (1959a) created a new genus Diplotrema barbi for adult diplozoids which had broad bilobed, symmetrical opisthaptors, with the two lobes generally folded on themselves and having 18-28 pairs of clamps. Larval hooks were not observed, the intestine was reticulate, and the egg oval with a thick shell. He soon (1959b) changed the name of genus Diplotrema to Neodiplozoon as he found that the name Diplotrema had already been given to another group of parasites. Price (1967) separated these two genera Diplozoon and Neodiplozoon as follows:

" - Genito-intestinal canal present, two adults permanently fused together in the form of an<<x>>:

1. Eight pairs of attaching clamps on hapter of each adult member ..... Diplozoon
2. Numerous pairs of attaching clamps on hapter of each adult member ..... Neodiplozoon"

Later on, Khotenovskiĭ in 1978, 1981 and 1982 re-ranked most of the species of Diplozoidae into a new genera. In 1978 he stated that D. aristichthysi from Aristichthys sp. in China was a synonym of

D. inustiatus from Hypophthalmichthys molitrix in the Far East of the U.S.S.R. At the same time he proposed the genus Inustiatus with I. inustiatus n. comb. as the type and only species. The diagnostic features of this genus were: a lateral uterus in the middle of the anterior body with the ova lying in the uterus with backwardly directed filaments. He included both species under I. inustiatus. A comparison between the drawings of D. inustiatus (Fig. 4.2 A13) and that of D. aristichthysi (Fig. 4.2 C2) reveals that they both have the uterus and the genital apertures located in the anterior parts of the body. The shapes of the posterior parts of the bodies of the two species are also the same, with the ovarys and testes placed adjacent to the invagination in both. However, the eggs of the two species differ, a feature discussed later.

In 1981 he proposed that the subgenus D. (Paradiplozoon) created by Akhmerov (1974) be raised to generic status. So in his opinion, all Diplozoon species which look similar to D. homoion should be placed in this new genus. Khotenovskiĭ (1981) also made a revision of the Diplozoidae from all parts of the world and rearranged them into 5 genera as shown in Fig. 4.9 A-E. He suggested the following key for these proposed genera depending on presence or absence of the expansion (the invagination) on the posterior part, the arrangement of the intestinal branches and the position of the opening of the genital pores.

Fig. 4.9. The posterior parts of the five genera of Diplozoidae as propsed by Khotenovskii, 1981

A. Diplozoon

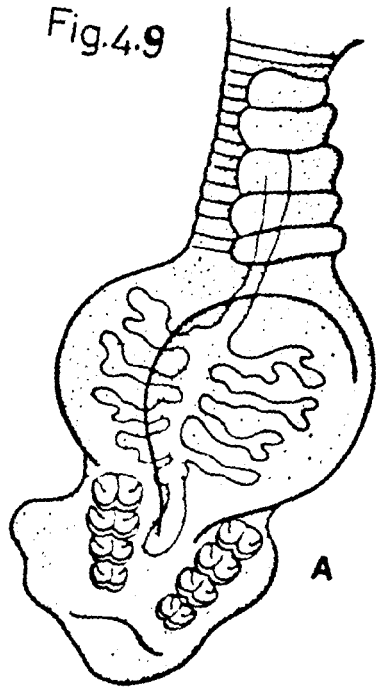
B. Sindiplozoon

C. Paradiplozoon

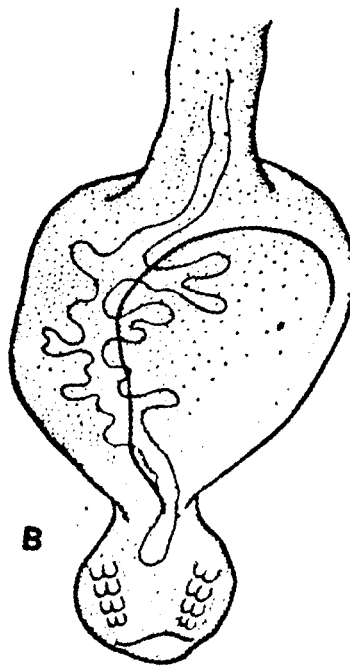
D. Neodiplozoon

E. Inustiatus

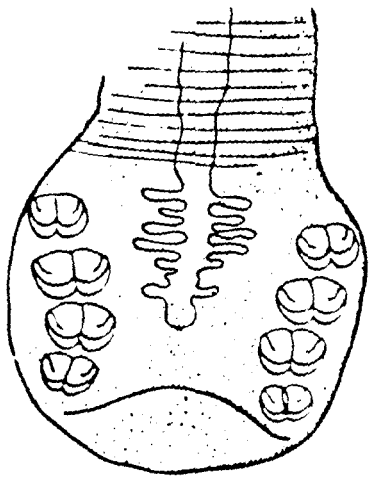
Fig.4.9



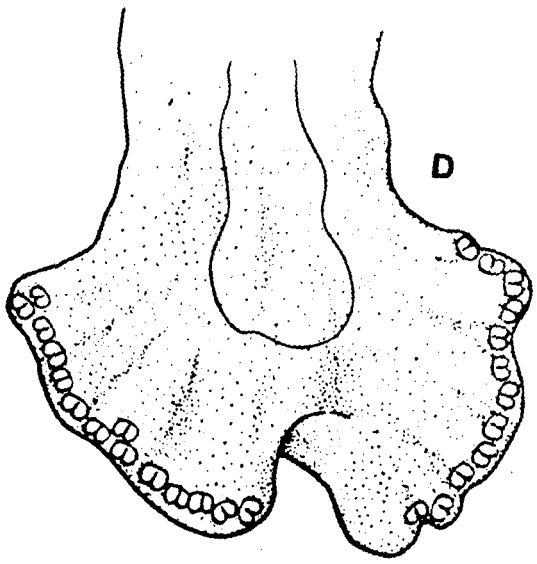
A



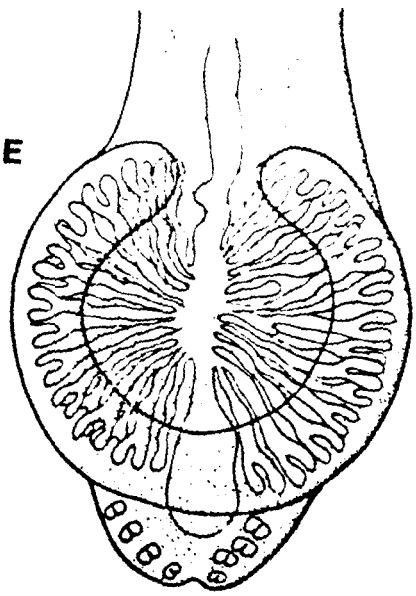
B



C



D



E

Key to the genera of Diplozoidae of Khotenovskiĭ, 1981 (Translated from the Russian)

1(4). Number of clamps numerous

2(3). Number of clamps more than 15 pairs, arranged along the edge of posterior portion, Indian ..... Neodiplozoon Tripathi, 1960

3(2). Number of clamps less than 15 pairs, arranged on both sides of hind part, African ..... Afrodiplozoon gen. n.

4(1). Number of clamps 4 pairs

5(6). Median portion of posterior part has no expansion ..... Paradiplozoon Achmerov, 1974

6(5). Median portion of posterior part has expansion.

7(8). Expansion has cup-like shape parasites of Palearctic fishes ..... Diplozoon Nordmann, 1832

8(7). Expansion has different shape. Parasites of Amur - Chinese fishes.

9(10). Sexual pore opens at the middle level of anterior part. Expansion covers all the intestinal diverticulae... Inustiatus Khotenovskiĭ, 1978

10(9). Sexual pore opens at the junction area of front and hind parts. Intestinal diverticulae partially cover expansion ..... Sindiplozoon gen. n.

Khotenovskii (1981) did not classify D. nipponicum in his key to genera. He also separated these genera on the relationship of the length of

the posterior part of the body in relation to the diameter of the third clamp of the opisthaptor. He also divided the genus Neodiplozoon into two genera, one, Neodiplozoon, from Indian localities, with more than 15 pairs of clamps on the opisthaptor of adult worms and the other, Afrodiplozoon, on African fishes, with less than 15 pairs of clamps.

Later, Kornakova (1983) studied in detail the histology of the frontal parts especially the mouth structure and the gland system of five species of Diplozoidae. She depended mainly on the Khotenovskiĭ (1978, 1981 and 1982) nomenclature. She reported D. nipponicum in this study as Eudiplozoon nipponicum, but I have been unable to find the author who proposed this new genus for D. nipponicum.

Before making a detailed assessment of the genera suggested by Khotenovskiĭ in the light of Karnakova's observations and my own studies, it seems relevant to state that these genera have closely similar characters. Khotenovskiĭ, in his taxonomic study gave priority to the host specificity and highly variable morphological characters to create differences between species of the same genus, while he considered constant characters like that of the invagination, the ridges on the posterior part of the body and the branches of the intestine in the posterior part to represent generic characters. However, Mayr (1949) stated that it will usually be found, on closer examination, that good characters are correlated to a number of other characters. Where there is a break between two systematic categories, this break will generally affect a whole group of different characters. It is thus important, according to his opinion, to base systematic categories and classification schemes on as many characters as possible. He believed that the fewer the characters used, the greater is the danger that mistakes in the classification will be made. I think that the genera Paradiplozoon and Sindiplozoon

are invalid, their species belonging to the genus Diplozoon. The taxonomic position of the species of these genera will be discussed later. Both the genera Inustiatus and Eudiplozoon appear valid as they have characters of taxonomic value to separate them from the other genera. For example, in D. nipponicum (which it has been proposed should be placed in the genus Eudiplozoon), the adults, the eggs and the larvae show significant differences from other species. The adults of D. inustiatus (= Inustiatus inustiatus) have very significant distinguishing characters. I agree with the proposals to keep them as two separate genera rather than in the genus Diplozoon.

The value of the characters used to define the genus Neodiplozoon (Tripathi, 1959a and b), which include N. barbi from India and N. polycotyleus and N. grassitrema from Africa have been discussed in the literature. Oliver and Reichenback-Klinke (1973) found that there were abnormalities in the number of and structure of the clamps on the diporpal stages of D. homoion gracile. The number of clamps reached 5-6 clamps on each side of some unpaired diporpa, whereas in the normal development they do not normally exceed 4 pairs of clamps in the members of genus Diplozoon. They attributed the abnormalities to climatic conditions, particularly water temperature. It is relevant to state that all Neodiplozoon species have been recovered from localities of high water temperatures (Indian and African territories). In addition, the numbers of pairs of clamps in the adult stages of these species are not constant on the two halves of the same specimen as they are in other Diplozoon species. The number of pairs of clamps also differed between the young and gravid worms as explained earlier (see also Fig. 4.2 D3a and b). Owing to this information, it seems probable that the members of the

genus Neodiplozoon are close to and developed from the genus Diplozoon. This hypothesis should be tested by critical experimental studies on the life cycle stages of these parasites at different water temperatures. The broad, bilobed distal portion of the opisthaptor described on the adult of Neodiplozoon barbi, with numerous clamps (Figs. 4.2 B5 and 4.3 B3) may be the result of an assymetrical arrangement of the blocks of clamps on the opisthaptor.

V. EVALUATION OF SPECIES OF DIPLOZOIDAE

From this review, it is clear that the systematic work on Diplozoidae species during last 40 years has been concentrated on the worthless taxonomic characters of the adult stage, but has ignored other stages of the life cycle which in most species are still unknown.

My review of the taxonomic characters used for the identification of these parasites, together with the presentation of the original drawings, and, by contrast with the variation and value of each character to taxonomy as shown by the detailed study of D. homoion given in Chapter 3, indicates that the following species of Diplozoidae are probably valid:

1. European and Russian Species

   A. D. nipponicum. The characters of the adult, egg and larval stages suggest it is a valid species. It has recently been placed in a new genus Eudiplozoon. I have been unable to trace a description of this genus, but agree with the separation from the genus Diplozoon.

   B. D. diplodiscus, D. bychowskyi, D. strelkowi and D. (Diplozoon) mylopharyngodonis which have the invagination on the posterior part without large ridges (deep folds) appear to be identical and are synonyms. Priority should be given to the name D. diplodiscus Nagabina, 1965.

   C. D. paradoxum, D. nagibinae and D. balleri which have the invagination and the large ridges (deep folds) on the posterior part of the adult worm also appear to be synonyms. They should be included under the name D. paradoxum Nordmann, 1832 which has priority. They are specific to the genus Abramis (see

Chapter 2, Table 2.1).

D. D. inustiatus and D. aristichthysi which have the genital pores opening on the anterior part of the worm. This character is quite distinct from all other species. The presence of an invagination on the posterior part and the position of the reproductive organs in relation to it as well as the manner of egg laying in D. aristichthysi and the egg shape of D. inustiatus confirm they are distant from the other members of the genus Diplozoon. Thus, these characters justify the removal of these two species into the genus Inustiatus. The two species should be separated from each other as I. inustiatus and I. aristichthysi on the basis of their egg shapes.

E. D. homoion and all other species and sub-species indicated in this chapter which lack the invagination and the large ridges (deep folds) on the posterior part all are one species, except D. megan, D. markewitschi and D. rutili. Priority should be given to the name D. barbi Reichenbach-Klinke, 1951.

F. Although D. megan, D. markewitschi and D. rutili have a similar morphological appearance to the adults of D. homoion (= D. barbi), they had eggs with a fusiform (rugby-ball) shape. I suggest they represent one species D. megan Bychowsky and Nagibina, 1959.

2. In the Indian, other Asian and African species of Diplozoon, the morphology of the adult and egg stages seem to be identical except for the size of the eggs of D. minutum and D. soni. The presence of a marked constriction separated the opisthaptor from the region of fusion on the adult of

D. ghanense (Thomas, 1957). This constriction also seems to occur on some Indian species, D. indicum and D. cauveryi (Fig. 4.3 B1 and 3 respectively). The shape of the intestine in the posterior region of these species was similar extending as a single caecum behind the testis or even closer to the fourth clamp. No egg filament was seen on some Indian as well as African species. It can be suggested that the species of these two geographic regions are close to each other and may belong to one or two species of Diplozoon. It is impossible without detailed taxonomic study to suggest whether or not these species represent one or two of the European and Russian species. All the Indian and African species are without the invagination and the large ridges (deep folds) on the posterior part of the paired worms which confirms their position in the genus Diplozoon. Neodiplozoon species were also found on both Indian and African fishes. The systematic position of these species of this genus was discussed earlier.

In D. minutum, the presence of the large ridges (deep folds) on the posterior part was similar to D. paradoxum, but the size of the eggs was significantly different from D. paradoxum. It is difficult without seeing specimens to make any further comment about the origin and its relationships to the other species of Diplozoidae. We also need details about the life cycle of this species.

VI. CLASSIFICATION AND PHYLOGENY OF SPECIES OF DIPLOZOIDAE IN RELATION TO OTHER GROUPS OF MONOGENEA

A. Previous Position of Species of Diplozoidae

When D. paradoxum was discovered in 1832, there was no clear understanding about the position of this species in relation to the other monogenean groups.

The Order Monogenea was divided by Odhner (1912) into two sub-orders, the Monopisthocotylea and the Polypisthocotylea, characterized by the presence or absence of a genito-intestinal canal, i.e. a connection between the oviduct and the right caecum, rarely the left.

Price (1936) proposed the Superfamily Diclidophoroidea. The parasites belonging to this Superfamily have a pair of buccal suckers on the prohapter and an opisthaptor comprising four or more pairs of clamps, or a few suckers, each organ having cuticular skeletal supports or sclerites.

Price (1936) named a new family Discocotylidae in which the parasites are characterised by possessing a terminal haptor, usually Linguiform, with 4 pairs of clamp-like suckers, and with 1 or 3 pairs of terminal hooks. The genital atrium is small, and unarmed. Vaginae, when present, have marginal openings located in the anterior part of the body a short distance posterior to the level of the genital aperture. Price (1936 and 1943) also divided this family into 3 subfamilies: Anthocotylinae, their parasites with an armed cirrus; Discocotylinae, in which the parasites have the haptor armed with a single pair of hooks, testes postovarial; and Vallisinae, in which the hapter is armed with 1 to 3 pairs of hooks unlike those of Discocotylinae, testes preovarial. He ranked the genus Diplozoon with 2 other genera Octomacrum and Discocotyle in the subfamily Discocotylinae. The Key for these genera given by him was:

Key to genera of Discocotylinae proposed by Price (1943)

1. Mature individuals fused in form of letter X ..... Diplozoon Nordmann 1832

   Mature individuals not fused as above 2

2. Genital sucker present; single testis, extensively lobed..... Octomacrum Mueller 1934

   Genital sucker absent, follicular testes ..... Discocotyle Diesing 1850

Later Palombi (1949) proposed that the Genus Diplozoon should be removed from Discocotylinae as proposed by Price (1943) and placed into a new subfamily Diplozooninae in which the intestine was not bifurcate. According to Palombi the name of subfamily vallisinae change into Microcotylinae of Monitcelli. Tripathi (1959a) suggested that on the basis of the number of clamps, the two genera Neodiplozoon and Diplozoon should be placed in the Microcotylidae, but they were precluded from being placed there owing to their body form and typically discocotylid clamps. The genera Diplozoon and Neodiplozoon are peculiar in having all the genital organs confined to the posterior parts of the body behind the site of fusion. The genital aperture in all the other genera of Discocotylidae and Microcotylidae is in anterior part of body behind the pharynx. Therefore, Tripathi proposed the removal of Diplozoon from Discocotylidae and placing it along with Neodiplozoon in Diplozooidae. He also reported that Diplozooidae was more nearly related to Discocotylidae than to Microcotylidae in the structure of its clamps, and, as far as the unlimited number of the clamps was concerned it shared a common character with Microcotylidae. All genera of Discocotylidae and Microcotylidae have many testicular follicles but the genera of the

family Diplozooidae have only one testis behind the ovary.

Yamaguti (1961) agreed with the transfer of the genus Diplozoon to the family Diplozooidae (Tripathi, 1959a), but with an emended spelling from Diplozooidae to Diplozoidae. But Lambert (1980b) put these parasites under the family Discocotylidae.

Price (1967) suggested that it was quite likely that the genera Diplozoon and Neodiplozoon were closely related and he rejected Tripathi's opinion that Neodiplozoon was related to Microcotyle because of the presence of numerous pairs of clamps, and because the host-parasite relationships did not indicate any such affinities. So Price (1967) proposed that Diplozoon was ancestral to Neodiplozoon, the additional pairs of clamps in the latter arising as a secondary phenomenon.

Khotenovskiĭ (1981) created a new subfamily Neodiplozoinae for the genus Neodiplozoon and put all the other genera Inustiatus, Sindiplozoon, Paradiplozoon and Diplozoon under the subfamily Diplozoinae (with one 'o' after 'z') which had already been proposed by Palombi (1949). Khotenovskiĭ was incorrect when stated Palombi (1949) was an author for the family Diplozoidae. He also agreed with Tripathi's (1959a) opinion to separate Diplozoidae from Discocotylidae. But he suggested that Diplozoidae and Discocotylidae had a similar evolutionary origin.

B. Notes on the Origin and the Evolution of Species of Diplozoidae

Lambert and Denis (1982) described in detail the structure of the oncomiracidia of D. nipponicum and found a significant difference between some structures of this larva and larvae of other species of Diplozoidae. Firstly, the presence of two additional pairs of hooklets fixed laterally above the two clamps (see Fig. 4.8 H1-6) has been described earlier.

Secondly, according to Lambert and Denis (1982) the arrangement

of the sixth ciliated epidermal cell in the median lateral group of the oncomiracidium of D. nipponicum (Fig. 4.8 H1) indicated that this species was closely similar to the larva of Discocotyle sagittata (Fig. 4.10B as given by Owen, 1970), a solitary monogenean belonging to the family Discocotylidae while in other Diplozoon it is as in Figure 4.10A. The dissimilarity between these two larvae in the number of posterior ciliated cells can be seen from Fig. 4.10A and B. However Kamegai (1968) stated that there was a pair of adhesive glands on the larva of D. nipponicum located near to the oral sucker. These are exactly similar in position to the glands found on the adult stages of this parasite as observed by many authors (see earlier). But Khotenovskiĭ (1975) and Lambert and Denis (1982) could not see these glands on the oncomircidium of D. nipponicum.

Many taxonomists believe that the larval stage is the most important stage in the systematics of the monogeneans. The different structures of this stage should be a great help to facilitate the study of evolution of these parasites. Lambert (1980a) believed that the larval haptor should be the basis in the taxonomy because of the attachment systems having strong adaptative variations. Llewellyn (1963) suggested that the osmo-regulatory system may be of more use for the identification of species than as a guide to phylogenetic relationships while the development of the haptor has yielded the most useful clues to the probable evolutionary trends in monogeneans.

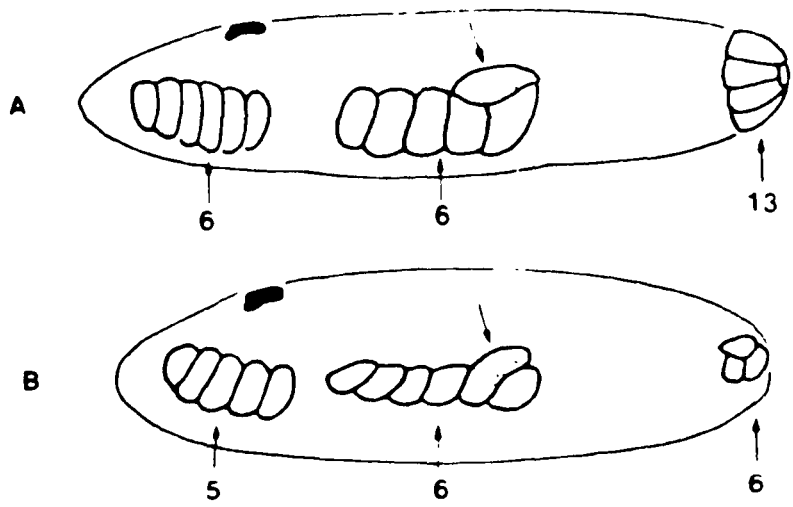
Kearn (1978) found that the oncomiracidia of Diplozoon taken from Abramis brama, Phoxinus phoxinus, Gobio gobio, had two laterally directed cups, each of which contained a single rhabdomere, and in addition, the larvae possessed two other eyes, each located near the lateral border of the head region. The presence of a single pair of eyes has been

Fig. 4.10. Lateral view of the oncomiracidial stages of:

A. D. paradoxum

B. Discocotyle sagittata (according to Owen, 1970)

From Lambert (1980b)



proposed as a distinctive feature of the larvae of many polypisthocotylinean monogeneans with most monopisthocotylinean larvae having two pairs of eyes (Llewellyn, 1963; Lyons, 1972). Lambert and Denis (1982) confirmed the findings of Kearn (1978) when they also found the second additional pair of lateral photoreceptors on the oncomiracidium of D. nipponicum. During my study the oncomiracidium of D. homoion stained with haemotoxylin also showed the presence of the second pair of photoreceptors.

According to all these data regarding the structure of oncomiracidium, as well as about the shape and structure of eggs of Diplozoon species, including D. nipponicum, and Discocotyle sagittata, Lambert and Denis (1982) proposed the scheme of evolution of species of Diplozoon as shown in Fig. 4.11 A-E, in which they suggested that Discocotyle was ancestral to Diplozoon.

As a result of my review on the morphological appearance of these parasites, I agree with Tripathi (1959a) that Diplozoidae should be in a separate family. Also there are two additional factors which need to be considered in the evolution of these parasites:

Firstly, the absence of the filaments from eggs of some species of Diplozoidae, especially the Indian and African ones. If correct, it means that the eggs of some Diplozoon species are quite similar to the eggs of Discocotyle. Also the eggs of Inustiatus inustiatus have one filament in each pole, which is similar to some eggs of species of Microcotylidae.

Secondly, the position of the genital aperture about the middle of the anterior region of adult I. inustiatus and I. aristichthysi (Fig. 4.2 A13 and Fig. 4.2 C2) may be primitive compared to its position at the junction in all other Diplozoidae. This would mean that the species of Diplozoidae are closely similar to Microcotylidae in relation to

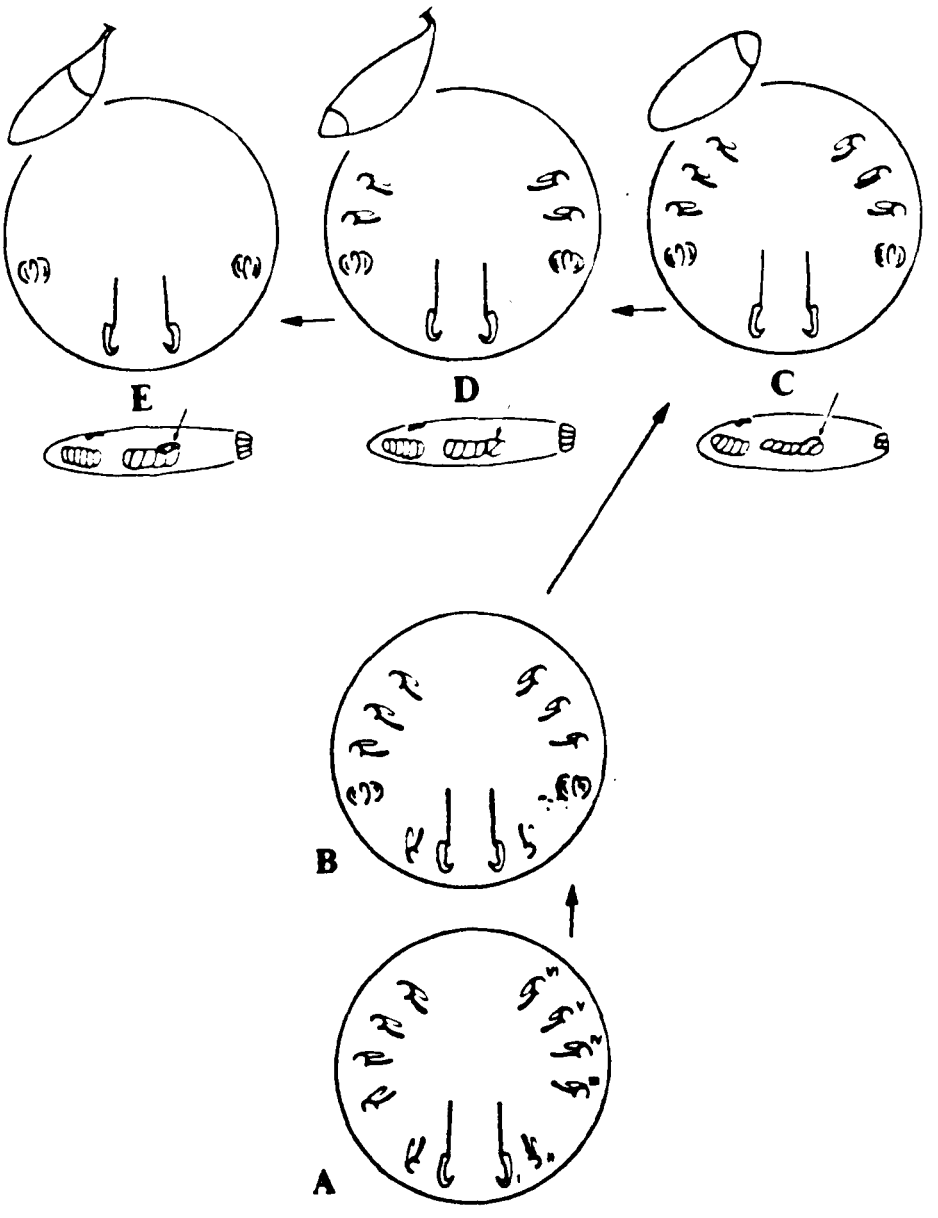
Fig. 4.11. The line of evolution of Discocotylidae proposed by Lambert and Denis (1982), in relation to the structure of the larval adhesive organs and the eggs of these groups of parasites.

A and B, hypothetical haptors

C, Discocotyle sagittata

D, Diplozoon nipponicum

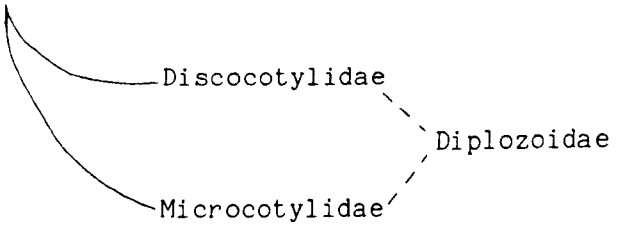
E, other Diplozoon spp.



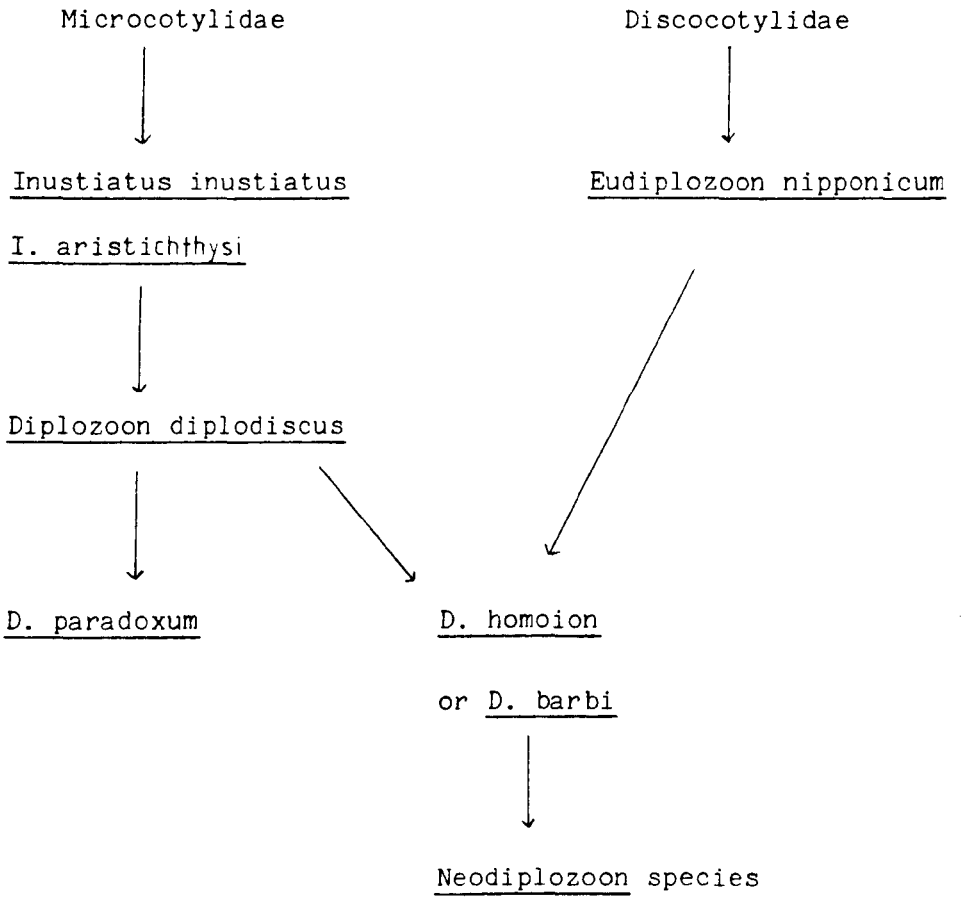
these characters. The position of the reproductive organs in I. inustiatus and I. aristichthysi and the numerous clamps on Neodiplozoon species indicate the close relationship between the Diplozoidae and Microcotylidae.

