

HEMATOZOA OF TELEOSTS FROM LIZARD ISLAND, AUSTRALIA, WITH SOME COMMENTS ON THEIR POSSIBLE MODE OF TRANSMISSION AND THE DESCRIPTION OF A NEW HEMOGREGARINE SPECIES

Nico J. Smit, Alexandra S. Grutter*, Robert D. Adlard,† and Angela J. Davies‡

Department of Zoology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa. e-mail: njs@rau.ac.za

ABSTRACT: Little is known of the blood parasites of coral reef fishes and nothing of how they are transmitted. We examined 497 fishes from 22 families, 47 genera, and 78 species captured at Lizard Island, Australia, between May 1997 and April 2003 for hematozoa and ectoparasites. We also investigated whether gnathiid isopods might serve as potential vectors of fish hemogregarines. Fifty-eight of 124 fishes caught in March 2002 had larval gnathiid isopods, up to 80 per host fish, and these were identified experimentally to be of 2 types, *Gnathia* sp. A and *Gnathia* sp. B. Caligid copepods were also recorded but no leeches. Hematozoa, found in 68 teleosts, were broadly hemogregarines of 4 types and an infection resembling *Haemohormidium*. Mixed infections (hemogregarine with *Haemohormidium*) were also observed, but no trypanosomes were detected in blood films. The hemogregarines were identified as *Haemogregarina balistapi* n. sp., *Haemogregarina tetraodontis*, possibly *Haemogregarina bigemina*, and an intraleukocytic hemogregarine of uncertain status. Laboratory-reared *Gnathia* sp. A larvae, fed experimentally on brushtail tangs, the latter heavily infected with the *H. bigemina*-like hemogregarine, contained hemogregarine gamonts and possibly young oocysts up to 3 days postfeeding, but no firm evidence that gnathiids transmit hemogregarines at Lizard Island was obtained.

Although hemogregarines (Apicomplexa) are common parasitic protozoans of the blood of many vertebrates (Davies and Johnston, 2000), little is known of their occurrence in coral reef fishes (see Saunders, 1960; Burrenson, 1989). Hemogregarines may have 2 or more hosts in their life cycles, but traditionally, they have a vertebrate intermediate host, e.g., reptile, amphibian, fish, and an invertebrate definitive host vector, e.g., arthropod, leech (see Davies and Johnston, 2000). However, the vectors of hemogregarines of coral reef fishes, and indeed of many other fishes, are unknown.

Hemogregarines likely play an important role ecologically and physiologically as they invade the white and red blood cells (RBCs) of vertebrates, an aspect of their biology that is rarely considered, and some can affect their hosts negatively in this respect. They can modify RBC counts, the size and general morphology of RBCs, and their oxygen carrying capacity (Combes, 1995; Davies, 1995; Davies and Johnston, 2000). Infected lizards, for example, have reduced hemoglobin levels, oxygen consumption, and running speed (Oppliger and Clobert, 1997) and increased RBC counts (Oppliger et al., 1996). Although some hemogregarine infections in fishes are light and chronic, others appear acute and possibly pathogenic, such as *Haemogregarina bigemina* Laveran and Mesnil, 1901, infections in young *Ericentrus rubrus*, where 85% of the lymphocytes and monocytes can be involved (Laird, 1953). Hemogregarines have also been associated with a leukemia-like condition in turbot, and kidney and spleen disease in Atlantic mackerel (Davies, 1995). However, little is known about the pathogenicity of most hemogregarines of fish.

In fishes, it has been often assumed that leeches are the main vectors of hemogregarines (Davies, 1995). Davies (1982, 1995) and Davies and Johnston (1976), however, proposed that gna-

thiids, rather than leeches, are vectors of *H. bigemina*, and this was supported by subsequent observations (Davies et al., 1994; Smit and Davies, 1999; Davies and Johnston, 2000). Gnathiid isopods are one of the most common parasitic arthropods of coral reef fishes (Grutter and Poulin, 1998). The 3 larval stages are parasitic and engorge on fish blood before returning to the benthos to digest their meal and molt to the next stage (Smit, Basson, and Van As, 2003; Smit and Davies, 2004). Recently, Smit and Davies (2001) found that hemogregarines completed both sexual and subsequent asexual reproduction in gnathiids. This indicates strongly that gnathiids may be the vectors of at least some of these blood-borne parasites. Transmission experiments, however, have yet to be achieved, so it is not known whether gnathiids transmit these hemogregarines between fishes in the wild. In addition, most of the work on this topic has been done in temperate areas. Gnathiids in the tropics, e.g., Great Barrier Reef, Australia, however, are likely more dynamic and abundant than gnathiids in temperate waters and, thus, are ideal for laboratory studies (Grutter, 2003).

Almost nothing is known of the blood parasites of coral reef fish on the Great Barrier Reef. To our knowledge, 5 reports exist of marine fish hematozoa from Australia (Laveran, 1908; Mackerras and Mackerras, 1925, 1961; Laird, 1958; Burrenson, 1989). The blood infections recorded from marine teleosts include 4 species of hemogregarines (*Haemogregarina bigemina*, *Haemogregarina gilbertae* Mackerras and Mackerras, 1925, *Haemogregarina parmae* Mackerras and Mackerras, 1925, and *Haemogregarina tetraodontis* Mackerras and Mackerras, 1961), 2 forms of *Haemohormidium* (*Haemohormidium aulopi* [Mackerras and Mackerras, 1925] Laird and Bullock, 1969, and *Haemohormidium* sp. Burrenson, 1989), and 3 species of flagellates (*Trypanosoma pulchra* Mackerras and Mackerras, 1925, *Trypanosoma aulopi* Mackerras and Mackerras, 1925, and *Trypanoplasma parmae* Mackerras and Mackerras, 1925). These infections were located in blood samples from fishes of eastern Australia and its offshore islands, namely Norfolk Island (Laird, 1958), Sydney Harbour, Broken Bay, and Mornington Island (Mackerras and Mackerras, 1961) and Heron Island on the Great Barrier Reef (Burrenson, 1989).

To determine which coral reef fishes are infected with blood

Received 7 October 2005; revised 25 January 2006; accepted 25 January 2006.

* School of Integrative Biology, University of Queensland, Brisbane, Queensland, 4072, Australia.

† Protozoa Section, Queensland Museum, P.O. Box 3300, South Brisbane, Queensland, 4101, Australia.

‡ School of Life Sciences, Faculty of Science, Kingston University, Kingston upon Thames, KT1 2EE, United Kingdom.

parasites, the prevalence and identity of hematozoans among these vertebrates, and the potential vectors of hemogregarines among such fishes, we surveyed the hematozoan and ectoparasite populations of a wide range of reef fishes off Lizard Island, Great Barrier Reef. We also investigated the potential pathogenicity of hemogregarines to fish by examining the number and morphology of infected blood cells. Furthermore, in an effort to discover the possible route of transmission of fish hemogregarines at this location, we conducted laboratory experiments to determine whether these protozoans could be detected in gnathiids that had fed on hemogregarine-positive fish and, if so, whether they developed further in the isopod. Finally, to establish how long fish retain hemogregarines in the absence of potential vectors (leeches and gnathiids), we held infected fish in captivity for periods up to 1 yr.

MATERIALS AND METHODS

Fishes

Four-hundred ninety-seven teleost fishes (Table I) were collected from reefs off Lizard Island, Great Barrier Reef, during May 1997; March, May, and October 2002; and March and April 2003. Fishes in May 1997 were collected on hook and line or by spear, then taken to the laboratory. The remaining fishes were caught by coaxing them into a barrier net, then trapping them with a hand net and immediately transferring individuals to sealed plastic bags. The latter method ensured that few ectoparasites attached to fishes escaped detection (see Grutter, 1995).

In the laboratory, each fish was removed from its transport bag, placed in a bucket (10 L) of aerated seawater and left for approximately 24 hr. This is sufficient time for gnathiids to feed and drop off the host, a duration of usually 1 hr (Grutter, 2003). Individuals were then examined as described below. Subsequently, some brushtail tangs *Zebbrasoma scopas* (Cuvier, 1829), captured in March and May 2002, were held collectively in 1,000-L tubs in the laboratory (see diagram in Grutter, 2001) and were examined twice yearly (October 2002, March 2003) to determine whether blood infections persisted in the absence of possible vectors.

