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SHORT NOTE

Potential dispersal of tardigrades by birds through endozoochory: evidence from Sub-Antarctic White-bellied Seedsnipe (*Attagis malouinus*)

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Abstract

Tardigrades are potentially dispersed by birds, but the extent of the interactions between birds and tardigrades is virtually unknown. We discovered nine tardigrades within feces of White-bellied Seedsnipe (*Attagis malouinus*) collected from high Andean tundra on Navarino Island, Chile. Eight of the tardigrade specimens began moving once rehydrated. Two specimens belonged to the genus *Adropion* (Hypsibiidae), one to the *Macrobiotus* (Macrobiotidae), and five could not be identified. A ninth specimen was a species of *Isohypsibius* in an embryonic egg state. These tardigrades could have passed through the avian digestive tract after incidental ingestion or burrowed into the feces post-defecation to feed on microorganisms and undigested plant matter present in the feces. To our knowledge, this is the first discovery of tardigrades in bird feces and may have implications for tardigrade distributions if birds transport tardigrades endogenously.

Keywords Bird feces · Cape Horn region · Herbivory

Introduction

Tardigrades are microscopic metazoans that inhabit terrestrial and aquatic (freshwater and marine) environments throughout the world (Ramazzotti and Maucci 1983; Schill 2018). The phylum Tardigrada is ubiquitous, cosmopolitan, and currently consists of *ca*. 1200 species (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Vicente et al. 2013; Degma et al. 2009–2019). Some tardigrades can enter a cryptobiotic tun state upon desiccation, which allows them to survive various environmental stressors. While in this protective form of suspended animation, they are thought to disperse passively by wind over great distances (Kristensen 1987; Bertolani et al. 1990; Pugh and McInnes 1998).

Tardigrades occur in bird nests, suggesting that birds may disperse tardigrades by carrying them in their feathers or transporting them altogether with collected material for their nests, e.g., mosses or lichens (Kaczmarek et al. 2018; Mogle et al. 2018). Here, we present the discovery of tardigrades inhabiting the droppings of White-bellied Seedsnipe (*Attagis malouinus* Boddaert, 1783) and propose two possible explanations for their presence: ingestion and endogenous dispersal or post-defecation colonization.

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Materials and methods

This study was conducted during a three-month period from January to March 2018. We collected 26 White-bellied Seedsnipe droppings opportunistically from Andean alpine habitats along the Dientes de Navarino trail on Navarino Island, Chile. Samples were air dried and kept undisturbed at room temperature until examination. The dried external crust surface of the droppings was removed with a razor blade prior to observation to exclude airborne contaminants. Droppings were sliced into 1 mm-wide discs and diluted with filtered

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spring water for examination under a dissecting microscope. Live tardigrades recovered from the feces were placed in filtered spring water and unresponsive tardigrades were placed in 90% ethanol. Each tardigrade was mounted on a glass slide using de Faure's mounting media and observed under differential interference contrast at 400 × (using an Olympus BX20 microscope). Most individuals were identified to genus or species (Ramazzotti and Maucci 1983; Degma et al. 2009–2019). Permanent mounts of the specimens were retained by SJM at the British Antarctic Survey.

Results and discussion

Tardigrades have previously been recorded on Navarino Island by Richters (1908) and Rahm (1931, 1932) who reported four taxa *Echiniscus testudo* (Doyère, 1840), *Pseudechiniscus suillus* (Ehrenberg, 1853), *Mesobiotus furciger* (Murray, 1907), and *Macrobiotus hufelandi* (C.A.S. Schultze, 1833), all now considered species complexes. We found nine tardigrade specimens in seedsnipe feces, representing at least four species from three genera. One of the recovered tardigrades was unresponsive after hydration and was presumed dead. All tardigrades were recovered from the interior of two different White-bellied Seedsnipe droppings collected from the same location above tree line (Navarino Island; 579 m a.s.l., 55° 01′ 17″ S 67° 43′ 07″ W). We confirmed two distinct species of the genus *Adropion* (Hypsibiidae). The first *Adropion* specimen had three placoids, a microplacoid, and a septulum reminiscent of *Adropion greveni* (Dastych 1984) or *A. onorei* (Pilato et al. 2002), both recorded from South America (Kaczmarek et al. 2015). The differences included the two anterior macroplacoids being of equal size, while in both *A. greveni* and *A. onorei* the second macroplacoid was the shortest; a much larger septulum and larger claws than either *Adropion greveni* or *A. onorei*; and lacked the distinct spines or crenulation at the claw base. The second *Adropion* had three macroplacoids, but no microplacoid or septulum, robust claws with an obvious accessory spine on the inner claws, no bars, lunules, or teeth at the base of the claws, and did not match any *Adropion* recorded from South America. These two specimens of *Adropion* may represent new species, but more material should be collected to confirm their distinction and variation before a formal taxonomic proposal is made. One specimen is believed to belonged to the genus *Macrobiotus* (Macrobiotidae). This genus includes several species complexes that require careful taxonomic analysis, including examining egg morphology, to properly differentiate the species. Without eggs, we were unable to identify the species group, which could belong to the *hufelandi*, *pallari* or *echinogenitus* groups. The last specimen, an *Isohypsibius* sp., was an individual observed in an embryonic egg stage and therefore, the species could not be determined. The remaining five tardigrades were unidentifiable due to degradation while traveling between laboratories.

All but one of the tardigrades discovered regained vigor upon hydration, suggesting that they were in a cryptobiotic tun state due to desiccation (Wełnicz et al. 2011). We suggest two possible reasons for the presence of tardigrades in droppings of White-bellied Seedsnipe: (1) incidental ingestion and endogenous dispersal or (2) post-defecation colonization.

