Journal of Helminthology

cambridge.org/jhl

Ocular spirorchiidiosis in sea turtles from Brazil

H. Jerdy[1], M. Werneck[2], D. Goldberg[3], P. Baldassin[4], R. Feriolli[1], A. Maranho[5], R. Ribeiro[1], M. Bianchi[1], L. Ribeiro[1], E. Shimoda[1] and E. Carvalho[1]

[1]Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF, Campos dos Goytacazes, RJ CEP 28013-602, Brazil; [2]BW Consultoria Veterinária LTDA ME, Est. Da Praia Seca n. 12.143 (L. 41), CEP 28.970-000, Praia Seca, Araruama, RJ, Brazil; [3]Rede Rio Doce Mar - RRDM, Campus Carreiros Rio Grande, Av. Itália km 8 S/N, CEP 96203-900, RS, Brazil; [4]Serviços em Meio Ambiente, CTA, Rua Saturnino Rangel Mauro 283, Pontal de Camburi, Vitória, ES CEP 29062-030, Brazil and [5]Instituto Argonauta, Estrada Guarujá Bertioga, Km 13.5, Guarujá-SP, CEP 11680-000, Brazil

Short Communication

Cite this article: Jerdy H *et al* (2020). Ocular spirorchiidiosis in sea turtles from Brazil. *Journal of Helminthology* **94**, e56, 1–4. https://doi.org/10.1017/S0022149X1900049X

Received: 22 February 2019
Accepted: 14 May 2019

Key words:
Spirorchiidiosis; choroiditis; visual capacity; optic nerve; cachexia

Author for correspondence: H. Jerdy,
E-mail: hjerdy@hotmail.com

Abstract

The causes of the beaching and death of sea turtles have not been fully clarified and continue to be studied. Mild, moderate and severe lesions caused by spirorchiidiosis have been seen for decades in different organs and were recently defined as the cause of death of a loggerhead turtle. In the present study, eyes and optic nerves were analysed in green sea turtles with spirorchiidiosis and no other debilitating factors. Injuries to the optic nerve and choroid layer were described in 235 animals (90%) infected with spirorchiids. Turtles with ocular spirorchiidiosis are approximately three times more likely to be cachectic than turtles with spirorchiidiosis without ocular involvement.

Introduction

Pathological changes caused by cardiovascular flukes (Spirorchiidae) in sea turtles stem mainly from the deposition and subsequent dissemination of eggs through the circulatory system, with descriptions of occurrence in various organs (Santoro *et al.*, 2007; Flint *et al.*, 2010; Stacy *et al.*, 2010; Chen *et al.*, 2012). Tissue injuries in hosts range from mild to severe (Santoro *et al.*, 2017). The histological examination of tissues provides valuable data and is particularly useful for cases in which small egg granulomas cannot be observed macroscopically (Glazebrook *et al.*, 1989; Gordon *et al.*, 1998; Stacy *et al.*, 2010). The main finding is an inflammatory reaction composed of multinucleated giant cells, although eggs can be found without an inflammatory response in some cases (Jerdy *et al.*, 2016). The severe consequences of spirorchiidiosis are aneurysm, endocarditis, arteritis, embolism and thrombosis (Glazebrook *et al.*, 1989; Gordon *et al.*, 1998; Stacy *et al.*, 2010; Werneck *et al.*, 2015).

Adult cardiovascular flukes live in the cardiovascular system of the host, where they lay eggs that can travel to practically all tissues and organs. Injuries in the spleen, intestine, lung, stomach and pancreas due to the presence of eggs are common findings (Stacy *et al.*, 2010; Chen *et al.*, 2012).

Despite the many reports of eggs from the family Spirorchiidae in hosts throughout the world, there have only been two scientific reports describing eggs in the choroid layer of the eyes (Glazebrook *et al.*, 1981, 1989). Moreover, the consequences and severity of these injuries were not addressed in the studies cited.

Therefore, the aim of the present study was to report 235 cases of ocular spirorchiidiosis, as well as describe the different lesions and possible consequences.