Ectoparasites

Ectoparasites that might act as vectors for fish hematozoans were studied, particularly during March 2002 (Table I), but also in October 2002, and March and April 2003. Around 24 hr after capture, fishes in buckets were anesthetized with clove oil (Munday and Wilson, 1997), identified from Randall et al. (1990), measured (total length [TL]), and scanned under a dissection microscope for ectoparasites on their gills, external surfaces, and buccal cavity. Ectoparasites were also detected by rinsing fishes with fresh seawater for 2 to 5 min and by filtering (62 μ m-mesh sieve) the bucket water in which fishes had spent 24 hr. In addition, ectoparasites were filtered from the bag water in which the fishes had been transported from the reefs to the laboratory.

Initially, ectoparasites (gnathiids) captured by these means were kept singly or collectively in clear jars (50 ml) of seawater. Subsequently, some gnathiids were used to establish laboratory cultures and then used in feeding experiments, as described below and elsewhere (see Grutter, 2001).

Hematozoans

For the majority of fishes, blood was taken from the caudal vein of anesthetized specimens by needle and syringe (see Grutter and Pankhurst, 2000), smeared on clean, glass slides (at least 3 smears per fish), fixed in absolute methanol, and Giemsa-stained (see Smit and Davies, 1999). Finally, blood smears were examined with a Zeiss Axioskop 20 photomicroscope (Carl Zeiss, Oberkochen, Germany) and images were captured and measured by Nikon (Tokyo, Japan) DN100 digital camera and Nikon Eclipse Net image analysis system (see Davies and Smit, 2001; Smit and Davies, 2001). After a sufficient recovery period, most fishes were released to the wild. Fish held in captivity long-term were

held singly or in pairs in 1,000-L tubs with running seawater and containing 3 plastic pipes (15 cm diameter and 30 cm long) for shelter, and fed daily with fish flakes and frozen *Artemia* sp. Fishes from the May 2002 collection, however, were captured as part of another study on the digenean parasites of coral reef fishes, and so, blood smears were made from heart blood. These smears were processed and screened as above.

Experiments with gnathiid larvae

Gnathiid larvae that had fed on hemogregarine-positive fish were crushed and smeared from 1 day postfeeding (dpf) to 20 dpf, using previously established protocols (see Smit and Davies, 1999; Davies and Smit, 2001). In March 2002, gnathiid larvae for taxonomy were taken from hemogregarine-negative fish and maintained in 50-ml jars following the methods of Smit and Basson (2002) and Smit et al. (2002). Because the taxonomy of gnathiids relies on the morphology of adult males (see Smit and Davies, 2004), stage 3 larvae were maintained until they molted into adult males or females. Two different types of gnathiids were identified and, for the purposes of this study, these adults were designated *Gnathia* sp. A (Fig. 1A) and *Gnathia* sp. B (Fig. 1B).

To test whether it was possible to establish hemogregarine infections in hemogregarine-free gnathiid larvae, experiments were conducted using laboratory-reared larvae. Two laboratory-reared, female gnathiids of *Gnathia* sp. A, obtained from third-stage (praniza 3) larvae that had fed on apparently hemogregarine-free fishes, were mated with a male, laboratory-reared *Gnathia* sp. A. Females released their young 15 days later, and 10 larvae from each female were placed in turn in a bucket (10 L) with a single *Z. scopas* known to be heavily infected with hemogregarines (25/1,000 infected erythrocytes). Larvae were allowed to feed for 5 hr and then retrieved from the bucket using a plastic pipette. Ten and 8 larvae were recovered from each of the 2 trials. The 18 fed-larval gnathiids were subsequently crushed, smeared, stained, and examined over a period up to 8 dpf. In October 2002, 40 larval gnathiids (unknown species) taken from 24 wild-caught fishes (Table I) were crushed and examined within 1 dpf. In March 2003, a further 53 larval gnathiids that had been laboratory-reared (*Gnathia* sp. A) were fed on wild-caught *Z. scopas*. These gnathiids were examined over a longer period (up to 20 dpf), using the same protocols.

RESULTS

Fishes

We sampled 497 fishes from 22 families, 47 genera, and 78 species during May 1997; March, May, and October 2002; and March and April 2003 (Table I). March and April 2003 catches are combined in Table I because they relate to the end of 1 mo and the beginning of the next and are, therefore, hardly separated in time compared with the other samples.

Ectoparasites

In March 2002, the month in which ectoparasite prevalence was studied most closely, 17 of 29 fish species collected harbored larval gnathiid isopods, involving a total of 58 infected fishes of 124 captured (46.8% overall prevalence). Infestation levels of gnathiid larvae on fishes ranged from 1 to 80 per host (see Table I). During this same period, it was observed that fishes of the Acanthuridae were parasitized by *Gnathia* sp. A, and those of the Balistidae and Caesionidae by *Gnathia* sp. B. The Labridae were infested by larvae of both *Gnathia* sp. A and *Gnathia* sp. B. Both of these new species of *Gnathia* will be described elsewhere.

Two species of fishes were parasitized by caligid copepods in March 2002. One of 2 black-spotted pufferfish, *Arothron nigropunctatus* (Bloch and Schneider, 1801) (TL = 210 mm), bore 5 caligids, and 2 of 28 *Z. scopas* (TL = 135 mm and 154 mm) had 2 caligids each. No leeches were found on any of the fishes collected.

TABLE I. Families and species of teleost fish collected from Lizard Island, Australia, and examined for hematozoans and gnathiid isopod larvae (infected/sample size).

Families and species of teleosts collected from Lizard Island	No.	Hematozoa detected					Gnathiids mean burden (range) March 2002	Gnathiid load per catch March 2002
		May 1997	March 2002	May 2002	October 2002	March and April 2003		
Acanthuridae								
1 <i>Acanthurus nigrofuscus</i> (Forsskål, 1775)	11		2/10		1/1		0.4 ± 0.52 (0–1)	4/10
2 <i>Ctenochaetus binotatus</i> Randall, 1955	1		0/1				0	0/1
3 <i>Ctenochaetus striatus</i> (Quoy & Gaimard, 1825)	7		1/7				3.4 ± 5.74 (0–16)	4/7
4 <i>Naso annulatus</i> (Quoy & Gaimard, 1825)	1		0/1				0	0/1
5 <i>Zebrasoma scopas</i> (Cuvier, 1829)	59	3/5	22/28		7/7	18/19	1.1 ± 1.12 (0–3)	18/28
6 <i>Zebrasoma veliferum</i> (Bloch, 1797)	4	0/1	1/3				10.0 ± 9.54 (4–21)	3/3
Apogonidae								
7 <i>Apogon cyanosoma</i> Bleeker, 1853	8			0/8				
8 <i>Apogon fragilis</i> Smith, 1961	4			0/4				
Balastidae								
9 <i>Balistapus undulatus</i> (Park, 1797)	3	1/1		0/2				
10 <i>Sufflamen chrysopterum</i> (Bloch & Schneider, 1801)	28	0/23	0/2		3/3		2 & 2	2/2
Caesionidae								
11 <i>Caesio cuning</i> (Bloch, 1791)	7	0/3	0/2	0/2			1 & 0	1/2
Carangidae								
12 <i>Carangoides fulvoguttatus</i> (Forsskål, 1775)	1			0/1				
Chaetodontidae								
13 <i>Chaetodon auriga</i> Forsskål, 1775	2			0/2				
14 <i>Chaetodon trifasciatus</i> Park, 1797	12	0/12						
Gobiidae								
15 <i>Amblygobius phalaena</i> (Valenciennes, 1837)	3		0/3				0.3 ± 0.58 (0–1)	1/3
16 <i>Signigobius biocellatus</i> Hoese & Allen, 1977	2		0/2				0	0/2
17 <i>Valenciennea puellaris</i> (Tomiyama, 1956)	1		0/1				0	0/1
Haemulidae								
18 <i>Diagramma labiosum</i> Macleay, 1883	1	0/1						
19 <i>Diagramma pictum</i> (Thunberg, 1792)	3			0/3				
20 <i>Plectorhinchus goldmanni</i> (Bleeker, 1853)	7		0/7				0	0/7
Labridae								
21 <i>Bodianus mesothorax</i> (Bloch & Schneider, 1801)	1			0/1				
22 <i>Cheilinus chlorourus</i> (Bloch, 1791)	5	0/3	0/2				14 & 17	2/2
23 <i>Cheilinus fasciatus</i> (Bloch, 1791)	8	0/4	0/3	0/1			13.3 ± 2.89 (10–15)	3/3
24 <i>Cheilinus trilobatus</i> (Lacépède, 1802)	4			0/4				
25 <i>Epibulus insidiator</i> (Pallas, 1770)	5		0/3	0/2			29.7 ± 43.59 (4–80)	3/3
26 <i>Halichoeres melanurus</i> (Bleeker, 1851)	1			0/1				
27 <i>Halichoeres ornatissimus</i> (Garrett, 1863)	2		0/2				1 & 0	1/2
28 <i>Hemigymnus melapterus</i> (Bloch, 1791)	53	0/41	0/12				6.0 ± 9.03 (0–33)	8/12
29 <i>Labroides bicolor</i> Fowler & Bean, 1928	1			0/1				
30 <i>Labroides dimidiatus</i> (Valenciennes, 1839)	33		0/10	0/3			0	0/10
31 <i>Oxycheilinus digrammus</i> (Lacépède, 1801)	4		0/4			0/20	2.5 ± 2.08 (0–5)	3/4
32 <i>Stethojulis bandanensis</i> (Bleeker, 1851)	1		0/1				0	0/1
33 <i>Thalassoma janseni</i> (Bleeker, 1856)	1		0/1				1	1/1
34 <i>Thalassoma lunare</i> (Linnaeus, 1758)	27	0/24	0/3				0.7 ± 0.58 (0–1)	2/3

