Molecular detection of apicomplexan blood parasites of coral reef fishes from free-living stages of ectoparasitic gnathiid isopods

Article *in* Parasitology Research · April 2020

DOI: 10.1007/s00436-020-06676-6

6 authors, including:

Paul Sikkel
University of Miami
110 PUBLICATIONS **1,588** CITATIONS
SEE PROFILE

Juan Andres Pagan
CIBIO Research Center in Biodiversity and Genetic Resources
4 PUBLICATIONS **22** CITATIONS
SEE PROFILE

Joana L. Santos
University of Basel
24 PUBLICATIONS **113** CITATIONS
SEE PROFILE

Gina Hendrick
University of Miami
7 PUBLICATIONS **39** CITATIONS
SEE PROFILE

Molecular detection of apicomplexan blood parasites of coral reef fishes from free-living stages of ectoparasitic gnathiid isopods

Paul C. Sikkel, J. Andres Pagan, Joana L. Santos, Gina C. Hendrick, Matthew D. Nicholson & Raquel Xavier

Parasitology Research
Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113

Parasitol Res
DOI 10.1007/s00436-020-06676-6

Springer

Parasitology Research
https://doi.org/10.1007/s00436-020-06676-6

FISH PARASITOLOGY - SHORT COMMUNICATION

Molecular detection of apicomplexan blood parasites of coral reef fishes from free-living stages of ectoparasitic gnathiid isopods

Paul C. Sikkel[1,2] · J. Andres Pagan[3] · Joana L. Santos[3] · Gina C. Hendrick[1] · Matthew D. Nicholson[1] · Raquel Xavier[3]

Received: 18 December 2019 / Accepted: 24 March 2020

Abstract

Gnathiid isopods are marine ectoparasites that feed on the blood of fishes that have been implicated as vectors of blood parasites, with transmission possibly occurring through biting during their parasitic life-stages, or through ingestion by fishes. However, evidence for their role as vectors is limited, reflecting the small number of research groups working on them. Here, we used a molecular barcode approach to identify fish hosts and apicomplexan parasites in free-living gnathiids from the eastern Caribbean Sea, with the goal of further evaluating their potential role as reservoirs and/or vectors for these parasites. Apicomplexa were only identified in 8% of the *Gnathia* analyzed, and in four cases we could identify both Apicomplexa and fish host DNA. The results further suggest that Gnathia spp. in this region may serve as reservoirs for Apicomplexa, but whether they are vectors for this parasite remains uncertain.

Keywords DNA barcoding · Pathogen reservoir · Pathogen vector · Caribbean Sea · *Gnathia*

Introduction

Parasites are represented in all phyla and comprise about half of all living organisms globally (e.g., Holmstad et al. 2005). Parasitism can influence hosts by inhibiting growth rate, altering normal behavior, and decreasing fitness/reproductive success (Wood and Johnson 2015), consequently impacting population and community dynamics (Holmstad et al. 2005). Therefore, understanding how parasites exploit their hosts and characterizing parasite life cycles is essential for understanding population and community processes.

Assessing patterns of host-exploitation in ecological communities is complicated by the myriad life history strategies employed by parasitic organisms. For example, some parasites are host specialists remaining permanently associated with an individual host, whereas others are host-generalists exploiting multiple hosts during their life cycle. Some parasites live inside their host (endoparasites), whereas others reside on the outside (ectoparasites). Among the latter, some spend most or all of their life attached to a host, while others may associate only temporarily.

Understanding the magnitude of parasite biodiversity, life history and ecological impacts is more difficult in marine ecosystems compared with most other environments (Poulin et al. 2016). This is particularly so for highly diverse systems such as coral reefs (e.g., Cook et al. 2015), and is due in large part to the sheer diversity of potential hosts and thus their associated parasites that must be sampled. Moreover, there is a paucity of skilled taxonomists and ecologists working in these systems able to identify and characterize the parasite community (Poulin 2014).

