

Novel Dermatophilosis and Concurrent Amyloidosis in Sanderlings (*Calidris alba*) from Louisiana, USA

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ABSTRACT: We observed Sanderlings (*Calidris alba*) with facial growths in coastal Louisiana, US during summer of 2016. Severe lesions were associated with lethargy and lack of a flight response. We determined that the skin growth etiology was a bacterium of the genus *Dermatophilus*, rarely reported infecting birds. Sanderlings also exhibited severe amyloidosis.

We observed signs of illness in a population of Sanderling (*Calidris alba*) on Whiskey Island (29°3'18"N, 90°52'5"W) in Louisiana, US during the summer of 2016. Affected birds had disarranged facial feathers and pale-yellow growths on the feathered parts of the face and wings. In severe cases, growths appeared to occlude the eye and one bird had bloody discharge (Fig. 1A, B). Affected birds shook their heads repeatedly, appeared lethargic, foraged infrequently relative to healthy birds, tended to not associate with other birds, and often allowed observers to approach closely. We observed affected Sanderlings from 10 June to 8 August 2016 during weekly, 9.7-km transect surveys recording all shorebirds present and individuals with visually apparent lesions. The maximum apparent prevalence on a survey was 45% (27/60 visually affected). Mildly-affected Sanderlings were observed in proximity to other shorebirds. We observed 22 species and the only other affected species we observed was one Dunlin (*Calidris alpina*) with mild lesions that was behaving normally.

We captured three moribund Sanderlings, one on 6 July and two on 21 July 2016, and euthanized them via cervical dislocation (US Geological Survey Wetlands and Aquatic Research Center Animal Care and Use Committee WARC-L Schulz 2016-01). We performed necropsies on these Sanderlings

including gross examination, bacterial culture, parasitologic, and histopathologic exams. The Sanderlings had scant epicardial fat, moderately atrophied pectoral muscle, and enlarged, pale, and friable livers. All three had multinodular, waxy, yellow exudative growths on the feathered skin craniodorsal to the maxillary beak and extending to the submandibular skin. Feathers protruded irregularly through the surface of the growths. We observed blepharitis with no abnormalities of the conjunctivas and corneas. On cut surface, the growths were linearly striated in the same orientation as the feathers. Similar, but smaller, growths were bilaterally present on the medial surface of the wings near the carpal joint (Fig. 1C). Fleas (*Siphonaptera* sp.) and lice (*Menoponidae* sp.) were present on the skin in small numbers.

We performed histopathology on skin, eyes, brain, liver, lung, spleen, heart, kidney, pancreas, small and large intestine, esophagus, proventriculus, and gizzard. The skin growths were characterized by acanthosis with superficial and feather follicular epithelial orthokeratotic and parakeratotic hyperkeratosis (Fig. 2A). The dermis was edematous with moderate multifocal lymphoplasmacytic perivascular infiltrates. Large numbers of branching septate filamentous bacteria arranged in parallel rows, typical of *Dermatophilus congolensis*, were invading the surface and follicular epithelium (Fig. 2B). There was no evidence of parasites, fungi, or viral infections in the skin. All three birds had hepatic and splenic amyloidosis. There was moderate to marked distortion of the hepatic architecture by extracellular eosinophilic hyaline material replacing hepatocytes (Fig. 2C). The extracellular material stained red and showed bire-



FIGURE 1. Sanderlings (*Calidris alba*) photographed in June–July 2016 on Whiskey Island, Louisiana, USA. (A) Flock with at least six diseased birds; (B) individual with severe facial lesions, and (C) lesions near the carpal joint of the wing caused by dermatophilosis.

fringe with a Bennhold's Congo Red stain (Newcomer Supply, Middleton, Wisconsin, USA). Amyloid was also present in the walls of sheathed capillaries, and in the ellipsoids of the spleen and there was marked splenic plasmacytosis. Other histopathologic abnormalities included intestinal acanthocephaliasis and cestodiasis, infection of the superficial koilin layer of the gizzard with small yeasts consistent with *Candida* sp. fungi (3–5 μ m with pseudohyphae), and larval nematodiasis in the mucosa of the proventriculus. No abnormalities were present in the brain, lung, heart, eyes, pancreas, esophagus, proventriculus, or kidneys of any of the birds.

Bacterial culture of the skin growths using tryptic soy agar with 5% sheep blood at 37 C

failed to yield *Dermatophilus*. We extracted DNA from affected skin and amplified a portion of the bacterial 16S rRNA gene using primers fd2 (Weisburg et al. 1991) and 519R (Turner et al. 1999). We sequenced amplicons in both directions with the same primers used for amplification. The sequences from two Sanderlings most closely matched *D. congolensis* (98–99%) in GenBank. We could not interpret the sequence from the third sanderling, likely because of the presence of other bacteria. We cloned this PCR product (TOP-O[®] TA Cloning[®] Kit, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and sequenced 20 bacterial transformants. The consensus sequence from eight of these clones was identical to those from the first two birds

