SHORT COMMUNICATION

A breeding record of the Ryukyu Scops Owl on Okinoshima, in northernmost Fukuoka, Japan

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Abstract A nest of the Ryukyu Scops Owl Otus elegans was found on Okinoshima (Okino Island), Fukuoka Prefecture, Japan (34.24°N, 130.10°E), in the Tsushima Strait, on 28 July 2013. The breeding pair and their three owlets were caught and their identity confirmed genetically using the BOLD System for COI in the mitochondrial genome. Their calls and external morphological measurements also accorded with what is known of the species. We estimated that at least 23 territorial males inhabit the Island. Okinoshima lies 490 km beyond the previously known northern limit of the species’ distribution.

Key words External morphological identification, Genetic identification, Northern limit of distribution, Otus elegans, Spectrographic identification

The Ryukyu Scops Owl Otus elegans is known to range from the Tokara Islands off southwestern Kyushu, Japan, to small islands north of Luzon, Philippines (Ornithological Society of Japan 2012; Gill & Donsker 2013). The species is known only from relatively small islands ranging in area from 1.15 km² (Geruma Island) to 1,206 km² (Okinawa Island) in Japan (Haruka Ito personal communication; Takagi 2011). The total distribution spans a straight-line distance of approximately 1,000 km (Fig. 1A). Although Nakanoshima has been considered to be the northern limit of the species’ distribution (Seki et al. 2011; cf. Ornithological Society of Japan 2012), it has long been believed that Ryukyu Scops Owls also inhabit Okinoshima, lying 490 km away beyond the known northern limit of distribution (Environmental Agency Japan 1975; Munakata City 2008). The population on Okinoshima had not previously been designated as belonging to the Ryukyu Scops Owls because the birds there had not been identified scientifically, and there were no breeding records from the Island (Ornithological Society of Japan 2012).

On 28 July 2013 we located the nesting cavity of a pair of owls on Okinoshima, and caught the breeding pair and their three owlets. In this paper, we describe our genetic identification of the species using the BOLD Identification System (IDS). IDS for the mitochondrial Cytochrome c oxidase subunit I (COI) accepts sequences from the 5’ region of the COI gene and returns a species-level identification when one is possible (Ratnasingham & Hebert 2007). In addition, we describe calls, external morphological measurements, and the breeding cavity. We also discuss the peculiarity of the species’ distribution.

METHODS

1) Study site

This study was conducted on Okinoshima (N34°14′, E130°06′), an island located some 57 km off the northeastern Kyushu coast, in Fukuoka Prefecture, Japan. The island is located in the middle of the eastern route of the Tsushima Strait (Fig. 1A).
Okinoshima has an area of just 0.69 km$^2$ and measures about 1.5 km from east to west, and about 1 km from north to south. There is a ridgeline from the northeast to the southwest reaching an altitude of 243.6 m. The island is covered with primary laurel forest consisting mainly of *Machilus thunbergii* and *Arisaema ringens*, and in some areas by shrubs, grasslands, and wasteland. The Tsushima Warm Current influences the plant community on the island, thus various sub-tropical species, such as *Pandanus odoratissimus* and *Asplenium antiquum* are also native to the island (Munakata City 2008; Ministry of the Environment Japan 2013).

2) **General field procedures**

On 27 July 2013 we walked a forest trail from 15:00 to 17:00, then again from 18:00 to 23:00 listening for owls (Fig. 1B). A typical Ryukyu Scops Owl call recorded on Nakanoshima was played back using the built-in-speakers of a Marantz PMD 661 solid-state recorder (D&M Holdings Inc., Tokyo, Japan) to stimulate owls to respond. We located calling owls at distances of less than 100 m while conducting playback experiments. When an owl responded, playback was stopped and calls were recorded at a range of about 10 m. Responses were recorded at 44.1 kHz, using a 16-bit sampling rate and Audio-Technica AT4073A directional microphones (Audio-Technica Japan 2013).
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Corporation, Tokyo, Japan). Streaked Shearwaters Calonectris leucomelas also breed on the island (Munakata City 2008), and after dusk their calls are extremely noisy making it difficult to record owls clearly.

On 28 July, we mist-netted two adult owls in front of their breeding nest cavity, and caught three owlets in the nest by hand. All five birds were banded with metal rings sequentially numbered by the Japanese Ministry of the Environment, and a unique combination of colored reflective tapes was attached around the band facilitating easy visual identification in the field. We looked for the presence of a brood patch in the two adults.

Eight morphological traits were measured using a digital caliper (Absolute Digimatic CD-30C, precision of ±0.03 mm, Mitsutoyo, Kanagawa, Japan), basically following Svensson (1984): (1) length of unflattened wing, (2) tail length, (3) length of bill from the tip of the bill to the skull, (4) bill depth and (5) width at anterior edge of nostril opening, (6) total length of head (the maximum length from the tip of the bill to the back of the skull), (7) tarsus length, (8) horizontal minimum diameter of tarsus at 7 mm from the base of the tarsus. All of the morphological measurements were repeated three times for each owl. The measurements for each trait were averaged over the three values for each of the adult owls, and a single value for each trait was used per individual.