Materials and methods

A total of 256 *Chelonia mydas* juveniles (curvilinear carapace length (CCL): 35–70 cm; weight: 4.3–20 kg), one adult *Caretta caretta* (CCL: 116 cm; weight: 100 kg) and one adult *Lepidochelys olivacea* (CCL: 72 cm; weight: 39.8 kg) with spirorchiidiosis were examined. All sea turtles were either found dead, stranded ($n = 239$) in the states of Santa Catarina, São Paulo and Rio de Janeiro, Brazil, or died at rehabilitation centres ($n = 17$) between 2015 and 2017. CCL (in centimetres) and weight (in kilograms) were determined. For each sea turtle, the body condition was classified using the method described by Stacy *et al.* (2010) based on the examination of the pectoral muscles (i.e. poor, intermediate or robust). A reciprocating saw was used to open the skull for the removal of the eyes and part of the optic nerve, following the method described by Wyneken (2001). The samples were collected, fixed in 10% neutral formalin solution for 24 h and sent to the Laboratory of Animal Pathology at the Darcy Ribeiro Northern Fluminense State University in the state of Rio de Janeiro, Brazil. The samples were cleaved and stored in disposable plastic tissue cassettes. The paraffin blocks were cut into 5-μm sections using a rotary microtome. The sections were stained with haematoxylin and eosin and mounted on slides for histopathologic examination under a light microscope. The severity of the injuries was classified based on Santoro *et al.* (2017).

Turtles were classified as juveniles or adults based on CCL measurements, considering the smallest measures of curved carapace length for nesting females in Brazil (see Kotas *et al.*, 2004; Grossman *et al.*, 2007; Marcovaldi & Chaloupka, 2007; Silva *et al.*, 2007; Thomé *et al.*, 2007; Sales *et al.*, 2008; Santos *et al.*, 2010; Lima *et al.*, 2012 for reference values).

To determine changes in thickness, the width of the choroid layer was measured in 40 animals with affected and normal areas. Eggs from eyes were digested for analysis. The study by Wolke *et al.* (1982) was used for the determination of eggs in the ocular layer and optic nerves: type 1 (fusiform eggs with polar processes), type 2 (oval-shaped eggs with one process) and type 3 (rounded eggs with no processes). Morphometric data on normal and affected portions of the choroid layer and eggs (expressed in µm and mean ± standard deviation (range)) were determined with the aid of a Nikon Eclipse 80i microscope (Kurobane Nikon Co., Ltd., Otawara, Tochigi, Japan) using the NIS Elements BR software programme.

Statistical analysis involved the binary logistic equation to obtain odds ratios for animals with mild, moderate or severe injuries presenting cachexia compared to animals without injuries. The analyses were performed in the R software with the level of significance set to 5% ($P < 0.05$).

Results and discussion

Among the green sea turtles with spirorchiidiosis, 233 of 256 had eggs with a giant-cell inflammatory reaction in the choroid layer. Among these 233, 146 were microscopically considered mild cases, 48 were considered moderate cases and 39 were considered severe cases of ocular spirorchiidiosis (substitution of choroid layer by parasitic granuloma). In this group of 233 turtles with ocular spirorchiidiosis, the body classification of 133 (57%) was cachectic, 55 (24%) were thin and 45 (19%) had a good body score. For 23 green turtles with spirorchiidiosis, but without ocular spirorchiidiosis, six (32%) were cachectic, five (20%) were thin and 12 (48%) had a good body score. Macroscopically, all eyes and optic nerves exhibited no abnormalities at the time of collection.

Cases of severe, moderate and mild ocular spirorchiidiosis were associated with body condition. In mild cases ($n = 146$), 83 (56%) were cachectic, 33 (22%) were thin and 30 (20%) had a good body score. In moderate cases ($n = 48$), 29 (60%) were cachectic, ten (21%) were thin and nine (19%) had a good body score. In severe cases ($n = 39$), 23 (59%) were cachectic, 11 (28%) were thin and five (13%) had a good body score.

