Agreement on the Conservation of Albatrosses and Petrels

Eleventh Meeting of the Seabird Bycatch Working Group

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Development of DNA markers to resolve uncertainties of seabird bycatch using feathers collected from dead seabirds

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SUMMARY

Incidental mortality in fisheries is a major driver of population changes for albatrosses and petrels globally. However, inaccurate identification of impacted species can hinder monitoring efforts due to visual similarities of closely related species and/or degradation of specimens. Genetic methods can be powerful diagnostic tools, but require appropriate genetic markers and reference databases to identify the target species. A range of genetic markers were designed, tested and evaluated to assist in the identification of the albatross and petrel species listed in Annex 1 to ACAP and in Australia's Threat Abatement Plan for the incidental catch (or bycatch) of seabirds during oceanic longline fishing operations. Analyses found that the combination of two genetic markers could identify 97% (n=35) of 36 target seabird species to either species (n=32) or sister species (n=3), while for one petrel species there were no reference sequences. Genetic methods provide a streamlined framework for the molecular identification of seabird bycatch in fisheries to corroborate and/or correct logbook entries, observer reports and audits of imagery captured by electronic monitoring systems.

RECOMMENDATIONS

That the Seabird Bycatch Working Group

1. Encourages the collection of feather or tissue samples from dead seabirds bycaught in fisheries.
2. Encourages the incorporation of genetic identification of seabird bycatch samples into fishery monitoring programs to improve the accuracy of species assignment.
3. Recommends Parties work towards creating a repository of known provenance samples and making these available to improve the accuracy and confidence in genetic data.
4. Recommends Parties work on developing and maintaining a curated reference sequence database to improve diagnostic species identification.

Desarrollo de marcadores de ADN para resolver incertidumbres sobre la captura secundaria de aves marinas utilizando plumas recogidas de aves marinas muertas

RESUMEN

La mortalidad incidental en las pesquerías es una de las principales causas de los cambios poblacionales de albatros y petreles en todo el mundo. Sin embargo, la identificación inexacta de las especies afectadas puede dificultar los esfuerzos de seguimiento debido a las similitudes visuales de especies estrechamente relacionadas o a la degradación de los especímenes. Los métodos genéticos pueden ser potentes herramientas de diagnóstico, pero requieren marcadores genéticos adecuados y bases de datos de referencia para identificar las especies objetivo. Se diseñó, probó y evaluó una serie de marcadores genéticos para ayudar en la identificación de las especies de albatros y petreles incluidas en el Anexo 1 del ACAP y en el plan de reducción de amenazas de Australia para la captura secundaria (o incidental) de aves marinas durante las operaciones de pesca con palangre oceánico. En los análisis se observó que la combinación de dos marcadores genéticos podía servir para identificar el 97 % (n=35) de las 36 especies de aves marinas objetivo con especies (n=32) o especies hermanas (n=3), mientras que para una especie de petrel no había secuencias de referencia. Los métodos genéticos proporcionan un marco racionalizado para la identificación molecular de las aves marinas capturadas incidentalmente en las pesquerías con el fin de corroborar o corregir las entradas de los libros de a bordo, los informes de los observadores y las auditorías de las imágenes captadas por los sistemas de monitoreo electrónico.

RECOMENDACIONES

Que el Grupo de Trabajo sobre Captura Secundaria de Aves Marinas tome las siguientes medidas:

1. Fomentar la recolección de muestras de plumas o tejidos de aves marinas muertas capturadas incidentalmente en pesquerías.
2. Alentar la incorporación de la identificación genética de las muestras de aves marinas capturadas incidentalmente en los programas de monitoreo de las pesquerías para mejorar la precisión de la asignación de especies.
3. Recomendar a las Partes que trabajen para crear un repositorio de muestras de procedencia conocida y ponerlo a disposición para mejorar la precisión y la confianza en los datos genéticos.
4. Recomendar a las Partes que trabajen en el desarrollo y mantenimiento de una base de datos de secuencias de referencia para mejorar la identificación diagnóstica de especies.