SMIT ET AL.—HEMATOZOA OF AUSTRALIAN TELEOSTS

TABLE I. Continued.

Families and species of teleosts collected from Lizard Island	No.	Hematozoa detected					Gnathiids mean burden (range) March 2002	Gnathiid load per catch March 2002
		May 1997	March 2002	May 2002	October 2002	March and April 2003		
Lethrinidae								
35 <i>Lethrinus atkinsoni</i> Seale, 1909	2	0/2						
36 <i>Lethrinus lentjan</i> (Lacépède, 1802)	2			0/2				
37 <i>Lethrinus nebulosus</i> (Forsskål, 1775)	4			0/4				
38 <i>Lethrinus variegatus</i> (Valenciennes, 1830)	3	0/3						
Lutjanidae								
39 <i>Lutjanus bohar</i> (Forsskål, 1775)	1	0/1						
40 <i>Lutjanus carponotatus</i> (Richardson, 1842)	1			0/1				
41 <i>Lutjanus carponotatus</i> (Richardson, 1842)	25	0/25						
42 <i>Lutjanus fulviflamma</i> (Forsskål, 1775)	3	0/3						
43 <i>Lutjanus quinquelineatus</i> (Bloch, 1790)	1	0/1						
44 <i>Lutjanus russelli</i> (Bleeker, 1849)	1	0/1						
Mullidae								
45 <i>Parupeneus barberinus</i> (Lacépède, 1801)	1			0/1				
46 <i>Parupeneus ciliatus</i> (Lacépède, 1802)	1			0/1				
47 <i>Parupeneus cyclostomus</i> (Lacépède, 1801)	1			0/1				
48 <i>Parupeneus heptacanthus</i> (Lacépède, 1802)	2			0/2				
49 <i>Parupeneus multifasciatus</i> (Quoy & Gaimard, 1825)	2		0/1	0/1			1	1/1
50 <i>Parupeneus pleurostigma</i> (Bennett, 1831)	1			0/1				
Nemipreridae								
51 <i>Scolopsis bilineatus</i> (Bloch, 1793)	17	0/17						
52 <i>Scolopsis monogramma</i> (Cuvier, 1830)	5	0/3		0/2				
Ostraciidae								
53 <i>Ostracion cubicus</i> Linnaeus, 1758	3	0/3						
Pinguipedidae								
54 <i>Parapercis hexophthalma</i> (Cuvier, 1829)	2	0/2						
Pomacentridae								
55 <i>Abudefduf whitleyi</i> Allen & Robertson, 1974	2	0/2						
56 <i>Acanthochromis polyacanthus</i> (Bleeker, 1855)	20	0/20						
57 <i>Amblyglyphidodon curacao</i> (Bloch, 1787)	5	0/2		0/3				
58 <i>Chrysiptera rollandi</i> (Whitley, 1961)	2			0/2				
59 <i>Neoglyphidodon melas</i> (Cuvier, 1830)	1			0/1				
60 <i>Neopomacentrus azysron</i> (Bleeker, 1877)	5			0/5				
61 <i>Pomacentrus coelestis</i> Jordan & Starks, 1901	4			0/4				
Pseudochromidae								
62 <i>Pseudochromis fuscus</i> Muller & Troschel, 1849	7		0/7				0	0/7
Scaridae								
63 <i>Chlorurus sordidus</i> (Forsskål, 1775)	5			1/1	2/4			
64 <i>Scarus psittacus</i> Forsskål, 1775	6			1/1	3/4	1/1		
65 <i>Scarus rivulatus</i> Valenciennes, 1840	1		0/1				0	0/1

TABLE I. Continued.

Families and species of teleosts collected from Lizard Island	No.	Hematozoa detected					Gnathiids mean burden (range) March 2002	Gnathiid load per catch March 2002
		May 1997	March 2002	May 2002	October 2002	March and April 2003		
Serranidae								
66 <i>Cephalopholis boenak</i> (Bloch, 1790)	2			1/2				
67 <i>Cephalopholis cyanostigma</i> (Valenciennes, 1828)	11	0/6		0/1	0/4			
68 <i>Epinephelus fasciatus</i> (Forsskål, 1775)	1	0/1						
69 <i>Epinephelus merra</i> (Bloch, 1793)	2	0/1			0/1			
70 <i>Epinephelus quoyanus</i> (Valenciennes, 1830)	1	0/1						
71 <i>Plectropomus leopardus</i> (Lacépède, 1802)	3	0/1		0/2				
Siganidae								
72 <i>Siganus doliatus</i> Cuvier, 1830	3	0/1	0/1	0/1		0	0/1	
73 <i>Siganus lineatus</i> (Valenciennes, 1835)	18	0/18						
74 <i>Siganus puellus</i> (Schlegel, 1852)	1			0/1				
Synodontidae								
75 <i>Synodus variegatus</i> (Lacépède, 1803)	1			0/1				
Tetraodontidae								
76 <i>Arothron nigropunctatus</i> (Bloch & Schneider, 1801)	2	0/1	0/1			12	1/1	
77 <i>Canthigaster bennetti</i> (Bleeker, 1854)	3		0/3			0	0/3	
78 <i>Canthigaster valentini</i> (Bleeker, 1853)	2		0/2			0	0/2	
Totals (prevalence %)	497	4/233 (1.7)	26/124 (21)	3/76 (3.9)	16/24 (67)	19/40 (47.5)	58/124 (46.8)	

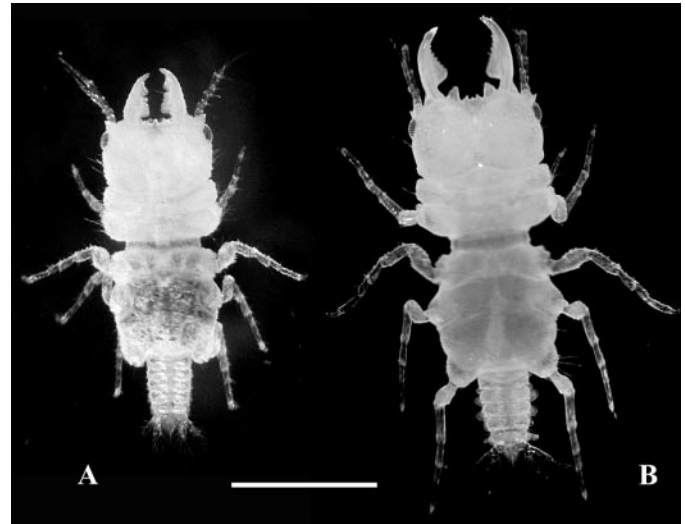


FIGURE 1. Light micrographs of the males of the 2 species of gnathiids found parasitizing Lizard Island fishes. (A) *Gnathia* sp. A. (B) *Gnathia* sp. B. Bar = 1 mm.

