S3 Fig. Alignment of selected exons with point mutations

- Open reading frame disrupting mutations are marked on red background
- Start codon (ATG), splice donor (gt), and splice acceptor (ag) sequences are on yellow background
- Exon sequences are in uppercase letters
- Amino acid sequence of the rifleman MOXD2 protein is shown at the top

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A. Multiple alignment of exon 1 sequences from 11 birds

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 1 Rifleman (aa)                                  M  M  A  I  V  F  S  R  I  K  R
 1 Rifleman                gactcaggaattgcagtggtgctgttgATGATGGCAATTGTCTTCTCGAGAATCAAGAGG    60
 2 Golden-collared manakin gactcagaaattgaagtggtactgttggtgATGGCAGTGTCCTTCTCAAGAATCCAGGGT    60
 3 American crow           gactcagaaactgcag---------tgATGATGGTGATGCTCTTCTCAGGGATCAAGAGA    51
 4 Hooded crow             gacacagaaactgcag---------tgATGATGGTGATGCTCTTCTCAGGGATCAAGAGA    51
 5 Ground tit              gtaccagagactgcag---------agATGATAGTGATGTTCTTTTCAGGAATCAAGAGA    51
 6 Collared flycatcher     gactcagagactgcag---------agATGATGGTTTTGTTCTTCTCAGGAATCAAGAAA    51
 7 Zebra finch             gactcagagactgcag---------agATGATGGTGATC---TTTTCAGGAATCAAGAGA    48
 8 Atlantic canary         gactcagagactgcag---------agATGATGGTGATGTTCTACTCAGGAATCAAGAGA    51
 9 Medium ground finch     gacttggagactg-ag---------agATGATGTTGATGTTCTTCCCAGGAATCAAGAGA    50
10 White-throated sparrow  gactcagagactgcag---------agattATGGTGATGTTCTTCTCAGAAATCAAAGAA    51
47 Rock pigeon             gactccgaaattgcagtggtcctggtgataATGGCAGTTCTTTTCTCAAGTATCAAGGGT    60
48 Yellow-throated sandgrouse gactcagaaactgccgtggtcctgttgATGATGGCAGTTGTCTTCTCAAGAACCAAGGGT 60
                           *     *  * **  *          * * **     *    *   *    * * *

 1 Rifleman (aa)           M  F  F  L  L  F  F  P  C  F  C  S  G  Q  L  A  P  P  P  L
 1 Rifleman                ATGTTCTTCCTCTTGTTCTTCCCATGCTTTTGTTCTGGTCAGCTTGCACCTCCACCGCTG   120
 2 Golden-collared manakin ATGCTCTTCCTCTTGTTCC----------------GGTCAGCCTGCAGCTCCACTACTG   103
 3 American crow           ATGCTCTTCCTCTTGTTCCTCCCATGCTTTTGTTCTGGCCAGCTTGCACCTCCACTGCTG   111
 4 Hooded crow             ATGCTCTTCCTCTTGTTCCTCCCATGCTTTTTTTCTGGCCAGCTTGCACCTCCACTGCTG   111
 5 Ground tit              ATGCTCTTCCTCTTGTTCCTCCCATGCTCTTGTTCTGGCCAGCTTGCATCTCTGCTGCTG   111
 6 Collared flycatcher     ATGCTCTTCCTCTAGTTCCTCTCATGCTTTTGTTCTGGCCTGCTTGCATCTCCCCTGCTG   111
 7 Zebra finch             ATGCTCTTCCTTTTGTTCT---CTTGCCTCTGTTCTGGCCAGCTTGCATCTCCACTGCTG   105
 8 Atlantic canary         CTGCTCTTCCTCTTTTTCCTTCCATGCTTTTGTTCTGGCCAGCTTGCATTTCCACTGCCG   111
 9 Medium ground finch     ATGCTCTTCCTCTTGTTCCTCCCATGCTTTTGTTCTGGCCAGCTTGCATCTCCTCTGATG   110
10 White-throated sparrow  ATGCTCTTCCTCTTGTTCCTCCCATGCTTTTGTTCTGGCCAGCTTGCATTTCCACTGCTG   111
47 Rock pigeon             ATGCTCTTCCTCTTGCTCCTCCCATACTTTTGTTTTGGTCAGCCTGCACCTTCACTGCTG   120
48 Yellow-throated sandgrouse ATGCTCTTCCTCTT-TTCTCCCCATGCTTTTATTATGATCAGCCTGCAACTCCATAGCTG 119
                            ** ******* *   **                 *  * ** ****  *       *

