Subspecific Identification of the Great Lakes' First Brown Booby (*Sula leucogaster*) Using DNA

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The first Brown Booby (*Sula leucogaster*) recorded in the Great Lakes basin was discovered on Lake Erie near the source of the Niagara River on 7 October 2013 by J. P. Morphologic evidence suggested that this bird was an adult female of the nominate Atlantic subspecies. We obtained genomic DNA from feces left by the bird. Mitochondrial DNA from the control region (CR2) was sequenced and compared with extensive CR2 data for Brown Booby available in GenBank; this corroborated the morphologic hypothesis. This is the first time that a vagrant bird in Canada has been identified using DNA extracted from feces.

Key Words: Brown Booby; *Sula leucogaster*; Great Lakes; distribution; vagrant; mitochondrial DNA; mtDNA; new record; Ontario; DNA extraction; feces; fecal sample; DNA identification

Introduction

On 7 October 2013, J. P. discovered a Brown Booby (*Sula leucogaster*) on Lake Erie at the source of the Niagara River. This was the first time the species had been observed on the Great Lakes. The bird was most frequently observed with Double-crested Cormorants (*Phalacrocorax auritus*) at Donnelly's Pier (42.884485°N, 78.903401°W) on the Buffalo, New York, side of the lake and on the historic Horseshoe Reef Lighthouse just inside United States waters (42.881273°N, 78.915133°W) (Burrell 2013). The bird was regularly observed venturing far out into the open lake to feed and was often seen crossing over to the Ontario side. Eighty observations made from the Buffalo side of the Niagara River and 79 from the Fort Erie side were entered into eBird, which is an online platform for reporting bird checklists (Sullivan *et al.* 2009). Undoubtedly many more observations of the bird were made, but were not added to the database. The last known observation of the Brown Booby at this location was made on 24 October (Pawlicki 2014).

What was presumably the same bird (based on the sex, age, and facial markings) was rediscovered 99 km to the southwest at the tip of Long Point, Ontario (42.549816°N, 80.043848°W) on 31 October by Ken Burrell (Pawlicki 2014). It was seen again at that location on the morning of 1 November before being rediscovered in the afternoon 55 km to the northeast at Mohawk Point, Ontario (42.849085°N, 79.467712°W). It was last seen on 2 November 2013 at Mohawk Point, when observers noted that it looked moribund (Jacklin 2013; Watson 2013). J. H. S. requested feathers or tissues if the bird succumbed (Jacklin 2013); however, it revived and disappeared out over the lake (D'Anna and Potter 2013). It was never seen again but M. J. collected fecal samples from where the bird had been sitting and sent them to J. H. S. for analysis. The fecal samples were easy to isolate, as they were the only excrement on the rock where the booby had been sitting. The samples were collected with a cotton swab and sealed into a zip-lock bag.

There are four recognized subspecies of Brown Booby (Schreiber and Norton 2002). Brewster's Brown Booby (*Sula leucogaster brewsteri*) breeds in the Gulf of California; Columbian (*S. l. etesiaca*) breeds along the Pacific coast from Honduras to Colombia; Forster's (*S. l. plotus*) breeds across the central, west and southern Pacific Ocean; and the nominate Atlantic (*S. l. leucogaster*) breeds in the tropical Atlantic from the Bahamas and Caribbean to the Central American coast east to Cape Verde Island. In a typical year, any east-coast vagrant Brown Booby would be assumed to be the nominate subspecies. However, an unprecedented northward influx of Brown Boobies and Blue-footed Boobies (*Sula nebouxii*) along the Pacific coast (eBird 2013), combined with Ontario's first record of another Pacific coastal species (Elegant Tern, *Thalasseus elegans*) later in the autumn (20–24 November 2013; eBird n.d.) led to speculation that the Lake Erie Brown Booby might have been from the eastern Pacific subspecies *brewsteri*.