Hematozoans

Hemogregarines and a *Haemohormidium*-like infection were the only types of hematozoa detected (Table II), and these are described in detail below. Single species of hemogregarines or of *Haemohormidium*-like infections often occurred in several genera or species of fishes, and in other instances, mixed infections were found within the blood of a single fish (Table II). Trypanosomes, however, were not observed.

Four of the 233 fishes collected in 1997 were infected (Tables I, II). Among these 4 fishes, the erythrocytes of 3 of 5 *Z. scopes* (TL = 135 mm, 136 mm, and 177 mm) were parasitized with a *Haemogregarina bigemina*-like infection (see Burreson, 1989). A single orange-lined triggerfish, *Balistapus undulatus* (Park, 1797) (TL = 250 mm), harbored another species of he-

TABLE II. Families and species of teleosts with *Haemogregarina balistapi* (Hb), *Haemogregarina bigemina*-like (Hbl), *Haemogregarina tetraodontis* (Ht), intraleukocytic hemogregarine (Ih), and *Haemohormidium*-like (Hal) infections collected from Lizard Island, Australia.

	Hb	Hbl	Ht	Ih	Hal
Acathuridae					
<i>Acanthurus nigrofuscus</i>		3/11			
<i>Ctenochaetus striatus</i>		1/7			
<i>Zebrasoma scopes</i>		50/59			*1/59
<i>Zebrasoma veliferum</i>		1/4			
Balistidae					
<i>Balistapus undulates</i>	1/3				
<i>Sufflamen chrysopterum</i>	3/28				
Scaridae					
<i>Chlorurus sordidus</i>					3/5
<i>Scarus psittacus</i>			*1/6		5/6
Serranidae					
<i>Cephalopholis boenak</i>				1/2	

* Mixed infection.

mogregarine currently unknown to science (described below as *Haemogregarina balistapi* n. sp.).

During the March 2002 collection, 26 of 124 fishes examined had hemogregarines and the *Haemohormidium*-like infection. A *H. bigemina*-like infection similar to that found in *Z. scopas* in 1997 was detected in 22 of 28 *Z. scopas* (mean TL = 142 ± 13.7 mm; range 106–161 mm); 1 of 3 sailfin tangs, *Zebrosoma veliferum* (Bloch, 1797) (TL = 155 mm); 2 of 10 brown surgeonfish, *Acanthurus nigrofuscus* (Forsskål, 1775) (TL = 155 mm and 160 mm); and 1 of 7 lined bristletooth surgeonfish *Ctenochaetus striatus* (Quoy and Gaimard, 1825) (TL = 195 mm). In one of the *Z. scopas*, the *H. bigemina*-like infection was mixed with an unusual intraerythrocytic parasite resembling, in some respects, a species of *Haemohormidium* Henry, 1910 (see Burrenson, 1989) (see below and Table II). Interestingly, all of the fishes found with hematozoa in March 2002 belonged to the Acanthuridae (see Table I).

In the May 2002 material, 3 of 76 fishes were infected with hematozoans. The same *Haemohormidium*-like infection that had formed a mixed infection in *Z. scopas* in March 2002 occurred as a single infection in the blood of a bullethead parrotfish, *Chlorurus sordidus* (Forsskål, 1775) (TL = 91 mm), and a palenose parrotfish *Scarus psittacus* (Forsskål, 1775) (TL = 158 mm). In addition, in May 2002, an intraleukocytic hemogregarine was observed in 1 of 2 brown-barred rockcod, *Cephalopholis boenak* (Bloch, 1790) (TL = 232 mm) (Table II).

In the October 2002 collection, the *H. bigemina*-like and *Haemohormidium*-like infections that had been found previously were observed in some of the same hosts (*Acanthurus nigrofuscus*, *C. sordidus*, *S. psittacus*, and *Z. scopas*), but none of these formed mixed infections. However, on this occasion, 1 of the *Haemohormidium*-like infections found in *S. psittacus* appeared to be mixed with an infection strongly resembling *Haemogregarina tetraodontis* (see Burrenson, 1989) (Table II). Another hemogregarine found at the same time, but in the half-moon triggerfish, *Sufflamen chrysopterum* (Bloch and Schneider, 1801), was considered to be the same as the infection (*H. balistapi*) found in *B. undulatus* in the 1997 collection (Table II).

In the March and April 2003 collections, infected fishes were *Z. scopas* and a single *S. psittacus*. These contained the *H. bigemina*-like and *Haemohormidium*-like infections noted earlier, but no mixed infections (Tables I, II).

Examination of 6 *Z. scopas* in October 2002, held in the laboratory from March 2002, showed that they had retained their *H. bigemina*-like hemogregarine infections for 8 mo. Ten captive fish of the same species examined in March 2003 showed that the hemogregarine had persisted for 1 yr. However, all infection levels in these captive fish were low (<0.1 infected cells/10,000 erythrocytes).

Gnathiid larvae

A total of 18 of the 58 wild-caught gnathiid larvae in March 2002, all of them *Gnathia* sp. A, that had fed on fishes with a hemogregarine infection was smeared within 1 dpf. Two of these had fed on *A. nigrofuscus*, 4 on *C. striatus*, and 12 on *Z. scopas*. In smears of 3 of 12 larvae that had fed on *Z. scopas*, stages of a hemogregarine, similar to those found in the blood

of their fish host, were found. In October 2002, a further 40 unidentified gnathiids were processed using the same techniques 1 dpf. Over half of these gnathiids (21/40) were from fishes that appeared to lack hematozoans, and no hemogregarines were detected in them. Fifty-three gnathiid smears taken from *Z. scopas*, between 1 and 20 dpf during April 2003, were also negative, except for 2 smears containing stages like those seen in the blood of fishes. However, blood films from the host *Z. scopas* from which these gnathiids were taken showed that all fishes had very light infections (<0.01 infected cells/10,000 erythrocytes).

Experiments with gnathiid larvae

Hemogregarines were found readily in 3 of the 18 laboratory-reared larvae (*Gnathia* sp A) that had fed on a *Z. scopas* with a heavy *H. bigemina*-like infection (Fig. 2A). These larvae had been smeared at 1, 2, and 3 dpf. The stages observed were gamonts (Fig. 2A), like those seen in the fish blood (12.2 ± 1.77 µm × 1.3 ± 0.35 µm, n = 15), and possibly young oocysts (7.3–9.0 µm across, n = 4).

Descriptions of the hematozoans

The intraerythrocytic and intraleukocytic hematozoans observed in blood films from fishes may constitute as many as 5 morphometrically distinct species, but it is difficult to be certain in all cases. Four of the 5 are detailed below, and at least 1 of these can be considered a new species. *Haemogregarina tetraodontis*, however, appears identical to some illustrations of Burrenson (1989) and material deposited by him in the Queensland Museum (see below). We found it as a light infection in only 1 parrotfish, *Scarus psittacus* (as a mixed infection); no new stages were discovered, and we have nothing further to add to previous descriptions (Mackerras and Mackerras, 1961; Burrenson, 1989). For these reasons, we feel that it is unnecessary to redescribe *H. tetraodontis* from Lizard Island, but we merely note its presence. All measurements are in micrometers unless otherwise noted.

Haemogregarina balistapi n. sp.

(Figs. 2B–2H)

Diagnosis: Stages in blood films included merozoites in mature erythrocytes, and gamonts in the same cells, as well as in small and large lymphocytes. Intensity of infection: from 1 parasite in approximately 250 erythrocytes in 1 *S. chrysopterum*, to <1 parasite in 5,000 blood cells (erythrocytes and lymphocytes) in the remaining fishes.

Groups of 4, 6, or 8 merozoites located in erythrocytes, but only rarely (Fig. 2B, D); a few extracellular merozoites also observed. Merozoites had a marked effect on host cells, especially when 6–8 were present, making erythrocyte broader, often shorter, noticeably rounder, and displacing host nucleus to one side. Uninfected erythrocytes: 10.9 ± 0.79 µm long × 7.2 ± 0.75 µm wide (n = 25); an erythrocyte with 4 merozoites, 11.1 µm long × 9.7 µm wide; another with 6 merozoites, 10.11 µm long × 10.6 µm; and a third with 8 merozoites, 9.50 µm long × 9.39 µm wide.

