

BLACK-VENTED SHEARWATER *PUFFINUS OPISTHOMELAS* NESTING IN THE GULF OF CALIFORNIA: A MAJOR EXTENSION OF BREEDING RANGE

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SUMMARY

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The endangered Black-vented Shearwater has been reported nesting at only three islands in the Mexican Pacific Ocean, one of which contains ~95% of their world population. We report on the nesting of this species at a new site, Isla Rasa in the Gulf of California, in 2010 and 2011, and on other signs of possible nesting in 2008 and 2009. Evidence includes a nesting burrow; a genetically identified addled egg at a burrow entrance; nest activity, indicated by tracks and photographs of the nesting individuals; and, in several years, vocalizations, which were monitored to determine prevalence. Although the number of nests at Isla Rasa has not been determined, our observations confirm the nesting of this species, which is the first such report for the Gulf of California. We also report on the presence of the species at Isla Partida Norte, as well as on complementary records during marine surveys in the Midriff Islands Region. Because of the endangered status of the Black-vented Shearwater and of the presence of introduced predators at islands where it is known to breed, the confirmed record of an alternative nesting site, which has now been freed of introduced rodents, bodes well for the conservation of the species. It will be important to explore other potential nesting sites in the Gulf of California and to promote nesting of the species at Isla Rasa through the use of artificial burrows (nest boxes), vocalization playbacks or other methods.

Key words: Black-vented Shearwater, Gulf of California, Isla Rasa, new nesting site record, spontaneous reintroduction

We dedicate this work to the memory of our dear friend Jesús Ramírez-Ruiz, who achieved the eradication of introduced rodents at Isla Rasa in 1995.

INTRODUCTION

Black-vented Shearwater *Puffinus opisthomelas* (BVSH) is considered to be in danger of extinction: “*En Peligro de Extinción*” (*Endangered*, in English), which under Mexican environmental law (NOM-059-SEMARNAT-2010) is the category that calls for the highest level of protection. It is considered Imperiled (G2) in North America (Natureserve 2014) and Near Threatened by the IUCN (2015). Although the species’ non-breeding distribution extends from central California (USA) to central Mexico (Fig. 1), it has been reported thus far to nest only at three islands off the western coast of Baja California, Mexico. About 95% of the known population nests on Isla Natividad, and the other 5% at Isla San Benito and small islets off Guadalupe Island; all of these locations have permanent human settlements (Everett 1988, Everett & Pitman 1993, Keitt *et al.* 2000). Some authors suggest that other small colonies may exist elsewhere (Anderson 1983, Everett 1988, Everett & Anderson 1991). The nesting population estimated in the late 1990s for Isla Natividad was 76 570 SD 18 411 pairs (Keitt *et al.* 2000). The BVSH population at this island has experienced much disturbance due to permanent

human habitation, hunting, burrow destruction or competition by introduced sheep, goats, rabbits and rats, predation by introduced cats and collision with lighted objects. The additive effect of all these factors has caused a severe decline in the nesting population of around 4% per year (Keitt 1998). All introduced species have recently been removed from Isla Natividad, and the nesting population seems to be increasing in response (Keitt *et al.* 2000).

Although BVSHs have been reported within the Gulf of California (Anderson 1983, Everett 1988, Everett & Pitman 1993) and sightings have been recorded during marine surveys among the San Lorenzo Archipelago from late 1970s to present, particularly in the vicinity of Rasa and San Pedro Martir islands (D.W. Anderson 1983 & pers. comm., E. Velarde pers. obs.), no existing reports indicate nesting in the Gulf of California. However, there is a single, generally ignored report of shearwater nesting burrows at Isla Rasa (28°50’N, 113°00’W) by Bancroft (1927). No reference is made as to where he observed these burrows, how many there were, whether they were active, nor which particular species were present. This is the only confirmed report of shearwater-nesting burrows in any Gulf

of California islands, and it has been confirmed by some of the most knowledgeable researchers working in the area, such as Boswall & Barrett (1978) and Anderson (1983). However, Anderson (pers. comm.) and two of us (J.P.G. & E.V.) have observed BVSHs in the waters of the Midriff Island Region on several occasions during the last four decades; and in 1982, Anderson (pers. comm.) and his collaborators saw one flying off Isla San Pedro Martir toward the sea, suggesting that shearwaters may be nesting there.

