Supporting Information

Thomson et al. 10.1073/pnas.1407207111

miRNA 1791

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Gallus    TGAAGCACCATGTTGGGCTGCATCAGTCATGCCATGTTATGAAACCTAACGCGATGTGACTGATGCAGGCTGACGTGATGTGTCA
Alligator TGATGCACCATGTTGGGCTGCCTCAGTCATGCCATGTTATGAAACCTAATGCAATGTGACTGATGCAGGCTGACGTGATGTGTCA
Chrysemys TGATGCACCATGTTGGGCTGCATCAGTCATGCCATGTTATGAAACCTAACACGATGTGACTGATGCAGGCTGACATGACATGTCA
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```
Gallus ΔG = -40.60
U| A   C     UG G              CCA     UGA
 GA GCA CAUGU  G CUGCAUCAGUCAUG   UGUUA   \
 CU UGU GUGCA  C GACGUAGUCAGUGU   GCAAU   A
A^ G   A     GU G              AGC     CCA

Alligator ΔG = -37.20
U      C     UG G    C         CCA|  U  GAA
 GAUGCA CAUGU  G CUGC UCAGUCAUG   UGU AU   A
 CUGUGU GUGCA  C GACG AGUCAGUGU   ACG UA   C
A      A     GU G    U         A--^  -  AUC

Chrysemys ΔG = -38.30
U|   CAC     UG G              CCA     UGA
 GAUG   CAUGU  G CUGCAUCAGUCAUG   UGUUA   \
 CUGU   GUACA  C GACGUAGUCAGUGU   ACAAU   A
A^   ACA     GU G              AGC     CCA
```

miRNA 1641

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Gallus    GATGCAGGGCATTTCCTGAGGATTAATGACTGTCTGGGGTCATCATCTCCTCCCAGTTAGTTATTAATCCCC-AGGAAATACTCTGTGCCTTGATC
Alligator AATGCAGGGCATTTCCTGGGGATTAATGACTGTCTGGGGTCATCATCTCCTCCCAGTTAGTTATTAATCCCC-AGGAAATACTTTATACCTTGATC
Chrysemys GACGCAGGGCATTTCCTGGGGATTAATGACTGTCTGGGGTCATCATCTCCTCCCAGTTAGTTATTAGTCCCCCAGGAAATACTGTGTGCCTTGATC
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```
Gallus ΔG = -47.80
GAU------|     C        A             U      UCAUC
         GCAGGG AUUUCCUG GGAUUAAUGACUG CUGGGG     \
         UGUCUC UAAAGGAC CCUAAUUAUUGAU GACCCU     A
CUAGUUCCG^     A        C             U      CCUCU

Alligator ΔG = -48.40
AAUGC------|   C                      U      UCAUC
           AGGG AUUUCCUGGGGAUUAAUGACUG CUGGGG     \
           UUUC UAAAGGACCCCUAAUUAUUGAU GACCCU     A
CUAGUUCCAUA^   A                      U      CCUCU

Chrysemys ΔG = -49.60
A-------|   G  C           -           U      UCAUC
        CGCA GG AUUUCCUGGGG AUUAAUGACUG CUGGGG     \
        GUGU UC UAAAGGACCCC UGAUUAUUGAU GACCCU     A
CUAGUUCC^   G  A           C           U      CCUCU
```

miRNA 1743

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Gallus    AAAGTAGGCTCCACTGTGTTAATCCTCTTGGAATGCAGCAATTATCACCTACCATATAGGTGTTAAGTGCTGAATTTCAAAGGGATTATCACTTTCCTCCTTT
Alligator CAAGTGGGTTCTATTGTGCTAATCCTCTTGGAATGCAGCAATTATCACCTACCATATAGGTGTTAAGTGCTGAATTTCAAAGGGATTATCACTTTTCCCCCTT
Chrysemys NNNNNNNNNNCACTGTGCTAATCCTCTTGGAATGCAGCAATTATCACCTACCATATAGGTGTTAAGTGCTGACTTTCAAAGGGATTATCACTTTCCCCCCTT
```

```
Gallus ΔG = -38.40
|   U   CUCCACU   U      UC       G     A   U      CC
AAAG AGG       GUG UAAUCC  UUGGAAU CAGCA UUA CACCUA  \
UUUC UCC       CAC AUUAGG  AACUUUA GUCGU AAU GUGGAU  A
^   C   UUU----   U      GA       A     G   U      AU

Alligator ΔG = -40.60
C|  U   UUCUAUU   C      UC       G     A   U      CC
 AAG GGG       GUG UAAUCC  UUGGAAU CAGCA UUA CACCUA  \
 UUC CCC       CAC AUUAGG  AACUUUA GUCGU AAU GUGGAU  A
-^  C   UUUU---   U      GA       A     G   U      AU

Chrysemys ΔG = -34.20
NNNNNNNNNNCACU|  C      UC      UG     A   U      CC
              GUG UAAUCC  UUGGAA  CAGCA UUA CACCUA  \
              CAC AUUAGG  AACUUU  GUCGU AAU GUGGAU  A
UUUCCCCCCUUU--^  U      GA      CA     G   U      AU
```

miRNA 2964

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Gallus    AATCTCTGCTACAGATGTCCAGACACAATTCTTGGTTCGTACGGCTCCAGCGCACAAGAATTGCGTTTGGACAATCAGGAGCAGAGATT
Alligator ACTCCCCGCCACAGATGTCCAGACACAATTCTTGGTTTGTACGTTTCCACGGCACAAGAATTGCGTTTGGACAATCAGGAGCGGAGACT
Chrysemys AATCTCTGCCACAGATGTCCAGACACAATTCTTGGTTTGTATGATTCCATTGCACAAGAATTGCGTTTGGACAATCAGGAGCAGAGATT
```

```
Gallus ΔG = -43.10
          ACA  -|        A         GUU   ACGG
AAUCUCUGCU   GA UGUCCAGAC CAAUUCUUG   CGU    \
UUAGAGACGA   CU ACAGGUUUG GUUAAGAAC   GCG    C
          GGA  A^        C         AC-   ACCU

Alligator ΔG = -35.90
A--|   CCG  ACA   -        A         GU    ACG
   CUCC   CC   GAU GUCCAGAC CAAUUCUUG  UUGU   U
   GAGG   GG   CUA CAGGUUUG GUUAAGAAC  GGCA   U
UCA^   CGA  A--   A        C         AC    CCU

Chrysemys ΔG = -39.70
         CACA  -|        A         GUU    -  A
AAUCUCUGC    GA UGUCCAGAC CAAUUCUUG   UGUA UG U
UUAGAGACG    CU ACAGGUUUG GUUAAGAAC   ACGU AC U
         AGGA  A^        C         ---    U  C
```

Fig. S1. Sequence alignment and predicted secondary structure for four microRNA families that were detected in the *Alligator* and *Chrysemys* genomes via BLAST similarity searches. The mature miRNA sequence from miRBase is underlined in the sequence and secondary structure of the reference species (*Gallus*). Substitutions relative to the reference sequence are highlighted in red. miRNA 1743 sits at the end of a contig in the *Chrysemys* genome assembly and is truncated by 10 bases on the 5′ end as a result. We represent these as ambiguous bases and make no attempt to predict secondary structure in this region.