DNA-based identification is a powerful tool that has been incorporated into many current biomonitoring studies and has resulted in the discovery of new taxa (reviewed in de Sousa et al. 2019). Using DNA barcoding for species identification is

Section Editor: Panagiotis Karanis

✉ Paul C. Sikkel
psikkel@astate.edu

✉ Raquel Xavier
raq.xavier@cibio.up.pt

[1] Department of Biological Sciences and Environmental Sciences Program, Arkansas State University, PO Box 599, State University, Jonesboro, AR 72467, USA

[2] Water Research Group, Unit for Environmental Sciences and Management, Potchefstroom Campus, North West University, Private Bag X6001, Potchefstroom 2520, South Africa

[3] CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

Published online: 24 April 2020

currently accessible, reliable, and widely adopted, supplementing and even replacing traditional morphology-based species identification (e.g., de Sousa et al. 2019), including for parasite species (e.g., Bakhoum et al. 2018). A variety of molecular barcodes have been developed for targeting different taxonomic groups, (e.g., de Sousa et al. 2019), with 18S rRNA often used to barcode Apicomplexan parasites, including those from fish (e.g., Renoux et al. 2017).

Apicomplexan parasites are obligate parasites that infect a variety of vertebrate species. Indeed, it is estimated that every vertebrate species is host to at least one apicomplexan parasite (Votýpka et al. 2017). Apicomplexans can have relatively minor impacts on their hosts or can cause severe damage or death (e.g., Kristmundsson et al. 2015). Most blood-borne apicomplexans 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 blood-feeding (hematophagous) invertebrate (definitive) host. Transmission of infective sporozoite stages from the infected invertebrate host occurs either through inoculation, such as in the haemosporidia (e.g., species of *Plasmodium*), piroplasms (e.g., species of *Babesia*) and some haemogregarines (e.g., species of *Haemogregarina*), or through ingestion of the infected invertebrate, as in most haemogregarines (e.g., species of *Hepatozoon*). Haemococcidia, such as species of *Lankesterella* and *Schellackia*, by comparison, complete development in their vertebrate host, with invertebrates acting only as paratenic or mechanical hosts when ingested by the vertebrate (O'Donoghue 2017). The vast majority of work on the phylum Apicomplexa has focused on *Plasmodium* and other genera of socioeconomic importance in terrestrial systems. Much less is known about apicomplexan parasites in marine fishes or in coral reef systems..

While little is known about apicomplexans or any other blood parasites of coral reef fishes, even less is known about their transmission (see Smit et al. 2006), with few attempts made at identifying vectors and experimental transmission (see Smit et al. 2006; Curtis et al. 2013). On land, hematophagous (blood-feeding) arthropods are perhaps the best-known and most thoroughly studied vectors of blood parasites. Their ecological equivalent in the marine environment are gnathiid isopods (Isopoda: Gnathiidae), which feed on the blood of a single fish host during each of their three juvenile life history stages (Smit and Davies 2004) and thus, also have the potential to transmit blood parasites between hosts. Like their terrestrial counterparts (e.g., mosquitoes and ticks), gnathiids associate only temporarily with their hosts, spending most of their life-history free-living on or near the substrate.

For a long time, leeches were assumed to be the main vectors of apicomplexan blood parasites in fishes (e.g., Smit et al. 2006). However, some investigators have proposed that gnathiid larvae may also be vectors of these pathogens (e.g., Davies and Johnston 1976). This suggestion was subsequently supported by observations made in temperate gnathiids (e.g., Smit et al. 2003) and more recent studies on the Great Barrier Reef (e.g., Curtis et al. 2013).

In a recent survey of hemoparasite biodiversity of reef-associated fishes of the eastern Caribbean, Cook et al. (2015) sampled 1298 individual fish from six 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 (Cook et al. 2015). The host fishes included members of the families Pomacentridae, Labrisomidae, Blenniidae, Scaridae, Carangidae, and Pomacanthidae, however, the invertebrate vector for any of these has yet to be identified.

