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Evolutionary Shifts in Three Major Structural Features of the Mitochondrial Genome Among Iguanian Lizards

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Abstract. A phylogenetic tree for major lineages of iguanian lizards is estimated from 1,488 aligned base positions (858 informative) of newly reported mitochondrial DNA sequences representing coding regions for eight tRNAs, ND2, and portions of ND1 and COI. Two well-supported groups are defined, the Acrodonta and the Iguanidae (sensu lato). This phylogenetic hypothesis is used to investigate evolutionary shifts in mitochondrial gene order, origin for light-strand replication, and secondary structure of $tRNA^{Cys}$. These three characters shift together on the branch leading to acrodont lizards. Plate tectonics and the fossil record indicate that these characters changed in the Jurassic. We propose that changes to the secondary structure of $tRNA^{Cys}$ may destroy function of the origin for light-strand replication which, in turn, may facilitate shifts in gene order.

Key words: Reptilia — Sauria — Iguania — Gene organization — Cystein transfer RNA — Mitochondrial DNA — Phylogenetics

Introduction

Structural organization of the vertebrate mitochondrial genome is evolutionarily stable except for three characteristics that are known to vary: gene order, position for the origin of light-strand replication (O_L), and secondary structure of tRNAs (for review see Macey et al. 1997a,b). Both shifts in gene order and the alterations of tRNA secondary structure have been suggested to result from errors in DNA replication (Macey et al. 1997a,b). Iguanian lizards, a group that dates at least to the fragmentation of Gondwanaland (Estes 1983), show variation for these three major structural features of the mitochondrial genome and provide an opportunity to examine the timing and relative phylogenetic positions of shifts in these characters.

Derived states of the three mitochondrial characters have been reported for an agamid lizard (Acrodonta: *Uromastix acanthinurus*) (Macey et al. 1997a,b). This lizard contains a unique gene arrangement in which the $tRNA^{Ile}$ and $tRNA^{Gln}$ genes are switched in order relative to the more typical vertebrate arrangement. The origin for light-strand replication (O_L) is absent in *Uromastix* from the usual vertebrate position between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes, and $tRNA^{Cys}$ lacks a dihydrouridine (D) stem and instead contains a D-arm replacement loop (Macey et al. 1997a,b). These novel features were not observed in an iguanid lizard, *Sceloporus occidentalis* (Kumazawa and Nishida 1995). See Appendix 1 for taxonomy used.

Iguanian lizards comprise eight iguanid (sensu lato) groups (Etheridge and de Queiroz 1988) and four acrodont groups (Frost and Etheridge 1989; Moody 1980). Systematics of iguanian lizards has been highly controversial, and the monophyly of the Iguanidae (sensu lato) with respect to the Acrodonta has been questioned (Frost and Etheridge 1989). With iguanian phylogenetics un-

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certain, the position for the evolutionary shift of the three mitochondrial characters remains unclear.

We assessed the order of genes for one-third of the mitochondrial genome, located between ND1 and COI, and the presence or absence of a recognizable O_L in representatives of all major iguanian lineages. The evolution of these characters and the secondary structural alternatives for $tRNA^{Cys}$ (Macey et al. 1997b) were examined on a phylogenetic estimate derived from 1,488 bases of aligned sequence corresponding to genes for eight tRNAs, ND2, and portions of ND1 and COI.

Methods

Specimen Information. Museum numbers and approximate localities for voucher specimens from which DNA was extracted and GenBank accession numbers are presented in phylogenetic order. Acronyms are CAS for California Academy of Sciences, San Francisco; MVZ for Museum of Vertebrate Zoology, University of California at Berkeley; MZUSP for Museu de Zoologia, University of São Paulo, Brazil; TCWC for the Texas Cooperative Wildlife Collection, Texas A&M University; and USNM for United States National Museum, Washington D.C. Acronyms followed by a dash RM represent field numbers of the first author for uncatalogued specimens being deposited in either the California Academy of Sciences or the Museum of Vertebrate Zoology. **Iguanidae:** *Anolis paternus* Cuba (USNM 498070; U82679); *Basiliscus plumifrons* Costa Rica (MVZ 204068; U82680); *Crotaphytus collaris* Texas, USA (TCWC 72206; U82681); *Gambelia wislizenii* Nevada, USA (MVZ-RM2464; U82682); *Hoplocercus spinosus* Mato Grosso, Brazil (MZUSP 907931; U82683); *Liolaemus tenuis* Chile (MVZ 162076; U82684); *Oplurus cuvieri* Madagascar (CAS-RM10468; U82685); *Phrynosoma douglassi* New Mexico, USA (MVZ 180332; U82686); *Sauromalus obesus* California, USA (MVZ 144194; U82687). **Acrodonta:** *Chamaeleo fischeri* Tanzania (CAS 168965; U82688); *Uromastix acanthinurus* Morocco (MVZ 162567; U71325) (Macey et al. 1997a,b); *Leiolepis belliana* Thailand (MVZ 215497; U82689); *Physignathus cocincinus* Vietnam (MVZ 222159; U82690); *Phrynocephalus raddei* Turkmenistan (CAS 179770; U82691). **Anguidae:** *Elgaria panamintina* California, USA (MVZ-RM1641; U82692).

Laboratory Protocols. Tissue samples were collected and frozen in liquid nitrogen. Genomic DNA was extracted from muscle or liver using the Qiagen QIAamp tissue kit. Amplification of genomic DNA was conducted using a denaturation at 94°C for 35 s, annealing at 45–53°C for 35 s, and extension at 70°C for 150 s with 4 s added to the extension per cycle, for 30 cycles. Amplified products were purified on 2.5% Nusieve GTG agarose gels and reamplified under similar conditions. Reamplified double-stranded products were purified on 2.5% acrylamide gels (Maniatis et al. 1982). Template DNA was eluted from acrylamide passively over 3 days in which Maniatis elution buffer (Maniatis et al. 1982) was replaced each day. Cycle-sequencing reactions were run using the Promega fmol DNA sequencing system with a denaturation at 95°C for 35 s, annealing at 45–53°C for 35 s, and extension at 70°C for 1 min for 30 cycles. Sequencing reactions were run on Long Ranger sequencing gels for 5–12 h at 38–40°C.

Amplifications used primer H5934 and one of the following: L3881, L4160, L4178a, or L4178b. DNA samples from some taxa were amplified also with L3002 and either H4419a or H4419b (Table 1). Both strands were sequenced using primers described in Table 1.

Sequence Alignment and Character Homology. DNA sequences encoding part of ND1, all of ND2, and part of COI were aligned by amino acid using MacClade (Maddison and Maddison 1992). Alignments of sequences encoding tRNAs were constructed manually based on secondary structural models (Kumazawa and Nishida 1993; Macey and Verma 1997). Secondary structures of tRNAs were inferred from primary structures of the corresponding tRNA genes using these models. Unalignable regions were excluded from phylogenetic analyses. See Fig. 1 for alignment.