The goal of this paper is to update the status of this species in the Gulf, reporting two records of BVSH nesting or, at the least, nesting attempts at Isla Rasa; to report on records of related observations, such as calls, the sighting of several individuals at Isla Partida Norte; and to report on several sightings of the species during marine surveys conducted in the Midriff Islands Region during the breeding season in the last two decades.

STUDY SITE AND METHODS

Isla Rasa is a small volcanic island (ca. 54 ha) in the Midriff Islands Region of the Gulf of California, where approximately 95% of the world population of the Heermann's Gull *Larus heermanni* and Elegant Tern *Thalasseus elegans* nest. A detailed description of the island is provided by Velarde *et al.* (2014). Black rats *Rattus rattus* and house mice *Mus musculus* are believed to have been introduced via transport boats to Isla Rasa during the guano mining period in the late 19th and early 20th centuries, and are believed to have been the cause of the extirpation of both the Craveri's Murrelet *Synthliboramphus craveri* nesting colony as well as the shearwater nesting populations (Bowen 2000, Bahr & Bourillón 2002, Velarde *et al.* 2007, 2011, Bowen *et al.* 2015). BVSHs are common in the Gulf of California during the seabird breeding season and, although they are not very abundant, they occur in the Midriff Islands Region and Guaymas Basin (Tershy *et al.* 2002), where they are sometimes observed in large rafts (E. Velarde pers. obs., J.P. Gallo pers. obs.). BVSH vocalizations had been heard sporadically at night at Isla Rasa since 1980 (Velarde pers. obs.), but they were always believed to be issued by birds overflying the island. Here we report definite evidence of this species nesting on Isla Rasa.

Between 2010 and 2014 we collected evidence of the presence of nesting BVSH on the island, such as increased frequency of calls heard at night, evidence of burrow activity, collection of an addled shearwater egg and photo images. For several years after the rodent eradication (1995), and even before that, BVSHs were seldom heard calling, although some individuals were sighted while boat cruises approached or left the island. On 25 April 2010, a shearwater addled egg was found at the entrance of a burrow (see Results). After this first positive evidence of shearwaters, almost daily monitoring of the burrow entrance was carried out in 2010 (25 April to 20 June) and 2011 (16 April to 17 June). On all occasions when we observed fresh tracks at the burrow entrance we recorded the date and took photographs. In 2011 we also monitored the entrance of that same burrow daily and recorded the dates when fresh tracks were found. In that year two movement-sensitive cameras (camera traps) were placed in two different locations about 1 m from the entrance of the burrow. The camera traps were Wildview Xtreme 4 (model STC-TGL4M), which is a digital scouting camera able to take high-resolution 4 MB images and store 600 MB in an SD card. These were left active between 18 April and 10 May and were checked every four days to change the camera memory card and batteries. During 2013 and 2014, we kept records of shearwater vocalizations

during one week in April around the new-moon period, between sunset and midnight. We chose these monitoring periods and hours to increase the probability of detection, because shearwaters are known to be active at the nest entrances (performing nest relief, etc.) almost exclusively during the hours of the night when there is no moonlight (Keitt 1998, Keitt *et al.* 2000), most likely to avoid predation by predominantly diurnal predators (Keitt *et al.* 2003). Another reason to concentrate our efforts during moonless nights was because we had heard their vocalizations a few times during those periods in previous years. We took note of all vocalizations heard from the field station in all directions, estimating that our hearing range extended over a radius of ~300 m. Although it was difficult to determine details in the dark, we made an effort to distinguish whether the location of the call changed and recorded whether the vocalization seemed to come from a bird flying overhead, a bird that seemed to be approaching to land, or a bird in a fixed position on land.

