MARINE FISH HAEMATOZOA FROM NEWFOUNDLAND WATERS

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by

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CONTENTS

ABSTRACT

Of 797 fish of 28 species, 159 of 12 species yielded blood parasites.

Eight species of protozoan parasites are reported - three each of Trypanosoma and Haemogregarina, and two of Haemohormidium.

Trypanosoma rajae Laveran & Mesnil is recorded from Raja radiata. Undetermined trypanosomes are reported from Glyptocephalus cynoglossus and Gadus morhua respectively.

Haemogregarina myoxocephali Fantham et al. was found in Myoxocephalus octodecemspinosus. The occurrence of haemogregarine sporozoites (perhaps of this species) in the gut of a piscicolid leech (Malmiana nuda) from Myoxocephalus scorpius represents the first discovery of a potential vector of any fish haemogregarine. New hosts are listed for H. delagei Laveran & Mesnil and H. platessae Lebailly.

Haemohormidium terraenovae n. sp. is described from six hosts:- Ammodytes americanus, Urophycis tenuis, Melanogrammus aeglefinus, Limanda ferruginea, Glyptocephalus cynoglossus and Hippoglossoides platessoides; and Haemohormidium beckeri n.sp. from Myoxocephalus octodecemspinosus.

Intraerythrocytic inclusions of unknown origin were common in *Clupea harengus* and *Argentina silus*, and myxosporidans occurred as blood film contaminants in *Raja radiata* and *Gadus morhua* (in the latter case the parasite was referable to *Kudoa* sp.). Other artifacts reported were bacterial contaminants from *Squalus acanthias* and *Limanda ferruginea*.

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INTRODUCTION

The history of our knowledge of blood-inhabiting protozoans is intimately connected with their discovery in poikilothermic vertebrates. Numerous important "firsts" originated from the interest of the early investigators in parasites of fish, amphibians and chelonians. Thus, the first observation of a blood parasite was that of Valentin (1841), who saw trypanosomes in trout, Salmo fario (= Salmo trutta Linnaeus, 1758). In the following two years frog trypanosomes were recorded from Germany, Belgium and France. Mammalian trypanosomes were first recorded from European field mice and moles by Gros (1845), but another quarter of a century was to go by before Lewis (1878) adequately described related parasites from the blood of rats in India.

In North America, the first report of any blood parasite from marine fish was that of Mavor (1915). He recorded Haemogregarina sp. from the squirrel hake, Urophycis chuss (Walbaum), while searching for protozoan parasites in specimens from Passamaquoddy Bay. His material was collected at or near the mouth of the St. Croix river, St. Andrew's, New Brunswick. Several years later, Kudo (1922) found that of 50 fish representing 25 species from Woods Hole, Mass., only one winter skate (Raja ocellata Mitchill) was infected. This fish was scantily infected with haemoflagellates which

Kudo referred to Trypanosoma rajae[1] Laveran & Mesnil, a parasite of European skates. Ellis (1930) subsequently examined blood smears from an unstated number of fish of nine species at St. Andrew's, N. B., without finding any haematozoa.

Following the examination of marine fish collected from St. Andrew's, N. B., Halifax, Nova Scotia, and Montreal, Fantham et al. (1942) described a number of blood parasites. These included a haemoflagellate from the yellow perch, Perca flavescens (Mitchill), which they designated as a new variety (canadensis) of Trypanosoma percae Brumpt, 1906, of the European perch, Perca fluviatilis. Two new species, T. myoxocephali and Haemogregarina myoxocephali, were described from the longhorn sculpin, Myoxocephalus octodecemspinosus[2] (Mitchill) and H. urophysis[3] from the white hake, Urophycis tenuis (Mitchill). These authors also identified H. aeglefini[4] Henry from the Atlantic cod (Gadus callarias) = G. morhua L. caught off Labrador. Other undesignated haemogregarines were also discussed by Fantham et al. (1942). One of these, a haemogregarine

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[1]Spelt as T. raiae by Kudo.

[2]Spelt as M. octodecimspinosus by Fantham et al. (1942).

[3]Emended to H. urophycis by Laird and Bullock, 1969.

[4]Spelt as H. aeglifini by Fantham et al. (see Laird and Bullock, 1969).

closely allied to H. platessae Lebailly, was recorded from the winter flounder, Pseudopleuronectes americanus (Walbaum). Another, probably H. bigemina, was observed in the blood of the ocean pout, Macrozoarces americanus (Bloch & Schneider). Still another, which was described as a haemosporidian different from but probably allied to H. bigemina, was reported from the black sea bass, Centropristes striatus (L.). The last two parasites were found in fish from the Montreal market.

Ten years later, during the winter of 1952, Bullock (1952, 1953) discovered a species of Cryptobia in the blood of a young winter flounder taken through ice in Greenland Bay, New Hampshire. In the summer of the following year, Bullock surveyed the blood protozoans of marine fish from southern New England. He (1958) reported T. rajae from a little skate, Raja erinacea Mitchill, and undesignated haemogregarines from summer flounder, Paralichthys dentatus (L.), northern puffer, Sphaeroides maculatus (Bloch & Schneider), and black sea bass, Centropristes striatus. The summer flounder parasite was identified by Laird & Bullock (1969) as Haemogregarina platessae. In C. striatus, the white blood cells held division stages of a parasite recognized as Haemogregarina bigemina by Laird & Bullock (1969). Bullock (1958) also

encountered a myxosporidan in the blood of Pseudopleuronectes americanus and a mummichog, Fundulus heteroclitus (L.). This parasite was afterwards identified as Kudoa sp. by Laird & Bullock (1969).

In the late 1950's, Laird made an extensive study of haematozoa in the Bay of Fundy. His results were published together with Bullock's earlier ones from Woods Hole, Mass. (Laird & Bullock, 1969). Their joint account included various records of haematozoa additional to those already given by Bullock (1952, 1953, 1958). Haemogregarina delagei Laveran & Mesnil was reported from spiny dogfish (Squalus acanthias L.) and the skates Raja erinacea Mitchill and R. radiata Donovan, the latter also proving positive for Trypanosoma rajae.

Haemogregarina aeglefini was reported from three species of gadid fish, Melanogrammus aeglefinus (L.), Pollachius virens (L.) and Urophycis tenuis (Mitchill). This parasite is not known from hosts outside the family Gadidae. Laird & Bullock (1969) relegated H. urophycis to synonymy with H. aeglefini, and also considered the organism seen by Mavor (1915) to be referable to this species. Laird & Bullock (1969) obtained fresh records of Haemogregarina myoxocephali from the longhorn sculpin, and described H. mavori as new from Macrozoarces americanus.

They also extended the host range of H. platessae to Pseudopleuronectes americanus, and noted undesignated haemogregarines in a rock gunnel, Pholis gunnellus (L.) and a seasnail Liparis atlanticus (Jordan & Evermann). A particularly interesting finding of Laird & Bullock was that of the babesioid Haemohormidium sp. from the sea raven, Hemitripterus americanus (Gmelin), and the American plaice, Hippoglossoides platessoides (Fabricius). These authors designated as Piscine Erythrocytic Necrosis an apparent viral disease causing destruction of the red cell nucleus in four fish representing three genera (one Gadus morhua, one Liparis atlanticus and two Myoxocephalus octodecemspinosus). Cryptobia bullocki Strout (1965) was found in a number of hosts.

From the mid-fifties to the mid-sixties, in warmer waters to the south, Saunders made extensive surveys for haematozoa of marine fish. All in all, she recorded six species of haemogregarines and one of Trypanosoma. In particular, she found Haemogregarina in numerous hosts in Florida (Saunders 1955, 1958a, 1964), Bahamas (Saunders, 1958b), and Puerto Rico (Saunders, 1966). In Florida, she described H. achiri from the hogchoker, Achirus fasciatus Lacépède (Saunders, 1955). An undesignated haemogregarine recalling the European H. polypartita Neumann was observed in the blood of the spotted sea trout, Cynoscion nebulosus (Cuvier) (Saunders, 1954). Two other haemogregarines were

also reported from the same area. H. mugili Carini (for which the first record had been obtained from Brazilian waters by Carini, 1932) was found in a striped mullet, Mugil cephalus L., and H. brevoortiae Saunders, 1954 was described from three menhaden, Brevoortia tyrannus (Latrobe). Elsewhere in the Bahamas, Saunders (1958b) described a new haemogregarine, H. dasyatis from the southern stingray, Dasyatis americana (Hildebrand & Schroeder). The same author also described a flagellate, Trypanosoma balistes from the common trigger fish, Balistes capriscus Gmelin, and a great barracuda, Sphyraena barracuda (Walbaum), in the Florida Keys (Saunders, 1958a, 1959).

Little work has been done in the northern and western regions of the continent. Laird (1961a) described H. irkalukpiki from the arctic char, Salvelinus alpinus (L.) in southern Ungava Bay, Quebec. Later in the same year the same author (1961b) reported H. bigemina from the padded sculpin, Artedius fenestralis Jordan & Gilbert, at Nanaimo, Vancouver Island. Other fish blood parasites reported from North America are from freshwater hosts and need not concern us here.

The above paragraphs, then, sum up the major work on the haemotozoa of North American marine fish. Most of the information presently available concerns the Atlantic

coast of the mainland. In Newfoundland, at the threshold of one of the world's major sea fisheries and at the easterly limit of the continent, no previous work had been undertaken on marine fish haemotozoa. The purpose of the present survey was threefold -

1. To describe the fish haemotozoa found in Newfoundland waters, if any.

2. To compare them with those described from other areas.

3. To search for vectors of any blood parasites discovered.

MATERIALS AND METHODS

Blood films of fish were made on the Grand Banks of Newfoundland aboard the "A. T. Cameron" of the St. John's Biological Station, Fisheries Research Board of Canada during November, 1967 and May, 1968. The specimens were obtained by otter trawl, from depths ranging from 25 to 150 fathoms. Additional fish were collected by handline and throw net along the eastern coast of Conception Bay at St. Phillips and Portugal Cove, also on the open Atlantic coast of the Avalon Peninsula at Middle Cove. All three localities are within a few kilometers of St. John's.

Blood was always obtained from live fish. The area posterior to the isthmus was first slit open with a sharp pair of scissors or scalpel, exposing the pericardial cavity. Pericardial fluid often proved abundant, particularly in elasmobranchs. Care was taken to sponge this away, and then to wipe dry the surface of the heart before making the incision, to avoid contaminating the smears. After first allowing a brief free flow from the cut, the corner of a clean slide was touched against the welling blood. The end of another clean slide was then used to make a thin smear in the standard fashion.

Immediately after air-drying, the blood smears were fixed in absolute methyl alcohol. They were always brought back to the laboratory for further processing. Giemsa's stain was used, in a 1 : 40 dilution in distilled water buffered to a slight alkalinity (pH between 7.2 - 7.4 was found most satisfactory). Smears were stained for 45 minutes, then washed in running tap water for three minutes and allowed to dry. The most satisfactory staining results were obtained by turning the fixed smears face down on racks in a shallow tray which was then filled with the staining solution until it touched the lower surface of the slides. This prevented the deposition of stained particles on the smears. Good results were also obtained by overnight staining with Giemsa (1 : 80 dilution) in an incubator at 37°C. All preparations were left uncovered, and searched for parasites under a 8x ocular and 40x or 100x oil immersion objective for at least 15 minutes. Whenever time allowed the whole area of each blood smear was carefully examined, in view of the lightness of many trypanosome infections in fish.

While collecting at sea it proved quite impracticable to examine fresh drops of blood for moving trypanosomes, the rough waters of the Grand Banks not being conducive to sustained high power microscopy.

Drawings were made with the aid of a Zeiss drawing apparatus. A 12.5x ocular was substituted for the 8x used in searching for parasites, and a magnification of 1250x was obtained. The photographs were taken with a Zeiss Photomicroscope I at a magnification of 1400x.

All measurements were made from camera lucida outline drawings, the results (in millimeters) being converted into microns with the assistance of a stage micrometer. The length of trypanosomes was measured by tracing out the course of the midline on the sketch with a piece of thread, as described by Minchin (1909a).

Table 1 lists the fish examined, and indicates those found to be parasitized. Table 2 classifies these parasites. It should be noted that the common and scientific names used for North American fishes are those listed by the American Fisheries Society (Bailey, 1960).

Table 1. Study material in present survey

Scientific name	Common name	Number examined	positive	Locality
CHONDRICHTHYES				
Squaliformes				
Squalidae (dogfish sharks)				
Squalus acanthias L.	Spiny dogfish	24	1	G.B. (Grand Banks)
Rajiformes				
Rajidae (skates)				
Raja radiata Donovan	Thorny skate	87	65	G.B.
Raja senta Garman	Smooth skate	2	2	G.B.
OSTEICHTHYES				
Clupeiformes				
Clupeidae (herrings)				
Clupea harengus harengus L.	Atlantic herring	34	0	G.B.
Osmeridae (smelts)				
Mallotus villosus (Müller)	Capelin	17	0	Middle Cove
Argentinidae (argentines)				
Argentina silus Ascanius	Atlantic argentine	39	0	G.B.

Table 1. Study material in present survey (contd.)

Scientific name	Common Name	Number examined	& positive	Locality
Anguilliformes				
Anguillidae (freshwater eels)				
Anguilla rostrata (LeSueur)	American eel	2	0	G.B.
Gadiformes				
Gadidae (codfishes and hakes)				
Gadus morhua L.	Atlantic cod	180	4	123 Grand Banks 57 Portugal Cove, C.B.
Melanogrammus aeglefinus (L.)	Haddock	11	3	G.B.
Micromesistius poutassou (Risso)[5]	Blue whiting	5	0	G.B.
Urophycis chesteri (Goode & Bean)[6]	Longfin hake	4	0	G.B.
Urophycis tenuis (Mitchill)	White hake	40	4	G.B.
Macrouridae (grenadiers)				
Nezumia bairdi (Goode & Bean)	Marlin-spike	2	0	G.B.
Perciformes				
Labridae (wrasses)				
Tautogolabrus adspersus (Walbaum)	Cunner	26	0	St. Phillips, C.B.

Table 1. Study material in present survey (contd.)

Scientific name	Common name	Number examined	& positive	Locality
Scorpaenidae (scorpionfishes and rockfishes)				
Sebastes marinus (L.)	Redfish	121	0	G.B.
Cottidae (sculpins)				
Hemitripterus americanus (Gmelin)	Sea raven	26	0	G.B.
Myoxocephalus octodecemspinosus (Mitchill)	Longhorn sculpin	12	8	G.B.
Myoxocephalus scorpius (L.)	Shorthorn sculpin	4	0	St. Phillips, C. B.
Cyclopteridae (lumpfishes and snailfishes)				
Cyclopterus lumpus L.	Lumpfish	1	0	G.B.
Ammodytidae (sand lances)				
Ammodytes americanus DeKay	American sand lance	23	19	G.B.
Anarhichadidae (wolffishes)				
Anarhichas lupus L.	Atlantic wolffish	1	0	G.B.
Anarhichas minor Olafsen	Spotted wolffish	1	0	G.B.
Pleuronectiformes				
Bothidae (lefteye flounders)				
Scophthalmus aquosus (Mitchill)	Windowpane	4	2	G.B.

