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## **Ovodefensins, an Oviduct Specific Antimicrobial Gene Family Have Evolved in Birds and Reptiles to Protect the Egg by Both Sequence and Intra Six Cysteine Sequence Motif Spacing**

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A)

**OvoDA C-X5-C-X3-C-X11-C-X3-CC**

|                                     |  |
|-------------------------------------|--|
| <i>Gallus gallus</i> OvoDA1         | LVLK---YCPKI <b>G</b> YCSNTCSKTQIWATSHGCKMYCCLPASWKWK  |
| <i>Meleagris gallopavo</i> OvoDA1   | QVLK---YCPKI <b>G</b> YCSSKCSKAEVWAYS PDCKVHCCVPANQKWK |
| <i>Meleagris gallopavo</i> OvoDA1 2 | QVLK---YCPKI <b>G</b> YCSSKCSKAEVWAYS SDCKVHCCVPANRKWK |
| <i>Anas platyrhynchos</i> OvoDA1    | QVRK---YCPKV <b>G</b> YCSSKCSKADVWSLSSDCKFYCCLPPGWKWK  |
| <i>Taeniopygia guttata</i> OvoDA1   | KFRK---TCAPM <b>G</b> YCSPKCRVMDLKYTS GDCKYSCCIPTAWKWK |
| <i>Cygnus atratus</i> OvoDA1        | QVRK---YCPKV <b>G</b> YCSSKCSKADVWSLSSDCKFYCCLPPGWK--  |
| <i>Columba livia</i> OvoDA1         | QVKKYWTYCPKE <b>G</b> HCSSKCDKMYTWTSSDCKFYCCIPYSWNGK   |
| <i>Ficedula albicol</i> OvoDA1      | -IRK---TCAPV <b>G</b> YCSPKCNVLDLKYTSADCKYSCCI PNPWKWK |
| <i>Geospiza fortis</i> OvoDA1       | -VRK---TCAPV <b>G</b> YCSPKCRVMDLKYTSADCKYSCCI PPSWKWK |
| <i>Geospiza fortis</i> OvoDA1 2     | -VRK---TCAPV <b>G</b> YCSPKCRVMDLKYTSADCKYSCCI PPSWKWK |
| <i>Geospiza fortis</i> OvoDA1 3     | -VRK---TCAPV <b>G</b> YCSPKCRVMDLKYTSADCKYSCCI PPSWKWK |
|                                     | . * * . * : ** . * * . ** ** : * :                     |

**OvoDB C-X3-C-X3-C-X11-C-X4-CC**

|                                       |   |
|---------------------------------------|---|
| <i>Gallus gallus</i> OvoDB1           | KRKGTC <b>K</b> GYCAPTCNKKDEWSFHQSCCKMYCCLLPLKKGK   |
| <i>Meleagris gallopavo</i> OvoDB1     | QHKGTC <b>K</b> GYCASACNKKDEWTFHQSCCKMYCCLPPPCKKGK  |
| <i>Melopsittacus undulatus</i> OvoDB1 | QFKGSCA <b>G</b> YCSHMAKRDEWTFHSCGKMYCCIPPPCKKGK    |
| <i>Columba livia</i> OvoDB1           | QYKGVCP <b>G</b> YCSNMCDKMDEWGFSSRSCRKMYCCIPLPCKKGK |
| <i>Anas platyrhynchos</i> OvoDB1      | QKKGFCA <b>G</b> YCSYSCAKTDEWTFHQTCGKMYCCIPPPCKKGK  |
| <i>Ficedula albicol</i> OvoDB1        | ----FCD <b>G</b> YCAHACGETEWSFSPYCEELHCCIPSPCKKGK   |
| <i>Chrysemys picta bellii</i> OvoDB1  | --KGGCP <b>G</b> RCAKSCDKHEVASKTFDCKYMKCCT-YPKKG-   |
| <i>Pelodiscus sinensis</i> OvoDB1     | ---KGCAG <b>R</b> CDWKCCKYEEYETYTYDCPKSYCCLPLFKWK-  |
| <i>Pelodiscus sinensis</i> OvoDB1 2   | ---KGCAG <b>R</b> CDWKCCKYEEYETYTYDCPKSYCCLPLFKWK-  |
| <i>Caretta caretta</i> OvoDB1         | --EKKCP <b>G</b> RCTLKCGKHERPTLPYNCGKYICCVV-VKVK-   |
| <i>Taeniopygia guttata</i> OvoDB1     | QPKRSCR <b>G</b> HCSRTCCKGEREEHTEDCGGMHCCLTHRKRK-   |
|                                       | * * * * : : * ** *                                  |