Here, we applied a molecular barcoding strategy to free-living individuals of *Gnathia* from the north-east Caribbean, to identify both their recent fish hosts and Apicomplexa parasites. Using this methodology, we gain insight into the possible role of *Gnathia* as reservoirs and as potential vectors for pathogens. To do so, we used the portions of the *cytochrome oxidase subunit I* to identify *Gnathia* species and fish hosts, and a portion of the *18S rRNA* to identify apicomplexan blood parasites.

Methods

Gnathiid isopods ($n = 109$) were collected as part of a broader study on population genetics of a gnathiid species from four sites in the north-east Caribbean Sea. These included shallow (< 10 m) reefs in Lindquist Beach (18°20′20.5"N 64°51′ 20.2"W), St. Thomas and Maho Bay (18°21′37.7"N 64°44′ 43.7"W), St. John, US Virgin Islands, two deeper reefs (25-150 m) off the south side of St. Thomas, US Virgin Islands (18°12′11.7"N 64°57′50.6"W), and one site off Punta Cana, Dominican Republic (18°32′24.9"N 68°20′49.9"W). We refer to these reefs below as "Lindquist Beach", "Maho Bay", "Meso", "90 ft", and "DR", respectively.

Gnathiids were collected using light traps as described in Artim and Sikkel (2016), which capture both fed and unfed free-living gnathiids. Fed gnathiids are easily distinguished from unfed gnathiids by their distended abdomen that is full of host blood or body fluids. Traps were set in the late afternoon and retrieved the following morning. Contents were then sorted under a dissecting microscope, and gnathiids were sorted between pranizae (fed, $n = 57$) and zuphea (unfed, $n = 52$) individuals. Fed individuals were collected in Maho Bay ($n = 20$), Lindquist Beach ($n = 20$), and Meso Reef ($n = 17$). Unfed individuals were collected in the DR ($n = 28$) and 90 ft. reef ($n = 24$). Whole specimens were placed individually in eppendorfs and genomic DNA (gDNA) was extracted from

entire specimens using the Invitrogen PureLink genomic DNA mini extraction kit. Briefly, individuals were digested using Proteinase K, and genomic DNA was purified using a spin-column based centrifugation procedure, following the manufacturer's protocol (Invitrogen, Carlsbad, California).

The DNA of each specimen was used in three Polymerase Chain Reaction protocols (PCR) described in Table 1. The first PCR was performed to identify gnathiids to species; the second PCR aimed at barcoding fish hosts by sequencing gnathiid blood meals; and the final PCR targeted Apicomplexa parasites using primers which are able to amplify taxa across the different groups (*Eimeria*, *Goussia*, *Haemohormidium*-like, among others, Xavier et al. 2018). PCR products were sent for sequencing to a commercial company. Sequences were manually checked and edited using Codon Code Aligner V.8.0.1 (Codon Code Corporation, Centerville, Massachusetts) and BLAST algorithm was used to find the best sequence match in NCBI's GenBank database.

Results and discussion

The initial PCR to identify gnathiids to species successfully amplified all individuals and Protein BLAST results revealed *Gnathia trimaculata* as the closest match (maximum divergence 23.89%) and the presence of three species (interspecific divergence ranging from 24.0% to 27.1%). One of the species we were able to morphologically identify as *Gnathia marleyi*, but for the other two species identification remains unknown (Table 2). PCRs that aimed to identify fish hosts yielded 102 amplicons, however, after sequencing, only 10 were identified as fish DNA (percent identity ranging between 99 and 100%) (Table 2), with the remainder mostly displaying low quality or belonging to non-target organisms, such as bacteria. The final PCR that aimed to screen blood parasites yielded 25 amplicons, all from fed gnathiids. Of these, 9 were a ≥ 97% match to an Apicomplexa sp. reference sequence on GenBank, all of which correspond to *Haemohormidium*-like lineages previously found in fish blood from the Caribbean region and detailed in Renoux et al. (2017) and Sikkel et al. (2018) (Table 2). None of these apicomplexans has yet to be described. In total, two individuals collected from Lindquist Beach and seven individuals from Maho Bay tested positive for Apicomplexa. Fish hosts were successfully identified for four of the samples positively tested for Apicomplexa: one individual from Lindquist Beach matched to Lambrisomus sp., and three individuals from Maho Bay had fish host DNA that matched Lambrisomus sp., *Holocentrus rufus* and *Ocyurus chrysurus*.