Most protein-coding regions were alignable (1,062 positions) but some regions were excluded from phylogenetic analyses because of questionable alignment. In ND1, sequences encoding amino acids that extended more than one amino acid beyond the sequence for *Gallus gallus* (Desjardins and Morais 1990) were excluded from analyses as well as stop codons, which sometimes overlap with adjacent tRNAs (positions 82–97). In ND2, a gap was placed in the *Leiolepis* sequence at the position corresponding to amino acid 249 in *Gallus* (positions 1076–1078). Sequences that encoded ND2 beyond amino acid 316 of *Gallus* were unalignable and therefore excluded (positions 1280–1376). In COI, sequences corresponding to the third amino acid of *Gallus* appear only in *Physignathus* and *Phrynocephalus;* therefore, a gap was placed in the other sequences (positions 1809–1811).

Genes for $tRNA^{Ile}$ and $tRNA^{Gln}$ (positions 98–258) are switched in order in acrodont lizards and were aligned to the other sequences at the end of the alignment (positions 1839–1989; Fig. 1). Among tRNA genes, most loop regions were unalignable as were noncoding sequences between genes. The dihydrouridine (D) and TψC (T) loops were excluded from genes for $tRNA^{Ile}$ (positions 1852–1858, 1891–1899), $tRNA^{Met}$ (positions 272–278, 310–316), $tRNA^{Trp}$ (positions

Table 1. Primers used in this study[a]

Human position	Gene	Sequence
L3002	16S	5′-TACGACCTCGATGTTGGATCAGG-3′
L3881	ND1	5′-TTTGACCTAACAGAAGGAGA-3′
L4160	ND1	5′-CGATTCCGATATGACCARCT-3′
L4178a	ND1	5′-CARCTWATACACYTACTATGAAA-3′
L4178b	ND1	5′-CAACTAATACACCTACTATGAAA-3′
L4221	$tRNA^{Ile}$	5′-AAGGATTACTTTGATAGAGT-3′
H4419a	$tRNA^{Met}$	5′-GGTATGAGCCCAATTGCTT-3′
H4419b	$tRNA^{Met}$	5′-GGTATGAGCCCGATAGCTT-3′
L4437a	$tRNA^{Met}$	5′-AAGCTTTCGGGCCCATACC-3′
L4437b	$tRNA^{Met}$	5′-AAGCAGTTGGGCCCATRCC-3′
L4645	ND2	5′-ACAGAAGCCGCAACAAAATA-3′
L4831	ND2	5′-TGACTTCCAGAAGTAATACAAGG-3′
H4980	ND2	5′-ATTTTTCGTAGTTGGGTTTGRTT-3′
L5002	ND2	5′-AACCAAACCCAACTACGAAAAAT-3′
H5540	$tRNA^{Trp}$	5′-TTTAGGGCTTTGAAGGC-3′
L5556	$tRNA^{Trp}$	5′-GCCTTCAAAGCCCTAAA-3′
L5551	$tRNA^{Trp}$	5′-GACCAAAGGCCTTCAAAGCC-3′
H5617a	$tRNA^{Ala}$	5′-AAAATRTCTGRGTTGCATTCAG-3′
H5617b	$tRNA^{Ala}$	5′-AAAGTGTCTGAGTTGCATTCAG-3′
L5638a	$tRNA^{Ala}$	5′-CTGAATGCAACYCAGAYATTTT-3′
L5638b	$tRNA^{Ala}$	5′-CTGAATGCAACTCAGACACTTT-3′
H5692	$tRNA^{Asn}$	5′-TTGGGTGTTTAGCTGTTAA-3′
L5706	$tRNA^{Asn}$	5′-TAGTTAACAGCTAAACAC-3′
H5934	COI	5′-AGRGTGCCAATGTCTTTGTGRTT-3′
H5937	COI	5′-GTGCCAATGTCTTTGTG-3′

[a] Primers are designated by their 3′ ends, which correspond to the position in the human mitochondrial genome (Anderson et al. 1981) by convention. H and L designate heavy-strand and light-strand primers, respectively. Positions with mixed bases are labeled with their standard one-letter codes: R = G or A; Y = T or C; W = A or T. All primers are from Macey et al. (1997a) except L4160 (Kumazawa and Nishida 1993), L4437b (new to this study) and L5551 (Macey et al. 1997b)

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1-100          ND1.........................................................................................STP.......ILE
               1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1.........AA>
Gallus         AACTTCCTCCCCCTAACCCTAGCCTTATGCCTCTGACATACCAGCATACCAATCAGCTACGCCGGCCTCCCCCCAATC---------TAA-------GGA
Elgaria        CAATTCCTACCAATTACCCTCGCCTTATGTCTATGACACATCTCACTTCCAGTTTTCTTATCAGGAACCCCTCCAACAAAC------TAGAC-----GGA
Anolis         CAATTTCTCCCAATTACTTTAGCTTTATGCCTATGACACACAACACTTTTAACGTCCATATCTTTTATTCCTCCACAA---------T---------GAA
Basiliscus     CAATTTTTACCAATCACCCTAGCCCTCTGCCTATGACACGTAGCCCTTCCAATCTCCACATTTGCCCTCCCCCCACAACTA------TAAA------GGA
Crotaphytus    CAATTTTTACCATTAACACTAGCCTTCTGCCTATGACACATTTCTTTACCAATCTCAACGTTCTCTTTACCCCCTCAATCA------TAAAC-----GGA
Gambelia       CAATTCCTACCGTTAACACTAGCCCTATGCCTGTGACACATCTCCATGCCAATTTCTATGTTCTCTTTACCACCCCAGTCG------TAAAA-----GGA
Hoplocercus    CAATTTCTGCCCCTTACCCTAGCCCTATGCCTATGGCACATCTCCATTCCAACCATAACATTTGCCCTGCCACCACTCCCA------TAAC------GGA
Liolaemus      CAATTTTTACCATTAACACTAGCAATGTGTCTATGACACACCTCAATACCAATTTCACTACTAGCCACCCCCCCCATAACA------TA--------GGA
Oplurus        CAATTTCTTCCAATCACATTAGCCCTATGCCTATGGTATACCTCACTCCCAACAATAACCTTCACCCTACCCCCACAACAA------TA--------GGA
Phrynosoma     CAATTTTTACCAATAACACTAGCACTATGCCTATGACACACATCATTACCAATCTCAACATTCGGACTACCCCCACAACAA------TAAT------GGA
Sauromalus     CAATTTCTGCCCCTCACACTAGCCCTATGCCTATGACACATCTCACTCCCAACCTCAACATTCGCACTACCCCCAATTAAC------TAGCTCAAAAGGA
               ND1.........................................................................................STP....GLN-ST
               1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1.........*<<
Gallus Rearranged  AACTTCCTCCCCCTAACCCTAGCCTTATGCCTCTGACATACCAGCATACCAATCAGCTACGCCGGCCTCCCCCCAATC---------TAA-------CTA
Chamaeleo      CAATTTCTACCCCTAACCCTAGCCATATGCCTACTCTACACAACACTGCCGCTAGCACTCACAGCCCTTCC-------------------------ATA
Leiolepis      CAATTCCTACCAATGACTTTAACAATATGCCTACTGTACACTATTGCTCCTACAGCCCTAGCCTCTCTACCACCAAACAC-----------------TTA
Uromastix      CAATTCCTACCCCTGACCTTAGCCATATGCCTAATATACACAAACCTCCCATCAGCCCTAGCTGCTCTACCACCAGACAACCA--------------TTA
Phrynocephalus CAATTCCTACCAATAACCCTAGCATCTTGCTTATTATACACAATAATACCTACATCAATTATAGCTCTCCCCCCAACAACCCTAAA-----------TTA
Physignathus   CAATTCCTTCCAATAACCCTAGCCTCCTGCCTTCTATATACAACTATACCTCTAGCCATAGCTTCACTCCCACCAAACAACCTCGC-----------ATA