Therefore, I think that the genus Inustiatus is another ancestral form of Diplozoidae as well as the genus Eudiplozoon. Furthermore, Kornakova (1983) studied the mouth region and gland system of many species of Diplozoidae and found that Eudiplozoon nipponicum and Inustiatus inustiatus represented one morphological group while the other species from another group. As a consequence, there is a need for a detailed study of the life cycles of these two species because of their importance in the evolution of Diplozoidae. It must be remembered that most members of the Microcotylidae and Discocotylidae are parasites of marine fishes while all members of the Diplozoidae are parasites of freshwater fishes, specific to Cyprinidae and some Characidae.

In the light of this study it is suggested that the Discocotylidae, Microcotylidae and Diplozoidae have the same origin, but species of Diplozoidae may have developed from both families as follows:



The evolutionary relationships of the genera and some species of Diplozoidae may be as follows:


Microcotylidae
Discocotylidae
Inustiatus inustiatus
Eudiplozoon nipponicum
I. aristichthysi
Diplozoon diplodiscus
D. paradoxum
D. homoion
or D. barbi
Neodiplozoon species

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CHAPTER 5

THE LIFE CYCLE OF *D. HOMOION*

I INTRODUCTION

The life cycle of D. paradoxum was first described by Nordmann (1832). Later, many of his observations were found to be incorrect. Siebold (1851) and Zeller (1872) made observations on the establishment of the larvae of these parasites on the gills of the host and demonstrated the conjugation of two individuals to form the compound worm.

At present, unfortunately, very few experiments on the life cycle stages of Diplozoon species are available. The majority of the work is mostly confined to estimation of the longevity of some stages (Bovet, 1959 and 1967; Sterba, 1957; Khotenovskii, 1977a and b; Bychowsky, 1957). Furthermore, few have attempted to correlate observations on the life cycle stages in the laboratory with the establishment of the new generation of parasites in the field.

In Britain, there has been only one study on egg-laying and hatching rhythms in D. homoion gracile (Macdonald and Jones, 1978) although others have investigated the development of vitelline cells and the Mehlis gland (Halton et al., 1974 and 1976; Stranock and Halton, 1975).

Owing to this lack of information, the opportunity has been taken to investigate the longevity period of each stage of the life cycle of D. homoion under controlled laboratory conditions, using for this purpose the advantage that infection of D. homoion are transferable between various cyprinid species as shown in Chapter 3.

It is thought that the current work also contributes to a clarification of the systematic position of D. homoion.

II MATERIALS AND METHODS

A. Seasonal Variation of the Size of Adult Parasites

The adult *D. homoion* collected from the gills of *Rutilus rutilus* from Llyn Tegid every month (as described in Chapter 6) were carefully measured. Details of fixation were described in Chapter 3. The measurements were carried out with the aid of light microscope using a cavity slide containing the parasite in a drop of fixative.

B. Development of Reproductive Organs of *D. homoion*

Adult specimens of *D. homoion* collected from various sizes of fishes and months were stained with Gower's carmine (Gower, 1939) to show the reproductive organs, especially the ovary. The number of parasites used each month was dependent on the number of infected fishes available and on the abundance of parasites at each period of time. The developmental stage of the ovary and the number of shelled-eggs in each gravid parasite was recorded every month.

C. Fecundity and Formation of Clusters of Eggs

The 5 infected fishes used in these experiments were obtained from Llyn Tegid at different periods of time. For this reason the experiments could not be carried out at the same time.

Four experiments were designed to demonstrate the fecundity and formation of clusters of eggs. The live fishes used in each experiment were collected by gill netting and brought back to the laboratory in an aerated water tank. In the laboratory, the fishes were placed into half-filled tanks containing clean, previously aerated tap water at a temperature between 18^{o}-21^{o}C. To maintain this range of temperatures, aquarium thermostats and heaters were used.

At the end of each experiment the fishes were killed and their

fork length and weight measured, then their gills were carefully checked for any gravid adults or larval (diporpa) stages which were settled there. The examination of the gills was carried out immediately after killing the fish by isolating the individual gills into separate small dishes containing tap water or fish saline and were then carefully checked under the dissecting microscope. The water of the tank was filtered first through a hand net to retain the large egg clustered, immediately followed by a test sieve with pores of an aperture 150 μm (approximately similar to the length of the eggs of D. homoion) in order to collect the individual eggs. Then, the walls of the tank, the sieve and the hand net were washed several times and the washing water checked for eggs. The eggs of D. homoion collected were distributed into many small dishes containing tap water. The number of unhatched and hatched eggs were all counted under a dissecting microscope. Some clusters of eggs were mounted in glycerine jelly for further microscopic studies.

The number of fishes used in each experiment and the period of incubation were:

Experiment 1, one fish was used for 3 days;

Experiment 2, one fish was used for 11 days;

Experiment 3, one fish was used for 14 days;

Experiment 4, two fishes were used for 14 days.

D. Egg Hatching

Hatching rhythms of the eggs were determined by collecting the eggs daily from a tank containing two infected Rutilus rutilus caught from Llyn Tegid by gill net. The procedure for filtering the water and collecting the eggs was described in Section C. The eggs were cleaned carefully each day and used as follows:

1. At laboratory conditions (16 hours light, 8 hours dark photoperiod) at 18°-21°C air temperature:
   a. 5 small dishes with tap water, each containing 4 eggs
   b. 5 small dishes with distilled water, each with 4 eggs
   c. 5 small dishes with distilled water mixed with mucus material from the outer surface of a dead fish, each with 4 eggs.
   d. 5 small dishes with tap water, each containing 4 eggs collected from the faecal material of the fishes.
2. At 10°C with a similar light exposure (16 hours light, 8 hours dark), another 5 small dishes were used each with tap water and 4 eggs. At the end of the experiment (20 days later), the 5 dishes with all the unhatched eggs were transferred to the laboratory conditions to see if the eggs were still viable.

In all the experiments listed above, the development and hatching of the eggs were examined and recorded daily until the last egg in each dish had hatched.

E. Longevity of the Oncomiracidium of D. homoion

For each of these experiments twenty oncomiracidia hatched during a known period of time were necessary. To obtain these, clusters of eggs were gathered from the tanks in which infected R. rutilus were maintained. The clusters were observed under a stereomicroscope and oncomiracidia hatching over a 20 minutes period were collected. Normally this time was sufficient to obtain the required number of larvae, but if not, the process was repeated until twenty oncomiracidia were collected within the one period. In total, eight groups of twenty larvae each were used for the experiments.

For each group, 4 small dishes containing tap water were used and 5 larvae were transferred to each dish making the grand total for

every period 20 larvae. The dishes were kept under controlled conditions (16 hours day light, 8 hours dark, 18^{o}-21^{o}C).

These larvae were examined after periods of: 24 hours, 12 hours, 6 hours, 5 hours, 4 hours, 3 hours, 2 hours and 1 hour. As it was not possible to continuously observe the larvae in the dishes, the first period of time used was the longest (24 hours). The subsequent periods were progressively reduced until the minimum period of 1 hour was reached. By so doing the average longevity of the oncomiracidia was determined.

The number of live and dead larvae were counted at the end of each period. The modes of swimming of these larvae were also recorded during their short life span and especially the sluggish movements when they approached the end of their life. All other morphological changes were also recorded during their lives. To achieve this larvae were collected at various times during their life span and either treated with silver nitrate to show their ciliature and papillae or prepared for SEM observation. These procedures are described in detail in Chapter 3.

F. Longevity of the Diporpa and Juvenile Stage and the Commencement of Sexual Maturity of Adult D. homoion

The main host used in this study was the fry of Leuciscus leuciscus (see Chapter 3, Table 3.1) because these fishes were readily available from the fisheries unit within the department when this experiment began. These fishes also proved easy to handle in large numbers in the same tank which facilitated this part of the study. This decision was reached after it had been shown that L. leuciscus could be infected by D. homoion as demonstrated in Chapter 3.

Before they were used for the experiments, 30 of these fishes were carefully examined and were found not to be infected with Diplozoon parasites. Then, the experiment was begun by adding more than 150 fry altogether to the tank containing 2 infected Rutilus rutilus with D. homoion producing a massive number of eggs. The infections on the Rutilus rutilus had been maintained in the tank for 14 days to ensure the presence of infective oncomiracida. A sample of eggs from the water of this tank was checked before the experiment began to ensure that oncomiracidia were being released in the conditions prevailing within the tank. The water temperature of the tank was kept between 18^{o}-21^{o}C by using aquarium thermostats and heaters.

Then, during the first 5 days, 20 fry were randomly collected from the tank every day by a hand net. They were killed and their gills examined very carefully for any larval (diporpa) stages. The number of each stage was recorded. As these stages were highly active it was impossible to determine the number settled on each particular gill arch. This was owing to small size of the gills of these fry. The rest of the fry were left for 5 days further exposure to the infection so that sexually mature parasites could be obtained. On the tenth day, another 20 fry were examined, and any infection recorded and determined, especially the sexual maturity of the newly adult individuals.

The behaviour of these life cycle stages on the gills was also recorded. Other observations were also made on the unattached diporpae which were seen on the bottom of the dish after finishing the examination of the gills. Some unpaired diporpae on the gills of fry and an unattached one were prepared for SEM examination while other life cycle stages were stained with haematoxylin and trichrome stains for permanent preparations as described by Chubb (1962).

III RESULTS

A. Longevity of Adult D. homoion

Although a wide range of size variation was seen within the parasite population recovered from different fork lengths of Rutilus rutilus (Chapter 3), the mean size of parasites (length x width of anterior and posterior regions) from five length classes remained relatively constant during all months as shown in Table 5.1. No data were available for the mean sizes of parasites during July 1983 (as indicated in Chapter 6). The only comparable data available were the sample of infected Rutilus rutilus with D. homoion collected during July 1984. It was found that 7 infected fishes (23-25.5cm long) contain 43 parasites. The length of each of seven parasite specimens was more than 5.0mm while the rest (36) were less than 2.5mm. Also many other small specimens of parasites were recovered during August, September and October 1983.

B. Development of Reproductive Organs of D. homoion

Only the ovary was used during this study. The main ovarian stages identified throughout the year were:

Stage 1, Ovary very small and difficult to identify, seen only as a group of cell rudiments near the junction region. The posterior part of the adult worm where the ovary is located is not enlarged (Fig. 5.1);

Stage 2, The ovary is large and well-developed, occupying most of the area between the junction region and the testis. The ovum cells have a clear outline. They are large, rounded to square in shape, and contain a big circular nucleus placed in the middle. The posterior region of the adult worm where the ovary is located is enlarged (Fig. 5.2);

Stage 3, The ovary is more developed than in stage 2, the cells become

Table 5.1 Seasonal mean dimensions of adult *D. homoion* collected from certain fork lengths of *Rutilus rutilus* from Llyn Tegid. Measurements are given in mm.

Date	19-19.9 cm long fishes			20-20.9 cm long fishes			21-21.9 cm long fishes			22-22.9 cm long fishes			23-23.9 cm long fishes		
	Total number of Parasites	Anterior region Length x width	Posterior region Length x width	Total number of Parasites	Anterior region Length x width	Posterior region Length x width	Total number of Parasites	Anterior region Length x width	Posterior region Length x width	Total number of Parasites	Anterior region Length x width	Posterior region Length x width	Total number of Parasites	Anterior region Length x width	Posterior region Length x width
Feb. 1983	2	2.8x0.7	1.6x0.3	0	-	-	2	2.4x0.9	1.5x0.4	2	2.1x0.6	1.3x0.3	3	3.3x0.9	1.8x0.4
Mar.	2	3.3x0.9	1.9x0.4	9	2.8x0.9	1.6x0.4	9	3.3x1.0	2.0x0.4	8	3.1x1.1	1.9x0.4	3	3.8x1.1	2.1x0.4
April	7	2.5x0.8	1.5x0.27	12	2.9x0.8	1.8x0.3	0	-	-	3	2.4x0.8	1.6x0.2	3	3.5x1.0	2.0x0.4
May	0	-	-	4	2.6x0.8	1.5x0.3	15	3.3x1.1	2.0x0.4	8	3.6x1.1	2.2x0.4	8	3.7x1.0	2.4x0.4
June	0	-	-	0	-	-	1	3.1x1.1	1.7x0.4	7	3.8x1.3	2.2x0.4	2	4.3x1.2	2.4x0.4
Aug.	0	-	-	0	-	-	1	2.1x1.1	1.4x0.3	4	1.4x0.5	1.1x0.3	1	2.7x0.7	1.6x0.3
Sept.	0	-	-	0	-	-	0	-	-	0	-	-	8	3.2x0.8	2.0x0.3
Oct.	8	2.6x0.6	1.6x0.3	6	2.9x0.6	1.8x0.3	11	2.9x0.7	1.7x0.3	4	2.8x0.7	1.8x0.3	3	3.2x0.8	2.0x0.4
Nov.	0	-	-	0	-	-	4	2.8x0.8	1.7x0.4	0	-	-	6	3.0x0.8	1.6x0.4

Fig. 5.1. The posterior region of adult D. homoion showing the ovary at stage 1. jr, junction region; ov, ovary; t, testis.

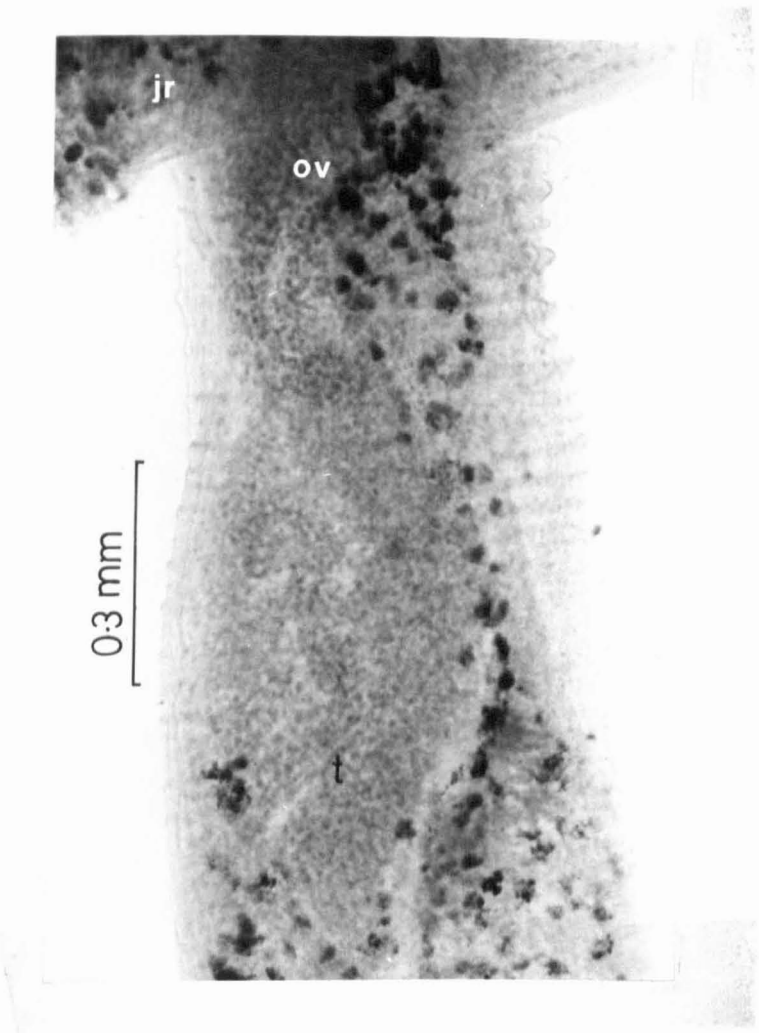
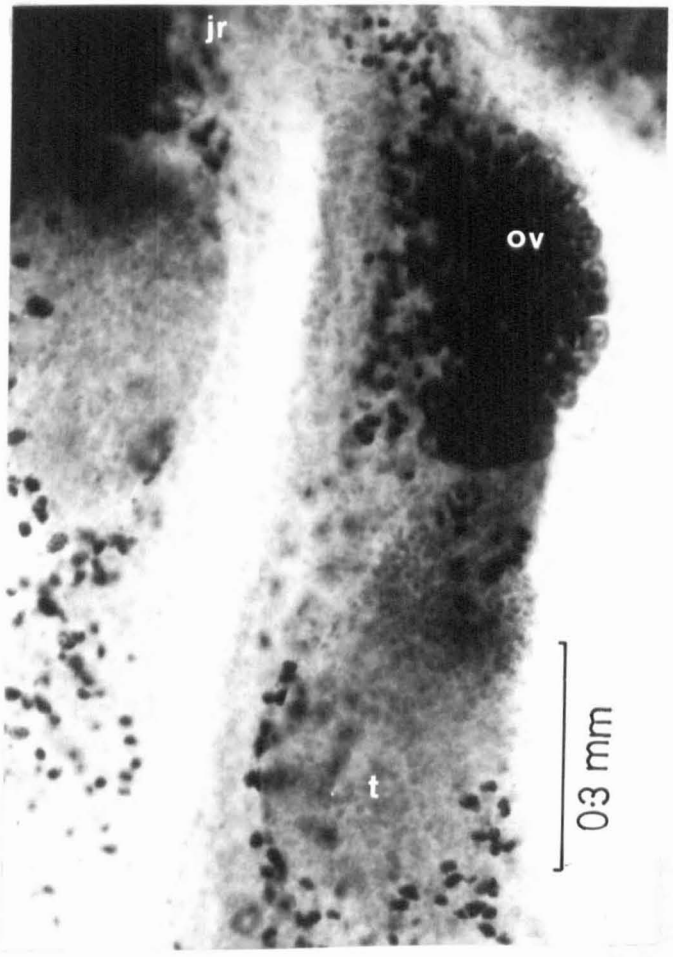


Fig. 5.2. The posterior region of adult D. homoion showing the ovary at stage 2. jr, junction region; ov, ovary; t, testis.



larger and occupied one-third of the posterior region of worm. This stage can also be identified by the presence of the reservoir containing the mature vitellocytes which are crowded into it (Fig. 5.3);

Stage 4, The ovaries of both posterior regions of the adult parasite become fully developed. Fertilization and egg shell formation have occurred. The stage can be exactly identified by one or two eggs at the junction area or near to the two ovaries (Fig. 5.4);

Stage 5, The ovaries and vitelline glands are at highest level of activity in this stage. A peak of fertilization and shelled-egg production takes place. The adult parasite has 3 or 4 eggs near the ovaries or around the junction area (Fig. 5.5).

Many D. homoion were seen with their ovaries at intermediate conditions between stages 1 and 2. Most of these intermediates were grouped with stage 1 especially during the winter, but others approaching stage 2 during autumn and spring were grouped with this latter stage.

The seasonal changes in the development of the ovaries of adult D. homoion on the gills of Rutilus rutilus from Llyn Tegid are shown in Table 5.2. It is obvious from these results that the main period of egg production (ovaries at stages 4 and 5) occurred between May to October during 1983. Only one specimen out of 20 parasites examined during December 1983 was found with one egg. Also 3 parasites out of 22 (13.6%) examined during October 1982 were found each with one egg. The gravid parasites with 3-4 shelled-eggs in the uteri were observed only during June 1983, but together with specimens with one and two eggs. Other specimens from May, August, September, October and December 1983 were all found with one or two eggs (stage 4). The highest percentage of gravid individual parasites (84.8%) was also seen during June 1983, and the minimum level was 5% during December

Fig. 5.3. The posterior region of adult D. homoion showing the ovary at stage 3. jr, junction region; ov, ovary; t, testis; vr, vitellocytes reservoir.

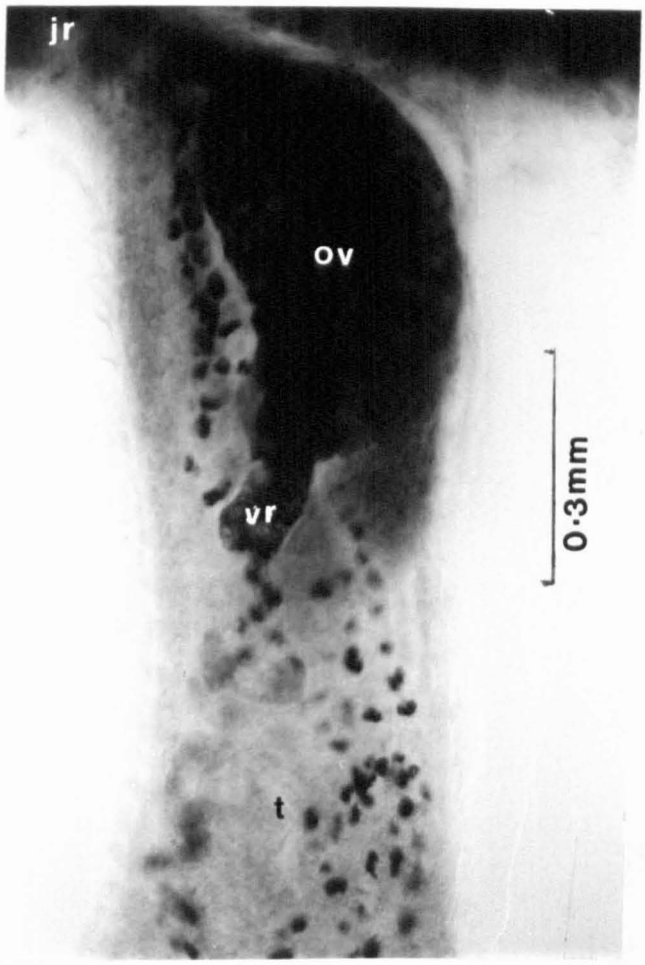


Fig. 5.4 The posterior regions of adult D. homoion showing the ovary at stage 4 with one egg near the junction region. e, egg; ov, ovary; t, testis.

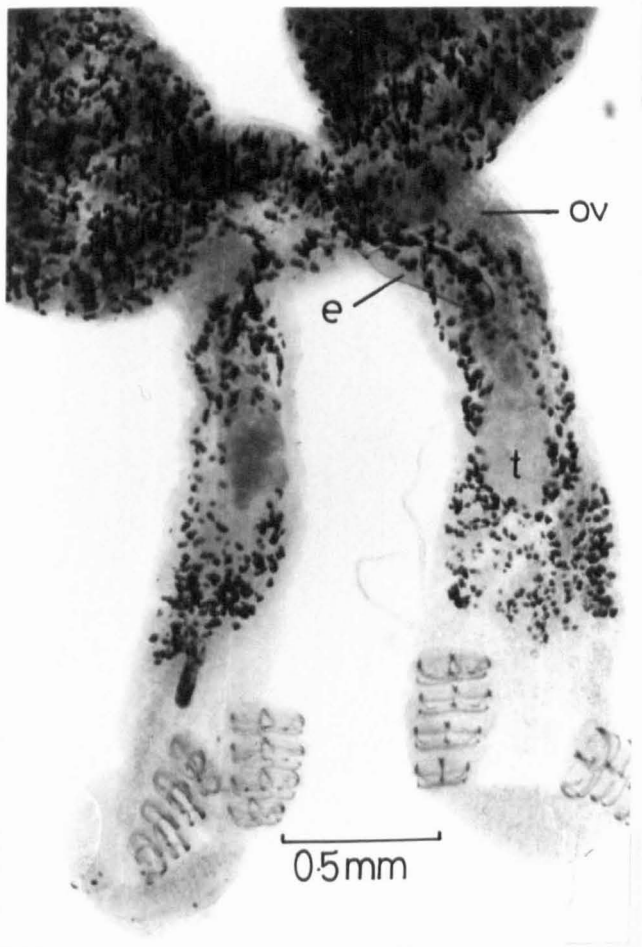


Fig. 5.5 The posterior regions of adult D. homoion showing the ovary at stage 5 with 3 eggs near the ovaries and junction regions. e, eggs.

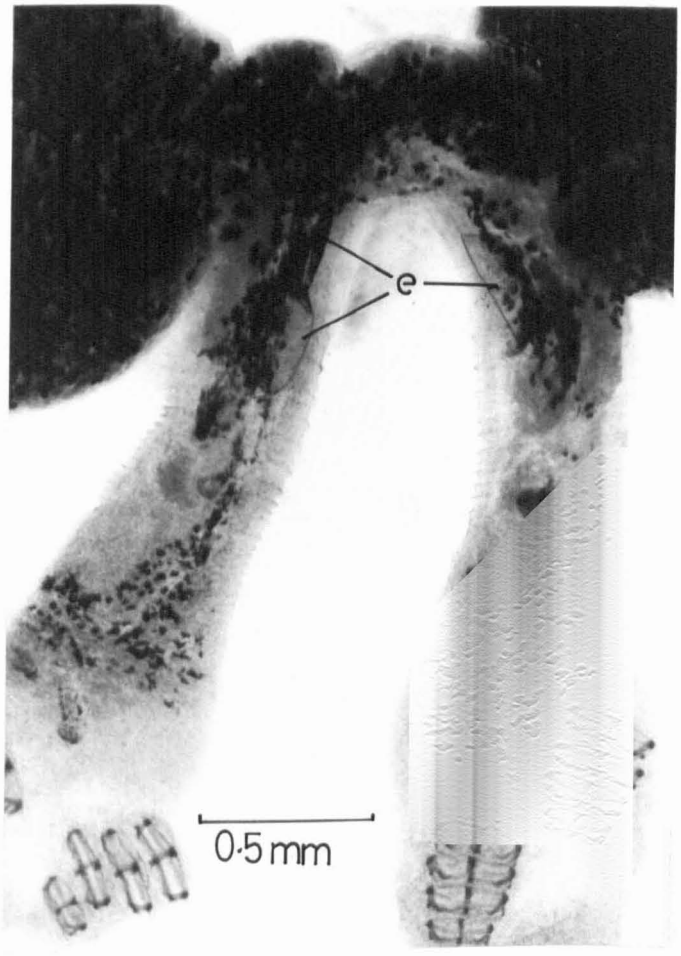


Table 5.2 Seasonal changes in the developments of ovaries of adult D. homoion.

Date	Nos. of parasites			Stage of ovarian development				
	Examined	Containing eggs	%	1	2	3	4	5
Sept. 1982	17	0	-	0	14	3	0	0
Oct.	22	3	13.6	0	8	11	3	0
Nov.	8	0	-	0	5	3	0	0
Dec.	14	0	-	4	7	3	0	0
Jan. 1983	22	0	-	16	5	1	0	0
Feb.	22	0	-	10	9	3	0	0
Mar.	48	0	-	0	31	17	0	0
April	49	0	-	0	23	26	0	0
May	62	26	41.9	0	0	36	26	0
June	33	28	84.8	0	5	0	14	14
Aug.	11	4	36.4	0	3	4	4	0
Sept.	14	2	14.3	0	4	8	2	0
Oct.	65	6	9.2	5	35	21	4	0
Nov.	29	0	-	8	14	7	0	0
Dec.	20	1	5	1	7	11	1	0

1983. Unfortunately, no data were available during July 1983 for comparison. The maximum number of eggs seen in the adult parasite was 4. Parasites with slightly developed ovary (stage 1) were observed during December 1982 to February 1983. Adult parasites with ovaries at stages 2 and 3 were detected during all months of the study. Table 5.2 also shows that the parasites had closely similar levels of ovarian development during September to December of both 1982 and 1983.

C. Fecundity and Formation of Clusters of Eggs of D. homoion

Experiment 1:

One Rutilus rutilus with 4 sexually mature adult D. homoion was kept in a tank under laboratory conditions for 3 days. One hundred and sixty-two eggs were collected from the tank and many unpaired diporpae and paired diporpae with different numbers of pairs of clamps were recovered from this fish. Therefore, the number of eggs laid by each parasite per day must have been about 14. It was found that 78 of these eggs were laid individually (not attached by their filaments) and the rest were formed into clusters each with 2-70 eggs (Fig. 5.6).

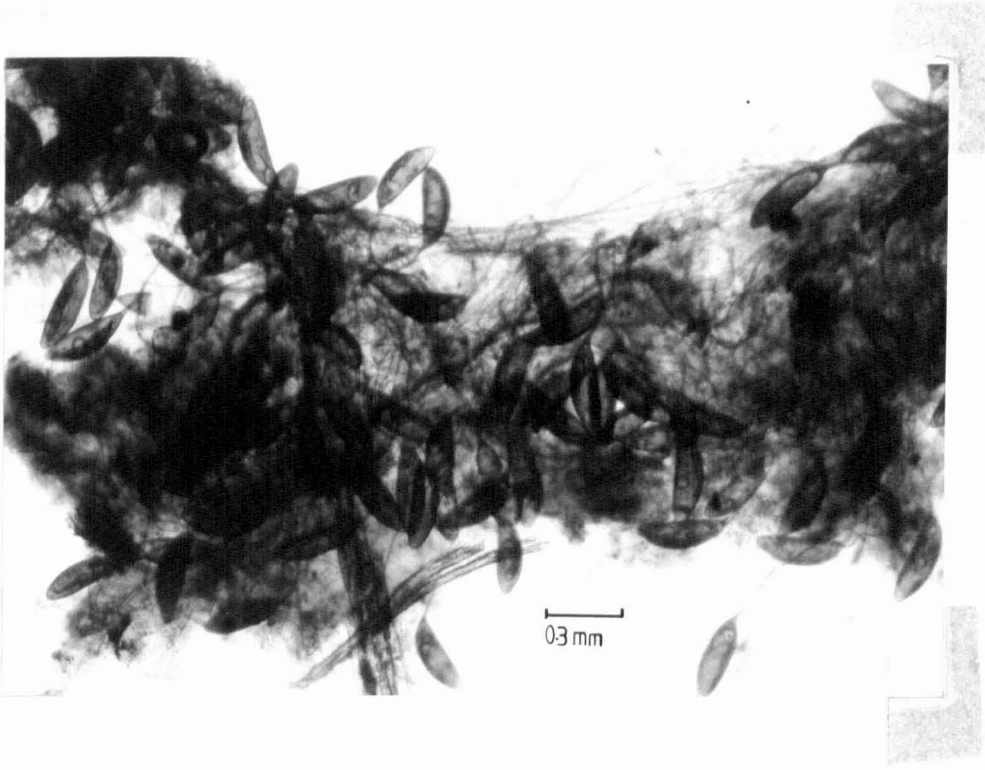
Experiment 2:

One fish was used in this experiment over a period of 11 days. The number of eggs collected was 509 and the number of sexually mature adults found on the gills of this fish was 2. Therefore 23 eggs per parasite must have been laid per day (the number of young adult, juvenile stages were all ignored for determining the fecundity of the parasite). One hundred and nineteen eggs out of the total were found free in the water and 390 eggs were found forming clusters ranging between 2-50 eggs in each.