The odds ratio of cachexia among animals with mild lesions compared to those without lesions was 3:73 (confidence interval (CI) = 1.39 to 10.01, *P*-value = 0.005), meaning that the presence of mild injury increased the chance of an animal being cachectic by 273%. The odds ratio for cachexia among animals with moderate injuries was 4.32 (CI = 1.45 to 12.94; *P*-value = 0.006) compared to those without injury, meaning that animals with moderate injuries are 332% more likely to be cachectic than those without injury. The odds ratio for cachexia in animals with severe injuries was 4.07 (CI = 1.32 to 12.59; *P*-value = 0.011). Thus, the chance of animals with severe injuries being cachectic is 307% higher compared to animals without injuries.

Microscopically, affected eyes were compared to a normal ocular bulb (fig. 1a). Vascular tunic vessels, especially those of the choroid, were multifocal, distended and thick due to granulomatous inflammation causing partial to complete obstruction. Inflammation was caused by eggs of the family Spirorchiidae enveloped by multinucleated giant cells that formed coalescing granulomas (fig. 1b). Loose reactive connective tissue surrounded the clusters and melanocytes of the choroid layer. Only a few vessels were affected in milder cases. In more severe cases ($n = 39$), all vessels of the choroid layer were affected by embolisms and thrombi formed by massive clusters of eggs enveloped by multinucleated giant cells that coalesced with adjacent clusters of eggs and ruptured the walls of the vessels, forming extensive parasitic giant-cell granulomas. In severe cases, the choroid layer was completely disorganized and largely replaced by giant-cell granulomas, scarce loose reactive connective tissue, rare blood capillaries and adult parasites in the vascular lumen (fig. 1c). The infection found in *C. caretta* involved multifocal clusters (five eggs per cluster) of eggs from the family Spirorchiidae and was classified as moderate. In *L. olivacea*, infection consisted of multifocal individualized eggs and was classified as mild (fig. 1d).

Among the 30 optic nerves analysed, five had type 3 eggs (most likely a species of Neospirorchis) enveloped by multinucleated giant cells, characterizing parasitic granulomatous neuritis (fig. 1e).

In all affected eyes, the choroid layer with severe lesions was thicker than foci with mild lesions. Even with mild injury, the choroid layer was thicker than sites without injury. In the 40 animals for which the choroid layer was measured, the width of the choroid layer was increased by an average of 213 µm in areas with a granulomatous inflammatory response (fig. 1f). The greatest thickness was 717 µm.

Only egg types 1 and 3 were found in the eyes and optic nerves of the animals studied. Among the 30 animals infected, six were cases of infection by type 1 eggs, 11 were cases of concomitant infection by type 1 and type 3 eggs, and 13 were cases of infection by type 3 eggs.

In the morphometric analysis, 83 type 1 eggs had a mean length (without the processes) of 324 ± 9 (range: 313–346 µm) and mean width of 47 ± 3 (range: 41–54). One hundred and thirty-four type 3 eggs had a mean length of 42 ± 3 (range: 36–47 µm) and mean width of 33 ± 2 (range: 31–39 µm).

Although the histological findings described by Glazebrook (1981, 1989) are similar to those of the present report, the author did not describe an increase in the thickness of the choroid layer or the coalescence of egg masses. Therefore, the present study may involve more severe cases of ocular spirorchiidiosis.

Numerous arteries and veins separated by a stroma compose the choroid layer. These vessels constitute an important source of oxygen and nutrients for the retina, pigmented epithelium and photoreceptors (Walls, 1942). Unlike what occurs physiologically, the choroid layer loses this function in cases of severe ocular spirorchiidiosis and also releases inflammatory factors through macrophages and lymphocytes capable of harming the cells and structures of the retina.

Inflammation of the optic nerve can cause disturbances in the transmission of nerve signals. Choroiditis and neuritis are extremely painful conditions that cause considerable discomfort (Maggs *et al.*, 2017).