101-200        ............................................................................ILE.....GLN.....................
               >>>>..D>>>.......D>>>.AC>>>..COD..AC>>>.....T>>>>.........T>>>>AA>>>>>*.....*<<<<<AA<<<<T.......<<<<
Gallus         AGCGTGCCTGAACAAA-AAGGATCACTATGATAAAGTGAACAT-AGAGGTATAACAACCCTCTCACTTCCTTAATCCTAGAAAAGTAGGAATCGAACCTA
Elgaria        AGTGTGCCTGAACTA--AAGGACTACCTTGATAGAATAGACAACAGAGGTTAAAAT--CCTCTCACTTCC-------TAGAAAGACAGGAATTGAACCTG
Anolis         AATGTGCCTGAAATTT-AAGGATAACTTTGATAGAGTTAACAATAGAGGCTAGAAC--CCTCTCATTTTC-------TAGAAAACCAGGACTCGAACCTG
Basiliscus     AATGTGCCTGAACACA-TAGGGCTACTTTGATAGAGTAGAATATAGGGGTTAAAAT--CCACTCATTTCCC------TAAAAGAACAGGACTCGAACCTG
Crotaphytus    AATGTGCCCGAAATTT-AAGGATTACTTTGATAGAGTAAATCACAGAGGTTTAAAT--CCTCTCATTTCCT------TAGAAGAATGGGACTTGAACCCA
Gambelia       AACGTGCCTGAACTACTAAGGATTACTTTGATAGAGTAAACAATAGAGGTTAAAAT--CCTCTCGCTTCCT------TAGAAGAATGGGACTTGAACCCA
Hoplocercus    AGCGTGCCTGAACTA--AAGGACTACTTTGATAGAGTAAAACATAGCGGTTTAAGC--CCGCTCACTTCC-------TAGAAGAACAGGGCTCGAACCTG
Liolaemus      AATGTGCCCGAGATACTAAGGGATACTTTGATAGAGTATATTACAGAGACTAAAC---CCTCTCATTTCCT------TAGAAGGACAGGACTCGAACCCG
Oplurus        AATGTGCCTGAGTATTTAAGGGTTACTTTGATAGAGTAAACCACAGGGACTAAAC---CCCCTCATTTCCT------TAGGAAGACAGGATTCGAACCTG
Phrynosoma     AGCGTGCCCGAATTTC-AAGGATTACTTTGATAGAGTAAACAACAGAGGTTAAAAT--CCTCTCACTTCC-------TAAAAGAACAGGACTTGAACCTG
Sauromalus     AATGTGCCCGAGCATC-AAGGATTACTTTGATAGAGTAAATTACAGAGGTTTAAAT--CCTCTCATTTCC-------TAGAAGAACAGGACTTGAACCTG
               P.........................................................................GLN.............ILE.............
               <<<AA<<<<T.......<<<<T....<<<AC..COD..<<<AC.<<<D.........<<<D..<<<<<AA..............AA>>>>>..D>>>....
Gallus Rearranged  GAAAAGTAGGAATCGAACCTACACAGAAGAGATCAAAACTCTTCATACTCCCTC--TATATTATTTTCTA.............GGAAGCGTGCCTGAACA
Chamaeleo      GGGGGGAAGGGATTGAACCAACATCAAAAAACTCAAAATTTCACGCATTCCAT---TATACTACCCCCTA-TAATACCTTC--GGAAGTGTGCCCGAGAA
Leiolepis      GAAGAATGGGACTCGAACCCACAATAAAAAATCCAAAATCTTTTGTACTCCCAA-CTATACTATCTCCTACCTACAAGTCTATGGAGACGTGCCCGAGAA
Uromastix      GCAGGGAAGGAATCGAACCAACACCGAAAAACCCAAAATATTTCATACCTCCA--CTATACTACCTGCTA--TACAA------GGAAGCGTGCCTGATTA
Phrynocephalus GAGAAAGAGGACTCGAACCTCCATAAAAAAATCCAAAATTTTTTGCACTACCAC-CTATGCTACTCTCCA---CTTGAAC---AGAAGTGTGCCCGAGAT
Physignathus   GAGAAGAAGGACTCGAACCAACACCTAAAAACCCAAAATTTTTAGTACTCCCAC-CTATACTACTCTCTA----CAA------GGAAGCGTGCCCGAAAC

