The distribution and host-association of a haemoparasite of damselfishes (Pomacentridae) from the eastern Caribbean based on a combination of morphology and 18S rDNA sequences

Paul C. Sikkela,b,*, Courtney A. Cookb, Lance P. Renouxb, Courtney L. Bennettc, Lillian J. Tuttd, Nico J. Smitb

a Department of Biological Sciences and Environmental Sciences Program, Arkansas State University, State University, AR, USA
b Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa
c Sarasota High School, 2155 Bahia Vista St, Sarasota, FL 34239, USA
d Pacific Biosciences Research Center, University of Hawai‘i at Mānoa, Honolulu, HI, USA

ARTICLE INFO

Keywords:
Stegastes
Blood parasite
Haemoooccidia
Apicomplexa
Coral reefs

ABSTRACT

Coral reefs harbor the greatest biodiversity per unit area of any ecosystem on earth. While parasites constitute the majority of this biodiversity, they remain poorly studied due to the cryptic nature of many parasites and the lack of appropriate training among coral reef ecologists. Damselfishes (Pomacentridae) are among the most abundant and diverse fishes on coral reefs. In a recent study of blood parasites of Caribbean reef fishes, the first ever apicomplexan blood parasites discovered in damselfishes were reported for members of the genus Stegastes. While these blood parasites were characterized as “Haemohormidium-like”, they appear to be distinct from any other known apicomplexan. In this study, we examined host associations, geographic distributions, and provide further insights on the phylogenetic affiliation of this parasite. A combination of morphological characteristics and 18S rDNA sequences suggest that this parasite may be the same species at multiple sites and occurs from the southern to the northern extreme of the eastern Caribbean, although it appears rare in the north. At present it appears to be limited to members of the genus Stegastes and infects all life history stages. It is most common in benthophagous species that occur in high population densities and appears basal to a major monophyletic clade containing species of coccidia, distinct from the Piroplasmida, the order to which Haemohormidium spp. have been assigned. These findings suggest a possible fecal-oral mode of transmission.

1. Introduction

Near-shore scleractinian coral reefs harbor the greatest biodiversity found in the world’s oceans (e.g., Roberts et al., 2002), and in fact contain more species per square meter than any other ecosystem on the planet (Knowlton et al., 2010). This high biodiversity contained within a relatively small area facilitates a multitude of complex interactions between components of the biotic and abiotic community (Dornelas et al., 2006). Parasites compose the majority of biodiversity on coral reefs (Rhode, 1992, 1999; Poulin and Morand, 2000; Muñoz et al., 2007; Knowlton et al., 2010), and provide a key link in coral reef ecosystems, providing both a food source and selective pressure on hosts that influence the behavior of the coral reef inhabitants (Hudson et al., 2006). Along with providing key ecological links in coral reefs, parasites also cause and/or act as vectors for disease (Lefèvre and Thomas, 2007).

Most research on parasitic diseases in coral reef systems has focused on diseases of the corals themselves as a major cause of coral decline (e.g., Harvell et al., 2004; Correa et al., 2009). Research on diseases of fishes has mainly focused on species that are of economic or recreational importance, and/or diseases impacting the aquaculture industry (Arkoosh et al., 1998; Johnson et al., 2004; Masson et al., 2013). This research has been further biased towards bacterial and fungal infections affecting large top-trophic level fish (Cahill, 1990; McVicar, 1997). Given that diseases can have a large impact on population structure and thus knock-on effects at the community or ecosystem level, a broader understanding of potential disease-causing organisms in coral reef fishes seems important.

Apicomplexan hemoparasites are obligate parasites of many species of vertebrates (Davies and Johnston, 2000). Apicomplexans can exist within their host with relatively little impact or can cause catastrophic damage resulting in death. The majority of blood-borne apicomplexans appear to be limited to members of the genus Stegastes and infects all life history stages. It is most common in benthophagous species that occur in high population densities and appears basal to a major monophyletic clade containing species of coccidia, distinct from the Piroplasmida, the order to which Haemohormidium spp. have been assigned. These findings suggest a possible fecal-oral mode of transmission.
require two hosts to complete their development. Asexual development, which leads to the formation of gamont stages in the peripheral blood, occurs in a vertebrate (intermediate) host, and sexual development, initiated by the uptake of gamont stages, occurs in a haematopagous invertebrate (definitive) host. Transmission of infective sporozoite stages from the infected invertebrate host occurs either through inoculation as in the case of the haemosporidia (e.g. species of *Plasmodium*) and piroplasms (e.g. species of *Babesia*), and some haemogregarines (e.g. species of *Haemogregarina*), or through ingestion of the infected invertebrate as in the case of most haemogregarines (e.g. species of *Hepatozoon*). Haemococcidia, however, such as species of *Lankesterella* and *Schellackia*, complete their development in their vertebrate host, invertebrates acting only as paratenic or mechanical hosts when ingested by the vertebrate (*O唐Donoghue*, 2017). The vast majority of work on the phylum Apicomplexa has focused on *Plasmodium* and other genera of socioeconomic importance (*Wozniak et al.*, 1994; *Bejon et al.*, 2006; *Sant’Anna et al.*, 2008; *Ogedengbe et al.*, 2013; *Heddergott et al.*, 2012) in terrestrial systems. Much less is known about apicomplexan parasites in coral reef systems or in marine fishes.