Approximately 8% of the gnathiid isopods screened hosted Apicomplexa and these we identified only from fed gnathiids, confirming that *Gnathia* can serve as potential reservoirs for pathogens, but whether gnathiids may be vectors for these pathogens still remains unknown. In cases where both the apicomplexan and the fish host could be identified from the Pranizae (fed) stages, in two cases (Labrisomus spp.), the host was among the species previously reported by Cook et al. (2015) to harbor apicomplexans in this region. However, in the other two cases (*Holocentrus rufus*, and *Ocyurus chrysurus*), the identified most recent host was not among those previously known to harbor apicomplexan blood parasites. Among 170 Holocentrid species sampled by Cook et al.

Table 1 Primers and PCR protocols used in the present study

Gene	Target species	Primer pair, reference	PCR protocol (*Taq* used)	Amplicon size
COI	Gnathia sp.	Forward: gmar5COIfor (GGGATTTTTAGAGAATGAGCA) Pagán et al. 2020 Reverse: jgHCO2198 (TAIACYTCIGGRTGICCRAARAAYCA), Geller et al. 2013	95 °C for 15 mins; 35 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s; 72 °C for 10 mins (QIAGEN master mix, Venlo, Netherlands)	650 bp
COI	Fish hosts	Primer cocktail COI-3 (Ivanova et al. 2007)	95 °C for 15 mins; 35 cycles of 95 °C for 45 s, 54 °C for 45 s, 72 °C for 90 s; 72 °C for 10 mins (HOT FIREPol® DNA Polymerase, Solis BioDyne, Estonia)	679 bp
18S rRNA	Apicomplexa	Forward: HepF300 (GTITCTGACCTATCAGCTTICGACG), Ujvari et al., 2004 Reverse: Hep900 (CAAATCTAAGAATTICACCTCTGAC), Ujvari et al., 2004	95 °C for 15 mins; 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 90 s; 72 °C for 10 mins (HOT FIREPol® DNA Polymerase, Solis BioDyne, Estonia)	600 bp

Table 2 List of samples for which positive indentifications of Apicomplexa and/or fish hosts were retrieved. The closest matches on Genbank are depicetd. Genbank accession numbers for the sequences produced in the present study are depicted in the following order: *Gnathia* spp., Apicomplexa sp. and fish host

Locality	Sample code	Gnathiid species	Apicomplexan sequences BLAST result (Reference GenBank Accession)	Fish sequences BLAST result (Reference GenBank Accession)	Gnathiid status	Sequence accession number
Lindquist	Lindquist 1_17	Gnathia sp1	–	*Gerres cinereus* (KT005474)	Fed	MT317108, -, MT308640
Lindquist	Lindquist 3_17	Gnathia sp1	Apicomplexa sp. (KY940307)	*Labrisomus* sp. (JQ839805)	Fed	MT317109, MT296588, MT308641
Lindquist	Lindquist 15_17	Gnathia sp1	Apicomplexa sp. (MH401640)	–	Fed	MT317110, MT296587, -
Maho Bay	Maho 4–17	Gnathia sp1	Apicomplexa sp. (MH401640)	*Labrisomus* sp. (JQ840554)	Fed	MT317101, MT296593, MT308645
Maho Bay	Maho 5–17	Gnathia sp1	–	*Haemulon flavolineatum* (JQ842143)	Fed	MT317102, -, MT308646
Maho Bay	Maho 6–17	*G. marleyi*	–	*Ctenogobius saepepallens* (AY077609)	Fed	MT186562, -, MT308647
Maho Bay	Maho 7–17	Gnathia sp1	Apicomplexa sp. (MH401640)	–	Fed	MT317103, MT296594, -
Maho Bay	Maho 14–17	Gnathia sp1	Apicomplexa sp. (MH401640)	*Holocentrus rufus* (JQ840538)	Fed	MT317104, MT296589, MT308642
Maho Bay	Maho 15–17	*G. marleyi*	Apicomplexa sp. (MH401640)	*Ocyurus chrysurus* (MK297435)	Fed	MT186562, MT296595,M-T308643
Maho Bay	Maho 16–17	*G. marleyi*	Apicomplexa sp. (MH401640)	–	Fed	MT186562, MT296590, -
Maho Bay	Maho 17–17	Gnathia sp2	–	*Lutjanus synagris* (JQ839827)	Fed	MT317105, -, MT308644
Maho Bay	Maho 18–17	*G. marleyi*	Apicomplexa sp. (MH401640)	–	Fed	MT186562, MT296591, -
Maho Bay	Maho 19–17	Gnathia sp2	–	*Lutjanus synagris* (KF461200)	Fed	MT317106, -, MT308648
Maho Bay	Maho 20–17	Gnathia sp1	Apicomplexa sp. (MH401640)	–	Fed	MT317107, MT296592, -
Meso Reef	Meso 15–17	Gnathia sp2	–	*Gymnothorax moringa* (MF041684)	Fed	MT317100, -, MT308649