Experiment 3:

One infected fish was separated for 14 days; the number of eggs

Fig. 5.6 Cluster of eggs of D. homoion.



collected was 1021 and the number of sexually mature adult parasites was 2 with many other life cycle stages. So the number of eggs laid per parasite per day was 36. The number of eggs found in the different sizes of clusters ranged between 2-300 eggs.

Experiment 4:

Two infected fishes were observed for 14 days. The number of eggs collected from the tank was 4352 and the number of sexually mature adults found on the two fishes was 6. Many newly-formed adults and juveniles with other diapora stages were seen. Therefore the number of eggs laid by each of the 6 gravid parasites each day was about 52. The number of eggs in one cluster formed during this experiment approached 1000. Other cluster sizes were between 32-600 eggs. Very few eggs were found singly.

It was noted during these experiments that whenever the period of incubation of Diplozoon infection was prolonged, the number of clusters and the number of eggs in each cluster increased. Some of these eggs were recovered from the faecal material of the fishes. When eggs are exposed to the air for a few seconds, they show resistance to wetting and will only sink thereafter by the application of slight pressure. There was also a tendency for the eggs to become attached to vegetation, especially long thin weeds. Very few eggs were found attached to animal materials (dead maggots and other animal tissues). In a few adult worms collected from the fishes after the experiments were finished from 2 to 7 eggs were seen in a cluster close to the body of the parasite but with the terminal part of two of these egg filaments still retained within each of the genital systems via the genital apertures. The filament of a newly laid egg which was still attached to its adult was more flexible than those of other eggs which

had already been laid and formed the cluster. Eggs which were still inside the uterus of a worm had the filament spirally coiled around itself. During the process of egg deposition, the body of the egg came out from the genital pore first and was followed by the filament. The length of the filament was about 1cm long.

D. Embryonic Development and Egg Hatching of D. homoion

The observations on the embryonic development and hatching rhythms of the eggs kept in different types of water and at different temperatures can be seen in Table 5.3. The results show that the embryonation period was closely similar regardless of the type of water at temperatures between 18°-21°C. After 3-8 days a fully developed larva with eyespots, clamps and larval hooks was visible through the egg capsule. Slight movement of the clamps and cilia of the larvae inside the egg capsule were seen during this period. The eggs took 8-10 days to hatch under the conditions shown in Table 5.3. It was also noticed that the time needed for hatching in tap, distilled water and distilled water mixed with mucus was relatively similar. A few eggs remain unhatched even after 15 days of incubation. All eggs taken from faecal material were found with fully developed larvae and 50 percent of them hatched within 3-5 days.

Embryonation and hatching times of eggs in tap water at 10°C were longer (7-20 days) than at 18°-21°C. The fully developed larvae inside the egg capsules were seen within 7-10 days while the escape of larvae from the capsules occurred between 15-20 days. Most of these eggs (13) remained unhatched even after 25 days of incubation, but when they were warmed to 18°-21°C they all hatched within 7 days.

E. Longevity of the Oncomiracidium of D. homoion

The morphology of this stage is described in Chapter 3. All

Table 5.3 Hatching time for eggs of *D. homoion* in different water types and temperatures.

Type of experiment	Nos. eggs used for each experiment	Time (days)		Nos. eggs not hatched 5 days after the last previous hatching
		Eggs containing fully developed larvae	Eggs hatched	
1. At 18°-21°C using day/night light				
A. Tap water	20	5 to 7	8 to 10	3
B. Distilled water	20	3 to 7	8 to 11	0
C. Distilled water + mucus	20	3 to 8	8 to 10	2
D. Distilled water + eggs from faecal material	20	0	3 to 5	10
2. At 10°C using day/night light				
Tap water	20	7 to 10	15 to 20	13

20 larvae were dead after 24, 12, 6, 5 and 4 hours of incubation at 18°-21°C while after 3 hours of incubation 11 larvae were still alive and 9 dead. After 70 minutes of incubation 17 larvae were still active but there were 3 dead. It was found that the movement of oncomiracidia decreased as the time of death approached. Phototropism was not seen in active larvae. They swam in straight lines, in all directions, often went up to the surface of the water and then down again. At intervals they stopped swimming for a few seconds of rest. At the end of their life-span, they settled at the bottom of the dish making sluggish movements of the body or without any movement at all. But in all these stages, even when the oncomiracidia were stationary, the cilia were still beating. A critical change in the shape as well as in the size of the oncomiracidium was noted at this stage. This is described in full in Chapter 3. The area of the posterior ciliated cells became cone-like in structure and some ciliated cells from the other groups disappeared as shown in Chapter 3, Fig. 3.36. The next stage was when the ciliated epidermal cells disappeared altogether. In the experiments this happened when the ocomiracidia died.

F. Longevity of the Diporpa and Juvenile Stages and the Commencement of Sexual Maturity of Adult D. homoion

The longevity of different diporpa, juvenile and sexually mature adult stages can be seen in Table 5.4. During the first 2 days of study of fry Leuciscus leuciscus with D. homoion infections, the unpaired diporpae and paired diporpae were found on the gills (Figs. 5.7, 5.8, 5.9, 5.10, 5.11 and 5.12). Some of these stages became detached during examination of the fishes. Single diporpae were very small of length 0.23mm (Fig. 5.7) while others of the same stage were longer, 0.48mm (Fig. 5.8). All the six unpaired diporpae from the first day of study

Table 5.4 Longevity of diporpa, juvenile stages and the commencement of sexual maturity of adult *D. homoion*.

Period of Study (day)	Nos. of fry Examined	Infected	Unpaired diporpa	Paired diporpae	Juvenile	Young adult	Sexually mature adult with egg
1	20	2	6	2	0	0	0
2	20	6	29	7	0	0	0
3	20	13	25	13	47	14	0
4	20	10	18	7	20	6	0
5	20	15	35	10	13	25	0
10	20	13	21	17	3	27	1
Total	120	59	134	56	83	72	1

Fig. 5.7 Early stage of unpaired diporpa of D. homoion with one pair of clamps, a few hours after hatching. c, clamps; i, intestine; lh, larval hook; os, oral suckers; p, pharynx.

Fig. 5.8 Unpaired diporpa of D. homoion with one pair of clamps, 1-2 days after hatching. c, clamp; os, oral sucker.

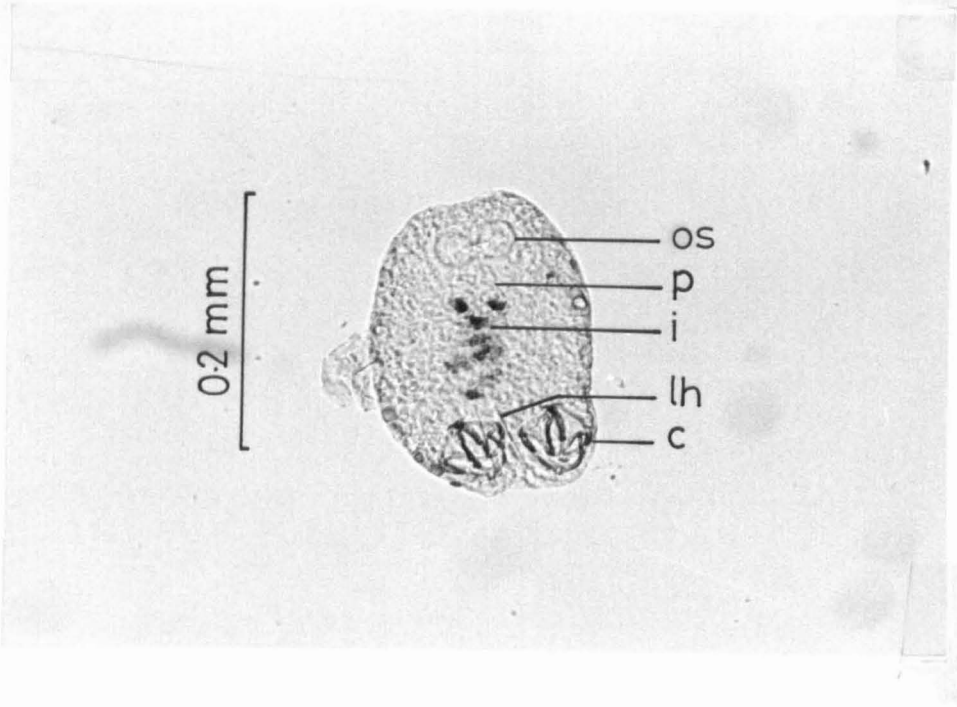


Fig.5·7

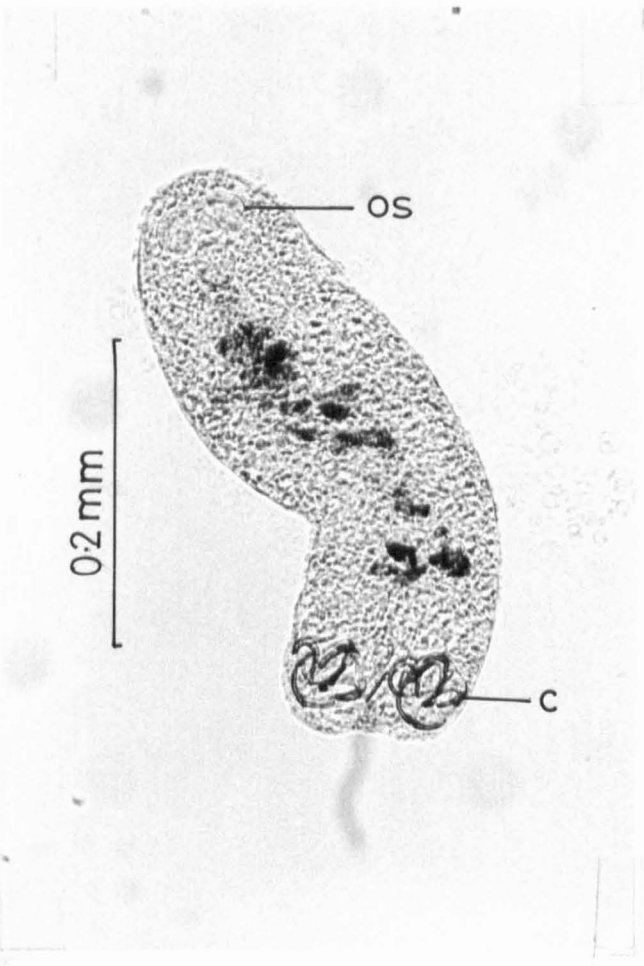


Fig.5·8

Fig. 5.9 Unpaired diporpa of D. homoion with one pair of clamps but with early appearance of the second pair of clamps. 1-2 days after hatching. ms, median sucker; os, oral sucker.

Fig. 5.10 Unpaired diporpa of D. homoion with 2 pairs of clamps, 1-2 days after hatching.

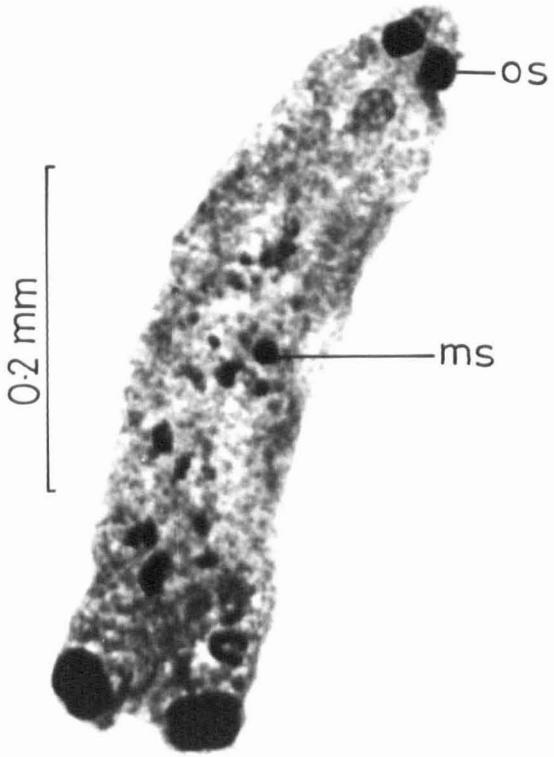


Fig. 5·9

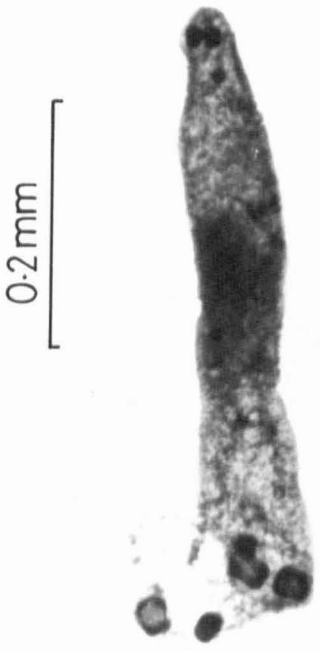


Fig. 5·10

Fig. 5.11 Paired diporpae of D. homoion with one pair of clamps. 1-2 days after hatching. c, clamp; lh, larval hook; ms, median sucker; os, oral sucker.

Fig. 5.12 Paired diporpae of D. homoion with 1-2 pairs of clamps. 1-2 days after hatching. c, clamp; os, oral sucker.

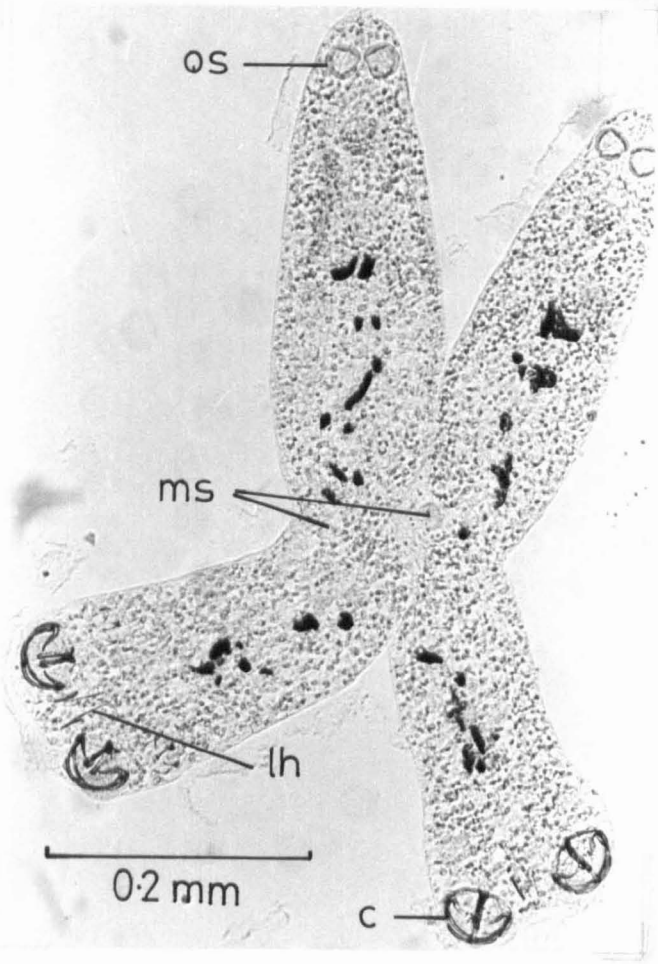


Fig. 5.11

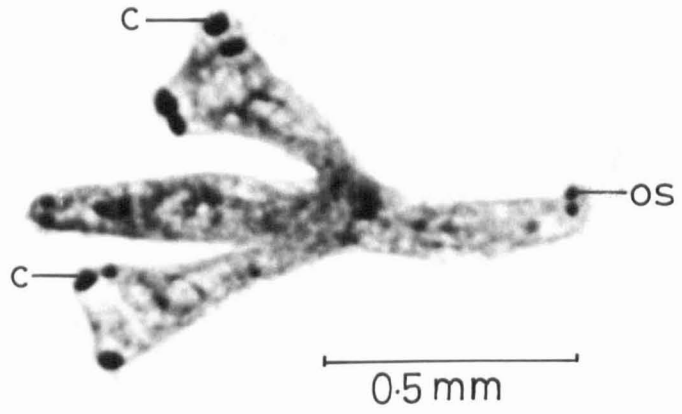


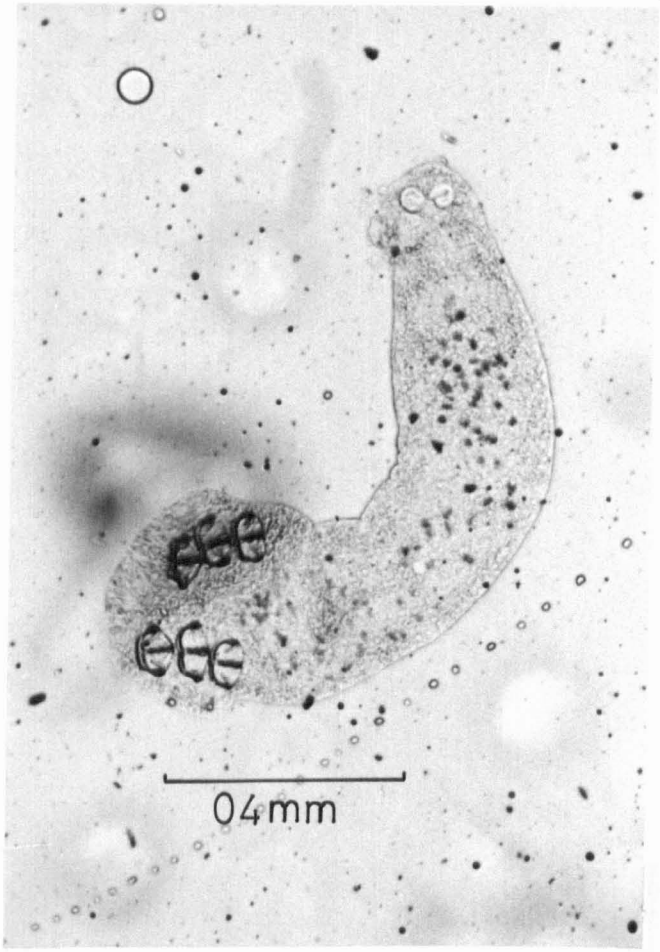
Fig. 5.12

had one or two pairs of clamps with one pair of hooks. These stages were found to be very active and a few dark patches represented the first branches of the intestine. A circular structure was visible at the middle part of the body on these stages. It was thought to be the precursor of the oral sucker (Fig. 5.9) and was usually seen on the unpaired diporpa stages with one and two pairs of clamps. In the living larvae, this structure was opened and closed periodically. Fig. 5.9 also shows the early formation of one of the second pair of clamps. The other two paired diporpae had one and two pairs of clamps respectively (Figs. 5.11 and 5.12), and their length was about 1mm. Their morphological appearances were relatively similar to single diporpae and the presence of the pair of median suckers could be observed at the junction area (see Fig. 5.11). The stage of development of clamps in the two partners of the paired diporpae was different. For example, one partner had 2 pairs of clamps and the other had one pair and the third clamp of the second pair was just becoming visible. The beginning of a few dark patches representing the intestine was seen on most of these stages during the first day and the patches increased in the paired diporpae compared to those in the unpaired ones.

On the second day of study 29 unpaired diporpae and 7 paired diporpae were recovered from the gills of 6 infected fry. The development of both these larval stages and the number of clamps were similar to those described for the first day of study, except for the appearance in one unpaired diporpa of 1.1mm long with 3 pairs of clamps (Fig. 5.13) and two paired diporpae had 3 pairs of clamps.

It was most common to find on each gill of infected fishes an even number of unpaired diporpae, with one or two pairs of clamps, and unusual to find odd numbers of unpaired larvae. These unpaired

Fig. 5.13 Unpaired diporpa of D. homoion with 3 pairs of clamps. 1-2 days after hatching.



and double diporpae, with one and two pairs of clamps, were readily able to detach from the gills and to re-attach as described in Chapter 7. Observations on the unattached single and paired diporpae indicated that their clamps and oral suckers were highly active in grasping any tissue near to them, even their own bodies.

The mode of attachment of unpaired diporpa on the gill of fry can be seen in Fig. 5.14. It is clear from this photograph how the first pair of clamps was accommodated to the size of the secondary gill lamellae of the fish. Both unpaired and paired diporpae also used their oral suckers for temporary attachment and to facilitate movement on the primary gill lamellae. The unpaired diporpa with two pairs of clamps can be seen in Fig. 5.15. The sizes of both 1st and 2nd pairs of clamps were relatively similar at this stage of development. Fig. 5.15 also shows the facultative arrangement of clamps in the diporpa stages.

An adhesive-like material was found surrounding the body of unpaired and paired diporpae with one and two pairs of clamps only as shown in specimens stained with trichrome (Figs. 5.12 and 5.16). Also living individuals of these particular stages showed a similar substance, so that when a needle touched their bodies, they tended to become attached to the needle.

On the third day of incubation the juvenile and young adult stages were observed (Fig. 5.17 and Chapter 3, Fig. 3.4) as well as other diporpian stages. Most of the worms were unpaired diporpae or juveniles and young adults. The length of the juvenile stages was 2-2.7mm and the young adults were over 3mm long. The branches of the intestine had increased in size and number and the early appearance of vitelline follicles could be seen in the anterior

Fig. 5.14 Unpaired diporpa of D. homoion with one pair of clamps showing the temporary attachment to the primary lamella using these clamps and the oral suckers. This stage was observed 1-2 days after hatching. Markers = 10μm.

Fig. 5.15 Unpaired diporpa of D. homoion with 2 pairs of clamps. 1-2 days after hatching. Markers = 8μm.

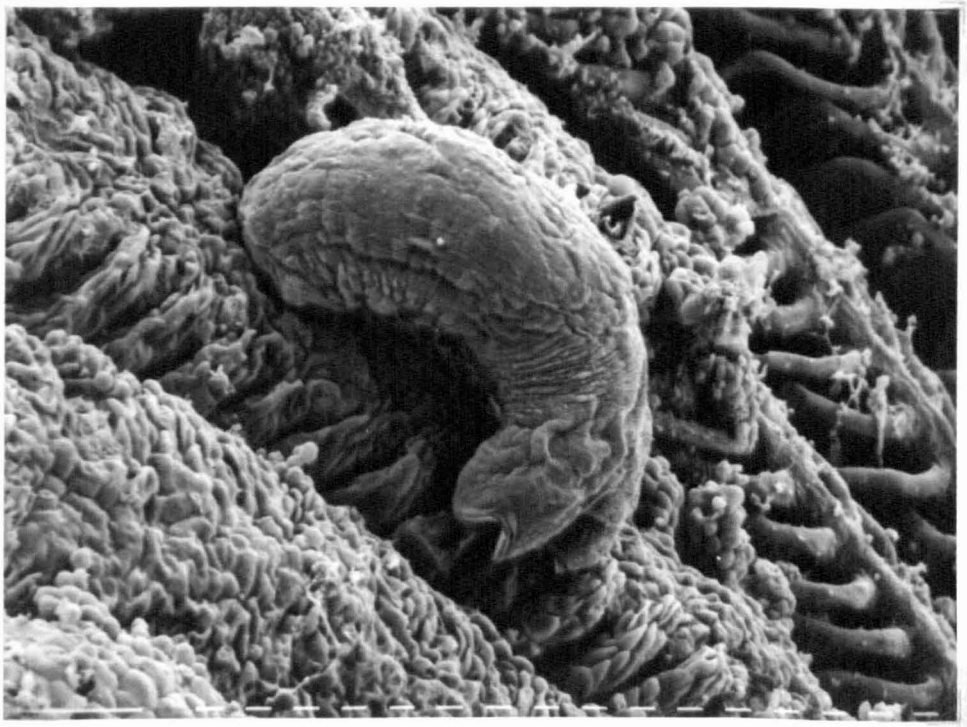


Fig.5.14

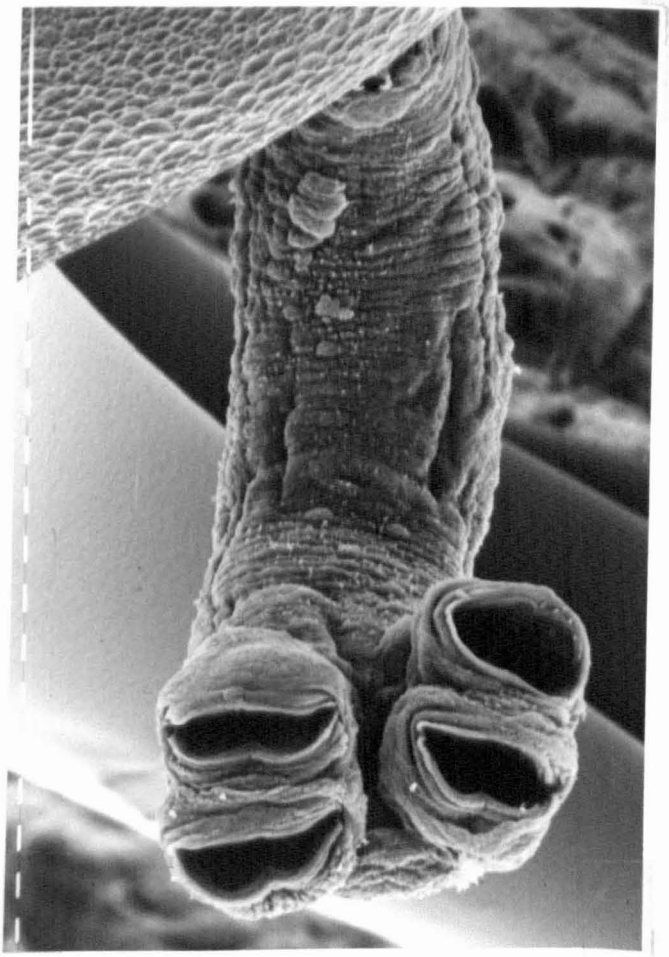


Fig.5.15

Fig. 5.16 Paired diporpæ of D. homoion with 2 pairs of clamps covered by mucous-like material.

Fig. 5.17 Juvenile stage of D. homoion with 4 pairs of clamps and early appearance of vitelline follicles on the anterior parts. vt, vitelline follicles.

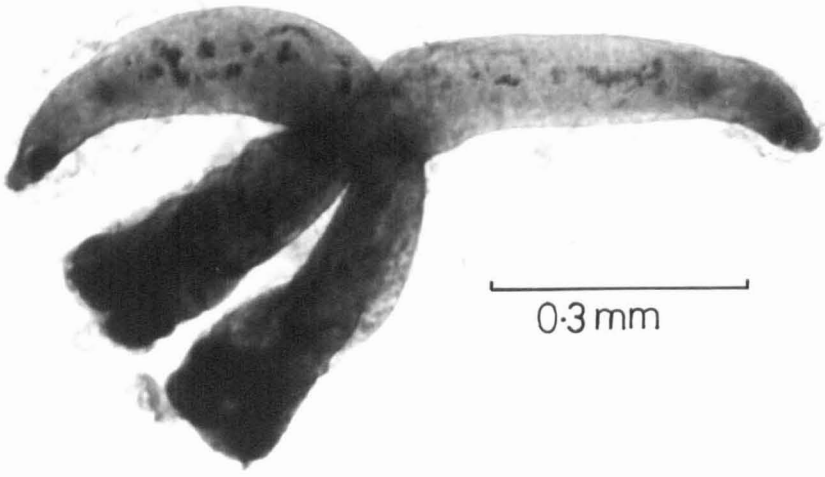


Fig.5·16

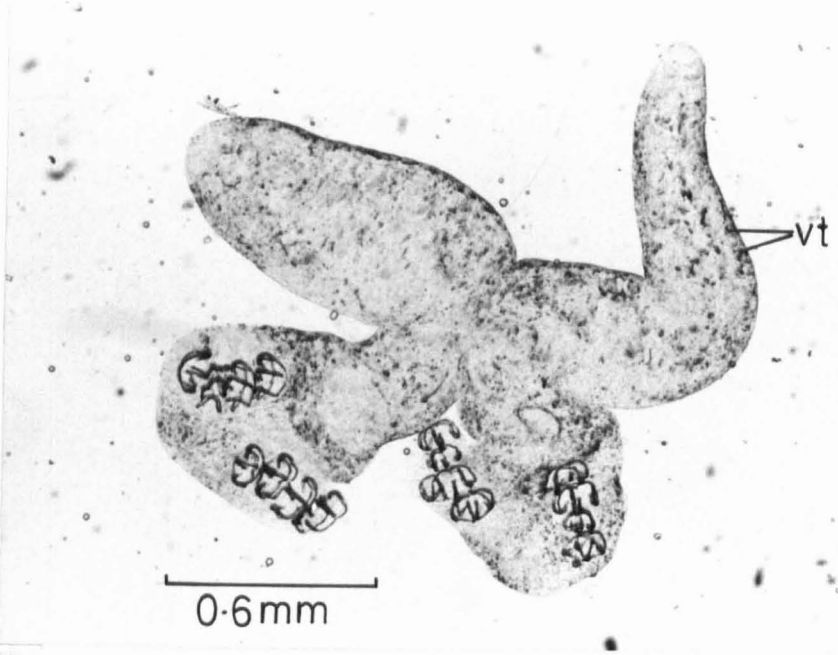


Fig.5·17

parts of the body. Formation of the pairs of clamps was completed in these stages and no indication of the presence of adhesive material was observed around their bodies.