201-300        ..............................................GLN.........MET.......................................
               T....<<<AC..COD..<<<AC.<<<D.........<<<D..<<<<<AA---------AA>>>>>..D>>>.......D>>>.AC>>>..COD..AC>>>
Gallus         CACAGAAGAGATCAAAACTCTTCATACTCCCTC--TATATTATTTTCTA----------GTAAGGTCAGCTAACT--AAGCTATCGGGCCCATACCCCGA
Elgaria        CACCAAAAAACTCAAAATCTTCTGTACTCCCAT--TATACTACTTCCTA----------GTAAAGTCAGCTAAAT--AAGCTTTCGGGCCCATACCCCGA
Anolis         TACCTAAAAACTCAAAATCATTTGTACTTCTT---TATACTATTTTCTA----------GTAAAGTCAGCTAAAA--AAGCTATTGGGCCCATACCCCAA
Basiliscus     CACCAAAAGACTCAAAATCTTTAGTACTCCAC---TATACTATCTTCTA----------GTAAAGTCAGCTAAAA--AAGCTATCGGGCCCATACCCCGA
Crotaphytus    CACCAAAAGACTCAAAATCTAAGGCACTTCAT---TATGCTATCCTCTA----------GTAAAGTCAGCTAACT--AAGCTATCGGGCCCATACCCCGA
Gambelia       CACCAAGAGACTCAAAATCTCAAGCACTCCAT---TATGCTACCCTCTA----------GTAAAGTCAGCTAATT--AAGCTATCGGGCCCATACCCCGA
Hoplocercus    CACCAAAAGACTCAAAATCCTTTGCACTCCAT---TATGCAACCTCCTA----------GTAAAGTCAGCTAAAA--AAGCTATTGGGCCCATACCCCAA
Liolaemus      CACCTAGAAGCTCAAAACTTCCAGCACTCCAT---TATACTACCATCTA----------GTAAAGTCAGCTAAAC--AAGCTATCGGGCCCATACCCCGA
Oplurus        CACTTAGAAACTCAAAATTACTTGTACTCCATT--ACTACAACCTCCTA----------GTAAAGTCAGCTAAAC--AAGCTATCGGGCCCATACCCCGA
Phrynosoma     CACACAAAGACTCAAAATCTTCAGTACTACAT---TATACTATCTTCTA----------GTAAAGTCAGCTAATT--AAGCTATCGGGCCCATACCCCGA
Sauromalus     CACCTAAAGACTCAAAATCTCCAGCACTTCAT---TATACTATCTTCTA----------GTAAAGTCAGCTAACCC-AAGCTATCGGGCCCATACCCCGA
               ......................................................ILE..MET.......................................
               ...D>>>.AC>>>..COD..AC>>>....T>>>>.........T>>>>AA>>>>>*..AA>>>>>..D>>>.......D>>>.AC>>>..COD..AC>>>
Gallus Rearranged  AA-AAGGATCACTATGATAAAGTGAACATAGAGGTATAACAACCCTCTCACTTCCT---GTAAGGTCAGCTAACT--AAGCTATCGGGCCCATACCCCGA
Chamaeleo      CT-AGGGGCTACTTTGATAGAGTAGAAACAGAGACAGAAA---ACTCTCCCTTCC---ACTAGAATCTGCTACATT-AAGCAATTGGGCCCATGCCCCAA
Leiolepis      TC-AGGGACTATTTTGATAAAATAGACACAGAGACCAACC---CCTCTCGTCTCCCA-ATTAGGATCTGCTACGG--AAGCAGTTGGGCCCATACCCCAA
Uromastix      ---AAGGGCTGTCTTGATAAGACAAACATAGAGGGCCAACAT-CCTCTCGATTCCCCCATTAGGGTCTGCTACGTCTAAGCAATTGGGTTCATGCCCCAA
Phrynocephalus ---AGGGGTTATTTTGATGAAATAAACACAGAGTAACATT---ACTCTCACTTCCA--ATTAGGATCTGCTACAT--AAGCAGTTGGGCCCATGCCCCAA
Physignathus   CACAAGGACTACTTTGATAAAGTAAACATGGAGCTCACAAT--ACTCCCACTTCCC--GCTAGGGTATGCTACATTTAAGCAATTGGGCTCATGCCCCAA

301-400        .........................MET..ND2...................................................................
               ....T>>>>.......T>>>>AA>>>>>*..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..
Gallus         AAATGATGGTTTAACCCCTTCCCCTACTA--ATGAACCCCCATGCAAAACTAATCTGCACAGTAAGCCTCATCATGGGAACCAGCATCACAATCTCCAGC
Elgaria        AAATGTTGGTTTAAACCCCTCCTTTATCA--ATGAGCCCAATAATTACTTCAATCCTAATCCTCAACCTAGCAACAGGAACTATTATTACCATATCAAGC
Anolis         AAATGTTGGTTTAAACCCTTCCTATACTA--ATGAGCCCATTTATTCTTACAATTATTTTTTCAAGCTTAGCAACAGGCACAATTATTACTATAACCAGC
Basiliscus     AAATGTTGGTTTAATCCCTTCCTATACTA--ATGAGCCCCATCACAACAACAATTATTATTACAAGCTTAGCAACTGGCACAGTCATTACATCCACCAGC
Crotaphytus    AAATGTTGGTTTAAATCCCTCCTGTACTA--ATGAGCCCCACCACATTAATAATTCTAATTTCAAGCCTAGCAACCGGCACCATTATCACATTATCGAGC
Gambelia       AAATGTTGGTTTAAACCCCTCCTCTACTA--ATGAGCCCAACCGCAACTATAATCCTAATTTCAAGCTTAGCAACAGGCACCATTATTACTACATCAAGC
Hoplocercus    AAATGCTGGTGCAACTCCTTCCTTTACTA--ATGAGCCCACTAACAACCTCAATTGTCCTCTCCAGCTTAGCCACCGGCACTATCATCACAATATCCAGC
Liolaemus      AAATGTTGGTTCAACTCCTTCCTATACTA--ATGAGCCCAACAACCACAACAATCTTAATCTCAACCCTAACCATAGGAACAATTATTACAGCATCAAGC
Oplurus        AAATGTTGGTTTAAATCCCTCCTCTACTA--ATGAGCCCAATAACAACTACACTACTAATCTCAAGCCTTGCTACAGGAACAATCATCACAGCATCAAGT
Phrynosoma     AAATGTTGGTTTAAACCCCTCCTCTACTA--ATGAGCCCTATAACAAAAATAATCCTAATCTCAAGCCTTGCTACCGGCACAATCATCACAGCCTCTAGC
Sauromalus     AAATGTTGGTTTAAATCCTTCCTCTACTA--ATGAGCCCTACCACAACATCCCTACTTCTCTCAAGCCTTGCAATAGGCACCATCATCACCGCATCCAGC
Chamaeleo      AAACGATACCTCA---ATATCTTCTAGCA--TTGAACCTACTAACCCAACTAATCGCCTCATTTAACCTCATCCTTGGAACTACCATTACCACCTCAAGC
Leiolepis      AAACGGTGATT-----TCACCTCCTAATATCTTGCCCCCATTAATACTCCCAATTTTAATCCTCGGCGTCACCATAGGCACCATCTTCGCTGCATCCAGT
Uromastix      AAACGGTGCCA-----CCACCCCCTAATA--TTGCCACCATCCACCACCCTAATCTTCTACACAAGCCTCGTATCAGGCACACTAATTGTCATATCAAGC
Phrynocephalus AAACGGTGAC------ACACCTCCTAATAC-ATTTCCCCCACATCTATCGTAATCATTACCATAAGTATTACCATAAGCACTATTATAGTTATATCAAGC
Physignathus   AAACGGTGAAA-----ACACCCCCTACCAA-ATTTCAGCCCTTACAACCCTAGCCCTTACAACCAGCATCACCACCAGCATACTAATCACAGCCTCAGCA
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Fig. 1. Aligned DNA sequences for the length-variable regions as used in the phylogenetic analyses and showing major shifts in genomic structure. Positions 401–1299 from the ND2 gene are not shown, and this region had no length variation except for a gap in *Leiolepis* for positions 1076–1078 corresponding to amino acid 249 in *Gallus.* Sequences are presented as light-strand sequence and tRNA secondary structure is designated above the sequence. Stems are indicated by *arrows* in the direction encoded: AA = amino-acid-acceptor stem, D = dihydrouridine stem, AC = anticodon stem, T = TψC stem. The tRNA anticodons are designated *COD. Asterisks* indicate the unpaired 3′ tRNA position 73. *Periods* represent bases located outside stem regions; *1* depicts the first codon position of protein-coding sequences. *STP* represents stop codons. In acrodonts, *GLN-STP* denotes the stop codon (TAG) of ND1 which overlaps the first three bases of the AA-stem in the $tRNA^{Gln}$ gene. The stems of the origin for light-strand replication are *underlined,* but note that the stems in acrodonts are atypical and presumed nonfunctional. Two secondary structural models of $tRNA^{Cys}$ are shown for acrodonts from Macey et al. (1997b). Note that from ND1 to $tRNA^{Met}$ the acrodonts are aligned to *Gallus,* which is shown in the derived acrodont gene order rather than its true order (*Gallus* rearranged). The rearranged $tRNA^{Gln}$ and $tRNA^{Ile}$ genes of acrodonts are aligned to the tRNA genes of taxa having the typical vertebrate gene order at the end of the sequence as used in the phylogenetic analysis (positions 1839–1989).

