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Haemogregarina bigemina (Protozoa: Apicomplexa: Adeleorina) – past, present and future

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Abstract. This paper reviews past, current and likely future research on the fish haemogregarine, *Haemogregarina bigemina* Laveran et Mesnil, 1901. Recorded from 96 species of fishes, across 70 genera and 34 families, this broad distribution for *H. bigemina* is questioned. In its type hosts and other fishes, the parasite undergoes intraerythrocytic binary fission, finally forming mature paired gamonts. An intraleukocytic phase is also reported, but not from the type hosts. This paper asks whether stages from the white cell series are truly *H. bigemina*. A future aim should be to compare the molecular constitution of so-called *H. bigemina* from a number of locations to determine whether all represent the same species. The transmission of *H. bigemina* between fishes is also considered. Past studies show that young fish acquire the haemogregarine when close to metamorphosis, but vertical and faecal-oral transmission seem unlikely. Some fish haemogregarines are leech-transmitted, but where fish populations with *H. bigemina* have been studied, these annelids are largely absent. However, haematophagous larval gnathiid isopods occur on such fishes and may be readily eaten by them. Sequential squashes of gnathiids from fishes with *H. bigemina* have demonstrated development of the haemogregarine in these isopods. Examination of histological sections through gnathiids is now underway to determine the precise development sites of the haemogregarine, particularly whether merozoites finally invade the salivary glands. To assist in this procedure and to clarify the internal anatomy of gnathiids, 3D visualisation of stacked, serial histological sections is being undertaken. Biological transmission experiments should follow these processes.

Haemogregarines are apicomplexan protozoa, broadly distributed among vertebrate hosts, including fishes (Davies and Johnston 2000). They are especially common in marine fishes, where they are recognised in circulating erythrocytes, but also in cells of the leukocytic series (Davies 1995). Siddall (1995), in a partial taxonomic revision of the haemogregarine complex, placed fish haemogregarines in the genera *Cyrilia* Lainson, 1981 (one species), *Desseria* Siddall, 1995 (41 species) and *Haemogregarina* (sensu lato) Danilewsky, 1885 (13 species). *Haemogregarina* (sensu stricto) was reserved for species infecting chelonians. This classification was later adopted by Barta (2000) in his general account of the apicomplexan suborder Adeleorina Léger, 1911, although at least some *Desseria* spp. are now known to belong to other genera (see Negm-Eldin 1999, Davies and Johnston 2000, Smit et al. 2003a).

Cyrilia spp. and *Haemogregarina* spp. (sensu lato) are characterised by intraerythrocytic merogony in the fish host, whereas *Desseria* spp. lack this process (see Barta 2000, Davies and Smit 2001). *Cyrilia* spp. and *Desseria* spp. also undergo sporogony in leeches (see Davies and Smit 2001). In *Cyrilia* spp., sporogonic development produces numerous sporozoites and two life cycles are reported (Lainson 1981, Negm-Eldin 1999). In *Desseria* spp., sporogony yields more than 16 sporozoites, this is followed by primary merogony in the same leech hosts, and one life cycle is described in detail (see Siddall 1995). Evidence for invertebrate stages among *Haemogregarina* spp. (sensu lato) is, according to Siddall (1995), limited to just one fish haemogregarine, *Haemogregarina* (s.l.) *uncinata* (Khan, 1978) Siddall, 1995, which undergoes development in leeches, although this species was considered a member of the genus *Cyrilia* by Lainson (1981).

The marine fish haemogregarine *Haemogregarina* (s.l.) *bigemina* Laveran et Mesnil, 1901 appeared first in Siddall's list of *Haemogregarina* (sensu lato) and its development in an invertebrate host was not reported (Siddall 1995). However, it is a remarkable haemogregarine because of its apparent cosmopolitan distribution among marine fishes. It also appears to be the only apicomplexan of its type transmitted by arthropods rather than leeches (Davies and Smit 2001). This paper reviews past and current knowledge of *H. bigemina*, highlights some anomalies concerning the organism, and suggests what is still to be determined.

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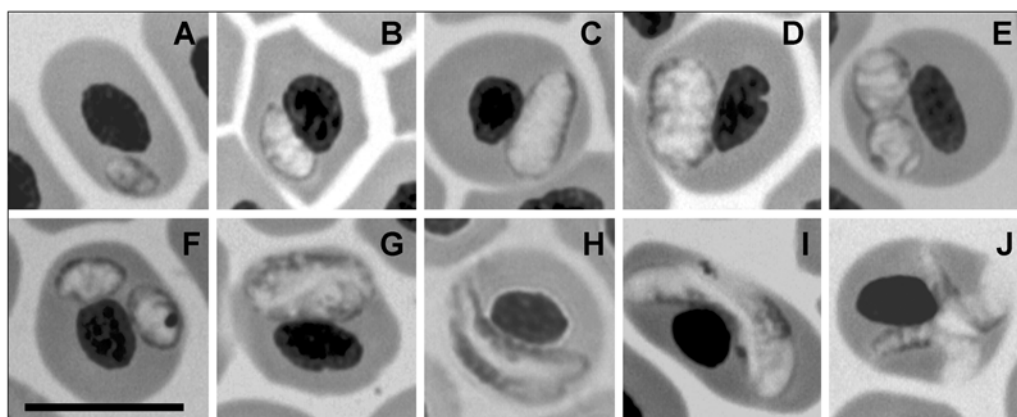
Fig. 1. Giemsa-stained erythrocytes from *Lipophrys pholis* with stages of *Haemogregarina bigemina*. **A** – trophozoite; **B, C** – developing meronts; **D–F** – meronts undergoing transverse binary fission; **G–I** – longitudinal binary fission of meronts; **J** – mature paired gamonts. Scale bar = 10 μm.