Merozoites in groups of 4, each measured 5.8–6.1 µm long × 1.4–1.8 µm wide (n = 8), and were elongate with rounded

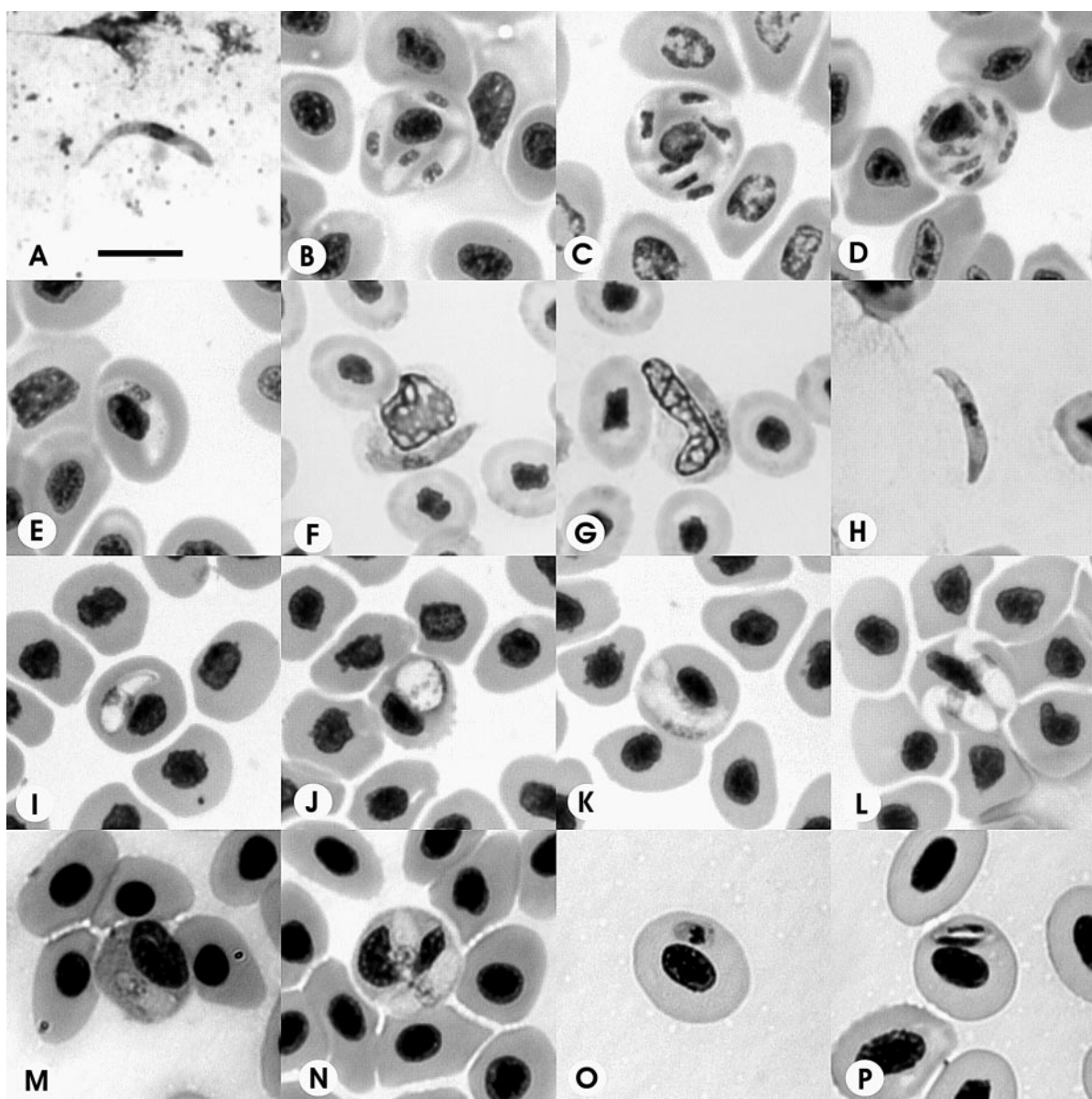


FIGURE 2. Light micrographs of different Giemsa-stained life-cycle stages of 4 species of haematozoa found in Lizard Island, Australia, fishes and gnathiids. (A) Gamont stage of *Haemogregarina bigemina*-like haemogregarine in the gut of a *Gnathia* sp. A juvenile fed on *Z. scopas*. Group of 4 (B), 6 (C), and 8 (D) intraerythrocytic merozoites of *H. balistapi* n. sp. from *Balistapus undulatus*. (E) Intraerythrocytic gamonts of *H. balistapi* from *B. undulatus*. (F, G) Gamonts of *H. balistapi* attached to the remnants of host cells from *Sufflamen chrysopterum*. (H) Free gamont of *H. balistapi* from *S. chrysopterum*. Trophozoite (I), meront (J), dividing meront (K), and paired adult gamonts (L) of *H. bigemina*-like organism, all in erythrocytes of *Zebrasoma scopas*. One (M) and 2 (N) intraleukocytic merozoites in *Cephalopholis boenak*. Undivided (O) and dividing stages (P) of a *Haemohormidium*-like parasite in *Scarus psittacus*. Bar = 10 μ m.

ends; nuclei is proximately one-half to one-third of the way along the parasite body, with chromatin loosely arranged (Fig. 2B). Parasite nuclei are oval, 2.0–2.4 μ m long \times 1.1–1.4 μ m wide ($n = 8$); cytoplasm is pale-stained and without granules. Merozoites in groups of 6 are also elongate, but shorter and narrower than those occurring in groups of 4, and measure 4.5–5.8 μ m long \times 1.1–1.6 μ m wide ($n = 6$). Nuclei are longer, 2.3–3.4 μ m long \times 1.0–1.2 μ m wide ($n = 6$), than those of merozoites in groups of 4, and are also rather strip-like in structure, with the chromatin more condensed (Fig. 2C). Cytoplasm is pale-stained and without granules. Merozoites in groups of 8

are similar to those occurring in groups of 6, with elongate nuclei and condensed chromatin (Fig. 2D), 4.2–5.1 μ m long \times 1.0–1.4 μ m wide ($n = 8$). Their nuclei are 2.0–3.3 μ m long \times 1.0–1.1 μ m wide ($n = 8$).

Mature gamonts are single, monomorphic, and in close proximity to host erythrocyte or lymphocyte nucleus, often curving around it. In some instances, particularly in *S. chrysopterum*, but also in *B. undulatus*, host cells are changed so that only remnants of host-cell cytoplasm or nucleus remained, making it difficult to determine whether it was originally from red or white cell series. Gamonts are broad, particularly anteriorly,

with pointed or slightly rounded ends, 10.4 ± 0.31 (9.0–11.6) μm long \times 2.2 ± 0.34 (1.7–2.9) μm wide ($n = 25$) at broadest, and are dominant forms in blood films (Fig. 2E). Deep purple-stained nucleus is situated in posterior half of body. Chromatin is mostly loose, but occasionally compacted; each nucleus is 2.3 ± 0.39 (1.8–3.1) μm long \times 1.4 ± 0.36 (1.0–1.9) μm wide ($n = 25$). Cytoplasm of mature gamonts stained pale blue, except for some purplish staining toward the posterior pole in some instances. No deep staining caps are seen, and a parasitophorous vacuole is not visible. Free gamonts are observed in infected blood smears or are attached to remnants of host cells, as described above (Figs. 2F–H).

Taxonomic summary

Type host: One of 3 *Balistapus undulatus* (Park, 1797)

Other host: Three of 28 *Sufflamen chrysopterum* (Bloch & Schneider, 1801)

Type locality: Lizard Island (23°27'S, 151°55'E), Australia.

Specimens deposited: Holotype: Queensland Museum (QM G464750), paratype: Queensland Museum (QM G464751).

Etymology: The species is named for the generic name of the type host.

Remarks

Haemogregarina parmae commonly produces 1–4 parasites in each infected red cell and, rarely, 6 or 7. We have examined the Mackerras and Mackerras (1961) paratype material of *H. parmae* deposited in the Queensland Museum and have found the merozoites to be much more slender than those of the parasite found in *B. undulatus* and *S. chrysopterum*. Furthermore, the intracellular gamonts of *H. parmae* in the paratype material are smaller and shorter than those found in *B. undulatus*. They also have curved tails, prominent caps, and a nucleus in the anterior half of the gamont. The hemogregarine found in our smears is, therefore, very different from *H. parmae*.