Members of the family Pomacentridae are small-to medium-sized fishes that exhibit a circumtropical distribution and include some subtropical and warm temperate species (*Allen*, 1991; *Helfman et al.*, 2009). They include herbivores, planktivores, and omnivores that inhabit all areas from shoreline to deep-reef structures (*Allen*, 1991; *Helfman et al.*, 2009). Some species defend permanent multipurpose territories while in others only the males are territorial when defending nests. Members of this family are present in high numbers on reefs, and are prey for larger predators (e.g., *Greenfield and Johnson*, 1990; *Wilson and Meekan*, 2002; *Mumby et al.*, 2012).

In the Caribbean, pomacentrids are represented by members of the genera *Abudelfaf*, *Chromis*, *Stegastes*, and *Micropathodon*. The most common species of *Abudelfaf* (*A. saxatilis*) and *Chromis* (*C. multilineata*) are midwater shoalers that spend their time feeding on zooplankton during the day and retire to the reef at night (e.g., *Randall*, 1968; *Allen*, 1991). In contrast, *Abudelfaf taurus* is solitary and inhabits shallow, high surge areas. Both sexes of species of *Stegastes* and *Micropathodon* maintain permanent territories and occupy a wide range of shallow coral reef habitats (*Waldner and Robertson*, 1980; *Itzkowitz et al.*, 1995).

As in other systems where top-level predators have been removed, parasitic diseases often replace them as the primary regulators of populations (*Packer et al.*, 2003; *Lafferty et al.*, 2008; *Raffel et al.*, 2008). Thus, identifying actual or potential disease-causing organisms and how they are transmitted becomes essential to understanding coral reef community dynamics.

In a recent survey of hemoparasite biodiversity of reef-associated fishes of the eastern Caribbean, *Cook et al.* (2015) sampled 1298 individual fish from 6 eastern Caribbean islands, representing 27 families, 57 genera and 103 species. In all, members of 14 species from 8 families were infected with 8 distinct types of blood parasites, 6 of which were apicomplexan. These included a newly discovered intraerythrocytic parasite that was tentatively referred to as *Haemohormidium*-like and was common in adults of three species of *Stegastes* damselfishes (*Pomacentridae*) including *S. adustus*, *S. diencaeus* and *S. leucostictus* (*Cook et al.*, 2015). This blood parasite was rare or absent in three other species of *Stegastes* and was absent in *A. saxatilis* and both Caribbean *Chromis* spp. sampled. However, variation among *Stegastes* and apparent absence in *A. saxatilis* may have been attributable to small sample sizes and/or sampling from a single site. In a subsequent study, *Renoux et al.* (2017) developed an apicomplexan DNA barcoding system, targeting the 18S rDNA gene, to detect infections of the *Haemohormidium*-like parasites in *Stegastes* spp. Phylogenetic analysis of this parasite by *Renoux et al.* (2017) placed it at the base of a major monophyletic clade containing species of coccidia, suggesting it to be more closely related to this group than to the pirosplasms, the group to which the Haemohormidiidae have been assigned pending molecular support (see *O唐Donoghue*, 2017). As a follow-up to the work of *Cook et al.* (2015) and *Renoux et al.* (2017), the aim of the current study was to determine the geographic distribution and host-association of this parasite in damselfishes in the eastern Caribbean. Specifically, we: 1) further quantify which damselfish species and life history stages are infected by the *Haemohormidium*-like blood parasite, increasing the sample size for under-sampled species and including juvenile life history stages; and 2) further elucidate the geographic distribution and phylogenetic affiliation of this blood parasite in the eastern Caribbean.