 1 Rifleman (aa)           R  F  S  T  F  L  D  P  S  N  M  V  Y  L  H  W  D  H  D  D
 1 Rifleman                CGTTTCTCCACTTTCCTGGATCCTTCCAACATGGTCTACCTCCACTGGGACCATGATGAT   180
 2 Golden-collared manakin CATTTCTCCACCTCCCTGGATCCTTCCAACACAATCCACCTCCGCTGGGACCATGATGAA   163
 3 American crow           CGTTTCCCCACCTTATTGGATCCTTCCAGCATGTTCTACCTCCACTGGGACCACGAGGAA   171
 4 Hooded crow             CGTTTCCCCACCTTATTGGATCCTTCCAGCATGTTCTACCTCCACTGGGACCACGAGGAA   171
 5 Ground tit              AGTTTCCCCACCTTCCTGGATCCTTCCAGCATGTTCTACCTCCGCTGGCACTATAAGGAA   171
 6 Collared flycatcher     CGTTTCCCCACCTTCCTGGATCCTTCCAGCATGTTCTACCTCTGCTGGGACCACAAGGAA   171
 7 Zebra finch             CGTTTCTCCACCTTCCTGGATCCTTCTAGCATGTTCCACCTCTGCTGGGACCATGAGGAA   165
 8 Atlantic canary         CGTTTCCCCACCTTCCTGGATCCTTCCAGCATGTTCTACCTCTGCTGGGACCATGAAGAA   171
 9 Medium ground finch     CTTTTCCCCACCTTCCTGGATCCTTCCAGCATGTTCTACCTCTGCTGGGAGCACAAGGAA   170
10 White-throated sparrow  CATTTCCCTGCCTTCCTGGATCCTTCCAGCATGTTCTGCCTC----------TGAGGAA   160
47 Rock pigeon             CATTTCTCCATCTTCCTGGAGCCTACAATCATGGTTTACCTCTGCTGAGACCATGACGAA   180
48 Yellow-throated sandgrouse CGTTTCTCCATCTTCCTGGATCCTTCAAACATGGTCTACCTCCGCTGGGACCATGACGAA 179
                              **** *    *   **** *** * * **   *   ****           * **

