Electronic appendix: Molecular methods

The Sapayoa tissue sample is documented by a voucher specimen in Museo Ecuatoriana de Ciencias Naturales in Quito. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN). We utilized two nuclear introns in the analyses, one in the myoglobin gene (intron 2) and one in the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene (intron 11). Myoglobin intron 2 was amplified and sequenced as described in Irestedt et al. (2002). Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) intron 11 was amplified using primers G3P13b and G3P14b (5’- TCC ACC TTT GAT GCG GGT GCT GGC AT -3’ and 5’- AAG TCC ACA ACA CGG TTG CTG TA -3’, both modified from van Tuinen et al. 2001) and anchored in exons 11 and 12, respectively. The thermocycling conditions were: initial denaturation of 5’ at 94 °C, 40 cycles of 40'' at 94 °C, 40” at 57 °C, 60” at 72 °C, final extension of 5’ at 72 °C. Sequencing was performed using primers G3PintL1 (5’- GAA CGA CCA TTT TGT CAA GCT GGT T -3’) and G3P14b.

New sequences were deposited in GenBank (myoglobin: XXXX-XXXX; G3PDH: XXXX-XXXX; for previously published myoglobin sequences see Ericson and Johansson 2003; Irestedt et al. 2002; Johansson and Ericson 2003). The DNA sequences were aligned manually. This was unproblematic for myoglobin. However, the G3PDH gene evolves faster than myoglobin (according to larger pair-wise distances observed between taxa), which makes sequence alignment less straightforward in certain regions. For example, it proved impossible to unambiguously align the passerine sequences with a non-passerine outgroup. We also excluded two portions of the alignment of passerines from the analyses of G3PDH (positions 33 to 42 and 324 to 339, respectively). The alignments are deposited in EMBL-EBI (acc.nos. XXX and XXX).

References