## SHORT NOTE

## Molecular analysis confirms the occurrence of *Thalassarche steadi* in Argentinian waters

JUAN PABLO SECO PON\* MAGDALENA GRAZIANO ROCÍO MARIANO-JELICICH Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata-CONICET. Rodríguez Peña 4046, nivel 1 (B7602GSD), Argentina.

Before the current availability of molecular methods for separating shy (Thalassarche cauta) and whitecapped albatrosses (T. steadi) (Abbott & Double 2003), it was accepted that these two 'shy-type albatrosses' (see Brooke 2004, Penhallurick & Wink 2004; Onley & Scofield 2007) along with Chatham albatross (T. eremita) and Salvin's albatross (T. salvini) were historically placed under a single polytypic species: the shy albatross (Diomedea cauta) (Marchant & Higgins 1990; Carboneras 1992). In modern days the shy albatross complex is divided into four species placed in the genus Thalassarche based on morphometric, phylogenetic, and population genetics studies (Robertson & Nunn 1998; Abbott & Double 2003; Sangster et al. 2015). Albeit this taxonomy has been adopted by leading organizations such as the Agreement on the Conservation of Albatrosses and Petrels - ACAP (Taxonomy Working Group 2006; ACAP 2011) and BirdLife International (2022), some authors show discrepancies (see Brooke 2004; Onley & Scofield 2007). In this paper we follow ACAP (2011) and BirdLife International (2022) in considering T. steadi as a separate species.

Thalassarche steadi, a New Zealand breeding endemic species, breeds on Auckland Islands, Antipodes Islands, and occasionally on the Chatham Islands. The vast majority of these birds (up to 90%) breed on Disappointment Islands (50°44'S, 166°06'E). Thalassarche steadi is regarded as a biennial breeder with a total population estimated at 203,600 mature individuals (BirdLife International 2022). Population trends of the species show strong inter-annual fluctuations, and despite further data needed to confirm the population trend, this parameter is currently considered as declining (BirdLife International 2022). Currently, Thalassarche steadi is listed as 'Near Threatened' due to a combination of at-sea (mainly fisheries bycatch) and on land (chiefly invasive non-native species) threats (Taylor 2000; Baker et al. 2007; Francis 2012).

*Thalassarche steadi* has an almost circumpolar distribution outside the breeding season, showing westerly dispersal directions (Shirihai 2008; Howell & Zufelt 2019), mainly reaching productive waters off South Africa, particularly on the Atlantic side, a well-established key foraging area for overwintering and non-breeding individuals (Baker *et al.* 2007; Petersen *et al.* 2009 and references therein). There, the species is bycaught in large numbers by longline fisheries operating off South Africa (Baker *et al.* 2007; Petersen *et al.* 2009). Still, other

*Received 14 May 2022; accepted 3 September 2022* \*Correspondence: *secopon@mdp.edu.ar* 

productive areas included in the range of T. steadi during its early stages of life and/or during the non-breeding season are the Argentine Continental Shelf and its shelf-break (White et al. 2002; Favero & Silva Rodríguez 2005; Seco Pon & Tamini 2013). This area has long been regarded as an ecosystem of global importance, offering abundant food for a number of local and migratory marine megafauna, including seabirds (Croxall & Wood 2002; Favero & Silva Rodríguez 2005). In other areas of the southwestern Atlantic, the species may reach waters off Uruguay (Jiménez et al. 2009) and southern Brazil (Pereira et al. 2016). Further south, white-capped albatrosses have been sighted at Malvinas/Falkland Islands (White et al. 2002) and at Georgias del Sur/ South Georgia (Phalan et al. 2004).

Although T. steadi is listed within known Argentine bird checklists (de la Peña & Rumboll 1998; Narosky & Izurieta 2010; MADYS 2017; Pearman & Areta 2020), to date the occurrence of the species is based on at-sea observations of birds attending commercial fisheries (Seco Pon & Tamini 2013) and a single specimen found stranded in northern Patagonia (40°45'S, 64°57'W) (Savigny & Carabajal 2015). Due to the great resemblance between white-capped and shy albatrosses, especially at sea (see Abbott & Double 2003; Double et al. 2003), the study by Seco Pon & Tamini (2013) did not attempt to identify individuals at the specific level. On the other hand, the study by Savigny & Carabajal (2015) suggests the occurrence of the species based solely on biometric data and plumage colouration. According to Double et al. (2003), T. steadi is on average larger than *T. cauta*, and discriminant functions involving body measurements may assist in the identification. However, T. steadi can be accurately separated by a fixed substitution in Domain I of the mitochondrial DNA control region (Abbott & Double 2003). In this paper, we confirm the occurrence of the species in Argentina based on molecular techniques.