2. Materials and methods

2.1. Host blood collection

This study was conducted between May 2013 and August 2016. Fish used in this study were collected on nearshore reefs from 0 to 7 m depth by free divers or scuba divers using modified cast nets or large monofilament hand nets. In order to further assess host associations among Caribbean damselfishes, and life history associations among *Stegastes* species, we sampled a total of 627 damselfish from sites at or near where infected fish had previously been found in at least one species in addition to two new sites (Fig. 1). These sites were: Great Lameshur Bay, St. John, United States Virgin Islands (USVI; 18.33° N, 64.73° W), two sites (Brewers Bay, Fortuna Bay) on St. Thomas (18.33° N, 64.91° W), USVI; White Bay, Guana Island, British Virgin Islands (BVI; 18.50° N, 65.30° W); Culebra, Puerto Rico (18.30° N, 65.30° W); La Parguera, Puerto Rico (17.97° N, 67.04° W); and Frederiksted St. Croix, USVI (17.71° N, 64.87° W). Collections from these sites included 39

![Fig. 1. Map of the Eastern Caribbean region showing collection sites for the current study and *Cook et al.*, 2015.](image-url)
**DNA extraction, PCR and phylogenetic analysis of 18S rDNA**

Fishes from the supplemental samples for molecular analysis (Supplement Table 3) and identified microscopically as infected with the *Haemohormidium*-like parasite with intensities of ≥1 infection per 500 erythrocytes were preferentially used for DNA extraction following a rapid DNA extraction method as detailed in the Kapa Express Extract Kit (Kapa Biosystems, Cape Town, South Africa). Molecular characterisation of the *Haemohormidium*-like parasite was performed via PCR amplification, amplifying approximately the full 18S rRNA gene using forward primer EF (5'-GAAACCTGGAAATGCTATT-3') and reverse primer ER (5'-CTTGGGCCCTACATTGGCACAT-3') (Kvičerová et al., 2008). Conditions for PCR were as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles, entailing a 95 °C denaturation for 30 s, annealing at 55 °C for 30 s with an end extension at 72 °C for 2 min, and following the cycles a final extension of 72 °C for 10 min.

All PCR reactions were performed with volumes of 25 μl, using 12.5 μl Thermo Scientific DreamTaq PCR master mix (2×), 2× DreamTaq buffer, 0.4 mM of each dNTP, and 4 mM MgCl₂, 1.25 μM of each primer (10 μM), and at least 25 ng of DNA. PCR grade nuclease free water (Thermo Scientific, Vilnius, Lithuania) was used to make up final reaction volume. Reactions were undertaken in a Bio-Rad C1000 Touch™ Thermal Cycler PCR machine (Bio-Rad, Hemel Hempstead, UK). An agarose gel (1%) stained with gel red was used to visualise resulting amplicons under UV light. Two PCR products from each sample were sent to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa) for purification and sequencing in both directions. Quality of resultant sequences was assessed using Geneious Ver. 7.1 (http://www.geneious.com, Karse et al., 2012) before consensus sequences were generated from both forward and reverse sequence reads. Sequences were identified using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/), and deposited in the NCBI GenBank database under the accession numbers: MH401637, MH401638, MH401639, MH401640, MH401641, MH401642 or MH401643-42.

For the phylogenetic analysis sequences generated of the *Haemohormidium*-like parasite from the different species of damselfish and from the different sites were compared. Comparative sequences of coccidia (with reference to the findings of Renoux et al., 2017) with *Adelina dimidiana* (GenBank: DQ096835) as outgroup (following Barta et al., 2012; Xavier et al., 2018), were downloaded from GenBank and aligned to the sequences generated within this study. Sequences were aligned using the Clustal W alignment tool (Thompson et al., 1994) implemented in Geneious Ver. 7.1. The alignment consisted of 43 sequences and was 1100 nt in length, with the exception of six sequences (MF468290, MF468291, MF468292, MF468293, MF468323, MF468328) being ~500 nt. These shorter sequences were included as they represent species of coccidia recently isolated from marine fish hosts by Xavier et al. (2018), two of these falling with a *Haemohormidium*-like parasite isolated by Renoux et al. (2017) (see Xavier et al., 2018). To infer phylogenetic relationships of the aligned dataset a Bayesian inference (BI) method was used. A model test was performed to determine the most suitable nucleotide substitution model, according to the Akaike information criterion (AIC) using jModelTest 2.1.7 (Guindon and Gascuel, 2003; Darriba et al., 2012). The best model identified was the General Time Reversible model with estimates of invariable sites and a discrete Gamma distribution (GTR + I + F). The BI analysis was performed using MrBayes software (ver. 3.2.6).
generations, log-likelihood scores were plotted, and only the Markov chain Monte Carlo (MCMC) chains were run for 10,000,000
of trees were used to produce the consensus trees by setting the ‘burn in’ parameter at 2500.