We have also examined the type material of *H. tetraodontis* (QM G2516). The description of *H. tetraodontis* is based solely on the merozoite stages, with Mackerras and Mackerras (1961) recording up to 6 merozoites within a single red cell. Unfortunately, the type material has lost much of its staining and quality since 1961, but even so, it suggests that the hemogregarine from the Balistidae is different from *H. tetraodontis*, although superficial similarities exist between merozoites and their effects on host cells. However, our parasite also produces up to 8 merozoites per host cell, and the nuclei are rather strip-like in structure. Furthermore, we have gamonts in the erythrocytes and in lymphocytes, which have not been described for *H. tetraodontis*.

We have also examined the Burrenson (1989) slide of *H. tetraodontis* from the Queensland Museum (QM GL10152) and found his material to be rather different from that found in *B. undulatus* and *S. chrysopterus*. Our merozoites occurring in groups of 4 are generally shorter and a little wider than those of the Burrenson (1989) *H. tetraodontis*, and they do not occur in compact bundles. Burrenson reported 2, 4, or 6 merozoites per red cell, and in our material, there were 4, 6, or 8 merozoites per cell. His material also lacks the characteristic gamonts found in the blood of *B. undulatus*. Finally, material that we do consider is *H. tetraodontis* was found by us as a mixed infection

in one of our fishes (*Scarus psittacus*) from Lizard Island (Table II), further supporting our conviction that *H. balistapi* is quite different from *H. tetraodontis*.

Infection of *Haemogregarina bigemina* Laveran and Mesnil, 1901

Diagnosis: This infection (Fig. 2I, L) found in members of Acanthuridae, including *Acanthurus nigrofuscus*, *Ctenochaetus striatus*, *Zebrasoma scopas*, and *Zebrasoma veliferum*. Intensity of infection similar to that seen for *H. balistapi* among Balistidae; ranged from very light infection to a level approaching 1 parasite per 250 erythrocytes.

Late trophozoites, meronts, and gamonts were found in mature erythrocytes. Commonly, erythrocyte nuclei were displaced, and host cells slightly enlarged, 19.5 ± 4.60 (15.1–22.4) \times 16.7 ± 3.66 (14.3–19.1) μm ($n = 25$) compared with uninfected erythrocytes of 18.1 ± 4.31 (15.3–21.1) \times 14.1 ± 5.35 (12.8–17.1) μm ($n = 25$). Trophozoites were seen only rarely, $4.4\text{--}6.2 \times 1.7\text{--}2.5 \mu\text{m}$ ($n = 4$) (Fig. 2I). Meronts are 6.0 ± 1.56 (4.6–8.5) μm long \times 3.3 ± 0.48 (2.8–4.3) μm wide ($n = 12$) and elongated with 1 end slightly broader than the other; cytoplasm stained pale blue and without granules (Fig. 2J). Meronts are normally single in erythrocytes but are occasionally in pairs. In trophozoites, nuclear chromatin is loosely arranged, but in meronts, chromatin is often difficult to discern, especially before division. Mature meronts are larger, oval, or rounded, and divided into 2, 3, or 4 individual merozoites/pre-gamonts. Although division into 2 (by longitudinal binary fission) is most common (Fig. 2K), it is not unusual to observe 3 or 4 individuals in erythrocytes. Red cells with both paired pregamonts and trophozoites are also occasionally seen. Gamonts are 9.9 ± 0.53 (8.6–12.7) μm long \times 2.1 ± 0.30 (1.3–2.8) μm wide ($n = 25$) and are mostly in pairs in erythrocytes (Fig. 2L), with broad anterior ends, mostly rounded, occasionally pointed, but without caps. Posterior ends are often recurved and rather narrow (Fig. 2L). Nucleus is situated roughly halfway down the body. Nucleus, with loosely arranged chromatin, is sometimes wider than long, 2.1 ± 0.38 (0.9–2.3) μm long \times 2.0 ± 0.21 (1.8–3.6) μm wide ($n = 12$), though not invariably. Parasitophorous vacuole is not visible. Free gamonts, resembling those in erythrocytes, are also observed in infected blood smears.

Remarks

The parasite from *Z. scopas* is different from *Desseria gilbertiae*, *H. parmae*, and *H. tetraodontis* in its patterns of division and in the characteristics of its gamonts. The hemogregarine is also different from *Desseria (Haemogregarina) rubrimarensis* (Saunders, 1960) Siddall, 1995, another hemogregarine from reef fish of the Acanthuridae, found in the Red Sea. Although the mature gamonts of the Australian parasite fall within the length range of those of *D. rubrimarensis*, they do not have the sharply pointed anterior ends and small, circular-to-oval nuclei with compact chromatin, characteristic of the Red Sea hemogregarine. The cytoplasm of *D. rubrimarensis* gamonts also occasionally has granules, which have not been seen in the parasite from *Z. scopas*, and furthermore, the Red Sea parasite does not demonstrate the division patterns seen in the Australian material.

The hemogregarine from Lizard Island is, however, similar in size and appearance to the parasite that Burreson (1989) identified as resembling *Haemogregarina bigemina*, in *Ecsenius bicolor* from Heron Island. Infections reported as *H. bigemina* have been found at other sites in the southern hemisphere, namely in New Zealand and the South Pacific (Laird, 1953, 1958) and in South Africa (Smit and Davies, 1999; Davies and Smit, 2001; Smit, Van As, and Davies, 2003; Davies et al., 2004). However, Burreson (1989) was rightly cautious in his identification of *H. bigemina* from Heron Island. Davies et al. (2004) have recently questioned whether this hemogregarine can truly exist in the remarkable 96 species of host fishes, from which it is supposedly reported, worldwide. The longitudinal division of the parasite seen in *Z. scopas* into 2 individuals is certainly similar to that which occurs in *H. bigemina*, although we found no transverse division. *Haemogregarina bigemina* in its type hosts *Lipophrys pholis* and *Coryphoblennius galerita* (see Davies, 1982, 1995; Davies et al., 2004) frequently demonstrates both longitudinal and transverse binary fission and does not readily exhibit division into more than 2 individuals. Multiple individuals within red cells were, however, relatively common in our material from Lizard Island. The gamonts of *H. bigemina*, though paired, are also generally more slender, and the nuclei are more posterior, usually narrower and have more condensed chromatin than those of the parasite found in *Z. scopas*. Gamonts of *H. bigemina* typically also have a characteristic posterior granule, lacking in our parasite from Lizard Island. Another feature uncharacteristic of *H. bigemina* is the relatively long, narrow, recurved tail, seen especially in the extracellular gamonts of the hemogregarine from Australia.

We conclude that the hemogregarine from Lizard Island is very close in appearance to the Burreson (1989) parasite from Heron Island and is “*H. bigemina*-like” in some phases of its pregamontic division, i.e., when it forms 2 individuals. However, in Australian samples, the hemogregarine also exhibits a number of features not typical of *H. bigemina* in its type hosts, but at present, we are uneasy about naming it a new species.

Intraleukocytic infection

Diagnosis: Intraleukocytic hemogregarine (Fig. 2M, N) is present in small and large lymphocytes and in eosinophils of 1 specimen of *Cephalopholis boenak*. Infection is in 6 % of white cells; of these, 2% with 1 parasite, 3% with 2 parasites, 1% with 4. No hemogregarine were detected in erythrocytes.

Stages observed were all presumed to be merozoites; merozoites were rather stout with rounded ends, monomorphic, without features normally associated with gamonts (much longer than wide, broad anterior sometimes with cap, narrow posterior sometimes recurved, characteristic granules, etc.) (Fig. 2M, N). Nucleus in these forms was more or less centrally placed, or slightly nearer to one pole; chromatin was coarsely granular. Nuclear division, indicative of merogony, was not observed; cytoplasm stained pale blue with Giemsa, otherwise unremarkable. Merozoites were single within white cell, 7.1 ± 0.66 (6.0–7.9) μm long \times 2.3 ± 0.20 (2.0–2.2) μm wide; nuclei were 2.3 ± 0.31 (1.9–2.9) μm \times 1.8 ± 0.34 (1.2–2.2) μm (n = 10) (Fig. 2M). When in pairs, they were 6.1 ± 1.86 (2.4–8.7) μm long \times 2.3 ± 0.34 (2.0–3.0) μm wide, with nuclei of 2.1 ± 0.31 (1.7–2.7) \times 1.7 ± 0.23 (1.5–2.0) μm (n = 10) (Fig. 2N). Those

in groups of 4 were 6.1 ± 0.80 (5.3–7.3) μm long \times 2.1 ± 0.13 (1.9–2.2) μm wide, with nuclei, 2.1 ± 0.10 (1.9–2.2) \times 1.6 ± 0.17 (1.5–1.9) μm (n = 8).