On 09 March 2019, a dead shy-type *Thalassarche* albatross on the beach at Punta Mogotes ( $38^{\circ}02'55''S$ ,  $57^{\circ}31'52''W$ ), Mar del Plata city, located in the southeastern Buenos Aires Province, Argentina was reported to one of the authors (JPSP). Prior to the necropsy, morphometric measurements were taken (following Double *et al.* 2003) (using Vernier calliper with an accuracy of ±0.1 mm and digital scale with precision ±1 g), and primary, tail, and body (head, back, and belly) moult was evaluated (after Ginn & Melville 1983). Body moult was defined as active if at least five growing feathers were found; otherwise body moult was treated as inactive (see Bugoni *et al.* 2015). Upon necropsy, muscle tissue samples were preserved in absolute ethanol.

The carcass was found showing a slight decomposition (i.e. with intact internal organs,

some rotten smell). No oil stains were noted on feathers or skin of the specimen. The bird had a grey head and neck; its crown and chin were white with little to no pale grey. The rump and undersides were white, and the upper wings black; white underwings with narrow black margins and pale primary flight feather bases (Fig. 1). The bill was pale grey with a blackish tip on the mandible. No primary or tail feathers showed active moult, but body feathers were moulting.



**Figure 1.** *Thalassarche steadi* found stranded in Mar del Plata, Buenos Aires Province, Argentina. Photographs: Marco Favero.

Morphometric measurements of the specimen are as follows: head length, 77.2 mm; maximum head width, 65.6 mm; culmen length, 131.5 mm; upper bill depth, 31.5 mm; basal bill depth, 50.1 mm; basal bill width, 35.7 mm; middletoe (without nail), 126.2 mm; middletoe nail, 19.0 mm; tarsus, 90.3 mm; wing cord, 584.0 mm; and tail length, 245.0 mm. By applying the discriminant function developed by Double *et al.* (2003) – which correctly identifies both the sex and species of 84% of specimens from a sample size of 70 birds previously recognized using molecular techniques - the bird was classified as a female *T. steadi*. The specimen was then sexed by dissection and direct examination of gonads as an immature female.

Total genomic DNA was isolated from a portion of pectoral muscle using an adaptation of Chelex DNA extraction method (Walsh *et al.* 1991; Phillips et al. 2012). A small fraction of muscle, c. 5 x 5 mm, was placed in a 1.5 ml tube with 200 µl of Chelex 100 resin<sup>™</sup> Matrix (BioRad) 5%. The sample was then incubated at 55°C for 30 minutes, followed by 8 minutes at 100°C. Molecular species identification was done through the PCR amplification of a 325bp fragment of the Domain I of the mitochondrial control region following Abbott & Double (2003). Primers GluR7 and SPECF2 (Abbott & Double 2003) were used. The PCR product was visualized on a 1.5% agarose gel, posterior purification of the PCR product and sequencing was performed at Macrogen Inc. (Seoul, Korea). The sequence was aligned with MUSCLE within the program Mega X (Kumar et al. 2018) together with 15 and 23 sequences for shy and white-capped albatrosses, respectively, uploaded from GenBank. The individual was identified as Thalassarche steadi based on a single nucleotide polymorphism (SNP), an Adenine to Guanine substitution, detected at the 121 nucleotide position, which corresponds to a fixed difference diagnostic for the species (Abbott & Double 2003). The sequence is deposited in GenBank under accession number OP832372.

From molecular determination we can confirm that the stranded bird analyzed corresponded to a *Thalassarche steadi* individual. This identification method has been previously implemented in the molecular determination of shy-type albatrosses stranded on coastal areas and/or by-caught in fisheries operating in the region (Jiménez *et al.* 2009; 2015; Pereira *et al.* 2016). Molecular techniques as the one used here have been largely applied to the identification of seabirds incidentally killed in fisheries and/or stranded on coastal areas (Abbott *et al.* 2006; Burg *et al.* 2017; Baetscher *et al.* 2022; among others). To our knowledge this would be the first study to identify *T. steadi* as a non-breeding visitor in Argentinian waters.

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