Développement de marqueurs ADN pour lever les incertitudes sur les captures accessoires d'oiseaux de mer à l'aide de plumes prélevées sur des spécimens morts

RÉSUMÉ

La mortalité accidentelle due à la pêche est l'un des principaux facteurs d'évolution des populations d'albatros et de pétrels dans le monde. Cependant, le manque de précision dans l'identification des espèces touchées peut entraver les efforts de surveillance en raison de similitudes visuelles entre des espèces étroitement apparentées et/ou de la dégradation des spécimens. Les méthodes génétiques peuvent constituer des outils de diagnostic puissants, mais elles nécessitent des marqueurs génétiques appropriés et des bases de données de référence permettant d'identifier l'espèce cible. Un ensemble de marqueurs génétiques a été conçu, testé et évalué pour faciliter l'identification des espèces d'albatros et de pétrels inscrites à l'annexe 1 de l'ACAP et dans le Plan australien de réduction des menaces pour les captures accidentelles (ou captures accessoires) d'oiseaux de mer lors des opérations de pêche océanique à la palangre. Les analyses ont montré que la combinaison de deux marqueurs génétiques permettait d'identifier 97% (n=35) des 36 espèces d'oiseaux marins ciblées à une espèce en particulier (n=32) ou à des espèces sœurs (n=3). Aucune séquence de référence n'était disponible pour une espèce de pétrel. Les méthodes génétiques fournissent un cadre rationalisé pour l'identification moléculaire des captures accessoires d'oiseaux de mer dans les pêcheries, afin de corroborer et/ou de corriger les entrées des journaux de bord, les rapports des observateurs et les audits des images capturées par les systèmes de suivi électronique.

RECOMMANDATIONS

Que le Groupe de travail sur les captures accessoires

1. Encourage la collecte d'échantillons de plumes ou de tissus sur les oiseaux de mer morts capturés accidentellement par les pêcheries.
2. Encourage l'intégration de l'identification génétique des échantillons de captures accessoires d'oiseaux de mer dans les programmes de surveillance de la pêche afin d'améliorer la précision de l'attribution aux espèces.
3. Recommande aux Parties de travailler à la création d'un registre d'échantillons de provenance connue, qui seront rendus disponibles pour améliorer la précision et la confiance dans les données génétiques.
4. Recommande aux Parties de travailler à l'élaboration et à la mise à jour d'une base de données de séquences de référence soigneusement sélectionnées afin d'améliorer l'identification diagnostique des espèces.

1. INTRODUCTION

Incidental seabird bycatch in fisheries is a significant issue globally and one of the biggest threats facing seabird populations, particularly those for albatrosses, shearwaters and larger petrels (Phillips et al. 2016, Dias et al. 2019, Rodríguez et al. 2019). Effective development and evaluation of seabird bycatch mitigation requires precise information about which species are bycaught. The United Nation's Food and Agricultural Organisation (FAO) guidelines for reducing fisheries bycatch includes the need to conduct independent and effective monitoring programs (FAO 1999). Species identification is typically carried out by fisheries observers through the use of detailed species field guides (ACAP and NRIFSF 2015), retention of carcasses for necropsy, photography of dead animals for identification by experts, and electronic monitoring using image capture and subsequent auditing (FAO 2009). However, discrepancies still exist due to inter-specific phenotypic similarities (particularly of juvenile birds), poor specimen condition, and the prohibitive costs associated with transport and storage of samples when more detailed analyses are required.

Since 1998, Australia has implemented successive Threat Abatement Plans for the incidental catch (or bycatch) of seabirds during oceanic longline fishing operations (TAP-Seabirds, Commonwealth of Australia 2018). The Threat Abatement Plan applies to all Commonwealth-managed oceanic longline fisheries within Australia's jurisdiction and requires data to be collected on the bycatch of species, with accurate species determination prioritised. Efforts to improve species identification in these fisheries has included the introduction of a guide to collecting feather samples from dead birds for genetic analysis, based on the guide to collecting feather samples for DNA analysis developed by ACAP and the National Research Institute of Far Seas Fisheries (ACAP & NRIFSF 2015). Seabird interaction reports prepared by the Australian Fisheries Management Authority (AFMA) for Commonwealth-managed oceanic longline fisheries includes reporting of whether the birds were bycaught alive or dead (AFMA 2023). However, among 148 dead seabirds reported as bycatch in an oceanic longline fishery between 2019 and 2021, only 16% were identified to species level by AFMA. Birds are grouped into broad categories such as 'albatross' or 'bird', which does not allow the impact of species interactions to be fully assessed or quantified at a population or species level.

