



Molecular characterization and lesions associated with *Diomedinema diomedea* (Aproctoidea: Desmidocercidae) from grey-headed albatrosses (*Thalassarche chrysostoma*) on Subantarctic Marion Island

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ABSTRACT

The Prince Edward Islands are Subantarctic islands in the southwest Indian Ocean that are of global importance as seabird nesting sites, and are breeding grounds for five species of albatrosses (Procellariiformes: Diomedidae). In March–April 2016 numerous chicks of one of these species, the grey-headed albatross (*Thalassarche chrysostoma*), were found dead at colonies on Marion Island (46°57'S 37°42'E), the larger of the two Prince Edward Islands. Affected chicks were weak, prostrated, apathetic, had drooping wings, and many eventually died while sitting on the nest. Five carcasses were necropsied, and samples were obtained for pathological and parasitological analysis. Four chicks appeared to have died from starvation, and one died due to air-sac helminthiasis, with extensive hemorrhage in the air sacs and multifocal pyogranulomatous air-sacculitis. The air sac parasites were identified as *Diomedinema diomedea* (Aproctoidea: Desmidocercidae). Phylogenetic analysis of the nuclear 18S rRNA gene and mitochondrial COI gene confirmed that *D. diomedea* belongs to the suborder Spirurina and showed that it is most closely related to the Diplotriaeinae (superfamily Diplotriaeinoidea), a family of parasites that infect the air sacs and subcutaneous tissues of a variety of bird species. To our knowledge this is the first record of the occurrence of a nematode in the respiratory tract of an albatross and the first study to provide DNA sequences for a species of the superfamily Aproctoidea.

1. Introduction

Albatrosses (Procellariiformes: Diomedidae) are the world's most endangered family of seabirds (Croxall et al., 2012), and yet there is limited information on the pathogens and parasites affecting these birds (Weimerskirch, 2004; Quintana, 2011). The Prince Edward Islands are Subantarctic islands in the southwest Indian Ocean that are of global importance as seabird nesting sites (Williams et al., 1979). These islands being breeding grounds for five species of albatrosses (Procellariiformes: Diomedidae): wandering albatross (*Diomedea exulans*), grey-headed albatross (*Thalassarche chrysostoma*), Indian yellow-nosed albatross (*T. carteri*), dark-mantled sooty albatross (*Phoebastria fusca*), and light-mantled sooty albatross (*P. palpebrata*) (Ryan et al., 2009; Schoombie et al., 2016). Although no studies have examined the occurrence of metazoan parasites in albatrosses on these islands, it is

reasonable to assume that they are infected by the same species that have been recorded elsewhere considering the circumpolar distribution of these birds (Barbosa and Palacios, 2009; Quintana, 2011).

In April–May 2016, chicks of grey-headed albatrosses were found dead at colonies on Marion Island, the larger of the two Prince Edward Islands. Due to the history of avian cholera outbreaks (Cooper et al., 2009) and the recent occurrence of mouse scalpings (Dilley et al., 2016) and avian pox in seabirds at Marion Island (Schoombie et al., 2018), this mortality led to concern and prompted a parasitological and pathological investigation.

In this study we report the results from the post-mortem examination of five grey-headed albatross chicks, including the identification of the nematode *Diomedinema diomedea* in association in the air sacs of one bird. *D. diomedea* had not been recorded since its original description by Johnston and Mawson (1952) and belongs to the

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Fig. 1. A grey-headed albatross (*Thalassarche chrysostoma*) chick at Grey-headed Albatross Ridge on Marion Island with drooping wings. A chick sitting with a normal posture can be seen in the background. Photo: P.G. Ryan.

superfamily Aprotoidea (class Chromadorea, order Spirurida, suborder Spirurina), a group of parasites for which there are no publicly-available DNA sequences. We obtained sequences of the nuclear small ribosomal subunit RNA gene and the mitochondrial cytochrome c oxidase subunit I gene of this parasite, and employed Bayesian analyses to infer the evolutionary relationship of this parasite to other spirurid worms.

2. Materials and methods

During four nest checks between 3 April and 26 May 2016 of all accessible colonies on Grey-headed Albatross Ridge and along the coast to Rook's Hut on Marion Island (see Schoombie et al., 2016), grey-headed albatross chicks were observed to be weak, prostrated, and apathetic, with drooping wings (Fig. 1). Over time an increasing number of chicks became too weak to lift their heads. At the time, the affected colonies were also heavily impacted by mouse attacks on chicks (Dilley et al., 2016). However, many chicks died without any impact from mice or predators.

Five of these “dead on nest” chick carcasses were collected for post-mortem examination from Grey-Headed Ridge (46°57'18" S, 37°42'29" E; cases 1, 2, 4 and 5) and Rooks Bay West (46°57'50" S, 37°40'34" E; case 3). All chicks were near fledging, with only a few patches of downy feathers remaining. Case 3 was seen alive a few hours before it died, and appeared very weak, lethargic and with drooping wings. The remaining cases were not observed before death. Carcasses were still in *rigor mortis* when collected, but due to field constraints the carcasses had to be kept at ambient temperature (2–10 °C) until necropsies could be conducted 11 h (case 1) or 72 h (cases 2 and 3) after carcass retrieval, or carcasses had to be frozen for later examination (cases 4 and 5).

