

Phylogenetic placement of the critically endangered Townsend's Shearwater (*Puffinus auricularis auricularis*): evidence for its conspecific status with Newell's Shearwater (*Puffinus a. newelli*) and a mismatch between genetic and phenotypic differentiation

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Abstract Townsend's Shearwater (*Puffinus auricularis auricularis*) is a highly threatened bird and currently breeds on Socorro and Clarión Islands, México. This subspecies has minor differences in plumage patterns when compared to Newell's Shearwater of Hawaii (USA) (*Puffinus auricularis newelli*). These two forms are recognized as subspecies by the American Ornithologist's Union. However, some authors consider them as distinct species based on subtle plumage differences and different breeding chronologies. We used Bayesian and Maximum Likelihood methods to compare the *cytochrome b* and *cytochrome oxidase I* sequences from Townsend's Shearwaters with archived mitochondrial sequences from other taxa in the genus *Puffinus*. Townsend's and Newell's Shearwaters show little genetic differentiation; hence, there is no justification to consider them as different species. Additionally, differences in morphology and ecology might be the result of founder effects and phenotypic plasticity; proven migratory potential provides support to the current

taxonomic assessment that considers these birds as conspecifics. We recommend the continued treatment of Townsend's and Newell's Shearwaters as two subspecies of *P. auricularis*. We also advocate treating the Rapa Shearwater (*P. myrtae*) as a distinct species.

Keywords Townsend's Shearwater · *Puffinus auricularis auricularis* · Newell's Shearwater · *Puffinus auricularis newelli* · Socorro Island · Revillagigedo Archipelago · Genetic and phenotypic differentiation mismatch

Zusammenfassung

Stammesgeschichtliche Einordnung des hochgradig gefährdeten Townsendsturmtauchers (*Puffinus auricularis auricularis*): Nachweis für seinen engen Verwandtschaftsgrad zum Newellsturmtaucher (*Puffinus a. newelli*) und für die Diskrepanz zwischen genetischer und phänotypischer Abgrenzung

Der Townsendsturmtaucher ist ein hochgradig gefährdeter Vogel, der derzeit noch auf den Socorro und Clarion-Inseln, Mexiko, brütet. Zum Newellsturmtaucher auf Hawaii (USA) zeigt er nur kleine Unterschiede in der Gefiedermusterung; die beiden sind von der Union Amerikanischer Ornithologen als Unterarten anerkannt. Dennoch betrachten einige Autoren sie aufgrund der Unterschiede im Gefieder und in der Brutabfolge als eigenständige Arten. Wir setzten die Maximum Likelihood-Methode der Bayesschen Wahrscheinlichkeitsrechnung ein, um die Cytochrome b- und die Cytochrome Oxidasen I-Sequenzen miteinander zu vergleichen, die wir einerseits vom Townsendsturmtaucher, andererseits von archiviertem mitochondrialem Material anderer Sturmtaucher-Arten gewonnen und sequenziert hatten. Townsend- und Newellsturmtaucher zeigten nur geringe

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genetische Unterschiede, weswegen es keine Rechtfertigung dafür gibt, sie als eigene Arten anzusehen. Unterschiede in ihrer Morphologie und Ökologie könnten vom sog. „Gründereffekt“ und der phänotypischen Plastizität herrühren; das nachgewiesene Wander- und Ausbreitungspotential unterstützen die derzeitige taxonomische Bewertung, dass es sich hier um zwei Unterarten einer Art handelt. Wir empfehlen, Townsend- und Newellsturmtaucher auch weiterhin als zwei Unterarten von (*Puffinus auricularis*) zu betrachten. Darüber hinaus plädieren wir dafür, den Rapasturmtaucher (*Puffinus myrtae*) als eine eigene Art anzusehen.

Introduction

Townsend's Shearwater is a Manx-type Shearwater endemic to the Revillagigedo Archipelago, currently nesting on Socorro Island, (Brooke 1990; Ainley et al. 1997; Martínez-Gómez and Jacobsen 2004) and recently rediscovered on Clarión Island (J.A. Cervantes-Pasqualli pers. comm). This shearwater is one of the four medium-sized shearwaters in the Eastern Pacific with a dark dorsum and white ventral parts (Jehl 1982). Specimens of this species were first obtained from its breeding grounds on Clarión Island and described as *Puffinus auricularis* (Townsend 1890); additional specimens were obtained subsequently from San Benedicto (Anthony 1898) and Socorro Islands (McLellan 1926). A similar shearwater, described originally as a distinct species, *Puffinus newelli*, was discovered in Hawaii a few years later (Henshaw 1900). Both Townsend's and Newell's Shearwaters are considered as critically endangered, and endangered respectively (BirdLife 2000).