(2015), including 108 *H. rufus*, no apicomplexan blood parasites were found. Similarly, no apicomplexan blood parasites were found among 181 Lutjanid species, including 62 *O. chrysurus*. Given that only the most recent host can be detected (Hendrick et al. 2019); it is possible that the gnathiid was infected with the apicomplexan prior to feeding on the host. Although we did not find apicomplexans in unfed (z-stage) gnathiids, these were all collected from sites in which local fish have not been screened for the presence of apicomplexans.

In the Caribbean, blood-feeding Gnathia spp. are commonly found to infest over 40 different species of bony fishes, including species of Labrisomidae, Blenneidae, and Pomacentridae (e.g., Coile and Sikkel 2013, Hendrick et al. 2019). Species of pomacentridae S*tegastes* damselfish, as well as Labrisomid and Blenneid blennies, eat gnathiids (M. Nicholson and P. Sikkel unpublished data), and thus transmission of blood parasites can potentially occur either through *Gnathia* bites or by trophic transmission through ingestion of infected *Gnathia*. Initial efforts to identify vectors of eastern Caribbean haematozoans focused on blennoid and damselfish as hosts, and gnathiid isopods as potential vectors (Cook et al. 2015). While results for blennoids were largely consistent with other studies, suggesting gnathiid isopods as vectors of apicomplexans in coral reef fishes, Cook et al. (2015) did not find similar evidence for the haemohormidium-like parasite in *Stegastes* damselfishes.

Several previous studies have used molecular screening to detect the presence of apicomplexan parasites in ticks parasitizing reptiles and mammals (e.g., Harris et al. 2013; Greay et al. 2018; Hornok et al. 2017). While some just reported prevalence data (e.g., Harris et al. 2013), others led to

unexpected findings, such as the discovery of new species of Apicomplexa (Greay et al. 2018) or the inference on the connectivity of host populations based on the genetic structure of parasite populations (Hornok et al. 2017). Far more effort has so far been placed on identifying important human pathogens, such as *Plasmodium* species in *Anopheles* mosquitoes, employing PCR –based approaches (e.g., Murillo et al. 2019) due to the high specificity and accuracy of such assays. To the best of our knowledge, ours is the first study attempting to identify potential pathogens of fishes by screening their ectoparasites, with results showing that *Gnathia* may indeed be reservoirs of fish-infecting apicomplexans. In order to understand the role of *Gnathia* as vectors for apicomplexan parasites, future studies should focus on zuphea (unfed) stages in areas where fish are known to harbor apicomplexan blood parasites and on further experimental studies.