During the next 2 days of study (4th and 5th days) the same life cycle stages were detected on the infected fry. The unpaired diporpa stage was predominant on fry examined during the first 5 days of study. Double diporpae were rarely seen because this stage was rather transient. Juvenile and young adult stages were dominant during the third to fifth days of observations. Another unpaired diporpa with 3 pairs of clamps (Fig. 5.13) was recovered during these two days but never an unpaired diporpa with 4 pairs of clamps.

On the 10th day of study, one gravid but small adult worm (see Chapter 3, Fig. 3.8) was seen on the gills of fry in addition to the other life cycle stages indicated earlier. The largest numbers of larvae found were single diporpae and young adults, as these represent, respectively, undeveloped and developed worms. The morphology of the young adults has been described in Chapter 3.

Therefore the total numbers of life cycle stages of D. homoion recovered from 59 infected fry Leuciscus leuciscus during 10 days of study were: 134 unpaired diporpae with one and two pairs of clamps (except 2 unpaired diporpae with 3 pairs of clamps); 56 paired diporpae with one, two and 3 pairs of clamps; 83 juveniles; 72 young adults and 1 sexually mature adult. The numbers of clamps on each side of the opisthaptor of both single diporpae or paired diporpae were often disimilar.

It is evident from the characteristic features of the anatomy of adult D. paradoxum (invagination and ridges on the posterior parts of the body) that the points of union of two single diporpae at their

middle areas takes place at particular sites on each partner, namely ventral to ventral surfaces. This can be seen by comparing the arrangement of posterior parts, as shown in Chapter 3, Fig. 3.23, as well as here in Fig. 5.18. The adult parasite tends to twist one of its posterior parts (Fig. 5.18) in order to attach its opisthaptors to two consecutive primary lamellae (described in Chapter 7).

The position of the genital aperture on the ventral surface of each adult parasite appears to be placed to the left or right of the viewer depending on which individual is orientated on top of the other as shown in Fig. 5.19. This is also confirmed by the position of emergence of egg filaments from the two genital pores of adult D. homoion

Fig. 5.20 presents a summary of my observations of the life cycle of D. homoion, including information about the longevity of each stage. The life cycle was found to be as follows. Between 18^{o}-21^{o}C, the gravid worms laid their eggs in clusters within a few hours. The eggs needed 8-10 days to complete their embryonation and to hatch. The free swimming ocomiracidia live for a few hours (1-3) and search actively for their hosts. If they do not find their host within this time they will die without further development. If suitable hosts are available, the oncomiracida enter the gill chamber and actively grasp the gill tissue. There is a tendency for each larva to become attached near to another, so that even at this stage a potential partner with which a union may later be formed is available. Then follows on the disappearance of cilia, epidermal cells and eyespots and the body becomes longer. At this stage the worms change into unpaired diporpae on the gills with one pair of

Fig. 5.18 Adult D. paradoxum, with one of the posterior regions twisted to allow the clamps of the two opisthaptors to attach to two consecutive primary lamellae. Markers = 100μm.

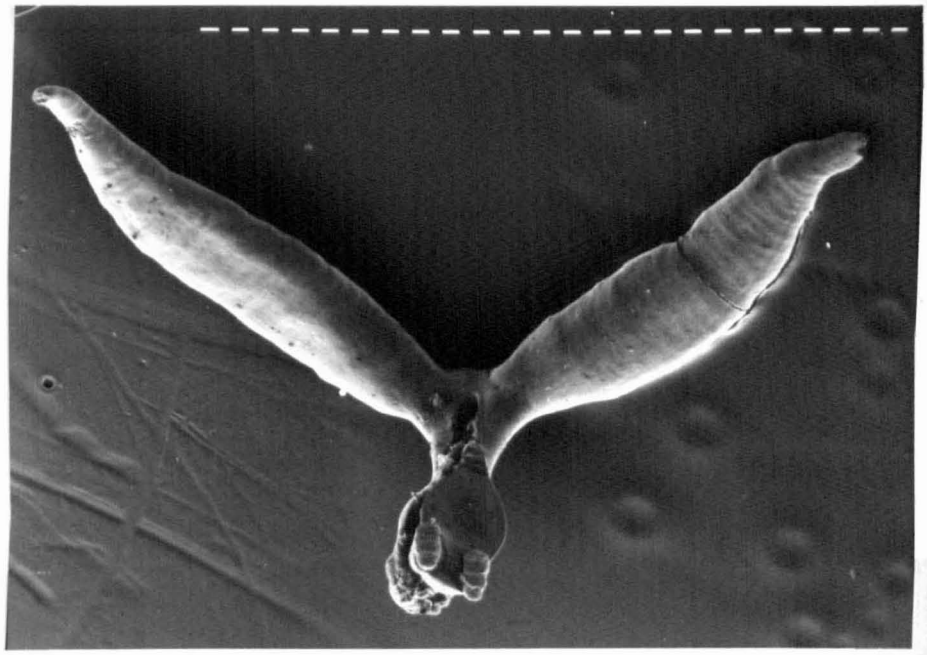


Fig. 5.19 The mode of orientation of the two partners in the union of D. homoion to form the adult parasite. In A the anterior end of the upper worm as seen by the viewer is to the left. The genital pore of the lower worm is also placed to the left. In B the anterior end of the upper worm as seen by the viewer is to the right. The genital pore of the lower worm is also placed to the right. ar, anterior region; gp, genital pore; por, posterior region

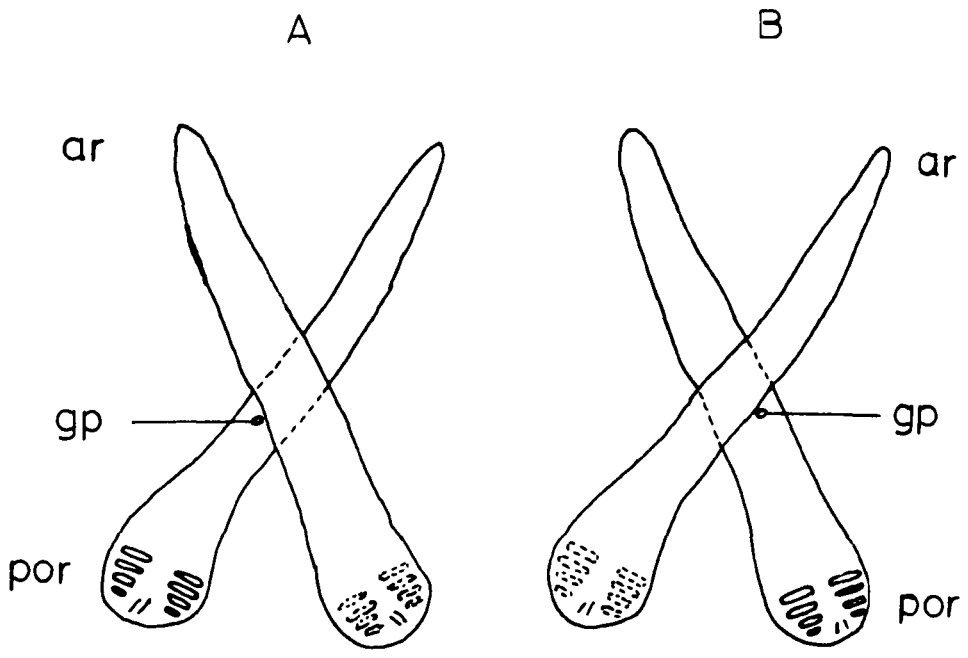
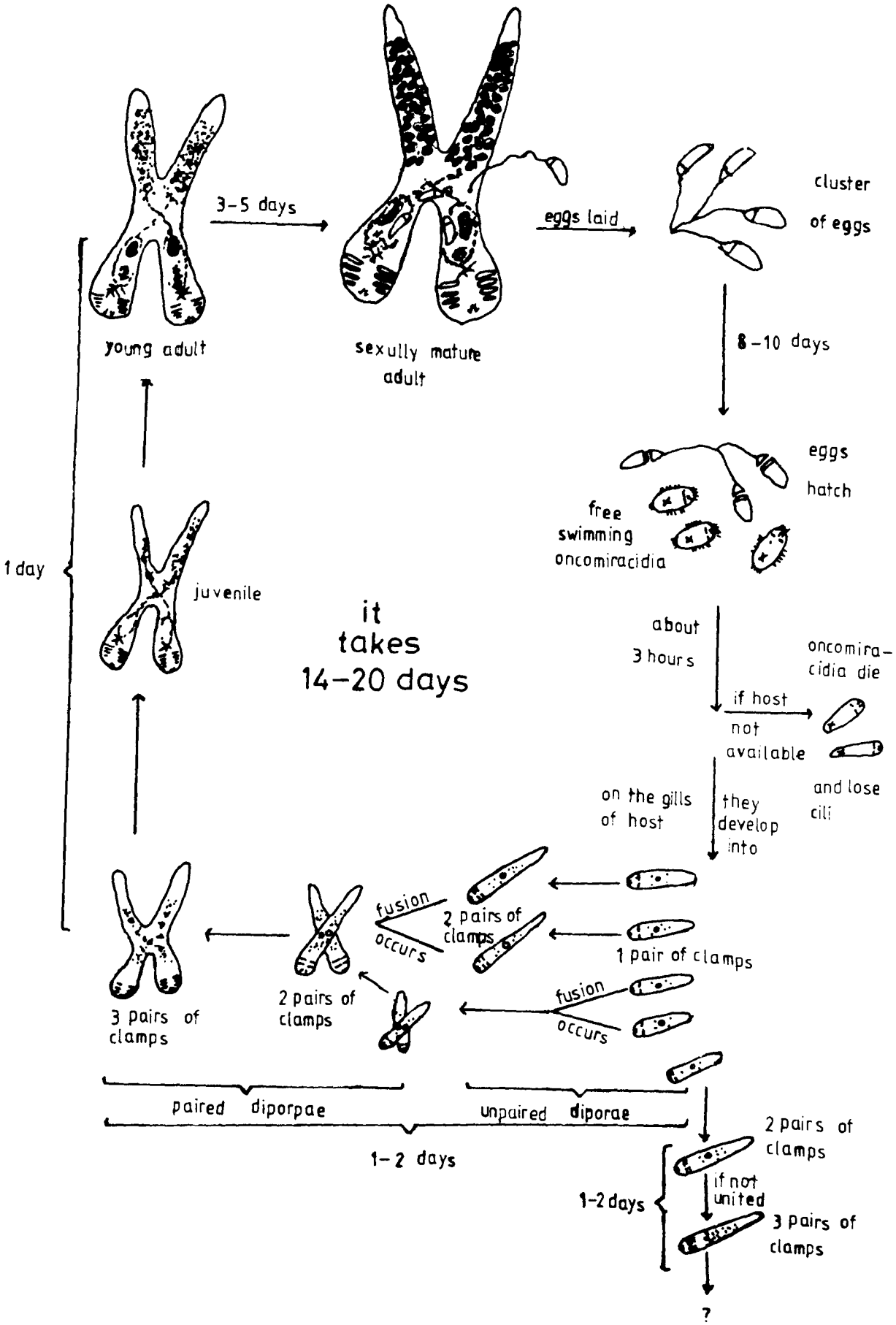


Fig. 5.20 The life-cycle of D. homoion at a water temperature of 18°-21°C.



clamps and one pair of larval hooks. The larvae either continue to develop and produce more pairs of clamps before two of them unite to each other, or the union occurs at an early stage of development when both of them have one pair of clamps. However, whenever the union between two partner diporpae occurs, they will complete their development by an increase in body size and addition of more pairs of clamps up to 4 in order to reach the juvenile stage. This process of development takes 1-2 days for completion. Sometimes, there are unpaired diporpae with one pair of clamps which have no chance to find a partner, so in this instance they continue their development as unpaired individuals up to the stage of 3 pairs of clamps. The fate of such stages is uncertain. The juvenile and young adult stages will appear later, within 2-3 day, while the gravid worms with shelled eggs require 3-5 days more to complete their development.

Therefore the period of the life-cycle of D. homoion is relatively short, taking 14-20 days from beginning to end, starting with egg laying by the old generation and finishing with the sexually mature adults of the next generation. The field data also confirmed these experimental observations about the period of life cycle, because some adult parasites of small size were recovered from infected fishes from Llyn Tegid during June 1983 when the onset of egg production by the overwinter parasites only started in May, as shown in Table 5.2.

IV DISCUSSION

It is very difficult in the field, if not impossible, to determine the exact longevity of adult D. homoion because of the overlapping between the overwinter and recently formed adult worms on the same fishes during summer. This is because when water temperatures are relatively high, only a short time is required for the completion of the life cycle and the production of newly gravid worms. The constancy of the mean length of parasites during most months even through the period of reproductive activity support the fact of the short period of development of mature adults. The majority of the small, newly developed adult worms recovered during July 1984 and the very few large worms present at that time may indicate that most of those surviving overwinter perished during the summer, particularly in July. The slight decrease in the number of infected fishes and in the number of parasites per fish during May to August, as shown in Chapter 6, may indicate that all the overwintering parasites die during this period and are replaced by new ones, but the rate of mortality of the overwintering individuals seemed slightly higher than the formation of new parasites. However, it was not possible to determine whether the parasites lived for less than one year, or one or even two years. As eggs continue to be shed during autumn, it is likely that the formation of new organisms can occur at a slow rate of development during this period and overwinter. Bychowsky (1957), Bovet (1967), Halvorsen (1969) and Anderson (1971) stated that D. paradoxum individuals lived at least one year after the oviposition. However, they had no evidence to support their suggestion.

The seasonal reproductive activity of D. homoion from Llyn Tegid was studied using mainly the development of the ovary and the number

of shelled eggs seen inside the uterus of gravid worms. It was found, after using a variety of different stains, that the testes of the parasites were not sufficiently clear to determine the seasonal levels of spermatogenesis.

According to the current observations, the ovaries were found well-developed during most months of the year (Ovary with stage 2 and 3), particularly in March and April. During December 1982 to February 1983 most of the specimens examined had their ovaries poorly developed (stage 1). The parasites with eggs (stages 4 and 5) were first seen in May 1983 and continued up to December 1983. But the peak of egg production and fertility of the parasites (stage 5) occurred during June 1983. A slight difference was also found between the numbers of sexually mature adults present during September to December 1982 and the same period of 1983. This may relate to the small size of the samples of parasites examined. The different ovarian stages were seen every month, from March to December, which clearly contributes to the range of variation in the development stages of the parasites found during this period.

Lyukshina (1977) indicated that the vitellaria of Diplozoon paradoxum, D. rutili, D. homoion, D. markewitchi and D. nipponicum showed intensive production of egg-shell building materials (essential proteins phenol and polyphenoloxidase) in specimens examined from May to June. Production of egg-shell precursors slowed down in September and stopped during the winter. Gläser (1967) mentioned that oviposition in D. rutili, Germany, occurs in the warm season. Kamegai (1970) stated that egg-laying in D. nipponicum from Cyprinus carpio, Japan, begins in the later part of April and continues during the summer and ends in September or October. He found the development of

reproductive organs of D. nipponicum begins in autumn for the testis and is complete by the end of winter, while the development of the ovary will be completed by the beginning of spring when oviposition starts and continues until the end of summer. In Russia, Khotenovskiĭ (1977b) found the ova for the first time in the uterus of D. homoion, D. nipponicum, D. markewitschi and D. rutili at the end of April and eggs hatched from mid-May. Most of these eggs were laid in June and July. He also stated that the gonad formation in D. paradoxum was virtually arrested during the winter, resuming from March onwards. Both testes and ovaries reached their maximum size in May when the ovaries were full of infective ova. Halvorsen (1972) found that the relative number of gravid worms of D. paradoxum on Rutilus rutilus and Abramis brama in Norway increased sharply from May to June then decreased. Egg production appeared to be restricted to the period between April and November. Wiles (1965) reported that D. paradoxum from British Cyprinidae actively produced shelled eggs from May to September. Mishra (1966) indicated that the onset of egg formation in D. homoion on the gills of Rutilus rutilus in the Shropshire Union Canal, began in March and the worms shed their eggs during April to July. He noted that most of parasites examined during April to May had eggs but during the remainder of the period of reproductive activity of these worms fewer eggs were seen.

Generally the present results agree with the previous findings. Commencement of egg production of D. homoion at Llyn Tegid also began early in the summer (May) but it continued during the autumn and early winter (until December). It seems likely that the fluctuations in water temperature during this period influenced the rate of egg formation.

The experimental observations revealed that the estimation of the fecundity of this parasite was very difficult because, most of the eggs collected from the bottom of the tanks formed a huge cluster joined by their filaments. Therefore, the loss of any of these clusters during the process of sampling them would clearly effect on the accuracy of the results. However, the four experiments on egg laying by these parasites indicated that under laboratory conditions each sexually mature adult of D. homoion could produce between 14-52 eggs daily. The difference between the two figures can be attributed to many reasons: 1. Period of incubation of infected fishes in each experiment; 2. Site and behaviour of infected fishes from which the parasites were recovered; 3. or perhaps missing some of these clusters during the process of egg collection from the water of the tank. The results of Macdonald and Jones (1978) on the egg-laying of D. homoion gracile in relation to photoperiod cannot be compared directly with the present finding because there was a probability that some of the parasites in their experiments had become sexually mature by the end of the 14 days of the experimental period, which would effect their data on the fecundity of these parasites. Unfortunately the photoperiod used in the current work (16 hours light) was different making the comparison between the current observations and those of other workers impossible.

It was noted that the number of eggs in each cluster increased whenever the period of egg laying of these parasites was prolonged. This may be associated with the possibility of two or more small clusters becoming attached together and forming new larger clusters. Only small groups of eggs were found in the waters of the tanks used for short experimental periods of time. The egg filaments play an

important role in this process. The laboratory observations on the newly-laid eggs show that the outer surfaces of their filaments were rather sticky and more flexible than those of old eggs. The cluster of eggs was also seen by Bovet (1959) in D. paradoxum. It seems that the presence of the filaments on the eggs of these monogenean parasites and the phenomenon of formation of clusters of eggs all reflect the evolution of these organisms to secure maximal survival by producing as many as possible active, infective larvae at each particular time and place to ensure the successful completion of the life cycle. It is important that at least two diporpa stages attach to the same host as if otherwise the formation of the dual-individual adult worm cannot occur. Further discussion of the other results which follow will also demonstrate this point. According to the present finding, it can be suggested that the larvae might not swim individually in the water but as a group.

The adhesion of the egg filaments to plant rather than animal materials may be an adaptation of the parasite to the feeding behaviour of its hosts which prefer plants during the parasite reproductive season, so that the infective larval stages are likely to be much closer to the hosts. This would increase the chances of successful invasion by larvae. Moreover the present observations show that the parasites themselves also take part in the process of formation of the clusters of eggs as described earlier.

The embryonation and egg hatching of D. homoion under controlled laboratory conditions at 18°-21°C were found to be similar in tap water, distilled water and in distilled water with mucus material added. The fully-developed oncomiracidia showed slight movement of their clamps inside the egg capsules during 3-7 days after the

day of egg laying by the gravid parasite. The eggs used in the experiments were collected daily from the tank and must therefore have been deposited by the parasites during the previous 24 hours. Therefore the time of the beginning of their embryonic development was known in theory. But in some the difference in the period of larval development time inside the egg capsule might be associated with the fact that some of the eggs might have been laid before the day of collection and have been eaten by the fish and have passed through their alimentary system before being passed with the faeces into the water again. In such an instance, some of embryonic development might take place inside the intestine of fish. This may also have been true in all experiments using the different water types. Hatching of the eggs occurred after 8-10 days at 18°-21°C in each of the three types of water. Sometimes a few eggs of D. homoion contained fully-developed oncomiracidia but hatching did not occur even after an extra 5 days incubation. The reasons for this are not known.

Sterba (1957) found that after the start of embryonation, the eggs of D. tetragonopterini took 13 days at 24-26°C to hatch, while Bovet (1959) reported that the eggs of D. paradoxum took 15 to 17 days for complete development into the larva presumably at laboratory temperature. In D. nipponicum, the eggs took 8 days to complete their embryonic development at 15°-20°C (Khotenovskiĭ, 1977b). However, Kamegai (1968) noted that the eggs of D. nipponicum took 8 days to complete the embryonic development and for the larva to emerge at 25.5°-28.5°C. In D. megan, the ova, somewhat similar to those of D. rutili, developed in 7 to 8 days in the laboratory at 18°-25°C (Khotenovskiĭ, 1977a). The results from these workers agree

with the present finding in that the eggs of D. homoion hatched within 10 days of incubation at nearly the same range of water temperature.

The eggs from faecal material hatched more quickly, in 3-5 days, presumably because most of embryonation took place inside the fish. This leads to the conclusion that the eggs of this parasite can survive and continue their development even under unusual conditions. Macdonald and Jones (1978) also found the same phenomenon for the eggs of D. homoion gracile.

The prolonged period required for the hatching of eggs in tap water at 10°C was quite expected, which demonstrates that water temperature is one of the main factors effecting the development of the oncomiracidium within the egg. However the period of embryonation of the oncomiracidium at both temperatures was found to be closely similar. Despite the long period of egg incubation (15-20 days) at 10°C, only 7 eggs hatched while the rest still did not hatch even after 25 days of incubation. This suggests that the lower temperature is unsuitable for hatching for most of the eggs. Transferring these unhatched eggs to 18°-21°C showed that within the next 7 days all of them hatched, which confirmed that the larvae inside them were viable and had not been adversely affected by the lower temperature during the period of incubation and that when normal conditions return the eggs hatch to give living larva. These laboratory observations demonstrated that the eggs produced by the parasite in the field during autumn and early winter, as shown in Table 5.2, could survive low water temperatures and perhaps some of them could hatch the following spring.

The results also revealed that the oncomiracidium of D. homoion had a short period of life, at 18°-21°C about 3 hours from the egg

hatching until they died. The few dead larvae after 70 minutes of life may have been caused by the process of transfer of these larvae to the petri dish used in the experiment. The high rate of egg production by the adult parasites and cluster formation may accommodate the short life-span of the oncomiracidia and the great risks involved in the successful completion of the life cycle. The shedding of cilia normally occurred when oncomiracidia established on their hosts, but it also was seen on those which failed to find a suitable host just prior to their death. However, Khotenovskiĭ (1977b) found that the free-swimming oncomiracidium of D. homoion and many other Russian species survived for 5 to 6 hours at 15°-20°C.

Light did not influence the behaviour of oncomiracidia of D. homoion. Such was also observed in other Diplozoon spp. and in many other related monogenean groups. For example Bovet (1959) reported a similar mode of behaviour of the oncomiracidium of D. paradoxum. Owen (1970) found comparable results for Discocotyle sagittata in the presence of light. The reasons for other changes in the morphology of these larvae during their short life period were not investigated. The change of the oncomiracidium into an unpaired diporpa can only occur when it reaches the gills of its host. To complete the life cycle on the fish two unpaired diporpae must unite to form the adult Diplozoon. Unpaired diporpae were often found near to each other on the gills of fry. In these instances the stage of development were usually closely similar with an even number of the unpaired diporpae being collected from the gills of one side of the fry. These events must result as a consequence of the large number of invasive oncomiracidia hatching at the same time and place from an egg cluster. Very few double diporpae were observed at any stage

of their development. No doubt this relates to their rapid growth once they form a permanent union to form the adult stage.

The results at 18°-21°C show that within 5 days, beginning from the attachment of the oncomiracidia to the gills of the fishes, small young adults were found on the gills. Most of the diporpal stages were seen after 3 days. Therefore it is likely that the change from the oncomiracidium to the unpaired diporpa will take place within a very short time. There was a tendency for most of the oncomiracidia to settle close together on the gills, as many of these stages were obtained from the same primary lamella. This facilitates the process of their fusion.

The size of unpaired diporpae just after settlement was very small; a well-developed pair of oral suckers, a small area of intestine, a small pair of clamps and pair of larval hooks can be seen. Subsequently the size of the single diporpa increased steadily with consequent morphological changes, but especially the addition of more pairs of clamps. Single diporpae which do not find a partner for any reason continue to develop and possess more than 2 pairs of clamps. The fate of these larvae is uncertain. The median sucker which is important for union was absent in these individuals. It is interesting to note that there may be a limited period during which single diporpae can form a union with one another. This is suggested by the fact that the median sucker, the organ necessary for the initial union between the two larvae, appeared to be present only for a short time during the one and two pair of clamps stages. After this period, in the unpaired single diporpae the sucker could no longer be seen, but the larva could develop another pair of clamps without union with another diporpa larva. The results indicate that occasionally

both individuals of a pair of diporpae differ in the number of pairs of clamps developed. The majority of the larval stages found during the 5 days of study were unpaired diporpae. The assymetrical disposition of the clamps on the opisthaptor was also found on both unpaired and paired diporpae in a similar way as in adult to ensure a firm hold of these stages on the gill. Both the unpaired and paired diporpae were very active and able to change their positions on the gills to greatly increase the chances for the life cycle to be completed. As shown in Chapter 7, these larval stages preferred the fourth gill arch more than any other which would enhance the likelihood of union occurring.

From my studies of Diplozoon paradoxum it was evident that in every instance the diporpae had united ventral surface to ventral surface. This was made clear in all the specimens studied by the orientation of the opisthaptor in relation to the body of the worms as well as to the manner of attachment of the opisthaptor to the primary lamellae.

The presence of mucus-like material around the unpaired diporpae and the early stage of paired diporpae, together with the disappearance of this material in the late larval stages and juveniles may suggest that this material is specific for each Diplozoon species in order to facilitate two unpaired diporpae of this same species to be attracted to each other and unite and perhaps also to discourage other diporpae from a different species. The presence of tiny pores on the outer surface of D. homoion and D. paradoxum as described in Chapter 3, might be responsible for the secretion of this material.

The process of union of one of single diporpa over the other, to form the adult, may take place in two different ways as shown

in Fig. 5.19. The type of arrangement of the diporpae at their junction areas can be identified easily on the adult parasite, especially on the recently joined pairs as the arrangement is responsible for the different positions of the genital pores at the sides of the junction area.

The juvenile and young adult stages were found on the third to the fifth day of study. They were the most common stages seen at this time which indicated that most of the larval stages had been completed during the first two days to become adults by the third day. The sexually mature adult stage was seen on the tenth day. Unfortunately, there were no data available from the 6th to the 9th day of study so that it was uncertain on which day the formation of the newly gravid worm was accomplished. Generally, it is possible that the life cycle period of D. homoion might be shorter than that which was estimated during this study, depending on the availability of the infective oncomiracidia and the hosts.

Nordmann (1832) incorrectly described the union of two larval forms into the adult stage. Siebold (1851) was the first who gave a description of the fusion of two worms. Zeller (1872) also demonstrated most of life cycle stages of Diplozoon recovered from Phoxinus phoxinus. He also mentioned the presence of the median sucker on the unpaired dipora and its role in the conjugation. Sterba (1957) also studied the joining and crossing of the two individual worms of D. tetragonopterini and he concluded that the primary reasons for such a union was not to facilitate cross fertilization (that was thought to be a secondary development) but rather to ensure the secure attachment to the gills.

The period of life cycle of D. homoion as shown by the laboratory

experiments is confirmed by the field observations made during May and June. The gravid parasites of the overwintering worms from Llyn Tegid were seen in May 1983, while a few newly formed, small adult parasites as well as the old one were recovered during June 1983. This indicates that the life cycle took about a month. In Fig. 5.20, the life span of each stage and the possible manners of conjugation of the diporpae are illustrated.

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CHAPTER 6

POPULATION DYNAMICS OF *DIPLOZOON HOMOION* ON *RUTILUS RUTILUS* (L.) FROM LLYN TEGID

I. INTRODUCTION

Relatively little is known about the seasonal dynamics of Diplozoon populations. Most previous accounts do little more than determining the level of infection in different cyprinid species. Nearly all of these studies have been concentrated in Russia and Europe while nothing has been done on the Indian or African species.

In Russia, Komarova (1964) studied the seasonal infection of Blicca bjoerkna with D. blicca in the Dnepr Delta. Izynmova (1964) determined the level of Diplozoon paradoxum infection on 3 cyprinids Abramis brama, Blicca bjoerkna and Rutilus rutilus in the Rybinsk Reservoir throughout the year. In Poland, Wierzbicka (1974) recorded the seasonality of D. gussevi, D. nagibinae and D. pardoxum infections on Blicca bjoerkna, Abramis ballerus and Abramis brama respectively in Lake Dabie. Halvorsen (1972) found that D. paradoxum was present during all months on Rutilus rutilus in the River Glomma, Norway.

In Britain, there was little work about seasonality of Diplozoon infections on various cyprinid species. In the Shropshire Union Canal, Mishra (1966) found D. homoion (as D. paradoxum) on Abramis brama and Rutilus rutilus during all the year. Wiles (1965, 1968) recorded the prevalence of infection and mean intensity of these worms on many cyprinids collected from various localities in the north west of England. Unfortunately, his study was based on samples collected at sporadic intervals.

In Llyn Tegid, no detailed account was available about Diplozoon homoion infection on the gills of Rutilus rutilus. Chubb (1963) had recorded the occurrence of this parasite on R. rutilus (as D. paradoxum). Cheyne (1977) reported on the level of infection of

R. rutilus with *D. homoion* (as *D. paradoxum*), but his study was based on small fish samples and continued over a short period only (October 1976 - February 1977).

The object of this study was to determine the seasonal dynamics of *D. homoion* on *R. rutilus* at Llyn Tegid and also to study the effect of length of host on the infection.

II. MATERIALS AND METHODS

A. Field Work

Llyn Tegid (Bala Lake) was visited several times each month. Fig. 6.1 shows the sampling area. Samples were collected between 15th September 1982 to 6th December 1983 (Table 6.1). Additional samples were also taken during June and July 1984. Gill netting was the main technique used for capturing fishes. More than one gill net was set each time at various sites within the sampling area. Different mesh sizes (19.5 mm, 26 mm and 32 mm knot to knot) were used to provide acceptable samples of fishes. Usually the nets were laid at different depths, between 3-20 m, and left over night before lifting. After removing the fishes from the nets, they were kept in polythene bags and brought back to the laboratory. The shade air and surface water temperatures were recorded using a mercury thermometer on each occasion. Table 6.1 shows the total number of males, females and unsexed fishes caught in each sample.