Ridgway (in Henshaw 1900) treated Townsend's and Newell's shearwaters as different species. He observed that “the *Puffinus (newelli)* which you sent for identification is without doubt a new species. It comes nearest to *P. auricularis* but differs in blacker color of upper parts, wholly white malar region, more extensive, more uniform and more abruptly white anterior, and central undertail coverts, more extensive and ‘solid’ blackish border to under-wing coverts region, and especially in the very abrupt line of demarcation along sides of neck between the black upper parts and white of under parts. *P. auricularis* also has the bill entirely black and also stouter”. A detailed examination confirming such differences is found in Howell et al. (1994).

Vocalizations of the two species are very similar, although minor differences probably due to recording conditions have been reported (Ainley et al. 1997; Baptista and Martínez-Gómez 2002). There are also differences in breeding chronology, Townsend's reproductive peak occurs in the spring while Newell's in the summer (Ainley et al. 1997). This parallels the case of two subspecies of

Oceanodroma leucorhoa on Guadalupe Island (Jehl and Everett 1985).

Murphy (1952) grouped *Puffinus puffinus*, *P. mauretanicus*, *P. yelkouan*, *P. gavia*, *P. huttoni*, *P. newelli*, *P. auricularis* and *P. opisthomelas* as subspecies of the former. This arrangement did not stand: the Mediterranean group was treated as separate species based on morphological and genetic differences (Bourne et al. 1988; Wink et al. 1993). The remaining subspecies were granted specific status in more comprehensive phylogenies of the genus (Heidrich et al. 1998, Austin et al. 2004). However, the forms from the Revillagigedo and Hawaiian islands were treated as *P. a. auricularis* and *P. a. newelli* based on their overall similarities in morphology and vocalizations (Jehl 1982; American Ornithologists' Union (AOU) 1983; American Ornithologists' Union (AOU) 1998). Austin et al. (2004) attempted to obtain DNA from a skin sample of Townsend's Shearwater; but were unsuccessful. Austin et al. (2004) also found that the Rapa Shearwater (*P. myrtae*) of Rapa, Austral Islands (French Polynesia) was a sister taxon to *P. a. newelli*. Overall, the taxonomy of the genus has remained controversial (Carboneras 1992). Townsend's shearwater has also been incorrectly referred to as *P. a. townsendi* by Ainley et al. (1997).

Contra AOU (1983, 1998), Brooke (2004) following Pratt et al. (1987) and Collar et al. (1992, 1994) proposed that Townsend's Shearwater from the Revillagigedo Islands and Newell's Shearwater from the Hawaiian Islands should be considered two species. Brooke (2004) admitted to “a hunch that, when molecular data become available, they will support this position”. Howell (2012) proposed a separate treatment based on their differences in morphology, breeding chronology (Ainley et al. 1997; Bourne et al. 1988), and breeding ecology (Spear et al. 1995). The IOC CheckList (Gill and Donsker 2014) follows Austin et al. (2004) in considering Townsend's and Newell's Shearwater as distinct species.

Also, it has been argued that Mexican island endemics, should be considered distinct species based on evolutionary and phylogenetic species concepts (Peterson and Navarro-Sigüenza 1999; Navarro-Sigüenza and Peterson 2004), and that morphologically distinct bird populations on islands should be treated as separate species (Gill 2014). Del Hoyo et al. (2014, following Tobias et al. 2010 scoring system) considered Townsend's and Newell's Shearwaters different species based on morphological and behavioral criteria. Recently, it has been shown that several island landbirds on Socorro and other islands conform to the above mentioned predictions based on endemism and insular condition (*Zenaidura graysoni*, Johnson and Clayton 2000; *Mimus graysoni*, Barber et al. 2004; *Troglodytes sissonii*, Martínez-Gómez et al. 2005; *Setophaga flavescens*, McKay et al. 2010; *Junco insularis*, Alexandre et al. 2013; *Setophaga*

graysoni, Evans et al. 2014), but each case needs to be examined separately.

Furthermore, a recent specimen of a Newell's Shearwater from coastal California, far from its breeding grounds, affords the possibility for occasional migratory movements between islands (Heindel and Garret 2008; Unitt et al. 2009). Consequently, ecological differences between Newell's and Townsend's shearwaters might represent behavioral plasticity resulting from different oceanic conditions in particular areas of their distributions, and observed minor plumage differences might be the outcome of founder effects, genetic drift or individual plasticity (Templeton 2006).

In this paper, we report the phylogenetic placement of Townsend's Shearwater and its relationship to Newell's and Rapa shearwaters based on molecular analyses derived from recently acquired tissue and museum specimens. Phylogeny based on cytochrome b (*cyt b*) and cytochrome oxidase I (*COI*) mitochondrial genes allowed comparisons between Townsend's and Newell's Shearwaters and other closely related species, shedding light on their taxonomic status.