PAST AND PRESENT

Distribution and development of *Haemogregarina bigemina* in fishes

Haemogregarina bigemina was first described from intertidal blenniid fishes *Lipophrys pholis* (Linnaeus, 1758) and *Coryphoblennius galerita* (Linnaeus, 1758) in northern France (Laveran and Mesnil 1901). In Giemsa-stained blood films from the type hosts, the smallest stages detectable are intraerythrocytic trophozoites. These increase in size and mature into meronts that undergo transverse or longitudinal pregamontic binary fission and produce, finally, the paired intraerythrocytic gamonts, characteristic of the species (Fig. 1 A–J). In *L. pholis*, transverse and longitudinal binary fissions of *H. bigemina* can occur in erythrocytes of the same blood smear, but the factors that govern these events and their significance are not clear.

Subsequent to its observation in northern France, *H. bigemina* has been recorded from a large number of fishes, worldwide (see the lists of Becker 1970, Levine 1988, Siddall 1995, and the updated list in Table 1). These reports include descriptions of *H. bigemina* from the white as well as the red blood cell series of fishes, and at face value they equate to the haemogregarine having been reported from a remarkable 96 species of host fishes, in 70 genera and 34 families (Table 1). However, if these records are scrutinized, they are very curious. In the type hosts (*L. pholis* and *C. galerita*), only intraerythrocytic development has been reported (Laveran and Mesnil 1901, Henry 1913, Davies and Johnston 1976, Davies 1982, Sarasquete and Eiras 1985, Eiras 1987, Eiras and Davies 1991, Davies et al. 1994) (see Table 1). Such intraerythrocytic development has also been observed in another member of the Blenniidae (*Parablennius cornutus*) in South Africa (Smit et al. 2003a) and in other members of this family elsewhere (Table 1). Two additional families of fishes (Clinidae, Gobiesocidae) from South Africa (Table 1) also exhibit

H. bigemina that develops as in the type hosts (Smit and Davies 1999, Davies and Smit 2001).

Intraleukocytic development of *H. bigemina* was first reported by Laird (1953) from New Zealand fishes of the Clinidae and Tripterygidae (Table 1). This process was illustrated from *Ericentrus rubrus* and involved a series of merogony and binary fission culminating in the production of six to eight merozoites in small and large lymphocytes, and monocytes. Intraerythroblastic and intraerythrocytic development (illustrated from several fishes), that apparently followed the intraleukocytic phase, was like that described for *H. bigemina* by Laveran and Mesnil (1901) in the type hosts. Subsequent to his observations in New Zealand, Laird reported (Table 1) intraleukocytic development of *H. bigemina* from fishes in the South Pacific and New England (Laird 1958, Laird and Bullock 1969).

By far the majority of sightings of *H. bigemina* are those of Saunders (1955, 1958a, 1958b, 1959, 1960, 1964, 1966) (Table 1). While largely supporting Laird's observations, many of Saunders' reports are of concern because they do not record which stages were present, or they identify *H. bigemina* from intraleukocytic forms, or from immature stages in the red cell series. The paired mature gamonts, characteristic of the species, appear largely absent. For example, a report from the Florida Keys does not record or illustrate the stages present in this material (Saunders 1958a). The account of *H. bigemina* from Bermuda notes development only in large leukocytes (Saunders 1959). Two further reports from Florida (Saunders 1955, 1964), and those from the Bahamas (Saunders 1958b) and Puerto Rico (Saunders 1966), record development in cells of the intraleukocytic series and immature stages of the haemogregarine, or "early gametocytes", in erythrocytes. Even among Red Sea samples (Saunders 1960) the report stated, "more early stages of the parasite were found than gametocytes".
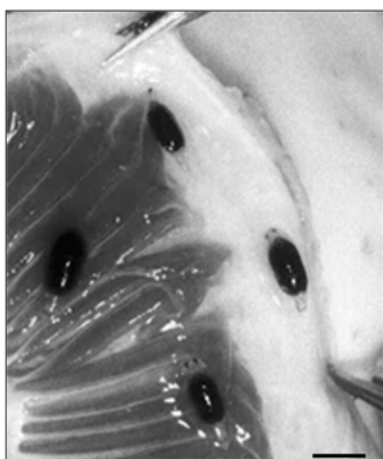
Fig. 2. Replete larvae of *Gnathia pantherina* Smit et Basson, 2002 attached to the gill septum and filaments of *Haploblepharus edwardsii* (Voight, 1832). Scale bar = 4 mm.