DNA barcoding is an established molecular technique for species identification that involves the identification of suitably diagnostic DNA sequences (Staats et al. 2016). Genetic markers have the potential to aid in the correct species identification of many phenotypically similar species (Ferrette et al. 2019). The ACAP Seabird Bycatch Identification Guide encourages the collection of feathers from dead bycaught birds for molecular species identification where a program is established (ACAP & NRIFSF 2015). However, feather DNA can often be degraded and the identification of appropriate genetic markers to identify albatross and petrel bycatch species have not been fully examined using feather samples.

The Australian Antarctic Division of the Department of Climate Change, Energy, the Environment and Water has developed an efficient molecular method for the accurate diagnostic determination of seabird species (see **SBWG11 Inf 09**).

2. ASSESSMENT AND DEVELOPMENT OF GENETIC MARKERS

Genetic markers were tested and developed for the diagnostic identification of albatross and petrel species listed in ACAP Annex 1 to the Agreement (www.acap.aq) and an additional five petrel species listed in Annex A to the TAP-Seabirds (Commonwealth of Australia 2018). The included species were those assigned under the ACAP Taxonomy Working Group and IOC World Bird List, respectively. Reference sequences were generated from the online genetic database GenBank, curated museum specimens and field samples.

We tested three genetic markers (Cytochrome B, Cytochrome Oxidase I and the Control Region) and found two markers that combined could identify 97% (n=35) of 36 target species to either species (n=32) or sister species (n=3). No reference DNA sequences were available for *Procellaria conspicillata*.

All albatrosses could be assigned to species using the Control Region marker except for *Diomedea epomophora / sanfordi.* The accuracy of assigning *Thalassarche steadi / cauta* was 97%, due to incomplete lineage sorting for one marker (Abbott et al. 2006). There was also one GenBank accession from an apparent *Thalassarche melanophris* that appeared to have an incorrect taxonomy. *Thalassarche impavida* and *Thalassarche melanophris* were considered conspecific when the sample was collected from a bycatch bird in 1996 (when they were treated as subspecies of *Diomedea melanophris*); this may explain this sample being coded to the 'nominate' taxon (Colin Miskelly, personal communication, March 2023). Unfortunately, the specimen was not retained and so its identification cannot be confirmed. The museum sample was collected from the Northland region of New Zealand, which is not a known breeding site of *Thalassarche melanophris* and the presence of lineage specific CR sequence (DiC GCRGCTGG, Burg et al. 2017) suggests it is in fact *Thalassarche impavida*. While there is evidence of some mixing of widespread and Falkland Islands (Islas Malvinas) *Thalassarche melanophris*, Burg et al. (2017) show that birds with mitochondrial DNA that matched *Thalassarche impavida* occurred only at Campbell Island, hence the provenance of individuals of this type can be assigned with a high certainty.

All target petrels and shearwaters were identified to species using a combination of Cytochrome B and Control Region markers, except for *Pterodroma macroptera* and *Puffinus mauretanicus*. Although these could be differentiated from other ACAP and TAP species, they were genetically similar to *Pterodroma gouldi* and *Puffinus yelkouan* respectively (Table 1). Due to a lack of reference material for some species, these could not be evaluated with all genetic markers.

3. DISCUSSION

The complexity and variation within the Procellariiformes mitochondrial region means that development of diagnostic markers is complicated and one genetic marker is insufficient to provide diagnostic capabilities across all species. The study found that two genetic markers could accurately identify the majority of albatross and petrel species listed in Annex 1 to the Agreement and the TAP-Seabirds. For albatross species and many petrel species, these results will be transferrable globally.

The reference database developed during this study is preliminary and still has considerable gaps. It was not possible to use known provenance samples for all species and data were missing entirely for some species. The ability to assess intraspecific variability was also limited due to a low number of sequences available for some species or unknown provenance. The

accuracy of future studies would be enhanced by the development of a repository of samples from known provenance species and the curation of an ACAP sequence reference database to improve the number and quality of sequences available for diagnostic genetic studies.

This study was designed to provide diagnostic genetic methods for attributing likely species to bycatch specimens, not to assess the taxonomy of species. The sequence lengths used were purposefully short to improve analysis success from degraded DNA and therefore may not provide resolution of broader taxonomic differences, as achieved with longer DNA sequences used for population genetics and differentiation of provenance.