Methods

We obtained a fresh sample of Townsend's Shearwater from a carcass obtained in a forested area northwest of Mount Evermann, the summit dome of Socorro Island (CNAV JCP20110401; DNA extract and photo record). Most likely it was preyed-upon by a Red-tailed Hawk (*Buteo jamaicensis socorroensis*) as suggested by the presence of plucked feathers on the ground (e.g. Veitch 1985, 1989; Martínez-Gómez and Jacobsen 2004). Special care was placed to avoid breaching the herbaceous layer that prevents cat predation in breeding areas and in minimizing disturbance to breeding activities. Remains were washed with distilled water several times; pectoral muscle was then cut into pieces and stored in lysis buffer (10 mM Tris-HCL pH8.0, 100 mM EDTA, 2 % SDS). Samples were kept at room temperature while on the island, then at -20°C 2 weeks later once on the mainland. DNA was extracted following animal tissue protocols of the Wizard SV Genomic DNA Purification kits (Promega Corporation, Madison, WI).

Also, DNA was extracted from toe pads and bones from three Townsend's Shearwater specimens at the Colección Nacional de Aves (CNAV P0000035) and one at the Museo de Zoología de la Facultad de Ciencias (MZFC 11573), Universidad Nacional Autónoma de México. Toe pads were washed with Sodium hypochlorite to 10 %; then digested for 3 days with 10 μl of *Proteinase K*, and 30 μl of Dithiothreitol (1 M) for 3 days. After that procedure, samples resulting from toe pads followed the extraction protocol of the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany).

We amplified partial sequences of the mitochondrial cytochrome b gene (*cyt b*) and cytochrome oxidase I (*COI*). We used mt-A, mt-Fr (Heidrich et al. 1998), L14841, and H15149 (Kocher et al. 1989) to amplify *cyt b*. For *COI* sequences we used the primer pair Ltyr-H8205, and BirdF1-BirdR1. For ancient DNA, we used internal primers in conjunction with those above: AwCintF2, AwCintR2, AwCintF4, and AwCintR4 (Patel et al. 2010; Lijtmaer et al. 2012). All polymerase chain reactions (PCR) were carried out in 25 μl reaction volumes according to the conditions described by Lijtmaer et al. (2012) and using the Platinum PCR Kit (Invitrogen Carlsbad, CA). Cycling PCR for *cyt b* had an initial 94°C hotstart step for 4 min, followed by 30 cycles with the following profile 94°C denaturing for 45 s, 50°C annealing for 60 s, extension at 72°C for 90 s, and terminal extension at 72°C for 4 min. For amplified ancient DNA, an initial denaturing step of 94°C for 2 min, 35–40 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 1 min, and a final extension of 72°C for 10 min. To amplify *COI* of modern samples the program recommended by Lijtmaer et al. (2012) was used. For ancient DNA we got better results with the following short program 94°C for 2 min followed by 35–40 cycles of 94°C for 30 s, $56\text{--}60^{\circ}\text{C}$ for 30 s, 72°C for 30 s, and a final extension of 72°C for 4 min, which was designed for modern samples (Patel et al. 2010). We also obtained partial *COI* sequences of the Black-vented Shearwater (*P. opisthomelas*) from four museum specimens (one sample of bone and three of toe pads, Table 1), according to the described protocol. PCR products were then sent to Elim Biopharmaceuticals Inc., Hayward, CA and High-Throughput Genomics Center, Seattle, WA for bi-directional sequencing. Robust DNA sequences were obtained by assembling DNA sequences from separate runs with Sequencher 4.8 (GeneCodes, Ann Arbor, MI). We obtained additional *cyt b* and *COI* sequences of other species in the genus *Puffinus* from GenBank Christmas Shearwater (*P. nativitatis*), Galapagos Shearwater (*P. subalaris*), Scopoli's Shearwater (*Calonectris diomedea*), and Streaked Shearwater (*C. leucomelas*) to be used as outgroups (Table 1).

The molecular model of evolution that best explained our data was selected with jModelTest (Darriba et al. 2012). The General Time Reversible model (GTR+G+I) was selected through the hierarchical likelihood ratio test for both genes with rate variation modeled by a gamma shape distribution 0.59 for *cyt b* and 0.31 for *COI*. Because sequences retrieved from Genbank did not belong to the same specimens and taxa, we ran separate analysis for *cyt b* and *COI* gene fragments. Results were incorporated to construct Bayesian and Maximum likelihood trees. In MrBayes version 3.1.2, two Markov Chain Monte Carlo (MCMC) simulations were run simultaneously for ten million generations with sampling every 200 generations

Table 1 List of cytochrome b (*cyt b*) and cytochrome oxidase I (*COI*) sequences of *Puffinus* and *Calonectris* used in the analyses

