PCR partial Control Region Chaetophractus 456 bp Version 2

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Abstract

We report a phylogeographic study of Chaetophractus villosus populations in Argentina. Control Region (CR) sequences (484 bp) were obtained for 76 C. villosus from 20 locations across the species whole distribution range. Seventeen new haplotypes were identified. The highest genetic variation and the earliest fossils were found in the Pampean Region, thus appearing as the most probable area of origin of the species. A general pattern of Contiguous Range Expansion (CRE) was revealed by Nested Clade Analysis (NCA) supported by mismatch analysis and Fu's test. The Pampean Region would have been the preexpansion area, while Patagonia would have been the main dispersal route of contiguous expansion, possibly after the Pleistocenic glaciations.

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Guidelines

Partial sequences of mtDNA Control Region (CR) are amplified using the universal primers Thr-L15926 (5´-CAATTCCCCGGTCTTGTAAACC-3´), located in the neighboring tRNA-pro gene and DL-H16340 (5´-CCTGAAGTAGGAACCAGATG-3´) (Vilá el al. 1999).

Protocol

DNA extraction

Step 1.

Extract DNA from fixed or dried tissues (liver, spleen, muscle and skin) using the sodium dodecyl sulphate–proteinase K/phenol/RNAse method (Sambrock et al., 1989), while extracting DNA from blood following John et al. (1991).

DNA extraction

Step 2.

Concentrate samples by ethanol precipitation.

Amplification

Step 3.

Perform the amplification of the double-stranded product in 25 ul volume of PCR mix containing 1.25 U of Taq DNA polymerase, 2.5 ul of 10 x Taq polymerase buffer, 3 mM of $MgCl_2$, 0,16 mM of dNTP´s and 5 pmol of each primer.

AMOUNT
25 µl Additional info: PCR mix
AMOUNT
2.5 µl Additional info: 10 x Taq polymerase buffer

Amplification

Step 4.

Perform the amplification in a Biometra T Personal thermocycler. With the thermal profile consisting of an initial denaturation at 94 ºC for 5 min, followed by 35 cycles of 94 ºC for 30 s, 56 ºC for 30 s and 72 ºC for 45 s with a final extension step of 72ºC for 8 min.

Sequencing

Step 5.

Purify double-stranded PCR products and concentrate by ethanol precipitation.

Sequencing

Step 6.

Examine on 1% agarose gels and directly sequence in both directions using the same primers as those used for amplification.

Sequencing

Step 7.

Conduct sequencing using an ABI PRISM 3130 XL automated sequencer (Applied Biosystems™) at the Sequencing Services of Universidad de Buenos Aires (Buenos Aires, Argentina).

Sequencing

Step 8.

Edit sequences with Chromas 2.3 (Technelysium Pty. Ltd., 1998–2004, http://www.technelysium.com.au) and align using the computer software package CLUSTAL W (Thompson et al., 1994).