While it is not impossible that Saunders found *H. bigemina* in a wide range of hosts, it is clear that caution is needed in interpreting her data. In our opinion intra-leukocytic and intraerythrocytic stages that are clearly immature should not be positively identified as *H. bigemina*. We have observed *H. bigemina* infections in seven species and six genera of fishes (*Blennioclinus brachycephalus, Chorisochismus dentex, Coryphoblennius galerita, Clinus cottoides, Clinus superciliosus, Lipophrys pholis, Parablennius cornutus*), across three families (Blenniidae, Clinidae, Gobiesocidae), from the UK, Portugal and South Africa (Table 1). We have also seen *H. bigemina*-like infections in four species of the Acanthuridae from Australia (unpublished data, not recorded in Table 1). Our reports in Table 1 are based on finding mature paired gamonts, morphometrically identical to those seen in the type hosts, in erythrocytes. Where stages similar to Laird's (1958) intraleukocytic stages have been observed in the absence of mature paired gamonts in erythrocytes, as for example in the intertidal wrasse *Symphodus* (*Crenilabrus*) *melops* from Wales (see Davies 1982), the identification of *H. bigemina* has not been confirmed.

Transmission

The apparent broad distribution of *Haemogregarina bigemina* raises questions concerning its transmission and it is interesting to note in Table 1 that most of its vertebrate hosts appear to be intertidal or reef-associated teleosts.

It has been known for some time that the infection can be detected in very young intertidal fish measuring under 4 cm in length (Laird 1953, Davies and Johnston 1976, Eiras 1987). In a study of almost 500 *Lipophrys pholis* (one of the type hosts) collected at Aberystwyth, Wales, UK the first patent infections of *H. bigemina* were seen in 26% of group 0 fish measuring 3.5 cm TL (total length) undergoing metamorphosis. Prevalence

was 46% of group 0 fish of 4.0 cm TL and in 94% of group 0 fish of 4.5 cm TL (Davies and Johnston 1976). Yearlings (fish of about 5 cm TL) and *L. pholis* from group 1 and upwards were all infected with *H. bigemina* (Davies and Johnston 1976). These same patterns of prevalence at Aberystwyth have now been observed many times at this site over a period spanning almost 30 years (Davies, personal observation). Similar prevalences for *H. bigemina* have also been detected among *L. pholis* from Foz do Douro, Portugal, except that the smallest infected fish was 3.2 cm long, prevalence was 87% in fishes of 5–5.9 cm in length and 100% prevalence was seen in fishes 7.0 cm long and upwards (Eiras 1987). Prevalence was, however, 100% in *L. pholis* of 6.0 cm and above at other sites in Portugal (Eiras 1987).

The question arises how transmission to such small fish is effected. Vertical transmission from adult to young fishes seems unlikely. When 234 hatchling *L. pholis* caught at Aberystwyth were kept in isolation for a period of two months, 42 that survived to reach 3.5 cm TL or more, did not reveal *H. bigemina* (see Davies and Johnston 1976). Faecal-oral transmission was also considered unlikely at this site (see Davies and Johnston 1976).

Two candidate haematophagous vectors for *H. bigemina* have been observed on *L. pholis* in the UK (see Davies and Johnston 1976). These are the leech *Oceanobdella blennii* (Knight-Jones, 1940) and the isopod *Gnathia maxillaris* (Montagu, 1804). However, *O. blennii* has never been observed at the study site in Aberystwyth where *H. bigemina* occurs commonly. Furthermore, *O. blennii* is not known to attach to fish as small as 3.5 cm TL, and does not feed at a time of year when young *L. pholis* are undergoing metamorphosis (see Davies and Johnston 1976). A general dearth of leeches has also been reported at other sites where *H. bigemina* has been investigated (Eiras 1987, Eiras and Davies 1991, Davies et al. 1994, Davies and Smit 2001). On the other hand, although *Gnathia* spp. adults are free-living, their blood-sucking larvae seem almost ideal vectors for *H. bigemina*.