 1 Rifleman (aa)           Q  E  L  M  T  F  E  L  Q  V  H  T  T  G  W  V  A   F  G  F
 1 Rifleman                CAGGAGCTGATGACATTTGAGCTGCAGGTCCATACAACTGGCTGGGTGGCA-TTTGGATT   239
 2 Golden-collared manakin CAGGAGCTGATGACATTTGAGCTGCAGGTCCATACAACTGGCTGGGTGGCA-TTTGGATT   222
 3 American crow           CAGGAGCTGATGAGG-TTGAGCTGCACATCCACACAACTGGCTGGATGGCA-TCTGGATT   229
 4 Hooded crow             CAGGAGCTGATGAGG-TTGAGCTGCACATCCACACAACTGGCTGGATGGCA-TCTGGATT   229
 5 Ground tit              CAGGAGCTGATGGGG-TTGAGCTGCACATCCACACAATTGGCTGGGTGGCA-CATGGA--   228
 6 Collared flycatcher     CAGGAGCTGATGGGG-TTGAGCTGCACATCCACACAACTGGCTGGGTGGCA-TCTGGATT   229
 7 Zebra finch             CAGGAGCTGATGGGG-TTGAGCTGCACATCCAC---GCTGCCTCATCCACACTGTAGATT   221
 8 Atlantic canary         CAGGACCTGATGGGG-TTGAGCTGCACATCCAC---ACGGGCTGGGTGGCA-CGTGGATT   226
 9 Medium ground finch     CAGGAGCTGATGGGG-TTGAGCTGCACATCCAC---ACTGGGTGGATGGCA-TGTGGATT   225
10 White-throated sparrow  CAGGAGCTGATGGGG-CTGAGCTGCACATCCAC---ACTGGCTGGGTGGCA-TGTGGATT   215
47 Rock pigeon             CAGGAGGTGATGACATTTGAGCCGCAAGTCCATACAACTGGCTGGGTGGCA-TTTGGATT   239
48 Yellow-throated sandgrouse CAGGAGCTGATGAAGTTTGAGCTGCAGGTCCATACGACTGGCTGGGTGGCA-TTTGGATT 238
                           *****  *****     ***** ***  ****       *  *      **   * **
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 1 Rifleman (aa)                S  P  H  G  E  L  P  G  S  D  I  V  I  G  G  V  F  P  N  G
 1 Rifleman                     CAGCCCTCATGGAGAGTTGCCTGGATCTGACATTGTGATAGGAGGTGTCTTCCCAAATGG   299
 2 Golden-collared manakin      CAGCCCTCATGGAGAGTTGCCTGGATCTGACATTGTGACAGGAGGTGTCTCCCCAAATGG   282
 3 American crow                CAGCCCTCATGGAGAGCTGCCTGGATATAACATTGTGACAGGAGGTGCCTCCCCAAATGG   289
 4 Hooded crow                  CAGCCCTCATGGAGAGCTGCCTGGATATGACATTGTGACAGGAGGTGCCTCCCCAAATGG   289
 5 Ground tit                   GAGCCCTCATGGAGAGCTGCCTGGATCTGACACTGTGTCAGGAGATGCCTACCGGAGTGG   287
 6 Collared flycatcher          CAGCCCTCATGGAGAGCTGCCTGGGTCTGACGTTGTGACAAGAGGTGCCTTCCAAAAGGG   289
 7 Zebra finch                  CAGCTATCATGGAGAGCTGCCTGGATCTGACATTGTGACAGGAGGTGCTTTCTGAAATGG   281
 8 Atlantic canary              CAGCCCTCACAGAAAGCTGCCTGGATCTGACATTGTGACAGGAGGTGTCTCCTGAAATGG   286
 9 Medium ground finch          CAGCCCTCATGGAGAGCTGCCTGCATCTGACATTGTGAGAGGAGGTGCCTTCTAAAATGG   285
10 White-throated sparrow       CAGCCCTCATGGAGAGCTGCCTGGATCTGACATTGTGCCAGGAGGTGCCTTCTGAAATGG   275
47 Rock pigeon                  CAGCCCTCATGGAGAGTTGCCTGGATCTGACATTGTGATAGGAAGTATCTTCCCCAATGG   299
48 Yellow-throated sandgrouse   CAGCCCTCACGGAGAATTGCCTGGATCTGATACTGTGATAGGAGGTGTCTTCCCAAATAG   298
                                 ***  ***  ** *  ******  * * *   ****  * **  *   * *   *   *

