# Resolving Chilean dolphin (*Cephalorhynchus eutropia*, Gray 1846) synonymy by sequencing DNA extracted from teeth of museum specimens

Resolviendo la sinonímia del delfín Chileno (*Cephalorhynchus eutropia*, Gray 1846) mediante secuenciamiento de ADN extraído desde dientes de especímenes de museo

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**Abstract.-** Mitochondrial DNA was amplified and sequenced from single teeth of six museum specimens described by Phillipi (1893) as three novel species, *Phocoena albiventris*, *Tursio platyrhinus* and *T. panope*. Subsequently, these specimens were reviewed and, with the exception of *T. panope*, were suggested as probable specimens of Chilean dolphin, *Cephalorhynchus eutropia*. DNA sequence from five of the six samples were compared to an existing database of odontocete type-sequences. These confirm that the specimens were Chilean dolphins. We conclude that *T. panope* is a Chilean dolphin, albeit of unusual size. These results demonstrate the utility of genetic testing of museum specimens to help resolve uncertainty in species identification.
Palabras Claves: Delfín chileno, especímenes de museo, taxonomía, ADNmt

**Resumen .-** Se amplificó y secuenció ADN mitocondrial a partir de muestras dentarias de seis especímenes de museo descritos por Phillipi (1893) como especies nuevas, *Phocoena albiventris*, *Tursio platyrhinus* y *T. panope*. Estos especímenes fueron revisados con posterioridad y, con la excepción de *T. panope*, fueron reidentificados como probables delfín Chileno, *Cephalorhynchus eutropia*. Secuencias de ADN de cinco de las seis muestras fueron comparadas con una base de datos existente de secuencias tipo de odontocetos. Esta comparación confirma que los especímenes corresponden a delfin Chileno. Por lo tanto, *T. panope* es un delfín Chileno, aunque de inusual tamaño. Estos resultados demuestran la utilidad de análisis genéticos en especímenes de museo para ayudar a resolver ambiguedades en la identificación a nivel específico.
Key words: Chilean dolphin, museum specimens, taxonomy, mtDNA

The classification of the Chilean dolphin *(Cephalorhynchus eutropia*, Gray 1846), based on a single skull (BMNH 936a) and without a physical description, resulted in considerably synonomy and confusion over the identity of museum specimens (as reviewed in Goodall *et al.* 1988). Based on a description of a dolphin caught in Valparaíso, Chile in 1882, Philippi (1893) proposed a new species *Phocaena* (or later: *Tursio*) *albiventris*. He subsequently examined several skulls from within Chilean waters some which he assigned to his proposed species (*T. albiventris*). Based on morphological variation between these skulls also he proposed a further two species, *T. platyrrhinus* (Philippi 1893) and *Turiso panope*. In his review of the skulls of Chilean dolphins, Philippi (1896) provided illustrations (Plates IV - VI) that appear to show significant morphological differences between skulls representing the three proposed species of dolphin (Philippi 1893). However, True (1903) suggested that the three species described by Philippi were Chilean dolphins. Using measurements of the same skulls made by RL Brownell Jr in 1973, Goodall & Cameron (1979) concurred with True that both *P. albiventris* and *T. platyrrhinus* were Chilean dolphins. As the condylobasal length of the type skull of *T. panope* (MNHM-S 584) was larger (at 379mm) than that found in Chilean dolphins (302 - 364mm), Goodall & Cameron (1979) suggested that the classification of this specimen could not be determined. Later examinations of the skull of *T. panope* (Brownell & Mead 1989, Van Waerebeek 1992) suggested that the specimen was a dusky dolphin, *Lagenorhynchus obscurus*. However, at the initiation of this study the identity of the *T. panope* skull was still uncertain, requiring further investigation.

Specimens of cetaceans that are held in museum collections contain DNA that can be used to provide significant insights into species identification (e.g. Dalebout *et al.* in review) and historic genetic structure (e.g. Rosenbaum *et al.* 1997, Pichler & Baker 2000). Depending on the treatment and storage of the specimen, bones (Dizon *et al.* 1995), baleen plates (Kimura *et al.* 1997, Rosenbaum *et al.* 1997) and particularly teeth (Pichler & Baker 2000) have been found to provide adequate DNA for genetic analyses. Only modest amounts of material are required from the specimen, such as single teeth from small odontocetes (Pichler & Baker 2000) or powder from holes drilled in the skull or teeth of larger cetaceans (Pichler *et al.* in review, Dalebout *et al.* in review). Here we attempt to identify the species of each of ten individual specimens, from museum collections in Chile, including six representatives of the three species described by Philippi (1893).

To preserve the maximum amount of museum material, a single tooth of each specimen (Table 1) was collected. Prior to freezing the tooth (in liquid nitrogen or at –80$^{o}$C overnight), the exterior surface of each tooth was lightly sanded to remove potential surface contaminants. The tooth was then placed into a sterile tooth-crushing device and reduced to fine powder (Pichler & Baker 2000). DNA was extracted from this powder (< 1g in weight) using a modified silica extraction procedure (Boom *et al.* 1990, Matisoo-Smith *et al.* 1997) as described in Pichler & Baker (2000). Sterile conditions and disposable equipment were used to minimise risk of contamination.