3. Results

3.1. Species and life history stage

3.1.1. Presence of blood parasites among damselfishes

A summary of infections among life history stages and species at sites used for species comparison is presented in Supplement Table 1. The intraerythrocytic Haemohormidium-like parasite found in this study was morphologically comparable to that described by Cook et al. (2015) (see Cook et al., 2015 Fig. 1a-e and present study Fig. 2a-c). Besides rare possible trophozoite stages (Cook et al., 2015 Fig. 2a) and possible meront stages of the parasite that appear to be undergoing transverse binary fission (Fig. 2b), the most common and characteristic stage of this parasite was what has been provisionally identified as a dividing meront stage with two to three slender nuclei (rarely four nuclei) (Fig. 2c). This stage measured 6.4 ± 0.4 μm (mean ± SD; range 5.6–7.6) × 1.9 ± 0.6 μm (mean ± SD; range 0.8–3.3) (n = 35) in the present study, compared to 5.7 × 1.5 μm (n = 10) in Cook et al. (2015).

No blood parasites were found in Microspathodon chrysurus (n = 39) or Abudefduf saxatilis (n = 23), even though these fish were collected from sites where the infection was common in Stegastes during this and/or a previous study (Cook et al., 2015). At localities where adult and juvenile Stegastes were sampled, blood parasites were found in both. These included S. leucostictus, S. planifrons and S. variabilis from White Bay, Guana Island; S. diencaeus, S. leucostictus, and S. planifrons from Lameshur Bay, St. John; and all 6 Stegastes species from St. Thomas. From La Parguera, both Stegastes adustus and Stegastes leucostictus juveniles harbored blood parasites. The smallest individual sampled in this study measured 2.6 cm, and the smallest that harbored blood parasites measured 2.9 cm. Of the five species-site combinations where sufficient numbers (n ≥ 10) of juveniles and adults of the same species were collected from the same site, four had blood parasites that were more prevalent in adults than juveniles.

Among the six Stegastes spp. at the six study sites with sufficient sampling (adults only), S. adustus had the highest proportion infected at 76.0% (95% CI 67.4–83.0%), followed by S. planifrons at 60.0% (95% CI 48.8–70.3%), S. diencaeus at 54.3% (95% CI 42.0–66.1%), S. leucostictus at 25.5% (95% CI 14.4–40.6%), S. variabilis at 14.3% (95% CI 7.1–25.9%), and S. partitus at 5.4% (95% CI 1.4–15.8%) (Fig. 3). Infection prevalences of S. adustus, S. planifrons, and S. diencaeus were each significantly greater than those of S. leucostictus, S. variabilis, and S. partitus (Table 1; GLMM: all pairwise comparisons with Tukey-adjusted p < 0.05). However, there were no significant differences in infection prevalence among the three species with higher prevalences (S. adustus, S. planifrons, and S. diencaeus; Table 1; GLMM: all pairwise comparisons with Tukey-adjusted p > 0.05), nor among the three species with lower prevalences (S. leucostictus, S. variabilis, and S. partitus; Table 1; GLMM: all pairwise comparisons with Tukey-adjusted p > 0.1). However, the lack of statistically significant differences between S. partitus and S. leucostictus and S. variabilis appears driven by one site in which three of four (75%) of S. partitus were infected (the only three infected fish among all adult S. partitus collected).