Table 1. Study material in present survey (concl.)

Scientific name	Common name	Number examined	& positive	Locality
Pleuronectidae (righteye flounders)				
Glyptocephalus cynoglossus (L.)	Witch flounder	19	10	G.B.
Hippoglossoides platessoides (Fabricius)	American plaice	60	28	G.B.
Hippoglossus hippoglossus (L.)	Atlantic halibut	2	0	G.B.
Limanda ferruginea (Storer)	Yellowtail flounder	47	13	G.B.
Lophiiformes				
Lophiidae (goosefishes)				
Lophius americanus Valenciennes	Goosefish	3	0	G.B.
	Totals:	797	159	

[5]Not listed by Bailey (1960).

[6]Listed as *Phycis chesteri* Goode & Bean by Bailey (1960).

(With these exceptions all scientific and common names are in accordance with the usage of the American Fisheries Society's "A list of common and scientific names of fishes from the United States and Canada".)

Table 2. Haematozoa found in present survey

Fish host	Number examined	Protozoan parasites			
		Trypanosoma	Haemogregarina	Haemohormidium	Others
Squalus acanthias L.	24				
Raja radiata Donovan	87	1	65		1
Raja senta Garman	2		2		1
Gadus morhua L.	180	1			3
Melanogrammus aeglefinus (L.)	11			3	
Urophycis tenuis (Mitchill)	40			4	
Myoxocephalus octodecemspinosus (Mitchill)	12		4	5	
Ammodytes americanus DeKay	23			19	
Scophthalmus aquosus (Mitchill)	4		2		
Glyptocephalus cynoglossus (L.)	19	1	7	3	
Hippoglossoides platessoides (Fabricius)	60			28	
Limanda ferruginea (Storer)	47			13	1

RESULTS AND DISCUSSION

SARCOMASTIGOPHORA: ZOOMASTIGOPHOREA

Trypanosoma Gruby

Trypanosoma rajae Laveran & Mesnil, 1902 (Figs. 1, 2, 98)

Eighty-seven thorny skates (Raja radiata) and two smooth skates (R. senta) were caught on the Grand Banks of Newfoundland. Only a single example of the former species sampled in May, 1968, was found to be parasitized by trypanosomes during the present study. The infection was light, only one parasite being found in the entire blood smear.

MORPHOLOGICAL ACCOUNT

Synopsis

Length of free flagellum	10.8 μ
Length of body	59.5 μ
Total length	70.2 μ
Width of body at centre of nucleus	8.0 μ
Greatest width of undulating membrane	3.8 μ
Length of nucleus	7.0 μ
Width of nucleus	7.0 μ
Distance of kinetoplast from posterior extremity	3.5 μ

The flagellate is quite tightly coiled (Figs. 2, 98), as is often the case with the larger fish and amphibian trypanosomes. Its broad membrane is well developed, 13 deep folds being evident around the outer margin. A relatively short free flagellum originates from the pointed anterior

end, and the posterior extremity is very blunt. Six distinct myonemes run longitudinally. The large, rounded nucleus occupies almost the entire breadth of the body, and exhibits scattered chromatic granules. The kinetoplast is small and compact, and is located very close to the posterior extremity.

Table 3. Dimensions of various skate trypanosomes

Species	Total length	Length of free flagellum	Body width with membrane	Reference
T. rajae Laveran & Mesnil, 1902	35–80 μ	6–20 μ	3–13 μ	Laveran & Mesnil (1902b); Kudo (1922)
T. giganteum Neumann, 1909	125–130 μ	25–30 μ	14 μ	Neumann (1909)
T. variabile Neumann, 1909	30–85 μ	10–15 μ	-	Neumann (1909)
T. gargantua Laird, 1950	66–132 μ	No free flagellum	5–17.5 μ	Laird (1950)

Among the trypanosomes so far known from skates, the organism described herein most closely resembles T. rajae Laveran & Mesnil, 1902, and T. variabile Neumann, 1909. Minchin & Woodcock (1910) believed that T. variabile is conspecific with T. rajae, which shows marked polymorphism.

Laird & Bullock (1969) have recorded T. rajae from a thorny skate (R. radiata) from the Bay of Fundy, N. B. Large trypanosomes of fish are often very polymorphic, and additional material of the present flagellate would certainly reopen the matter of its specific identity. However, there is nothing in the size and appearance of the one example available to justify its separation from T. rajae of European skates. Although this species has already been reported from North American Atlantic waters (Kudo, 1922; Bullock, 1958; Laird & Bullock, 1969), the present record is the most northerly to date and the first from the Grand Banks.

Many of the skates examined, including the example positive for T. rajae also harboured haemogregarines, which will be discussed later.

Table 4. A preliminary list of skate trypanosomes

Trypanosome	Host	Locality	Reference
Trypanosoma rajae Laveran & Mesnil, 1902	(R. mosaica) = R. undulata	Europe	Laveran & Mesnil, 1902b
"	R. macrorhynchus	"	"
"	R. clavata	"	"
"	(R. punctata) = R. asterias	"	"
"	R. batis	England	Coles, 1914
"	R. ocellata	N. America	Kudo, 1922
"	Raja sp.	Europe	Minchin & Woodcock, 1910
"	Raja sp.	Shetlands	Henry, 1913
"	R. erinacea	Woods Hole (N. America)	Bullock, 1958
"	R. erinacea & R. radiata	N. America (Woods Hole & St. Andrew's)	Laird & Bullock, 1969
Trypanosoma giganteum Neumann, 1909	R. oxyrhynchus	Europe	Neumann, 1909
Trypanosoma variabile Neumann, 1909	(R. punctata) = R. asterias	Europe	Neumann, 1909
Trypanosoma sp.	R. capensis	S. Africa	Fantham, 1919
Trypanosoma marplatensis Bacigalupo & Plaze, 1948	A South American ray	S. America	Bacigalupo & Plaza, 1948
Trypanosoma gargantua Laird, 1950	R. nasuta	New Zealand	Laird, 1950

Trypanosoma sp. (Figs. 3, 4, 99)

Of 19 specimens of Glyptocephalus cynoglossus (witch flounder) caught on the Grand Banks of Newfoundland in May, 1968, one proved to be parasitized by the trypanosome described herein. Once again, only one parasite was found in the thin smear.[7]

MORPHOLOGICAL ACCOUNT

Synopsis

Length of free flagellum	6.5 μ
Length of body	59.2 μ
Total length	65.8 μ
Width of body at centre of nucleus	3.2 μ
Greatest width of undulating membrane	1.5 μ
Length of nucleus	4.8 μ
Width of nucleus	3.2 μ
Distance of kinetoplast from posterior extremity	3.0 μ

The body of the trypanosome is very much coiled. There is a well defined undulating membrane showing five folds (Figs. 4, 99). The anterior end is pointed, while the posterior one is somewhat blunt. Its vacuolated

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[7] With regard to the "lightness" of many fish trypanosome infections, chronic infections can involve so few trypanosomes being in the circulation at the time of smearing that many apparently negative fish are in fact parasitized. "No parasites found" is thus a better diagnosis than "negative".

cytoplasm stains dark blue with Giemsa. The oval, pink-staining nucleus shows tiny chromatic granules. It occupies the entire width of the body and is situated some 70% of the total body length from the anterior extremity. The free portion of the flagellum accounts for 10% of the overall length of the trypanosome. A small and compact kinetoplast, staining dark red, is located 3.0 µ from the posterior end.

Table 5. Dimensions and other specific criteria of various flounder trypanosomes

	Total length	Free flagellum	Body width	Posterior end	References
T. soleae Laveran & Mesnil, 1901	40 µ	8 µ	-	pointed	Laveran & Mesnil, 1907
T. platessae Lebailly, 1904	52 µ	12 µ	3-3.5 µ	very attenuated	"
T. flesi Lebailly, 1904	55 µ	10 µ	5 µ	pointed	"
T. limandae Brumpt & Lebailly, 1904	45 µ	20 µ	2-2.5 µ	very attenuated	"
T. caulopsettae Laird, 1950	100 µ	18 µ	5-6 µ	very attenuated	Laird, 1950

The single trypanosome now described differs radically from these other species in having a bluntly rounded posterior end and a relatively shorter free flagellum. Nevertheless, while it is not readily assignable to any of the known trypanosomes from fish, polymorphism in these parasites is such that I do not propose to follow the example of some earlier authors in assigning a new specific name without having a reasonable range of examples before me. The situation is quite different from that encountered in connexion with the preceding species. Although only one trypanosome was located then too, it fell well within the range for a well-characterized species. Therefore, with the observation that the organism under discussion may prove to merit description as new when further material becomes available, it is simply designated for present purposes as Trypanosoma sp.

Trypanosoma sp. (Figs. 5, 6, 7, 100)

One hundred and eighty specimens (123 from the Grand Banks; 57 from Portugal Cove, Conception Bay) of Gadus morhua (the Atlantic cod) were examined. Only one (from Portugal Cove) caught in summer of 1969, yielded trypanosomes. The infection was quite heavy, 15 flagellates being seen on the thin smear. A myxosporidan was also found amongst the other cod smears from Portugal Cove (see p. 53).

MORPHOLOGICAL ACCOUNT

Synopsis (10 examples measured)

	Range	Average
Length of free flagellum	7.0–7.5 μ	7.3 μ
Length of body	62.5–81.5 μ	73.6 μ
Total length	69.5–88.5 μ	80.8 μ
Width of body at centre of nucleus	2.8–3.5 μ	3.0 μ
Greatest width of undulating membrane		1.0 μ
Length of nucleus	4.6–5.5 μ	4.8 μ
Width of nucleus	2.8–3.5 μ	3.0 μ
Distance of kinetoplast from posterior extremity	7.0–13.5 μ	10.8 μ

The flagellate is long, slender, and tapered at both ends, most of the examples showing a well-extended body (Figs. 6, 100), with a few coiled examples (Fig. 7). It has alveolar, rather granular cytoplasm, exhibiting many discrete vacuoles. The majority of the latter are anteriorly positioned. All but the posterior portion of the cytoplasm, which stains whitish-blue, appears sky blue with Giemsa. The oval nucleus stains a light pinkish-purple. Every example seen shows a prominent karyosome. The nucleus is situated 38% of the total body length from the root of the very short free flagellum, the length of which averages 7.3 μ and is only one-tenth that of the body proper. It originates from a basal granule just anterior to the nearly oval, red-staining kinetoplast, which is located some 11 μ (14% of the total body length) from the posterior extremity. The undulating membrane is not evident in most of the examples.

In suitably stained specimens, two or three myonemes (Figs. 6, 7, 100) are seen as dark streaks in the cytoplasm and across the nucleus.

Henry (1913) recorded T. aeglefini from "Gadus aeglefinus" - the haddock, Melanogrammus aeglefinus (L.) - without giving a recognizable description of his parasite. Nikitin (1927) described T. murmanensis from four of 15 Gadus callarias (= G. morhua). Staining dark blue with Giemsa, this species has a long, serpent-like body, measuring 50 - 60 μ. The cytoplasm exhibits a number of vacuoles, and the undulating membrane is not well developed. The kinetoplast is located not far from the posterior extremity, and the anterior extremity is said to be rounded. The violet-staining oval nucleus is situated at the middle of the body.

The present flagellate is somewhat longer than T. murmanensis as described by Nikitin (1927). It further seems to differ from the latter in tapering anteriorly; and in that the nucleus is located in the anterior half of the body (38% of the body length from the root of the free flagellum). Since piscine trypanosomes are often highly polymorphic, it is conceivable that T. murmanensis and the species under discussion are in fact identical. However, further material from both sources will be needed to settle this point.

Laird (1950) described T. coelorhynchi from Coelorhynchus australis (Richardson), the javelin fish, and Physiculus bachus (Forster), the red cod, in New Zealand waters. T. coelorhynchi shows a considerable degree of polymorphism. It has small, intermediate and large forms, the intermediate one being the commonest. Small and large forms comparable with those of the New Zealand trypanosome were not present in my material. A comparison of the intermediate form of T. coelorhynchi with the present species follows:-

	T. coelorhynchus	T. bacalaoi
Length of free flagellum	11.0 μ (av.)	7.3 μ (av.)
Length of body	55.8 μ (av.)	73.6 μ (av.)
Width of body at centre of nucleus	3.2 μ (av.)	3.0 μ (av.)
Position of nucleus	38% of total body length from root of free flagellum	38%
Shape of nucleus	Reniform to circular	Oval
Granules in cytoplasm	Absent	Present
Vacuoles in cytoplasm	Numerous	Numerous
General appearance of flagellate	Curled into a tight spiral	Well-extended

The present flagellate thus resembles the intermediate form of T. coelorhynchi in the following respects: body width, location of the nucleus, presence of numerous vacuoles in the cytoplasm and staining reaction with Giemsa. However, it has an appreciably shorter flagellum, and a decidedly longer

body, than the intermediate form of the New Zealand species. The presence of granules in the cytoplasm, the oval nucleus, the internal location of the kinetoplast and particularly the generally extended rather than coiled appearance of the trypanosome under discussion show it to be distinct from T. coelorhynchi.

In the absence of a large range of specimens it is felt inadvisable to describe the Newfoundland cod trypanosome as new. Neither can it be identified as T. murmanensis on the basis of existing information. It is therefore only determined to the generic level at this time.

The example illustrated in Fig. 6 is ringed on the slide, which has been deposited in the collection of the U. S. National Museum (catalogue number USNM Helm. Coll. 71800).

Mr. Ray Côté, a fellow graduate student, first noticed this trypanosome of Gadus morhua, and furnished the material from Portugal Cove.

SPOROZOA: TELOSPOREA

Haemogregarina Danilewsky

Haemogregarina delagei Laveran & Mesnil, 1902 (Figs. 8-16, 101-103)

This elongate, sausage-shaped haemogregarine, varies in shape from straight (Fig. 9) to crescentic (Figs. 10-12, 14-16, 101). One end is usually somewhat broader than the other. It was present in erythrocytes of most (65/87) of the thorny skates (R. radiata) examined, the only one sampled in November, 1967 and 64/86 of those taken in May, 1968, besides two smooth skates (R. senta), one from each of the two collecting dates. Although 73% of the skates were parasitized, infections were usually so light that only 1 - 5 haemogregarines could be detected in 15 - 20 minutes of observation under a high dry objective (oil being applied to the slide). Heavier infections were only seen in about a dozen instances where 10 - 15 haemogregarines were recorded in the same period of microscopic examination.

Morphological Account

Fifty intraerythrocytic haemogregarines were measured, and found to range from 9.5 - 19.0 μ (av. 12.8 μ) in length, and 2.2 - 4.5 μ (av. 3.5 μ) in breadth.