Acknowledgments We thank E. Brill, A. Hook, A. McCammon, A. Packard, S. Robles, C. Bennett, A. Coble, A. Savage, M. Luckin for assistance with data collection. We also thank the staff of the University of the Virgin Islands McLean Marine Science Center, Virgin Islands Environmental Resource Station, Guana Island Resort, and Isla Magueyes Marine Laboratory for logistic support. This is contribution # 214 from the University of the Virgin Islands Center for Marine and Environmental Studies.

Funding information Funding was provided by the US National Science Foundation grant OCE-1536794 (PC Sikkel, PI), the Falconwood Corporation, as well as the Portuguese Foundation for Science and Technology through FCOMP-01-0124-FEDER- 029939-PTDC/MAR-BIO/0902/2014 and POCI-01-0145-FEDER-016550 (RX as PI); PhD grant PD/BD/114357/2016 (to AP) and iFCT contract IF/00359/2015 (to RX). The authors declare no conflicts of interest.

References

Artim JM, Sikkel PC (2016) Comparison of sampling methodologies and estimation of population parameters for a temporary fish ectoparasite. Int J Parasitol Parasites Wildl 5:145–157. https://doi.org/10.1016/j.ijppaw.2016.05.003

Bakhoum MT, Sarr M, Fall AG, Huber K, Fall M, Sembène M, Seck MT, Labuschagne K, Gardès L, Ciss M, Gimonneau G, Bouyer J, Baldet T, Garros C (2018) DNA barcoding and molecular identification of field-collected *Culicoides* larvae in the Niayes area of Senegal. Parasite Vector 11:615. https://doi.org/10.1186/s13071-018-3176-y

Coile AM, Sikkel PC (2013) An experimental field test of susceptibility to ectoparasitic gnathiid isopods among Caribbean reef fishes. Parasitology 140(7):888–896

Cook CA, Sikkel PC, Renoux LP, Smit NJ (2015) Blood parasite biodiversity of reef-associated fishes of the eastern Caribbean. Mar Ecol Prog Ser 533:1–13. https://doi.org/10.3354/meps11430

Curtis L, Grutter AS, Smit NJ, Davies AJ (2013) *Gnathia aureamaculosa*, a likely definitive host of *Haemogregarina balistapi* and potential vector for *Haemogregarina bigemina* between fishes of the Great Barrier Reef, Australia. Int J Parasitol 43:361–370

Davies AJ, Johnston MRL (1976) The biology of Haemogregarina bigemina Laveran & Mesnil, a parasite of the marine fish Blennius pholis Linnaeus. J Protozool 23(2):315–320

de Sousa LL, Silva SM, Xavier R (2019) DNA metabarcoding in diet studies: unveiling ecological aspects in aquatic and terrestrial ecosystems. Environ DNA 1:199–214. https://doi.org/10.1002/edn3.27

Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol Ecol Res 13: 851–861. https://doi.org/10.1111/1755-0998.12138

Greay TL, Zahedi A, Krige A, Owens JM, Rees RL, Ryan UM, Oskam CL, Irwin PJ (2018) Endemic, exotic and novel apicomplexan parasites detected during a national study of ticks from companion animals in Australia. Parasite Vectors 11:197. https://doi.org/10.1186/s13071-018-2775-y

Harris DJ, Graciá E, Jorge F, Maia JPMC, Perera A, Carretero MA, Giménez A (2013) Molecular detection of *Hemolivia* (Apicomplexa: Haemogregarinidae) from ticks of north African *Testudo graeca* (Testudines: Testudinidae) and an estimation of their phylogenetic relationships using 18S rRNA sequences. Comp Parasitol 80:292–296. https://doi.org/10.1654/4594.1

Hendrick GC, Dolan MC, McKay T, Sikkel PC (2019) Host DNA integrity within blood meals of hematophagous larval gnathiid isopods (Crustacea, Isopoda, Gnathiidae). Parasite Vectors 12:316. https://doi.org/10.1186/s13071-019-3567-8

Holmstad PR, Hudson PJ, Skorping A (2005) The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. Oikos 111:377–391. https://doi.org/10.1111/j.0030-1299.2005.13640.x