3.1.2. Geographic range of blood parasites in Stegastes of the eastern Caribbean

Blood parasites were found in one or more Stegastes individuals at nine of the sites sampled (Fig. 1). This included White Bay (Guana Island), St. John, St. Thomas (both sites), St. Croix, Puerto Rico (both sites), Curaçao, and Key Largo. Interestingly, only two individuals (one S. planifrons and one S. variabilis) were infected from the Florida Keys (= 1.7%), and none of the 49 fish sampled (23 S. partitus and 26 S. diencaeus) from Eleuthera were infected. A summary of infections at sites used for supplemental geographic comparison is presented in Supplement Table 2.
Table 1
Simultaneous tests for general linear hypotheses from a binomial logistic regression (GLMM) of infection prevalence as a function of host species (fixed effect) nested within study site (random effect).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Estimate*</th>
<th>Std. Error</th>
<th>z</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. diencaeus - S. adustus = 0</td>
<td>-1.015</td>
<td>0.364</td>
<td>-2.789</td>
<td>0.055</td>
</tr>
<tr>
<td>S. leucostictus - S. adustus = 0</td>
<td>-2.275</td>
<td>0.457</td>
<td>-4.976</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S. partitus - S. adustus = 0</td>
<td>-4.022</td>
<td>0.647</td>
<td>-6.216</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S. planifrons - S. adustus = 0</td>
<td>-0.758</td>
<td>0.338</td>
<td>-2.244</td>
<td>0.206</td>
</tr>
<tr>
<td>S. variabilis - S. adustus = 0</td>
<td>-2.967</td>
<td>0.445</td>
<td>-6.665</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S. leucostictus - S. diencaeus = 0</td>
<td>-1.260</td>
<td>0.450</td>
<td>-2.798</td>
<td>0.053</td>
</tr>
<tr>
<td>S. partitus - S. diencaeus = 0</td>
<td>-3.006</td>
<td>0.670</td>
<td>-4.488</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S. planifrons - S. diencaeus = 0</td>
<td>0.258</td>
<td>0.368</td>
<td>0.700</td>
<td>0.961</td>
</tr>
<tr>
<td>S. variabilis - S. diencaeus = 0</td>
<td>-1.952</td>
<td>0.460</td>
<td>-4.245</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S. partitus - S. leucostictus = 0</td>
<td>-1.746</td>
<td>0.726</td>
<td>-2.405</td>
<td>0.145</td>
</tr>
<tr>
<td>S. planifrons - S. leucostictus = 0</td>
<td>1.518</td>
<td>0.455</td>
<td>3.333</td>
<td>0.010</td>
</tr>
<tr>
<td>S. variabilis - S. leucostictus = 0</td>
<td>-0.692</td>
<td>0.526</td>
<td>-1.314</td>
<td>0.766</td>
</tr>
<tr>
<td>S. planifrons - S. partitus = 0</td>
<td>3.264</td>
<td>0.655</td>
<td>4.985</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S. variabilis - S. partitus = 0</td>
<td>1.055</td>
<td>0.717</td>
<td>1.471</td>
<td>0.145</td>
</tr>
<tr>
<td>S. variabilis - S. planifrons = 0</td>
<td>-2.209</td>
<td>0.450</td>
<td>-4.907</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Natural log of estimates is the multiplicative change in the odds of infection between 2 spp.
**P-values adjusted with Tukey contrasts for multiple comparisons of means.

3.2. Molecular identification and phylogenetic analysis

Amplicons (> 1300 nt) of the Haemohormidium-like parasite were retrieved from 3 of the 5 (60%) infected damselfish species that formed part of the subset collected for the molecular analysis including S. adustus, S. diencaeus and S. planifrons from 4 of the 6 (67%) sites including Guana Island, La Parguera (Puerto Rico), St. Croix, and St. Thomas (Fig. 4). According to the 18S rRNA gene, parasite isolates represent either the same parasite species or two closely related species. Those isolated from Stegastes spp. from Guana Island (GenBank: MH401637-9) had a 2nd difference (both insertions) from those of the other three sites (GenBank: MH401637-9). Isolates of these three sites compared with those of a Haemohormidium-like parasite isolated by Renoux et al. (2017) from a S. adustus (KT806397) and S. diencaeus (KT806398) from St John. The Haemohormidium-like parasite was basal to a major monophyletic clade containing species of coccidia, a finding comparable to that of Renoux et al. (2017). Furthermore, amplicons retrieved in this study and in Renoux et al. (2017) formed a monophyletic clade with that of apicomplexans of unknown identity retrieved during a molecular survey from tissues of the liver of Solea senegalensis (MF468328) and the heart of Pagrus caeruleostictus (MF468323), both species of fish collected from the Northeast Atlantic (see Xavier et al., 2018).