The ovoid to irregular nucleus, which stains reddish purple with Giemsa, is usually centrally positioned in the larger examples. In smaller ones, it is sometimes subterminal. It measures 3.2 - 9.2 μ (av. 5.0 μ) by 1.0 - 3.0 μ (av. 2.4 μ), the chromatin granules numbering from 12 - 16 when individually distinguishable (Figs. 12, 16). However, these granules usually appear as an undifferentiated mass (Figs. 11, 13, 14, 15, 103). The alveolar cytoplasm, staining from light to dark blue, sometimes exhibits dark-staining granules (Figs. 9, 10, 14).

Fifty normal erythrocytes of R. radiata, measured 23.2 - 37.8 μ (av. 32.0 μ) by 14.5 - 20.0 μ (av. 17.2 μ). Fifty parasitized ones measured 24.5 - 36.0 μ (av. 31.2 μ) by 13.8 - 21.0 μ (av. 17.6 μ). The nuclei of the former ranged from 8.5 - 16.0 μ (av. 10.4 μ) by 7.0 - 9.2 μ (av. 7.8 μ); those of the latter, from 8.5 - 11.0 μ (av. 9.6 μ) by 6.5 - 8.8 μ (av. 7.5 μ). The parasite thus causes neither appreciable hypertrophy nor distortion of the host cells. Nor does it cause any obvious harm to the host cell nucleus.

Simple division of the parasite into three or four was detected on three occasions. In one instance, one of these products had become separated from the others (Fig. 13). These small haemogregarines measured 4.0 - 5.0 μ (av. 4.4 μ) by 1.0 - 1.5 μ (av. 1.5 μ), all having a subterminal nucleus.

Free haemogregarines were seen twice. They measured 15.0 μ by 4.5 μ, appearing slightly larger, more clearly defined and darker staining than intraerythrocytic ones (Figs. 15, 102). Double invasion in which two parasites at different stages of development occur within the same erythrocyte was observed twice only (Fig. 14). Fig. 103 illustrates an instance of double invasion in which the haemogregarines are of similar size and morphology.

In R. senta, the parasites found measured from 8.0 - 10.5 μ (av. 9.2 μ) in length by 2.8 - 4.0 μ (av. 3.2 μ) in breadth. The nucleus measured 4.0 - 4.5 μ (av. 4.3 μ) by 1.5 - 3.0 μ (av. 2.1 μ). Infections were again very light, yielding only four or five parasites in 15 - 20 minutes of searching. A single free haemogregarine (measuring 9.5 μ by 3.0 μ) was observed. Double invasion of the host erythrocyte was not recorded for this host.

None of the haemogregarines from either host showed polar caps.

Table 6. A preliminary list of haemogregarines from :

A. Skates

Parasite	Host	Locality	Reference
Haemogregarina delagei	Raja radiata	Europe	Laveran & Mesnil,1902a
	R. erinacea	St. Andrews, N. B.	Laird & Bullock,1969
	R. radiata	St. Andrews, N. B.	"

B. Rays

H. dasyatis Saunders, 1958	Dasyatis americana	Bahamas	Saunders, 1958
H. lobianci Kohl-Yakimoff & Yakimoff, 1915	Torpedo marmorata	Europe	Kohl-Yakimoff & Yakimoff,1915
H. torpedinis Neumann, 1909	Torpedo ocellaris	Europe	Neumann,1909

C. Other elasmobranchs

Parasite	Host	Locality	Reference
H. carchariasi	Carcharias sp.	Australia	Laveran,1908
H. delagei	Squalus acanthias	St. Andrews, N. B.	Laird & Bullock,1969
H. hemiscyllii	Hemiscyllium ocellatum	Australia	Mackerras & Mackerras, 1961

H. carchariasi Laveran (1908) was described from an Australian shark of the genus Carcharias. It measures 20 - 27 µ by 7 - 10 µ, and is thus altogether larger than the parasite under discussion. Besides, it causes radical elongation of the host cell. Normal host erythrocytes averaged some 26 µ in length, those containing H. carchariasi attaining as much as 34 µ.

H. delagei averages 13 µ by 2 µ, according to Laveran & Mesnil (1902a). Its nucleus consists of a mass of chromatin granules, and there are discrete volutin granules in the cytoplasm. No examples with a polar cap were seen. Double invasion was recorded. Laird & Bullock (1969) found the same parasite in a single dogfish and two species of skates. Their haemogregarines averaged 11.0 by 2.4 µ, the nucleus measuring some 3.0 by 1.9 µ. The latter structure exhibited up to 16 chromatin granules. Free examples (av. 11.3 by 3.7 µ) and double invasion of the host erythrocyte were observed. Polar caps were evident in some of the examples illustrated.

Saunders (1958b) described H. dasyatis from the southern stingray, Dasyatis americana, in the Bahamas. The maximum size of the mature gametocyte (13.0 by 3.8 µ), and the absence of polar caps in Giemsa-stained examples agreed with the organism under discussion. However, red cells apparently never held two parasites.

Another species, H. hemiscyllii Mackerras & Mackerras, 1961 occurs in the Australian dogfish, Hemiscyllium ocellatum. This haemogregarine is, however, slightly larger than the one under discussion. Some examples range up to 16 - 19 μ by 5 - 8 μ, and exhibit bluntly or smoothly rounded extremities.

Haemogregarines have been described from two members of the family Torpedinidae (electric rays). H. lobianci was described from Torpedo marmorata by Kohl-Yakimoff & Yakimoff (1915), and H. torpedinis from Torpedo ocellaris by Neumann (1909). The former species was said to have a "centrosoma" at one end. H. torpedinis, the mature gametocytes of which attain at least 18 by 4.5 μ, exhibits polar caps according to Neumann (1909).

The present organism is identified as H. delagei - it is considered that the brevity of the original description, and the parasite's occurrence in the type host (R. radiata) argue in favour of the general resemblances outlined outweighing minor dissimilarities. R. senta is a new host record.

One of the 65 R. radiata found positive harboured both Trypanosoma rajae and H. delagei.

Haemogregarina platessae Lebailly, 1904 (Figs. 17-22, 105)

A rather large haemogregarine, occupying the whole length of the host cell and half of its volume, was found in seven witch flounders (Glyptocephalus cynoglossus) and a single windowpane (Scophthalmus aquosus) caught in May, 1968. The body is slightly curved. Most of the examples have one of the extremities broadly rounded, the other tapering (Figs. 18, 19, 105) and sometimes pointed (Figs. 21, 22). All infections were light, only one or two parasites being encountered per host in 20 minutes of microscopic examination.

The organism measures 8.0 - 10.5 μ (av. 9.2 μ) in length, and 2.0 - 3.2 μ (av. 2.8 μ) in breadth. The large, oval nucleus, taking up the whole width of the parasite, occupies one-third to more than one-half of the body (Figs. 18, 19, 21). This structure, containing numerous chromatin granules, measures 2.8 - 4.8 μ (av. 3.5 μ) by 1.2 - 2.5 μ (av. 1.8 μ). It is sometimes centrally located (Fig. 19), but is usually somewhat closer to the broader extremity (Figs. 18, 105). The alveolar cytoplasm, staining light blue, frequently exhibits one or more terminal to subterminal vacuoles (Figs. 18, 19). Polar caps are usually lacking in examples from witch flounders, although not in those from Scophthalmus aquosus (Fig. 21).

Parasitized erythrocytes measured 8.8 - 13.0 μ (av. 11.0 μ) by 6.2 - 8.2 μ (av. 7.5 μ), as compared with the 9.8 - 12.5 μ (av. 10.6 μ) by 7.0 - 8.0 μ (av. 7.6 μ) of normal ones. The nuclei of the former ranged from 3.8 - 5.2 μ (av. 4.3 μ) by 2.5 - 3.0 μ (av. 2.7 μ), those of the latter from 3.8 - 4.8 μ (av. 4.5 μ) by 2.5 - 3.5 μ (av. 3.0 μ). Nuclei of parasitized cells seemed to be slightly reduced in size, and were often markedly displaced as well (Figs. 18, 19). Free haemogregarines were not observed. Neither were doubly invaded erythrocytes. Leucocytes were not seen to be parasitized.

A large and small form of the parasite were seen in Scophthalmus aquosus. The larger measured 9.5 by 1.5 μ, its nucleus (6.0 by 1.5 μ) being at the tapered extremity. A polar cap was clearly evident (Fig. 21). The smaller measured 3.5 by 1.0 μ, its round nucleus measuring 1.0 by 0.8 μ (Fig. 22). Infection was once again light, only one of the four examples of S. aquosus being parasitized.

Laveran & Mesnil (1901) first reported the occurrence of haemogregarines (their H. simondi) from a flatfish, the European Solea vulgaris (= Solea solea (L.)). This organism is a member of the bigemina group, undergoing schizogony in circulating erythrocytes. It is therefore quite different from the species under discussion.

Lebailly (1904) briefly described three species of haemogregarines from flatfish off the coast of France. Unfortunately, he furnished no illustrations. One of these three, H. platessae, was found in a plaice, Platessa vulgaris (= Pleuronectes platessae L.). The other two organismsin question were H. flesi Lebailly, 1904, found in Pleuronectes flesus (= Flesus vulgaris) and H. laternae Lebailly, 1904, from Platophrys laternae. H. platessae was described as measuring 9 by 2 μ (examples of smaller dimensions were also found), its body generally being crescentic, although only slightly curved. One extremity is rounded, the other bluntly pointed (and in larger examples, conspicuously vacuolated). The nucleus extends the whole width of the body and rather more than a third of its length, being usually located near the broad end of the body. This description suits the present organism very well, justifying its identification as H. platessae. Glyptocephalus cynoglossus and Scophthalmus aquosus are new host records.

H. platessae, or a form closely allied thereto, was first reported from North America by Fantham et al. (1942) from the winter flounder, Pseudopleuronectes americanus. However, no further information was given. Laird & Bullock (1969) described the same species from this host and another, Paralichthys dentatus, in North American waters (Woods Hole, Mass., and St. Andrews, N. B.).

Except for the smaller size of their haemogregarines (av. 7.7 by 1.4 μ), there is nothing in their description at variance with the present parasite.

Saunders (1955) reported another haemogregarine (H. achiri) from an American flatfish, the hog choker (Achirus fasciatus). Free merozoites and early invasive stages were observed. On the basis of an analysis of available data, Laird & Bullock (1969) suggested that H. achiri may be a synonym of H. platessae.

One example of Glyptoglossus cynoglossus, from which haemogregarines were not recorded, was parasitized by Trypanosoma sp. (p. 20). Three other G. cynoglossus harboured babesioids, which will be discussed later (p. 43). It should be noted that of these three examples, only one was infected with H. platessae.

Haemogregarina myoxocephali Fantham, Porter & Richardson, 1942. (Figs. 23-25, 104)

Another haemogregarine was found in the blood of the longhorn sculpin, Myoxocephalus octodecemspinosus. Four of 12 caught on the Grand Banks in May, 1968 were infected, none of them harbouring any ectoparasites. The same host is sometimes infected by babesioids (see p. 46).

All infections were light, not more than one haemogregarine being found per host in 20 minutes' examination of the smear. The parasite is sausage-like (Figs. 24, 25, 104) and sometimes slightly crescentic (Fig. 25). Both extremities may be smoothly rounded, one of them being slightly broader than the other (Figs. 24, 25). Overall dimensions of those examined were 9.2 - 10.0 μ (av. 9.5 μ) by 1.8 - 2.8 μ (av. 2.3 μ). The finely granular and alveolar cytoplasm stains light blue, the irregularly-shaped and deeply-staining nucleus exhibiting up to nine discrete chromatin granules (Fig. 25). It varies from 2.8 - 4.0 μ (av. 3.3 μ) by 1.2 - 1.8 μ (av. 1.5 μ) and is always displaced towards one pole.

Normal erythrocytes of the host measured 12.2 - 13.0 μ (av. 12.7 μ) by 8.0 - 10.8 μ (av. 9.4 μ), their nuclei varying from 4.5 to 5.2 μ (av. 4.9 μ) by 3.2 to 4.2 μ (av. 3.8 μ). Parasitized examples measured from 12.2 to 17.0 μ (av. 13.7 μ) by 7.5 to 8.5 μ (av. 7.5 μ), their nuclei ranging from 4.5 to 5.2 μ (av. 4.8 μ) by 2.5 to 3.5 μ (av. 2.9 μ). Thus parasitized erythrocytes are appreciably longer and narrower than normal, their nuclei being slightly reduced in size. Fifty percent of the parasitized red cells showed marked nuclear displacement (Figs. 24, 104). A polar cap was evident at the broader end of all the examples

seen. No free haemogregarines were found in any of the hosts, and double invasions were not observed.

The first haemogregarine ever found in a sculpin, H. cotti, was reported from Europe by Brumpt & Lebailly (1904), the host being Cottus bubalis Euphrasen. It was described as being a little more thick-set than H. callionymi Brumpt & Lebailly, (1904), which it otherwise closely resembles. The latter species has a curved body measuring 12 by 2.5 μ, its nucleus being somewhat closer to the more rounded extremity.

Fantham, Porter & Richardson (1942) described H. myoxocephali from a longhorn sculpin, M. octodecemspinosus, from Eastern Canada. This organism measures 5.5 to 9.2 μ by 2.2 to 2.8 μ. Its nucleus ranges from 1.5 to 4.5 μ by 1.5 to 2.8 μ. Laird & Bullock (1969) rediscovered this parasite in the type host, from adjacent waters. Their overall measurements were 4.9 - 9.6 μ (av. 7.9 μ) by 1.6 - 3.7 μ (av. 2.4 μ), the nucleus ranging from 1.8 - 4.1 μ (av. 2.7 μ) by 1.2 - 2.7 μ (av. 1.7 μ) and exhibiting 8 - 14 discrete chromatin granules. Polar caps were present in most of their examples. These dimensions are close to those of the haemogregarine under discussion, which further resembles H. myoxocephali in its general morphology and effect upon the host cell, and is therefore identified accordingly.

Laird & Bullock (1969) made the additional suggestion that H. myoxocephali might well in fact prove conspecific with H. cotti Brumpt & Lebailly, 1904, once sufficient data and illustrations of the latter species become available. Indeed, many of the older species of haemotozoa are so inadequately characterized as to preclude certain recognition from new material. Redescription of these older European species is urgently called for.

Possible Vectors of Fish Haemogregarines

Despite the many species of piscine haemogregarines described over the past 70 years, the method of transmission from one fish to another remains unknown. While there have been suggestions that leeches and/or parasitic copepods may serve as intermediate hosts, no proof has yet been brought forward.

Reichenow (1910) was the first to establish the transmission of a haemogregarine (H. stepanowi Danilewsky of the European water tortoise, Emys orbicularis (= Cistudo europea) by the leech, Placobdella catenigera). H. stepanowi supposedly begins its life cycle as a zygote (= ookinete) in the gut of the leech, P. catenigera. The zygote divides three times, forming eight sporozoites. When an infected leech takes blood from the turtle, E. orbicularis, the sporozoites pass to the vertebrate host and invade red

blood cells. Schizogony occurs there, leading to the production of merozoites, which are liberated from the host cells to infect further erythrocytes. Eventually, some of the merozoites which enter these host cells develop into gametocytes. If the red cells in question are ingested by a leech, the gametocytes are liberated. They then mature to gametes, zygote formation duly taking place.