Remarks

A number of hemogregarines have been reported to undergo merogony in leucocytes and development in leucocytes and erythrocytes (see Davies, 1995). *Haemogregarina bigemina* is reported to develop in both the white and red cell series of fishes from New Zealand (see Laird, 1953). Such development has also been observed at Norfolk Island, Australia (see Laird, 1958), but there are no further records from fishes caught off the Australian mainland or its other offshore islands. The current hemogregarine was observed on only 1 occasion, and it is uncertain whether it also multiplies in red cells. However, it is unlikely that it is related to the hemogregarine (*H. balistapi*) that exists in the leucocytes of the Balistidae (above) because that undergoes merogony in erythrocytes. Until more material from *C. boenak* is located, it is impossible to describe this species fully or name it.

Infection of *Haemohormidium*

Diagnosis: Infection (Fig. 2O, P) is indistinguishable from some stages reported by Burreson (1989) from *Pomacentrus melanochir* captured at Heron Island. All infections in the parrotfishes *Chlorurus sordidus* and *Scarus psittacus* at Lizard Island were light (<0.1 parasites per 10,000 erythrocytes); parasite stages are elongate, narrow, and almost all in division (see Fig. 2O, P). No stages were found in leucocytes.

Parasite nuclei stained deeply with Giemsa and were elongate and strip-like; cytoplasm, which stained pale or deeper blue, appeared free from granules or vacuoles. Undivided parasites were 3.7 ± 1.01 (2.9–4–9) \times 1.5 ± 0.42 (1.3–2.0) μm (n = 5) (Fig. 2O). Division stages are 7.0 ± 0.80 (6.1–7.1) long \times 1.9 ± 0.83 (1.2–2.1) μm wide (n = 25), with paired nuclei, 1.8 ± 0.52 (1.6–2.3) \times 1.3 ± 0.43 (1.0–1.6) μm (Fig. 2P).

Remarks

Parasites like those observed in *C. sordidus* and *S. psittacus* have been observed in species of *Chlorurus*, *Scarus*, and other genera from the Red Sea by Saunders (1960), who interpreted them as the merozoites and pregamonts (pregametocytes) of the hemogregarine *D. rubrimarensis*. However, in our material, we found no stages resembling the mature gamonts (gametocytes) of *D. rubrimarensis*. Identical parasites to those found in the present study were also observed by Burreson (1989) in *Pomacentrus melanochir* from Heron Island, but that author did not report any stages similar to *D. rubrimarensis* mature gamonts either. Here, the stages observed were interpreted as the development forms of a *Haemohormidium* sp. Burreson (1989) was, however, concerned that although the small stages resembled those of *Haemohormidium*, the elongate merozoites were more typical of those of species of *Haemogregarina*.

We conclude that our material is identical to Saunders' *D. rubrimarensis* pro parte (see Saunders, 1960) as well as the Burreson (1989) organism. We agree with Burreson (1989) that it is generally a small parasite, but our impression is that it may be too distinct generally to be a *Haemohormidium* sp. The

THE JOURNAL OF PARASITOLOGY, VOL. 92, NO. ?, MONTH 2006

amount of nuclear material, though not large, may be too great and the chromatin occasionally a little too loose, especially when compared with the type species *Haemohormidium cotti* Henry 1910 (see Davies, 1980, 1995; Davies et al., 2003). Furthermore, the cytoplasm is clearly defined and blue-stained, and the division stages are also very curious, with narrow forms undergoing longitudinal fission, again not typical of *Haemohormidium*. However, we agree with Burreson (1989) that this parasite cannot be readily identified as a hemogregarine either. Furthermore, because we also saw it on 1 occasion forming a mixed infections with the *H. bigemina*-like infection and with *H. tetraodontis*, it is difficult to be certain that any "hemogregarine-like" stages that might be present would not be connected with these rather than the *Haemohormidium*-like infection itself.

As we consider our parasite to be identical to *D. rubrimarensis*, in part, we suggest that the original *D. rubrimarensis* may well be 2 parasites (see Saunders, 1960). We recommend that the name *D. rubrimarensis* should be retained for the gamont stage of Saunders' parasite and suggest that her merozoites and pregamonts (pregametocytes), Burreson's parasite, and ours all represent another species. Because we cannot determine with certainty by light microscopy whether it is a species of *Haemohormidium*, a small hemogregarine, or another kind of parasite, we provisionally retain the Burreson (1989) designation and refer to it as "*Haemohormidium*-like", until we can study it in greater detail.

DISCUSSION

It is intriguing that among the numerous reef fishes of Lizard Island, it is apparently those in the Acanthuridae, Balistidae, Scaridae, and Serranidae that are parasitized by hematozoans. The blood parasites include predominantly hemogregarines but also a hematozoan of uncertain identity that we designate, for the present, *Haemohormidium*-like. The firm identity of this latter organism will require its study by transmission electron microscopy (TEM) because, at present, it is impossible to be certain whether it is truly a *Haemohormidium* sp. Related organisms and *Haemohormidium* spp. themselves lack the apicomplexan features characteristic of hemogregarines (see Davies et al., 2003). With the application of TEM, it should be possible, in the future, therefore, to determine to which group the enigmatic parasite belongs.

Some, normally common, blood parasites of marine fishes appear absent from Lizard Island samples, i.e., trypanosomes. Leeches are generally considered the vectors of fish hemoflagellates, both in marine and freshwater systems (see Lom and Dykova, 1992). Perhaps the absence of the trypanosomes from the current study samples reflects the absence of their annelid vectors. Burreson (1989) noted the apparent dearth of trypanosomes in teleosts and of marine leeches in tropical regions, and the current study appears to support that view.

The question then arises how fishes become infected with hemogregarines and the *Haemohormidium*-like organism at Lizard Island. The occurrence of mixed-blood infections in some fishes suggests a common vector. Davies et al. (2004) recently reviewed the biology of *Haemogregarina bigemina*. This organism is reported from 96 species of marine teleosts in 70 genera and 34 families, most of these being intertidal or reef-dwelling fishes and including many families of fishes

found at Lizard Island. Those authors also summarized the work that led to the conclusion that *H. bigemina* is transmitted by the larvae of the isopods of *Gnathia* spp., rather than by leeches, especially the evidence of Davies and Smit (2001). We have also located a *H. bigemina*-like parasite in some fishes at Lizard Island, but because it has unusual development patterns and morphometrics, we cannot identify the infection firmly as *H. bigemina* itself. This will likely require a molecular comparison of *H. bigemina* infections from the type hosts in Europe with those found in the fishes of Lizard Island. The fact remains, however, that *H. bigemina*-like hemogregarines, as well as other species of hemogregarines, are common in some families of fishes at Lizard Island, leeches appear absent, and blood-sucking gnathiid isopods are extremely common (Grutter and Poulin, 1998).

When we examined gnathiids generally from fishes by crush/smear techniques in the current study, we found few to contain recognizable hemogregarines. This can be explained either by the host fishes themselves having no hemogregarine infections or by hemogregarines being present in very low numbers in these hosts. However, when laboratory-reared gnathiid larvae were fed on a heavily infected *Zebrasoma scopas*, 3 of 18 contained hemogregarines. Clearly, if transmission of hemogregarines by gnathiid larvae is to be investigated at Lizard Island under laboratory conditions, these isopods must be allowed to feed on fish hosts heavily infected with hemogregarines. Perhaps, it will then be possible to demonstrate hemogregarine development in these invertebrate hosts as has been the case in Europe and South Africa (see Davies et al., 2004).

Of all fishes examined at Lizard Island, *Z. scopas* was most consistently infected with hemogregarines (50/59 fishes examined), although 1 specimen was also found to contain the *Haemohormidium*-like organism as a mixed infection. Laboratory experiments also demonstrate that this fish retains its hemogregarines for long periods in the absence of obvious vectors, although such infections tend to occur at low levels of intensity. *Zebrasoma scopas* would, therefore, appear to be an ideal candidate fish for transmission studies, but other members of the Acanthuridae and some Balistidae deserve consideration because they also act as hosts for hemogregarines.