The standardised inclusion of genetic methods by Range States and Regional Fisheries Management Organisations (RFMO) would improve their respective abilities to attribute species level impacts of fishing operations on seabirds, particularly threatened albatrosses and petrels. These methods have additional utility in advancing actions within the Concerted Action for the Antipodean Albatross (*Diomedea Antipodensis*) developed under the Convention on the Conservation of Migratory Species of Wild Animals (https://www.cms.int).

4. REFERENCES

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Table 1: Diagnostic species resolution, and the number of reference DNA sequences (seq) and haplotypes (hap- unique sequence variants) for the three tested primer sets for Procellariiformes species listed under either Annex 1 to ACAP and/or TAP-Seabirds.

	Cytochrome Oxidase 1			Cytochrome B			Control Region		
Species	Resolution	N Seq	N hap	Resolution	N Seq	N hap	Resolution	N Seq	N hap
Diomedea amsterdamensis[1,2 CR, L]	No reference sequences	0	0	Multi species – *D. amsterdamensis/ antipodensis /dabbenena / exulans*	1	1	Species	3	3
Diomedea antipodensis # [1,2 EN, L]	Multi species* - *D. antipodensis / dabbenena /* exulans	7	2	Multi species – *D.amsterdamensis/ antipodensis /dabbenena / exulans*	11	3	Species	7	6
Diomedea dabbenena[1,2 CR, L]	Multi species* - *D. antipodensis / dabbenena /* exulans	1	1	Multi species – *D. amsterdamensis/ antipodensis /dabbenena / exulans*	4	1	Species	2	2
Diomedea epomophora [1,2,VU, L]	Sister species* - *D. epomophora / sanfordi*	20	3	Sister species - *D. epomophora / sanfordi*	4	1	Sister species - *D. epomophora / sanfordi*	3	3
Diomedea exulans [1,2,VU, M]	Multi species*- *D. antipodensis / dabbenena /* exulans	11	2	Multi species – *D. amsterdamensis/ antipodensis /dabbenena / exulans*	4	2	Species	6	3
Diomedea sanfordi [1,2 EN, L]	Sister species* - *D. epomophora / sanfordi*	3	2	Sister species - *D. epomophora / sanfordi*	4	2	Sister species - *D. epomophora / sanfordi*	2	2
Phoebastria albatrus [1,VU]	Species*	4	1	Species	8	3	Species*	6	6

	Cytochrome Oxidase 1			Cytochrome B			Control Region		
Species	Resolution	N Seq	N hap	Resolution	N Seq	N hap	Resolution	N Seq	N hap
Phoebastria immutabilis [1,NT, L]	Sister species* - *P. immutabilis / nigripes*	11	2	Sister species - *P. immutabilis / nigripes*	7	2	Species*	5	5
Phoebastria irrorata[1, CR]	No reference sequences	0	0	Species	1	1	No reference sequences	0	0
Phoebastria nigripes [1,NT]	Sister species* - *P. immutabilis / nigripes*	6	1	Sister species - *P. immutabilis / nigripes*	5	2	Species*	7	7
Phoebetria fusca [1,2 EN, L]	Species	3	1	Species	18	1	Species	3	3
Phoebetria palpebrata [1,2,NT, L]	Species	6	1	Species	20	3	Species	2	2
Thalassarche bulleri [1,2,NT, L]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	8	1	Species	5	1	Species	4	4
Thalassarche carteri [1,2 EN, M]	Sister species - *T. carteri/chlororhynchos*	4	1	Sister species - *T. carteri / chlororhynchos*	5	1	Species	4	4
Thalassarche cauta [1,2,NT, M]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	16	3	Multi species – *T. cauta/eremita /salvini /steadi*	5	2	Species[+]	5	4
Thalassarche chlororhynchos [1,2 EN, L]	Sister species - *T. carteri / chlororhynchos*	3	1	Sister species - *T. carteri / chlororhynchos*	2	2	Species	1	1
Thalassarche chrysostoma [1,2 EN, M]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida /*	12	4	Species	6	2	Species	4	4