Wing formula was recorded in order of length of primaries. Body mass was determined with a spring scale (Medio 40300, precision of ±2 g, Pesola AG, Baar, Switzerland). For the three owlets we measured only (1), (3), (7), and body mass.

We obtained blood samples from the brachial veins of each bird using a fine-tipped needle (27 G) and collected about 100 μL blood into capillary tubes. Blood samples were stored in microtubes with 100% Ethanol.

3) Genetic analysis

DNA was extracted from stored blood samples using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Sex determination was conducted by analyzing the CHD gene. The following set of primers was used for the amplification of the CHD gene: 2550F (5’-GTTACTGATTCTACGAGA-3’) and 2718R (5’-ACGTGGGARATRATTCCAAATCCCTG-3’) following the experimental methodology described by Fridolfsson and Ellergen (1999).

We sequenced a partial region, the cytochrome c oxidase subunit I (COI) gene in the mitochondrial genome, which is used as a standard DNA barcoding region for most animals (Hebert et al. 2003). The barcoding region was amplified using the following primers: L6697Bird (5’-TCAACYAAC CACAAAGAYATCGGYAC-3’) and H7390Thrush (5’-ACGTTGGARATRATTCCAATCCCTG-3’) following the methodology described by Hebert et al. (2004). To identify species, we used a function “Identification Request” in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007; http://www.boldsystems.org/index.php/IDS_OpenIdEngine).

4) Spectrographic analysis

Spectrographic analysis of the calls were performed using Raven 1.3 (Cornell Laboratory of Ornithology, Bioacoustics Research Program, Ithaca, NY, USA) for Mac OS X. Spectrograms were calculated on an Apple MacBook Air (1.8 GHz Intel Core i7) with the following settings: window type hann, size 1,500 samples (76.8 ms); time grid, overlap 50%, hope size 17 ms; frequency grid, DFT size 65,536 samples, grid spacing 0.673 Hz. We introduced a “typological analysis” method to visually classify variation in the spectrograms according to the shape of the syllable of the owl calls (Takagi 2013). In the typological analysis, hoots, syllables, and element types were each defined according to the number of syllables, the degree of timescale overlap of the first and second elements in the second syllable, and the relative differences of duration and frequency of two elements in the second syllable.

5) Estimation of the number of territorial males

Because Ryukyu Scops Owls nest in tree cavities (Akatani et al. 2011; König & Weick 2008), it is inferred that the entire forest, composed of Machilus thunbergii, on Okinoshima is broadly available as nesting habitat for them (Fig. 1B, cf. Ministry of the Environment Japan 2009). We determined the area available for nesting according to responses to playback then estimated the number of territorial males on the Island.

RESULTS

By analyzing CHD genes we were able to determine that the individual without a brood patch (No. 7A-08556) was male, and that the individual with a brood patch free of down (No. 7A-08557)
was female (Table 1). We were able to determine the DNA sequences of each individual from 619 to 622 bp in the COI region. All of the sequences from the five birds (the breeding pair and their three owlets) were exactly the same. The sequence data from these five individuals were a 100% match for five published sequences (GenBank (accession No.: AB842979, AB842980, AB842985, AB842986, AB843638). In addition, 99.82 and 99.83% matched with two published sequences (GenBank accession No.: AB842977, AB842984), and 97.32–97.79% matched with six published individuals (GenBank accession No.: AB842978, AB842981, AB842982, AB842983, AB843639, AB843640). All five individuals were confirmed as Ryukyu Scops Owl using IDS. The DNA sequences for the five individuals were submitted to GenBank (Accession No.: AB898092, AB898093, AB898094, AB898095, AB898096).

The area in which seven different calling males were heard covered about 7 ha (Fig. 1B). The highest altitude above sea level at which we recorded a calling male was 140 m. No owl responded to playback between 140 m and 243.5 m, the highest point on the island.

The nest was in a natural cavity in an *Ilex integra* (Fig. 1B; Appendix 1). The tree was located in a forest at an altitude of 63 m (N34°14′45″, E130°06′18″). The height of the tree was about 15 m, and the diameter at breast height was 67 cm. The entrance of the cavity was 3.8 m above the ground, and the entrance was an oval shape (10 cm × 11 cm).