4. Discussion

Amplicomplex parasites of amphibians, reptiles and mammals are often characterized molecularly using the 18S rRNA gene. However, apicomplexans of fishes are almost exclusively identified morphologically, by comparing peripheral blood stages and their vectors (Davies and Johnston, 2000; Renoux et al., 2017). Here we combined morphological and molecular approaches. The distinctive morphological characteristics of this Haemohormidium-like species, particularly its small size and ‘meront’ stage development, support its identification in the six Stegastes species inhabiting the reefs of the eastern Caribbean, as the same species reported by Cook et al. (2015). Further evidence of this parasite’s presence in our samples is provided through molecular sequence data: highly similar sequences isolated from the two most frequently infected damselfish species, S. adustus and S. diencaeus, at four of our sites (five sites, if including those from Renoux et al. (2017)). If our morphological and molecular assessment is correct that this is the same parasite across sites and species, then the parasite has a wide geographic distribution and low host-specificity within the Stegastes genus; we have not yet detected it in any other Caribbean pomacentrids taken from the same sites, including Chromis spp. (n = 61, Cook et al., 2015), Abudelfalx saxatilis (n = 31, Cook et al., 2015 and this study), and Microspathodon chrysurus (n = 45, Renoux et al., 2017 and this study).

Based on morphological data alone, this Haemohormidium-like blood parasite appears to occur from the southernmost to the northernmost parts of the eastern Caribbean region. Outside of the Caribbean in the subtropical western Atlantic, the parasite was not found at our site in the Bahamas and was extremely rare in the Florida Keys. This may be because northern sites experience cool conditions in winter, which may reduce parasite and/or vector populations. We have yet to sample sites in the western Caribbean. The only other apicomplexan blood parasite of marine fishes recorded to date with a wide distribution and low host-specificity is the haemogregarine Haemogregarina (sensu lato) bigemina Laveran and Mesnil, 1901. H. bigemina has been recorded infecting fishes from 34 families across the world, but this distribution is based on morphology alone and has not yet been confirmed with molecular approaches (Davies et al., 2004; Cook et al., 2015).

Using parasite morphology alone, Cook et al. (2015) recorded similar Haemohormidium-like parasites as in the present study, except in another two families of Eastern Caribbean fishes. This included two larvid species, Nicholsina usta usta (n = 2 infected of 4 sampled) and Scarsus taeniopterus (n = 1 infected of 6 sampled), and one breamid Ophiolebias maculare (n = 9 infected of 14 sampled). However, the majority of infections reported by Cook et al. (2015) were from Stegastes spp. It would thus appear that this parasite may be genus-specific and the infections seen in the species of Labridae and Blenniidae an opportunistic case of host-switching or a different species entirely. Molecular analysis later revealed that the Haemohormidium-like parasite that infected O. macclueri was a different species than the one in Stegastes spp., even though the parasites were morphologically indistinguishable (Renoux et al., 2017).

The prevalence of this parasite in Stegastes spp. may be partially attributable to the variable feeding behaviors and ecologies within the genus. We found parasites in individuals as small as 3 cm in length. The highest prevalence, as mentioned above, was seen in S. adustus (nearly 80%) followed by S. planifrons and S. diencaeus (50–60%), then S. leucostictus and S. variabilis (20–25%), and S. partitus which was rarely infected. These differences track differences in social structure, feeding habits, and population density (Waldner and Robertson, 1980). The first three species are benthophagous, occupy hard reef structure with high algal growth, and occur in colonies of conspecifics that reach highest densities (Ferreira et al., 1998). While S. leucostictus and S. variabilis are also benthophagous, they tend to occur on rubble substrate and have larger territories, and thus occur in lower population densities. In contrast to the other five species, S. partitus is primarily planktivorous. A parasite’s mode of transmission is tied to host behavior. The benthophagous nature and high-density colonies of S. adustus facilitates exposure of the parasite to a number of new hosts on a continual basis. Similarly, if host behavior exposes the parasite to a wide variety of potential hosts, selection is inclined to favor host switching, that will in turn lead to a decrease in the host specificity of the parasite (Dick and Patterson, 2007), potentially explaining the wide distribution of this parasite, particularly in multiple species of Stegastes.