	Cytochrome Oxidase 1			Cytochrome B			Control Region		
Species	Resolution	N Seq	N hap	Resolution	N Seq	N hap	Resolution	N Seq	N hap
	melanophris / salvini / steadi								
Thalassarche eremita [1,2,VU, L]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	3	1	Multi species - *T. cauta / eremita / salvini / steadi*	4	1	Species	4	4
Thalassarche impavida [1,2,VU, H]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	4	1	Sister species - *T. impavida / melanophris*	5	2	Species[^%]	4	4
Thalassarche melanophris [1,2 LC, H]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	20	2	Sister species - *T. impavida / melanophris*	6	1	Species[^%]	4	4
Thalassarche salvini [1,2,VU, L]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	3	1	Multi species -*T. cauta / eremita / salvini / steadi*	4	1	Species	4	4
Thalassarche steadi [1,2,NT, M]	Multi species - *T. bulleri / cauta / chrysostoma / eremita / impavida / melanophris / salvini / steadi*	4	1	Multi species -*T. cauta / eremita / salvini / steadi*	5	1	Species[+]	5	5

	Cytochrome Oxidase 1			Cytochrome B			Control Region		
Species	Resolution	N Seq	N hap	Resolution	N Seq	N hap	Resolution	N Seq	N hap
Ardenna carneipes [2,NT, H]	Sister species - *A. carneipes / creatopus*	8	1	Sister species - *A. carneipes / creatopus*	150	10	Species*	4	4
Ardenna creatopus [1,VU]	Sister species - *A. carneipes / creatopus*	3	1	Sister species - *A. carneipes / creatopus*	2	1	Species*	1	1
Ardenna grisea [2,NT, L]	Species	5	2	Species	3	2	Species*	2	2
Ardenna pacifica [2,LC, M]	Species	14	2	Species	3	1	Species*	2	2
Ardenna tenuirostris [2,LC, L]	Species	9	1	Species	4	2	Species*	3	3
Macronectes giganteus [1,2,LC, L]	Sister species - *M. halli / giganteus*	9	3	Sister species - *M. halli / giganteus*	18	4	Species	2	1
Macronectes halli [1,2,LC, L]	Sister species - *M. halli / giganteus*	8	3	Sister species - *M. halli / giganteus*	12	5	Species	2	2
Procellaria aequinoctialis [1,2,VU, M]	Species*	10	3	Species*	2	1	No reference sequences	0	0
Procellaria cinerea [1,2,NT, M]	Species*	12	3	Species*	5	2	No reference sequences	0	0
Procellaria conspicillata [1,VU]	No reference sequences	0	0	No reference sequences	0	0	No reference sequences	0	0
Procellaria parkinsoni [1,2,VU, L]	Species*	5	1	Species*	1	1	No reference sequences	0	0
Procellaria westlandica [1,2 EN, L]	Species*	8	1	Species*	1	1	No reference sequences	0	0
Pterodroma macroptera [2,LC, M]	Multi species* *P. gouldi / macroptera / lessonii*	3	1	Multi species * *P. gouldi / macroptera / lessonii*	14	2	Sister Species* *P. gouldi/ macroptera*	2	2

	Cytochrome Oxidase 1			Cytochrome B			Control Region		
Species	Resolution	N Seq	N hap	Resolution	N Seq	N hap	Resolution	N Seq	N hap
Puffinus mauretanicus[1, CR]	No reference sequences	0	0	Sister Species* *P. mauretanicus / yelkouan*	106	9	No reference sequences	0	0

[1] ACAP.

[2] TAP-Seabirds.

ICUN Status: Least Concern [(LC)], Near Threatened [(NT)], Vulnerable [(VU)], Endangered [(EN)], Critically Endangered [(CR)]

Likely incidence in longline bycatch in the Australian Fishing Zone: Low [L], Moderate [M], High [H] (Commonwealth of Australia 2018)

* Some species within this genus have no reference sequences available on GenBank

[#] Includes *Diomedea antipodensis* and *Diomedea gibsoni* based on species assignment in Annex 1 to ACAP

^ A likely incorrect taxonomy for one GenBank accession (AY158677) makes species identification uncertain.

[%] Burg et al. (2017) assigned individuals to Falkland Islands (Islas Malvinas) *Thalassarche melanophris* (DmFI ATTGTCAG), widespread *Thalassarche melanophris* (Dm ATTGCTGA) and *Thalassarche impavida* (DiC GCRGCTGG) using lineage specific mtDNA markers.

[+] Species identification was based on a single nucleotide polymorphism (SNP) in the mitochondrial control region (Abbott and Double 2003). This method has an ~3% error at assigning species (Abbott et al. 2006)