The above life cycle of H. stepanowi was soon confirmed (Robertson, 1910) for H. nicoriae Castellani & Willey, 1904. This haemogregarine of the Ceylon lake tortoise, Nicoria trijuga, is transmitted by the leech, Ozobranchus shipleyi. Shortly after Reichenow and Robertson published their findings, Wenyon sectioned leeches obtained from Sternothaerus adansonii. Erythrocytes from this Sudanese water tortoise held looped haemogregarines considered possibly referable to H. stepanowi. The intestine of the leech, which Wenyon (1926) sectioned yielded haemogregarine life-history stages in every way comparable to this species. Many other haemogregarines of cold-blooded aquatic vertebrates will probably prove to have a similar life-history.

Piscicolid leeches (Malmiana nuda Richardson, 1970) were found on five shorthorn sulpins, Myoxocephalus scorpius, in November, 1969, maintained in tanks at the Marine Sciences

Research Laboratory, Logy Bay. Unfortunately it was impossible to obtain blood films from these fish, which were being kept alive for other studies in the laboratory. Upon examination of their gut smears, 80% of the leeches (8/10) showed polymorphic sporozoites, of oval to elongate form (Figs. 26-29, 106). Twenty were measured, and found to range from 5.0 - 15.0 μ by 2.0 - 3.2 μ, the irregular nucleus measuring from 1.8 - 3.0 μ by 1.2 - 2.5 μ. Their alveolar cytoplasm was stained very pale blue.

The presence of such sporozoites in leech gut smears combined with that of haemogregarines in the erythrocytes of closely related sculpins, strongly suggests that Malmiana nuda transmits Haemogregarina myoxocephali. Experimental proof will of course be necessary to test this hypothesis. Nevertheless, the present evidence represents the first actual clue towards elucidation of the transmission of any fish haemogregarines.

SARCOMASTIGOPHORA: PIROPLASMEA

Haemohormidium Henry

Haemohormidium terraenovae n.sp. (Figs. 30-71, 107-113)

Many of the fish examined were found to be parasitized by babesioids of the genus Haemohormidium Henry, 1910 (Wenyon, 1926; Laird & Bullock, 1969). Six hosts were involved (Table 2), three of them being very frequently infected - Ammodytes americanus (19/23), Hippoglossoides platessoides (28/60) and Limanda ferruginea (13/47). A few fish individually exhibited quite heavy infections. These were the exceptions, though, most hosts being only lightly infected (some 10 RBC per 10,000 parasitized). From one to six amoeboid babesioids may occur in an individual RBC, the central zone of which assumes a paler stain than the surrounding host cell cytoplasm. Indeed, many examples take up very little stain at all. The embayed periphery stains faint blue to dark blue with Giemsa.

Although approximately 80% of the Ammodytes americanus sampled in November, 1967, were positive, individual infections were light except in four cases. In these, as many as 10% of the red cells were parasitized. The majority of the RBC harboured only one trophozoite, but cells with two or three have also been encountered (Figs. 30-47). Overall measurement gave a

range of 1.0 - 3.2 μ by 0.3 - 1.0 μ. Small chromatic granules and particles often accompany these amoeboid bodies, the presence of which does not visibly affect the host cells.

Only 10% (4/40) of the Urophycis tenuis sampled in May, 1968, were found to harbour this babesioid. The infections were light, except in one host where some 10% of the RBC showed parasites. The organism measured 1.0 - 2.5 μ by 0.2 - 1.0 μ (Figs. 48-51, 108).

Three out of 11 Melanogrammus aeglefinus were parasitized, (3/6 sampled in November, 1967 were positive, but none of the five taken in May, 1968), one being heavily infected. The parasites measured 1.0 - 2.0 μ by 0.5 - 1.5 μ (Figs. 52-55, 110), and did not appear to affect the host erythrocytes.

Twenty-eight per cent of the Limanda ferruginea (13/47) were lightly infected, (3/8 sampled in November, 1967, and 10/39 taken in May, 1968), as many as six trophozoites being found in one erythrocyte (Fig. 61). The organism measured 1.2 - 3.2 μ by 0.4 - 2.2 μ (Figs. 56-61, 109), and caused no outward effect upon the host RBC.

Three out of 19 examples of Glyptocephalus cynoglossus caught in May, 1968, were found to be parasitized by this babesioid. Again the infections were light, but in this case the nuclei of the host erythrocytes were usually markedly displaced (Figs. 63-65). The babesioid measured 0.8-3.5 μ by

0.2 - 1.0 μ. It should be remembered that G. cynoglossus was also subject to infection by Trypanosoma sp. and Haemogregarina platessae. Of the three examples harbouring the babesioid, one was parasitized by H. platessae as well.

Some 50% (28/60) of Hippoglossoides platessoides proved positive (20/47 sampled in November, 1967, and 8/13 taken in May, 1968). In this host too, all the infections were very light. The overall measurements of the parasite were 0.5 - 4.0 μ by 0.1 - 2.5 μ. As in G. cynoglosus, the parasite was associated with marked displacement of the host cell nucleus (Figs. 68, 112). Furthermore, the host cells were sometimes distorted (Figs. 69, 70). It is possible, though, that such distortion might have come about during preparation of the smear. Horseshoe-shaped haemogregarines with a chromatic granule at either end and thought to be division stages were encountered in two of the American plaice (Figs. 71, 113).

The organism under discussion is clearly a babesioid. In both morphology and size it is in very close agreement with a haematozoan from British sculpins (Henry, 1913b). Henry had earlier (1910) proposed the name of Haemohormidium cotti for this organism, which was described as an irregularly round or oval body lying embedded in the protoplasm of the RBC, and measuring 2.0 - 4.5 μ by 1.0 - 3.0 μ. However, Henry (1913b) made no mention of the name that he had proposed only three years previously. He now apparently took the amoeboid bodies to be developmental stages of Haemogregarina cotti Brumpt & Lebailly, 1904. It remained

for Wenyon (1926) to validate Haemohormidium by publishing a recognizable account, although it can also be argued that the "indication" in Henry's (1910) paper was a sufficient validation under articles 11, 12 and 16 of the International Code of Zoological Nomenclature, 1961 (Mackerras & Mackerras, 1961; Laird & Bullock, 1969).

Laird & Bullock (1969) described as Haemohormidium sp. a morphologically similar organism from two Hemitripterus americanus and one Hippoglossoides platessoides (St. Andrew's, N. B.). These amoeboid bodies, as seen in a Giemsa-stained thin blood film, measured 2.2 - 4.8 μ by 0.8 - 3.2 μ.

The present organism resembles Haemohormidium in both size and morphology, and is therefore identified accordingly, all the host records except Hippoglossoides platessoides being new.

Neither H. cotti nor Haemohormidium sp. of Laird & Bullock (1969) are described in sufficient detail for new material to be assigned to them with any confidence. However, it is recognized that a full description of H. cotti from the type host and locality might well indicate the conspecificity of this species and mine (also that found by Laird & Bullock, which certainly seems to correspond closely with the Newfoundland material under discussion). In such an event, the earlier name would of course take

precedence over that proposed below. H. aulopi differs appreciably from the present parasite because of its larger size and general morphology - for example, its chromatin occurs as blocks at the periphery of the body (Mackerras & Mackerras, 1961).

For the reasons given, the Newfoundland babesioid is described as new. It is designated Haemohormidium terraenovae n.sp., having the characters outlined herein, the specific name being derived from the Latin for "Newfoundland".

Syntypes have been deposited in the collection of the U. S. National Museum (cat. no. USNM Helm. Coll. 71802).

Haemohormidium beckeri n.sp. (Figs. 72-79, 114-116)

Five of 12 longhorn sculpins, Myoxocephalus octodecemspinosus caught in May, 1968, were found to harbour babesioids of quite another kind than that described above. All infections were quite light, some 10 red cells per 1,000 being parasitized.

Amoeboid stages measured 2.0 - 5.0 μ by 0.5 - 2.5 μ. They differed from H. terraenovae in attaining a larger size and in the fact that cruciform division stages were seen (Figs. 79, 116). Rarely encountered, the latter measure 2.0 μ in diameter. They are fully comparable with the equivalent stages characterizing the genus Babesiosoma

as first described by Jakowska and Nigrelli (1956). Laird & Bullock (1969) relegated Babesiosoma Jakowska & Nigrelli, 1956 to synonymy with Haemohormidium Henry, 1910. Levine (1971) has followed their lead, emending the description of the family Dactylosomatidae Jakowska & Nigrelli, 1955, as follows. - "In erythrocytes of cold-blooded vertebrates; schizogony generally present, with formation of 4 - 16 merozoites; vectors unknown." He recognizes three genera - Dactylosoma Labbé, 1894, Haemohormidium Henry, 1910, and Sauroplasma du Toit, 1938 - in this family. Dactylosoma forms more than four merozoites at schizogony, Haemohormidium only four; while Sauroplasma undergoes only binary fission or budding into two daughter cells, without schizogony.

Table 7. Previous records of Haemohormidium spp.

(adapted from Levine, 1971)

Parasite	Host	Locality	Reference
Haemohormidium aulopi (Mackerras & Mackerras, 1925) Laird & Bullock, 1969	(Marine fish) Aulopus purpurissatus	Australia	Mackerras & Mackerras, 1961
Syn. Haemogregarina aulopi Mackerras & Mackerras, 1925	Parma microlepis		Laird & Bullock, 1969 Levine, 1971
H. clariae (Haiba, 1962) Levine, 1971 Syn. Cytauxzoon clariae Haiba, 1962	(Freshwater fish) Clarias lazera	Egyptian Nile	Levine, 1971
H. cotti Henry, 1910 (TYPE SPECIES)	(Marine fish) Cottus bubalis C. scorpius	U. K.	Henry, 1910
H. guglielmi (Carpano, 1939) Levine, 1971 Syn. Nuttallia guglielmi Carpano, 1939	(Turtle) Testudo campanulata	Europe	Levine, 1971

Table 7. Previous records of Haemohormidium spp. (contd.)

Parasite	Host	Locality	Reference
H. jahni (Nigrelli, 1929) Laird & Bullock, 1969 Syn. Dactylosoma jahni Nigrelli, 1929 Babesiosoma jahni (Nigrelli, 1929) Jakowska & Nigrelli, 1956	(Newt) Triturus (= Notophthalmus) viridescens	N. America	Nigrelli, 1929a Laird & Bullock, 1969 Levine, 1971
H. mariae (Hoare, 1930) Laird & Bullock, 1969 Syn. Dactylosoma mariae Hoare, 1930 Babesiosoma mariae (Hoare, 1930) Jakowska & Nigrelli, 1956	(Freshwater fish) Haplochromis spp., Tilapia spp., Labeo, Astatoreochromis	Africa	Hoare, 1930 Laird & Bullock, 1969 Levine, 1971
H. ophicephali (Misra, Haldar & Chakravarty, 1969) Syn. Babesiosoma ophicephali Misra, Haldar & Chakravarty, 1969	(Freshwater fish) Ophicephalus punctatus	India	Misra, Haldar & Chakravarty, 1969 Levine, 1971
H. rubrimarensis (Saunders, 1960) Laird & Bullock, 1969 Syn. Babesiosoma rubrimarensis Saunders, 1960	(Marine fish) Lethrinus xanthochilus, L. variegatus, Cephalopholis miniatus, C. hemistictus, Scarus harid, Mugil troscheli.	Red Sea	Saunders, 1960 Laird & Bullock, 1969

Table 7. Previous records of Haemohormidium spp.(concl.)

Parasite	Host	Locality	Reference
Haemohormidium sp. Laird & Bullock, 1969	(Marine fish) Hemitripterus americanus Hippoglossoides platessoides	St. Andrew's N. B.	Laird & Bullock, 1969
H. stableri (Schmittner & McGhee, 1961) Laird & Bullock, 1969 Syn. Babesiosoma stableri Schmittner & McGhee, 1961	(Frog) Rana pipiens pipiens	N. America	Schmittner & McGhee, 1961 Laird & Bullock, 1969 Levine, 1971
H. tetragonis (Becker & Katz, 1965) Laird & Bullock, 1969 Syn. Babesiosoma tetragonis Becker & Katz, 1965	(Freshwater fish) Catostomus sp. - sucker	N. America	Becker & Katz, 1965 Laird & Bullock, 1969 Levine, 1971

The present babesioid differs from H. terraenovae n.sp. in having rosette-shaped or cruciform schizonts in circulating erythrocytes. However, the total lack of such cruciform stages in the large amount of material reported upon with respect to H. terraenovae n.sp. leads me to suggest that there might be at least two different groups of babesioids in marine fish. One of these, characterized by conspicuous oval, ellipsoid or amoeboid trophozoites but lacking division stages in circulating RBC, might bear close comparison with Sauroplasma (of which only the type species, S. thomasi du Toit, 1938, of a lizard, is yet known). The other, somewhat larger, has more conspicuous chromatin/cytoplasm differentiation and exhibits rare rosette-shaped or cruciform schizonts in the RBC. However, the very rarity of schizonts in the latter group (to which the present species belong) suggests that the examination of additional material of H. terraenovae might still reveal schizonts. The matter clearly cannot be settled at this time, and must await further study.

Haemohormidium beckeri n.sp. having the characters described herein, is delicated to Dr. C. D. Becker[8] who has contributed much to the study of North American piscine

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haematozoa and who provided greatly appreciated advice during my visit to his laboratory in July, 1969. Syntypes have been deposited in the collection of the U. S. National Museum (cat. no. USNM Helm. Coll. 71801).

CNIDOSPORA: MYXOSPORIDA

Kudoa Meglitsch

(a) Kudoa sp. (Figs. 95, 96, 117)

Quadricapsulate chloromyxid myxosporidan spores distinctly quadrate as viewed from the anterior extremity (Fig. 95) were present in heart blood films from three Atlantic cod from Portugal Cove, Conception Bay. They measure 5.5 - 5.8 μ by 4.8 - 5.0 μ and closely resemble Kudoa clupeidae (Hahn) in both size and shape. They are only mentioned here as an addition to the already long list of organisms accidentally present in blood films, these histozoic parasites having presumably escaped from heart muscle slit during the smearing operation.

(b) Undetermined myxosporidan (Fig. 97)

A myxosporidan trophozoite measuring 20 μ by 21 μ was identified in a blood film from a thorny skate. The vegetative forms of myxosporidans in Giemsa-stained material show no features of taxonomic value, merely appearing as variously crumpled or creased sheets of purplish-staining protoplasm. However, it is noted that a form such as the present one could pass for the vegetative stage of a widespread myxosporidan known from the gall bladder of various European and eastern North American

elasmobranchs including Raja spp., Chloromyxum leydigi Mingazzini (Kudo, 1920). It is suggested that if indeed it is referable to the Chloromyxidae, though, a more likely explanation of its presence in a heart blood smear would be the one given above, namely, that it is a histozoic Kudoa sp. adventitiously released into the blood at the time of smearing.

Intraerythrocytic inclusions of unknown aetiology (Figs. 82-94, 118, 119)

Upon examining 34 Atlantic herrings, (Clupea harengus) and 40 Atlantic argentines (Argentina silus) caught in May, 1968, all the erythrocytes showed the inclusions illustrated.