Brown Boobies wander extensively away from their breeding islands but tend to stay in tropical waters (Schreiber and Norton 2002). There are scattered rec-

ords from up the east coast as far as Nova Scotia and up the west coast to British Columbia, but only five previous inland records in eastern North America and no previous observations in the Great Lakes basin (Sullivan *et al.* 2009). Of note, four of these five inland records occurred in the past year. The inland records are from Claytor Lake, Pulaski County, Virginia, 4–28 October 2008; White Lake Wildlife Management Area, Warren County New Jersey, 27–31 July 2012; Lake Norrell, Saline County, Arkansas, 9–21 August 2012; and Canyon Lake, Comal County, Texas, 25 August to 3 September 2012 (Pawlicki 2014). Figure 1 shows these records on a map along with all eBird Brown Booby records (Sullivan *et al.* 2009). Records from 2012 and 2013 are shown in a different colour to illustrate the apparent upsurge in recent vagrancy.

From its clean brown and white plumage, it was apparent that the Great Lakes bird was an adult and, from the yellowish face and gular with an isolated dark blue loral spot, a female (Figure 2; Pyle 2008). Pyle (2008) also states that females of *S. l. leucogaster* show a pale bluish iris and the brown head and breast slightly darker or more blackish than the back, whereas *S. l. brewsteri* has a pale yellowish iris with the brown head and neck slightly paler and grayer than back. Before Pyle (2008) published these characteristics, it was generally believed that field identification of Brown Boo-

FIGURE 2. Adult female Brown Booby (*Sula leucogaster*) sitting on Donnelly's Pier, Buffalo, New York, 9 October 2013. Photo by James Pawlicki.

by females to subspecies was impossible (Schreiber and Norton 2002). The characteristics are subtle and have not been tested adequately in the field. Based on photos and field observations, Pawlicki (2014) stated that the Great Lakes bird "appears to represent the nominate Atlantic subspecies."

In this paper we provide molecular evidence supporting this contention. Note that all four of the recent inland eastern North American records were believed to be of nominate adult female birds (Pawlicki 2014).