**OvoDC C-X3-C-X3-C-X13-C-X3-CC**

*Chrysemys picta bellii* OvoDC1  
*Chrysemys picta bellii* OvoDC1 2  
*Chrysemys picta bellii* OvoDC2

QKKGCPGRCTSKCGKYEYPSKITYPNCKTLCCIASKRK  
QKKGCPGRCTSKCGKYEYPSKITYPNCKTLCCIASKRK  
QKKGCPGRCTSKCGKYEYPSKIYPNCKALCCISSKKGK  
\*\*\*\*\*.\*\*\*\*\*:\*\*\*\*\*:\*\*\*:\*\*

**OvoDD C-X3-C-X3-C-X11-C-X3-CC**

*Alligator mississippiensis* OvoDD1  
*Alligator mississippiensis* OvoDD2

PKEPCGGRCVKKCDKDEVASSTYDCKFLCCTRPKQR  
PKEPCGGRCVKKCDKGEVASSTYDCKLLCCTRPKQR-  
\*\*\*\*\*.\*\*\*\*\*:\*\*\*\*\*

**OvoDE C-X6-C-X3-C-X11-C-X2-CC**

*Anolis carolinensis* OvoDE2 2  
*Anolis carolinensis* OvoDE2 3  
*Anolis carolinensis* OvoDE2  
*Anolis carolinensis* OvoDE1  
*Anolis carolinensis* OvoDE3

KPSCSVDGGRCAKYCEKTEYASYVHNCPLCCFKYK  
KPSCSVDGGRCAKYCEKTEYASYVHNCPLCCFKYK  
KPSCSVDGGRCAKYCEKTEYASYVHNCPLCCFKYK  
KPSCSVDGGRCAKYCEKTEYASYVHDCPLCCFKYK  
KPSCSVDGGRCAKYCEKTEYGYGYDCPLCCFKYK  
\*\*\*\*\*. \* :\*\*\*\*\*

**OvoDF C-X6-C-X4-C-X11-C-X2-CC**

*Anolis carolinensis* OvoDF1  
*Anolis carolinensis* OvoDF1 2  
*Anolis carolinensis* OvoDF2

KGPSCSTYGGSCNPKGCAKGYTSYSTHDCKYCCVSP  
KGPSCSTYGGSCNPKGCAKGYTSYSTHDCKYCCVSP  
KGPSCKEYGGICNPKSCKAGYSSYSTYDCKTCC---  
\*\*\*\*\*.\*\*\* \*\*\*\*\*.\*\*\*\*\*:\*\*\*\*\*:\*\*\* \*\*