White-bellied Seedsnipes belong to an endemic group of plovers (Charadriiformes: Thinocoridae) of Antarctic origin. They are associated with harsh high elevation and open landscapes of the southern Andes during the breeding season and migrate to lowland grasslands during the winter months (Ibarguchi 2011). Seedsnipes are found from 39° S (Bío-Bío) to 55° 57′ S (Cape Horn Island, eBird 2020), are specialized herbivores that resemble Ptarmigans (*Lagopus*) from the Northern Hemisphere. While little is known of their diet, Fjeldså and Kirwan (2020) concur with our observations (JEJ) that they consume crowberry (*Empetrum rubrum*) and grasses near mosses and lichens where tardigrades are present.

If ingested as adults or as eggs, the tardigrades could survive the passage through the birds' digestive tract (Fig. 1). Alternatively, the tardigrades could have burrowed into the already-voided droppings in search of microorganisms or undigested plant tissue as a food source. Indeed, we found numerous live microorganisms in White-bellied Seedsnipe feces during this research. In either case, as the droppings dried, the sunbaked and wind-blown outer crust of the feces may have prevented escape and forced the tardigrades to enter a cryptobiotic state where they lay dormant until their resurrection in the Petri dish where conditions were once again favorable.

In previous studies, tardigrades were found occurring in feces of land snails in Puerto Rico (Fox and García 1962; Fox 1966). In the case of the land snail species *Bulimulus exilis* (Gmelin, 1791), Fox and García (1962) and Fox (1966) believed the most likely explanation for the presence of live tardigrades in feces was that they were incidentally ingested by the snails and passed live though the animals' digestive system. This is the closest link to the work we present here and suggests the potential for tardigrades to move through another organism's digestive tract unscathed.

To our knowledge, our observations are the first of tardigrades inhabiting avian feces. In future studies, the examination of freshly defecated droppings may yield evidence to support the ingestion hypothesis. Experiments with captive birds could be used to determine whether tardigrades survive passage through the avian digestive tract

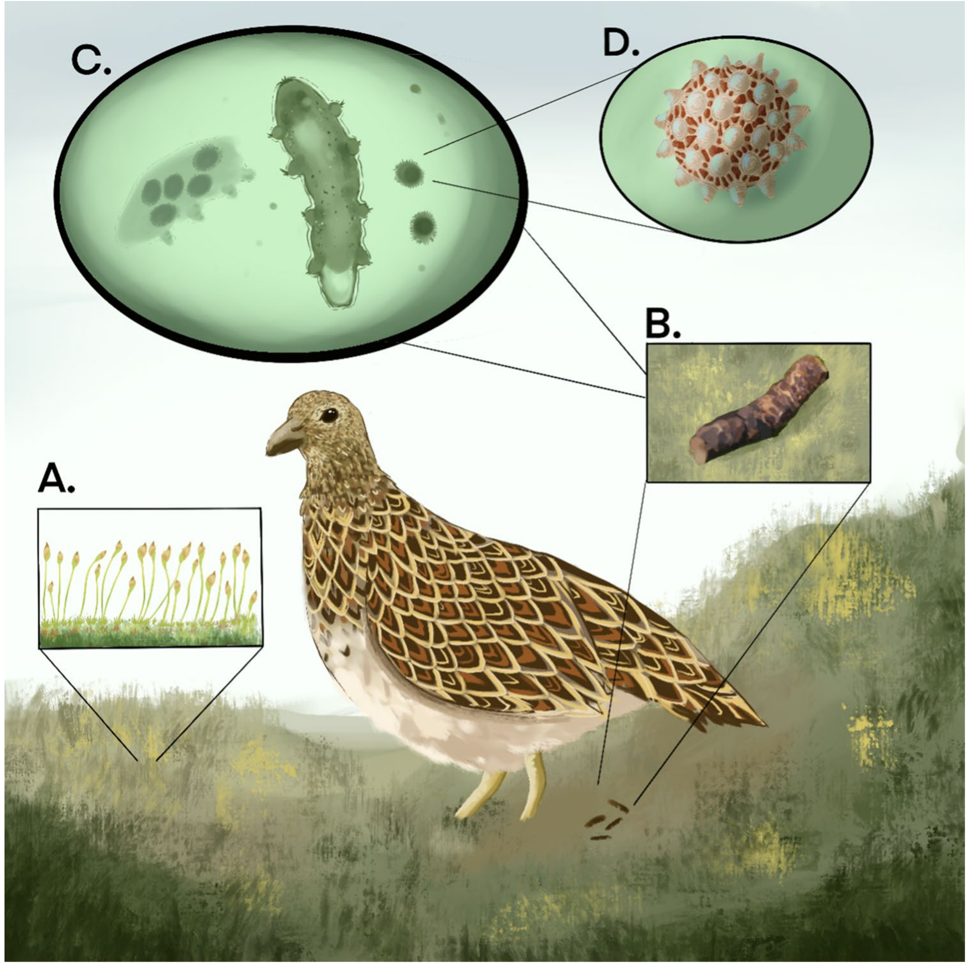

Fig. 1 Illustration depicting an example of the possible mechanism of endozoochorous dispersal of tardigrades by White-bellied Seedsnipe (*Attagis malouinus*). **a** Unseen tardigrades present in the environment are ingested by a bird during foraging. **b** Tardigrades pass through the digestive system from incidental consumption and are encapsulated within the feces. **c** Tardigrades could be present in various developmental stages: as adults, free laid eggs, or eggs present in the molted cuticle. **d** Ornamented tardigrade egg

(Hillerman et al. 1953). If tardigrades pass through the avian digestive system and remain viable post-defecation, this would add to the growing evidence that birds play a role as long-distance dispersers of tardigrades.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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