Although, Llyn Tegid was visited many times during July and the first week of August 1983, no R. rutilus were caught, even though a variety of different gill nets were used and set at various depths. For that reason the additional samples were collected during June and July 1984. Table 6.2 illustrates the monthly mean values of shade air and surface water temperatures provided by the Welsh Water Authority, Northern Division compared with my personal observations.

Fig. 6.1. Map of Llyn Tegid. The sampling area is enclosed by the dashed lines.

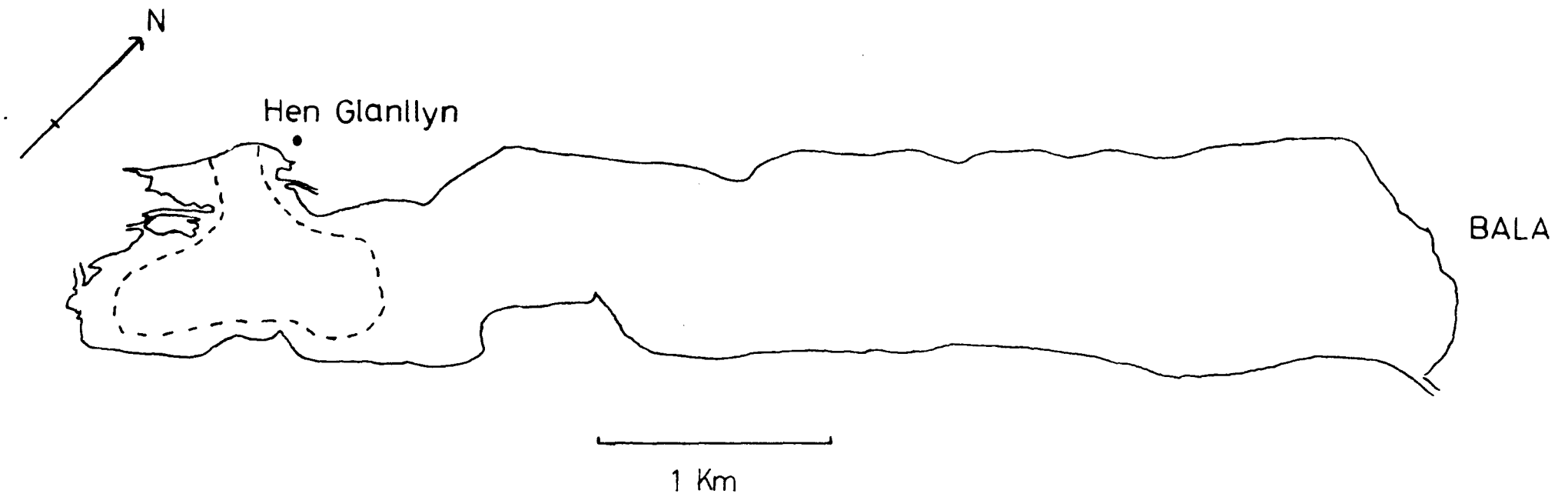


Table 6.1. Details of dates of visits to Llyn Tegid and samples of *Rutilus rutilus* collected.

Date of sample	Nos. of fishes			
	All	Male	Female	Unsexed
15th Sept. 1982	33	12	11	10
8th Oct.	11	8	3	0
12th Oct.	9	7	2	0
14th Oct.	7	5	2	0
2nd Nov.	18	10	8	0
1st Dec.	0	-	-	-
25th Dec.	16	12	4	0
18th Jan. 1983	26	15	11	0
3rd Feb.	10	9	1	0
15th Feb.	12	2	10	0
22nd Feb.	7	6	1	0
7th Mar.	44	32	11	1
7th April	35	25	9	1
5th May	60	30	30	0
1st June	31	21	10	0
20th June	28	21	7	0
12th July	0	-	-	-
19th July	0	-	-	-
26th July	0	-	-	-
2nd Aug.	0	-	-	-
17th Aug.	8	6	2	0

(contd..)

Table 6.1 (contd.)

Date of sample	Nos. of fishes All	Male	Female	Unsexed
23rd Aug.	15	8	7	0
25th Sept.	22	12	10	0
27th Sept.	4	2	2	0
3rd Oct.	10	7	3	0
7th Oct.	36	28	8	0
18th Oct.	20	14	5	1
25th Oct.	2	1	1	0
8th Nov.	6	4	1	1
29th Nov.	14	7	4	3
6th Dec.	6	6	0	0
14th June 1984	24	19	6	0
10th July	2	2	1	0
18th July	7	4	2	0
24th July	54	24	20	10
Total	578	359	192	27

Table 6.2. Air and surface water temperatures at Llyn Tegid for the period September 1982 - August 1983. The data in columns A and B are the mean temperatures obtained from the Welsh Water Authority, Northern Division. The data in columns C and D represent my personal observations.

Date	Mean Temperature (°C)			
	A	B	C	D
	Air	Water	Air	Water
Sept. 1982	12.6	14.2	17	14.5
Oct.	8.9	11.2	10	11.5
Nov.	7.3	9	9.5	10.5
Dec.	4	5.6	7	6
Jan. 1983	6.4	5.5	7	5.5
Feb.	5.3	3.3	2.5	2
Mar.	5.8	5	9.5	4.5
April	5.5	6.3	8.5	6.5
May	9.4	9.5	13.5	7.5
June	12.8	13.4	15.5	16
July	17.4	15.2	27	22
Aug.	15.4	14.6	18	19

B. Laboratory Work

Most fishes were examined as soon as they arrived at the Laboratory. Usually, the examination was completed over night, to avoid post-mortem effects. When the fish sample was large, some were kept in the deep freeze for later examination.

Every fish was weighed and its fork length was measured. A few scales were taken from the shoulder area dorsal to the lateral line. For determining the level of infection of the fish with D. homoion, the operculum was removed carefully from each side and kept for later cleaning and age determination. Then, the gills from each side of the fish were removed separately and kept in dishes of tap water or fish saline solution (0.65%). The number of parasites on each gill was checked carefully by using fine needles and forceps with the aid of a dissecting microscope. The details of localization of the parasites on the gills are discussed elsewhere (see Chapter 7). Diplozoon specimens collected from the gills were fixed either in 5% formaldehyde or 70% ethanol for later study. Then, the body cavity of the fish was opened and the sex and gonad condition were recorded. The stage of gonad development was determined according to the scheme of Nikolosky (1963). In some samples it was not possible to sex a number of R. rutilus owing to the immaturity of their gonads. These fishes are included in the tables of data, but will not be considered in detail in the text. Data for each fish were recorded on a card with an accession number. This card included the date of capture, fork length, weight, sex, gonad condition and the total number of D. homoion on the gills.

III. RESULTS

The total number of Rutilus rutilus collected during this study was 578: 359 (62.1%) were males, 192 (33.2%) were females and 27 (4.7%) could not be sexed (Table 6.1). The number of males was about twice that of the females, and males predominated in most months of the year.

A. Prevalence of Infection of Rutilus rutilus with D. homoion

Prevalence is used here as the number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined (Margolis et al., 1982).

The prevalences of infection of the total, males, females and unsexed Rutilus rutilus with D. homoion are shown in Table 6.3 and Fig. 6.2. Infections were found during all months of study.

In all fishes, the range of prevalence of infection was between 28.0% in June 1984 and 66.7% in December 1983 (Fig. 6.2). The value of prevalence of infection during July 1984 is introduced into Fig. 6.2 owing to the failure to sample R. rutilus during July 1983 as explained in the Materials and Methods section. Minor fluctuations of prevalence were seen throughout the seasons. The lowest levels of prevalence were especially in the summer and autumn months. For all fishes, 273 (47.2%) out of 578 were infected with D. homoion.

In the male fishes, the range of prevalence was between 21.4% in September 1983 and 66.7% in December 1983 (Table 6.3). The parasites were seen on males during all months. For the total male fishes, 176 (49.0%) out of 359 were found infected.

In females, the range of prevalence was between 12.5% in November 1982 and 63.6% in March 1983. The parasites were also found on

Table 6.3. The prevalance of infection of all, male, female and unsexed Rutilus rutilus with D. homoion from Llyn Tegid.

Date	Nos. of fishes Examined				Infected				Prevalence %			
	All	Male	Female	Unsexed	All	Male	Female	Unsexed	All	Male	Female	Unsexed
Sept. 1982	33	12	11	10	14	5	6	3	42.4	41.7	54.6	30.0
Oct.	27	20	7	0	15	12	3	-	55.6	60.0	42.9	-
Nov.	18	10	8	0	8	7	1	-	44.4	70.0	12.5	-
Dec.	16	12	4	0	7	6	1	-	43.8	50.0	25.0	-
Jan. 1983	26	15	11	0	13	7	6	-	50.0	46.6	54.6	-
Feb.	29	17	12	0	16	11	5	-	55.2	64.7	41.7	-
Mar.	44	32	11	1	29	21	7	1	65.9	65.6	63.6	100.0
April	35	25	9	1	15	11	3	1	42.9	44.0	33.3	100.0
May	60	30	30	0	28	14	14	-	46.7	46.7	46.7	-
June	59	42	17	0	19	15	4	-	32.2	35.7	23.5	-
Aug.	23	14	9	0	11	6	5	-	47.8	42.9	55.6	-
Sept.	26	14	12	0	8	3	5	-	30.8	21.4	41.7	-

(contd..)

Table 6.3 (contd.)

Date	Nos of fishes											
	Examined				Infected				Prevalence %			
	All	Male	Female	Unsexed	All	Male	Female	Unsexed	All	Male	Female	Unsexed
Oct.	68	50	17	1	30	23	7	0	44.1	46.0	41.2	0
Nov.	20	11	5	4	13	7	2	4	65.0	63.6	40.0	100.0
Dec.	6	6	0	0	4	4	-	-	66.7	66.7	-	-
June 1984	25	19	6	0	7	6	1	-	28.0	27.8	16.7	-
July	63	30	23	10	36	18	13	5	57.0	62.1	56.5	50.0
Total	578	359	192	27	273	176	83	14	47.2	49.0	43.2	51.9

Fig. 6.2. The prevalence of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

1982, 1983 ------- 1984

the gills of female R. rutilus during all months of study with many minor fluctuations (see Table 6.3). The overall prevalence of infection of female fishes was 43.2%; 83 fishes were infected out of 192.

Statistical comparison between prevalence of D. homoion on male and female R. rutilus for each month during the period of study revealed that there were no significant differences ($F = 1.1$, not significant at $p>0.05$ with 16 degrees of freedom). However, the results show that the prevalences of males in the samples were slightly higher than that of females.

The prevalence of infection of the total unsexed fishes (Table 6.3) was 51.9% (14 fishes out of 27 were found infected).

Generally, the prevalences of infection of the grand totals of male, female, unsexed and all Rutilus rutilus were similar to each other.

The prevalence of all male and female fishes can be seen with the data summarized into four monthly periods from September 1982 to December 1983 (Table 6.4).

In all fishes, there was no significant difference found between these values from the different quarterly period ($X^2 = 5.3$, not significant at $p>0.05$ with 3 degrees of freedom). The level of infection during January to April 1983 was slightly higher than in the other periods and the lowest were in May to August 1983. Also the percent of infection was relatively similar in both periods September to December 1982 and September to December 1983.

The prevalences of infection in both sexes during the 4 monthly periods followed that of the total fishes (Table 6.4).

The effect of fork length on the prevalence of infection of Rutilus rutilus with D. homoion is shown in Table 6.5 and Fig. 6.3.

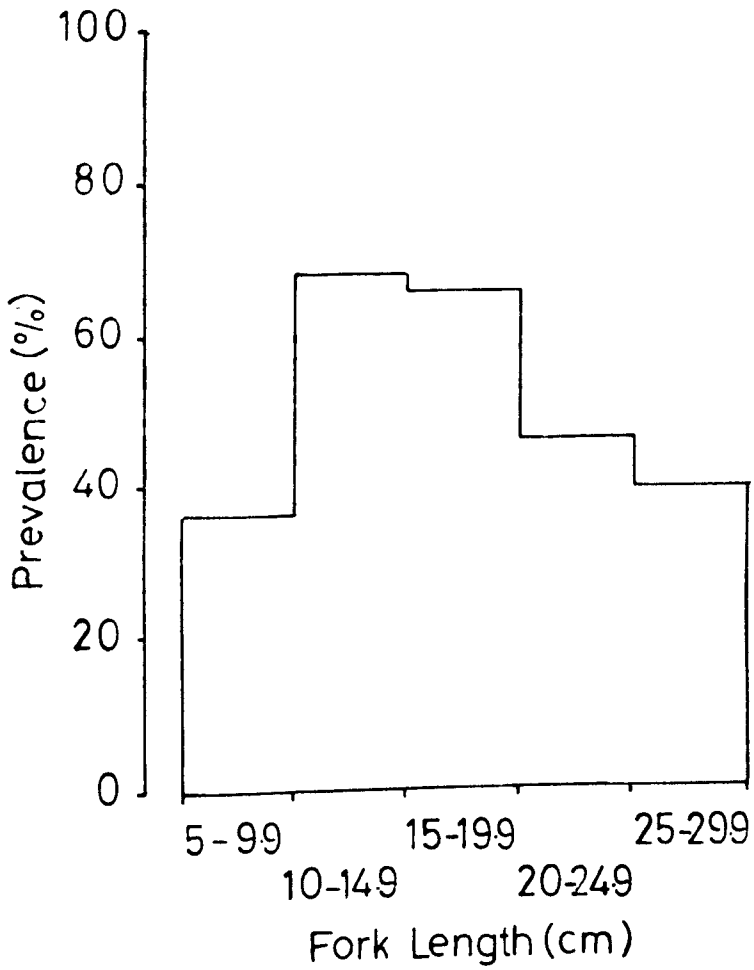
Table 6.4. The prevalence of infection of all, male and female Rutilus rutilus with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Nos. of fishes								
	Examined			Infected			Prevalence %		
	All	Male	Female	All	Male	Female	All	Male	Female
Sept.-Dec. 1982	94	54	30	44	30	11	46.8	55.6	36.7
Jan.-April 1983	134	89	43	73	50	21	54.5	56.2	48.8
May-Aug.	142	86	56	58	35	23	40.8	40.7	41.1
Sept.-Dec.	120	81	34	55	37	14	45.8	45.7	41.2

Table 6.5. The effect of fork length on the prevalence of infection of Rutilus rutilus with D. homoion from Llyn Tegid. Data for all months summed. A few fishes were excluded owing to caudal fin damage during netting.

Fork length class (cm)	Nos. of fishes		
	Examined	Infected	Prevalence (%)
5-9.9	11	4	36.4
10-14.9	41	28	68.3
15-19.9	26	17	65.4
20-24.9	399	183	45.9
25-29.9	94	37	39.4

Fig. 6.3. The effect of fork length on the prevalence of infection of Rutilus rutilus with D. homoion from Llyn Tegid.



The prevalences of infections were estimated for each 5 cm class. It is very clear from the Table and Figure that the prevalence of infection in small sizes of fishes was low (36.4%) and reached its highest level (68.3%) in fishes between 10-14.9 cm long. Then the prevalence gradually decreased as the size of fish increased and become 39.4% in fishes of 25-29.9 cm long, the longest length class. The Chi-Square test indicates that there was a highly significant difference between the values of infection with different fish sizes (X^2 = 39.87, highly significant at $p < 0.001$ with 4 degrees of freedom).

B. Relative Density of Infection of Rutilus rutilus with D. homoion

Relative density or abundance is the total number of individuals of a particular parasite species in a sample of hosts divided by total number of individuals of the host species (infected + uninfected) in the sample (Margolis, et al., 1982).

The relative density of infection for all, male, female and unsexed fishes is illustrated in Table 6.6 and Fig. 6.4. The monthly fluctuations of these values in general followed those of prevalence.

In all fishes, the range of relative density was between 0.7 in June 1983, September 1983 and June 1984, up to 2.8 in November 1983 (Fig. 6.4). The ratio of the mean of the relative density to the variance shows that the parasites were randomly distributed on the fishes throughout the period of study ($\frac{S^2}{\bar{x}}$ = 0.3). The relative density of infection for the grand total of fishes (578) was 1.5.

In male fishes, the range of abundance was between 0.7 in September 1983 up to 3.3 in July 1984 (Table 6.6). The relative density of grand total of males was 1.6. The ratio of the mean of the relative

Table 6.6. The relative density of infection of all, male, female and unsexed Rutilus rutilus with D. homoion from Llyn Tegid.

Date	Fishes examined				Parasites recovered				Relative density			
	All	Male	Female	Unsexed	All	Male	Female	Unsexed	All	Male	Female	Unsexed
Sept. 1982	33	12	11	10	32	20	9	3	1.0	1.7	0.8	0.3
Oct.	27	20	7	0	38	32	6	-	1.4	1.6	0.9	-
Nov.	18	10	8	0	16	14	2	-	0.9	1.4	0.3	-
Dec.	16	12	4	0	27	25	2	-	1.7	2.0	0.5	-
Jan. 1983	26	15	11	0	50	35	15	-	1.9	2.3	1.4	-
Feb.	29	17	12	0	33	19	14	-	1.1	1.1	1.2	-
Mar.	44	32	11	1	110	84	15	11	2.5	2.6	1.4	11.0
April	35	25	9	1	65	53	8	4	1.9	2.1	0.9	4.0
May	60	30	30	0	76	38	38	-	1.3	1.3	1.3	-
June	59	42	17	0	42	35	7	-	0.7	0.8	0.4	-
Aug.	23	14	9	0	21	16	5	-	0.9	1.1	0.6	-
Sept.	26	14	12	0	18	10	8	-	0.7	0.7	0.7	-
Oct.	68	50	17	1	88	60	28	0	1.3	1.2	1.6	-

(contd...)

Table 6.6. (contd.)

Date	Fishes examined				Parasites recovered				Relative density			
	All	Male	Female	Unsexed	All	Male	Female	Unsexed	All	Male	Female	Unsexed
Nov.	20	11	5	4	55	19	7	29	2.2	1.7	1.4	7.3
Dec.	6	6	0	0	15	15	-	-	2.5	2.5	-	-
June 1984	25	19	6	0	18	17	1	-	0.7	0.8	0.2	-
July	63	30	23	10	142	95	33	14	2.3	3.3	1.4	1.4
Total	578	359	192	27	844	587	198	61	1.5	1.6	1.0	2.3
Mean ($\bar{x}$)									1.5	1.7	0.9	
Variance (S^2)									0.5	0.5	0.2	
Ratio $\frac{S^2}{\bar{x}}$									0.3	0.3	0.9	

Fig. 6.4. The relative density of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

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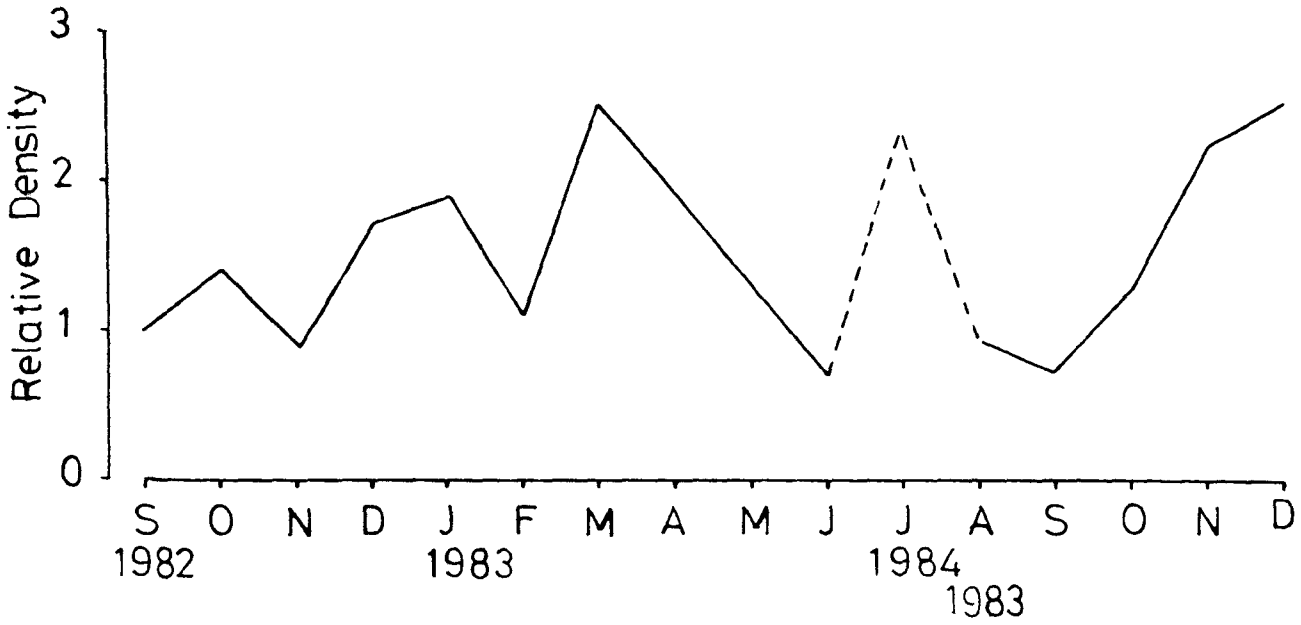

density to the variance shows that the abundance of these parasites in males was random as in the total fishes ($\frac{S^2}{\bar{x}}$ = 0.3).

In female fishes, the range was between 0.2 in June 1984 and 1.6 in October 1983 (Table 6.6). The relative density of the total females was 1.0, and the parasites were also distributed randomly throughout the female fishes during the whole year ($\frac{S^2}{\bar{x}}$ = 0.2).

A comparison between the relative density values for males and females during the period of study reveals that the difference between the values is significant (F = 2.5, significant at $p < 0.05$ with 16 degrees of freedom). Thus, in R. rutilus in Llyn Tegid the infection of males was slightly higher than in the females during most months of the year.

In the unsexed fishes, the relative density of total fishes (27) was 2.3 which is more or less higher than its comparable values of other sexes.

The fluctuation in the relative density of infection of all, male and female fish can be seen in data summarized into 4 monthly periods (Table 6.7). The pattern of relative density closely followed prevalence. As noted for the monthly analysis, there was a slight rise in relative density during the period January to April 1983 (1.9) compared with the three other periods. The lowest relative density was seen during May to August 1983 (1.0). These differences will be explained in Chapter 5 (life-cycle). The relative density for the period September to December 1982 and the comparable period of 1983 was almost the same.

In both sexes, the relative density for each of the 4 monthly periods was similar, with a slight rise in values during January to April 1983.

Table 6.7. The relative density of infection of all, male and female Rutilus rutilus with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Fishes examined			Parasites recovered			Relative density		
	All	Male	Female	All	Male	Female	All	Male	Female
Sept.-Dec. 1982	94	54	30	113	91	19	1.2	1.7	0.6
Jan.-April 1983	134	89	43	258	191	52	1.9	2.1	1.2
May-Aug.	142	86	56	139	89	50	1.0	1.0	0.9
Sept.-Dec.	120	81	34	176	104	43	1.5	1.3	1.3

From Table 6.7, it is interesting to note that the ratio of relative density of male compared with female R. rutilus for the periods September to December 1982 and January to March 1983 was approximately two to one, whereas by constrast, for the periods May to August and September to December 1983 it was one to one.

The effect of fork length on the relative density of infection of Rutilus rutilus with D. homoion can be seen in Table 6.8 and Fig. 6.5. The pattern of fluctuation in the values of occurrence of parasites in relation with fork length of fish follows that of the prevalence. The highest values of relative density (3.2 and 3.0) were observed on young fishes of both 10-14.9 cm and 15-19.9 cm length classes respectively, while the lowest levels (0.5 and 0.8) were found on small fishes (5-9.9 cm) and old ones (25-29.9 cm) respectively. Statistical analysis using Chi-square test shows the difference was real (X^2 = 53.0, significant at $p<0.05$ with 4 degrees of freedom).

C. Mean Intensity of Infection of Rutilus rutilus with D. homoion

Mean intensity is the total number of individuals of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in the sample (Margolis, et al., 1982).

The mean intensity of infection for all, male, female and unsexed fishes is presented in Table 6.9 and Fig. 6.6. They also followed the pattern of values of prevalences.

In all fishes, the range of the mean intensity was between 1.9 in August 1983 up to 4.3 in both April and November 1983. The ratio of the mean of the mean intensity to the variance shows that

Table 6.8. The effect of fork length on the relative density of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

Fork length class (cm)	Nos. of Fishes examined	Nos. of Parasites recovered	Relative density
5-9.9	11	6	0.5
10-14.9	41	133	3.2
15-19.9	26	79	3.0
20-24.9	399	543	1.4
25-29.9	94	75	0.8

Fig. 6.5. The effect of fork length on the relative density of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

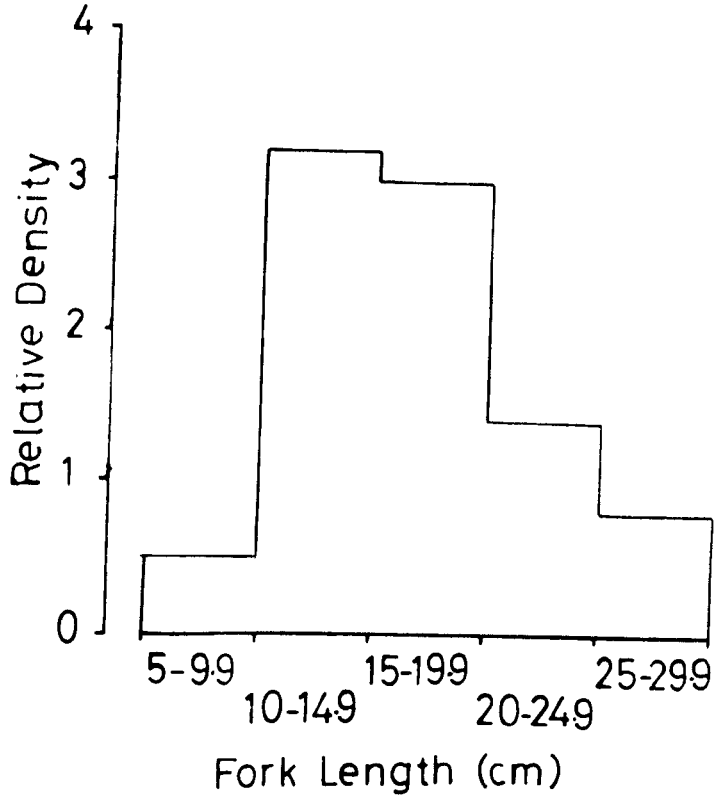

Table 6.9. The mean intensity of infection of all, male, female and unsexed *Rutilus rutilus* with *D. homoion* from Llyn Tegid.

Date	Fishes infected				Parasites recovered				Mean intensity			
	All	Male	Female	Unsexed	All	Male	Female	Unsexed	All	Male	Female	Unsexed
Sept. 1982	14	5	6	3	32	20	9	3	2.3	4.0	1.5	1.0
Oct.	15	12	3	0	38	32	6	-	2.5	2.7	2.0	-
Nov.	8	7	1	0	16	14	2	-	2.0	2.0	2.0	-
Dec.	7	6	1	0	27	25	2	-	3.9	4.2	2.0	-
Jan. 1983	13	7	6	0	50	35	15	-	3.9	5.0	2.5	-
Feb.	16	11	5	0	33	19	14	-	2.1	1.7	2.8	-
Mar.	29	21	7	1	110	84	15	11	3.8	4.0	2.1	11.0
April	15	11	3	1	65	53	8	4	4.3	4.8	2.7	4.0
May	28	14	14	0	76	38	38	-	2.7	2.7	2.7	-
June	19	15	4	0	42	35	7	-	2.2	2.3	1.8	-
Aug.	11	6	5	0	21	16	5	-	1.9	2.7	1.0	-
Sept.	8	3	5	0	18	10	8	-	2.3	3.3	1.6	-
Oct.	30	23	7	0	88	60	28	-	2.9	2.6	4.0	-

(contd.)

Table 6.9 (contd.)

Date	Fishes infected				Parasites recovered				Mean intensity			
	All	Male	Female	Unsexed	All	Male	Female	Unsexed	All	Male	Female	Unsexed
Nov.	13	7	2	4	55	19	7	29	4.2	2.7	3.5	7.3
Dec.	4	4	-	0	15	15	-	-	3.8	3.8	-	-
June 1984	7	6	1	0	18	17	1	-	2.6	2.8	1.0	-
July	36	18	13	5	142	95	33	14	3.9	5.3	2.5	2.8
Total	273	176	83	14	848	587	198	61	3.1	3.3	2.4	4.4
Mean $\bar{x}$									3.0	3.3	2.4	
Variance S^2									0.7	1.2	0.7	
Ratio $\frac{S^2}{\bar{x}}$									0.2	0.3	0.3	

Fig. 6.6. The mean intensity of infection of Rutilus rutilus with D. homoion from Llyn Tegid

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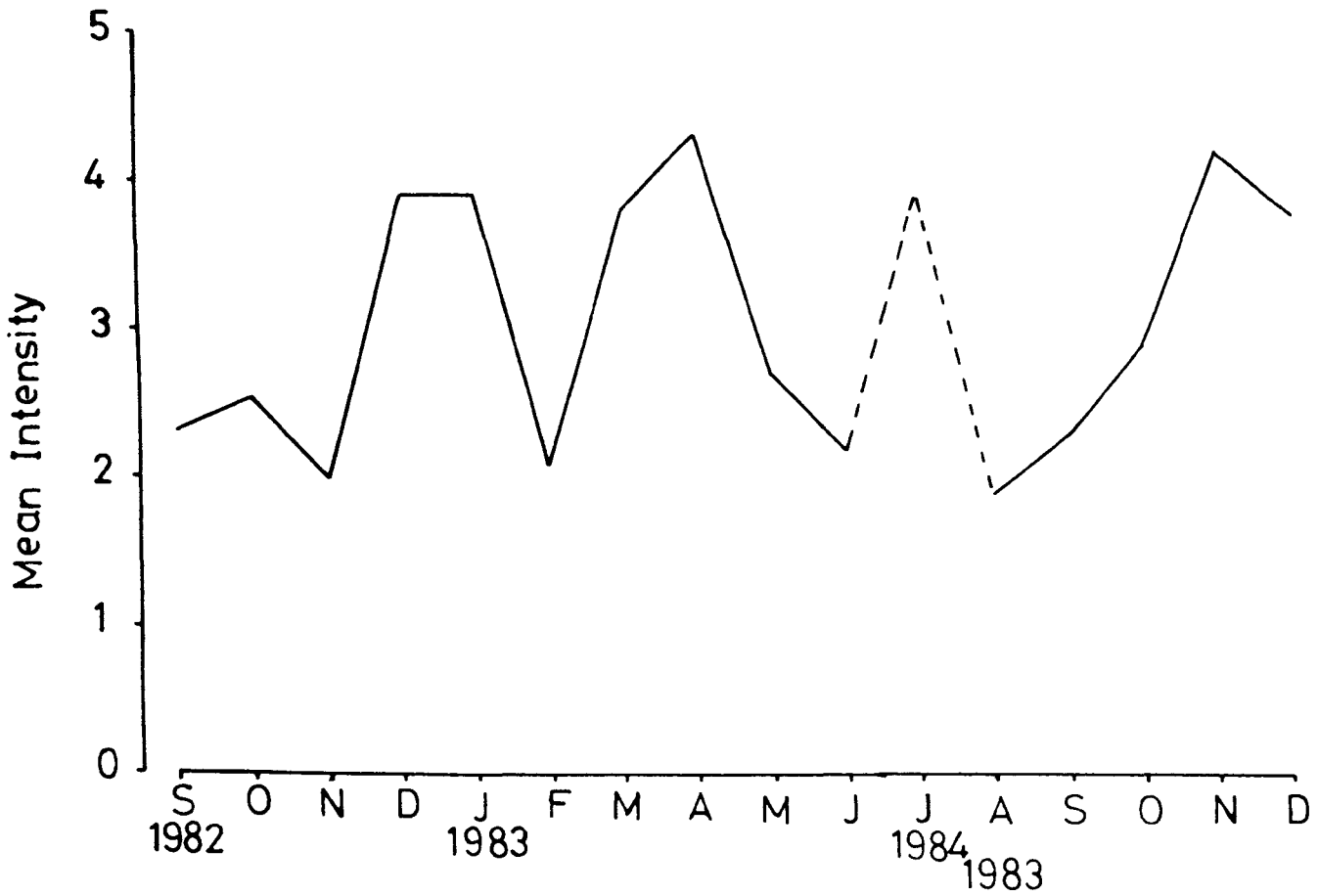


the parasites were randomly distributed on the infected fishes throughout the year ($\frac{S^2}{\bar{x}}$ = 0.2). The mean intensity of infection for the grant total of fishes was 3.1.

