Genetic identification of the diver *Gavia* sp. present on Lake Silvaplana in December 2015

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A diver *Gavia* sp. present during December 2015 at Lake Silvaplana, Canton Graubünden, Switzerland, was identified as a Pacific Diver *Gavia pacifica* based on plumage and structural features. The bird was collected after its death on 26th December. Based on muscle tissue samples collected at the Bündner Naturmuseum by U. E. Schneppat, the identification of the putative Pacific Diver was checked with DNA data.

A partial sequence of the mitochondrial gene Cytochrome Oxidase subunit 1 (COI hereafter) was chosen for species identification because sequences for all species in the genus *Gavia* were available for comparison on GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Total genomic DNA from the muscle tissue was isolated with the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. A partial sequence of the COI gene was amplified with polymerase chain reaction using published primers (BirdF1: TTCTCCAACCACAAAGACATTGGCAC and BirdR1: ACGTGGGAGATAATTCCAAATCCTG) (Hebert *et al.*, 2004). Total PCR reaction volumes was 25 µl containing 12.5 µl GoTaq_ Hot Start Green Master Mix (Promega), 2 µl genomic DNA, 2 µl of each primer with a concentration of 10 µM and 6.5 µl ddH2O. A negative control was used to check for potential contamination. PCR was performed on a Techne TC-512 thermo-cycler and amplification was performed with an initial denaturation at 94 °C for three minutes, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 55 °C , and extension at 72 °C for one minute, with a final extension at 72 °C for seven minutes. The PCR product was examined by gel electrophoresis to confirm amplification of the target fragment. PCR purification and sequencing were carried out by LGC Genomics (Berlin, Germany).

The resulting sequence was edited with Geneious Pro (Drummond *et al.*, 2013) and checked for quality by searching for apparent stop codons after the translation of sequences into amino acids.

For species identification, a gene tree was reconstructed using all available COI sequences of *Gavia* species present on GenBank. The emperor penguin *Aptenodytes forsteri* was used as outgroup (Jarvis *et al.*, 2014). Sequences were aligned using the MAFFT algorithm (Katoh *et al.*, 2002) implemented as a plug-in into Geneious Pro using default settings resulting in a final alignment of 662 base pairs. A maximum-likelihood (ML) search was then employed using RAxML 7.2.8 (Stamatakis, 2006) in GeneiousPro with 500 rapid bootstrap inferences (Stamatakis *et al.*, 2008) with all free model parameters estimated by the software (substitution rates, gamma shape parameter and base frequencies).

The *Gavia sp*. from Silvaplana showed an identical sequence to all *Gavia pacifica* available on GenBank with the exception of one individual with accession number DQ43666, which differed by the substitution of a C with a T at position 130 of the alignment. The *Gavia sp*. from Silvaplana differed from *Gavia arctica* by a minimum genetic distance of 5.7 % (corrected). Sequences DQ433670 (*Gavia pacifica*) and DQ137167 (*Gavia immer*) were not included in phylogenetic reconstruction due to a lot of missing data in those sequences.

In the ML reconstruction, the *Gavia sp.* from Silvaplana clustered with the other samples of *Gavia pacifica* and was clearly separated from samples of *Gavia arctica* (Figure 1). All species clades were supported by high bootstrap values (clades are usually considered as supported when bootstrap values are >70 (Hillis & Bull, 1993)). Note that the phylogenetic tree presented is just a gene tree based on one mitochondrial marker, hence the branching pattern does not necessarily indicate true relationships among taxa. Based on these results, there is no doubt that the mother of the *Gavia sp.* from Lake Silvaplana also had the mtDNA genome of a Pacific diver *Gavia pacifica*.
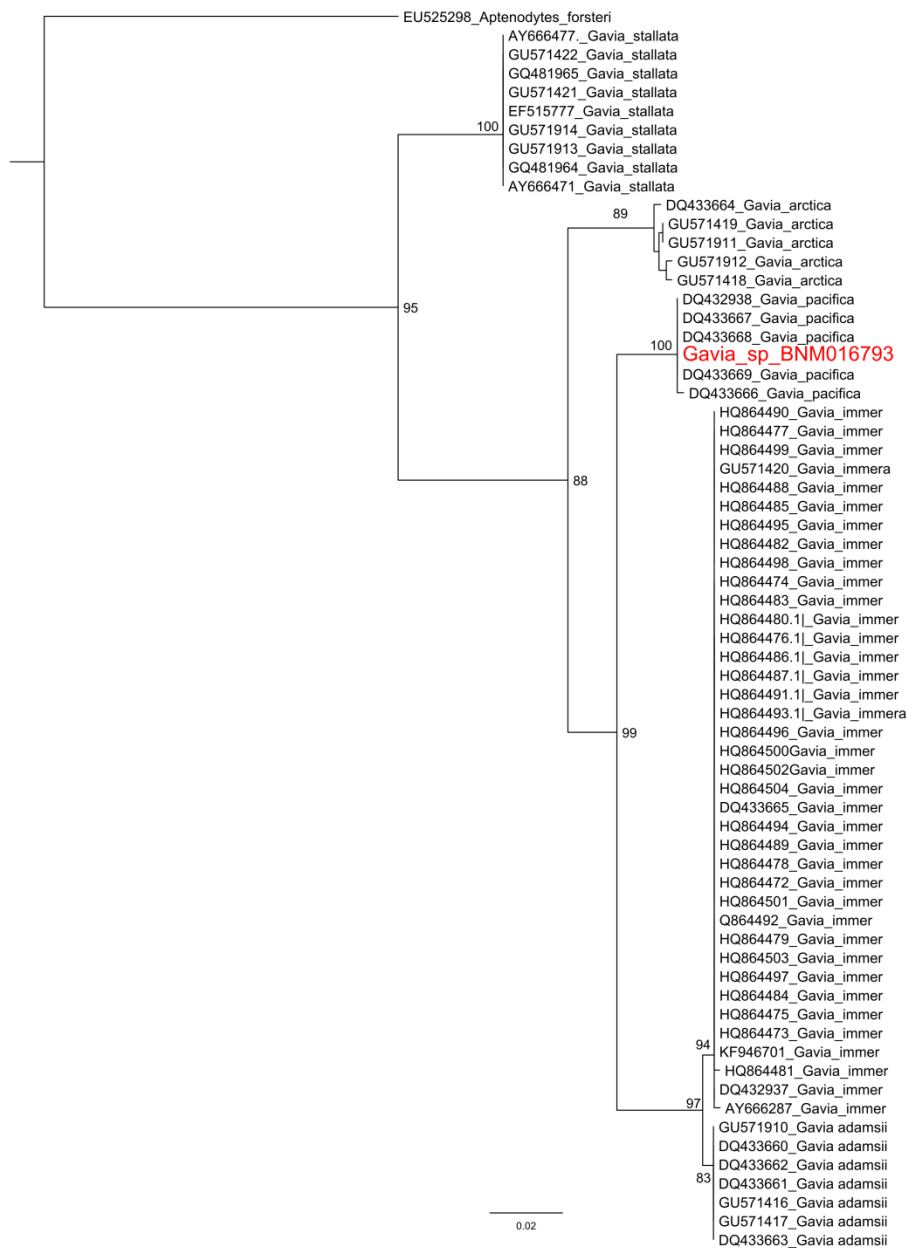

Figure 1. Results of the maximum likelihood analyses. Bootstrap values for species nodes and nodes above the species level are given. The Pacific diver form Lake Silvaplana is marked in red.

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