 1 Rifleman (aa)                S  I  Y  F  S
 1 Rifleman                     CAGCATCTACTTCTCTgtaagttgaaaatacttcctctgcatcccc                345
 2 Golden-collared manakin      CAGCATCTACTTCTCTggaagttgaaaagacttcctctgcaggctc                328
 3 American crow                CAGCATCTGATTCTCTgtaagttgaacagatttcctcttcatcccc                335
 4 Hooded crow                  CAGCATCTGATTCTCTgtaagttgaacagatttcctcttcatcccc                335
 5 Ground tit                   CAGCTTCTGCTTCCCTgtaagctgaacagatttcctcttcaacccc                333
 6 Collared flycatcher          CAGCATCTGCTTCTCTgtaagttgaacaggtttcctcttcgtccca                335
 7 Zebra finch                  CAGCATCTGCATCTCTgtaagttgaacagatatcctcttcatcctc                327
 8 Atlantic canary              CGGCATCTGCTTCTCTgtaagtggaacatagttcctcttcatctcc                332
 9 Medium ground finch          CAGCATCTGCTTCTCTgtaagttgaacagatttcctcttcaccccc                331
10 White-throated sparrow       CAGCATCTGCTTCTCCgtaagatgagcagattccctcttcatcccc                321
47 Rock pigeon                  CAGCATCTACTTCTCCgtaagttgaaaagacttcctctgcgtccct                345
48 Yellow-throated sandgrouse   CAGCATCTACTTCTCTgtaagctgaaaaaacttcctctgcatcctc                344
                                * ** ***   ** * * ***  **  *     ***** *
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B. Multiple alignment of exon 3 sequences from 3 birds

```
 1 Rifleman (aa)                                M  D  T  A  H  L  V  A  A  F
 1 Rifleman       agatttgccagtgaaacacttccatttcagATGGACACAGCCCATCTTGTTGCTGCATTT    60
35 Crested ibis   agactttccaatgaaacactcccattacagATGGACACAGCATGCCTTGTTACTGCCTTT    60
36 Little egret   agagtttccagtg-aatgttcccattactgATGGATACAGCACACTTCATTACTGCCTTT    59
                  *** ** *** ** **   * ***** * ****** *****     *  ** **** ***

 1 Rifleman (aa)  G  T  D  D  T  V  Q  F  F  K  S  Q  R  F  S  R  S  L  F  L
 1 Rifleman       GGCACTGATGACACAGTCCAATTCTTTAAAAGCCAAAGATTTTCCAGATCT-CTTTTCCT   119
35 Crested ibis   TGTGCTGATGACACAGTCCAATTCTTTAAAGGCCAAAGATTTTCCAAGTCTTTTTTTCTT   120
36 Little egret   GGTACTTATGACACAGTCTAATTCTTTAAAGGCCAAAGATTTTCTAAATGT-CTTTTCTT   118
                   *  ** *********** *********** ************* *  * *  ***** *

 1 Rifleman (aa)   M  R  Y  K  S  P  S  D  S  T  D  P  K  V  F  F  T  Y  D  L
 1 Rifleman       GATGAGGTACAAAAGCCCATCTGACTCAACTGACCCCAAAGTATTCTTCACCTATGACCT   179
35 Crested ibis   GATGAGGTACAGAGGCCCATCTGACTCAACTGACCCCAAAATATTCTTCACCTATGACCT   180
36 Little egret   GATGAGGTACAGAGGCCCATCTGACCCAACTGACCCCAAAATAATCTTCACCTATGACCT   178
                  *********** * *********** ************** ** ****************

 1 Rifleman (aa)   R  L  D  N
 1 Rifleman       GAGGCTGGACAACgtaagtattggacaagaactttgtttagta                  222
35 Crested ibis   GAGGCTGGACAATgtaagtactggacaagagctttacttggca                  223
36 Little egret   GAGGCTGGACAACataagtattggacaagagcattacttggca                  221
                  ************  ****** ********* * **  ** * *
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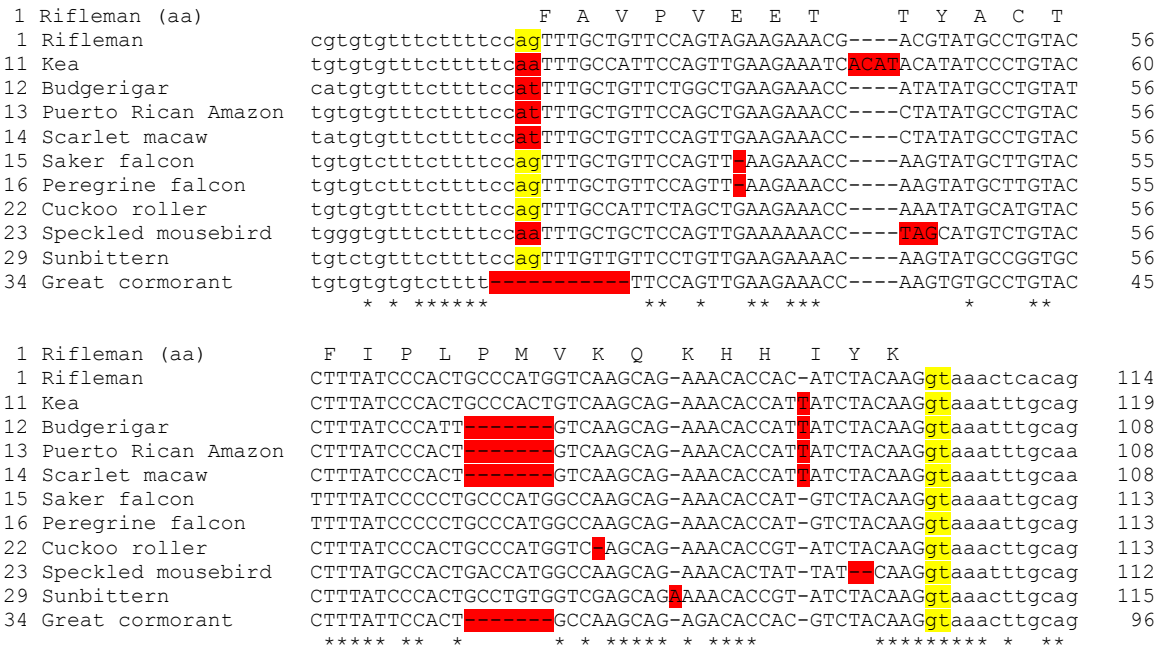
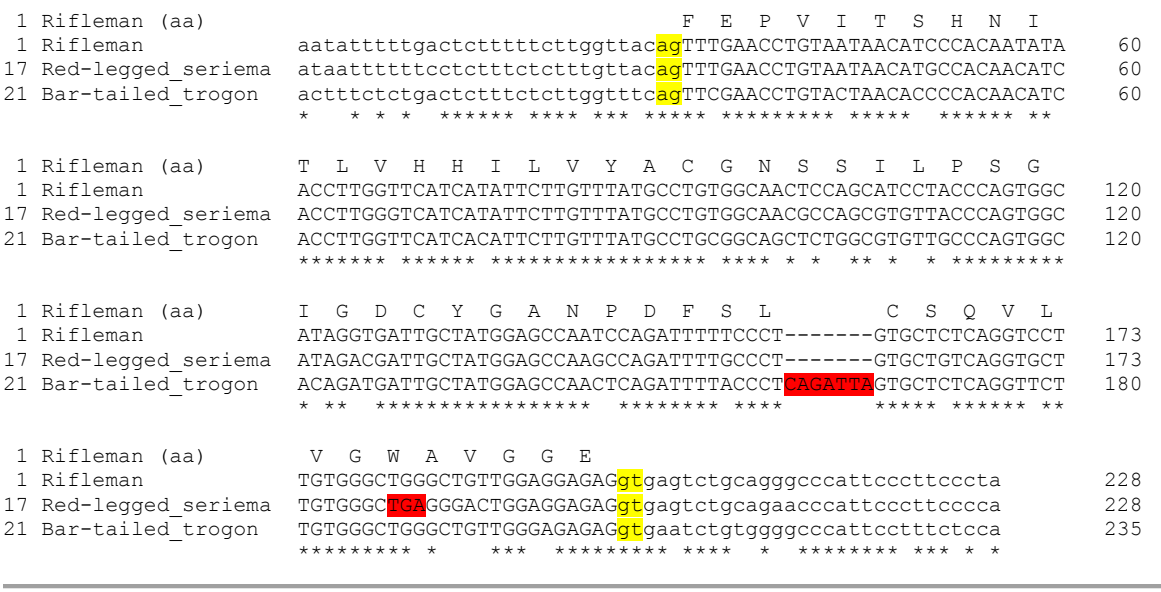
C. Multiple alignment of exon 4 sequences from 11 birds



D. Multiple alignment of exon 5 sequences from 3 birds



E. Multiple alignment of exon 7 sequences from 4 birds