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1301-1400
                 ND2.......................................................STP.TRP.....................
                 1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1..1...AA>>>>>..D>>>...........
Gallus           CCCACCGGCATTCTAACTGCGCTATCAACCACCCTATTGCCCCTTTCC---CCCCTAATTATTACCATACTAT---AGAAACTTAGGATTAACTGTCACC
Elgaria          TTACTACTCTCAACAACCCTCCCAATATCCATCATAGGACTACCTCTCGTACCACTAATAAAACCA------TAACAGAAATTTAGGTTACATA------
Anolis           ACCTTAACCTCTTCCACCACAACAATAACAATCTTCCTCCTACCACTTACACCCATAATTTTATTA------T---AAAAACTTAGGATCAAATA-----
Basiliscus       ACCCCTCTAACCCTGGCCCTCCCCCTATCACTCTTTATACTCCCAATTACCCCACTAATCTTGTCA------T---AAAAACTTAGGATAACTTA-----
Crotaphytus      CTACTGACCATCACACTTCCAGCCTCAACTATAATT---CTACCACTAACCCCAATAATACTGCTC------T---AGAAACTTAGGATTAAACCTA---
Gambelia         TTAATTACCATAACCCTCCCAGCCTCTACTATAATT---CTCCCACTAACCCCAATAATTTTACTC------T---AGAAACTTAGGATTAAACA-----
Hoplocercus      TTACTACTAACAACCTCATCCTCATTAATAACAATAGTACTACCACTTACCCCAATCCTATTATAC------TA--AGGAACTTAGGATAAACACA----
Liolaemus        CTCCTACTTACAACTTCATTACCAGCCACCATCCTATTCCTACCAATAACCCCCCTAATCTTAGCC------TA--AAGAGTTTAGGATAATTTACA---
Oplurus          CTATTACTGAGCATAACTGCCCCAATAACCATCTTAATACTACCAATCTCCCCACTAATCCTACAA------TAACAGAAACTTAGGTTAACTCA-----
Phrynosoma       CTTATACTCTCGCTAACTCTACCAGCATCTCTACTATTACTCCCAATCACCCCAATAATATGC---------T---AGAAACTTAGGATAACTTA-----
Sauromalus       ACCCTACTAACCACCACCCTACCCCTCACACTAATAATACTACCCATAACCCCCATAACACTTCTA------TAATAGAAGCTTAGGATAAACCA-----
Chamaeleo        CTAATAACAACGCTCATCCCACTGTCCCTTCTAACCCTTCCACTACTACTCCCCACCCTAATATCTATGACCT---AGAAGCTTAGGATACATCA-----
Leiolepis        CTTCCATTAGCAATCACCATAACAATAACCATAATAAGCCTCCCTATAACCCCTACATTTATCCCA------T---AAGGATTTAGGATAACATCA----
Uromastix        GCCACAATCGCCACACTGACCACAACCGCCCTATTGATGACCCCAACAGCCCCAATAATTACAATT------TA--CGAAGCTTAGGATTATCACCA---
Phrynocephalus   TTAGCAACAATAATAGCTCCAGTAGCACTATTCATTGCCCACCTAATTCCAGCAATCCCATTC---------AG--AGAAGCTTAGGATTAAATCTTA--
Physignathus     CTCACAACAATCCTAGCCCCAATAGCCCTATTTAACATGACAATAATACCCGCTTTAATTTCA---------T---AGAGACTTAGGATAGCTCA-----

1401-1500        ...................................................TRP..........ALA.................................
                 .D>>>.AC>>>..COD..AC>>>.....T>>>>.......T>>>>AA>>>>>*..........*<<<<<AA<<<<T.......<<<<T....<<<AC..C
Gallus           AAACCAAAGGCCTTCAAAGCCTTAAAT-AAGAGTTAAACTCTCTTAGTTTCTGCCCAAC----TAAGACCAACAGGACATTAACCTGTATCTCCTGAATG
Elgaria          -AACCAAGGGCCTTCAAAGCCCTAAAC-AAGGAACAC---TCCTTAATTTCTGA---------TAAGATCTGTAAGAATTTAACCTACATCTTCTGACTG
Anolis           -AACCAAGGGCCTTCAAAGCCCAAAAT-ATGGGTTAAACACCCATAGTTTTTG----------TAAGACTTGTGAAACACTATTACACATCTCCTGAATG
Basiliscus       -AACCATGGGCCTTCAAAGCCCAAAAC-AGGGATGAAACCACCCTAGTTTTTGCAC-------TAAAACCTGCGAAATATTATTACGCATCTTCTGAATG
Crotaphytus      -AACCAAGGGCCTTCAAATCCCAAAAC-AGGGGTGAAAACCCCCTAGTTTCTGCT--------TAAAACCTGTGGAACACTAAACCACATCTTCTGAATG
Gambelia         -AACCAAGGGCCTTCAAATCCCAAAAC-AGGAGTGAAAACCCCCTAGTTTCTGAAT-------TAAAACCTGTGGAACACTAAACCACATCTTCTGAATG
Hoplocercus      -AACCAAGAGCCTTCAAAGCTCAAAAT-ACGGGCGCAACCCCCGTAGTCCCTGC---------TAAAACCTGTGAAATACTA-AACACATCTCCTAAATG
Liolaemus        -AACCATAGACCTTCAAAGTCTAAAAA-AAAGGTGCAAACCCTCTAACTCTTGA---------TAAGACCTGTGTAACTCTATAACACATCTCCTGAATG
Oplurus          -AACCAAGGGCCTTCAAAGCCCTAAACGAAGCCAAGC---CCCTTAGTTCCTGC---------CAAGACCTGCGAATTACTATAACGCATCAACTGAATG
Phrynosoma       -AACCAAGGACCTTCAAAGCCCTAAAT-AGGGAGTAAACCCCCCTAGTTTCTGC---------TAAGACCTGCGAAAGACTAAAACGCATCTCCTGAATG
Sauromalus       -AACCAAGGACCTTCAAAGTCCAAAAT-AGGAGTGAAACCCCCCTAGCTTCTGC---------CAAGACCTGCGAAACATTAAAACGCATCTTCTGAATG
Chamaeleo        -AACCAGAAGCCTTCAAAGCTTCTAAA-AGGAGTATAAGCCTCCTAGCTTCTGATACCCCAAGGAAAGCCTGTTGAAACTA--TCAACATCTCCTGAATG
Leiolepis        -AACCAAGGGCCTTCAAAGTCCTAAAT-AAGAGACTGAC-CTCTTAATCCTTGACC-------TAAAACCTATAGAACTTTAATCTATATCATCCAAATG
Uromastix        -AACCGAGGGCCTTCAAAGCCCTAAAA-AAGAGTGCCAACCTCTTAGCTTCTGCTCAA-----TAAAACCTGTGAAACTCTA-ATCACATCTTCTGGATG
Phrynocephalus   -AACCAGTGGCCTTCAACACCACAAAT-AAGGGCGAA---CCCTTAGCTTCTG-----------AAGACCCATAGGACTTTAACCTACATCACCTGTATG
Physignathus     -AACCGAGGGCCTTCAAAGCCCTAAAC-AAGAGACCACC-CTCTTAGCCTCTGCA--------TAAAACCTATAGAACTTA--TCTACATCTTCTGAATG