Gnathia spp. larvae are isopods that behave much like underwater ticks, feeding from the general body surfaces, buccal cavity and the gills of fishes (Fig. 2). They become swollen with fish blood when feeding, normally drop off when replete and digest the blood meal, moult and then re-attach to fishes, feeding three times in this manner (see Smit et al. 2003b). Gnathiid larvae are therefore capable of drawing blood with *H. bigemina* from infected fishes and on at least three occasions during their development. Many of these isopods are widely distributed in marine environments, especially intertidally and on reefs, are apparently non-seasonal, and are readily eaten by many young and adult fishes (Monod 1926, Davies and Johnston 1976, Cohen and Poore 1994, Grutter and Poulin 1998, Arnal and Cote 2000, Sikkel et al. 2000, Davies and Smit 2001).
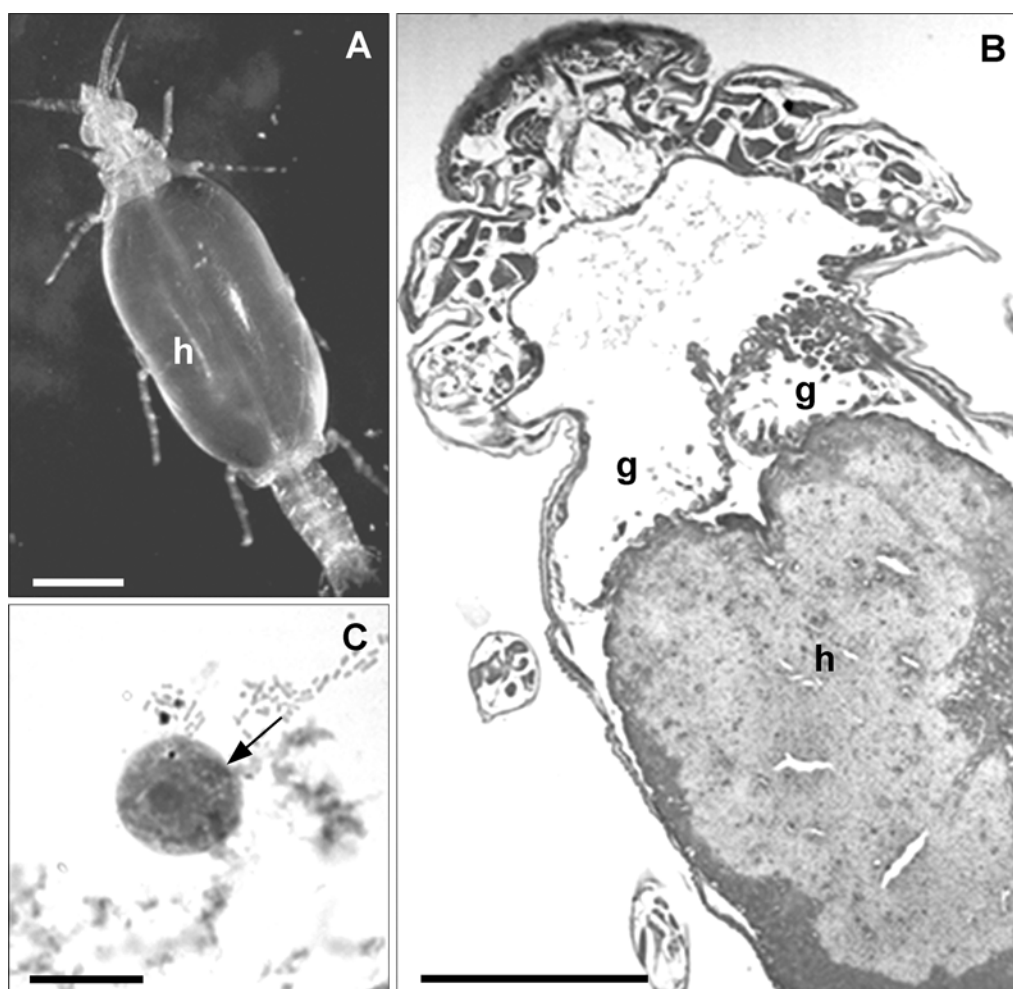
Fig. 3. A – Replete larva of *Gnathia* sp. Note the region of the inflated anterior hindgut (h). **B** – Longitudinal histological section through the posterior cephalosome and anterior pereon of larval gnathiid (*Gnathia africana*), stained with Masson's trichrome. Note the paired digestive glands (g) and the blood-filled anterior hindgut (h). **C** – Oocyst (arrow) from section through the digestive glands of *G. africana* shown in B. Scale bars: A = 1 mm; B = 0.2 mm; C = 10 μm.

Evidence that these isopods actually sustain the development of *H. bigemina* is supported by work on gnathiids and *L. pholis* in Wales and Portugal (Davies and Johnston 1976, Davies 1982, 1995, Davies et al. 1994). However, final proof has emerged from studying *H. bigemina*, *Gnathia africana* Barnard, 1914 and the intertidal fish *Clinus superciliosus* in South Africa (Davies and Smit 2001).

Stages observed by sequentially squashing *G. africana* that had fed on infected *Cl. superciliosus* revealed gamonts of *H. bigemina* like those seen in the fish host, normally from 1–6 days post feeding (d.p.f.). Syzygy also occurred up to 6 d.p.f., immature oocysts from 7 d.p.f. and mature oocysts (sporonts) were observed from 14 d.p.f. Sporogony yielding at least five sporozoites occurred around 11 d.p.f. and postsporogonic merogony forming three generations of corresponding merozoites also began close to 11 d.p.f., with second-generation merozoites appearing at 18 d.p.f. This developmental sequence in *G. africana*, with that observed in the fish host, formed the basis of a proposed life cycle for *H. bigemina* (see Davies and Smit 2001). However, questions about the cycle remain. The precise sites of haemogregarine development within gnathiids require identification, particularly whether merozoites settle in the salivary glands, and the mode of transmission from gnathiid to fish needs to be established.