The variation in infection prevalence among Stegastes combined with the phylogenetic relationship of this blood parasite to other Apicomplexa (basal position relative to that of known coccidia species) suggests that it may be transmitted via an oral-fecal route via oocysts. Species of coccidia that do not demonstrate blood-borne stages form infective stages (oocysts), which are disseminated into the environment along with the excretion of waste, particularly feces. These sporozoite-containing oocysts are infective upon ingestion by an appropriate host (Kheyssin, 1972). Species of Stegastes appear to defecate primarily outside territorial boundaries (M. Nicholson and P. Sikkels, unpublished...
Fig. 4. Phylogenetic analysis of the Haemohormidium-like parasite based on 18S rDNA sequences. Bayesian inference (BI) analysis showing the phylogenetic relationships for 8 Haemohormidium-like parasite isolates, 6 from the present study (GenBank:MH401637-42) (in bold) and 2 from Renoux et al. (2017), isolated from three species of Stegastes including Stegastes adustus, Stegastes diencaeus and Stegastes planifrons, from 5 sites in the eastern Caribbean. Comparative sequences representing known coccidia, with Adelina dimidiata (DQ096835) as outgroup, were downloaded from the GenBank database. Nodal support values > 50% are represented on the tree.
data), leading to a higher likelihood of ingesting fishes for benthopha-
gous species that live in dense colonies. However, the majority of
coccidia do not show peripheral blood stages, with the exception of two
genera: Lankesterella and Schellackia, in which the sporozoites are en-
countered in the blood cells (Mega-Palma et al., 2014). Also, merozoite
stages of species of the genus Isospora (formerly regarded as a species of
Ataxoplasma) have been recorded infecting blood cells. Therefore, a
second route of transmission may be ingestion by blood-feeding in-
vertebrates that in turn act as paratenic transport hosts, infecting new
vertebrate hosts upon being eaten (O’Donoghue, 2017). In the Car-
ibbean, the blood-feeding gnathiid isopod Gnaithia marleyi is commonly
found to infest over 20 different species of bony fishes, including spe-
cies of Stegastes (Farquharson et al., 2012; Coile and Sikkel, 2013;
Jenkins et al., 2017). As such, there is the potential for this ectoparasite
to act as a paratenic host of the Haemohormidium-like parasite. Stegastes
have been observed to consume gnathiids (PC Sikkel unpublished data).
However, if this is the case and the parasite infecting Stegastes spp. is
not genus-specific, we would have expected to find infestations in fishes
that feed primarily on small invertebrates, especially in those for
which gnathiids appear to form part of the diet. Artim et al. (2017) recorded
gnathiids from the gut contents of five genera of micro carnivorous
fishes. Two of these genera of fishes, Haemulon and Holocentrus, were
sampled by Cook et al. (2015) with no record of the Haemohormidium-
like parasite at sites where this parasite is common in species of Ste-
gastes. It is, however, still possible that G. marleyi does act as a route of
infection of the Haemohormidium-like parasite. Dessier et al. (1990)
demonstrated experimentally that a leech could act as the vector of the
hemococcidian Lankesterella minima (Chaussat, 1950), providing evi-
dence that species of this genus may not only use the ingestion of
parasitized hosts for transmission, but also inoculation. As G. marleyi
gnathiids, in contrast to leeches, are commonly encountered infesting
damsel fishes in the eastern Caribbean (PC Sikkel unpublished data), a
third route of transmission involving inoculation of the Haemohor-
mium-like parasite by gnathiids needs to be considered.
Currently, based on 18S rDNA, the Haemohormidium-like parasite is
distinct from known genera of the coccidia, as well as from genera of the
piroplasms for which there are available molecular data (this study; Renoux et al., 2017). Unfortunately, it is not possible at this time to
compare this parasite on a molecular level to species of Haemohormi-
dium, as no molecular data have been provided for known species of
this genus. However, based on morphology, the parasite of the present
study does not share the typical characteristics of the type species of
Haemohormidium cotti Henry, 1910 (Davies, 1995; Cook et al., 2015;
Renoux et al., 2017). Until this parasite’s uncertain taxonomic identity
has been resolved, we suggest referring to it as Apicomplexa sp. The
phylogenetic position of this parasite may be better resolved in the
future with the addition of molecular samples of other taxa of fish
apicomplexan blood parasites, including known Haemoproteus spp. of
fishes, and the use of other molecular markers such as mitochondrial
(mtDNA) in combination with the 18S rDNA gene (Ogedengbe et al.,
2015). Future research should further elucidate the transmission
pathway of this parasite under laboratory conditions. This will include
screening parasitized fishes for other developmental stages of the
parasite, and investigating gnathiids as potential hosts/ vectors by
studying gnathiid loads on the different species of Stegastes, as well as
examining gnathiids for parasite development.

Conflicts of interest
None.