A 550 base pair (bp) region of the mitochondrial (mt) DNA control region was amplified by Polymerase Chain Reaction (PCR, Saiki *et al.* 1988) with primers dlp1.5 (5'- TCA CCC AAA GCT GRA RTT CTA - 3') and dlp5 (5'- CCA TCG WGA TGT CTT ATT TAA GRG GAA - 3') following Pichler & Baker (2000). If this fragment failed to amplify, primer dlp5 was replaced with dlp4 (5'-CGG GTT GCT GGT TTC ACG-3') for amplification of a 400bp fragment. Finally, if both of these amplifications failed, a short 200 bp fragment was amplified using primers dlpFBP (5'–GTA CAT GCT ATG TAT TAT TGT GC–3') to dlp4. The PCR was prepared using dedicated "pre-PCR" pipettors with barrier tips, surfaces that had never been exposed to DNA extracts or PCR products and new reagents. The PCR amplicon was quantified using gel electrophoresis with DNA low-mass ladder (GIBCO BRL) then sequenced using BigDye (Applied Biosystems) chemistry and electrophoresed on an ABI 377 autosequencer.

Sequences of sufficient quality to determine species identification were obtained from a total of nine of the ten museum specimens. Successful amplification of 550 bp fragments was achieved from four of the samples. From the remaining five samples, two yielded 400 bp fragments and three yielded 200 bp fragments. The mtDNA "test" sequences obtained from the museum specimens were aligned by eye to a database of cetacean reference sequences (Baker *et al.* 1996) to confirm that the test sequences were Delphinids. The sequences were then imported into a database including representatives of 18 Delphinid species (unpublished data). The phylogeny of the Delphinids were reconstructed in PAUP v4.02b (Swofford 1998) using heuristic parsimony and including one test sequence at a time to establish the species identity of that sequence. A test sequence that fell within the clade of sequences representative of one species was considered to represent another individual from that species.

Using this method, all nine specimens for which we obtained sequences, including the type of *Tursio panope*, were identified as Chilean dolphins. No sequence differed from the nine existing Chilean dolphin sequence types, obtained from 13 beachcast Chilean dolphins, by more than two substitutions. Further, each sequence had an indel, at position "99" relative to the first bp of the control region, characteristic of the genus *Cephalorhynchus*, and of the same length found only in the Chilean and Commerson's dolphin (*C. commersonii*, unpublished data). The exact Chilean dolphin haplotype of seven of the nine sequences was determined despite four of the sequences being short fragments (Table 2). The sequence of *Tursio panope* was also short (200 bp), resulting in an incomplete fragment that allowed unequivocal identification of the species of the sample, but not to the precise haplotype.

The genetic evidence presented here confirm the morphological examinations of True (1903) and Goodall & Cameron (1979) that *P. albiventris* and *T. platyrrhinus* are junior synonyms of *C. eutropia*. Further, mtDNA sequence from the type specimen of *T. panope* supports True's (1903) suggestion that this skull was in fact a Chilean dolphin and not a dusky dolphin (Brownell & Mead 1989, Van Waerebeek 1992) or a new species (Philippi 1893). These results show that the three species proposed by Philippi arose from his difficulty in interpreting Gray's vague descriptions (Philippi 1896). Goodall *et al.* (1988) report that, due to damage to the type skull of *T. panope*, their measurement of the condylobasal length is an underestimate. Our finding that this skull was from a Chilean dolphin indicates that the skull may be the largest recorded for this species.

This report demonstrates the utility of genetic analysis in helping to resolve, or confirm, taxonomic

status of museum specimens. In the case of damaged or partial specimens, genetic identification may be the only method for determination of the species of origin for these materials. Museum collections are an invaluable resource of material for natural history studies. In particular, where a species is poorly known due to its rare or elusive nature in the wild, museum collections accrued over decades can help uncover answers to the taxonomy and current status of these species.

**Table 1**

**Specimen codes and species identification of the ten specimens examined. The two codes refer to the classification given to the species post identification at the University of Auckland (AUNZ) and the code attached to the specimen at the museum collection (CZI = Colección Zoología Instituto de la Patagonia, Punta Aremas, Chile; MNHN-S = Museo Nacional de Historia Natural de Santiago, Chile). The dates are as recorded on the specimens or as given in Goodall *et al* (1988). Otherwise dates are given as prior to the first reference of the specimen in a publication or report. The original classification of the specimen is given along with the subsequent DNA species identification. The haplotype of the specimen is noted in the final column. Novel haplotypes are shown in bold.**