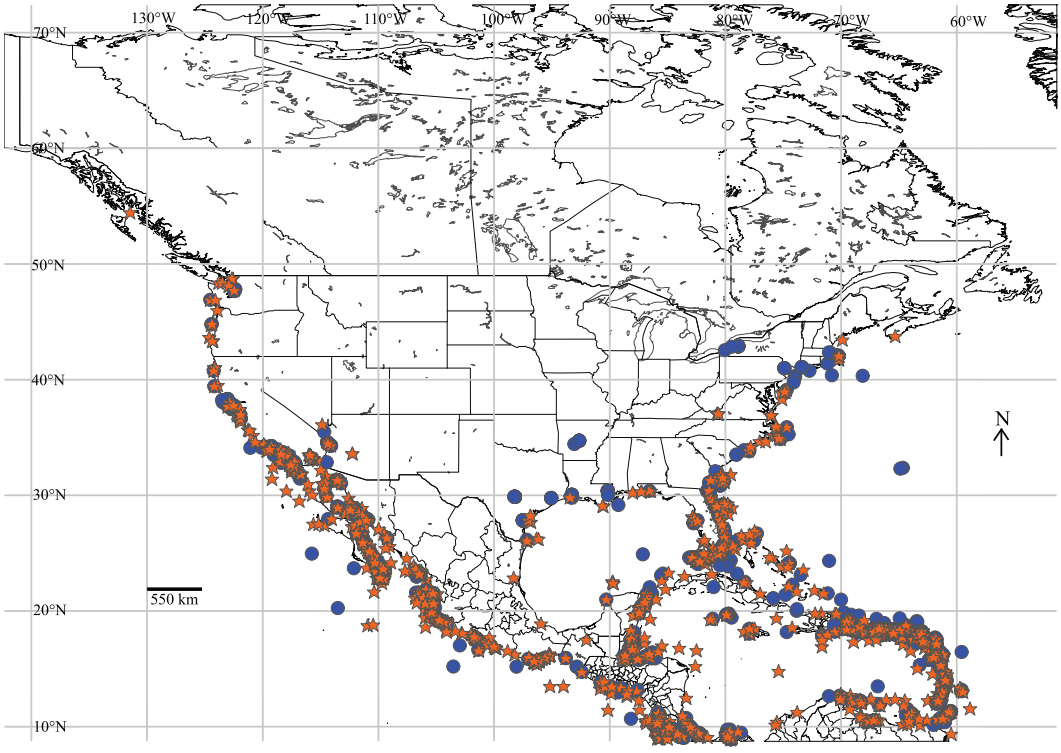

FIGURE 1. Distribution of Brown Booby (*Sula leucogaster*) based on eBird records compiled by Sullivan *et al.* (2009) and records noted by Morgan *et al.* (2009). Dots indicate records from 2012 and 2013; asterisks indicate older records.

Methods

DNA extraction

DNA was isolated from two separate fecal samples (obtained from the same location). The first sample was collected with a standard cotton swab. The feces-saturated cotton end was separated from the swab shaft and placed into a sterile 2.0-µL microfuge tube. The second sample, approximately 1 mg of dry feces, was transferred to a separate sterile 2.0-µL microfuge tube. DNA was extracted from each sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's "Isolation of DNA from Stool for Human DNA Analysis" protocol.

Polymerase chain reaction

Primers SlMCR-L160A and SdMCR-H750 (Steeves *et al.* 2005) were used to amplify the control region (CR2) in Brown Boobies. There are two paralogous copies of the control region and these primers specifically target the CR2 copy (Morris-Pocock, Taylot *et al.* 2010). Amplification was carried out in 25 µL reactions containing 14.7 µL distilled H_2O, 2.5 µL 10× ExTaq PCR buffer (containing 20 mM $MgCl_2$), 0.65 µL 25 mM $MgCl_2$, 1 µL of each 10 µM primers, 2 µL 10 mM dNTPs (Deoxyribonucleotide triphosphates), 0.15 µL ExTaq-HS (Hot Start) DNA polymerase (Takara Bio USA, Madison, Wisconsin, USA), and 3 µL total DNA template. Amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany).

DNA sequencing and editing

Amplified products were visualized on 1% agarose electrophoresis gels and purified using precast E-Gel CloneWell 0.8% SYBR Safe agarose gels (Invitrogen, Carlsbad, California, USA) following the protocol described by (Gibson *et al.* 2010). Sequencing reactions were performed in a total reaction volume of 10 µL, containing 2 µL double-distilled H_2O, 1.5 µL 5× sequencing buffer, 0.5 µL 10 µM primer, 1 µL BigDye Terminator (PE Applied Biosystems, Austin, Texas, USA), and 5 µL purified PCR product. Sequencing was performed at the Agriculture and Agri-Food Canada Eastern Cereal and Oilseed Research Centre Core Sequencing Facility, Ottawa, Ontario, Canada. Purification of sequencing reactions was performed using the Applied Biosystems (ABI) ethanol/ethylenediaminetetraacetic acid/sodium acetate precipitation protocol and reactions were analyzed on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, California, USA).

Raw sequence chromatograms were edited and contiguous consensus sequences (contigs) were generated using Sequencher 5.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequences from the two samples were identical. The CR2 contig obtained was published in GenBank under number KM491177.

All 119 published control region sequences for Brown Booby were downloaded from GenBank and aligned with our contig using automated alignment programs within Geneious v5.6.5 (Biomatters Ltd., Auckland, New Zealand). Muscle, Geneious and Clustal algorithms all produced the same alignment. This was checked for obvious errors using Mesquite version 2.75 (open-source software, Mesquite Project Team, 2010, http://mesquiteproject.org). Samples were from 12 widely separated populations (broadly including the Eastern Pacific, Eastern Atlantic, Caribbean, South Pacific, and the Gulf of California) and included all four recognized subspecies (Steeves *et al.* 2005; Morris-Pocock, Steeves *et al.* 2010).