Of those infections observed in fishes, hemogregarines appeared to affect the blood cells of the Balistidae most adversely, with mature gamonts reducing the host cells (erythrocytes and lymphocytes) to mere remnants. This is reminiscent of the effect of *Haemogregarina nototheniae* on the blood cells of Antarctic nototheniid fishes, where huge gamonts become extracellular and host cell nuclei remain attached terminally or stretched along the length of the gamont (Barber et al., 1987).

Lizard Island is separated from Heron Island by approximately 1,000 km, and yet, their hematozoans appear to be remarkably similar (see Burreson, 1989). These include *H. bigemina*-like and *Haemohormidium*-like infections, as well as *Haemogregarina tetraodontis*. There are also clear similarities between the blood parasites from both of these islands and from those of reef fishes of the Red Sea (see Saunders, 1960). Although all of the fish sampled have a pelagic larval stage, the adults are relatively sedentary (Sale, 2002). This raises questions about the identity of the vectors and their distribution, their host specificity, and the dispersal ranges of their hosts.

ACKNOWLEDGMENTS

Many thanks to the Lizard Island Research Station Staff, C. Fury, A. Kuris, and M. Johnson, for assistance in the field, P. Hayes for laboratory assistance, and the University of Queensland and the Australian Research Council for funding (A.S.G.), a Kingston University Post Doctoral Fellowship (N.J.S.), and a Leverhulm Trust Research Fellowship (A.J.D.). This work benefited greatly from discussions with A. Kuris, R.J.G. Lester, and H. McCallum.

LITERATURE CITED

- BARBER, D. L., J. E. MILLS WESTERMANN, AND P. STOROZ. 1987. *Haemogregarina nototheniae* n. sp. from the blood of Antarctic nototheniids. *Systematic Parasitology* **10**: 135–147.
- BURRESON, E. M. 1989. Hematozoa of fishes from Heron Island, Australia, with the description of 2 new species of *Trypanosoma*. *Australian Journal of Zoology* **37**: 15–23.
- COMBES, C. 1995. *Ecologie et evolution du parasitisme*. Masson, Paris. *Ecology* **26**: 286–191.
- DAVIES, A. J. 1980. Some observations on *Haemohormidium cotti* Henry 1910, from the marine fish *Cottus bubalis* Euphrasen. *Zeitschrift für Parasitenkunde* **62**: 31–38.
- . 1982. Further studies on *Haemogregarina bigemina* Laveran & Mesnil, the marine fish *Bleinnius pholis*, L. and the isopod *Gnathia maxillaris*. *Journal of Protozoology* **29**: 576–583.
- . 1995. The biology of fish haemogregarines. *Advances in Parasitology* **36**: 117–203.
- , J. C. EIRAS, AND R. T. E. AUSTIN. 1994. Investigations into the transmission of *Haemogregarina bigemina* Laveran & Mesnil, 1901 (Apicomplexa: Adeleorina) between intertidal fishes in Portugal. *Journal of Fish Diseases* **17**: 283–289.
- , AND M. R. L. JOHNSTON. 1976. The biology of *Haemogregarina bigemina* Laveran & Mesnil, a parasite of the marine fish *Bleinnius pholis* Linnaeus. *Journal of Protozoology* **23**: 315–320.
- , AND ———. 2000. The biology of some intraerythrocytic parasites of fishes, amphibia and reptiles. *Advances in Parasitology* **45**: 1–107.
- , C. C. REED, AND N. J. SMIT. 2003. An unusual intraerythrocytic parasite of *Parablennius cornutus*, from South Africa. *Journal of Parasitology* **89**: 913–917.
- , AND N. J. SMIT. 2001. The life cycle of *Haemogregarina bigemina* (Adeleina; Haemogregarinidae) in South African hosts. *Folia Parasitologica* **48**: 169–177.
- , ———, P. M. HAYES, A. M. SEDDON, AND D. W. WERTHEIM. 2004. *Haemogregarina bigemina* Laveran & Mesnil, 1901 (Protozoa: Apicomplexa: Adeleorina)—Past, present and future. *Folia Parasitologica* **51**: 99–108.
- GRUTTER, A. S. 1995. Comparison of methods for sampling ectoparasites from coral reef fishes. *Marine and Freshwater Research* **46**: 897–903.
- . 2001. Parasite infection rather than tactile stimulation is the proximate cause of cleaning behaviour in reef fish. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **268**: 1361–1365.
- . 2003. Feeding ecology of the fish ectoparasite, *Gnathia* sp. (Crustacea: Isopoda), from the Great Barrier Reef, Australia and its implications for fish cleaning behaviour. *Marine Ecology Progress Series* **259**: 295–302.
- , AND N. W. PANKHURST. 2000. The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus* (Labridae). *Journal of Fish Biology* **56**: 391–401.
- , AND R. POULIN. 1998. Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. *Marine Ecology Progress Series* **164**: 263–271.
- LAIRD, M. 1953. The protozoa of New Zealand intertidal zone fishes. *Transactions of the Royal Society of New Zealand* **81**: 79–143.
- . 1958. Parasites of south Pacific fishes, 1: Introduction and haematozoa. *Canadian Journal of Zoology* **36**: 153–165.
- LAVERAN, A. 1908. Sur une hémogregarine, un trypanosome et un spirille, trouvés dans le sang d'un requin. *Bulletin de la Société de Pathologie Exotique* **1**: 148–150.
- LOM, J., AND I. DYKOVÁ. 1992. Protozoan parasites of fishes. *In* Developments in aquaculture and fisheries science. Elsevier, Amsterdam, The Netherlands, p. 1–315.
- MACKERRAS, I. M., AND M. J. MACKERRAS. 1925. The haematozoa of Australian marine teleostei. *Proceedings of the Linnean Society of New South Wales* **50**: 359–366.
- , AND ———. 1961. The haematozoa of Australian frogs and fish. *Australian Journal Zoology* **9**: 123–139.
- MUNDAY, P. L., AND S. K. WILSON. 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. *Journal of Fish Biology* **51**: 931–938.
- OPPLIGER, A., M. L. CELERIER, AND J. CLOBERT. 1996. Physiological and behavioural changes in common lizards parasitized by haemogregarines. *Parasitology* **113**: 433–438.
- , AND J. CLOBERT. 1997. Reduced tail regeneration in the common lizard, *Lacerta vivipara*, parasitized by blood parasites. *Functional Ecology* **11**: 652–655.
- RANDALL, J. E., G. R. ALLEN, AND R. C. STEENE. 1997. *The complete diver's and fishermen's guide to fishes of the Great Barrier Reef and Coral Sea*. Crawford House Publishing, Bathurst, Australia, 557 p.
- SALE, P. F. 2002. *Coral reef fishes: Dynamics and diversity in a complex system*. Academic Press, San Diego, California, 534 p.
- SAUNDERS, D. C. 1960. A survey of the blood parasites in the fishes of the Red Sea. *Transactions of the American Microscopical Society* **79**: 239–252.
- SMIT, N. J., AND L. BASSON. 2002. *Gnathia pantherina* sp. n. (Crustacea: Isopoda: Gnathiidae), a temporary ectoparasite of some elasmobranch species from southern Africa. *Folia Parasitologica* **49**: 137–151.
- , ———, AND J. G. VAN AS. 2003. The life cycle of the temporary fish parasite, *Gnathia africana* (Crustacea: Isopoda: Gnathiidae). *Folia Parasitologica* **50**: 135–142.
- , AND A. J. DAVIES. 1999. New host records for *Haemogregarina bigemina* from the coast of southern Africa. *Journal of the Marine Biological Association of the U.K.* **79**: 933–935.
- , AND ———. 2001. An encapsulated haemogregarine from the evileye pufferfish in South Africa. *Journal of the Marine Biological Association of the U.K.* **81**: 751–754.
- , AND ———. 2004. The curious life-style of the parasitic stages of gnathiid isopods. *Advances in Parasitology* **58**: 289–391.
- , J. G. VAN AS, AND L. BASSON. 2002. Redescription of female of *Gnathia africana* (Gnathiidae: Crustacea: Isopoda) from southern Africa. *Folia Parasitologica* **49**: 67–72.
- , ———, AND A. J. DAVIES. 2003. Taxonomic re-evaluation of the South African fish haemogregarine, *Desseria fragilis*. *Journal of Parasitology* **89**: 151–153.