Acknowledgements
Funding for this project was provided by the National Research
Foundation (NRF) of South Africa (NRF project IFR170210222411, NJ
Smit, PI), the US National Science Foundation (grant number NSF OCE-
121615 and OCE-1536794, PC Sikkel, PI), Puerto Rico Sea Grant (grant
number R-31-1-14, PC Sikkel, PI), and the Falconwood Corporation.
Opinions expressed, and conclusions arrived at, are those of the authors
and are not necessarily those of the NRF, NSF, or Puerto Rico Sea Grant.
We thank M. Nicholson, A. Hook, E. Brill, G. Hendrick, H. Grattil, T.
Santos, and J. Sellers for assistance with collection and processing of
fishes. We are also grateful to the staff of Isla Maguayes Marine
Laboratory, McLean Marine Science Center, Cape Eleuthera Institute,
and Guana Island. This contribution 196 from the University of the
Virgin Islands Center for Marine and Environmental Studies and con-
tribution 255 from the North-West University-Water Research Group.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.
doi.org/10.1016/j.ijpaw.2018.05.004.

References
Arkouth, M.R., Casillas, E., Clemons, E., Kaygle, A.N., Olson, R., Reno, P., Stein, J.E.,
isopods on coral reefs: a comparison of Caribbean cleaning gobies with non-cleaning
Barta, J.R., Ogendengbe, J.D., Martin, D.S., Smith, T.G., 2012. Phylogenetic position of the
parasite, and investigating gnathiids as potential hosts/vectors by
studying gnathiid loads on the different species of Stegastes, as well as
eating gnathiids for parasite development.

Conflicts of interest
None.

Acknowledgements
Funding for this project was provided by the National Research
Foundation (NRF) of South Africa (NRF project IFR170210222411, NJ
Smit, PI), the US National Science Foundation (grant number NSF OCE-
121615 and OCE-1536794, PC Sikkel, PI), Puerto Rico Sea Grant (grant
number R-31-1-14, PC Sikkel, PI), and the Falconwood Corporation.
Opinions expressed, and conclusions arrived at, are those of the authors
and are not necessarily those of the NRF, NSF, or Puerto Rico Sea Grant.
We thank M. Nicholson, A. Hook, E. Brill, G. Hendrick, H. Grattil, T.
Santos, and J. Sellers for assistance with collection and processing of
fishes. We are also grateful to the staff of Isla Maguayes Marine
Laboratory, McLean Marine Science Center, Cape Eleuthera Institute,
and Guana Island. This contribution 196 from the University of the
Virgin Islands Center for Marine and Environmental Studies and con-
tribution 255 from the North-West University-Water Research Group.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.
doi.org/10.1016/j.ijpaw.2018.05.004.

References
Arkouth, M.R., Casillas, E., Clemons, E., Kaygle, A.N., Olson, R., Reno, P., Stein, J.E.,
isopods on coral reefs: a comparison of Caribbean cleaning gobies with non-cleaning
Barta, J.R., Ogendengbe, J.D., Martin, D.S., Smith, T.G., 2012. Phylogenetic position of the
parasite, and investigating gnathiids as potential hosts/vectors by
studying gnathiid loads on the different species of Stegastes, as well as
eating gnathiids for parasite development.

Conflicts of interest
None.

Acknowledgements
Funding for this project was provided by the National Research
Foundation (NRF) of South Africa (NRF project IFR170210222411, NJ
Smit, PI), the US National Science Foundation (grant number NSF OCE-
121615 and OCE-1536794, PC Sikkel, PI), Puerto Rico Sea Grant (grant
number R-31-1-14, PC Sikkel, PI), and the Falconwood Corporation.
Opinions expressed, and conclusions arrived at, are those of the authors
and are not necessarily those of the NRF, NSF, or Puerto Rico Sea Grant.
We thank M. Nicholson, A. Hook, E. Brill, G. Hendrick, H. Grattil, T.
Santos, and J. Sellers for assistance with collection and processing of
fishes. We are also grateful to the staff of Isla Maguayes Marine
Laboratory, McLean Marine Science Center, Cape Eleuthera Institute,
and Guana Island. This contribution 196 from the University of the
Virgin Islands Center for Marine and Environmental Studies and con-
tribution 255 from the North-West University-Water Research Group.
Jenkins, W.G., Demopoulos, A.W.J., Sikkel, P.C., 2017. Effects of host injury on sus-
dx.doi.org/10.1007/s13199-017-0518-z.
ological and morphological features. Parasitology 135, 443–452.
McVicar, A.H., 1997. Disease and parasite implications of the coexistence of wild and cul-
Murch, G., Gutter, A.S., Cribb, T.H., 2007. Structure of the parasite communities of a coral reef fish assemblage (Labridae): testing ecological and phylogenetic host fac-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-
O'Donoghue, P., 2017. Haemoprotozoa: making biological sense of molecular phylo-
Newcombe, R.G., 1998. Two-sided confidence intervals for the single proportion: com-