Analyses

PAUP (Phylogenetic Analysis Using Parsimony, v. 3.1, David L. Swofford, Illinois Natural History Survey, Champaign, Illinois) was used to produce a neighbour-joining tree (Figure 3, Appendix 1) and to calculate pairwise distances.

Parsimony analysis was conducted using TNT version 1.1 (Goloboff *et al.* 2008). Parsimony searches with tree bisection–reconnection branch swapping and a random stepwise addition of taxa was repeated 1000 times, followed by ratcheting, tree-fusing, sectorial searches, and tree-drifting with default settings.

Results and Discussion

The control region sequence obtained from the Lake Erie fecal samples conclusively clusters with the nominate Caribbean population of Brown Booby, *Sula leucogaster leucogaster* (Figure 3). Note that parsimony analysis found the same groups as those shown in Figure 3. The only difference is that the backbone of the tree (i.e., relationships between clades) collapses under strict consensus.

The sequence obtained was nearly identical (0.2% pairwise variation) to sequences from birds nesting on Isla Monito (18.083°N, 67.883°W) (Morris-Pocock, Steeves *et al.* 2010). Intraspecific pairwise distances within *S. l. leucogaster* vary from 0.2% to 7.8% (average 3.9%, $n = 33$). This is relatively consistent with data from other subspecies: *S. l. brewsteri*, 0.0–3.7%, average 1.7%, $n = 36$; *S. l. etesiaca*, 0.2–2.4%, average 1.3%, $n = 8$; *S. l. plotus*, 0.2–9.2%, average 5.4%, $n = 41$). Pairwise distance within subspecies averages 3.8%, and between subspecies it averages 7.1%.

We, thus, support the contention of Pawlicki (2014) that the Brown Booby found in the Great Lakes originated from the Atlantic population and refute the hypothesis that it was part of the extensive pattern of Pacific Booby vagrancy witnessed in 2013.

DNA identification from feces is still relatively novel. It has been used mostly for mammal identification, particularly in conservation-related projects (e.g., Reed *et al.* 1997; Davison *et al.* 2002; Dalén *et al.* 2004; Gompper *et al*. 2006; Napolitano *et al.* 2008; Michalski *et al.* 2011; Chaves *et al.* 2012). Only a few papers have reported using this technique for birds. For example, Cheung *et al.* (2009) surveyed avian influenza