The eggshell was transported to the University of California, Merced, to be analyzed and to confirm the species' identity. We sequenced a portion of the mitochondrial cytochrome b gene and compared this sequence with a reference panel of bird species known to nest in Isla Rasa and other species of the same genus. Previous studies have found eggshells to contain DNA suitable for amplification via polymerase chain reaction (PCR) (Schmaltz *et al.* 2006, Eglob *et al.* 2009, Oskam *et al.* 2010, Velarde *et al.* 2011). To ensure that no foreign DNA would contaminate the sample and to avoid incorporating sediments and other surface contaminants, eggshell fragments were cleaned on both the inner and outer surfaces with 100% alcohol. Following this, eggshell fragments were weighed and ground with a mortar

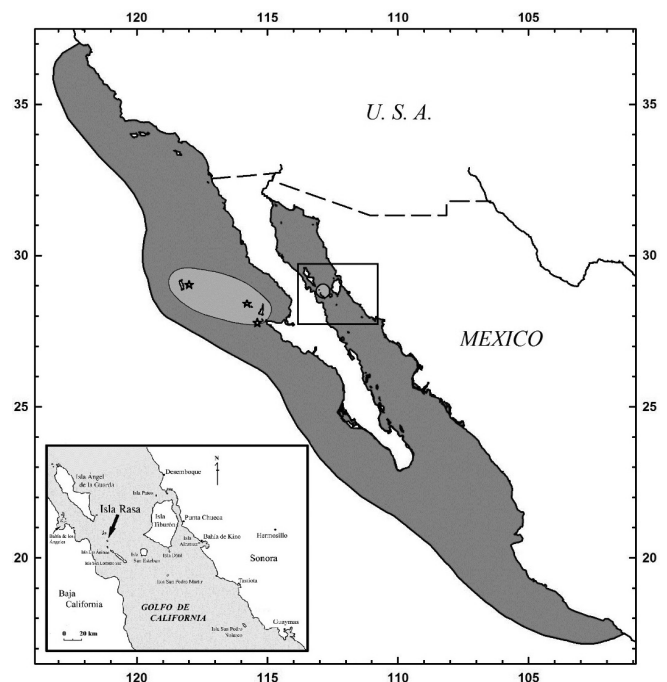


Fig. 1. Map indicating area of Black-vented Shearwater non-breeding distribution (dark gray), known breeding distribution (light gray), with stars indicating known nesting sites: Isla Natividad, Isla San Benito and Isote Negro, off Guadalupe Island and the new site reported in this work, located in the Gulf of California (Isla Rasa). Insert showing detail of Midriff Islands Region drawn by Cathy Moser Marlett.

and pestle. Powder was subsequently transferred to a 2 mL sterile microcentrifuge tube and stored at room temperature for digestion. All laboratory protocols were done in a dedicated clean room, and the sampling area and tools were decontaminated between processing of the samples to prevent sample contamination. To extract the DNA from eggshell powder, we followed the method of Oskam *et al.* (2010) with slight modifications. We used 500 mg of ground eggshell instead of 100 mg.

The cytochrome b gene was chosen because it has been sequenced for most of the extant avian species nesting on Isla Rasa, allowing for an easier and more comprehensive comparison. PCR amplification of the approximately 1 045 bp fragment of the cytochrome b gene was carried out using primers L14841 and H16065 (Friesen *et al.* 1996). Amplification was performed using an Applied Biosystems Veriti gradient thermocycler (Applied Biosystems, Foster City, CA) with the following temperature profile: initial denaturation at 94 °C for 5 min; followed by 35 cycles at 94 °C for 1 min, at 51 °C for 1 min, at 72 °C for 1 min; and a final extension at 72 °C for 5 min. Each PCR reaction contained 50–100 ng of DNA, 1× PCR buffer (Applied Biosystems, Foster City, CA), 0.167 μmol/L of each primer, 0.3 mmol/L of each dNTP, 2.5 mmol/L MgCl₂ and 0.5 U of Amplitaq (Applied Biosystems) in a 30 μL reaction. We included negative and extraction controls to detect possible contamination. Amplicons were sequenced with BigDye chemistry (Applied Biosystems) on an Applied Biosystems 3730 DNA Analyzer. Sequences were imported into Malign v2.7 (Wheeler & Gladstein 1994) and aligned by eye.