Current research

Attempts to locate the development sites of *H. bigemina* in gnathiids now involve sequential histological studies from conventional wax-embedded material rather than serial squashes. Initial results indicate that gamonts, as they are released from the fish erythrocytes during digestion, accumulate in the inflated anterior hindgut and some young oocysts, similar to those seen
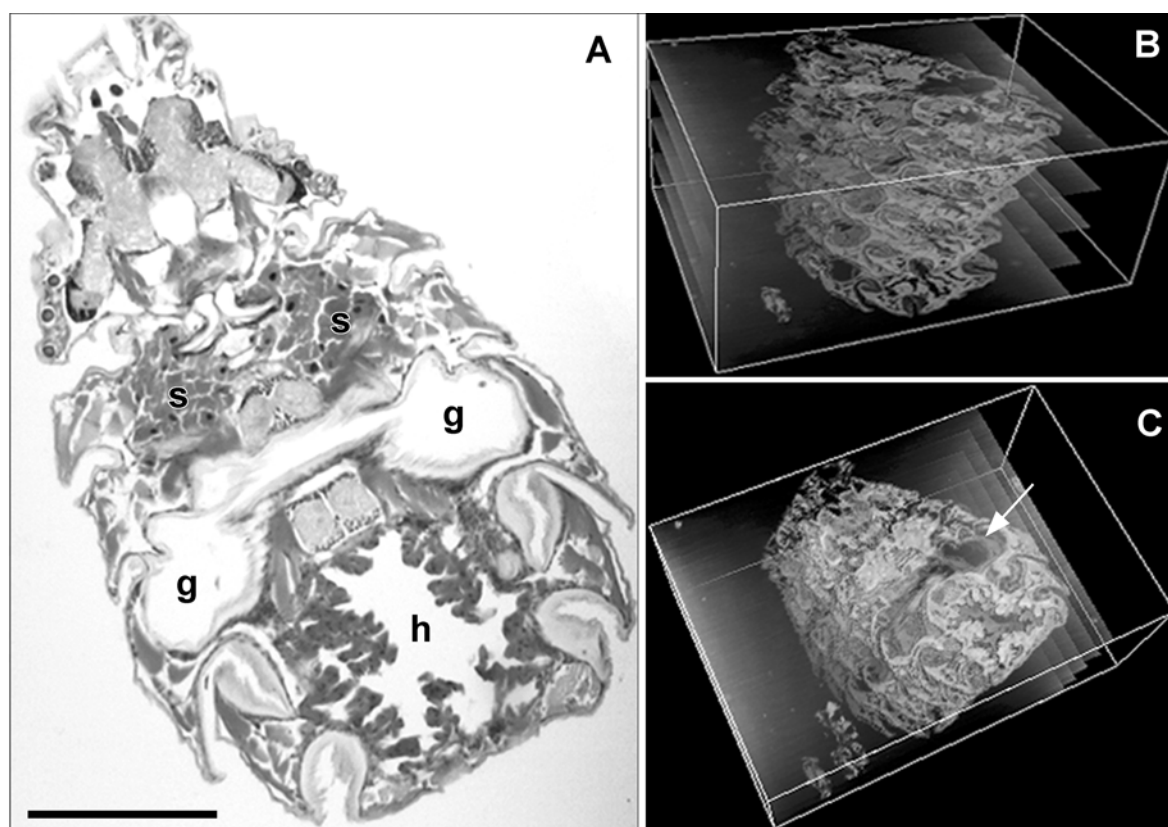
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Fig. 4. Serial histological sections through the cephalosome and anterior pereon of larva of *Gnathia maxillaris*, later in digestive cycle than *G. africana* larva shown in Fig. 3B. **A** – Uppermost histological section stained with haematoxylin and eosin. Note salivary glands (s), paired digestive glands (g) and anterior portion of anterior hindgut (h). **B** – Simple stack of five serial histological sections (uppermost section, as in A) captured and visualised in IRIS Explorer. **C** – Rotation of stack imparts depth to salivary and particularly digestive glands (arrow), beginning the 3D effect. Scale bar: A = 0.2 mm; B and C are not to scale.

by Davies and Johnston (1976) and Davies and Smit (2001), appear in the paired digestive glands that lie anterior to the hindgut (Fig. 3). However, if the digestive processes in gnathiids are similar to those of typical isopods, then the occurrence of oocysts in the digestive glands is problematic. In a typical isopod, the contents of the anterior hindgut (essentially a storage organ) pass forward to the digestive glands as digestion proceeds. However, only liquids or fine material can pass the primary and secondary filtering system of the stomach to reach the digestive glands (see Wägele 1992). If this type of digestion also occurs in parasitic gnathiids, then the developing stages of *H. bigemina* would be unlikely to survive passage through such a filtering mechanism. Clearly, careful study of the internal anatomy and digestive processes within larval gnathiids is required to discover how oocysts form in the paired digestive glands.

