

Report of the presence of *Hapalotrema synorchis* and *H. postorchis* (Digenea: Spirorchiiidae) in marine turtles (Reptilia: Cheloniidae) in Northern Territory waters

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Abstract

The spirorchiid digenean *Hapalotrema synorchis* was recovered from the heart of a juvenile Hawksbill Turtle (*Eretmochelys imbricata*) found deceased on a local Darwin beach. The turtle was in poor condition, showing many characteristics associated with spirorchiid infection. A second necropsied turtle showed signs of infection with spirorchiiids but adult specimens were not recovered. Examination of specimens held at the Berrimah Veterinary Laboratory found another *E. imbricata* infected with *H. synorchis* and a Green Turtle (*Chelonia mydas*) infected with the related *H. postorchis*. Despite previous reports of infected turtles, this is the first confirmed identification of *H. synorchis* and *H. postorchis* from Northern Territory waters.

Marine turtles are hosts to spirorchiid digeneans that inhabit the cardiovascular system, including the tissues and blood vessels of all the major organs (Glazebrook *et al.* 1989; Chapman *et al.* 2015; Flint *et al.* 2015). Spirorchiiids have been implicated as causes of strandings and mortality in turtles around the world, with infected turtles often showing muscle wastage, plastron shrinkage and general listlessness (Glazebrook *et al.* 1989; Stacy *et al.* 2010).

Clinical signs of infection with spirorchiiids include sunken eyes, plastron shrinkage and generalised muscle wastage (Glazebrook *et al.* 1981; Gordon *et al.* 1998) and in some cases, neurological symptoms (Jacobsen *et al.* 2006). However, the direct correlation of spirorchiid infection with disease in turtles is poorly understood (Stacy *et al.* 2010). Apparently healthy turtles were found to harbour significant spirorchiid infections by Stacy *et al.* (2010), while Glazebrook *et al.* (1989) reported only 26% of infested turtles displayed any clinical signs, which were non-specific. Gordon *et al.* (1998) found that 98% of stranded turtles showed evidence of infection with spirorchiiids, although only

45% contained adult digeneans. Flint *et al.* (2010) found that 75% of turtles presented for necropsy were infected with spirorchids, which were deemed the primary cause of death in around 42% of cases. Egg granulomas can be found in high numbers in turtles where adult digeneans are apparently absent (Gordon *et al.* 1998; Flint *et al.* 2010).

Although reports of cardiovascular digeneans in stranded marine turtles from Northern Territory waters are known (Limpus 2009; Mackouras & Griffiths 2014), no species-level identification has been undertaken. Mackouras & Griffiths (2014) reported on the pathological examination of eleven marine turtles stranded from June 2012 to June 2014 in Northern Territory waters. One of six Green Turtles (*Chelonia mydas*) was found to have died as a result of severe cardiovascular digenean infection; two of five Hawksbill Turtles (*Eretmochelys imbricata*) were infected with cardiovascular digeneans, but these were not considered the cause of death.

A juvenile *E. imbricata*, was submitted to the Berrimah Veterinary Laboratories (BVL) for post-mortem assessment after it was found deceased on a local beach (in the suburb of Nightcliff (12.3783°S, 130.8453°E) on 10 July 2014. The turtle weighed 2.5 kg and externally appeared thin and in poor condition. During necropsy it was noted that the abdominal fat was absent except for remnants attached to the plastron (lower shell) and around organs and there was extensive clear fluid present within the abdomen and pericardial sac. Granulomas containing digenean eggs were present in the blood vessels associated with the lungs, liver, heart, intestine and fat. Digeneans were recovered from the heart and placed into a vial of 70% ethanol.

A second (female) *E. imbricata* was submitted to the BVL after it was found deceased entangled in a net at Lee Point (12.3292°S, 130.8844°E) on 8 January 2015. The turtle weighed 3.5 kg and had a curved carapace length of 35 cm. The turtle was in good body condition. No digeneans were recovered from the heart or lungs. A small number of egg granulomas were observed in the blood vessels of the intestinal system.

A subsequent search of the BVL parasite collection found two vials of digeneans identified as *Hapalotrema* sp. The first came from an *Eretmochelys imbricata* collected from Bare Sand Island, Fog Bay (12.5369°S, 130.4189°E) in 1997 and the second from a *Chelonia mydas* (collection data listed as Darwin) in 2003.