In males, the range of these values was between 1.7 in February 1983 up to 5.0 and 5.3 in January 1983 and July 1984 respectively (Table 6.9). The ratio of the mean of the mean intensity to the variance shows that the distribution of parasites among the male infected fishes was random throughout the whole year ($\frac{S^2}{\bar{x}}$ = 0.3). The mean intensity of grand total males was 3.3.

In females, the range of mean intensity of infection was between 1.0 in August 1983 and in June 1984 up to 4.0 in October 1983 (Table 6.9). Also the parasites were randomly distributed throughout the infected female fishes during the year ($\frac{S^2}{\bar{x}}$ = 0.3). The mean intensity of the grand total females was 2.4.

A comparison between the mean intensity of infection of males and females during the period of study indicates that there was no significant difference between them ($F = 1.7$, not significant at $p>0.05$ with 16 degrees of freedom).

In the unsexed fishes, the mean intensity of the total fishes was higher (4.4) than that of its comparable values of males and females.

The fluctuations in the levels of mean intensity of all, male and female fishes are summarized into 4 monthly periods in Table 6.10. These values also follow the pattern shown by prevalence of infection for the 4 monthly periods. The mean intensity was closely similar throughout with a slight rise in the period January to April 1983 in all, male and female fishes. A closely similar

Table 6.10. The mean intensity of infection of all, male and female Rutilus rutilus with D. homoion. Data summarized into 4 monthly periods.

Date	Fishes infected			Parasites recovered			Mean intensity		
	All	Male	Female	All	Male	Female	All	Male	Female
Sept.-Dec. 1982	44	30	11	113	91	19	2.6	3.4	1.7
Jan.-April 1983	73	50	21	258	191	52	3.5	3.8	2.5
May-Aug.	58	35	23	139	89	50	2.4	2.5	2.2
Sept.-Dec.	55	37	14	176	104	43	3.2	2.8	3.1

distribution of the parasites per infected fishes was seen between the period September to December of 1982 and 1983 for all or male fishes. But in female fishes, these values were slightly different (Table 6.10).

The effect of fork length on the mean intensity of infection can be seen in Table 6.11 and Fig. 6.7. The relation between the fork length of fishes and the number of parasites per infected fish has the same pattern as in prevalence and relative density. The highest values, 4.8 and 4.6, were seen on fishes 10-14.9 cm length and 15-19.9 cm long respectively and the lowest values, 1.5 and 2.0, were found on small fishes 5-9.9 cm long and the largest fishes, 25-29.9 cm long respectively. The Chi-square test indicates a significant difference between these values (X^2 = 130.5 significant at $p<0.05$ with 4 degrees of freedom).

D. Intensity of Infection of Rutilus rutilus with D. homoion

The intensity is the number of individuals determined directly or indirectly of a particular parasite species in each infected host in a sample (Margolis et al., 1982).

The frequency distribution for intensity of infection of all Rutilus rutilus for the period of September 1982 to December 1983 is shown in Table 6.12 and Fig. 6.8. The data are summarized into 4 monthly periods. It is obvious from these results that the numbers of fishes with different intensities were relatively constant during the year (X^2 = 15.02, not significant at $p>0.05$ with 12 degrees of freedom) with slight rise in these values during January to April 1983. The fish with maximum intensity (18) was seen in March 1983.

Table 6.11. The effect of fork length on the mean intensity of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

Fork length Class (cm)	Nos. of Fishes infected	Parasites recovered	Mean intensity
5-9.9	4	6	1.5
10 -14.9	28	133	4.8
15-19.9	17	79	4.6
20-24.9	183	543	3.0
25-29.9	37	75	2.0

Fig. 6.7. The effect of fork length on the mean intensity of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

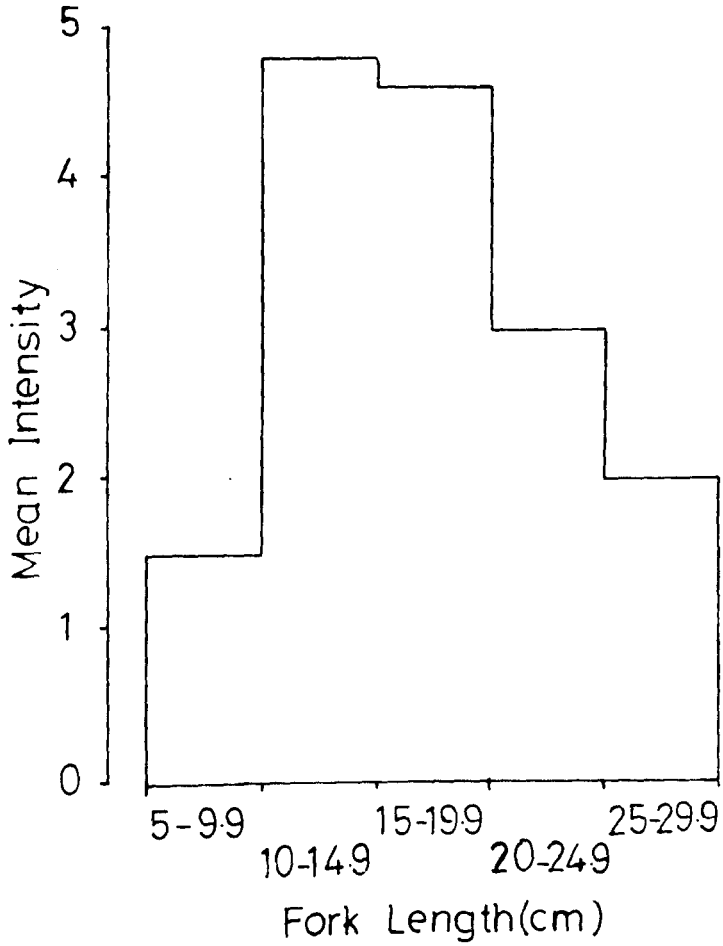
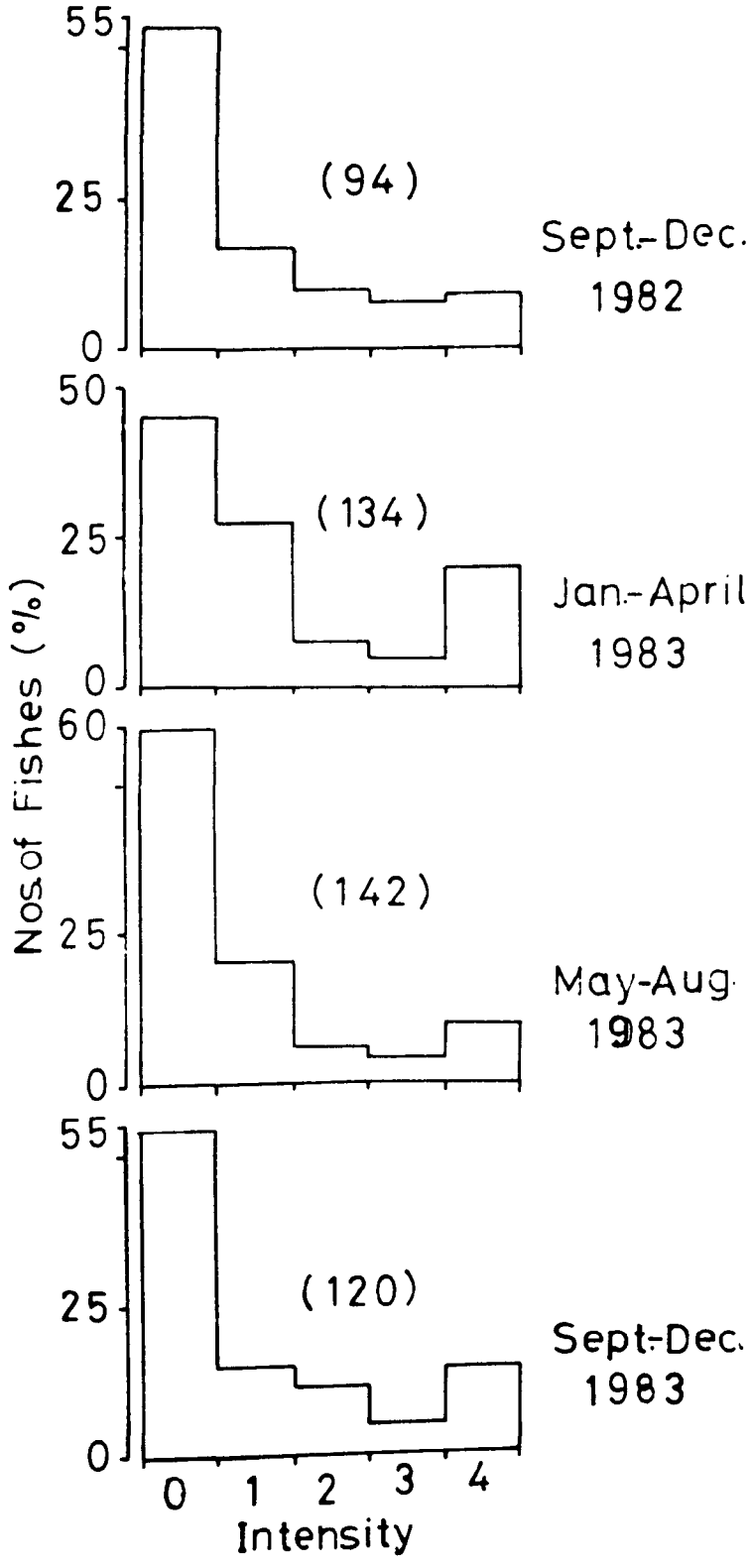


Table 6.12. Frequency distribution of all Rutilus rutilus with different intensities of infection with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Nos. of fishes Examined	Intensity 0 (%)	1 (%)	2 (%)	3 (%)	4 or more (%)	Maximum nos. of parasites on one fish and the month
Sept.-Dec. 1982	94	50 (53.2)	17 (18.1)	10 (10.6)	8 (8.5)	9 (9.6)	12, September
Jan.-April 1983	134	61 (45.5)	27 (20.1)	11 (8.2)	7 (5.2)	28 (20.9)	18, March
May-Aug.	142	84 (59.2)	28 (19.7)	9 (6.3)	6 (4.2)	15 (10.6)	8, May
Sept.-Dec.	120	65 (54.2)	18 (15.0)	14 (11.7)	6 (5.0)	17 (14.2)	15, October

Fig. 6.8. Frequency distribution of Rutilus rutilus with different intensities of infection with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods. The number of fishes for each period are shown in parentheses.



The frequency distribution of the intensities estimated for the total fishes collected (578 fishes) during the period of study were: 53.3% (308 fishes) free from infection, 18.3% (106 fishes) with intensity 1, 8.1% (47 fishes) with intensity 2, 5.5% (32 fishes) with intensity 3 and 14.7% (85 fishes) with intensity 4 or more parasites. The negative binomial distribution test (Kolmogorov-Smirnov test) shows that there is no significant difference between the observed and expected numbers of fishes (K-S = 0.0117, not significant at $p>0.05$ and 0.01 with n = 578).

In both males and females, the frequency distribution of numbers of fishes with different numbers of parasites are illustrated in Table 6.13 and 6.14 and Fig. 6.9. Generally,these results followed the pattern for the intensity of infection for all fishes.

In males, the numbers of fishes with various levels of intensity during each 4 monthly period were nearly identical (X^2 = 20.46, not significant at $p>0.05$ with 12 degrees of freedom) with a slight rise in the maximum intensity (4 or more parasites) during January to April 1983 (Table 6.13 and Fig. 6.9). The male fish with a maximum number of parasites (18) was caught in March 1983. In the grand total of males (359) for the whole period of study, the frequency distribution of fishes with different intensities was 51.0% (183 fishes) without infection, 16.2% (58 fishes) with intensity 1, 8.9% (32 fishes) with intensity 2, 6.4% (23 fishes) with intensity 3 and 17.5% (63 fishes) with intensity 4 or more. The negative binomial distribution test shows no difference between observed and expected values (K-S = 0.0199, not significant at $p>0.05$ and 0.01 with n = 359).

In females, the frequency distribution of their numbers with

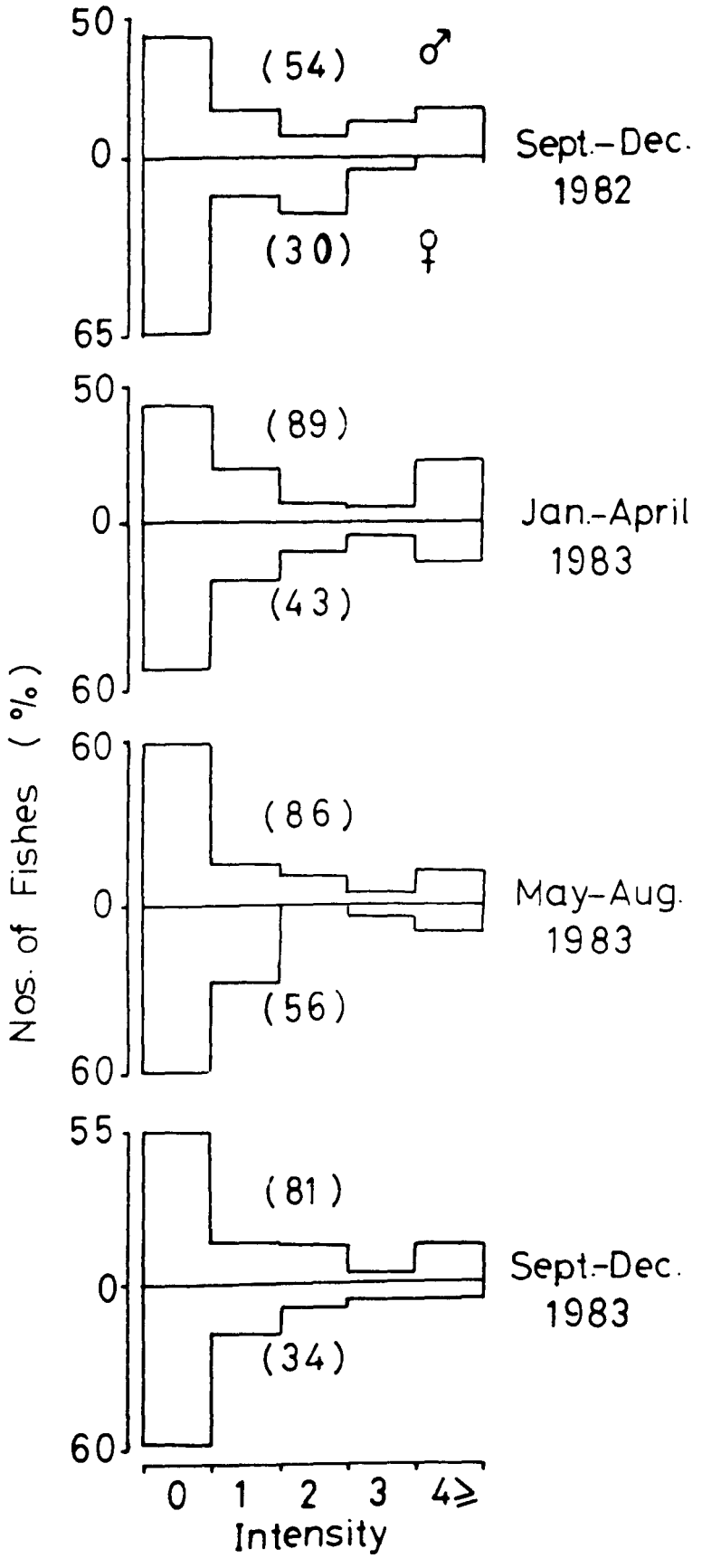
Table 6.13. Frequency distribution of males Rutilus rutilus with different intensities of infections with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Nos. of fishes Examined	Intensity 0 (%)	1 (%)	2 (%)	3 (%)	4 or more (%)	Maximum nos. of parasites on one fish and the month
Sept.-Dec. 1982	54	24 (44.4)	10 (18.5)	4 (7.4)	7 (13.0)	9 (16.7)	12, September
Jan.-April 1983	89	39 (43.8)	18 (20.2)	7 (7.9)	5 (6.0)	20 (22.5)	18, March
May-Aug.	86	51 (59.3)	12 (14.0)	9 (10.5)	4 (4.7)	10 (11.8)	7, May
Sept.-Dec.	81	44 (54.3)	12 (14.8)	11 (13.6)	3 (3.7)	11 (13.6)	7, October and December

Table 6.14. Frequency distribution of females Rutilus rutilus with different intensities of infections with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Nos. of fishes Examined	Intensity 0 (%)	1 (%)	2 (%)	3 (%)	4 or more (%)	Maximum nos. of parasites on one fish and the month
Sept.-Dec. 1982	30	19 (63.3)	4 (13.3)	6 (20.0)	1 (3.3)	0	3, September
Jan.-April 1983	43	22 (51.2)	9 (20.9)	4 (9.3)	2 (4.7)	6 (14.0)	7, January
May-Aug.	56	33 (59.0)	16 (28.6)	0	2 (3.6)	6 (10.7)	8, May
Sept.-Dec.	34	20 (58.9)	6 (17.6)	3 (8.8)	2 (5.9)	2 (5.9)	15, October

Fig. 6.9. Frequency distribution of males and females Rutilus rutilus with different intensities of infections with D. homoion from Llyn Tegid. Data summarized into 4 monthly periods. The number of fishes for each period are shown in parentheses.



different levels of intensity of infection during the 4 monthly periods is illustrated in Table 6.14 and Fig. 6.9. The results show no significant difference between the numbers of females with different intensities in relation with various periods of the year (X^2 = 17.93, not significant at $p>0.05$ with 12 degrees of freedom). The female with the maximum number of parasites (15) was seen in October 1983. In the grand total females for the period of study (192 fishes), the frequency distribution of the numbers of fishes with different levels of intensities was: 56.8% (109 fishes) with intensity 0, 22.9% (44 fishes) with intensity 1, 7.8% (15 fishes) with intensity 2, 3.6% (7 fishes) with intensity 3 and 8.9% (17 fishes) with intensity 4 or more. These results fit with the negative binomial distribution (K-S = 0.03, not significant at $p>0.05$ and 0.01 with n = 192).

A comparison between the males and females intensity of infection during different periods of time (Tables 6.13 and 6.14 and Fig. 6.9) indicates no difference between them. Also the Chi-square test shows no difference in intensities between the pattern of distribution for the total numbers of males and females (X^2 = 12.16, not significant at $p>0.204$ with 9 degrees of freedom).

In the total unsexed fishes (27), the frequency distribution of the numbers of fishes with different intensities was: 59.3% (16 fishes) without infection, 14.8% (4 fishes) with intensity 1, no fish was observed with intensity 2, 7.4% (2 fishes) with intensity 3 and 18.5% (5 fishes) with intensity 4 or more. The two unsexed fishes had the maximum number of parasites (11) were caught in March and November 1983. The statistical test confirmed that the distribution

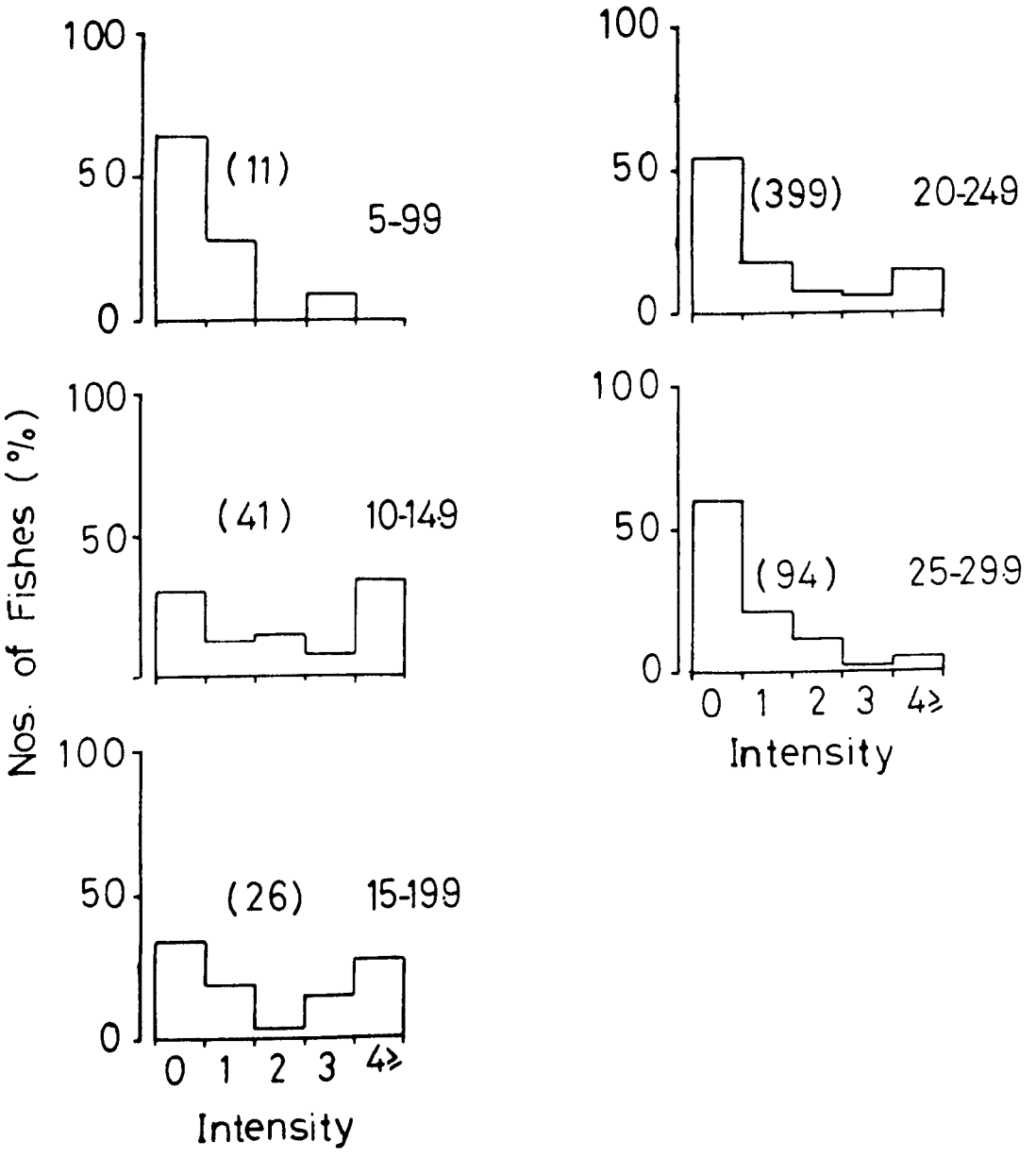
of unsexed fishes with different intensities fitted the negative binomial distribution (K-S = 0.07, not significant at $p > 0.05$ and 0.01 with n = 27).

The effect of fork length on the intensity of infection of Rutilus rutilus with D. homoion is shown in Table 6.15 and Fig. 6.10. The results again follow the pattern shown for the fork length of fish in relation to the mean intensity of infection. It appears that the number of fishes with each intensity of infection increases with the increase of fork length class up to 15-19.9 cm, and then decreases as the size of the fishes becomes higher (X^2 = 42.33, highly significant at $p < 0.05$ with 16 degrees of freedom). The length of fish with maximum number of parasites (18) was about 18 cm long.

Table 6.15. The effect of fork length on the intensity of infection of Rutilus rutilus with D. homoion from Llyn Tegid.

Class of fork length (cm)	Examined	Nos. of fishes Intensity					Maximum nos. of parasites on one fish
		0 (%)	1 (%)	2 (%)	3 (%)	4 or more (%)	
5-9.9	11	7 (63.6)	3 (27.3)	0	1 (9.1)	0	3
10-14.9	41	13 (31.7)	5 (12.2)	6 (14.6)	3 (7.3)	14 (34.1)	12
15-19.9	26	9 (34.6)	5 (19.2)	1 (3.8)	4 (15.4)	7 (26.9)	18
20-24.9	399	216 (54.1)	73 (18.3)	28 (7.0)	23 (5.8)	59 (14.8)	17
25-29.9	94	57 (60.6)	20 (21.3)	11 (11.7)	1 (1.1)	5 (5.3)	10

Fig. 6.10. The effect of fork length on the intensity of infection of Rutilus rutilus with D. homoion from Llyn Tegid. Number of fishes examined shown in parentheses.



IV. DISCUSSION

Rutilus rutilus is the only species of cyprinid which has been caught from Llyn Tegid during the period of this study. The predominance of males in the samples for most months of the year at Llyn Tegid may be attributed either to the fact that males are more numerous than females at this locality or perhaps to the feeding behaviour of females which may prefer deep water (below 20 m). The disappearance of Rutilus rutilus during the period 12th July to 2nd August 1983 was likely to have been influenced by unusually high air and water temperatures. Table 6.2 columns A and B show a considerable rise in mean temperature during July to August 1983. However my personal record of air and surface water temperatures shows that they were very high during this period (Table 6.2 columns C and D). The surface water temperatures were 22°C and 19°C during July and August respectively.

My investigations show that D. homoion infections were found during all months of the year on both male and female fishes. For either sex nearly 50% of fishes were found to be infected. The prevalence of infection of the total, male and female fishes was a little higher during January to April 1983 while the number of parasites per fish remained at about the same level during the whole year.

These results contradict the findings of other workers who stated that the levels of Diplozoon species infection were higher on most cyprinids species during the summer, with a decline in winter. Komarova (1964) found the prevalence of D. markewitschi on Vimba vimba vimba in Dnepr Delta increased from 20% in February to 66.6% during June to July and fell to 13.2% in October. She recorded the highest prevalence and mean intensity in March to July. She

also found D. blicca on Blicca bjoerkna in the Dnepr Delta from February to June and October with a maximum prevalence of 31.2% and a mean intensity of 7 in June. In Poland, Wierzbicka (1974) found that the prevelance of D. gussevi infection on Blicca bjoerkna was 62% and the mean intensity 3.2 with a maximum prevalence of 100% in July and August. The lowest prevalence of 10% was seen in autumn and early winter. The infection of Abramis ballerus with D. nagibinae was 81.3% and the mean intensity was 5.34 while the peak of prevalence was seen in the autumn (100%), and lowest (18.7% and 29.4%) in November to December. In Britain, Mishra (1966) recorded a maximum prevalence of infection of Rutilus rutilus and Abramis brama with D. homoion during July to August.

The present results are attributable to the onset of reproductive activity of D. homoion at Llyn Tegid from May onward. From this time there is a high mortality rate of the overwinter worms, which is rapidly replaced by young worms formed during summer. The mortality is thought to explain the decrease in prevalence, relative density and mean intensity which was seen from May to August. It should be noted that this decrease was not statistically significant, nevertheless the decrease occurred at a biologically significant period in the life of the D. homoion.

Although not statistically significant, nonetheless the slightly higher prevalence of infection on the fishes and the slight increase of the number of parasites per fish during January to April were perhaps a result of the availability of the eggs of D. homoion in water until late autumn and early winter (see Chapter 5) which may have developed throughout winter and have allowed infection of fishes

to continue. This was also true for male and female fishes considered separately.

The results also show that the prevalence, mean intensity and intensity of infection of males and females were closely similar except for relative density where it was higher in males than in females. Also a small difference was noticed between the sexes in the number of infected fishes and the number of parasites per fish during some of the four month periods of time. These differences may attributed to sampling errors to variation in behaviour between the sexes. Paling (1965) found that males of Salmo trutta harboured more Discocotyle sagittata than females. However in cestode infections in which females were more infected than males, Thomas (1964) and Kennedy (1968) reported that the differences were usually confined to the breeding season of the host.

The present results also show a close similarity of prevalence and number of parasites per fish for the 4 month periods September to December 1983 and 1983 for all fishes and both sexes. These results agree with the opinions of Hopkins (1959), Walkey (1967) and Kennedy (1969) who believed that the pattern of occurrence in a host-parasite system at any locality varied little from year to year.

My results indicated that the distribution of the numbers of fishes with different intensities of D. homoion fitted the negative binomial distribution for all males and females during all the year. There was no significant difference between males and females which means that the distribution of parasites on each fish was not affected by its sex.