1501-1600        ...............................ALA........ASN.......................................................
                 OD..<<<AC.<<<D......<<<D..<<<<<AA........*<<<<<AA<<<<T.......<<<<T.......<<<AC..COD..<<<AC.<<<D.....
Gallus           CAAATCAGACGCTTTAATT-AAGCTAAGGCCTCTAC-----CTAGACAGATGGGCTTCGATCCCATACAAT--TTTAGTAAACAGCTAAATGCCAACA-C
Elgaria          CAACCCAGACACTTTAATT-AAGCTAAAACCTCA-------CTAGACAGACGGGCCTCGATCCCGTAAACA--GTTAATTAACAGCTAATCACTCAAT-C
Anolis           CAACTCAAGCACTTTTATT-AAGCTAAAGCCTCA-------CTGAATAGGCGGGCTTTGATCCCACGACAA--ATTAATTAACAGCTAATTACCCAAA-C
Basiliscus       CAACTCAGACACTTTAATT-AAGCTAAGGCCTCA-------CTGGACAGGAGGGCCTTGATCCCACAATTAAACCTAGTTAACAGCTAGGCACCCTAG-C
Crotaphytus      CAAATCAGACACTTTAATT-AAGCTAAGGCCTCT-------CTAGATAGGAGGGCCTTGATCCCACAAAAA--TTTAGTTAACAGCTAAACACCCAAT-C
Gambelia         CAACTCAGACACTTTAATT-AAGCTAAGGCCTCC-------CTAGACAGGAGGGCCTCGATCCCACAAAAA--CTTAGTTAACAGCTAAGCACCCAAT-C
Hoplocercus      CAACTCAGGCACTTTAATT-AAGCTAAGGCCTC--------CTGGACAGGTGGGCCTCGATCCCACAAAA---ATTAGTTAACAGCTAAACACCCAAC-C
Liolaemus        CAACTCAGACACTTTAATT-AAGCTAAGGCCTCC-------CTAGATAGGCGGGCCTTGATCCCACAATAA--CTTAGTTAACAGCTAAACACCCAAA-C
Oplurus          CAACCCAGACACTTTAATT-AAGCTAAGGCCTCC-------CTAGATAGGAGGGCCTTGATCCCACGAACA--ATTAGTTAACAGCTAAACACCCAAA-C
Phrynosoma       CAAACCAGACACTTTAATT-AAGCTAAGGCCTCAA------CTAGATAGGCGGGCCTTGATCCCACGAAAA--CTTAGTTAACAGCTAAACACCCAAA-C
Sauromalus       CAACTCAGACACTTTAATT-AAGCTAAGGCCTCC-------CTAGACAGGCGGGCCTCGATCCCACAAAAA--ATTAGTTAACAGCTAAACGCCCCAG-C
Chamaeleo        CAACCCAGACACTTTAATT-AAGCTAAGACCTCACCACATACAGGACCAGCGGGCCTCGATCCCACAAAA---ACTAGTTAACAGCTAGCCGCCCAAA-C
Leiolepis        CAACCCGGACACTTTCATT-AAGCTAAGATTTTA-------CAAGACCGGTGGGCCTCGATCCCACAAAA---ACTAGTTAACAGCTAGCCGCTCAAA-C
Uromastix        CAACCCAAATGTTTTAATTAAAACTAAGGCCTTGCA-----CAGGGCTGGTGGGCTTCGACCCCACAACAC--ACCGGTTAACAGCCGATCGCCCAAA-C
Phrynocephalus   CAACTCAGACATTTTAATT-AAAATAAGGCCTA----------AAACCAGCGGGCCTTGATCCCGCAAAAT--AATAGTTAACAGCTATTCACCTAAAAC
Physignathus     CAACCCAGACATTTTAATT-AAACTAAGGCTTC---------GGAGTTAGAAGGCCTTGATCCTTCACAAC--ACTAGTTAACAGCTAGTTCACCCAC-C