To reconstruct phylogenetic relationships, we used the maximum-likelihood (ML) algorithm in the program PHYML v3.0 (Guindon *et al.* 2010). We determined the appropriate model of DNA evolution and model parameters using both the Akaike Information Criterion corrected for small sample sizes (AICc) and Bayesian Information Criterion (BIC) tests with jModeltest v0.1.1 (Posada 2008). Both tests supported the use of the Tamura-Nei model (Tamura & Nei 1993) with a gamma shape parameter (0.224). This model and parameter values, along with optimized base frequency and transition/transversion ratio, were used for phylogenetic reconstruction. One thousand bootstrap pseudoreplicates were performed to assess node support. The extant avian species nesting on Isla Rasa, plus other shearwaters of genus *Puffinus* (and GenBank accession numbers) used in the

phylogenetic analysis were *Corvus corax* (AY527270), *Falco peregrinus* (U83307), *Pandion haliaetus* (AY987232), *Thalasseus elegans* (AY631302), *Thalasseus maximus* (FJ356187), *Puffinus opisthomelas* (AF076087, AY219976), *P. pacificus* (AF076088), *P. bulleri* (AF076081), *P. tenuirostris* (U74352), *P. griseus* (U74353), *P. carneipes* (AF076082), *P. creatopus* (AF076083), *Larus livens* (AF268501), *L. heermanni* (AF268506), *Synthliboramphus craveri* (U37304), *Egretta rufescens* (U83153), *Nycticorax nycticorax* (AF193829), and *Ardea herodias* (AF193821, U83150). The *Falco peregrinus* sequence was used as outgroup.

Finally, we also included the results of 11 avifaunal surveys in the marine area between San Carlos, Sonora and the Midriff Islands Region, as far north as the end of Isla Angel de la Guarda. Surveys were carried out on different dates in 1996, 1997, 2006 and 2010–2013. Visual surveys were carried out during daylight (~12 h/d) by four observers positioned at the ship’s bridge, at an eye-height of about 5.5 m above the water surface, equipped with 10 × 50 binoculars. All birds, flying or on the water, in a 180° arc and 300 m radius in front of the observer were recorded. A Furuno (GP1850WF) dual 50–200 kHz GPS-echo sounder was used to determine the location of the boat. Date and time of the day were also recorded. Surveys varied in number of days and date of observation, although most were carried out in June, July, September and October of different years (Table 1). Records were collected while the boat was navigating at constant speed between two specific points (continent to island, island to continent or between two islands) where sea lion censuses would be carried out.

RESULTS

During the seabird nesting season of 2008 and 2009, E.V. observed an active burrow of a size suitable for a BVSH. Initially it was believed to be a chuckwalla *Sauromalus* sp. burrow, since a chuckwalla had recently been sighted on the island (Velarde *et al.* 2008). On 25 April 2010, the first time that the burrow was inspected during the field

Table 1
Number of Black-vented Shearwaters observed in the 12 surveys carried out from 1996 to 2015 in the Midriff Islands Region; all survey days consisted of constant navigation for 12 daylight hours

Date	No. survey days	No. individuals
2–14 July 1996	10	38
9–16 August 1997	5	1
16–20 July 2006	4	1
7–13 June 2010	6	8
26 September 2010	1	8
9–16 July 2011	7	2
16–19 July 2012	4	7
1–4 July 2013	4	25
16–19 September 2013	4	12
23–29 September 2013	7	5
29–31 October 2013	3	12
13–22 July 2015	9	36
Totals	64	155



