

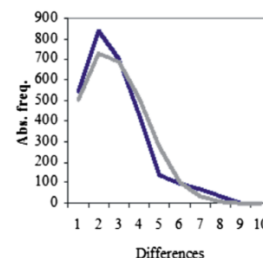
Nov 06, 2017

Version 2

PCR partial Control Region Chaetophractus 456 bp V.2

DOI

dx.doi.org/10.17504/protocols.io.kmncu5e



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Protocol Citation: Sebastian Poljak 2017. PCR partial Control Region Chaetophractus 456 bp. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kmncu5e>

**Manuscript citation:**

Phylogeography of screaming hairy armadillo *Chaetophractus vellerosus*: successive disjunctions and extinctions due to cyclical climatic changes in southern South America.

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Protocol status: Working

Created: November 05, 2017

Last Modified: February 04, 2018

Protocol Integer ID: 8590

Keywords: Chaetophractus vellerosus, cyclical climatic changes, disjunct distribution, phylogeography, screaming hairy armadillo, southern South America., phylogeographic study of chaetophractus villosus population, chaetophractus villosus population, locations across the species, main dispersal route of contiguous expansion, nested clade analysis, phylogeographic study, partial control region chaetophractus, species, control region chaetophractus 456 bp, main dispersal route, pcr partial control region chaetophractus 456 bp, pleistocenic glaciation, probable area of origin, earliest fossil, new haplotype, highest genetic variation, general pattern of contiguous range expansion, pampean region, contiguous range expansion, contiguous expansion

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.

Guidelines

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

Troubleshooting

DNA extraction

- 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).
- 2 Concentrate samples by ethanol precipitation.

Amplification

- 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.

 25 µL PCR mix

 2.5 µL 10 x Taq polymerase buffer

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

Sequencing

- 5 Purify double-stranded PCR products and concentrate by ethanol precipitation.
- 6 Examine on 1% agarose gels and directly sequence in both directions using the same primers as those used for amplification.
- 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).
- 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).