No.	Taxon	Gen	Gen Bank Accession	Sample	Clade ^b	References
1	<i>P. a. auricularis</i>	<i>cyt b</i>	KP938301	MZFC 11573	Central Pacific	This study
1	<i>P. a. auricularis</i>	<i>COI</i>	KP938302	MZFC 11573	Central Pacific	This study
2	<i>P. a. auricularis</i>	<i>cyt b</i>	KP938300	CNAVJCP2011N0401	Central Pacific	This study
2	<i>P. a. auricularis</i>	<i>COI</i>	KP938303	CNAVJCP2011N0401	Central Pacific	This study
3	<i>P. a. auricularis</i>	<i>COI</i>	KP938304	CNAV P0000035	Central Pacific	This study
4	<i>P. a. newelli</i>	<i>COI</i>	JF498893	USNM 643474	Central Pacific	Kerr and Dove (unpublished)
5	<i>P. a. newelli</i>	<i>COI</i>	JF498894	USNM 643473	Central Pacific	Kerr and Dove (unpublished)
6	<i>P. a. newelli</i>	<i>cyt b</i>	L43008	PUFMTCTBG. Panw108	Central Pacific	Austin (1996)
7	<i>P. a. newelli</i>	<i>cyt b</i>	AY219974	Panw106	Central Pacific	Austin et al. (2004)
8	<i>P. a. newelli</i>	<i>cyt b</i>	AY219975	Panw108	Central Pacific	Austin et al. (2004)
9	<i>P. a. newelli</i>	<i>cyt b</i>	HQ589356	NESH000000MI1	Central Pacific	Pyle et al. (2011)
10	<i>P. myrtae</i>	<i>cyt b</i>	AY219938	MNHN 1975.1788	Central Pacific	Austin et al. (2004)
11	<i>P. myrtae</i>	<i>cyt b</i>	AY219939	MNHN 1975.1787	Central Pacific	Austin et al. (2004)
12	<i>P. opisthomelas</i>	<i>cyt b</i>	AY219976	BMNH 1949.64.56	Baja California	Austin et al. (2004)
13	<i>P. opisthomelas</i>	<i>cyt b</i>	AF076087	PUFOPIS-1	Baja California	Nunn and Stanley (1998)
14	<i>P. opisthomelas</i>	<i>COI</i>	KP938305	CNAV 19957	Baja California	This study
15	<i>P. opisthomelas</i>	<i>COI</i>	KP938306	CNAVE 20101	Baja California	This study
16	<i>P. opisthomelas</i>	<i>COI</i>	KP938307	CNAV P000038	Baja California	This study
17	<i>P. opisthomelas</i>	<i>COI</i>	KP938308	CNAV P000801	Baja California	This study
18	<i>P. bryani</i>	<i>cyt b</i>	HQ589355	USNM 492974		Pyle et al. (2011)
19	<i>P. atrodorsalis</i>	<i>cyt b</i>	AY219965	DNSM 36093	Indian and Pacific	Austin et al. (2004)
20	<i>P. bailloni</i>	<i>cyt b</i>	AY219963	Plba_EP2	Indian and Pacific	Austin et al. (2004)
21	<i>P. bailloni</i>	<i>cyt b</i>	AY219964	Plba_VB	Indian and Pacific	Austin et al. (2004)
22	<i>P. colstoni</i>	<i>cyt b</i>	AY219961	Plco_EP4	Indian and Pacific	Austin et al. (2004)
23	<i>P. colstoni</i>	<i>cyt b</i>	AY219962	Plco_EP6	Indian and Pacific	Austin et al. (2004)
24	<i>P. dichrous</i>	<i>cyt b</i>	AY219953	Pldi_EP10	Indian and Pacific	Austin et al. (2004)
25	<i>P. dichrous</i>	<i>cyt b</i>	AY219954	Pldi_EP11	Indian and Pacific	Austin et al. (2004)
26	<i>P. nicolae</i>	<i>cyt b</i>	AY219960	BMNH 1957.16.2	Indian and Pacific	Austin et al. (2004)
27	<i>P. nicolae</i>	<i>cyt b</i>	AY219957	Plco_EP1	Indian and Pacific	Austin et al. (2004)
28	<i>P. persicus</i>	<i>cyt b</i>	AY219967	BMNH 1962.9.2	Indian and Pacific	Austin et al. (2004)
29	<i>P. persicus</i>	<i>cyt b</i>	AY219966	BMNH 1976.1.27	Indian and Pacific	Austin et al. (2004)
30	<i>P. polynesiae</i>	<i>cyt b</i>	AY219955	BMNH 1948.59.29	Indian and Pacific	Austin et al. (2004)
31	<i>P. temptator</i>	<i>cyt b</i>	AY219980	RMCA 83.43.A.756	Indian and Pacific	Austin et al. (2004)
32	<i>P. baroli</i>	<i>cyt b</i>	AJ004207		North Atlantic and Caribbean	Heidrich et al. (1998)
33	<i>P. baroli</i>	<i>cyt b</i>	AY219936	Pabr91	North Atlantic and Caribbean	Austin et al. (2004)
34	<i>P. boydi</i>	<i>cyt b</i>	AY219937	BMNH 1936.2.21.87	North Atlantic and Caribbean	Austin et al. (2004)
35	<i>P. lherminieri</i>	<i>cyt b</i>	AF076085		North Atlantic and Caribbean	Nunn and Stanley (1998)
36	<i>P. lherminieri</i>	<i>cyt b</i>	AY219943	LSM B20918	North Atlantic and Caribbean	Austin et al. (2004)
37	<i>P. loyemilleri</i>	<i>cyt b</i>	AY219946	BMNH 1959.31.1	North Atlantic and Caribbean	Austin et al. (2004)
38	<i>P. lherminieri</i>	<i>COI</i>	AB843721	YIO-63294	North Atlantic and Caribbean	Saitoh et al. (2014)
39	<i>P. lherminieri</i>	<i>COI</i>	AB843722	YIO414-10	North Atlantic and Caribbean	Saitoh et al. (2014)
40	<i>P. lherminieri</i>	<i>COI</i>	AB843723	YIO415-10	North Atlantic and Caribbean	Saitoh et al. (2014)
41	<i>P. lherminieri</i>	<i>COI</i>	JQ176049	USNM:Birds:607634	North Atlantic and Caribbean	Schindel et al. (2011)
42	<i>P. lherminieri</i>	<i>COI</i>	JQ176050	USNM:Birds:607633	North Atlantic and Caribbean	Schindel et al. (2011)
43	<i>P. lherminieri</i>	<i>COI</i>	JX297489		North Atlantic and Caribbean	Zarzoso-Lacoste (unpublished)