Fig. 2. Shearwater leaving its burrow on Isla Rasa, 30 April 2011 at 21h53, taken by a movement-sensitive camera (camera trap) placed in front of the burrow entrance.

season that year, an addled white egg was found inside the burrow, very near its entrance. At the same time, several seabird tracks were observed indicating an individual coming out of the burrow. The tracks resembled those of the Heermann's Gull, thousands of which step on the soft terrain of the island (the top layer of guano on Isla Rasa has a soft powdery texture), but these slightly narrower tracks were in a location and position that indicated the bird was coming out of the burrow (a most unlikely action for a gull) and showed the mark of the tarsus/metatarsus section of the leg behind the foot track. This suggested they belonged to a seabird that was walking in a squatting position. Remembering Bancroft's report (1927), the possibility of this being a shearwater nesting burrow was immediately considered. The egg was collected and daily monitoring of the entrance indicated fresh tracks every 2.7 d on average until May 22. The burrow entrance was monitored daily until 20 June, but no further activity was noticed after May 22. Thus, we suspected that any chick must have fledged.

In 2011 the burrow was checked for the first time on 16 April, and tracks were again noticed. Two camera traps placed close to the burrow's entrance produced a few pictures of a shearwater, and it could be verified that it was attending the burrow regularly. Cameras recorded a shearwater entering or exiting the burrow every 2.5 d on average. In that year the activity lasted until 6 May, ceasing completely thereafter. Some of the clearest images of the shearwater were sent to Brad Keitt and Sophie Webb for identification (Fig. 2).

They both agreed that the images were of a BVSH. The dark bill and feet and the mottling of the neck were the most useful characteristics for identification.

The 2013 and 2014 auditory records of vocalizations revealed some very irregular activity. For those two years we observed for two hours (generally from 21h00 to 23h00) during 7 nights in April and recorded a mean of 1.36 SD 0.62 vocalizations/h (range = 0–7 vocalizations/h and mode = 0.6 vocalizations/h).

Our phylogenetic analysis of the cytochrome b sequences supported the following clades: Falconiformes, Ciconiiformes, Passeriformes, Accipitriformes, Charadriiformes and Procellariiformes (Fig. 3). Despite the low support for basal clades (values lower than 70% are not shown), the phylogeny closely resembles other previously published avian phylogenetic reconstructions (Gibb *et al.* 2007, Pacheco *et al.* 2011). Within the Procellariiformes clade, all the shearwaters of genus *Puffinus* group together, and this clade contains the sequence from our eggshell sample (PUFCYTB eggshell, GenBank accession number KT266869, Fig. 3). The eggshell sequence grouped with the two BVSH cytochrome b sequences obtained from GenBank with high bootstrap support (100%). All other *Puffinus* species formed a sister group to *P. opisthomelas* sequences, both from GenBank and our eggshell sample. This result demonstrated that the eggshell was from a