In C. harengus, some RBC exhibited one or two dark blue-staining spots (0.5 μ diameter) in their cytoplasm (Fig. 82). Others showed many (up to 40 or 50) oval or rod-like bodies of smaller size. Staining uniformly light blue with Giemsa, these were sometimes scattered throughout the cytoplasm and sometimes quite regularly arranged around the nucleus (Figs. 85, 87, 118). The presence of these bodies was not associated with hypertrophy of the RBC, but in about 2% of the cells, the nucleus showed marked displacement (Fig. 84).

In A. silus, similar bodies were found in all erythrocytes. It was tempting to hypothesize a developmental sequence from the material studied.

However, a more critical assessment provided jarring notes. For example, some of the bodies stained uniformly pinkish, others blue. Also, the majority (in A. silus) were of irregular outline (Figs. 90, 92, 96), some of them being markedly elongated (Fig. 96). There was no definite pattern of arrangement around the nucleus as in the Atlantic herring.

The first few slides examined were merely thought to be contaminated by bacteria or some other extraneous microorganisms. It was also speculated that the fish concerned were dead when the films were made. However, the field notes showed that all fish were still alive at the time of smearing. Moreover, further slides subsequently showed precisely the same apparent infection, although other species of fish collected at the same time and treated in the same way had RBC that stained normally and were altogether devoid of such bodies.

At first sight, these bodies resemble Grahamella sp. (Brumpt, 1911) - found in many small mammals - and Bartonella sp. (Strong, Tuzzer, Brues, Sellards & Gastiaburu, 1915) - responsible for Oroya fever in South America - in

their shape and tiny size. Nevertheless, their staining reaction and incidence (all RBC exhibiting them) separates them from the two organisms mentioned. The possibility of their being rickettsiae was also considered.

Due to the extremely small size of these bodies (0.5 - 1.5 μ by 0.2 - 0.5 μ) and the shortcomings of the Giemsa method, it was not possible to make out any structural details. Because of their uniform incidence it is suspected that they might prove to be merely a structural peculiarity of the RBC of clupeid fish. They are therefore, at this time, simply noted and referred to as intraerythrocytic inclusions of unknown origin.

Unidentified artifacts (Figs. 80, 81, 120, 121)

Bacteria-like entities were found in blood smears from one spiny dogfish and one yellowtail flounder. In both instances, only a single groups of organisms was encountered. Their regular arrangement and uniform staining reaction with Giemsa, suggested that they were bacterial artifacts the presence of which was accidental and associated with the preparation of the smears.

In the spiny dogfish, these organisms appeared to be intraerythrocytic (Figs. 80, 120) and in the same plane of focus as the blood cell. In the yellowtail flounder,

on the other hand, they were both intra- and extraerythrocytic and were rather obviously superimposed upon the smear (Figs. 81, 121).

GENERAL DISCUSSION AND SUMMARY

Three species of trypanosomes were recorded in this survey. Trypanosoma rajae has already been reported from North American waters in Raja radiata (Kudo, 1922; Bullock, 1958; Laird & Bullock, 1969). While Trypanosoma sp. from Glyptocephalus cynoglossus is the first record of trypanosome found in this host, and the single example found showed differences from trypanosomes previously described from flounders, determination of the species must obviously await additional material. The Atlantic cod, Gadus morhua, has once previously been found to harbour trypanosomes. The species in question, Trypanosoma murmanensis Nikitin, 1927, differs in detail from Trypanosoma sp. described herein from only one of 180 cod-fish examined. This record is felt to be of particular interest in view of the desirability of our gaining an exhaustive knowledge of the pathogens and parasites of a fish of such economic importance. Nothing whatsoever being known about the role of fish trypanosomes in causing disease in their hosts, it is submitted that this cod trypanosome might be thought of as a particularly appropriate candidate for intensive laboratory investigations. Such studies would of course demand early elucidation of the vector, and piscicolid leeches have been implicated in this connexion with respect to some other fish trypanosomes (Laveran & Mesnil, 1907).

Three known species of Haemogregarina were recorded. H. delagei was found in Raja radiata and R. senta, the latter being a new host for this parasite. Glyptocephalus cynoglossus and Scophthalmus aquosus are new host records for H. platessae. H. myoxocephali was recorded from the type host, Myoxocephalus octodecemspinosus.

Free sporozoites in every way comparable with those described from hirudinid vectors of tortoise haemogregarines by earlier workers, were found in gut smears of piscicolid leeches (Malmiana nuda) attached to Myoxocephalus scorpius. This, together with the fact that so close a relative to M. octodecemspinosus might well be expected to harbour the same species of haemogregarine, strongly suggests that this leech is in fact a vector of H. myoxocephali. Although a number of investigators have devoted considerable efforts to searching for vectors of piscine haemogregarines since the first description of these parasites 70 years ago, this is the first evidence (circumstantial though it is) ever obtained to suggest that the cycle is the same as that elucidated for H. stepanowi and H. nicoriae in the early part of the century (Reichenow, 1910; Robertson, 1910).

Haemohormidium terraenovae n.sp. was recorded from six hosts:- Ammodytes americanus, Hippoglossoides platessoides,

Limanda ferruginea, Urophycis tenuis, Melanogrammus aeglefinus, and Glyptocephalus cynoglossus. While this babesioid might eventually prove conspecific with H. cotti Henry (1910) and Haemohormidium sp. Laird & Bullock (1969) more detailed descriptions of the latter organisms are prerequisite to final clarification. The common occurrence of this parasite in several species of fish on the Grand Banks of Newfoundland, and its rather high incidence in individual hosts, are of very considerable interest. Aside from the fact that the record constitutes only the fifth for babesioids from marine fish anywhere in the world, the facts that this group of haematozoa are responsible for serious diseases of some mammals (and have recently been confirmed to be parasites of man), and that the type locality is one of the world's major fishing grounds, demand follow-up investigations.

A second babesioid, Haemohormidium beckeri n.sp. was recorded from M. octodecemspinosus. While cruciform and/or rosette-shaped schizonts characterize H. beckeri n.sp., they have yet to be reported from H. terraenovae n.sp. The possibility of future separation at the subgeneric level must therefore be kept in mind. All in all, the combination of a good range of unusually interesting haematozoan material, the proximity of a major fishery of great economic importance to several countries and the

facilities of the Marine Sciences Research Laboratory at Logy Bay make Memorial University of Newfoundland a singularly appropriate centre for the field and laboratory studies that will be necessary for experimental elucidation of the problems mentioned above.

Some of the negative hosts in the present project had been found to be hosts for other haematozoans by earlier workers. Thus, Squalus acanthias is known to harbour Haemogregarina delagei off the North American mainland (Laird & Bullock, 1969), where the same authors found Melanogrammus aeglefinus and Urophycis tenuis to be parasitized by H. aeglefini; Anarhichas lupus by H. anarhichadis Henry, 1912 (spelling emended from anarrhichadis by Fantham et al., 1942); and Hemitripterus americanus by Haemohormidium sp. The reasons for different incidences of marine fish haematozoan as between different localities may be basically ecological (for example, the type of substrate influencing the incidence of potential vectors such as leeches, which thrive on hosts frequenting sandy and muddy bottoms but not on stony ones). Sample size is of obvious importance too (e.g. my single record of a species of Trypanosoma from 180 cod), and again, the extremely low incidence of presumably chronic infection characteristic of most fish haematozoa must inevitably lead to some infections being overlooked.

Intraerythrocytic inclusions of unknown origin were seen in all the herrings and argentines examined. The prevalence of these bodies and their indeterminate nature suggested that they might simply represent staining artifacts in normal red cells the cytoplasmic structure of which responds to Giemsa in an unusual way. Bacterial artifacts were noted in slides from a spiny dogfish and a yellowtail flounder, and chloromyxid myxosporidan protozoans from three cod and a thorny skate. The two latter parasites may well have been histozoic organisms accidentally introduced into the blood in the act of slitting the heart wall.

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Fig. 1. Erythrocyte, *Raja radiata*.

Fig. 2. *Trypanosoma rajae*, from *R. radiata*.

Fig. 3. Erythrocyte, *Glyptocephalus cynoglossus*.

Fig. 4. *Trypanosoma* sp., from *G. cynoglossus*.

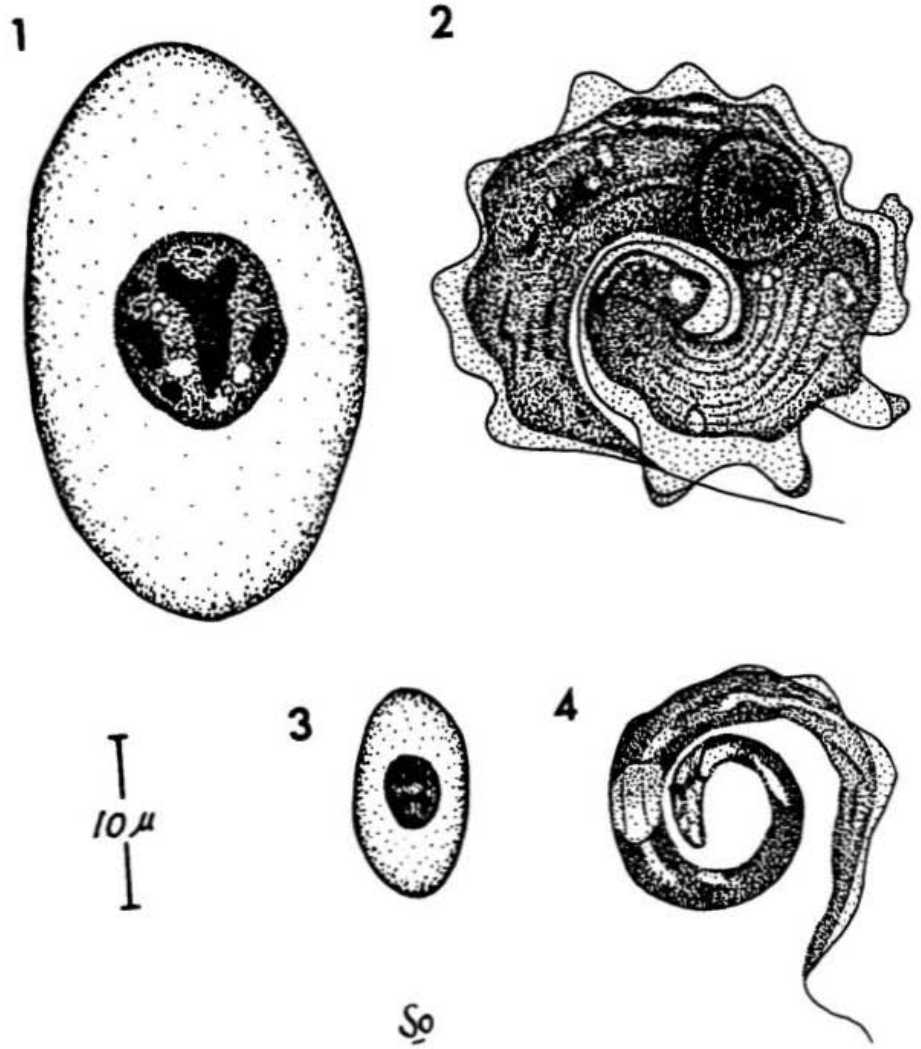
1
2
3
4
10 µ
So

Fig. 5. Erythrocyte, Gadus morhua.

Figs. 6-7. Trypanosoma sp. from G. morhua.

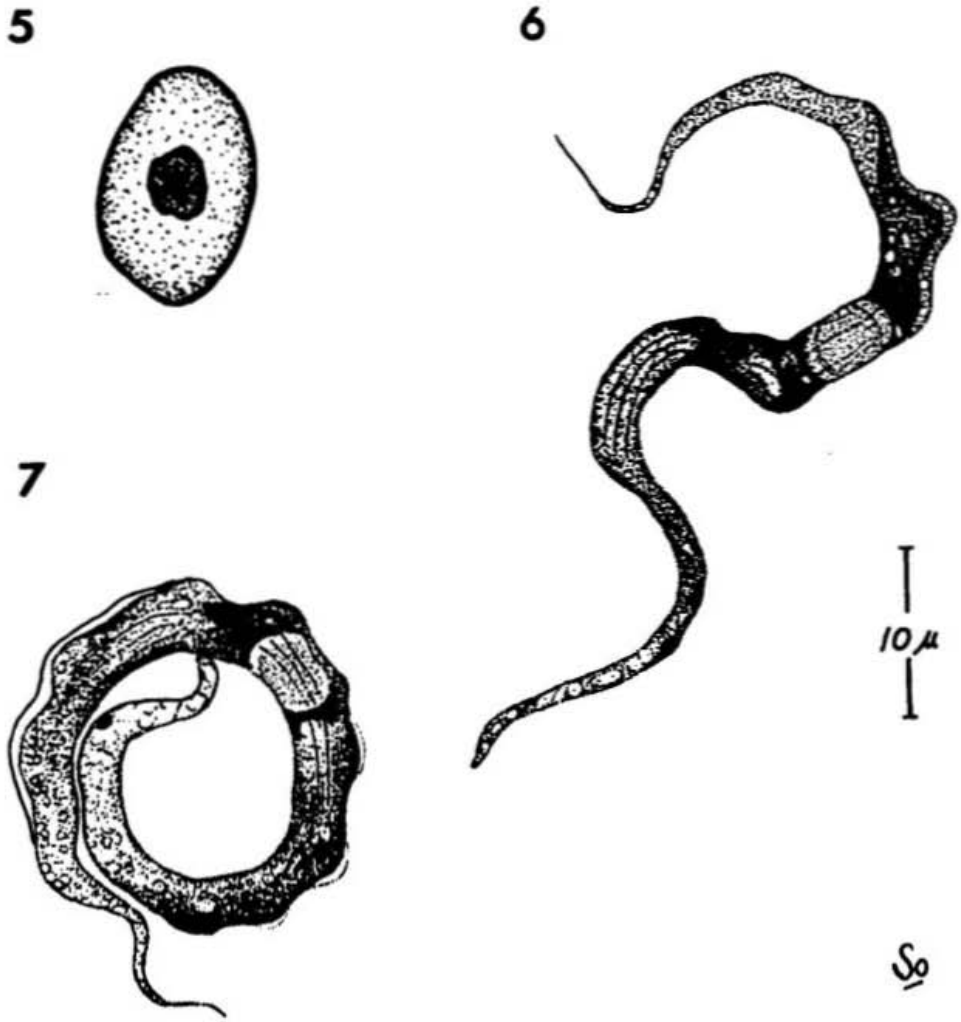
5
6
7
10 μ
So

Fig. 8. Erythrocyte, Raja radiata.

Figs. 9-11. Haemogregarina delagei, R. radiata.