Código e identificación específica de los diez especímenes examinados. Los dos códigos se refieren a la clasificación dada a las especies luego de la identificación en la University of Auckland (AUNZ) y al código original en los museos (CZI = Colección Zoología Instituto de la Patagonia, Punta Aremas, Chile; MNHN-S = Museo Nacional de Historia Natural de Santiago, Chile). Las fechas son aquellas registradas en los especímenes o aquellos dados por Goodall *et al* (1988). De otra manera, las fechas se refieren a aquellas de la primera referencia hecha para el especimen en una publicación o reporte. La clasificación original de los especímenes está dada luego de la identificación mediante ADN. El haplotipo de los especímenes está anotado en la columna final. Nuevos haplotipos están mostrados en negrita.

| Code | | | | | |
|---|---|---|---|---|---|
| **AUNZ** | **Museum** | **Date** | **Original classification** | **DNA identification** | **Haplotype** |
| Ceut10 | CZIP0529 | ? | *C. eutropia* | *C. eutropia* | A |
| Ceut11 | MNHN-S 581 | <1904 | *Phocaena albiventris* | *C. eutropia* | **J** |
| Ceut12 | MNHN-S 582 | <1886 | *Phocaena albiventris* “dibujado” | *C. eutropia* | I |
| Ceut13 | MNHN-S 583 | <1893 | *Phocaena albiventris* | *C. eutropia* | **K** |
| Ceut14 | MNHN-S 584 | 1887 | *Tursio panope* Philippi | *C. eutropia* | A or B |
| Ceut15 | MNHN-S 585 | 1882 | *Tursio albiventris* Perez (1893) | *C. eutropia* | D or E |
| Ceut16 | MNHN-S 587 | 1894 | *Tursio platyrrhinus* “dibujado” | fail | Fail |
| Ceut17 | MNHN-S 592 | <1904 | *C. eutropia* | *C. eutropia* | **L** |
| Ceut18 | MNHN-S 594 | <1978 | *C. eutropia* | *C. eutropia* | **L** |
| Ceut19 | MNHN-S 1493 | ? | ? | *C. eutropia* | **M** |

*Specimen information for Philippi’s skulls.:*

MNHN-S 581 *Phocaena albiventris*, collected at Chiloé (42ºS; 74ºW) by the 1880's.
MNHN-S 583 *Phocaena albirostris* and *Tursio albiventris*, collected at Talcahuano (36º40'S; 73º10'W) by 1880's.
MNHN-S 582: The skull illustrated in Philippi 1896.
MNHN-S 584: type of *Tursio panope*.
MNHN-S 585, *Tursio albiventris* collected at Río Valdivia (39º50'S; 73º25'W) or Valparaíso (33º00'S; 71º35'W) in 1882.

**Table 2**

**Comparison of mitochondrial haplotypes obtained from Chilean dolphin specimens (n=13) with the test sequences from the museum samples. The consensus fragment begins at position 15 relative to the first base pair of the mtDNA control region. The numbering of sites of substitutions is relative to the first base pair of the t-pro end of the control region. There are two gaps (not shown) when aligned to all Delphinids, the first gap extends from position 85 to 101 and the second from 109 to 111. These gaps are identical in length to that of the outgroup but no other species.**

Comparación de los haplotipos mitocondriales obtenidos de especímenes de delfines Chilenos (n=13) con las secuencias de referencia obtenidas de muestras de museo. El fragmento consenso comienza en la posición 15 relativa a la primera base de la región control del ADN mitocondrial. El número de los sitios de sustitución es relativo al rpimer par de bases del extremo t-pro de la región control. Existen dos inserciones (no mostrado) al alinear esta secuencias con aquellas de los demás delfínidos, el primero se extiende desde la posición 85 a 101 y la segunda desde la posición 109 a la 111. Esas inserciones son idénticas en longitud a aquella del outgroup pero no a otras especies.

| Haplotype | Length | 42 | 56 | 252 | 239 | 297 | 349 | 352 | 396 | 482 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 550 | A | A | T | T | T | G | A | T | T |
| B | 550 | . | . | . | . | . | . | . | C | . |
| C | 550 | . | . | . | . | C | . | . | C | C |
| D | 550 | . | . | G | . | . | . | . | . | . |
| E | 550 | . | . | G | . | . | . | . | . | C |
| F | 550 | . | . | G | C | . | . | . | . | . |
| G | 550 | . | . | G | C | . | . | . | . | . |
| H | 550 | . | . | G | C | . | . | T | . | . |
| I | 550 | . | . | . | . | . | . | T | . | . |
| Ceut10 | 550 | . | . | . | . | . | . | . | . | . |
| Ceut11 | 550 | G | G | . | . | . | . | . | . | . |
| Ceut12 | 200 | ? | ? | . | . | . | . | T | ? | ? |
| Ceut13 | 400 | . | . | G | C | . | A | T | ? | ? |
| Ceut14 | 200 | ? | ? | . | . | . | . | . | ? | ? |
| Ceut15 | 200 | ? | ? | G | . | . | ? | ? | ? | ? |
| Ceut17 | 400 | . | . | . | . | . | . | . | . | C |
| Ceut18 | 550 | . | . | . | . | . | . | . | . | C |
| Ceut19 | 550 | . | . | . | . | . | . | . | C | C |

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