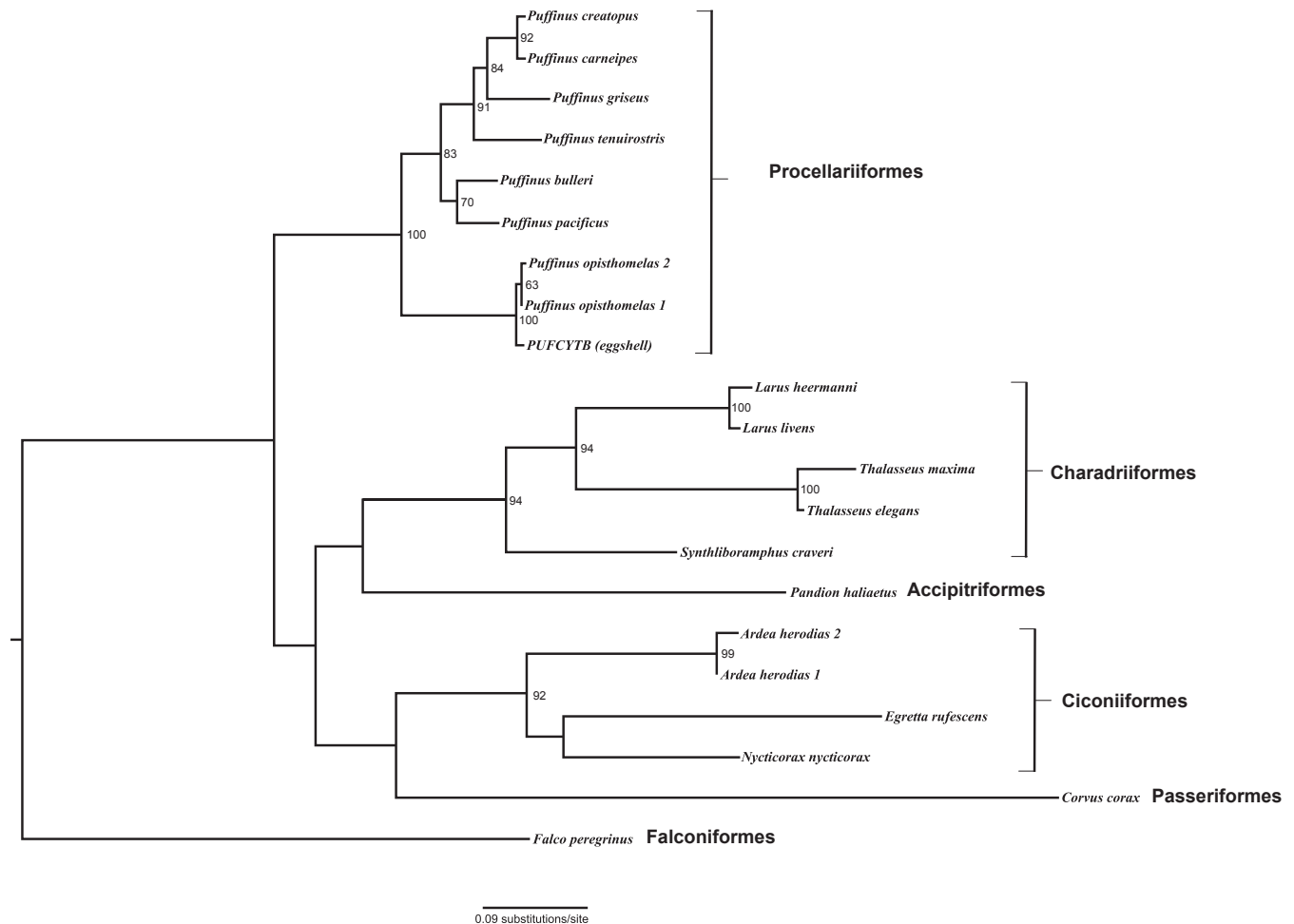


Fig. 3. Maximum likelihood tree based on 1 045 bp of the mtDNA cytochrome b gene of nesting birds from Isla Rasa plus other shearwaters of the genus *Puffinus*, using program PHYML v3.0. Bootstrap values greater than 70 are shown at nodes.

BVSH and adds conclusive evidence that this species is using Isla Rasa for nesting.

A total of 47 records of BVSHs were obtained during the boat surveys, with a total of 155 individuals. The records showed that BVSHs were concentrated mainly in the vicinity of Isla San Pedro Nolasco, and secondarily the portion of ocean that stretches between the southwestern tip of Isla Tiburón and Punta San Francisquito on the Baja California Peninsula; some points also occurred around Isla Angel de la Guarda and in the vicinity of Isla Rasa (Fig. 4). It is interesting that 40% of the sightings and 59% of the individuals were recorded in a maximum radius of about 13 nautical miles (or approximately 24 km) around Isla San Pedro Nolasco.