All Ryukyu Scops Owls on Okinoshima gave hoots consisting of two syllables (see Fig. 2 for spectrograms of calls from three males) and were classified as HT-1. Hoot syllables were classified as MST-E, SST-CL, and SST-CL (see Fig. 2A, B, and C, respectively). Hoot elements were classified as RL-RH. Characteristics of the calls of three males recorded on Okinoshima essentially coincided with those of Ryukyu Scops Owls recorded elsewhere. Hoot type HT-1 is common on islands north of the Kerama Gap (Takagi 2013). Element type RL-RH is the most common type given by Ryukyu Scops Owls (Takagi 2013). In contrast, the shapes of two syllables (see Fig. 2 for spectrograms of calls from three males) and were classified as HT-1. Hoot syllables were classified as MST-E, SST-CL, and SST-CL. All Ryukyu Scops Owls gave hoots consisting of two syllables (see Fig. 2; Takagi 2013). Element type RL-RH is the most common type given by Ryukyu Scops Owls (Takagi 2013). In contrast, the shapes of two syllables (see Fig. 2 for spectrograms of calls from three males) and were classified as HT-1. Hoot syllables were classified as MST-E, SST-CL, and SST-CL.
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of the species in the Ryukyu Islands, wing length was a little shorter than the smallest individual there, and was within the range of the endemic subspecies *O. e. interpositus* occurring on Minami-daito Island (see Appendix (a)–(h)).

The density of male Ryukyu Scops Owls was 0.9 individual per ha in a restricted area of habitat (Fig. 1B). The entire forest area available for the owls measures ca. 36 ha (Fig. 1B). However, because owls did not respond to playback at altitudes above 140 m (an area of ca.10 ha), we estimate that the area available for the owls on the island is of about 26 ha, and that at least 23 territorial males could inhabit the island.

**DISCUSSION**

We concluded, based on vocalizations, morphometric and genetic data, that the *Otus* owl population on Okinoshima consists of Ryukyu Scops Owls *Otus elegans elegans*. Our sequence data were an almost perfect match for seven previously published sequences, except that six published sequences showed a slight genetic difference amounting to 2.21–2.68% when compared with the five owls studied on Okinoshima. This distinct genetic group is mainly found in the Yaeyama Islands, in the southern Ryukyu Islands (Masaoki Takagi & Takema Saitoh unpublished data), and rarely occurs on other islands, especially the northern Ryukyu Islands (Masaoki Takagi & Takema Saitoh unpublished data; cf. Saitoh et al. 2014).

Prior to this study, BOLD only had 13 data sets for the Ryukyu Scops Owl. Unfortunately, we were unable to conduct inferential statistics for the relationship (similarities and differences) between the external morphometrics and vocalizations of different islands due to the small sample size obtained in this study, although we did not find any discrepancies in any of the characteristics of the Ryukyu Scops Owl. A more extensive examination of the genetic structure of the Ryukyu Scops Owls would be invaluable.

Okinoshima has a very small land area of only 0.68 km², and suitable forest habitat available for owls there is smaller still, indicating that the breeding population must be very small and difficult to maintain because of its isolation and the small area available to it.

The smallest island previously known to be inhabited by Ryukyu Scops Owls was Geruma Island (1.15 km²) (Haruka Ito personal communication); however the population there is considered to be a sub-population of meta-population occurring on Geruma Island and two adjacent islands: Aka (3.82 km²), and Zamami (6.66 km²). Because Aka and Zamami Islands lie within about 0.2 km and 2 km of Geruma Island, respectively, and because several smaller islands are situated between Zamami and Aka Islands, Ryukyu Scops Owls may disperse readily among the three islands. Ryukyu Scops Owls also live on two spatially isolated oceanic islands, Minami-daito, Japan (31 km²) and Lanyu Islands, Taiwan (48 km²). The population about 300+ individuals on Minami-daito is considered to be an endemic subspecies *O. e. interpositus* (Masaoki Takagi et al. unpublished data; cf. Takagi et al. 2006, 2007) while the population of approximately 1,000 individuals on Lanyu is recognized as a different endemic subspecies *O. e. boterensis* (König & Weick 2008). It is inferred that the two islands are each large be enough to maintain a separate population each. It would be very interesting to understand how the population on Okinoshima maintains itself, making phylogeographic analyses using other genetic markers, quantitative spectrographic and morphometric analyses, and understanding their breeding biology invaluable.

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**Fig. 2.** Spectrograms of the calls of three different male Ryukyu Scops Owls (a), (b), and (c) corresponding to the same letters on Fig 1B) made using Raven® 1.3 software (Cornell Laboratory of Ornithology).
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REFERENCES


Appendix 1. Photographs of Ryukyu Scops Owls and of a nesting tree on Okinoshima: male (ID No. 7A-08556; a, c, e, g) and female (7A-08557; b, d, f, h. Images a and b, c and d, e and f, and g and h, show full face, span of right wing, rectrices, and flanks respectively. Image i shows the nesting tree, and the arrow indicates the entrance of the nest cavity, while image j shows one of the three banded owlets (7A-08560).