Among the 233 *C. mydas* with ocular spirorchiidiosis, the majority 158 (81%) were cachectic or thin. Animals with severe ocular infection had severely affected choroid layers. Less severe cases could also cause focal visual loss by the thickening of the choroid layer. Spirorchidiosis exerts a direct influence on the body score (Flint *et al.*, 2010). Indeed, no other cause of cachexia was found during necropsy or the microscopic analysis. Ocular

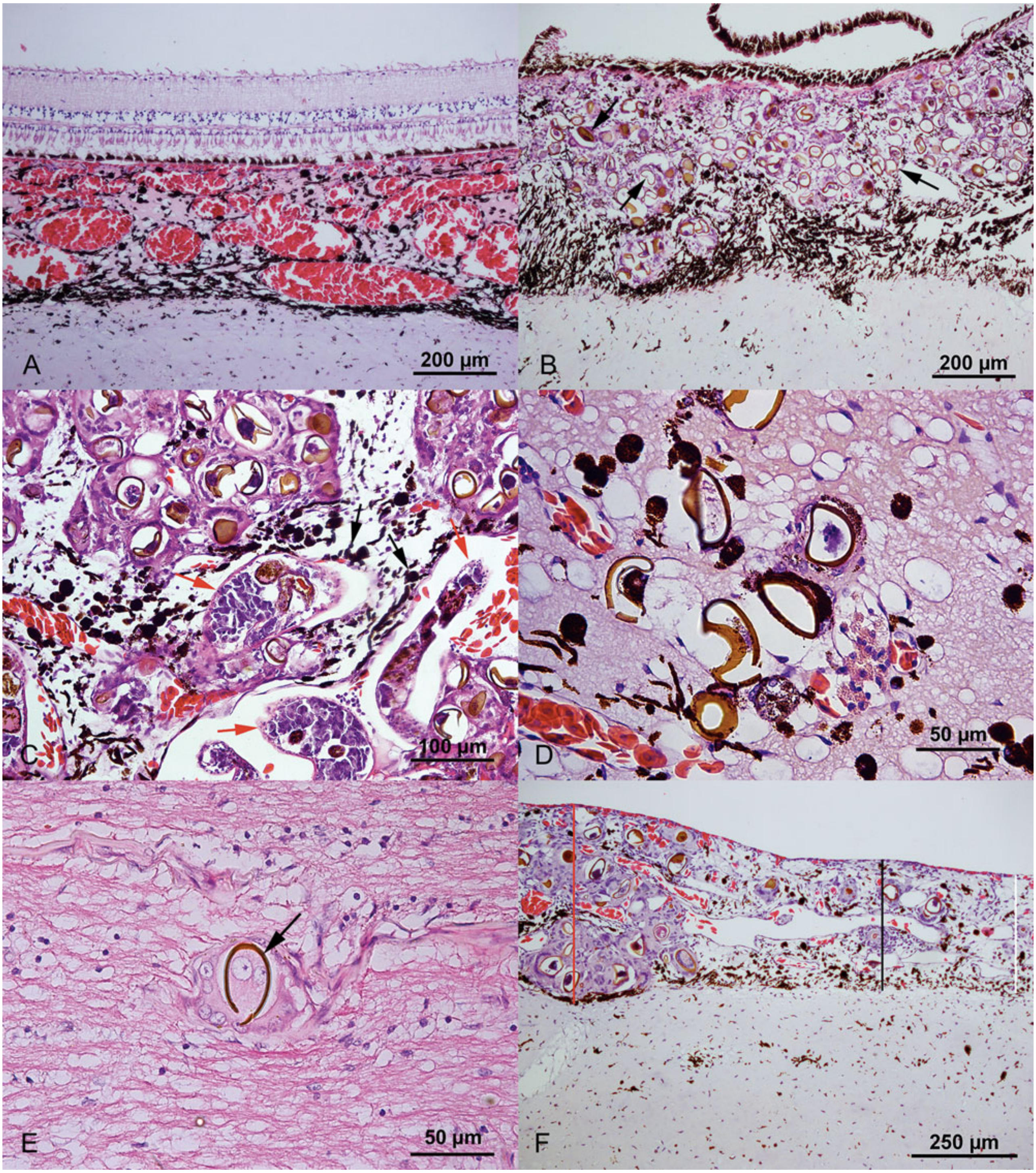

Fig. 1. (A) Normal *C. mydas* eye, presenting sclera (bottom), choroid layer (middle-vascular) and retina (top); (B) Choroid replete with golden-brown trematode eggs (arrow). Blood vessels severely replaced by coalescing large granulomas that involved Spirorchiidae eggs in *C. mydas*. Contrast with (A) with same magnification; (C) Severe spirorchiid infection in choroid exemplified by numerous clusters of golden-brown trematode eggs surrounded by histiocytes and giant cells admixed with dysplastic melanocytes (black arrows) that largely efface vascular architecture. Note adult spirorchiids (red arrows); (D) Mild ocular spirorchiidiosis associated with few eggs in choroid layer of *L. olivacea*. 'Contrast with (C)'; (E) Spirorchiidae egg (black arrow) surrounded by giant multinucleated cells in *C. mydas* optic nerve; (F) Large granuloma (red bar) with 460 µm length, mild granuloma (black bar) with 310 µm length and normal choroid layer (white bar) with 280 µm length in *C. mydas*. Note the difference in thickness of the choroid layer with different lesion degrees: severe (red bar), moderate (black bar) and mild (white bar).

lesions may increase the occurrence of cachexia in turtles with spirorchidiosis, since animals with moderate to severe lesions had higher percentages of thinness and cachexia. The statistical analysis showed that turtles with ocular lesions caused by spirorchiidiosis are about 300% more likely to be thin or cachectic than those with spirorchidiosis but without ocular injury.

Reports of parasitic granulomatous choroiditis caused by eggs of parasites from the family Spirorchiidae are scarce and this

https://doi.org/10.1017/S0022149X1900049X Published online by Cambridge University Press

phenomenon is not well understood. Green turtles with severe choroiditis have increased choroid layer thickness and are more likely to be cachectic. The injuries found in the present study were severe, with the complete loss of function of the choroid layer, tissue architecture and normal thickness in many cases. Based on the present findings, it is plausible to think that ocular spirorchiidiosis may cause vision disturbance, with the partial or total loss of sight