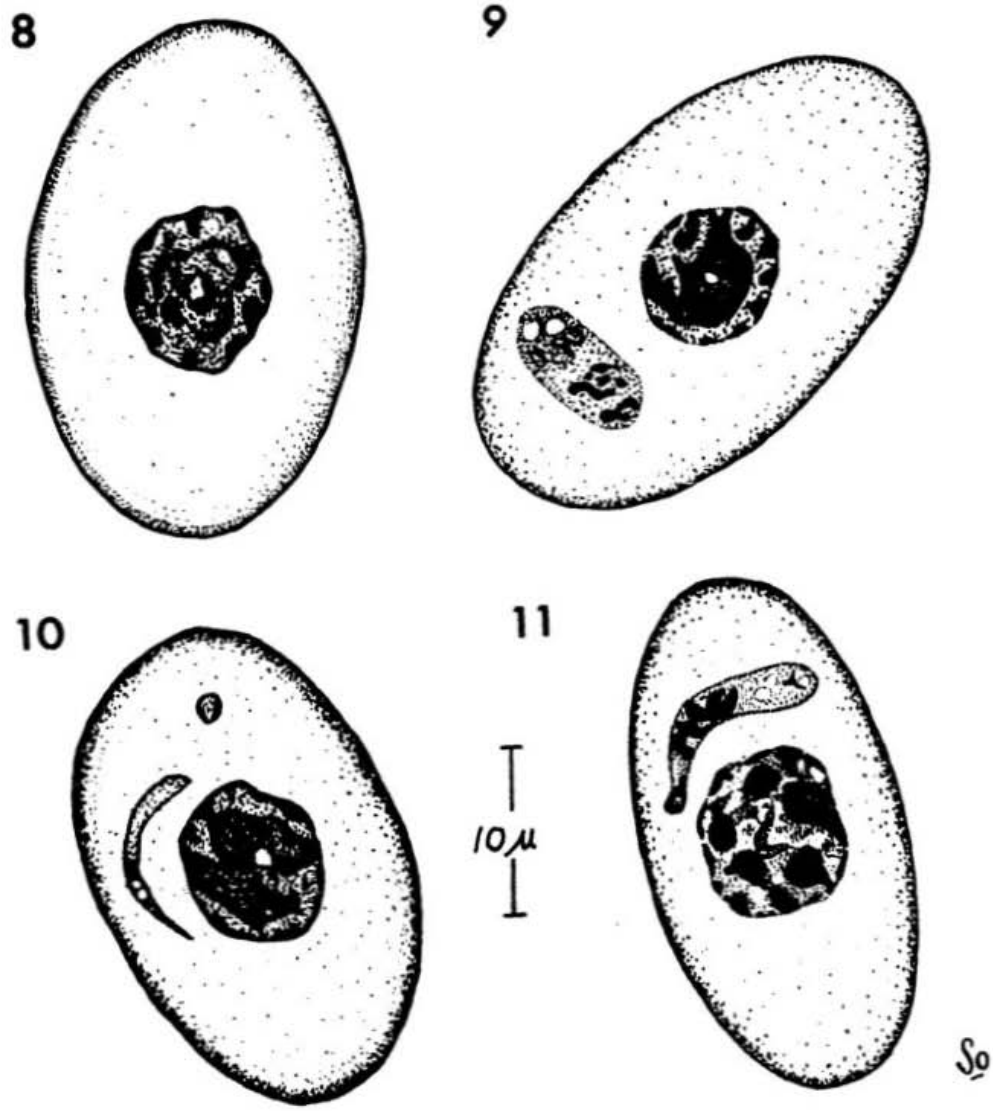
8
9
10
11
10 µ

Fig. 12. *Haemogregarina delagei*, *Raja radiata*.

Fig. 13. Dividing *H. delagei*, from *R. radiata*.

Fig. 14. *H. delagei* from *R. radiata*, showing double invasion of host erythrocyte.

Fig. 15. Free vermicule of *H. delagei*, from *R. radiata*.

Fig. 16. *H. delagei* from *Raja senta*.

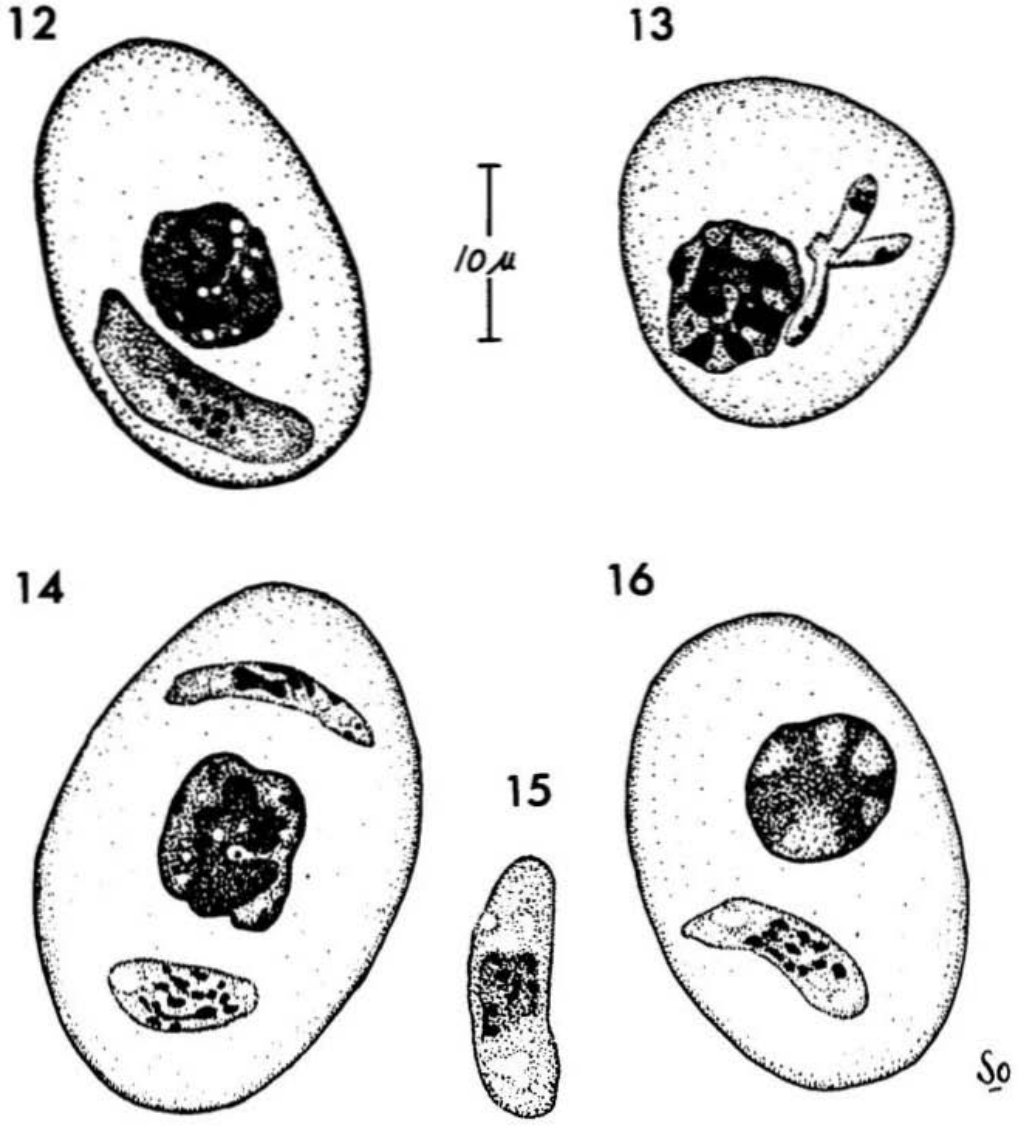
12
13
10 µ
14
15
16
So

Fig. 17. Erythrocyte, *Glyptocephalus cynoglossus*.

Figs. 18-19. *Haemogregarina platessae*, from *G. cynoglossus*.

Fig. 20. Erythrocyte, *Scophthalmus aquosus*.

Figs. 21-22. *H. platessae*, from *S. aquosus*.

Fig. 23. Erythrocyte, *Myoxocephalus octodecemspinosus*.

Figs. 24-25. *H. myoxocephali*, from *M. octodecemspinosus*.

Figs. 26-29. Sporozoites from gut of the leech, *Malmiana nuda*.

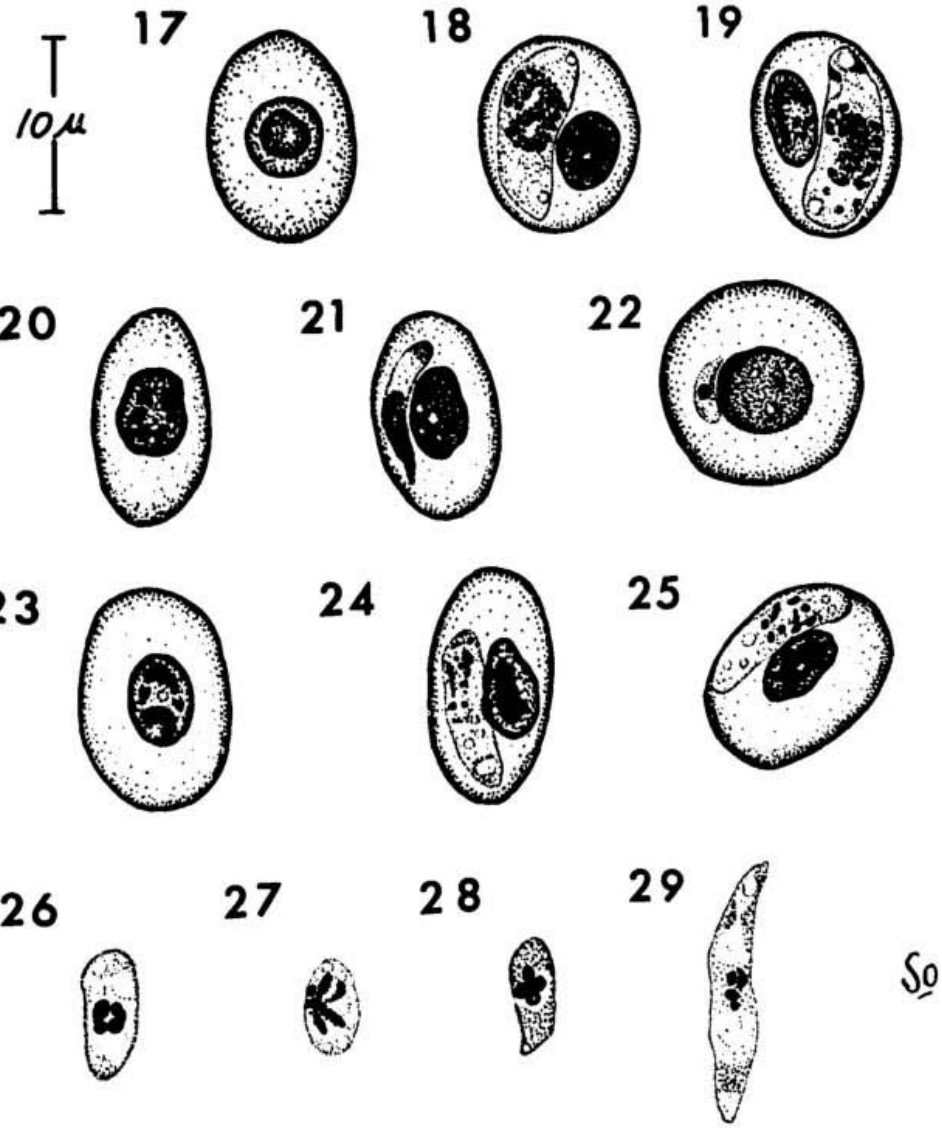
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Fig. 30. Erythrocyte, *Ammodytes americanus*.

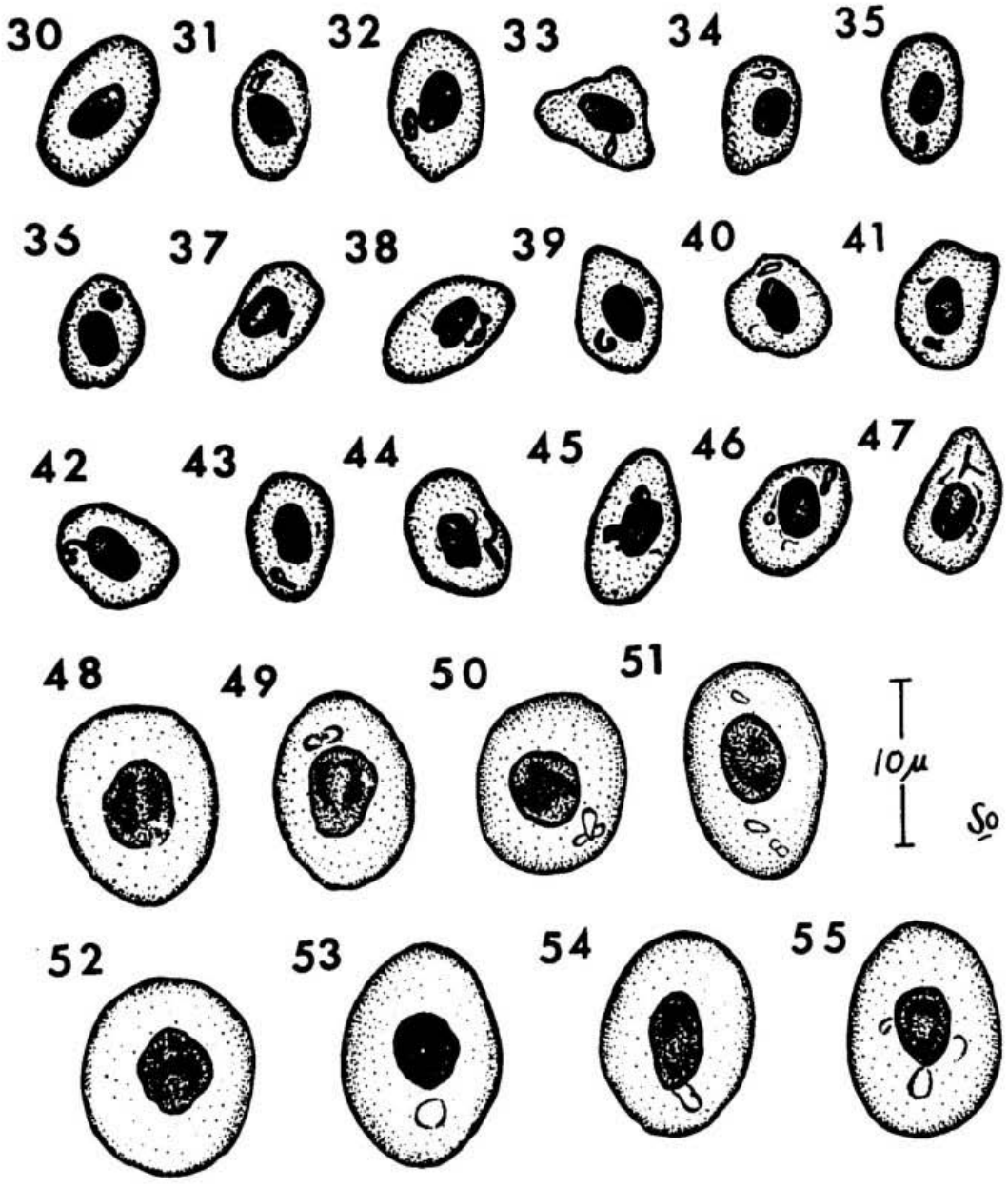
Figs. 31-47. *Haemohormidium terraenovae* n.sp. from *A. americanus*.