From each sample, the anterior end (anterior to the ventral sucker) of one specimen was removed, placed into 100% ethanol, and used for genetic analysis. The remaining portion of that specimen was stained with aceto-orcein, dehydrated through a graded ethanol series and mounted in Canada Balsam. The 1997 *E. imbricata* vial contained a number of specimens, so an intact specimen was also mounted. Further specimens were left intact as unmounted specimens.

The digeneans recovered from the 2014 *E. imbricata* were identified as *Hapalotrema synorchis* through a combination of morphological and genetic analyses. The specimens recovered from the 1997 *E. imbricata* were also identified as *H. synorchis* and the specimens from



Fig. 1. *Hapalotrema synorchis*. Lateral view of whole specimen orientated upside-down as is Fig. 2, the convention for digeneans. Abbreviations: OS, oral sucker; VG, vitelline (= yolk) glands; VS, ventral sucker. Scale bar = 1 mm. (Adam Bourke)

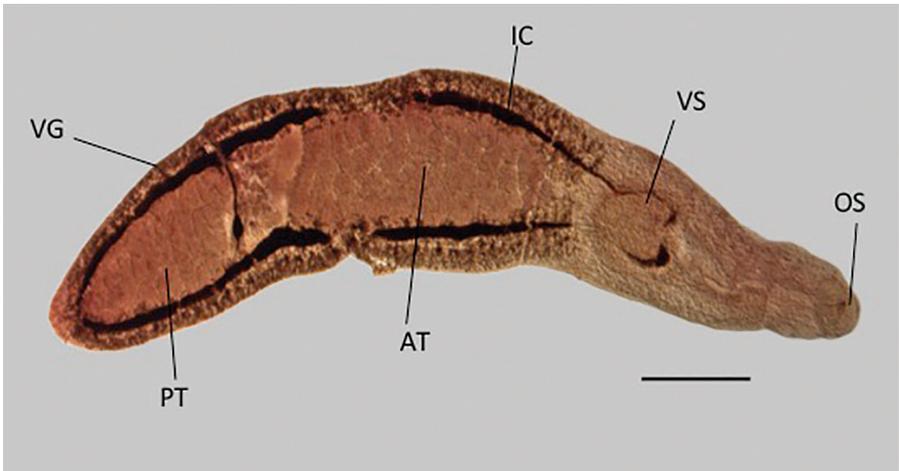


Fig. 2. *Hapalotrema synorchis*. Ventral view of stained whole mount specimen. Abbreviations: AT, anterior testes; IC, intestinal caecum; OS, oral sucker; PT, posterior testes; VG, vitelline (= yolk) glands; VS, ventral sucker. Scale bar = 1 mm. (Adam Bourke)

the *Chelonia mydas* were identified as *H. postorchis* through morphology; unfortunately the genetic analysis was unsuccessful on these specimens, possibly because they may have been initially preserved in formalin.

The specimens collected in this study were easily identified to the genus *Hapalotrema* based on the morphological criteria of the genus (see Platt 2002). Morphologically, the specimens from *E. imbricata* exhibited the testes arrangement as indicated in Platt and Blair (1998) for *H. synorchis*, with testes forming compact anterior and posterior masses. Species identification was confirmed via genetic analysis using the PCR and sequencing methodology described in Chapman *et al.* (2015) for one specimen. A BLAST search was conducted on the resulting sequence, which indicated that the closest match (99% similarity) was *H. synorchis*. The specimens from *Chelonia mydas* were identified

as *H. postorcbis* due to a similar number and arrangement of the testes (16 counted; 9 posteriorly, 7 anteriorly) and the vitelline glands as described by Dailey *et al.* (1993).

Hapalotrema synorchis was described by Luhmann in 1935, from a Loggerhead Turtle (*Caretta caretta*) from Tortugas, Florida (Platt & Blair 1998). Subsequent records of *H. synorchis* occurred from *Chelonia mydas* in the Gulf of Mexico (Caballero y Caballero 1962) and from *E. imbricata* in Puerto Rico (Fischthal & Acholonu 1976). Takeuti (1942) described *H. orientalis* from *E. japonica* (= *E. imbricata*) in Japanese waters, which was subsequently synonymised with *H. synorchis* by Platt & Blair (1998).