A small number of unsexed Rutilus rutilus 5 - 9.9 cm was examined. They were infected with D. homoion. Details of prevalence, relative density and mean intensity can be seen in Tables 6.5, 6.8 and 6.11 respectively.

Lucký (1981) also mentioned that 20% of fry Hypophthalmichys molitrix at Pohořelice were infected with D. homoion. Kawatsu (1978) recorded D. nipponicum infection on small Carassius carassius in Japan. On the other hand, Wiles (1970), Bychowsky (1957) and Anderson (1974) did not find Diplozoon infection on the fry of the cyprinid species they studied.

The current study shows that there was a positive relationship between the fork length of fishes and the prevalence of infection and the number of parasites per fish. The level of infection was lowest on the small fishes, 5-9.9 cm and was higher in the fishes 10-19.9 cm group but fell again as length increased thereafter (Tables 6.5, 6.8 and 6.11), which means that small fishes and large fishes have lower infection with small numbers of parasites. In other monogenean parasites, Remley (1942), Frankland (1955), Wiles (1965) and Anderson (1974) found that the numbers of parasites rose with increasing age. Wiles (1965) noted this positive relationship with Diplozoon infections in Rutilus rutilus and Phoxinus phoxinus but not in Abramis brama. He reported that in gill parasites, one of the causal factors involved in the increase of parasite burdens possibly included the larger volumes of water drawn in during respiration and the increase in the available attachment area on the gills. But Chappell (1969) found Gyrodactylus rarus in Gasterosteus aculeatus decreased in abundance with increasing host age. Chapter 7 examines

the distribution of *D. homoion* on the gills of *Rutilus rutilus* from Llyn Tegid in more depth.

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CHAPTER 7

FREQUENCY DISTRIBUTION OF *DIPLOZOON HOMOION* ON THE GILLS OF *RUTILUS RUTILUS* (L.) FROM LLYN TEGID

I. INTRODUCTION

The distribution and mechanisms of attachment of many monogeneans on the gills of their hosts have been described. For instance, Cerfontaine (1896) was the first to describe the disposition of Diclidophora denticulata on the gills of Gadus virens. Later Sproston (1945) and Llewellyn (1956a) demonstrated the attachment of Kuhnia scombri on the gills of Scomber scrombrus. Llewellyn (1956b) also described the distribution of Plectanocotyle gurnardi on the gills of Trigla cuclus and T. lineata. The preferential settlement of Discocotyla sagittata on the gills of Salmo trutta was studied by Llewellyn and Owen (1960) and Paling (1969).

In the genus Diplozoon, Owen (1963a and b), Bovet (1959 and 1967), Wiles (1968), Cheyne (1977) and Khotenovskiĭ (1980) described the adhesive attitude and orientation of these compound worms in many cyprinid species. The asymmetrical alignment of clamps on the posterior parts of these parasites was incorrectly used for identification of some species of Diplozoon (Reichenbach-Klinke, 1951 and 1954; Sterba, 1957). Most of the previous studies were based on small samples of parasites for investigating the process of attachment of Diplozoon species on the gills of their hosts.

Therefore, in the current study it was considered essential to examine large samples of fishes to determine whether or not the biology of the species of host might have a significant effect on the mode of settlement of the parasite on the gills of R. rutilus in Llyn Tegid.

II. MATERIALS AND METHODS

Collecting and examination of the fishes are detailed in chapter 6. The exact site of attachment of all the Diplozoon on the gills was checked with the aid of stereomicroscope for each infection. The distribution was recorded according to: 1-the serial number of the gill arches; 1, 2, 3 and 4: 2 - the left and right sides of the fish; 3 - the outer and inner hemibranchs: 4 - the mode of attachment either on one or two primary lamellae; 5 - the occupation of the dorsal, median or ventral segment of the hemibranch. The dorsal surface of primary gill lamella represents the side which is nearest to the roof of the buccal cavity. The disposition of D. homoion on the gills of fry Leuciscus leuciscus and the alignment of the clamps on the opisthaptors of unattached worms were studied using scanning electron microscope. Specimens of unattached worms of D. paradoxum and D. rutili were also used for comparison. Techniques for preparation of the materials are mentioned earlier (Chapter 3).

For examining live adults and post larval stages (unpaired diporpa and paired diporae), 10 live Rutilus rutilus, 15-22.5 cm long, were captured by gill nets and brought back from Llyn Tegid in an aerated water tank. In the laboratory, these fishes were transferred to another 2 tanks containing tap water which had been well-oxygenated and which also included many clusters of eggs of D. homoion. The clusters of eggs were laid by stock D. homoion which were already maintained in the laboratory as described elsewhere (Chapters 3 and 5). The tanks were kept for a month at a water temperature of 18°-21°C, then the fishes were killed and the gills carefully examined for the number and behaviour of the infective stages on them. To achieve this, each gill was immersed in a dish

with tap water and immediately checked. As far as possible, care was taken to avoid clots of blood which formed on the gill surfaces as they influenced attachment of larval stages. The clots were removed gently using fine needles under the dissecting microscope. In order to check the ability of living adult and diporpal stages to reattach to the gill tissues, a fine needle was used to carefully detach some of the clamps of these stages from the secondary lamellae without causing damage to the parasites.

III. RESULTS

A. Orientation of Clamps on the Opisthaptors of adult D. homoion

Adult worms of the genus Diplozoon normally use 8 pairs of clamps and 2 pairs of hooks to secure their position on the gills of the host. Often, each 4 pairs of clamps are asymmetrically aligned on the left and right sides of the opisthaptor during its attachment to the secondary gill lamellae (Fig. 7.1). In this Figure, the arrangement of eight clamps of one opisthaptor (which is in the lower part of the picture) is more or less in one series so each clamp can grasp a single secondary lamella, e.g. the eight clamps attach to eight consecutive secondary lamellae of the same face of primary lamella. The other type of clamp arrangement is a bilateral symmetry where every pair of clamps on one opisthaptor will attach to the same surface of one secondary lamella as shown by the second opsithaptor at the upper right of the picture (Fig. 7.1). This means that 4 consecutive secondary lamellae are involved with one pair of clamps from each half of the opisthaptor attached to each. The asymmetrical disposition can also be seen in an unattached adult worm (Fig. 7.2). The 8 clamps on each of the posterior regions of one Diplozoon are asymmetrically arranged. The scanning electron microscope studies on the tissues surrounding the 4 clamps on each side of the opisthaptor (Fig. 7.1 and 7.2) and observations on living specimens on the gills strongly suggest that the 4 clamps on the left and right sides of each opisthaptor can move freely as one segment around its axis to a considerable angle. It seems that the tissues of the 4 clamps were relatively isolated from the inner surface of either sides of the opisthaptors. This characteristic feature was also seen on dead

Fig. 7.1. Attachment of the opisthaptors of D. homoion to the corresponding dorsal surfaces of two consecutive primary lamellae. Damaged areas of primary lamellae arrowed. c, clamps; ds, dorsal surface of primary lamella; md, muscular disc; pl, primary lamella; sl, secondary lamella; vs, ventral surface of primary lamella. Markers = 10μm.

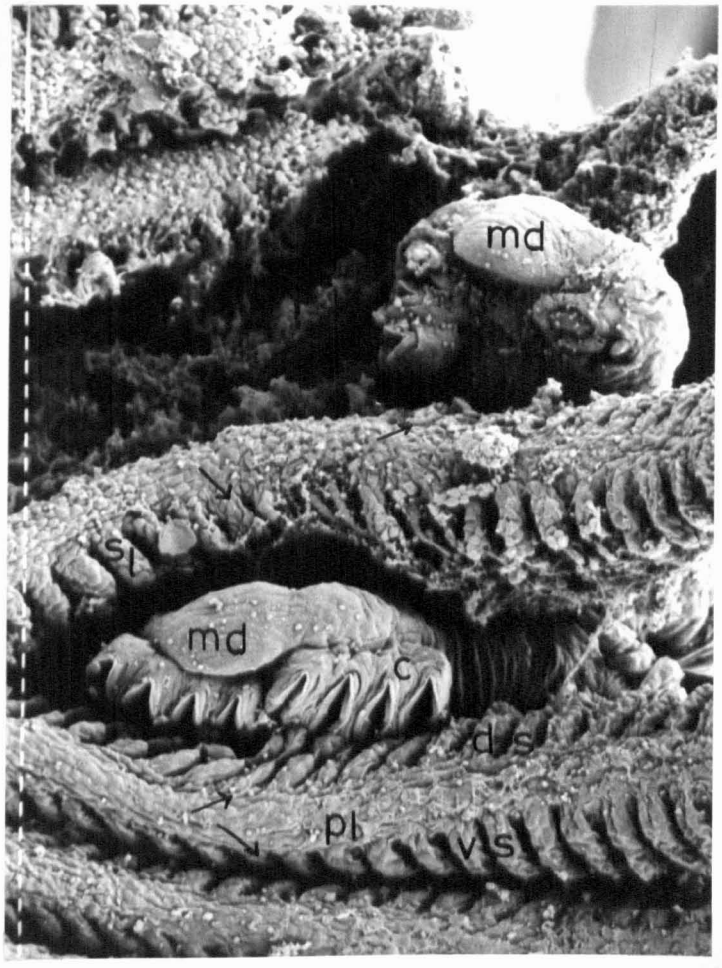
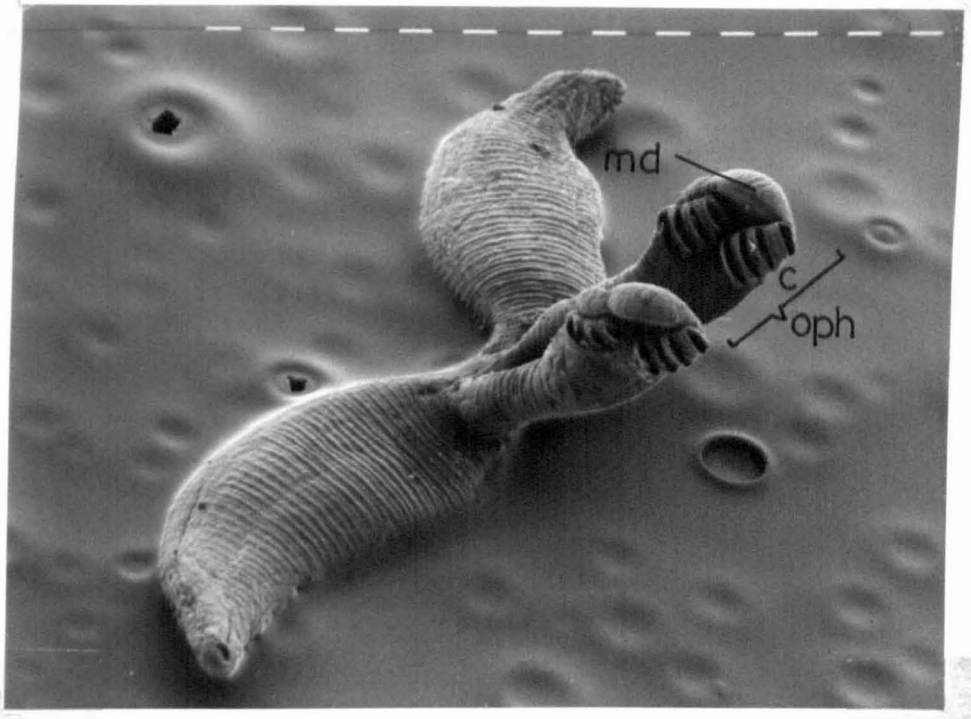


Fig. 7.2. Arrangement of clamp faces of the two opisthaptors of *D. homoion* (ventral to dorsal faces) when the parasite was attached to two consecutive primary lamellae.
c, clamps ; md, muscular disc; oph, opisthaptor.
Markers = 100 µm.



specimens of D. paradoxum and D. rutili as shown in Chapter 3 (Figs. 3.12, 3.14 and 3.16 respectively).

B. Mode of Attachment of D. homoion to the Gills of Rutilus rutilus

All Diplozoon specimens examined on the gills showed that the adult worms always occupied the space between the outer and inner hemibranchs of the gill in a way so that the longitudinal axis of the parasite lay parallel to the primary lamellae with its anterior ends towards the outer free end of the gill and posterior ends of the parasite near the interbranchial septum (Fig. 7.3).

The attachment of the adhesive organs of the adult parasite to the gill usually took place on one or two consecutive primary lamellae. When only one primary lamella was involved both posterior ends of the worm were attached to the opposing surfaces of the same lamella (dorsal and ventral surfaces) as appears in Fig. 7.4. The clamps, in this case, must face each other on the posterior parts of the worm as is shown in the dead specimen (Fig. 7.5). The second type was when the pair of opisthaptors attached to the corresponding sides of two consecutive primary lamellae from the same hemibranch (Fig. 7.1). Consequently, there are two possibilities of attachment. The parasite was attached either to two corresponding dorsal (dorsal and dorsal) or two ventral (ventral and ventral) surfaces of two consecutive primary lamellae. The arrangement of the ventral (clamp) sides of the two opisthaptor is likely to be as in Fig. 7.2. These were the only types of attachment which were seen during this study.

The attachment of the parasite also varied according to outer and inner hemibranch as well as to the dorsal, median or ventral

Fig. 7.3. Position of parasite between the two hemibranchs of the gill. ar, anterior region of the parasite; ibs, interbranchial septum; ih, inner hemibranch; oh, outer hemibranch; por, posterior region of parasite. Markers = 100µm.

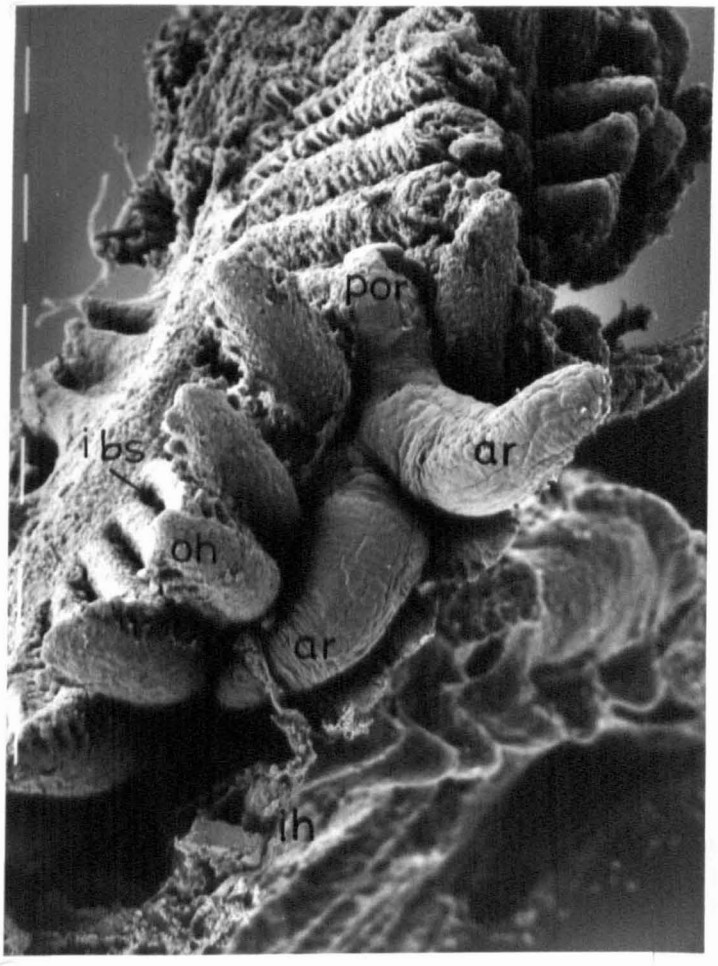


Fig. 7.4. Attachment of two opisthaptors of an adult parasite to the two opposing sides (dorsal and ventral) of one primary lamella. Damaged areas of primary lamellae arrowed. ds, dorsal surface of primary lamella; vs, ventral surface of primary lamella. Markers = 10µm.

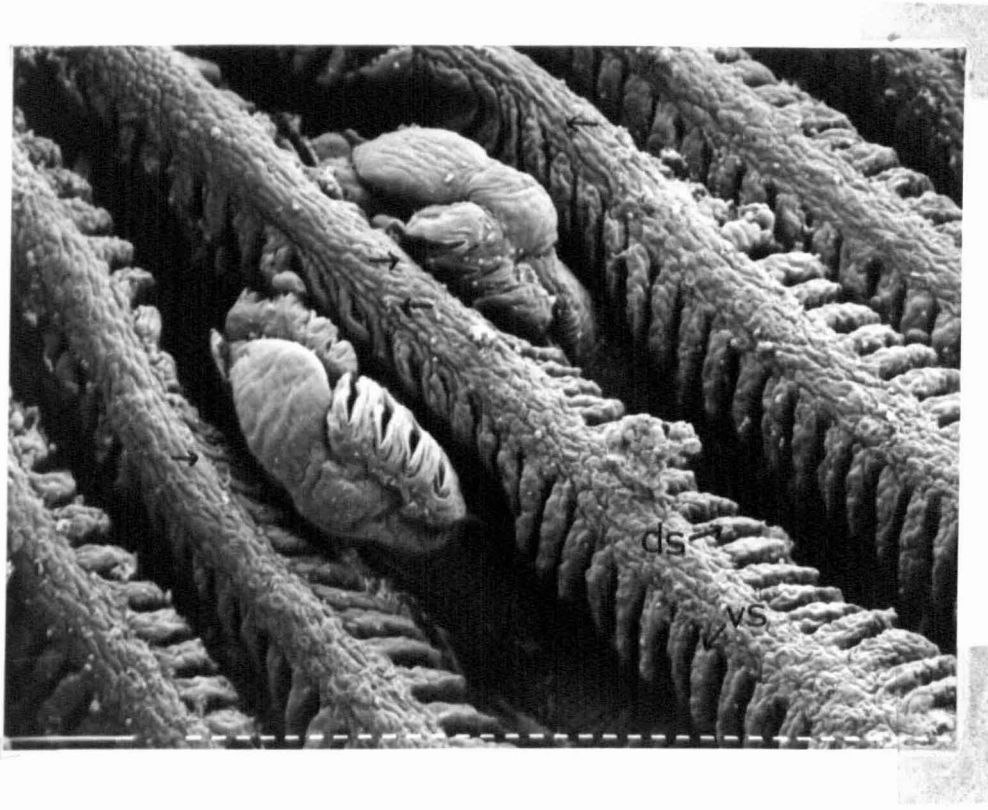
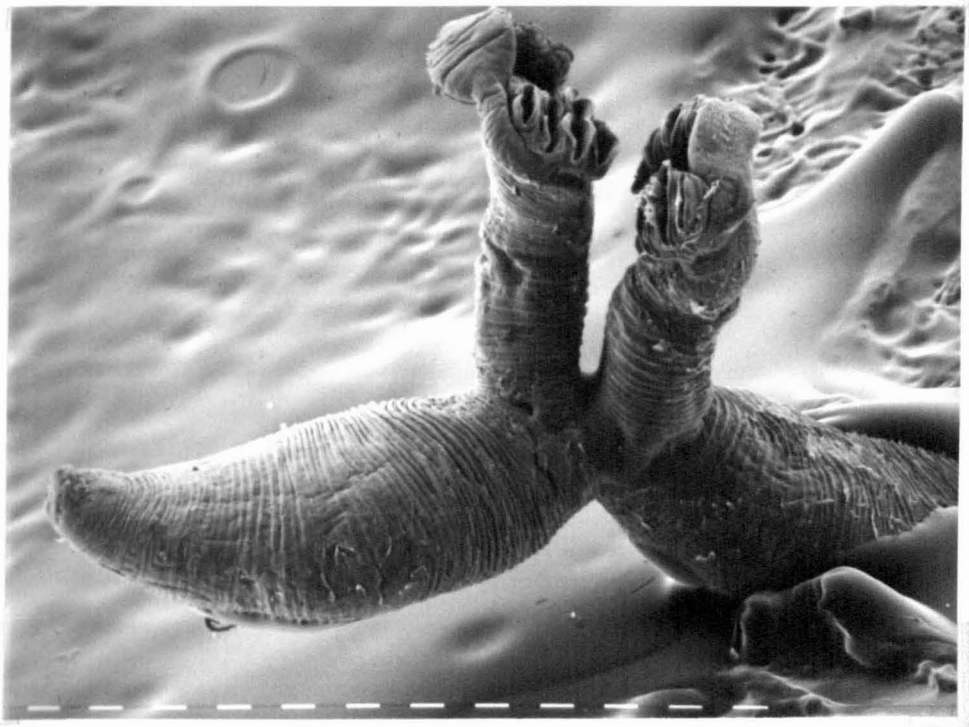


Fig. 7.5. Arrangement of the ventral (clamp) face of the two opisthaptors face to face in a dead adult parasite where dorsal and ventral attachment was to the same primary lamella. Markers = 71.4μm.



sectors of the gill arch. Fig. 7.6 shows the attachment of the clamps to the outer hemibranch of the left gill. The parasite occupied the dorsal third of the gill. The clamps were arranged on the corresponding dorsal surfaces of two consecutive primary lamellae (dorsal and dorsal). In Fig. 7.7 the parasite was attached to the inner hemibranch of the left gill, occupying the median segments and the clamps grasped the opposing sides of the same primary lamellae (dorsal and ventral).

The laboratory observations on the live adults indicated that they were unable to reattach or change their positions on the gill, but only the anterior regions were able to move freely from time to time in different directions. By contrast observations on living specimens of unpaired and early stages of paired diporpae (all with one or two pairs of clamps on the opisthaptors) showed the ability of these stages for reattaching to the gills by using the oral suckers for temporary attachment. Living specimens of unpaired diporpa, with one or two pairs of clamps, and two paired diporpae, with one pair of clamps, available during this study showed their ability to change their positions by very active movement on the same primary lamella in different directions. Other living life cycle stages, including unpaired diporpa with three pairs of clamps, paired diporpae with 2-3 pairs of clamps and juveniles were not examined during this study.

In the adult stages, the eight clamps of each opisthaptor on the surface of a primary lamella was usually aligned in such a way that each clamp was able to grasp one secondary lamella (Figs. 7.1 and 7.4).

Most adult specimens examined were found to be attached to the middle part of the primary lamella or close to the interbranchial

Fig. 7.6. Attachment of clamps of the two opisthaptors to the outer hemibranch of the left gill. The parasite occupied the dorsal sector of the gill. ar, anterior region; ibs, interbranchial septum; oph, opisthaptor. Markers = 66.7 μm.

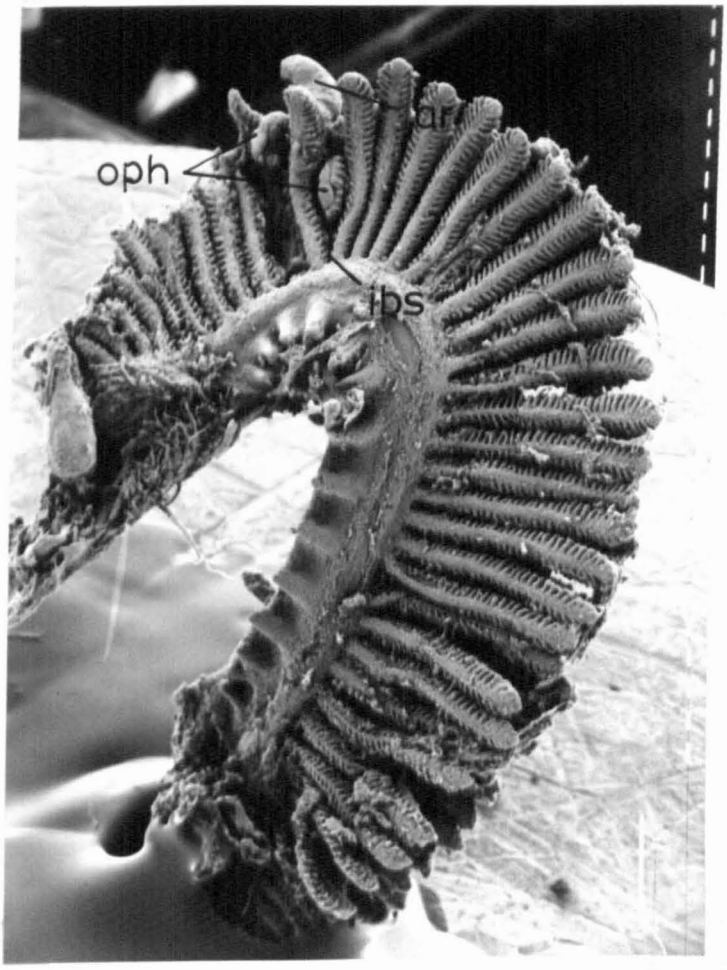
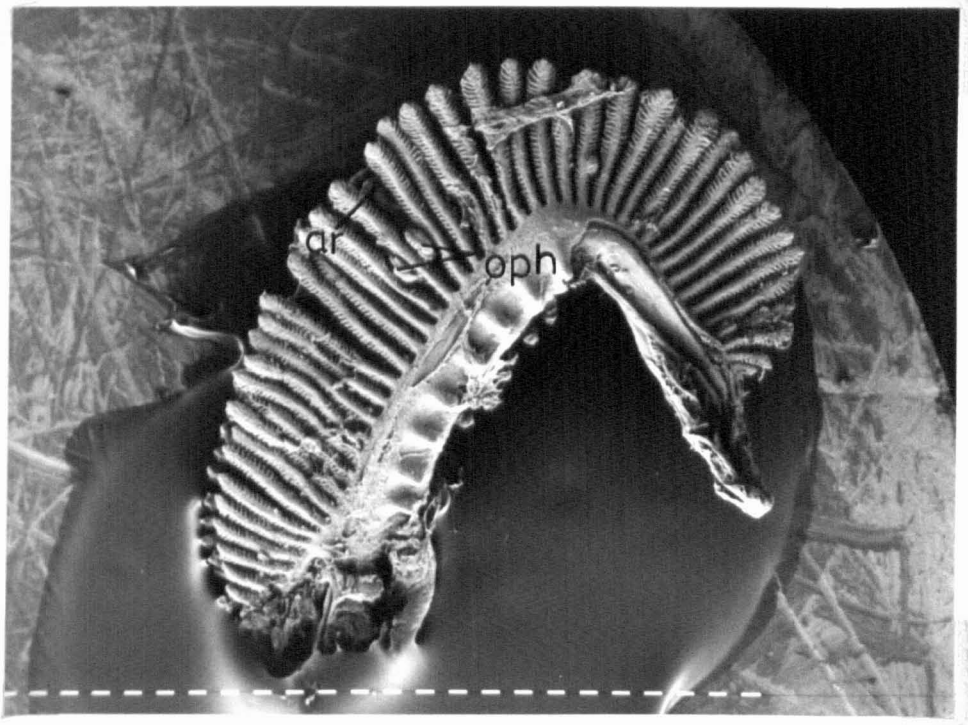


Fig. 7.7. Attachment of clamps of the two opisthaptors to the inner hemibranch of the left gill. The parasite occupied the median sector of the gill. ar, anterior region; oph, opisthaptor. Markers = 100µm.



septum (Figs. 7.6 and 7.7).

The damage on the primary lamellae tissues caused by these parasites is clearly shown in Figs. 7.1 and 7.4.

C. Frequency Distribution of D. homoion on the Gills of Rutilus rutilus

1. Distribution according to the gill arch number

Table 7.1 shows the monthly distribution of parasites on the four gill arches. In most months, there was a tendency for the parasites to be attached to all gill arches randomly except for the fourth gill, where they existed at low numbers. The distribution of the total parasites (671) on the gills was: 216 (32.2%) on the first, 186 (27.7%) on the second, 173 (25.8%) on the third and 96 (14.3%) on the fourth. The statistical analysis showed that the significant result between the distribution of parasites on different gill arches was brought about by the small numbers settled on the 4th gill (X^2 = 46.7, highly significant at $p < 0.001$ with 3 degrees of freedom), while the Chi square test showed a random distribution of parasites on the first three gill arches (X^2 = 5.1, not significant at $p > 0.05$ with 2 degrees of freedom).

The maximum number of parasites on one gill arch was four, but this maximum was reached only in three gills during the period of study (on a first gill arch, January; a third in March and a second in October, 1983).

The laboratory observations showed that diporpae stages of D. homoion (both unpaired diporpa and paired diporpae) preferred the fourth gill arch for attachment more than the others (Table 7.2).

Table 7.1. Frequency distribution of *D. homoion* according to the gill arch number of *Rutilus rutilus* from Llyn Tegid.

Date	Total	Parasite nos. Gill arches 1 (%)	2 (%)	3 (%)	4 (%)
Sept. 1982	32	12 (37.5)	10 (31.2)	6 (18.8)	4 (12.5)
Oct.	38	10 (26.3)	11 (28.9)	12 (31.6)	5 (13.2)
Nov.	16	5 (31.2)	3 (18.8)	8 (50.0)	0
Dec.	27	8 (29.6)	10 (37.0)	7 (25.9)	2 (7.4)
Jan. 1983	50	13 (26.0)	15 (30.0)	14 (28.0)	8 (16.0)
Feb.	33	9 (27.2)	11 (33.3)	7 (21.2)	6 (18.2)
Mar.	110	31 (28.2)	35 (31.8)	28 (25.5)	16 (15.5)
April	65	18 (27.7)	15 (23.0)	21 (32.3)	11 (17.0)
May	73	21 (28.8)	23 (31.5)	16 (21.8)	13 (17.8)
June	42	14 (33.3)	9 (21.4)	10 (23.8)	9 (21.4)
Aug.	21	8 (38.0)	4 (19.0)	6 (28.7)	3 (14.3)
Sept.	18	9 (50.0)	2 (11.1)	3 (16.7)	4 (22.2)
Oct.	87	26 (29.9)	24 (27.6)	27 (31.0)	10 (11.5)
Nov.	44	22 (50.0)	13 (29.5)	5 (11.4)	4 (9.0)
Dec.	15	10 (66.7)	1 (6.7)	3 (20.0)	1 (6.7)
Total	671	216 (32.2)	186 (27.7)	173 (25.8)	26 (14.3)

Table 7.2. Frequency distribution of diporpae, juvenile and adult D. homoion according to the gill arch number of Rutilus rutilus. Data obtained from 20 infected roach maintained in the laboratory.

Stage	Total	Parasite nos. Gill arches			
		1	2	3	4
		(%)	(%)	(%)	(%)
1. Unpaired and paired diporpae	142	19 (13.4)	31 (21.8)	35 (24.6)	57 (40.1)
2. Juveniles and adults	119	39 (32.8)	25 (21.0)	35 (29.4)	20 (16.8)

The minimum numbers of these stages were seen on the first gills. The laboratory observations agreed with the field data in that juvenile and adult stages were more numerous on the first three gills (X^2 = 23.5 significant at $p<0.05$ with 3 degrees of freedom).