While the internal anatomy of one gnathiid, *Paragnathia formica* (Hesse, 1864) is reasonably well understood (see Monod 1926, Charmantier 1982), that of *Gnathia* spp. is not well known and neither are their digestive cycles. Serial and sequential histological sec-

tions, used to locate *H. bigemina*, are therefore being investigated by 3D visualisation through a system developed using IRIS Explorer (NAG Ltd., Oxford, UK). A series of contiguous images is stacked and a 'render' module allows adjustment of the angle of viewing and zoom. A simple stack of five serial sections is shown in Fig. 4 A, B and on rotation, this gives some depth to the paired digestive glands (Fig. 4 C). Stacks of 20 or more serial histological sections are also currently under investigation. These may well permit the entire internal anatomy of the gnathiid to be represented in this manner, allowing the sites of development of the haemogregarine to be clearly defined. It is anticipated that this technique will also help determine the mode of passage of *H. bigemina* from gnathiid to fish. Although young fishes are known to eat gnathiids, suggesting that they acquire *H. bigemina* by predation on these isopods, transmission to fish by bite from gnathiids cannot yet be ruled out. It is therefore important to examine the salivary glands of these isopods in histological sections and 3D representations (Fig. 4) for merozoites of *H. bigemina*.

Table 1. Reports of *Haemogregarina bigemina* with current, valid names of fish hosts (according to Froese and Pauly 2000), location in which each host was caught and associated habitat. Also noted are infections (Inf.) in host erythrocytic series (E), leukocytic series (L), or both (E/L), and the author(s) who first reported the haemogregarine at each location.

Host fish	Location	Habitat	Inf.	Author(s)
A c a n t h u r i d a e				
Ctenochaetus strigosus (Bennett, 1828)	Red Sea	Reef	E/L	Saunders 1960
B a l i s t i d a e				
Balistes capriscus Gmelin, 1789	Florida Keys	Reef		Saunders 1958a
Balistes vetula Linnaeus, 1758	Bahamas	Reef	E/L	Saunders 1958b
B e l o n i d a e				
Strongylura notata notata (Poey, 1860)	Bahamas; Southwest Florida	Reef	E/L	Saunders 1958b, 1964
B l e n n i i d a e				
Coryphoblennius galerita (Linnaeus, 1758)	France; Portugal	Intertidal	E	Laveran and Mesnil 1901[1], Davies et al. 1994
Ecsenius bicolor (Day, 1888)	Heron Island, Australia	Reef	E	Burreson 1989
Lipophrys pholis (Linnaeus, 1758)	France; UK; Portugal	Intertidal	E	Laveran and Mesnil 1901, Henry 1910, Eiras 1984
Parablennius cornutus (Linnaeus, 1758)	South Africa	Intertidal	E	Fantham 1930[2]
Parablennius gattorugine (Linnaeus, 1758)	UK	Intertidal	E	Henry 1913
Blenniella periophthalmus (Valenciennes, 1836)	Fiji	Reef	E	Laird 1951[3]
C a r a n g i d a e				
Carangoides bartholomaei (Cuvier, 1833)	Puerto Rico	Reef	E	Saunders 1966
Carangoides ruber (Bloch, 1793)	Bermuda	Reef	L	Saunders 1959
Caranx crysos (Mitchell, 1815)	Florida Keys; Bahamas	Reef	E/L	Saunders 1958a, b
Caranx hippos (Linnaeus, 1766)	Florida Keys; Puerto Rico	Reef	E	Saunders 1958a, 1966
Seriola dumerili (Risso, 1810)	Florida Keys	Reef		Saunders 1958a
Trachinotus falcatus (Linnaeus, 1758)	Bermuda	Reef	L	Saunders 1959
C l i n i d a e				
Blennioclinus brachycephalus (Valenciennes, 1836)	South Africa	Intertidal	E	Davies et al.[4]
Clinus cottoides Valenciennes, 1836	South Africa	Intertidal	E	Smit and Davies 1999
Clinus superciliosus (Linnaeus, 1758)	South Africa	Intertidal	E	Smit and Davies 1999
Ericentrus rubrus (Hutton, 1872)	North Island, New Zealand	Intertidal	E/L	Laird 1953
Heteroclinus perspicillatus (Valenciennes, 1836)	Norfolk Island, South Pacific	Reef	E/L	Laird 1958
C o r y p h a e n i d a e				
Coryphaena hippurus Linnaeus, 1758	Florida Keys; Bermuda	Pelagic	L	Saunders 1958a, 1959
C o t t i d a e				
Artedius fenestralis Jordan et Gilbert, 1883	Vancouver Island, Canada	Intertidal	E	Laird 1961
G e r r e i d a e				
Eucinostomus gula (Quoy et Gaimard, 1824)	Bahamas	Reef	E/L	Saunders 1958b
Gerres cinereus (Walbaum, 1792)	Southwest Florida	Reef	E/L	Saunders 1964
G o b i e s o c i d a e				
Chorisochismus dentex (Pallas, 1769)	South Africa	Intertidal	E	Davies and Smit 2001
Trachelochismus melobesia Phillips, 1927	North Island, New Zealand	Intertidal	E	Laird 1953
G o b i i d a e				
Amblygobius albimaculatus (Rüppell, 1830)	Red Sea	Reef	E/L	Saunders 1960
Bathygobius soporator (Valenciennes, 1837)	Bahamas	Intertidal	E/L	Saunders 1958b
H a e m u l i d a e				
Haemulon album Cuvier, 1830	Florida Keys; Bahamas	Reef	E/L	Saunders 1958a, b
Haemulon aurolineatum Cuvier, 1830	Puerto Rico	Reef	E	Saunders 1966
Haemulon flavolineatum (Desmarest, 1823)	Florida Keys	Reef		Saunders 1958a
Haemulon plumierii (Lacépède, 1801)	Florida Keys	Reef		Saunders 1958a
Haemulon sciurus (Shaw, 1803)	Florida Keys; Bahamas	Reef	E/L	Saunders 1958a, b

Table 1. Continued.