Platt & Blair (1998) reported *C. caretta* as a host for *H. synorchis* at Shark Bay, Western Australia (4 turtles), Heron Island (2) and Mon Repos (1) in central Queensland waters. Chapman *et al.* (2015) subsequently reported *H. synorchis* from *E. imbricata* from Redland Bay and Buddina Beach in south-east Queensland. In the study by Chapman *et al.* (2015), *H. synorchis* was found in two of three *E. imbricata*, but no *Chelonia mydas* from Queensland (of 22) or Hawaiian (of 10) waters. Stacy (2008) also reported *H. synorchis* from *C. caretta* from Florida (originally described as *H. pambanensis*, however later amended to *H. synorchis* when further sequences became available for comparative molecular analysis (see Chapman *et al.* 2015).

Hapalotrema postorcbis was described by Rao from *Chelonia mydas* in the Gulf of Manar, India (Dailey *et al.* 1993). It has subsequently only been reported from the same host species in Hawaii (Dailey *et al.* 1993; Chapman *et al.* 2015: one of ten hosts examined) and various locations in south-east (Moreton Bay; Coolum) and central Queensland (Quoin Island, Gladstone Harbour) (Cribb & Gordon 1998: three infections recorded from an unstated total number of hosts examined; Gordon *et al.* 1998: three infections from 96 hosts examined; Chapman *et al.* 2015: total of three from 22 hosts examined).

Reports of infections with *Hapalotrema* from Australian waters are confusing as many reports of digeneans infecting turtles were undertaken prior to the review of the genus (see Cribb & Gordon 1998). Glazebrook *et al.* (1981) reported a *Hapalotrema* sp. from a heavily infected *Chelonia mydas* off Townsville, North Queensland. Although they stated that it was a different species to *H. synorchis*, Glazebrook *et al.* (1989) subsequently listed it as *H. synorchis* in their literature records of cardiovascular digeneans recovered from sea turtles. However, *H. synorchis* has never been reported from another *Chelonia mydas* in Australia, despite a large number of this turtle species being examined (see Chapman *et al.* 2015). Morphologically, *H. synorchis* is very similar to *H. pambanensis* (a synonym of *H. mebrai*), so it is more likely, without genetic confirmation, that the original record from Glazebrook *et al.* (1981) belonged to this species.

Four species of marine turtles are found in the waters of the Northern Territory (Mackouras & Griffiths 2014). During the period 2012–2014, a total of 60 marine turtles were reported as stranded in the Northern Territory; the most common species was the Green Turtle (*Chelonia mydas*; 26) followed by the Hawksbill (*E. imbricata*; 16) (Mackouras & Griffiths 2014). The vast coastline of the Northern Territory and the array of large

marine predators (saltwater crocodiles and sharks) unfortunately limits the access to fresh carcasses required for necropsy for a systematic survey of diseases of marine turtles.

During the 2012–2014 reporting period, 11 turtles were necropsied at the BVL. Of these, one (of six) *Chelonia mydas* was determined to have died from a severe cardiovascular digenean infection and one (of five) *E. imbricata* contained a heavy cardiovascular digenean infection, but this was not the suspected cause of death (Mackouras & Griffiths 2014). A further *E. imbricata* contained possible cardiovascular digenean egg granulomas, but no adult digeneans were observed (Mackouras & Griffiths 2014). Unfortunately, no digeneans specimens were available from these necropsies for examination in this study. A study into stranded Green Turtles from Gladstone, central Queensland, found that nine (of twelve) turtles were infected with spirorchiid digeneans at a level that could have contributed to their death (Flint *et al.* 2015). All turtles, however, were infected with a variety of spirorchiiids that belonged to five genera, including *Hapalotrema*.

Hapalotrema synorchis is a widespread parasite that has been positively identified from three species of marine turtles in waters of Queensland and Western Australia, Japan, the Gulf of Mexico, Florida and Puerto Rico. *Hapalotrema postorchis* has only been reported in *Chelonia mydas*, with a distribution in waters of India, Queensland and Hawaii. This is the first confirmed report of the presence of *H. synorchis* and *H. postorchis* in waters of the Northern Territory.

The following voucher specimens have been deposited in the collections of the Museum and Art Gallery of the Northern Territory: *H. synorchis* from *E. imbricata* (2014) (D1532); *H. synorchis* from *E. imbricata* (1997) (D1545); *H. postorchis* from *Chelonia mydas* (D1546). The Genbank accession number for the *H. synorchis* is KT361641.

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