Author ORCIDs. H. Jerdy, 0000-0002-2373-2453

Acknowledgements. Biological samples were obtained through the 'Phase 1' beach monitoring project in the states of Santa Catarina and São Paulo and the 'Phase 2' beach monitoring project in the state of Rio de Janeiro. Analyses of the parasites were authorized by federal licenses for activities with scientific purposes (SISBIO 30600-1 and 9329-1). This project is part of the requirements established by the federal environmental licensing process of the Brazilian Environmental Agency (IBAMA) for the exploration of oil and gas by PETROBRAS in the Santos Basin pre-salt layer.

Financial support. This work was conducted during a scholarship supported by the International Cooperation Program CAPES/COFECUB at the University of Northern Rio de Janeiro, financed by CAPES – Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil.

Conflicts of interest. None.

Ethical standards. No experimentation was performed in this study.

References

Chen H, Kuo RJ, Chang TC, Hus CK, Bray RA and Cheng IJ (2012) Fluke (Spirorchiidae) infections in sea turtles stranded on Taiwan: prevalence and pathology. *Journal of Parasitology* **98**, 437–439.

Flint M, Patterson-Kane JC, Limpus CJ and Mills PC (2010) Health surveillance of stranded green turtles in Southern Queensland, Australia (2006-2009): an epidemiological analysis of causes of disease and mortality. *EcoHealth* **7**, 135–145.

Glazebrook JS, Campbell RSF and Blair D (1981) Pathological changes associated with cardiovascular trematodes (Digenea: Spirorchidae) in a green sea turtle *Chelonia mydas* (L). *Journal of Comparative Pathology* **91**, 361–368.

Glazebrook JS, Campbell RSF and Blair D (1989) Studies on cardiovascular fluke (Digenea: Spirorchiidae) infections in sea turtles from the great barrier reef, Queensland, Australia. *Journal of Comparative Pathology* **101**, 231–250.

Gordon AN, Kelly WR and Cribb TH (1998) Lesions caused by cardiovascular flukes (Digenea: Spirorchiidae) in stranded green turtles (*Chelonia mydas*). *Veterinary Pathology* **35**, 21–30.

Grossman AP, Mendonça MRC and Bellini C (2007) Morphometrics of the green turtle at the Atol das Rocas Marine Biological reserve, Brazil. *Marine Turtle Newsletter* **118**, 12–13.