Samples of organs and tissues were fixed in 10% neutral buffered formalin for 48 h, then transferred to 70% ethanol. Parasites were collected in 70% ethanol. Tissues were then embedded in paraffin and 3–5 µm sections were obtained, stained with hematoxylin-eosin and examined under light microscopy. Parasites (19 females and two males)

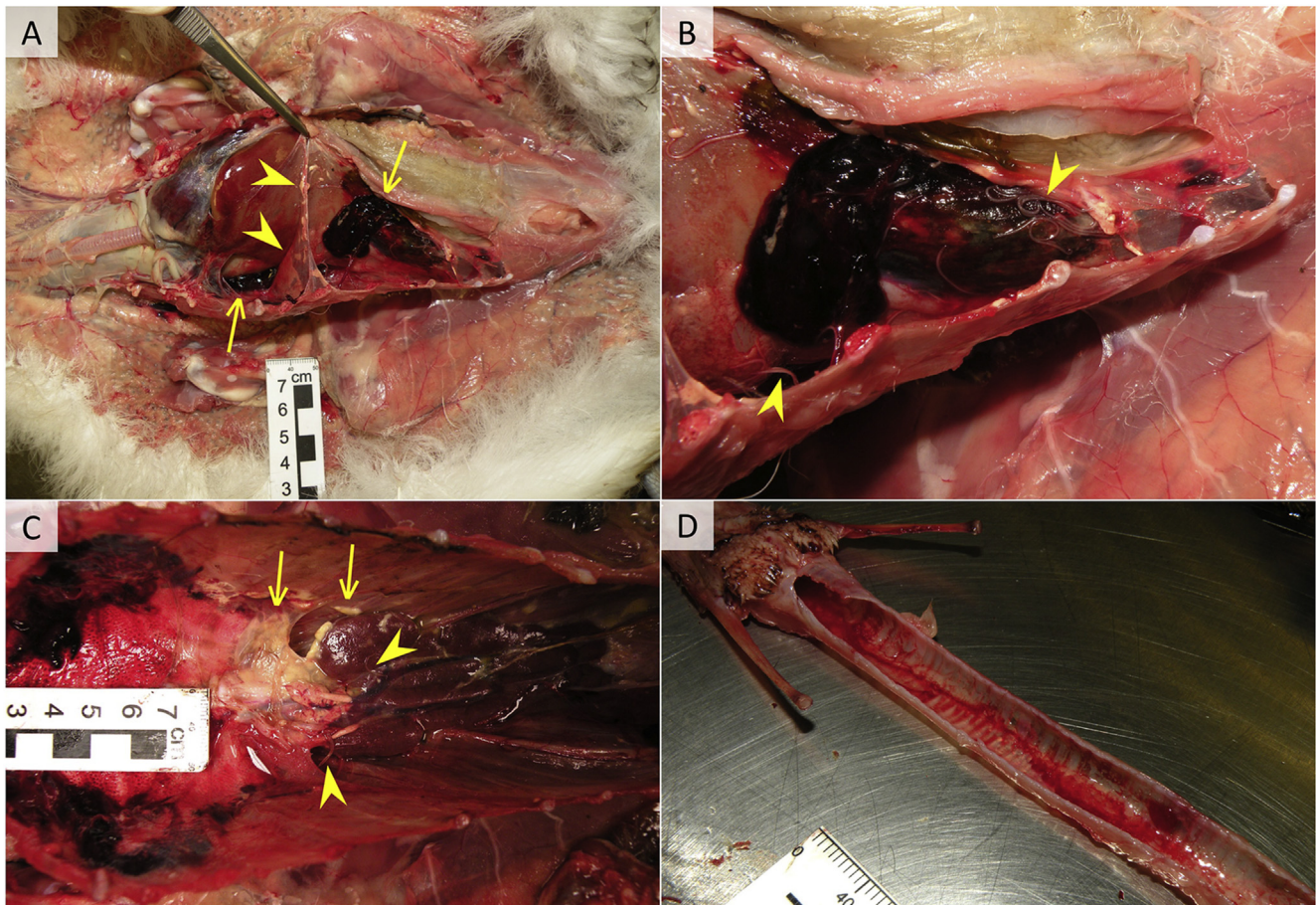


Fig. 2. Lesions associated with *Diomedeenema diomedee* infection in a grey-headed albatross chick (*Thalassarche chrysostoma*). Legend: (A) blood clots (arrows) and masses of pus (arrowheads) in the right thoracic air sac; (B) close-up of the blood clot and nematodes (arrowheads) in the right thoracic air sac; (C) nematodes (arrowheads) and masses of pus (arrows) in the abdominal air sacs; (D) tracheal hemorrhage.

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