Host fish	Location	Habitat	Inf.	Author(s)
H e m i r a m p h i d a e				
Hemiramphus brasiliensis (Linnaeus, 1758)	Puerto Rico	Reef	E	Saunders 1966
Hyporhamphus unifasciatus (Ranzani, 1842)	Bermuda	Reef	L	Saunders 1959
I s t i o p h o r i d a e				
Istiophorus albicans (Latreille, 1804)	Florida Keys	Pelagic		Saunders 1958a
K y p h o s i d a e				
Kyphosus bigibbus Lacépède, 1801	Red Sea	Reef	E/L	Saunders 1960
L a b r i d a e				
Cheilinus trilobatus Lacépède, 1801	Red Sea	Reef	E/L	Saunders 1960
Halichoeres bivittatus (Bloch, 1791)	Bermuda	Reef	L	Saunders 1959
Lachnolaimus maximus (Walbaum, 1792)	Florida Keys	Reef		Saunders 1958a
Pteragogus pelycus Randall, 1981	Red Sea	Reef	E/L	Saunders 1960
Thalassoma bifasciatum (Bloch, 1791)	Bahamas	Reef	E/L	Saunders 1958b
Thalassoma purpureum (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
L e t h r i n i d a e				
Lethrinus mahsena (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Lethrinus nebulosus (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Lethrinus variegatus Valenciennes, 1830	Red Sea	Reef	E/L	Saunders 1960
L u t j a n i d a e				
Lutjanus apodus (Walbaum, 1792)	Bahamas; Puerto Rico	Reef	E/L	Saunders 1958b, 1966
Lutjanus bohar (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Lutjanus griseus (Linnaeus, 1758)	Florida Keys	Reef		Saunders 1958a
Lutjanus synagris (Linnaeus, 1758)	Florida Keys; Bahamas	Reef	E/L	Saunders 1958a, b
Ocyurus chrysurus (Bloch, 1791)	Florida Keys; Bahamas; Puerto Rico	Reef	E/L	Saunders 1958a, b, 1966
M a l a c a n t h i d a e				
Malacanthus plumieri (Bloch, 1786)	Florida Keys; Bahamas	Reef	E/L	Saunders 1958a, b
M u g i l i d a e				
Mugil trichodon Poey, 1875	Bahamas	Marine, brackish and freshwater	E/L	Saunders 1958b
M u l l i d a e				
Parupeneus forsskali (Fourmanoir et Guézé, 1976)	Red Sea	Reef	E/L	Saunders 1960
Upeneus tragula Richardson, 1846	Red Sea	Reef	E/L	Saunders 1960
M u r a e n i d a e				
Gymnothorax funebris Ranzani, 1840	Florida Keys	Reef		Saunders 1958a
P i n g u i p e d i d a e				
Parapercis hexophtalma (Cuvier, 1829)	Red Sea	Reef	E/L	Saunders 1960
P o m a c a n t h i d a e				
Pomacanthus maculosus (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
P o m a c e n t r i d a e				
Abudefduf saxatilis (Linnaeus, 1758)	Bahamas	Reef	E/L	Saunders 1958b
S c a r i d a e				
Chlorurus sordidus (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Scarus iseri (Bloch, 1789)	Puerto Rico	Reef	E	Saunders 1966
Sparisoma aurofrenatum (Valenciennes, 1840)	Puerto Rico	Reef	E	Saunders 1966
S c i a e n i d a e				
Bairdiella chrysoura (Lacépède, 1802)	Southwest Florida	Marine, brackish and freshwater	E/L	Saunders 1964
Menticirrhus littoralis (Holbrook, 1855)	Atlantic Coast Florida; Southwest Florida	Marine and brackish	E/L	Saunders 1955, 1964
S c o m b r i d a e				
Auxis thazard thazard (Lacépède, 1800)	Puerto Rico	Pelagic	E	Saunders 1966
Scomberomorus regalis (Bloch, 1793)	Florida Keys	Reef		Saunders 1958a
Scomberomorus cavalla (Cuvier, 1829)	Florida Keys	Reef		Saunders 1958a

Table 1. Continued.