```
 1 Rifleman (aa)                                        L  I  D  S  S  G  I  R  I
 1 Rifleman              ttgctaaaggagaggtcatttttcttttacagGCTTGATTGACAGCTCAGGGATACGAAT    60
20 Rhinoceros hornbill   tggtcattgctgaggtaatatttcttttacagCCTTGATTGACAGCTCAGGGGTACGAAT    60
44 Anna's hummingbird    atgatgaagaagtggcaatttttgttttacagATTTGATTGACAGTTCAGGGGTATGAAT    60
49 Brown mesite          cagctgaaggagtaggcatttttcttttacagGTTTGATCGACAGCTCAGGGGTACAAAT    60
                           *     *  *  *  ** *** ********  ***** ***** ****** **  ***

 1 Rifleman (aa)          Y  Y  T  P  E  L  R  K  Y  D  V  G  V  L  Q  T  G  V  F  I
 1 Rifleman              CTACTACACACCAGAGCTACGGAAATATGATGTGGGGGTTCTGCAAACAGGTGTCTTCAT   120
20 Rhinoceros hornbill   CTACTATACTCCGGAGATACGAAAATATGATGTTGGGATTCTGCAAACAGGCATCTTCAT   120
44 Anna's hummingbird    --------CACCAGAGCTACGGAAATATGATGTGGGGATTCTGCAAACAGGTGTCTTCAT   112
49 Brown mesite          CTAAAACACTTCAGAGCTACGGAAATATGATGTGGGGGTTCTGCAAACAGGCATCTTCAC   120
                              *  * *** **** *********** *** *************  ******