## B) Global Alignment

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Anolis carolinensis OvoDF1      -KGP---SCSTYG--GSCNPKGCAKGYTSY--STHDC--KYCCVSP-----
Anolis carolinensis OvoDF3      -KGP---SCSTYG--GSCNPKGCAKGYTSY--STHDC--KYCCVSP-----
Anolis carolinensis OvoDF2      -KGP---SCKEYG--GICNPKSCAKGYSSY--STYDC--KTCC-----
Ficedula albicol OvoDA1         -IRK---TC---APVGYCSPK-CNVLDLKY--TSADC-KYSCCIPNP--WKGK
Taeniopygia guttata OvoDA1       KFRK---TC---APMGYCSPK-CRVMDLKY--TSGDC-KYSCCIPTA--WKGK
Geospiza fortis OvoDA1          -VRK---TC---APVGYCSPK-CRVMDLKY--TSADC-KYSCCIPTS--WKGK
Geospiza fortis OvoDA1 2        -VRK---TC---APVGYCSPK-CRVMDLKY--TSADC-KYSCCIPTS--WKGK
Geospiza fortis OvoDA1 3        -VRK---TC---APVGYCSPK-CRVMDLKY--TSADC-KYSCCIPTS--WKGK
Gallus gallus OvoDA1           LVLK---YC---PKIGYCSNT-CSKTQIWA--TSHGC-KMYCCLPAS--WKWK
Columba livia OvoDA1           QVKKYWTYC---PKEGHCSSK-CDKMYTWT--TSSDC-KFYCCIPYS--WNGK
Cygnus atratus OvoDA1          QVRK---YC---PKVGYCSSK-CSKADVWS--LSSDC-KFYCCLPPG--WK--
Anas platyrhynchos OvoDA1       QVRK---YC---PKVGYCSSK-CSKADVWS--LSSDC-KFYCCLPPG--WKGK
Meleagris gallopavo OvoDA1      QVLK---YC---PKIGYCSSK-CSKAEVWA--YSPDC-KVHCCVPANQKWK--
Meleagris gallopavo OvoDA1 2    QVLK---YC---PKIGYCSSK-CSKAEVWA--YSSDC-KVHCCVPANRKWK--
Ficedula albicol OvoDB1        -----FC---D---GYCAHA-CGETEELS--FSPYCEELHCCIPSP--KKGK
Gallus gallus OvoDB1           KRKG---TC---K---GYCAPT-CNKKDEWS--FHQSCKKMYCCLLPL--KKGK
Meleagris gallopavo OvoDB1      QHKG---TC---K---GYCASA-CNKKDEWT--FHQSCKKMYCCLPPP--KKGK
Columba livia OvoDB1           QYKG---VC---P---GYCSNM-CDKMDEWG--FSRSCRKMYCCIPLP--KKGK
Melopsittacus undulatus OvoDB1  QFKG---SC---A---GYCSHM-CAKRDEWT--FSHSCGKMYCCIPPP--KKGK
Anas platyrhynchos OvoDB1       QKKG---FC---A---GYCSYS-CAKTDEWT--FHQTCGKMYCCIPPP--KKGK
Anolis carolinensis OvoDE3      --KP---SCSVDG--GRCAKY-CEKTEYGY--YGYDC--PLCCFKY---KK--
Anolis carolinensis OvoDE1      --KP---SCSVDG--GRCAKY-CEKTEYAS--YVHDC--PLCCFKY---KK--
Anolis carolinensis OvoDE2      --KP---SCSVDG--GRCAKY-CEKTEYAS--YVHNC--PLCCFKY---KK--
Anolis carolinensis OvoDE2 2    --KP---SCSVDG--GRCAKY-CEKTEYAS--YVHNC--PLCCFKY---KK--
Anolis carolinensis OvoDE2 3    --KP---SCSVDG--GRCAKY-CEKTEYAS--YVHNC--PLCCFKY---KK--
Alligator mississippiensis OvoDD -PKE---PC---G---GRCVKK-CDKDEVAS--STYDC-KFLCCTRP---KKRQ
Alligator mississippiensis OvoDD -PKE---PC---G---GRCVKK-CDKGEVAS--STYDC-KLLCCTRPK--KR--
Chrysemys picta bellii OvoDB1   --KG---GC---P---GRCAKS-CDKHEVAS--KTFDCKYMKCCTYP---KKG-
Taeniopygia guttata OvoDB1      QPKR---SC---R---GHCSRT-CGKGEREE--HTEDCGGMHCCLTH---RKRK
Pelodiscus sinensis OvoDB1      ---K---GC---A---GRCDWK-CGKYEYET--YTYDCPKSYCCLPL---FKWK
Pelodiscus sinensis OvoDB1 2    ---K---GC---A---GRCDWK-CGKYEYET--YTYDCPKSYCCLPL---FKWK
Caretta caretta OvoDB1         --EK---KC---P---GRCTLK-CGKHERPT--LPYNCGKYICCVP----VKVK
Chrysemys picta bellii OvoDC1    -QKK---GC---P---GRCTSK-CGKYEYPSKITYPNC-KTLCCIAS---KRGK
Chrysemys picta bellii OvoDC1 2  -QKK---GC---P---GRCTSK-CGKYEYPSKITYPNC-KTLCCIAS---KRGK
Chrysemys picta bellii OvoDC2    -QKK---GC---P---GRCTSK-CGKYEYPSKIYPNC-KALCCISS---KKGK

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