Host fish	Location	Habitat	Inf.	Author(s)
S e r r a n i d a e				
Centropristis striata (Linnaeus, 1758)	Eastern Canada; New England	Reef	E/L	Fantham et al. 1942, Laird and Bullock 1969
Cephalopholis hemistiktos (Rüppell, 1830)	Red Sea	Reef	E/L	Saunders 1960
Cephalopholis miniata (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Epinephelus adscensionis (Osbeck, 1765)	Florida Keys	Reef		Saunders 1958a
Epinephelus fasciatus (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Epinephelus fuscoguttatus (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Epinephelus guttatus (Linnaeus, 1758)	Florida Keys; Bermuda	Reef	L	Saunders 1958a, 1959
Epinephelus morio (Valenciennes, 1828)	Florida Keys	Reef		Saunders 1958a
Epinephelus striatus (Bloch, 1792)	Florida Keys; Bermuda	Reef	L	Saunders 1958a, 1959
Epinephelus summana (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Epinephelus tauvina (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Mycteroperca bonaci (Poey, 1860)	Florida Keys; Bermuda	Reef	L	Saunders 1958a, 1959
Mycteroperca microlepis (Goode et Bean, 1879)	Florida Keys	Reef		Saunders 1958a
Plectropomus maculatus (Bloch, 1790)	Red Sea	Reef	E/L	Saunders 1960
Variola louti (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
S p a r i d a e				
Acanthopagrus bifasciatus (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Argyrops spinifer (Forsskål, 1775)	Red Sea	Range of marine habitats	E/L	Saunders 1960
Calamus bajonado (Bloch et Schneider, 1801)	Florida Keys; Bahamas	Reef	E/L	Saunders 1958a, b
Rhabdosargus haffara (Forsskål, 1775)	Red Sea	Reef	E/L	Saunders 1960
Lagodon rhomboides (Linnaeus, 1766)	Southwest Florida	Marine, brackish and freshwater	E/L	Saunders 1964
S p h y r a e n i d a e				
Sphyraena barracuda (Walbaum, 1792)	Florida Keys; Bahamas; Bermuda	Reef	E/L	Saunders 1958a, b, 1959
S y n o d o n t i d a e				
Synodus japonicus[5]	Red Sea		E/L	Saunders 1960
T r i p t e r y g i i d a e				
Bellapiscis medius (Günther, 1861)	North Island, New Zealand	Intertidal	E	Laird 1953
Enneapterygius rufopileus (Waite, 1904)	Norfolk Island, South Pacific	Reef	E/L	Laird 1958
Forsterygion varium (Forster, 1801)	North Island, New Zealand	Intertidal	E	Laird 1953
Notoclinus fenestratus (Forster, 1801)	North Island, New Zealand	Intertidal	E	Laird 1953
Z o a r c i d a e				
Zoarces americanus (Bloch et Schneider, 1801)	Atlantic Coast, Canada	Intertidal		Fantham et al. 1942 [6]
Zoarces viviparus (Linnaeus, 1758)	UK	Intertidal		Bentham 1917[7]

[1]Laveran and Mesnil (1901) reported *H. bigemina* from *Blennius pholis* (valid name *Lipophrys pholis*) and *Blennius gattorugine*. However, Laveran and Mesnil (1902) amended their original *B. gattorugine* to *Blennius montagui* (valid name *Coryphoblennius galerita*);

[2]Described by Fantham (1930) as *Haemogregarina fragilis*, this was formally recognised as *H. bigemina* by Smit et al. (2003);

[3]Described by Laird (1951) as *Haemogregarina salariasi*, this was formally recognised as *H. bigemina* by Siddall (1995);

[4]Paired mature gamonts of *H. bigemina* in the erythrocytes of *B. brachycephalus* were observed by AJD, NJS and PMH at De Hoop Nature Reserve, South Africa in 2003, a new host record for the haemogregarine;

[5]Appears to be a *nomen nudum*;

[6]A haemogregarine, probably *Haemogregarina bigemina*, according to Fantham et al. (1942);

[7]Reported in Laird (1953), but we were unable to trace the reference.

FUTURE RESEARCH

Future developments in research on *Haemogregarina bigemina* will obviously centre on continued attempts to reconstruct the internal anatomy of larval gnathiids from histological sections and to locate the development sites of the haemogregarine. However, does this haemogregarine really develop in as many fish hosts as Table 1 suggests? Biological transmission to clean fishes under laboratory conditions should be, therefore, another logical aim for the future. The apparent broad distribution of *H. bigemina* among fishes, the ease with which gnathiids can be persuaded to feed on these hosts (see Smit et al. 2003b) and the fact that many fishes eat gnathiids, may all aid attempts at transmission. Another important question is, does *H. bigemina* truly have an intraleukocytic phase or are mixed infections involved? Transmission studies may solve this problem, but another future aim should be to compare the molecular constitution of so-called *H. bigemina* from a number of locations to determine whether all samples represent the same species.

Haemogregarina bigemina is clearly an extraordinary and intriguing apicomplexan, seemingly unlike other fish haemogregarines in its distribution and transmission. Its known development most closely resembles that of *Haemogregarina* (sensu stricto), but the match is not perfect (see Davies and Smit 2001). When answers to some of the questions posed in this paper have been resolved, it may be that *H. bigemina*, instead of its current placing with *Haemogregarina* (sensu lato), will be deserving of a genus in its own right.

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