Table 1 continued

No.	Taxon	Gen	Gen Bank Accession	Sample	Clade ^b	References
44	<i>P. assimilis</i>	<i>cyt b</i>	AF076080		Southern Hemisphere-subtropical-subatartic	Nunn and Stanley (1998)
45	<i>P. assimilis</i>	<i>cyt b</i>	AY219925	Paas162	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
46	<i>P. elegans</i>	<i>cyt b</i>	AY219932	BMNH 1956.36.27	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
47	<i>P. elegans</i>	<i>cyt b</i>	AY219933	BMNH 1956.38.28	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
48	<i>P. haurakiensis</i>	<i>cyt b</i>	AY219930	Paha31	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
49	<i>P. haurakiensis</i>	<i>cyt b</i>	AY219931	Paha33	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
50	<i>P. kermadecensis</i>	<i>cyt b</i>	AY219928	Pakm2	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
51	<i>P. tunneyi</i>	<i>cyt b</i>	AY219926	BMNH 1949.64.60	Southern Hemisphere-subtropical-subatartic	Austin et al. (2004)
52	<i>P. mauretanicus</i>	<i>cyt b</i>	AJ004211		Mediterranean	Heidrich et al. (1998)
53	<i>P. mauretanicus</i>	<i>cyt b</i>	DQ230316	D.ORO5071350	Mediterranean	Genovart et al. (2007)
54	<i>P. yelkouan</i>	<i>cyt b</i>	AY219973	Ppyk61	Mediterranean	Austin et al. (2004)
55	<i>P. yelkouan</i>	<i>cyt b</i>	AJ004217		Mediterranean	Heidrich et al. (1998)
56	<i>P. yelkouan</i>	<i>COI</i>	AY567884		Mediterranean	Treutlein and Wink (unpublished)
57	<i>P. olsoni</i> ^a	<i>cyt b</i>	HQ651230		East Atlantic	Ramirez et al. (2010)
58	<i>P. puffinus</i>	<i>cyt b</i>	U74355	PPU74355 (P779E)	East Atlantic	Nunn and Stanley (1998)
59	<i>P. puffinus</i>	<i>cyt b</i>	AJ004215		East Atlantic	Heidrich et al. (1998)
60	<i>P. puffinus</i>	<i>COI</i>	DQ433152	AMNH DOT10101	East Atlantic	Kerr et al. (2007)
61	<i>P. puffinus</i>	<i>COI</i>	GU572067	BISE-Aves168	East Atlantic	Johnsen et al (unpublished)
62	<i>P. gavia</i>	<i>cyt b</i>	AY219977	Pgav1	New Zeland	Austin et al. (2004)
63	<i>P. huttoni</i>	<i>cyt b</i>	AF076084		New Zeland	Nunn and Stanley (1998)
64	<i>P. huttoni</i>	<i>cyt b</i>	AY219978	Phut10	New Zeland	Austin et al. (2004)
65	<i>P. nativitatis</i>	<i>cyt b</i>	AY219979	Pnat81	Outgroup	Austin et al. (2004)
66	<i>P. nativitatis</i>	<i>cyt b</i>	AF076086	CIS-O12	Outgroup	Nunn and Stanley (1998)
67	<i>P. subalaris</i>	<i>cyt b</i>	AY219969	MNHN 1970.854	Outgroup	Austin et al. (2004)
68	<i>P. subalaris</i>	<i>cyt b</i>	AY219970	MNHN 1970.855	Outgroup	Austin et al. (2004)
69	<i>P. pacificus</i>	<i>cyt b</i>	U70484	PPU70484	Outgroup	Bretagnolle et al. (unpublished)
70	<i>C. diomedea</i>	<i>cyt b</i>	AY139626		Outgroup	Garcia-Moreno et al. (2003)
71	<i>C. diomedea</i>	<i>COI</i>	DQ432808	USNM 620710	Outgroup	Kerr et al. (2007)
72	<i>C. diomedea</i>	<i>COI</i>	DQ433417	USNM 620711	Outgroup	Kerr et al. (2007)
73	<i>C. leucomelas</i>	<i>cyt b</i>	DQ372049		Outgroup	Gomez-Diaz et al. (2006)
74	<i>C. leucomelas</i>	<i>COI</i>	AB842604	BJNSM642-10	Outgroup	Saitoh et al. (2014)
75	<i>C. leucomelas</i>	<i>COI</i>	AB843388	YIO464-10	Outgroup	Saitoh et al. (2014)