 1 Rifleman (aa)          F  P  A  H  F  I  P  P  G  A  E  S  Y  R  S  Y  G  L  C  N
 1 Rifleman              TTTCCCTGCACATTTCATTCCTCCTGGAGCAGAATCCTACAGATCTTACGGTCTTTGCAA   180
20 Rhinoceros hornbill   TTT-CCTGTGCATTTCATTCCTCCTGGGGCACAATCCTACAAATCTTACGGCCTTTGCAA   179
44 Anna's hummingbird    TTTCCCTGTGCATTTCATTCCCCCTGGAGCAGAATCCTACAGATCTTATGGACTTTGCAA   172
49 Brown mesite          TCTCCCTGTGCATTTCATTCCTCTTGGAGCAGTATCCTACAGATCTCATGGTCTTTACAA   180
                         * * ****  *********** * *** ***  ******** **** * ** **** ***

 1 Rifleman (aa)          S  S  Q  F  D  E
 1 Rifleman              TTCCAGCCAGTTCGACGAAgtgagtgtggggaaagccatacaaaattct              229
20 Rhinoceros hornbill   TTCCAGCCAGTTTGATGAAgtgagtgtggggaaagccatac-aaattct              227
44 Anna's hummingbird    TTCCAGCCAGTTTGATGAAgtgagtgtggggacagccatacgagatcct              221
49 Brown mesite          TTCCAGCCAGTTTGATGAAgtgagtgtggggaaagacatgcaaaatcct              229
                         ************ ** ***************** ** *** * * ** **
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F. Multiple alignment of exon 8 sequences from 3 birds

```
 1 Rifleman (aa)                                  M  N  G         M  L  V  P  D
 1 Rifleman          ggctctgatgttctctgttttgaactgcagATGAATGGG-------ATGCTGGTTCCAGA    53
31 Emperor penguin   gactctgacctacttcattttgaactgcagATGAATGGTTCCAGATCTGCTGGTTCCAGA    60
32 Adelie penguin    gactctgacctacttcattttgaactgcagATGAATGGG-------ATGCTGGTTCCAGA    53
                     * ******  * **   **********************         *************

 1 Rifleman (aa)       L  H  V    F  A  Y  L  L  H  T  H  L  S  G  R  G  V  K  V
 1 Rifleman          TCTCCATGTC--TTTGCCTATCTGCTTCACACTCACCTGTCTGGCAGAGGAGTGAAAGTC   111
31 Emperor penguin   TCTGCATGTC--TTTGCCTACTTGCTTCATACCCACCTGTCTGGCAGAGGAGTGAAAGCT   118
32 Adelie penguin    TCTGCATGTCTTTTTGCCTACTTGCTTCATACCCACCTGTCTGGCAGAGGAGTGAAAGCT   113
                     *** ******  ********  ******* ** *************************

 1 Rifleman (aa)      V  Q  Y  R
 1 Rifleman          GTTCAATACCGgtaagagaaggaaacac--agtattgctggac                   152
31 Emperor penguin   GCTCAATACCGgtaagagaagcaaactc--ggtattgctgcct                   159
32 Adelie penguin    GCTCAATACCGgtaagagaagcaaactcatagtgttgctgcct                   156
                     * ******************** **** *   ** ******
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G. Multiple alignment of exon 9 sequences from 2 birds

```
 1 Rifleman (aa)                                        N  G  E  Q  L  R  I  I  C  E
 1 Rifleman                  cacacttctccctttgtttgtcctccccagGAACGGTGAGCAGCTGAGGATCATCTGTGA    60
19 Northern carmine bee-eater taactttcttcccttctctgtctttcccagGAATGGTGAGCAGCTAAGGATCATCTGTGA    60
                               *   **** ** ** * **** * ******** *********** **************

 1 Rifleman (aa)                D  N  K  Y  D  F  R  L  Q  E  I  R  D  T  K  E  I  L  T  I
 1 Rifleman                  GGACAATAAGTATGACTTCAGGCTGCAGGAAATTCGGGACACGAAGGAAATCCTCACAAT   120
19 Northern carmine bee-eater GGACAATAAGTATGACTTCAGG-------ACATTGAGGACATGAAGGAAATTGTGATAAC   113
                              **********************       * ***  ***** *********  * * **

 1 Rifleman (aa)                K  P
 1 Rifleman                  TAAACCAgtatgatcctctcctgggatgtagcactgg                      157
19 Northern carmine bee-eater CAAACCAgtaagatccttccctgtgacacagccacgg                      150
                               ********* ******  **** **   ***   **
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