Hornok S, Szőke K, Görföl T, Földvári G, Tu VT, Takács N, Kontschán J, Sándor AD, Estók P, Epis S, Boldogh S, Kováts D, Wang Y (2017) Molecular investigations of the bat tick *Argas vespertilionis* (Ixodida: Argasidae) and *Babesia vesperuginis* (Apicomplexa: Piroplasmida) reflect "bat connection" between Central Europe and Central Asia. Exp Appl Acarol 72:69–77. https://doi.org/10.1007/s10493-017-0140-z

Ivanova NV, Zemlek TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x

Kristmundsson Á, Erlingsdóttir Á, Freeman MA (2015) Is an Apicomplexan parasite responsible for the collapse of the Iceland scallop (*Chlamys islandica*) stock? PLoS One 10:e0144685. https://doi.org/10.1371/journal.pone.0144685

Murillo E, Muskus C, Agudelo LA, Vélez ID, Ruiz-Lopez F (2019) A new high-resolution melting analysis for the detection and identification of *Plasmodium* in human and *Anopheles* vectors of malaria. Sci Rep 9:1674. https://doi.org/10.1038/s41598-018-36515-9

O'Donoghue P (2017) Haemoprotozoa: making biological sense of molecular phylogenies. Int J Parasitol Parasites Wildl 6:241–256

Pagán JA, Veríssimo A, Sikkel PC, Xavier R (2020) Hurricane-induced disturbance increases genetic diversity and population admixture of the direct-brooding isopod, Gnathia marleyi. Sci Rep (in press)

Poulin R (2014) Parasite biodiversity revisited: frontiers and constraints. Int J Parasitol 44:581–589

Poulin R, Blasco-Costa I, Randhawa HS (2016) Integrating parasitology and marine ecology: seven challenges towards greater synergy. J Sea Res 113:3–10

Renoux LP, Dolan MC, Cook CA, Smit NJ, Sikkel PC (2017) Developing an Apicomplexan DNA barcoding system to detect blood parasites of small coral reef fishes. J Parasitol 103:366–376. https://doi.org/10.1645/16-93

Sikkel PC, Cook CA, Renoux LP, Bennett CL, Tuttle LJ, Smit NJ (2018) 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. Int J Parasitol Parasites Wildl 7:213–220. https://doi.org/10.1016/j.ijppaw.2018.05.004

Smit NJ, Davies AJ (2004) The curious life-style of the parasitic stages of gnathiid isopods. Adv Parasit 58:289–391. https://doi.org/10.1016/S0065-308X(04)58005-3

Smit NJ, Basson L, Van As JG (2003) Life cycle of the temporary fish parasite, Gnathia africana (Crustacea: Isopoda: Gnathiidae). Folia Parasit 50(2):135–142

Smit NJ, Grutter AS, Adlard RD, Davies AJ (2006) Hematozoa of teleosts from Lizard Island, Australia with some comments on their possible mode of transmission and the description of a new hemogregarine species. J Parasitol 92:778–788. https://doi.org/10.1645/GE-756R.1

Ujvari B, Madsen T, Olsson M (2004) High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in water pythons (*Liasis fuscus*) from tropical Australia. J Parasitol 90:670–672. https://doi.org/10.1645/GE-204R

Votýpka J, Modrý D, Oborník M, Šlapeta J, Lukeš J (2017) Apicomplexa. Handbook of the Protists. Springer, Cham

Wood CL, Johnson PT (2015) A world without parasites: exploring the hidden ecology of infection. Front Ecol Environ 13:425–434. https://doi.org/10.1890/140368

Xavier R, Severino R, Pérez-Losada M, Gestal C, Freitas R, Harris DJ, Veríssimo A, Rosado D, Cable J (2018) Phylogenetic analysis of apicomplexan parasites infecting commercially valuable species from the north-East Atlantic reveals high levels of diversity and insights into the evolution of the group. Parasit Vectors 11:63. https://doi.org/10.1186/s13071-018-2645-7