E.A.R. found and photographed a group of four BVSHs at Isla Partida Norte (also called Cardonosa, 28°53'N, 113°02'W), 4 nautical miles northwest of Isla Rasa, at about 20h43 on 17 April 2014. This happened during surveys unrelated to the work reported here. Based on the photographs, we were able to ascertain the identity of the species; individuals were heard vocalizing after dark and were located using a headlamp. The BVSHs showed no flight reaction when approached by humans. One of them was captured with relative ease by hand, and photographs were taken of the dorsal and ventral sides, with wings closed and spread. The individual was released immediately after and stayed at the same location while people were at the site.

DISCUSSION

The single encounter with an active nesting burrow for two years in a row at Isla Rasa, vocalizations heard during moonless nights and the wide range of variation in the frequency of vocalizations heard per hour of observation leads us to believe that several pairs nest on the island. Most vocalizations heard may belong to prospecting individuals, possibly attracted by conspecifics already nesting there. Alternatively, these individuals may have been hatched on Isla Rasa in previous years and were in the process of recruiting to the adult population.



Fig. 4. Google Earth image with small markers showing the 47 sites where the Black-vented Shearwater sightings occurred during the marine surveys performed from 1996 to 2015. Numbers by the markers, indicating the sites, show the number of individual shearwaters recorded. Larger markers show location of Isla Rasa, where nesting has been confirmed, as well as Partida Norte, San Pedro Mártir and San Pedro Nolasco islands, where nesting might occur (Image Landsat, Data SIO, NOAA, US Navy, NGA, GEBCO, © 2015 Google, © 2015 INEGI).

Although there is no way to be sure whether the burrows that Bancroft reported in his 1927 publication belonged to BVSH and whether those were excavated by nesting individuals of that species—who had endured the predation pressure of the introduced rodents—the present reoccupation (or continued occupation) of the island by the species, albeit in very low numbers, suggests that the BVSH may be relatively resilient to such disturbances, and given the new predator-free environment, this species population may be slowly increasing at the island. Furthermore, the few BVSHs seen at Isla Partida Norte by one of us (E.A.R.) may indicate that the species may also be prospecting or nesting there. Isla Partida Norte is an important breeding site for Least Storm-petrel *Oceanodroma microsoma* and Black Storm-petrel *O. melania* (Anderson 1983), as well as for Craveri's Murrelets *Synthliboramphus craveri* and for fish-eating bats *Myotis vivesi*, and the conditions are adequate for the breeding of burrow-nesting seabirds, especially due to the lack of land predators.

There is a possibility that the species is nesting at other islands besides Isla Rasa. The fact that we have recorded the species during nocturnal surveys on Isla Partida Norte and compiled numerous records in the vicinity of Isla San Pedro Nolasco may indicate that they might be nesting there too. Also, the fact that BVSHs prefer soft sandy soils where they can excavate a burrow for nesting (Everett 1988, Keitt 1998, Keitt *et al.* 2000) leads us to believe that, however scant the soil is on these three Gulf of California islands (Rasa, Partida Norte and San Pedro Nolasco), these locations may be appropriate for the species (Felger *et al.* 2011, and pers. obs.). This does not preclude the possibility that they may be nesting at some other islands where soil may accumulate in small areas, such as San Pedro Mártir, over which some observers have seen them flying (D.W. Anderson, pers. comm.).

Although future studies are needed to determine the number of breeding pairs that occur on Rasa or other islands and in what proportion these breeding events result in successful fledging, this confirmed nesting record demonstrates the reoccupation of a former nesting island by the species. This is important for the potential recovery of this endangered bird, particularly because this island is now protected and free of mammalian predators. The possibility that BVSHs are also nesting in Partida Norte and San Pedro Nolasco islands is also important for the conservation and recovery of this species. The former has no introduced predators, and the latter was recently subject to eradication of introduced rodents. According to recent surveys, only a few feral cats are found in Isla San Pedro Nolasco, and there are plans to remove them from the island (J.P.Gallo pers. obs.).

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