The frequency distribution of D. homoion on the different gill arches can be seen in data summarized into 4-monthly periods which are provided in Table 7.3. No difference was found between these values in regard to the different periods of time (X^2 = 12.8, not significant at $p>0.05$ with 9 degrees of freedom).

The effect of fork length of Rutilus rutilus on the frequency distribution of D. homoion on different gill arches is shown in Table 7.4. It is obvious that the number of parasites on any particular gill arch remained relatively constant in all length groups of fishes (X^2 = 15.09 not significant at p 0.0885 with 9 degrees of freedom).

The relationship between the sex of fish and frequency distribution of parasites per gill arch are shown in Table 7.5. The statistical test showed no significant difference between the sexes and the distribution of parasite on the respective gill arches (X^2 = 6.01 not significant at $p>0.05$ with 6 degrees of freedom).

The frequency distribution of gills of Rutilus rutilus with different numbers of D. homoion per gill is given in Table 7.6. 229 infected fish were used in this study. The results indicate that the number of gills with different numbers of parasites was relatively constant for the first 3 gill arches but differed for the 4th. The X^2 test showed a significant difference for this distribution of parasites (X^2 = 54.78 significant at $p<0.05$ with 12 degrees of freedom).

Table 7.3. Frequency distribution of *D. homoion* according to the gill arch number of *Rutilus rutilus* from Llyn Tegid. Data summarized into 4-monthly periods.

Date	Total	Parasite nos. Gill arches 1 (%)	2 (%)	3 (%)	4 (%)
Sept.-Dec. 1982	113	35 (31.0)	34 (30.1)	33 (29.2)	11 (9.7)
Jan.-April 1983	258	71 (27.5)	76 (29.5)	70 (27.1)	41 (15.9)
May-Aug.	136	43 (31.6)	36 (26.5)	32 (23.5)	25 (18.4)
Sept.-Dec.	164	67 (40.9)	40 (24.4)	38 (23.2)	19 (11.6)

Table 7.4. The effect of fork length of Rutilus rutilus on the frequency distribution of D. homoion on different gill arches

Fork length class (cm)	Total	Parasite nos. Gill arches			
		1	2	3	4
		(%)	(%)	(%)	(%)
5-9.9	6	2	3	0	1
		(33.3)	(50.0)		(16.7)
10-14.9	82	37	17	15	13
		(45.1)	(20.7)	(18.3)	(15.9)
15-19.9	75	24	18	27	6
		(32.0)	(24.0)	(36.0)	(8.0)
20-24.9	452	134	136	116	66
		(29.6)	(30.1)	(25.7)	(14.6)
25-29.9	49	17	14	12	6
		(34.7)	(28.6)	(24.5)	(12.2)

Table 7.5. **The effect of sex of Rutilus rutilus on frequency distribution of D. homoion on different gill arches.**

Sex of fish	Total	Parasite nos. Gill arches 1 (%)	2 (%)	3 (%)	4 (%)
Male	451	148 (32.8)	126 (27.9)	119 (26.4)	58 (12.9)
Female	165	47 (48.5)	47 (48.5)	46 (27.9)	25 (15.2)
Unsexed	33	9 (27.3)	11 (33.3)	5 (15.2)	8 (24.2)

Table 7.6. Frequency distribution of gills of Rutilus rutilus with different numbers of D. homoion per gill.

Gill arch nos.	Total	Nos. of gills with different nos. of parasites on each gill				
		0	1	2	3	4
		(%)	(%)	(%)	(%)	(%)
1	458	281	141	31	4	1
		(61.4)	(30.8)	(6.8)	(0.9)	(0.2)
2	458	306	123	26	2	1
		(66.8)	(26.9)	(5.7)	(0.4)	(0.2)
3	458	314	122	16	5	1
		(68.6)	(26.6)	(3.5)	(1.1)	(0.2)
4	458	374	73	11	0	0
		(81.7)	(15.9)	(2.4)		

2. Distribution according to the right and left gills

Data for this kind of distribution are given in Table 7.7. The monthly numbers of parasites show that the number on the left gills were slightly higher than on the right in most months. Of the total number of parasites (684), 313 (46.8%) were found on the right gills and 371 (54.2%) were found on the left. The X^2 test shows that this distribution was random (X^2 = 4.9, not significant at $p>0.027$ with 1 degree of freedom).

The distribution of parasites on the right and left gills of the fishes is given in Table 7.8 divided into 4 monthly periods. These results show that the distribution of the parasites on the left and right gills was random during these different periods of time (X^2 = 3.06, not significant at $p>0.05$ with 3 degrees of freedom).

The relationship between the fork length of fish and distribution of parasites on the left and right gills is given in Table 7.9. Again, no significant result was found between the distribution of parasite on both sides of fish and the fork length of the fishes (X^2 = 1.39, not significant at $p>0.8459$ with 4 degrees of freedom).

The effect of the sex of the fish on the frequency distribution of D. homoion either on the right or the left gills is introduced in Table 7.10. This distribution was also random without any effect caused by the sex of the fish (X^2 = 3.09 not significant at $p>0.2133$ with 2 degrees of freedom).

The frequency distribution of right and left gills of Rutilus rutilus with different numbers of D. homoion per gill is shown in Table 7.11. The total numbers of fishes examined from September 1982 to December 1983 was 229.

Table 7.7. Frequency distribution of *D. homoion* according to right and left gills of *Rutilus rutilus* from Llyn Tegid

Date	Total	Parasite nos. Site of branchial apparatus	
		Right	Left
		(%)	(%)
Sept. 1982	33	17 (51.5)	16 (48.5)
Oct.	38	15 (39.5)	23 (60.5)
Nov.	16	8 (50.0)	8 (50.0)
Dec.	27	12 (44.4)	15 (55.6)
Jan. 1983	50	28 (56.0)	22 (44.0)
Feb.	33	17 (51.5)	16 (48.5)
Mar.	110	50 (45.5)	60 (54.5)
April	65	26 (40.0)	39 (60.0)
May	76	29 (38.2)	47 (61.8)
June	42	15 (35.7)	27 (64.3)
Aug.	21	11 (52.4)	10 (47.6)
Sept.	18	7 (38.9)	11 (61.1)
Oct.	87	46 (52.9)	41 (47.1)
Nov.	53	22 (41.5)	31 (58.5)
Dec.	15	10 (66.6)	5 (33.4)
Total	684	313 (46.8)	371 (54.2)

Table 7.8. Frequency distribution of *D. homoion* according to right and left gills of *Rutilus rutilus* from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Parasite nos.		
	Total	Site of branchial apparatus	
		Right	Left
		(%)	(%)
Sept.-Dec. 1982	114	52	62
		(45.6)	(54.4)
Jan.-April 1983	258	121	137
		(46.9)	(53.1)
May-Aug.	139	55	84
		(39.6)	(60.4)
Sept.-Dec.	173	85	88
		(49.1)	(50.9)

Table 7.9. The effect of fork length of Rutilus rutilus on the frequency distribution of D. homoion on right and left side of the fishes.

Fork length class (cm)	Total	Parasite nos. Side of branchial apparatus	
		Right	Left
		(%)	(%)
5-9.9	6	3	3
		(50.0)	(50.0)
10-14.9	82	43	39
		(52.4)	(47.6)
15-19.9	75	34	41
		(45.3)	(54.7)
20-24.9	452	206	246
		(45.6)	(54.4)
25-29.9	49	23	26
		(46.9)	(53.1)

Table 7.10. The effect of sex of Rutilus rutilus on the frequency distribution of D. homoion on right and left sides of the fishes.

Sex of fish	Total	Parasite nos. Side of branchial apparatus	
		Right (%)	Left (%)
Male	442	219 (49.5)	223 (50.5)
Female	136	56 (41.2)	80 (58.8)
Unsexed	45	20 (44.4)	25 (55.6)

Table 7.11. Frequency distribution of right and left gills of Rutilus rutilus with different numbers of D. homoion per gill.

Gill side	Total	Nos. of right and left gills with different nos. of parasites on each gill				
		0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
Right	916	658 (71.8)	216 (23.6)	36 (3.9)	5 (0.5)	1 (0.1)
Left	916	635 (69.3)	230 (25.1)	43 (4.7)	6 (0.7)	2 (0.2)

No difference was observed between the numbers of right and left gills with the various numbers of parasites per gill (X^2 = 1.75 not significant at $p>0.6259$ with 3 degrees of freedom). Only two left and one right gills were found with the maximum number of parasites (4).

3. Distribution according to the inner and outer hemibranchs

The monthly numbers of parasites observed on inner and outer hemibranchs were approximately similar. Of the total number of parasites (681), 324 (47.6%) were on the inner hemibranch and 357 (52.4%) on the outer hemibranch (Table 7.12). The distribution was also random (X^2= 1.6, not significant at $p>0.206$ with 1 degree of freedom).

The frequency distribution of D. homoion on the inner and outer hemibranchs in different periods of time is shown in Table 7.13. Also these results were not significant for this kind of distribution and during the different seasons (X^2 = 4.45 not significant at $p>0.05$ with 3 degrees of freedom).

The effect of fork length on this kind of distribution is illustrated in Table 7.14. The distribution is random as shown by Chi test (X^2 = 7.69 not significant but approaching significant level at $p>0.0529$ with 3 degrees of freedom).

The effect of sex of the fish on the distribution of D. homoion on the inner and outer hemibranchs is shown in Table 7.15. Only here there was a highly significant result between the males and females on the one hand and the unsexed fish on the other (X^2 = 18.66 highly significant at $p<0.001$ with 2 degrees of freedom). In the unsexed fishes, the parasites settled more on the outer than on the inner hemibranchs, while in the males and females the distribution

Table 7.12. Frequency distribution of *D. homoion* according to the inner and outer hemibranchs of the gills of *Rutilus rutilus* from Llyn Tegid.

Date	Total	Parasite nos. Hemibranch	
		Inner (%)	Outer (%)
Oct. 1982	38	19 (50.0)	19 (50.0)
Nov.	16	9 (56.3)	7 (43.7)
Dec.	27	10 (37.0)	17 (63.0)
Jan. 1983	49	26 (53.1)	23 (46.9)
Feb.	33	16 (48.5)	17 (51.5)
Mar.	99	48 (48.5)	51 (51.5)
April	104	32 (30.8)	72 (69.2)
May	75	41 (54.7)	34 (45.3)
June	43	18 (41.9)	25 (58.1)
Aug.	21	13 (61.9)	8 (28.1)
Sept.	17	7 (41.1)	10 (58.9)
Oct.	86	37 (43.0)	49 (57)
Nov.	57	37 (64.9)	20 (35.1)
Dec.	16	11 (68.8)	5 (31.3)
Total	681	324 (47.6)	357 (52.4)

Table 7.13. Frequency distribution of *D. homoion* according to the inner and outer hemibranchs of the gills of *Rutilus rutilus* from Llyn Tegid. Data summarized into 4 monthly periods.

Date	Total	Parasite nos. Hemibranch Inner (%)	Outer (%)
Sept.-Dec. 1982	81	38 (46.9)	43 (53.1)
Jan.-April 1983	285	122 (42.8)	163 (57.2)
May-Aug.	139	72 (51.8)	67 (48.2)
Sept.-Dec.	176	92 (52.3)	84 (47.7)

Table 7.14. The effect of fork length of Rutilus rutilus on the frequency distribution of D. homoion on inner and outer hemibranchs of the fishes.

Fork length class (cm)	Total	Parasite nos. Hemibranch Inner (%)	Outer (%)
10-14.9	63	26 (41.3)	37 (58.7)
15-19.9	65	25 (38.5)	40 (61.5)
20-24.9	351	188 (53.6)	163 (46.4)
25-29.9	28	12 (42.9)	16 (57.1)

Table 7.15. The effect of sex of Rutilus rutilus on the frequency distribution of D. homoion on inner and outer hemibranchs of the fishes.

Sex of fish	Total	Parasite nos. Hemibranch	
		Inner (%)	Outer (%)
Male	360	185 (51.9)	175 (48.6)
Female	125	55 (44.0)	70 (56.0)
Unsexed	45	8 (17.0)	37 (82.2)

was random.

The frequency distribution of inner and outer hemibranchs of Rutilus rutilus with different numbers of parasites per hemibranch is shown in Table 7.16 (215 infected fish were studied). No significant difference was observed between the numbers of inner and outer hemibranchs with different numbers of parasites (X^2 = 3.23 not significant at $p>0.05$ with 4 degrees of freedom). The hemibranchs with the maximum numbers of parasites (4) were 1 inner and 2 outers.

4. Distribution according to the types of attachments to the surfaces of the primary lamellae

The results are illustrated in Table 7.17. Relatively similar numbers of parasites with each type of attachment were seen in most months. Of the total number of parasites (472), 152 (32.2%) were situated on the two dorsal surfaces of two consecutive primary lamellae (dorsal and dorsal), 160 (33.9%) were attached to the two ventral surfaces of two consecutive lamellae (ventral and ventral) and 160 (33.9%) were attached to the opposing surfaces of the same primary lamella (dorsal and ventral). There was no significant difference between these values (X^2 = 0.271 not significant at $p>0.873$ with 2 degrees of freedom). Generally, the parasites were attached to two consecutive primary lamellae twice rather than to a single lamella. The frequency distribution of D. homoion according to the types of attachments for one or two consecutive primary lamellae during 3 different periods is shown in Table 7.18. The Chi-square test shows no significant difference between these modes of attachment in different periods of time (X^2 = 1.968 not significant at $p>0.05$ with 4 degrees of freedom).

Table 7.16. Frequency distribution of inner and outer hemibranchs of Rutilus rutilus with different numbers of D. homoion per hemibranch.

Hemibranch	Total	Nos. of inner and outer hemibranchs with different nos. of parasites on each hemibranch				
		0	1	2	3	4
		(%)	(%)	(%)	(%)	(%)
Inner	1720	1478	220	20	1	1
		(85.9)	(12.8)	(1.2)	(0.1)	(0.1)
Outer	1720	1505	194	19	2	0
		(87.5)	(11.3)	(1.1)	(0.1)	

Table 7.17. **Frequency distribution of D. homoion according to the type of attachment to either one or two consecutive primary lamellae**

Date	Total	Parasite nos. Attached to one or two primary lamellae		
		1	2	
		Dorsal and ventral (%)	Dorsal and dorsal (%)	Ventral and ventral (%)
Jan. 1983	47	18 (38.3)	13 (27.7)	16 (34.0)
Feb.	27	7 (25.9)	9 (33.3)	11 (40.7)
Mar.	95	33 (34.7)	28 (29.5)	34 (35.8)
April	61	19 (31.1)	26 (42.6)	16 (26.2)
May	61	17 (27.9)	19 (31.1)	15 (41.0)
June	32	13 (40.6)	14 (43.8)	5 (15.6)
Aug.	20	6 (30.0)	7 (35.0)	7 (35.0)
Sept.	12	3 (25.0)	3 (25.0)	6 (50.0)
Oct.	68	28 (41.2)	17 (25.0)	23 (33.8)
Nov.	35	12 (34.3)	11 (31.4)	12 (34.3)
Dec.	14	4 (28.6)	5 (35.7)	5 (35.7)
Total	472	160 (33.9)	152 (32.2)	160 (33.9)

Table 7.18. **Frequency distribution of D. homoion according to the type of attachment either to one or two consecutive primary lamellae. Data summarized into 3 monthly periods.**

Date	Parasite nos.			
	Total	Attached to one or two primary lamellae		
		1	2	
		Dorsal and ventral (%)	Dorsal and dorsal (%)	Ventral and ventral (%)
Jan.-April 1983	230	77 (33.5)	76 (33.0)	77 (33.5)
May-Aug.	113	36 (31.9)	40 (35.4)	37 (32.7)
Sept.-Dec.	129	47 (36.4)	36 (27.9)	46 (35.7)

The effect of fork length of fish on the types of attachment of D. homoion to one or two primary lamellae is given in Table 7.19. Again no positive relationship was detected between these two variables and the distribution of parasites was random (X^2 = 5.71 not significant at $p>0.4564$ with 6 degrees of freedom).

5. Distribution according to the position on the gill arches

Data for this distribution are presented in Table 7.20. To avoid the effect of competition between parasites on the same hemibranch when two or more parasites attached to the same hemibranch occured they were ignored for the present analysis. It was quite obvious from the monthly samples that the order of settlement of the 468 parasites was dorsal, 214 (45.7%); median, 155 (33.1%) to ventral, 99 (21.2%). The difference between these results were statistically significant (X^2 = 212.53, highly significant at $p < 0.001$ with 2 degrees of freedom). The distribution of D. homoion according to different segments of the gill arches of Rutilus rutilus at different periods of time is shown in Table 7.21. The distribution was similar at all periods except that the aggregations of parasites on the ventral segments of the gills were more numerous during September to December 1983. This result was significant (X^2 = 13.422 significant at $p< 0.05$ with 4 degrees of freedom).

The effect of fork length of the fishes on the positions of parasites on the gill arches is given in Table 7.22. The results show that the parasites settled on the ventral segments of the gills in the small fishes less than 15 cm long while they aggregated on the dorsal segments of gills in the large fishes greater than 20 cm long. The settlement on the median sectors of the gills was between

Table 7.19. The effect of fork length of Rutilus rutilus on the type of attachment of D. homoion either to one or two consecutive primary lamellae

Fork length class (cm)	Total	Parasite nos. Attached to one or two primary lamellae		
		1	2	
		Dorsal and ventral (%)	Dorsal and dorsal (%)	Ventral and ventral (%)
5-9.9	2	1 (50.0)	1 (50.0)	0
10-14.9	57	21 (36.8)	16 (28.1)	20 (35.1)
15-19.9	55	14 (25.5)	23 (41.8)	18 (32.7)
20-24.9	314	105 (33.4)	100 (31.8)	109 (34.7)
25-29.9	30	14 (46.7)	9 (30.0)	7 (23.3)

Table 7.20. Frequency distribution of D. homoion according to their positions on the gill archs of Rutilus rutilus from Llyn Tegid.

Date	Total	Parasite nos. Segment of the gill		
		Dorsal	Median	Ventral
		(%)	(%)	(%)
Jan. 1983	46	23 (50.0)	15 (32.6)	8 (17.4)
Feb.	20	10 (50.0)	6 (30.0)	4 (20.0)
Mar.	103	42 (40.8)	44 (42.7)	17 (16.5)
April	62	32 (51.6)	16 (25.8)	14 (22.6)
May	69	32 (46.4)	25 (36.2)	12 (17.4)
June	34	21 (61.8)	11 (32.4)	2 (5.9)
Aug.	14	6 (42.9)	5 (35.1)	3 (21.4)
Sept.	12	6 (50.0)	5 (41.7)	1 (8.3)
Oct.	67	32 (47.8)	17 (25.4)	18 (26.8)
Nov.	31	8 (25.8)	8 (25.8)	15 (48.4)
Dec.	10	2 (20.0)	3 (30.0)	5 (50.0)
Total	468	214 (45.7)	155 (33.1)	99 (21.2)

Table 7.21. Frequency distribution of *D. homoion* according to their positions on the gill arches of *Rutilus rutilus* from Llyn Tegid. Data summarized into 3 monthly periods.

Date	Total	Parasite nos. Segment of the gill		
		Dorsal	Median	Ventral
		(%)	(%)	(%)
Jan.-April 1983	231	107	81	43
		(46.3)	(35.1)	(18.6)
May-Aug.	117	59	41	17
		(50.4)	(35.1)	(14.0)
Sept.-Dec.	120	48	33	39
		(40.0)	(27.5)	(32.5)

Table 7.22. The effect of fork length of *Rutilus rutilus* on the position of *D. homoion* on the gill arches of fishes.

Fork length class (cm)	Total	Parasite nos. Segment of the gill		
		Dorsal (%)	Median (%)	Ventral (%)
5-9.9	1	0	1 (100.0)	0
10-14.9	49	6 (12.3)	20 (40.8)	23 (46.9)
15-19.9	51	22 (43.1)	17 (33.3)	12 (23.5)
20-24.9	307	165 (52.1)	95 (30.9)	47 (15.3)
25-29.9	28	12 (42.9)	11 (39.3)	5 (17.8)

these two values in all fork length classes of fishes (X^2 = 38.92 very highly significant at $p < 0.0001$ with 6 degrees of freedom).

IV. DISCUSSION

The results show that the orientation of the clamps on the two opisthaptors of adult D. homoion was facultatively asymmetrical to *firmly* attach the parasite in its position on the gill against the ventilating current as well as to facilitate the feeding activity carried out by the anterior regions. This was true for diporpae as well as adults D. paradoxum and D. rutili. These results agree with those of Owen (1963a) and Khotenovskiĭ (1980) who found this feature on many Diplozoon species. Owen (1963a) believed that asymmetry was not random but was dependent upon the site of attachment and the incidence of the flow of the gill ventilating current at the position of attachment. Llewellyn and Owen (1960) indicated that many other factors seemed to effect on the direction of asymmetry, for example the position of the worm on the right or left gill, its attachment to the inner or outer hemibranch or the grasping of the primary lamella from either the dorsal or the ventral surface.

The adult worms of D. homoion were always found to settle between the inner and outer hemibranchs of the gills with their axis parallel to the primary lamellae. It seems that the asymmetrical disposition of the clamps on the opisthaptors facilitates the arrangement of parasite body in this way. Therefore, the parasite can avoid the effect of ventilation current which is strong enough to have influence on the adhesive attitudes and body shapes of gill-parasitic monogeneans as susggested by Llewellyn (1956b, 1957). The parallel arrangement of parasite body between the hemibranchs of gill was also seen in many solitary monogeneans, e.g. Discocotyle sagittata (Llewellyn and Owen, 1960) and Kuhina scombri (Llewellyn, 1957).

During the present observation, there were 3 types of attachments of the two opisthaptors clamps to the primary lamellae as described in the text (dorsal and ventral faces of the same primary lamella, dorsal to dorsal and ventral to ventral surfaces of two consecutive primary lamellae). But, Owen (1963a) stated that in one instances of an adult D. paradoxum one opisthaptor was attached to the dorsal surface and the other to the ventral surface of two consecutive primary lamellae. The settlement of all the other worms was similar to the types given above. Wiles (1968) also noticed that the attachment of opisthaptors of the adult worms were restricted to one or two consecutive primary lamellae.

The present observations on living parasites agree with the opinions of Bovet (1959 and 1967) and Owen (1963a) that adult Diplozoon was unable to reattach or change its position on the gills. Llewellyn and Owen (1960) suggested that adults of Discocotyle sagittata probably did not move their position once they were firmly attached. But in other monogenean parasites e.g. Tetraonchus monenteron from gills of the Esox lucius, they can move from place to place along the length of the primary gill lamella using the head region for temporary attachment (Kearn, 1966).

The alignment of clamps on each opisthaptor of D. homoion in a manner that each clamp can grasp only a single secondary lamella was also confirmed by Owen (1963a) who reported that the clamps of Diplozoon were small enough to grasp a single secondary lamella. It can be suggested that this kind of attachment would make the parasite cause minimum damage to its host. However, Bovet (1967) and Khotenovskiĭ (1980) showed that each clamp can grasp more than one secondary lamella.

Llewellyn (1957) found that attachment of Kuhnia scombri to their hosts was effected by the grasping of one or two secondary gill lamellae.

The tendency for adult D. homoion often to attach to the middle of a primary lamella or close to the interbranchial septum, as shown in the results and also as described as well in other monogeneans, was because these positions were more suitable for securing the attachment of the parasite where the ventilating current was least strong (Llewellyn, 1957 and Wiles, 1968).

The observations on the settlement of D. homoion on the different gill arch indicated that the adult parasites preferred the first three gills for attachment more than the fourth. This was seen virtually every month. The analysis of the results did not show any difference in this kind of arrangement during each 4 month period of the year. These results do not agree with the findings of other authors. Bovet (1959) found that D. paradoxum decreased in number from the first to the fourth pair of gills on Abramis brama. Owen (1963a) indicated that D. paradoxum was most prevalent on the second pair of gills in Rutilus rutilus. But Wiles (1968) found that the distribution of D. paradoxum on the four gill arches was random in Phoxinus phoxinus, Rutilus rutilus and Gobio gobio but not in A. brama, where the worms were significantly more numerous on the first two gill arches. Cheyne (1977) found D. homoion (as D. paradoxum) on Rutilus rutilus from Llyn Tegid to be more numerous on the first two gill arches. The difference between the findings of Owen (1963a), Wiles (1968), Bovet (1959) and Cheyne (1977) on the one hand and the present observations on the other may be associated with the

size of the samples. The distributions of other monogenean parasites on their host gills are also of relevance. Frankland (1955) noted an uneven distribution of Diclidophora denticulata over the gills of its host. Llewellyn (1956b), Llewellyn and Owen (1960) reported a preference of Discocotyle sagittata for the first gill arch of Salmo trutta. No reasons were given for the causes of differential distirbution of monogeneans on fish gill as reported by Llewellyn (1956b), Llewellyn and Owen (1960) and Owen (1963a). But Wiles (1968) considered that the differences in the ventilating mechanisms of the different fish species were important. Paling (1969) mentioned that the distribution of Discocotyle sagittata over the four pairs of gills of its host in nature was a reflection of the relative volumes of water flowing over the different gills. Llewellyn (1956b) indicated that the maximum incidence per gill arch of Diclidophora denticulata on Gadus virens may vary with the age of the parasites, either as a result of migrations of the parasites on the parts of the gills, or owing to a higher mortality of worms on certain gill arches.

In the present results, it was apparent that D. homoion did not have the ability to transfer from one gill arch to another. The initial attachment of oncomiracidia probably occurred to all of the four gill arches on each side of the fish, but the maximum number of diporpae were found on the fourth gill arch. It can be suggested that until the development of all 4 pairs of clamps on these larval stages was complete most of them could not tolerate the strength of the ventilating current on the first 3 gill arches so that they persisted longest on the 4th gill arch (which was the best place for them). However, owing to the fact that there were

very few juvenile and adult Diplozoon found on the fourth gill arch, this implies that the conditions on this arch were rather unsuitable for the development of the adults.

Owen (1963a) found two juveniles of D. paradoxum attached proximally to the margin of interbranchial septum. Also, in the present observations, most adult stages were found near the interbranchial septum. Very few were seen on either the middle part of primary lamellae or near their outer ends.

Observations on unpaired (with one and two pairs of clamps) and paired diporpae (with a pair of clamps) indicated that they also were able to reattach to the gill temporarily using their oral suckers. Unfortunately, no information is at present available about this kind of behaviour for the unpaired diporpae with 3 pairs of clamps, the paired diporpae with 2 or 3 pairs of clamps or the juvenile stages.

The study of disposition of these larval stages were concentrated on their distribution according to the gill arch number. Their attachment in relation to inner or outer hemibranchs and the surfaces of primary lamellae was not taken into account because of the frequent changes of their position on the primary lamellae.

The present observations also show that the distribution of parasites on the different gill arches of Rutilus rutilus was not effected by the size and sex of fish. Also the frequency distribution of gills with different numbers of parasites followed the distribution of the total number of parasites on the different gill arches in that the numbers of gills with different numbers of parasites were similar for the first three gills in contrast to the numbers of the fourth gill with no infection or only one parasite (Table 7.6).

The maximum number of Diplozoon on one gill was four.

For the grand total of D. homoion on R. rutilus there was a slightly higher number of parasites on the gills of the left side compared with those on the right. However, statistical analysis showed that the distribution of the worms on the gills of the two sides was random with regard to both season and total worms. Owen (1963a) also found that the left gills had a slightly heavier infection than those of the right. Wiles (1968) indicated a random distribution of the parasites on the left and right sides of Phoxinus phoxinus, Rutilus rutilus and Gobio gobio, while in Abramis brama, more worms were found on the right side. In addition the present results indicated no effect of fork length and sex of fish on the distribution of parasites on the right and left sides of fishes. Similarly, the numbers of gills of the right side with different numbers of parasites per gill were nearly the same as in the gills of the left side. The maximum number of parasites on the gill (4) was also seen on gills of both right and left sides.

The present results also showed a random distribution of D. homoion on the inner and outer hemibranchs either in total number of parasites or according to season. Wiles (1968) also found that the worms were randomly distributed between the outer and inner hemibranchs of Rutilus rutilus and Gobio gobio but in Phoxinus phoxinus and Abramis brama, greater numbers were present on the inner hemibranchs.

The distribution of D. homoion on the outer or inner hemibranchs was random for fishes of all fork lengths, as well as for male and female fishes separately. However, if the unsexed fishes were treated as a separate group , in this instance the outer hemibranchs were

more heavily infected than the inner. As noted earlier in this chapter the difference was statistically significant. This difference can probably be explained by the fact that all the unsexed fishes were of small size, less than 10 cm. It was also found that the numbers of inner and outer hemibranchs with one, two, three or four parasites respectively were closely similar, with a maximum bruden of 4 parasites in each instance.

The attachment of parasites to the single primary lamella (dorsal and ventral surfaces) and two primary lamellae (dorsal and dorsal or ventral and ventral) was random and was not affected by the season or the length of the fish. In general two-thirds of the total number of parasites were attached to two consecutive primary lamellae rather than to one. This was also seen by Owen (1963a) who suggested that adjacent primary lamellae would thus afford some protection from the ventilating current. Wiles (1968) suggested that attachment of D. paradoxum to two primary lamellae might provide a firmer hold by allowing more of the water to pass through unimpeded in the region of the opisthaptor, but when attached to one primary lamella, the opisthaptor virtually blocked all the space available for water passage.

The settlement of the parasites on the different segments of the gill arch, as shown in the results, indicates that more parasites occupied the dorsal segment of the gills, while fewer were settled on the ventral segment. Wiles (1968) found that the distribution of D. paradoxum between median, dorsal and ventral segments of the gills of Abramis brama was random. But in Phoxinus phoxinus and Rutilus rutilus significantly more worms were found in the median segment . In the current study more D. homoion were found in the

dorsal segment throughout the year except for September to December 1983, when the maximum number of parasites was found on the ventral sector rather than on the other parts of the gills. No reason can be offered for this result.

The results also showed that parasites aggregated more on the ventral segments of the gill arches of the smaller fishes while the infection was more numerous on the dorsal segments of the gills of the larger sizes of fishes.

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