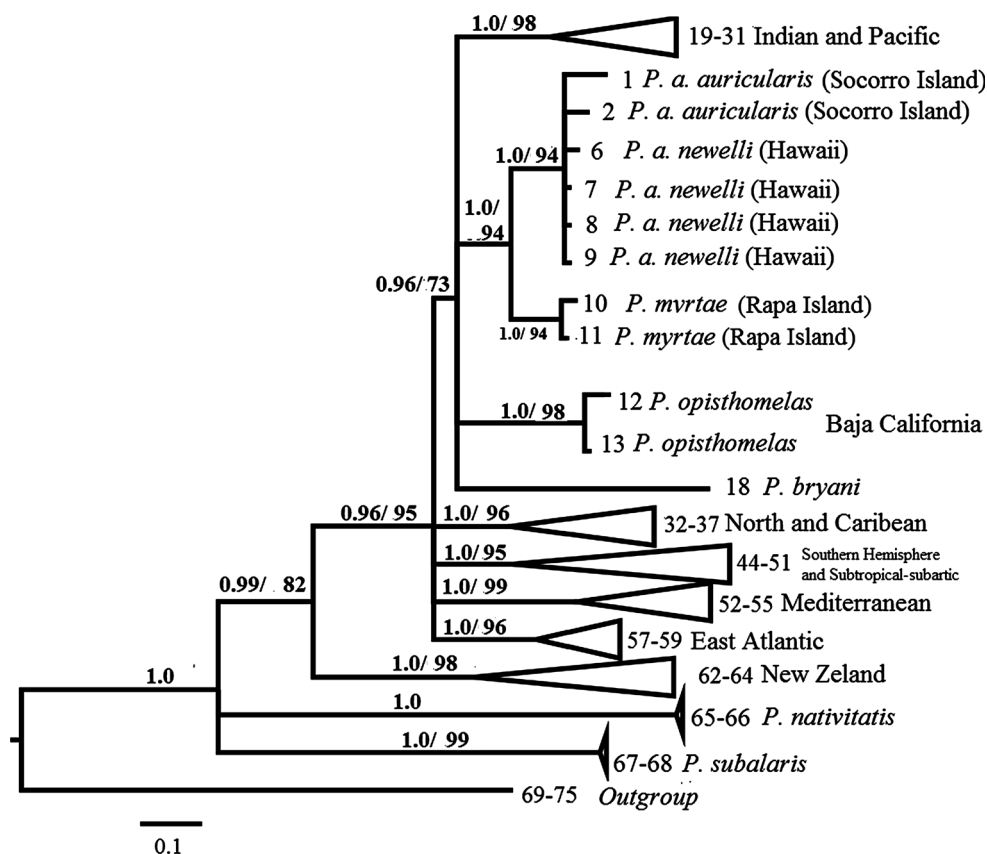
^a Extinct species

^b The name clade corresponding with Austin et al. (2004)

generating 100,000 trees (Huelsenbeck and Ronquist 2001). Convergence of runs was confirmed using Tracer V1.6 (Rambaut and Drummond 2013). The first 25,000

trees were discarded from the sample as the “burn-in” period that accounted for 25 % of the trees. The effective sample size values (ESS) of >200 confirmed a sufficient

Fig. 1 Tree topology based on Bayesian 50 % consensus majority rule derived from *cyt b* of *Puffinus auricularis* and close relatives. Numbers above nodes show posterior probabilities and bootstrap values. In parentheses, sample localities of Townsend's, Newell's, and Rapa Shearwaters are shown. Numbers before taxa correspond to those in Table 1



level of sampling. The remaining trees were used to construct a majority rule consensus tree and to calculate the posterior probabilities of the individual clades (Labarthe et al. 1998). Maximum likelihood analysis was conducted in PhyML Version 3.0 (Guindon et al. 2010). Statistical test for branch support was determined via 100 bootstrap replicates using the heuristic search option and the GTR+G+I model described above. Genetic distances between Townsend's and Newell's shearwaters were also calculated in Mega Version 6 (Koichiro Tamura et al. 2013) and DnaSP Version 5.10.01 (Librado and Rozas 2009).