1601-1700        ..............ASNOL...........................OLCYS.....................................................
                 ....<<<D..<<<<<AA..............................*<<<<<AA<<<<T........<<<<T.....<<<AC..COD..<<<AC..COD..<<<AC.<<<
Gallus           CAATTGGCTTCTGCCTAC-----------------------------AGACCCCGGCACACTTTAG--TGTACATCA-ACGAGTTTGCAACTCATTATG
Elgaria          CAGCGAGCTTCTATCTA-CTTCTCCCGTTAGAGAAAA-AACGGGAG--AAGTCCCGGAGATCTTAAC--GTCTCTTTT-CCAGGTTTGCAGCCTGATGTA
Anolis           CAGCGGGCTTCTATTCG-CTTCTCCCGTCGGTGAAGAA-ACGGGAG--AAGCCCCGGAGCCTTTAG---GGCTCTTCT-TCAAACTTGCATTTTGATGTG
Basiliscus       CAGCGGGCTTCTGTCCG-CTTCTCCCGTTGTAGAAGG--ACGGGAG--AAGCCCCGGAGCCTCTTAG--GGCTCTTCT-TCAAACTTGCAATTTGACATG
Crotaphytus      CAGCGGGCTTCTATCCG-CTTCTCCCGTCGAGAAAAA--ACGGGAG--AAGCCCCGGAACCCTTTAG--GGTTCTTCT-TAAAATTTGCAATTTTACGTG
Gambelia         CAGCGGGCTTCTATCCG-CTTCTCCCGTTAAGAAAA--AACGGGAG--AAGCCCCGGAACCCTTTAG--GGTTCTTCT-TAAAATTTGCAATTTTATGTG
Hoplocercus      CAGCGGGCTTCTATCCTGCTTCTCCCGTTAAGAAAA--AACGGGAG--AAGCCCCGGGACCTCTTAAG-GGTCCTTCT-TCAAACTTGCATTTTGACGTG
Liolaemus        CAGCGGGCTTCTATCCG-CTTCTCCCGTTGGTAAA---AACGGGAG--AAGCCCCGGAGCCTTTTGG--GGCTCGTCT-TCAAACTTGCATTTTGACGTG
Oplurus          CAGCGGGCTTCTATCCG-CTTCTCCCGTTAGTTAAAA-AACGGGAG--AAGCCCCGGAGCCTATTAG--GGCTCGTCC-TCGAATTTGCATTTCGATGTG
Phrynosoma       CAGCGGGCTTCTATCTG-CTTCTCCCGTGTTTTAAA---ACGGGAG--AAGCCCCGGACCCGTTT----GGGTCCTTCTTCAAATTTGCATTTTGACGTG
Sauromalus       CAGCGGGCTTCTGTCCG-CTTCTCCCGTCTTTTATTG--ACGGGAG--AAGCCCCGGAGCCTTTAG---GGCTCTTCT-TCAAACTTGCATTTTGACGTG
                 ..............ASNOL...........................OLCYS.....................................................
                 ...............................................*<<<<<<AA<<<<T......<<<<T.....<<<AC..COD..<<<AC.<<<
                 ....<<<D..<<<<<AA..............................*<<<<<AA<<<<T........<<<<T.....<<<AC..COD..<<<AC.<<<
Chamaeleo        CAGCGAGCATCAATCCA----GTCTCCCTTTAAAAAAA--AGGAGACT-AAGTCCAGGGTCTTCTA----CGACCTAAT-CCGGATTTGCAGTCTGGGCTG
Leiolepis        CAGCGAGCATCAACCTAACCCGCCCCGTCAAATAAAA--ACGGGGCGAAAAACCTCAGAGCCTTATG-AGCTCTTAT--TCGAATTTGCAATTCGAAACC
Uromastix        CAACAGGCATCAGCCCA-----------------------------GAAACCCACAAACATACTAA-GTTATTTCT-CCGAATTTGCACTCCGGAACA
Phrynocephalus   CAGCAGGCATCGGTTTATTAAATTTGCCCCCAACACAATCCT------TAGACCTACGAAAATCTA--CTTTCAAAA--CTAAACTTGCAATTTAGCATT
Physignathus     AATAGGTA-CTAACCCCACCCAAC-----------------------TAGACCAAAGAGCAGTAA--TACCCATCA--TCTGATTTGCACTCAGAATTT
```

Fig. 1. Continued.

1390–1401, 1434–1440), and tRNA^{Tyr} (positions 1782–1788, 1743–1749). In addition, the variable loop of the tRNA^{Tyr} gene was not alignable (positions 1755–1759). The D-loop was excluded from the tRNA^{Gln} gene (positions 1969–1976) and the T-loop from the tRNA^{Ala} (positions 1477–1483) and tRNA^{Asn} genes (positions 1567–1573). Noncoding sequences between the tRNA^{Ile} and tRNA^{Gln} genes (positions 1913–1917), the tRNA^{Trp} and tRNA^{Ala} genes (positions 1454–1463), the tRNA^{Ala} and tRNA^{Asn} genes (positions 1534–1541), and the tRNA^{Cys} and tRNA^{Tyr} genes (positions 1718–1729) were not used.

Two tRNAs, tRNA^{Asn} and tRNA^{Cys}, show unusual secondary structures in some taxa which made assessment of homologous sites questionable. In *Physignathus*, the amino-acid-acceptor (AA)-stem and

```
1701-1800
                  ..............CYS............TYR.....................................................................TY
                  D...<<<D..<<<<<AA............*<<<<<AA<<<<T.......<<<<T.....<<<AC..COD..<<<AC.<<<D.......<<<D..<<<<<A
Gallus            AACTTCACTACAGAGTC-------------GATAAGAAGAGGAATTGAACCTCTG-TAAAAAGGACTACAGCCTAACGCTTCAACACTCAGCCATCTTAC
Elgaria           T---TTACCTCGGGACC-------------TGATAAGAAAAGGAATTTAACCTCTA-TTATTAGGACTACAGCCTACCACCTGAACATTCGGCCATCTTAC
Anolis            GA--ACACTTCGGGACTT-----------TGATAAAGAAAGGAATTAAACCAATG-TAAATAGGACTACAGCCTACTGCCTAACA-CTCGGCTACTTTAC
Basiliscus        CTC-ACACCCCAGGGCTG-----------TGATAAAAAAAGGAATTTAACCCCTC-TGAGTAAGACTACAGCCTACCGCCTAAACACTCGGCCATTTTAC
Crotaphytus       ATC-ACACCCCGAGGCTT-----------TGGTAAAAATAGGAATTAAACCCATC-TAAATAGTTCTACAGACTACCACCTATACACTCGGCCATTTTAC
Gambelia          ATT-ACACCCCAAGGCTTA----------TGGAAAAAAGAGGAGTTGAACCCCTG-TGAGTAATTCTACAGACTACCGCCTAACA-CTCGGCCATTTTAC
Hoplocercus       AA--ACACCCCGGAGCTGTGTTG------TGGTAAAAGGGACATCT--AACCCCC-TAAGCAGATTTACAGCCTGCCACCTCT---CTCGGCCATTTTAC
Liolaemus         TA--ACACCTCGGGACTTAAATATAGCAATGGTAAAAAGAGGAATTAAACCCCTG-TTGATGGGACTACAGCCCACCACCTAAACATTCGGTCATTTTAC
Oplurus           ATA-ACACCCCGAGACTTA----------TGGTAAAAAAAGGAATTAAACCTCTC-TCAATAGGACTACAGCCTACCGCTTAAACACTCAGCCATTTTAC
Phrynosoma        AA--ACACCCCAAGGCTG-----------TGATAAAAAGAGGGGTTAAACCTCTA-TAAATAGGACTACAGCCTACCGCCTAAACGCTCGGCCATTTTAC
Sauromalus        TC--ACACCCCAGGGCTA-----------TGGTAAAAAAAGGAATTAAACCTTCC-TGAATAGGACTACAGCCTACCACCTAAACGCTCGGCCATTTTAC
                  ..............CYS............TYR.....................................................................TY
                  D..<<<D..<<<<<<AA.............................................................................
                  D...<<<D..<<<<<AA............*<<<<<AA<<<<T.......<<<<T.....<<<AC..COD..<<<AC.<<<D.......<<<D..<<<<<A
Chamaeleo         T---------CTGGGCTGTCTC-------TGGAACGGGGGGGAATCCAACCCCCATTA-ATAGATTTACAGTCTACCGCCTAACA-CTCGGCCGCCAAAC
Leiolepis         TT-------TGAGGCCT-------------GATAAGAGAGGGAGTTGAACCCCCATAAGGTGAACTTACAATTCATCGCCTACCACTTCAGCCTTCTTAC
Uromastix         CA--------TGGGTCT-------------GGAAAGGAGAGGACTT-AACCTCCATAA-GCAGAATTACAATCCGCCACCTAACA-CTCGGCCACCTTAC
Phrynocephalus    TCT------TTAGGCCT-------------GATAAGAGGAGCCTCA-CCACTCCATAA-ATGGAATTACAAACCACCGCCTAAAT--TCAGCCATCTTAC
Physignathus      TAT------TTTGGCCT-------------GAAAAGAGGGGCCATC---GCCCCATAA-ATGGAGTTACAACCCACCGCTTTAA--CTCAGCCATCTCTA