Fig. 48. Erythrocyte, *Urophycis tenuis*.

Figs. 49-51. *H. terraenovae* n.sp., from *U. tenuis*.

Fig. 52. Erythrocyte, *Melanogrammus aeglefinus*.

Figs. 53-55. *H. terraenovae* n.sp., from *M. aeglefinus*.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10μ
So
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54
55

Fig. 56. Erythrocyte, *Limanda ferruginea*.

Figs. 57-61. *Haemohormidium terraenovae* n.sp., from *L. ferruginea*.

Fig. 62. Erythrocyte, *Glyptocephalus cynoglossus*.

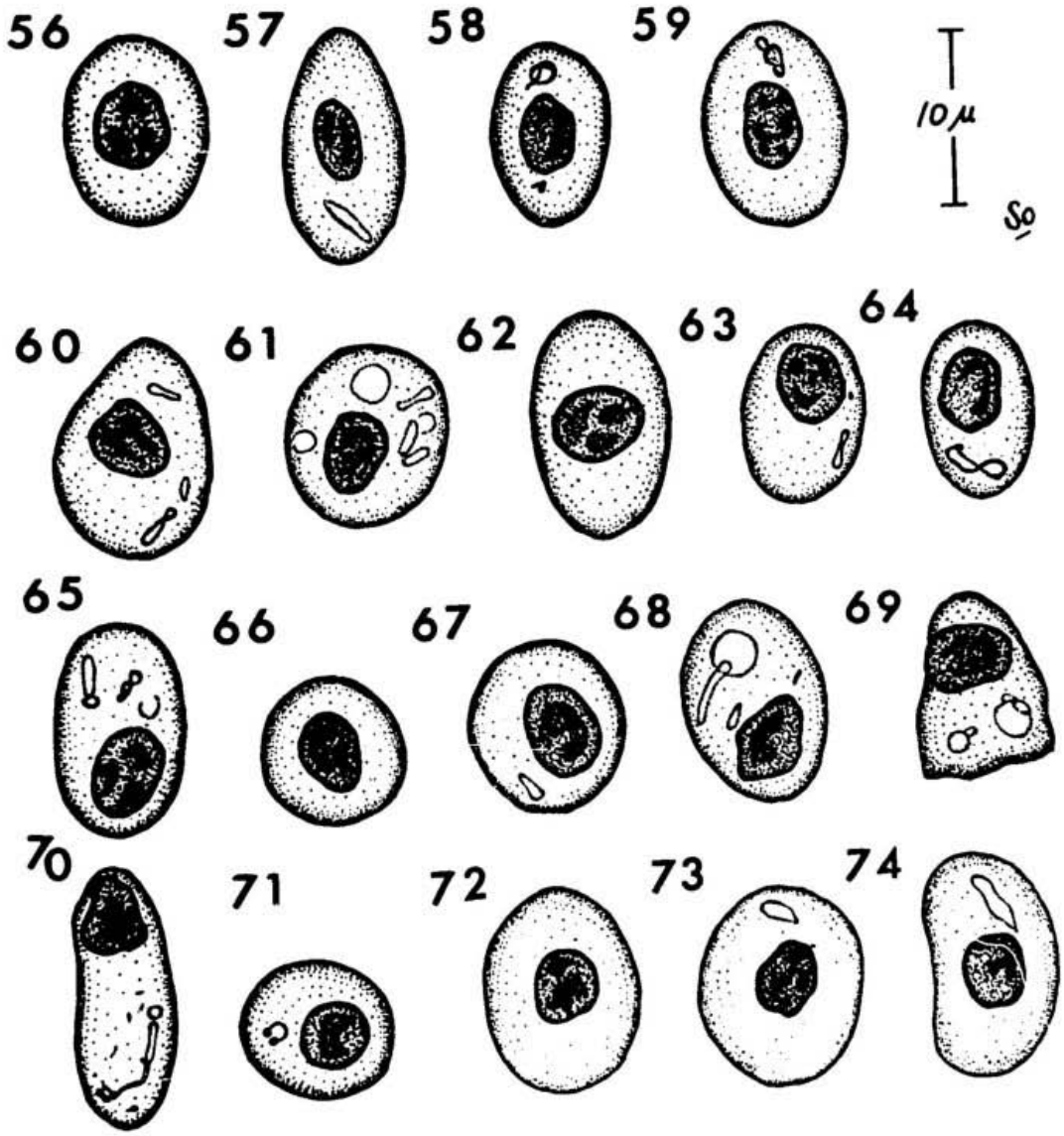
Figs. 63-65. *H. terraenovae* n.sp., from *G. cynoglossus*.

Fig. 66. Erythrocyte, *Hippoglossoides platessoides*.

Figs. 67-71. *H. terraenovae* n.sp., from *H. platessoides*.

Fig. 72. Erythrocyte, *Myoxocephalus octodecemspinosus*.

Figs. 73-74. *Haemohormidium beckeri* n.sp., from *M. octodecemspinosus*.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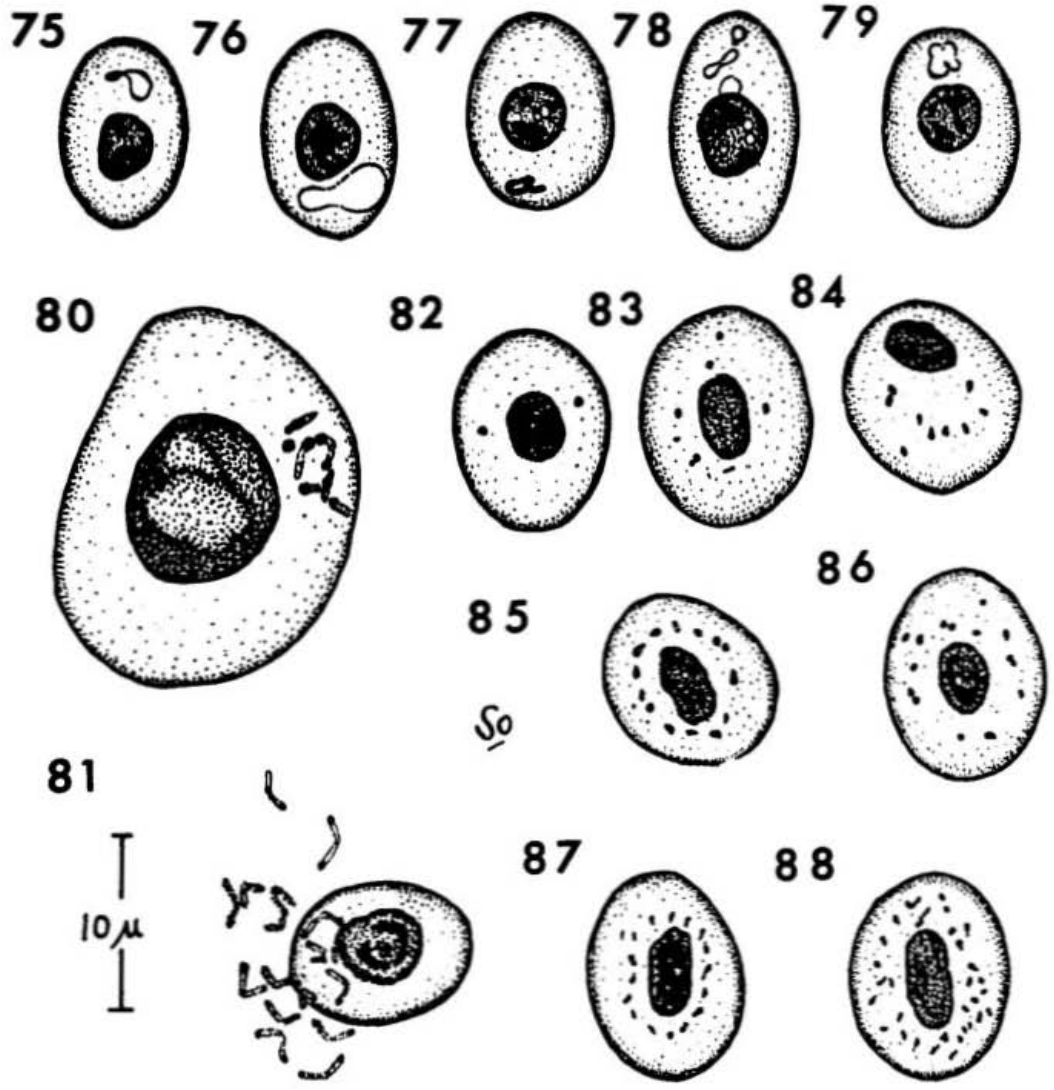
Figs. 75-79. *Haemohormidium beckeri* n.sp., from *Myoxocephalus octodecemspinosus*.

Fig. 80. Bacterial contaminants, from *Squalus acanthias*.

Fig. 81. Bacterial contaminants, from *Limanda ferruginea*.

Figs. 82-88. Intraerythrocytic inclusions of unknown aetiology, from *Clupea harengus*.


75
76
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So
86
81
10μ
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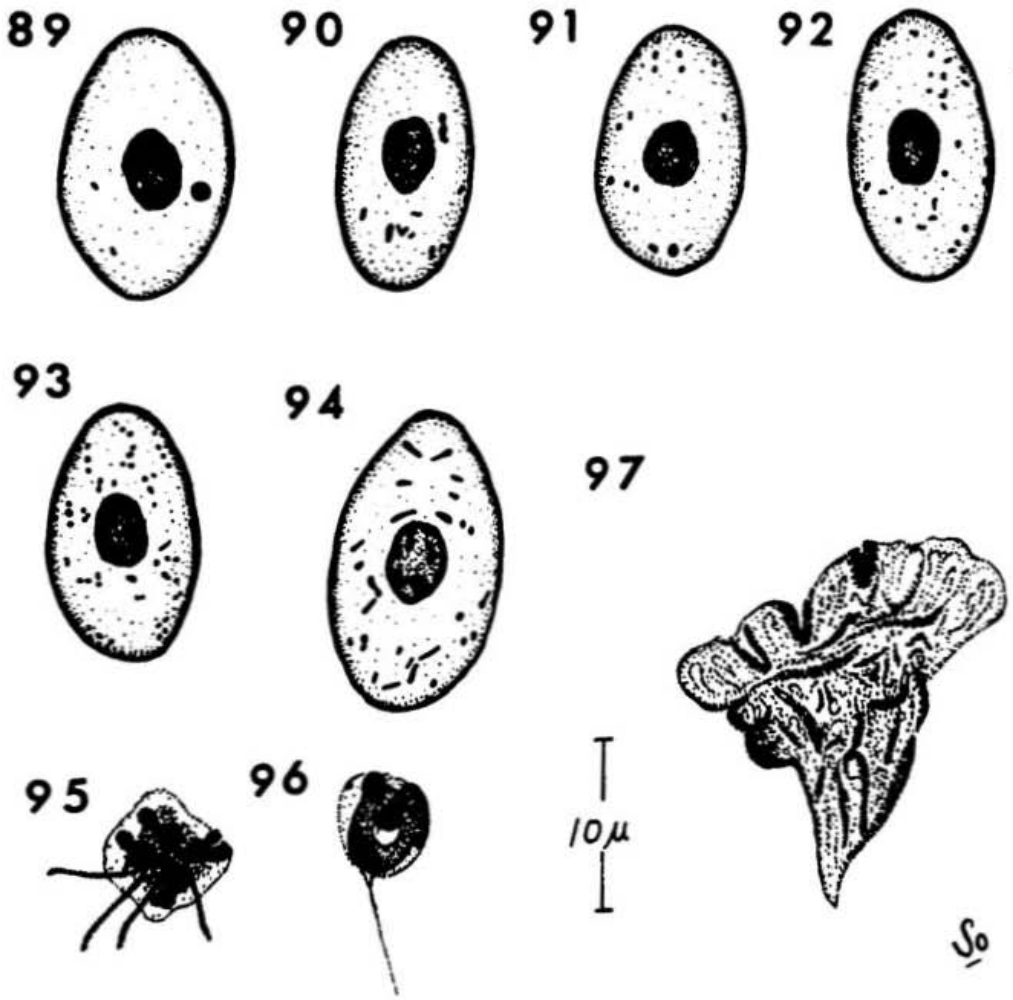
88

Figs. 89-94. Intraerythrocytic inclusions of unknown aetiology, from *Argentina silus*.

Figs. 95-96. *Kudoa* sp., as a contaminant in blood of *Gadus morhua*.

Fig. 97. Vegetative stage of unidentified myxosporidan present as contaminant in blood of *Raja radiata*.


89
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10μ
So

Fig. 98. *Trypanosoma rajae*, from *Raja radiata*.

Fig. 99. *Trypanosoma* sp., from *Glyptocephalus cynoglossus*.

Fig. 100. *Trypanosoma* sp., from *Gadus morhua*.

Fig. 101. *Haemogregarina delagei*, from *R. radiata*.

Fig. 102. *H. delagei*, from *R. radiata*, showing free vermicule.

Fig. 103. *H. delagei*, from *Raja senta*, showing double invasion of host erythrocyte.