Results

We successfully obtained, 917 base pairs (bp) of cytochrome b (*cyt b*), and 663 bp of cytochrome oxidase I (*COI*) from the fresh sample and three additional sequences of ancient DNA samples (327 pb of *cyt b* and two of 463 pb of *COI*) from Townsend's Shearwater. Four short *COI* sequences of Black-vented Shearwater were obtained from museum specimens (two of 253 and two 462pb). These sequences did not contain internal stop codons, and visual inspection of each sequence indicated that all the mutations

were in the third position, and represent synonymous substitutions. The proportion of invariant sites was 1.36 and 0.33 respectively for the two gene fragments. Townsend's and Newell's *cyt b* sequences contained 5 variable sites in the third position, and *COI* contained four; all of them non informative. Both Bayesian and Maximum Likelihood phylogenetic trees recovered the same topology corroborating that Townsend's and Newell's shearwaters conform a well supported monophyletic clade with posterior probabilities >0.99 and bootstrap values >94 % (Figs. 1, 2), with genetic distances among sequences ranging only from 0 to 0.006 for *cyt b* and 0 to 0.009 for *COI* (Table 2). The Rapa Shearwater (*P. myrtae*) appears as a sister taxa to the Townsend's and Newell's Shearwaters group. It is worth noting that genetic distances between *P. a. auricularis/newelli* and *P. myrtae* ranged from 0.015 to 0.023 for *cyt b* (Table 2); for the Rapa Shearwater there is no information available for *COI*.

Discussion

While genetic data indicate that Townsend's and Newell's Shearwaters are part of a unique clade, signs of incipient differentiation are noticeable in plumage characters,

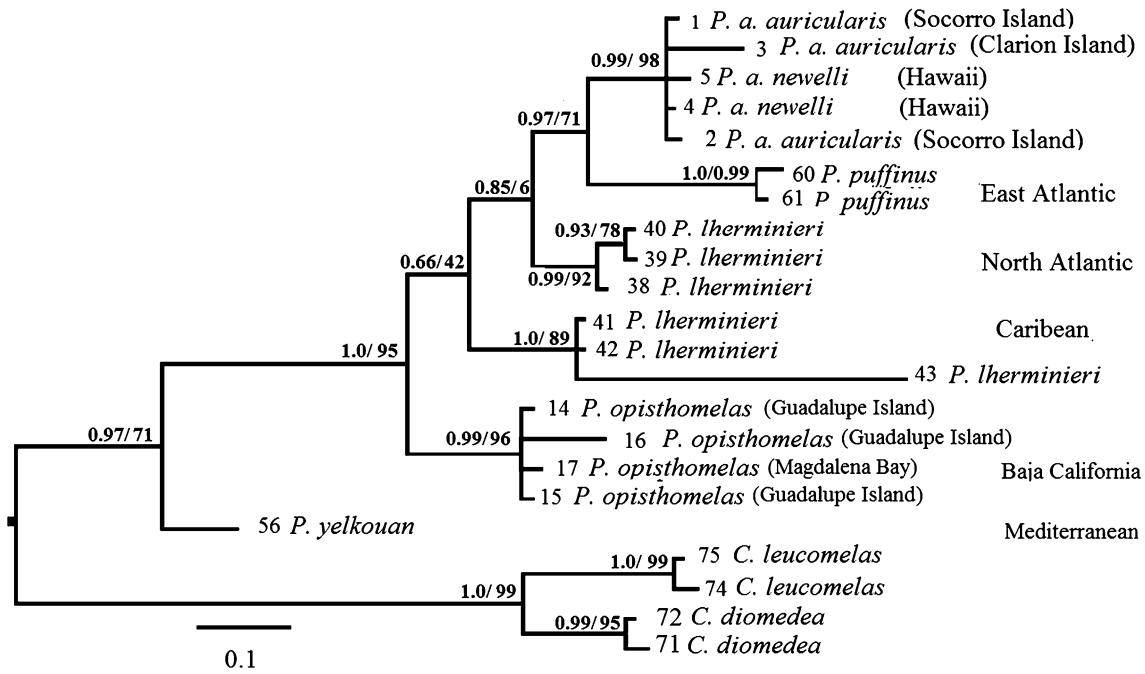


Fig. 2 Tree topology based on Bayesian 50 % consensus majority rule derived from *COI*. Numbers above nodes show posterior probabilities and bootstrap values. In parentheses, sample localities

Townsend’s, Newell’s and Black-vented Shearwaters are shown. Numbers before taxa correspond to those in Table 1