Jerdy H, Ribeiro RB, Silva MA, Medina RM, Werneck MR and Carvalho EC (2016) Spirorchiid infection in olive Ridley turtle, *Lepidochelys olivacea* (Eschscholtz, 1829) (Testudines: Cheloniidae), from Brazil. *Journal of Parasitology* **102**, 290–292.

Kotas JE, Dos Santos S, Azevedo VG, Gallo BMG and Barata PCR (2004) Incidental capture of loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) sea turtles by the pelagic longline fishery off southern Brazil. *Fishery Bulletin* **102**, 393–399.

Lima EPE, Wanderlinde J, Almeida DT, Lopez GG and Goldberg DW (2012) Nesting ecology and conservation of the loggerhead sea turtle (*Caretta caretta*) in Rio de Janeiro, Brazil. *Chelonian Conservation & Biology* **11**, 249–254.

Maggs DJ, Miller PE and Ofri R (2017) *Slatter's fundamentals of veterinary ophthalmology*. 6th edn. Missouri, Saunders/Elsevier.

Marcovaldi MA and Chaloupka M (2007) Conservation status of the loggerhead sea turtle in Brazil: an encouraging outlook. *Endangered Species Research* **3**, 133–143.

Sales G, Giffoni BB and Barata PCR (2008) Incidental catch of sea turtles by the Brazilian pelagic longline fishery. *Journal of the Marine Biological Association* **88**, 853–864.

Santoro M, Di Nocera F, Iaccarino D, *et al.* (2017) Pathology and molecular analysis of *Hapalotrema mistroides* (Digenea: Spirorchiidae) infecting a Mediterranean loggerhead turtle *Caretta caretta*. *Diseases of Aquatic Organisms* **124**, 10–18.

Santoro M, Morales JA and Rodriguez-Ortiz B (2007) Spirorchiidiosis (Digenea: Spirorchiidae) and lesions associated with parasites in Caribbean Green turtles (Chelonia mydas). *Veterinary Record* **161**, 482–486.

Santos AJB, Freire EMX, Bellini C and Corso G (2010) Body mass and the energy budget of gravid hawksbill turtles (*Eretmochelys imbricata*) during the nesting season. *Journal of Herpetology* **44**, 352–359.

Silva ACCD, Castilhos JC, Lopez GG and Barata PCR (2007) Nesting biology and conservation of the olive Ridley sea turtle (*Lepidochelys olivacea*) in Brazil, 1991/1992 to 2002/2003. *Journal of the Marine Biological Association* **87**, 1047–1056.

Stacy BA, Foley AM, Greiner E, Herbst LH, Bolten A, Klein P, Manire CA and Jacobson ER (2010) Spirorchiidiasis in stranded loggerhead *Caretta caretta* and green turtles *Chelonia mydas* in Florida (USA): host pathology and significance. *Diseases of Aquatic Organisms* **89**, 237–259.

Thomé JCA, Baptistotte C, Moreira LMP, Scalfoni JT, Almeida AP, Rieth DB and Barata PCR (2007) Nesting biology and conservation of the leatherback sea turtle (*Dermochelys coriacea*) in the state of Espírito Santo, Brazil, 1988–1989 to 2003–2004. *Chelonian Conservation & Biology* **6**, 15–27.

Walls GL (1942) *The vertebrate eye and its adaptive radiation*. New York, NY, Fafner Publishing Company.

Werneck MR, Souza GB, Berger BC*et al.* (2015) Pathological changes by Hapalotrema postorchis Rao 1976 (Digenea: Spirorchiidae) in a green turtle Chelonia mydas Linnaeus 1758 (Testudines, Cheloniidae) from Brazil. *Helminthologia* **52**, 148–154.

Wolke RE, Brooks R and George A (1982) Spirorchidiasis in loggerhead sea turtles (*Caretta caretta*): pathology. *Journal of Wildlife Diseases* **18**, 175–185.

Wyneken J (2001) *The anatomy of sea turtles*. US Department of Commerce. NOAA Technical Memorandum NMFS-SEFSC-470, 172.