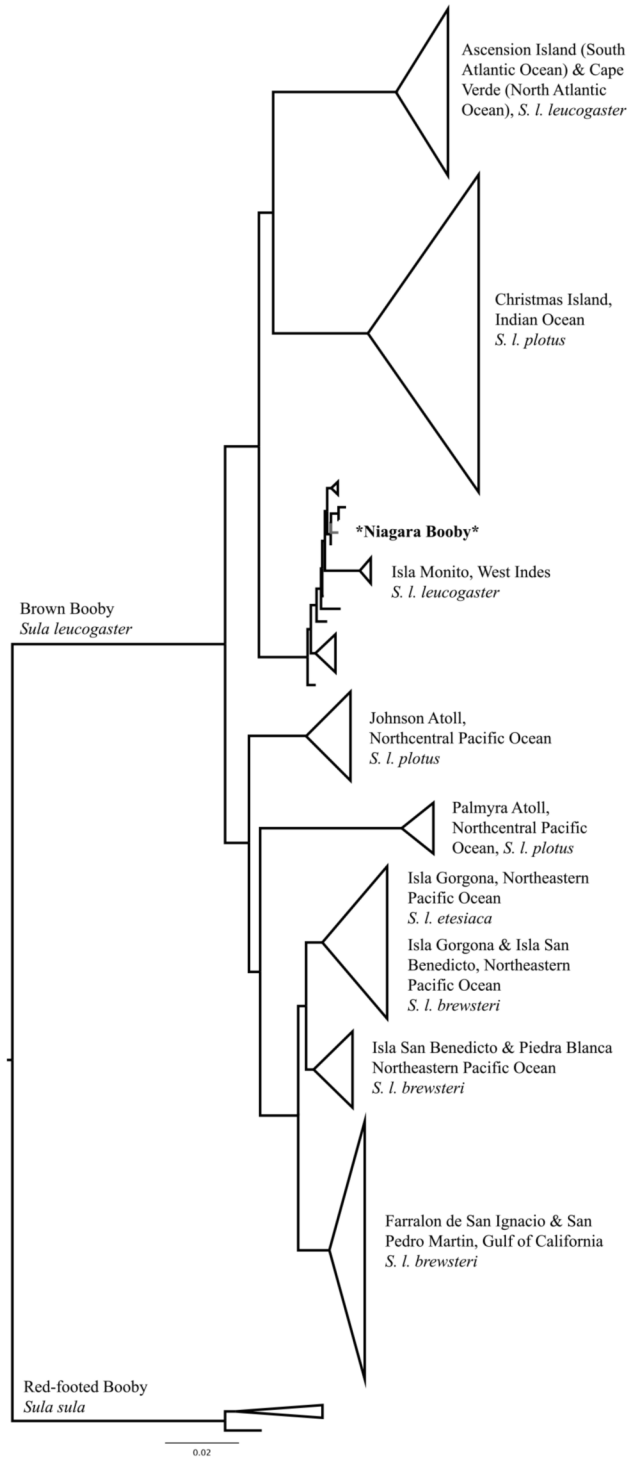

FIGURE 3. Neighbour-joining tree based on Brown Booby (*Sula leucogaster*) control region (CR2) sequences. Red-footed Booby (*Sula sula*) was used as the outgroup and to root the tree.

virus from feces and identified the bird species based on sequences of cytochrome oxidase subunit I (COI) from the same fecal matter. Joo and Park (2012) identified bird species and their prey using COI obtained from feces, and Marrero *et al.* (2008) distinguished between two threatened pigeon species by sequencing the control region from fecal samples.

We could only identify one previous study that used DNA retrieved from a fecal sample to identify a vagrant bird (Lindsay and Haas 2013). To the best of our knowledge, this paper documents the first time that DNA identification based on fecal material has been used to identify a vagrant bird in Canada. This approach has significant implications for future vagrants when morphologic identification or place of origin is unclear and a non-invasive approach is desired. For example, a vagrant *Elaenia* flycatcher turned up in Chicago in 2012, but has never been identified to species despite excellent photographic documentation (Brinkley 2012); a fecal sample might have supplied conclusive evidence for its identification. We encourage naturalists to carry a sterile cotton swab and baggie for future emergencies like this!

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APPENDIX 1. Neighbour-joining tree of *Sula leucogaster* isolates, *Sula sula* used to root tree. Numbers given on branches are GenBank numbers, followed by site abbreviations and isolate numbers. Abbreviations follow Morris-Pocock *et al.* 2010 but are repeated here for clarity. ***Sula leucogaster brewsteri*** (**Northeast tropical Pacific)**: Etp – Eastern Tropical Pacific (Clipperton and Piedra Blanca), Fsi – Farralon de San Ignacio, Pbl – Piedra Blanca, Sbe – Isla San Benedicto, Spm – San Pedro Mártin, ***Sula leucogaster etesiaca*** **(Central Eastern Pacific):** Gor – Isla Gorgona, ***Sula leucogaster leucogaster*** **(Caribbean and tropical Atlantic):** Mon – Isla Monito, Asn – Ascension, Cvd – Cape Verde, ***Sula leucogaster plotus*** (**Red Sea and West Indian Ocean to Central Pacific)**: Jon – Johnston Atoll, Pal – Palmyra Atoll, Xch – Christmas Island, ***Sula sula***: Ald – Aldabra Atoll (Indian Ocean), Asn (Ascension, Eastern Atlantic), Mon (Isla Monito, Caribbean).