Table 2 Genetic distances between Townsend’s, Newell’s and Rapa Shearwaters for *cyt b* and *COI*

<i>cyt b</i>	(1)	(2)	(6)	(7)	(8)	(9)	(10)
Townsend’s (1)							
Townsend’s (2)	0.007						
Newell’s (6)	0.000	0.000					
Newell’s (7)	0.006	0.002	0.000				
Newell’s (8)	0.006	0.002	0.000	0.000			
Newell’s (9)	0.006	0.002	0.000	0.000	0.000		
Rapa (10)	0.023	0.018	0.022	0.022	0.017	0.017	
Rapa (11)	0.020	0.018	0.018	0.018	0.015	0.015	0.005

<i>COI</i>	(1)	(2)	(3)	(4)
Townsend’s (1)				
Townsend’s (2)	0.000			
Townsend’s (3)	0.007	0.009		
Newell’s (4)	0.000	0.000	0.007	
Newell’s (5)	0.002	0.003	0.009	0.001

Numbers in parentheses correspond to sequences in Table 1. Genetic distances were calculated with the Tamura 3-parameter model with Mega 6.0

behavior and ecology. There are several examples of a mismatch between genetic and morphological evolution. On the one hand, there are cases like that of the White-

facéd Plover (*Charadrius alexandrinus dealbatus*) and the Kentish Plover (*C. alexandrinus*) where the two forms show marked phenotypic differentiation but lack genetic divergence between their populations (Rheindt et al. 2011). On the other hand, there are other island birds, potentially cryptic species, showing little differences in morphology but large genetic distances (e.g. Saitoh et al. 2014). Furthermore, there are species like the Rufous-tailed tairlorbird (*Orthothomus sericeus*) that have genetically identifiable subspecies with and without morphological divergence (Lim et al. 2014).

In the Procellariidae, genetic divergence within species ranges from 0 to 0.5 % and between species from 0.7 to 8.1 % (Austin et al. 2004), based on a *cyt b* molecular clock specific for this family with a variation of 0.9 % per million years (Nunn and Stanley 1998). These margins suggest that Townsend’s and Newell’s Shearwaters are conspecifics (genetic distances ranging from 0 to 0.6 % for *cyt b*), and that the Rapa Shearwater should be considered a distinct species from the sister group of *auricularis-newelli* (genetic distances ranging from 1.5 to 2.3 for *cyt b*). Moreover, the tree topology of the clade containing Newell’s and Townsend’s Shearwaters does not show reciprocal monophyly and is more consistent with a polyphyletic arrangement; thus indicating at the most a

very early stage in the speciation process (Avisé 2000). Thus, under a Phylogenetic Species Concept (PSB; e.g. De Queiroz 2007) Townsend's and Newell's Shearwaters show insufficient lineage separation to consider them distinct species. Morphological and ecological differences would represent incipient separation consistent with a subspecies ranking.

Based on mtDNA molecular clock approximations (e.g. Tarr and Fleischer 1993; Weir and Schluter 2008), Townsend's Shearwater likely arrived more recently than other endemic landbirds on Socorro Island (*Zenaida graysoni* 0.45 Ma, Johnson and Clayton 2000; *Mimus graysoni* ca 0.58–0.88 Ma, Barber et al. 2004, Lovette et al. 2012; *Setophaga graysoni* ca.1.15 Ma, Evans et al. 2014; *Troglodytes sissonii* ca. 3 Ma, Martínez-Gómez et al. 2005). Most likely Townsend's Shearwater did not colonize the Revillagigedo Archipelago when the islands emerged (ca. 3.0–1.8 Ma; Bryan 1966), but after current subaerial portions of the island were present (0.540 Ma; Bohrsen et al. 1996; Bohrsen and Reid 1998). It is worth noting that the specific status of these landbirds has been confirmed by molecular studies; while that was not the case for Townsend's and Newell's Shearwaters. Thus, patterns of speciation of island landbirds might differ from those of seabirds; namely, seabirds are capable of venturing across the ocean, while it constitutes a true barrier for landbirds.

The taxonomic affinity shown by Townsend's and Newell's Shearwaters must result in coordinated conservation strategies because of their similarities in several key aspects of their breeding cycles. Conservation teams in Hawaii and Mexico should work collaboratively to develop strategies for the mutual conservation of this species. Essential conservation actions should include (1) the restoration of degraded landscapes caused by feral pigs and sheep, (2) protection of the remaining nesting zones from excessive anthropogenic disturbance, (3) carefully planned removal of introduced feral species, (4) and nesting enhancement programs. With only ca. 75 breeding pairs left in the Townsend's population (J. E. Martínez-Gómez, unpublished field notes), these conservation efforts will be critical to ensure the continued existence of México's rarest seabird. Further sampling and monitoring should be conducted with extreme responsibility due to the small population size and associated demographic fragility of extant Townsend's Shearwater colonies.

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