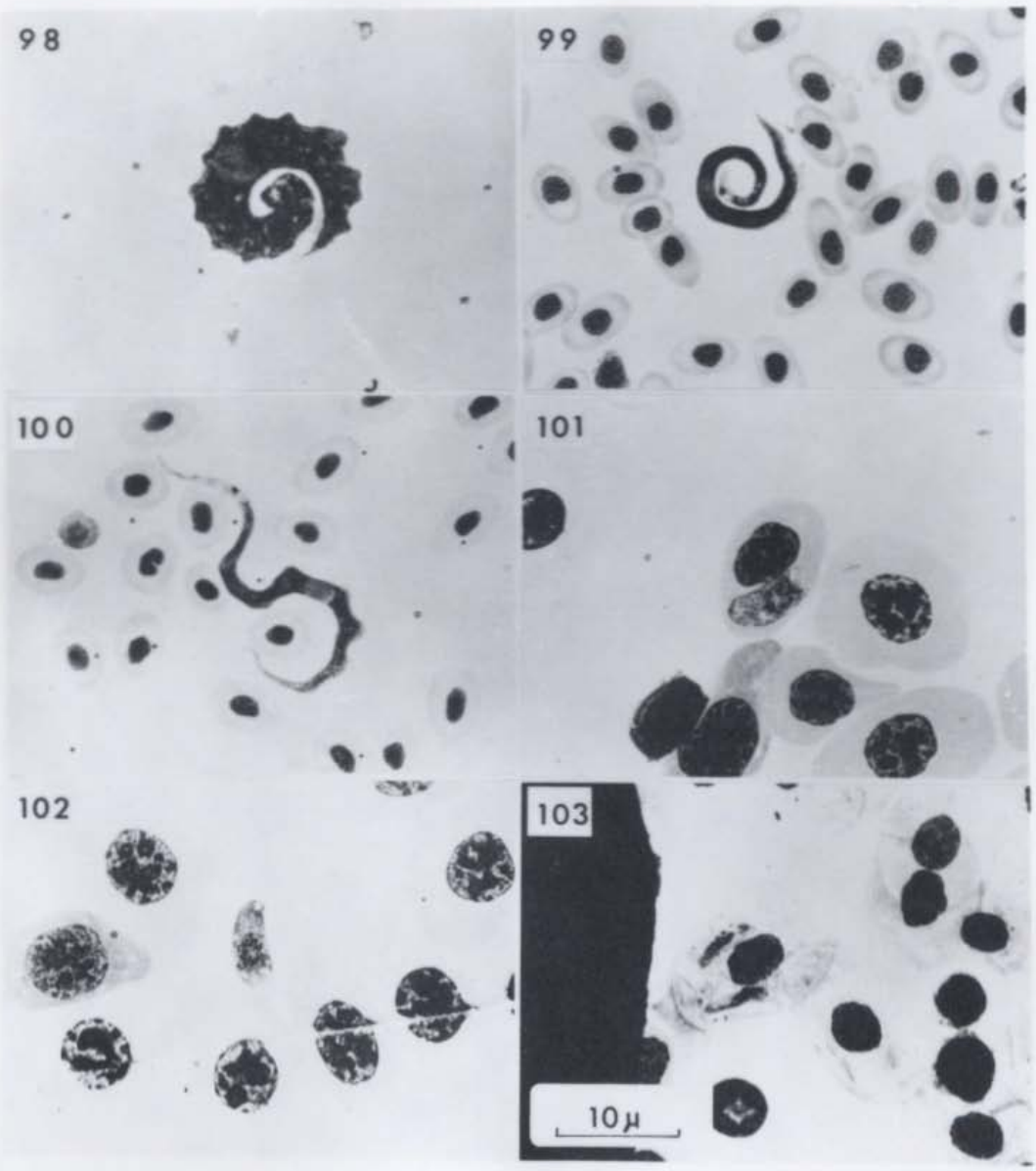
98
99
100
101
102
103
10µ

Fig. 104. Haemogregarina myoxocephali, from Myoxocephalus octodecemspinosus.

Fig. 105. Haemogregarina platessae, from Glyptocephalus cynoglossus.

Fig. 106. Free sporozoite from gut smear of piscicolid leech, Malmiana nuda.

Fig. 107. Haemohormidium terraenovae n.sp., from Ammodytes americanus.

Fig. 108. Haemohormidium terraenovae n.sp., from Urophycis tenuis.

Fig. 109. Haemohormidium terraenovae n.sp., from Limanda ferruginea.

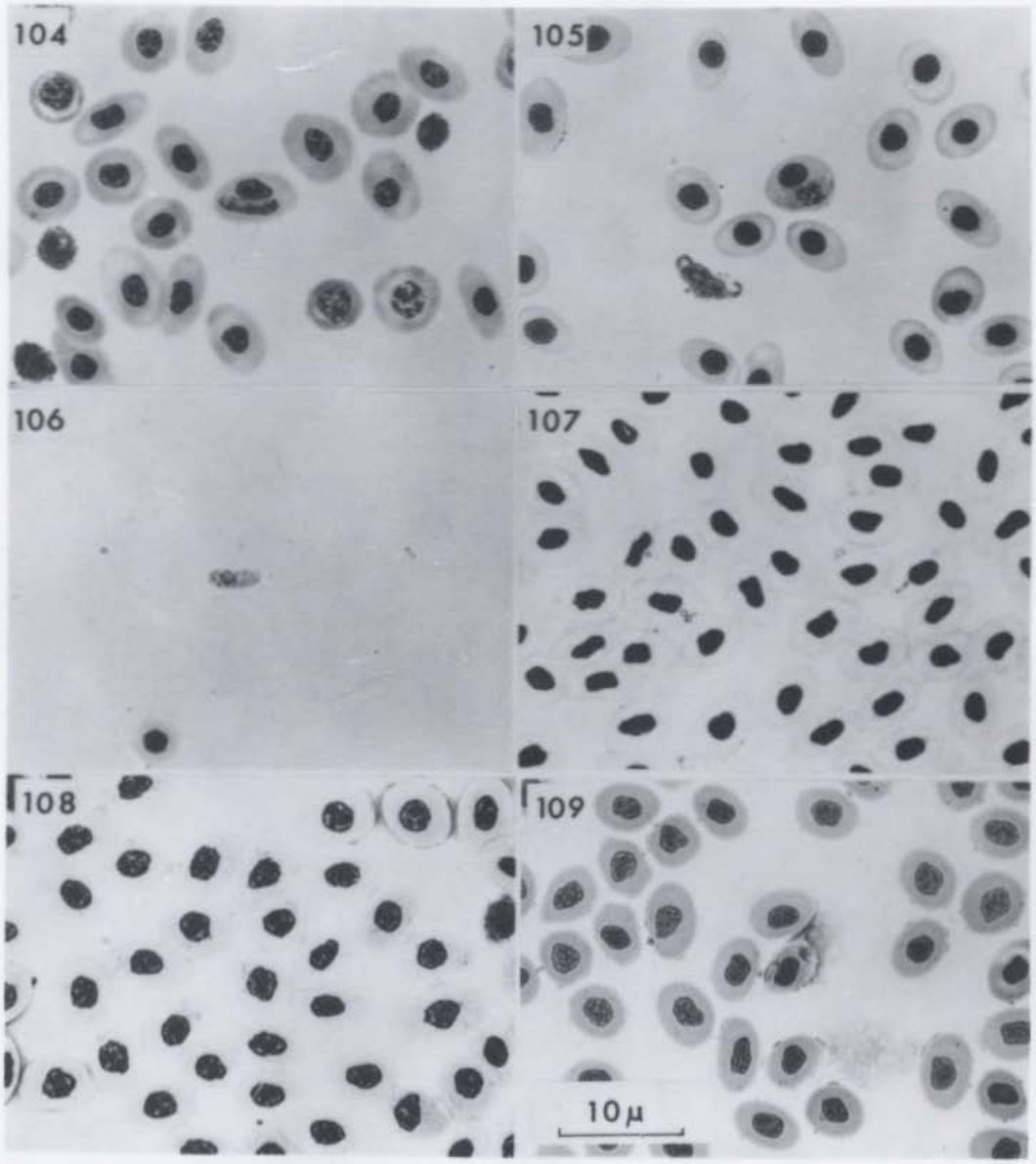
104
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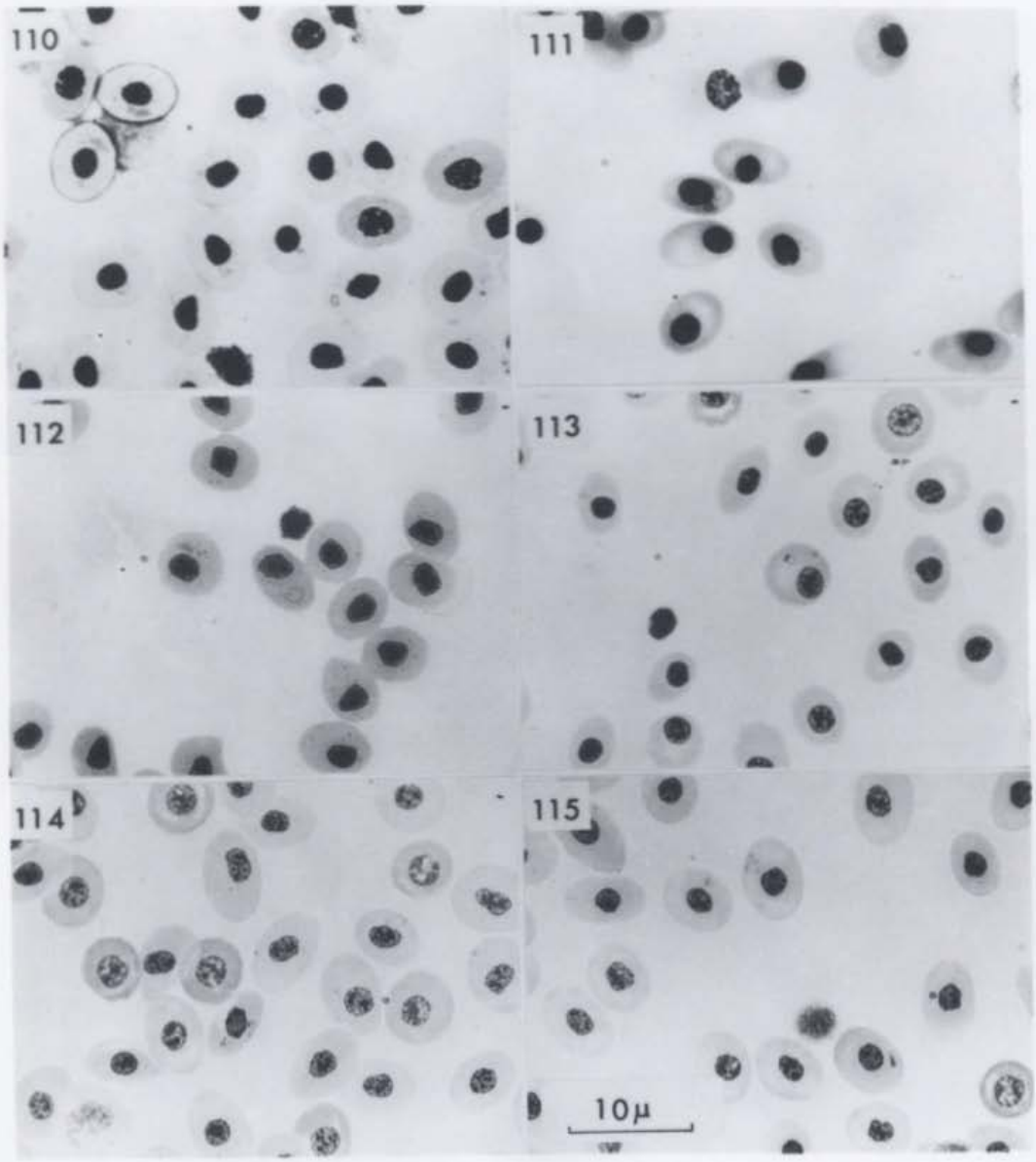
Fig. 110. Haemohormidium terraenovae n.sp., from Melanogrammus aeglefinus.

Fig. 111. Haemohormidium terraenovae n.sp., from Glyptocephalus cynoglossus.

Figs. 112-113. Haemohormidium terraenovae n.sp., from Hippoglossoides platessoides.

Figs. 114-115. Haemohormidium beckeri n.sp., from Myoxocephalus octodecemspinosus.


110
111
112
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10μ

Fig. 116. Haemohormidium beckeri n.sp., from Myoxocephalus octodecemspinosus, showing rosette schizont.

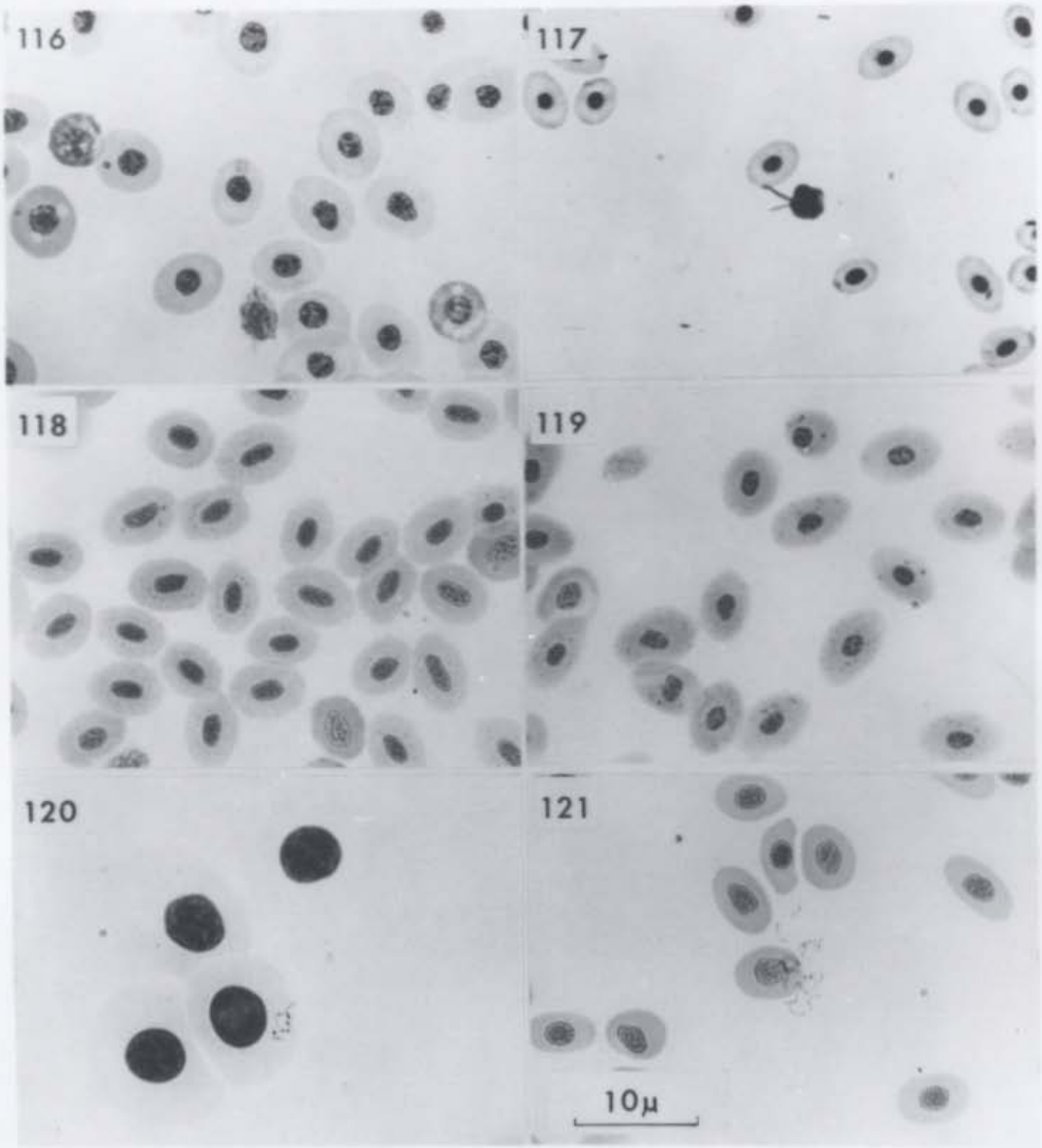
Fig. 117. Kudoa sp., from Gadus morhua.

Fig. 118. Intraerythrocytic inclusions of unknown aetiology, form Clupea harengus.

Fig. 119. Intraerythrocytic inclusions of unknown aetiology, from Argentina silus.

Fig. 120. Bacterial contaminants, from Squalus acanthias.

Fig. 121. Bacterial contaminants, from Limanda ferruginea.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