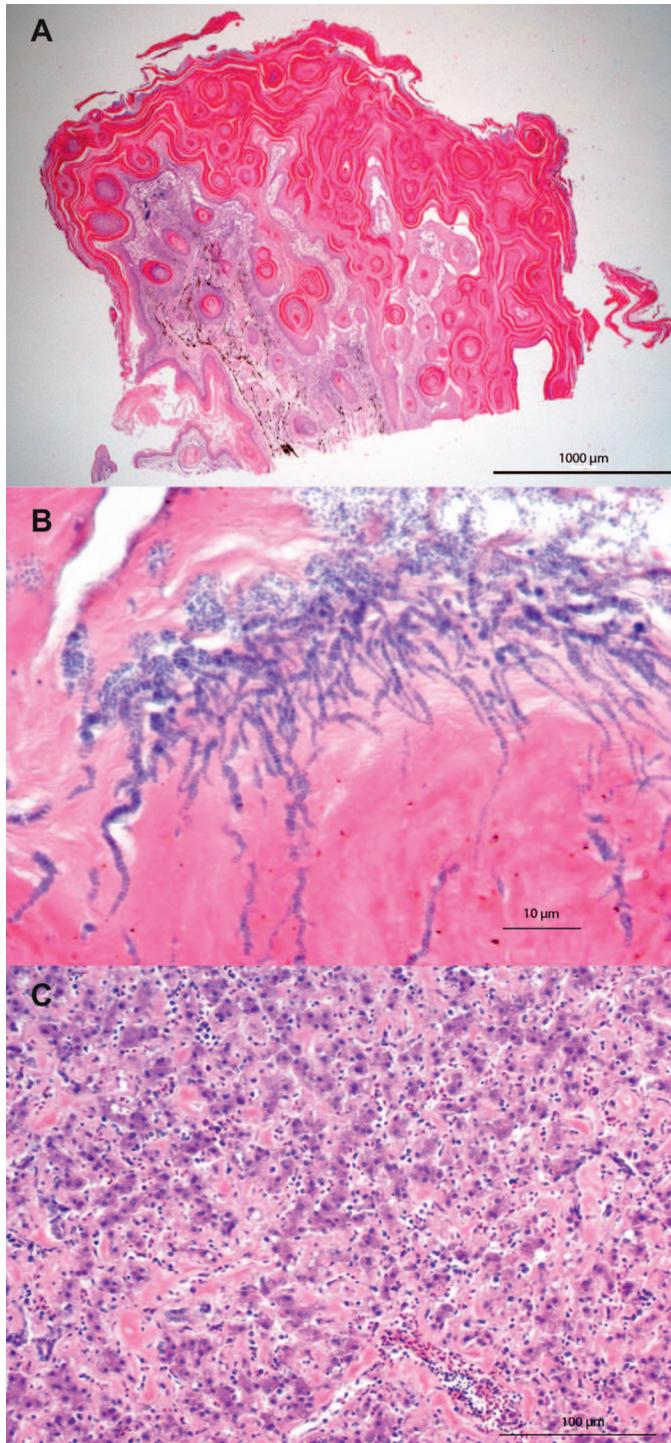


FIGURE 2. Histopathology of Sanderlings (*Calidris alba*) captured in June–July 2016 at Whiskey Island, Louisiana, USA that were infected with *Dermatophilus* sp. bacteria. (A) Marked acanthosis and hyperkeratosis in H&E-stained section of skin at 2× magnification. Bacteria are present in the hyperkeratotic epidermis and feather follicle epithelium. (B) *Dermatophilus* sp. bacteria invading epidermal keratin, Sanderling (H&E, 40×). Bacteria are filamentous and branching with transverse and longitudinal septation. (C) Liver, Sanderling, Bennhold's Congo Red stain (40×). Amyloid deposition in the space of Disse. Note the compression and atrophy of the hepatocellular plates.

(GenBank no. KY490016–KY490018). The remaining clones represented sequences that were likely skin surface contaminants because *Dermatophilus* appeared to be the primary histopathologic cause of the skin infections. Despite 99% sequence identity with *D. congolensis*, we did not obtain isolates to further characterize the bacterium and so we refer to the bacterium as *Dermatophilus* sp.

Dermatophilosis is a contagious zoonotic skin disease caused by bacteria of the genus *Dermatophilus*. Dermatophilosis is well described in mammals and has also been reported in lizards, chelonians, snakes, and farmed crocodiles (Jacobson 2007). We found one previous report of avian dermatophilosis affecting Hooded Crows (*Corvus cornix*) in Italy (Scaglione et al. 2016). In that instance, the infecting bacteria most-closely matched *Dermatophilus chelonae* (Scaglione et al. 2016), a species distantly related to *D. congolensis* (Hamada et al. 2010). The DNA sequences from the Sanderling skin shared only 93% identity with *D. chelonae*, suggesting that the Sanderling dermatophilosis had a different etiology than the case in Scaglione et al. (2016).

We found evidence of ecto- and endoparasite infection in the Sanderlings we examined as well as systemic amyloidosis indicating chronic inflammation. Avian amyloidosis can be a fatal and progressive disease in birds. It is most-commonly reported in waterfowl and gallinaceous birds (Landman et al. 1998). Systemic amyloidosis is thought to be secondary to chronic inflammation or enteric parasitism (Landman et al. 1998). In these Sanderlings, amyloidosis may have been secondary to dermatophilosis as well as to the presence of enteric parasites. Amyloid deposition in the liver in two of the three Sanderlings was severe enough that it may have led to death had the birds not been euthanized. In addition, some of the keratinous growths associated with the dermatophilosis likely impeded eyesight, decreasing the ability to forage and to escape predation. We

propose two nonexclusive hypotheses of impacts that dermatophilosis and amyloidosis could have on Sanderling ecology based on the severity of the infections and extent of observable signs of morbidity: 1) the physical effects of the lesions may decrease foraging efficiency and body condition, causing some infected individuals to not complete migration to breeding areas; and 2) mortality increases because of a direct effect of amyloidosis and dermatophilosis to increase susceptibility to starvation and predation mortality.

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