1801-1900         R.COI..........................COI...ILE.....................................................................
                  A.1..1..1..1..1..1..1..1..1..1.....AA>>>>>..D>>>.......D>>>.AC>>>..COD..AC>>>.....T>>>>.........T
Gallus            CTGTGACCTTCATCAACCGATGATTATTCTCAACC---GGAAGCGTGCCTGAACAAA-AAGGATCACTATGATAAAGTGAACAT-AGAGGTATAACAACC
Elgaria           CTGTGACC---ACAACTCGCTGATTATTCTCAACC---GGAAGTGTGCCTGAACTA--AAGGACTACCTTGATAGAATAGACAACAGAGGTTAAAAT--C
Anolis            CTGTGTTT---ATTAACCGTTGATTCTTCTCAACC---GAAAATGTGCCTGAAATTT-AAGGATAACTTTGATAGAGTTAACAATAGAGGCTAGAAC--C
Basiliscus        CTGTGTCC---ATCAACCGTTGACTCTTCTCAACT---GGAAATGTGCCTGAACACA-TAGGGCTACTTTGATAGAGTAGAATATAGGGGTTAAAAT--C
Crotaphytus       CTGTGTCC---ATTACCCGTTGATTCTTCTCAACC---GGAAATGTGCCCGAAATTT-AAGGATTACTTTGATAGAGTAAATCACAGAGGTTTAAAT--C
Gambelia          CAGTGTCT---ATCACCCGTTGATTCTTCTCAACA---GGAAACGTGCCTGAACTACTAAGGATTACTTTGATAGAGTAAACAATAGAGGTTAAAAT--C
Hoplocercus       CCGTGTTT---ATTTCCCGTTGACTATTCTCAACC---GGAAGCGTGCCTGAACTA--AAGGACTACTTTGATAGAGTAAAACATAGCGGTTTAAGC--C
Liolaemus         CCGTGTCT---ATTAATCGTTGATTCTTCTCAACT---GGAAATGTGCCCGAGATACTAAGGGATACTTTGATAGAGTATATTACAGAGACTAAAC---C
Oplurus           CTGTGTTT---ATTAATCGTTGATTTTTCTCAACA---GGAAATGTGCCTGAGTATTTAAGGGTTACTTTGATAGAGTAAACCACAGGGACTAAAC---C
Phrynosoma        CTGTGTTT---ATTAACCGTTGATTCTTCTCAACC---GGAAGCGTGCCCGAATTTC-AAGGATTACTTTGATAGAGTAAACAACAGAGGTTAAAAT--C
Sauromalus        CTGTGTCA---ATCACCCGTTGATTCTTCTCAACC---GGAAATGTGCCCGAGCATC-AAGGATTACTTTGATAGAGTAAATTACAGAGGTTTAAAT--C
Chamaeleo         C-ATGTGA---CTACTTCGCTGACTTTTATCAACA---GGAAGTGTGCCCGAGAACT-AGGGGCTACTTTGATAGAGTAGAAACAGAGACAGAAA----A
Leiolepis         C-ATGTCT---ATTCGTCGATGATTGTTTTCAACA---GGAGACGTGCCCGAGAATC-AGGGACTATTTTGATAAAATAGACACAGAGACCAACC----C
Uromastix         C-ATGATA---GCCCATCGATGACTCCTATCAACC---GGAAGCGTGCCTGATTA---AAGGGCTGTCTTGATAAGACAAACATAGAGGGCCAACAT--C
Phrynocephalus    C-ATGTCAACACTAACTCACTGATTCTTATCAACT---AGAAGTGTGCCCGAGAT---AGGGGTTATTTTGATGAAATAAACACAGAGTAACATT----A
Physignathus      CAATGTCTACCATAAATCGATGACTACTATCAACA---GGAAGCGTGCCCGAAACCACAAGGACTACTTTGATAAAGTAAACATGGAGCTCACAAT---A

1901-1989         .........ILE.....GLN.....................................................................GLN
                  >>>>AA>>>>>*.....*<<<<<AA<<<<T.......<<<<T....<<<AC..COD..<<<AC.<<<D........<<<D..<<<<<AA
Gallus            CTCTCACTTCCTTAATCCTAGAAAAGTAGGAATCGAACCTACACAGAAGAGATCAAAACTCTTCATACTCCCT-CTATATTATTTTCTA
Elgaria           CTCTCACTTCC-------TAGAAAGACAGGAATTGAACCTGCACCAAAAAACTCAAAATCTTCTGTACTCCCA-TTATACTACTTCCTA
Anolis            CTCTCATTTTC-------TAGAAAACCAGGACTCGAACCTGTACCTAAAAACTCAAAATCATTTGTACTTCTT--TATACTATTTTCTA
Basiliscus        CACTCATTTCCC------TAAAAGAACAGGACTCGAACCTGCACCAAAAGACTCAAAATCTTTAGTACTCCAC--TATACTATCTTCTA
Crotaphytus       CTCTCATTTCCT------TAGAAGAATGGGACTTGAACCCACACCAAAAGACTCAAAATCTAAGGCACTTCAT--TATGCTATCCTCTA
Gambelia          CTCTCGCTTCCT------TAGAAGAATGGGACTTGAACCCACACCAAGAGACTCAAAATCTCAAGCACTCCAT--TATGCTACCCTCTA
Hoplocercus       CGCTCACTTCC-------TAGAAGAACAGGGCTCGAACCTGCACCAAAAGACTCAAAATCCTTTGCACTCCAT--TATGCAACCTCCTA
Liolaemus         CTCTCATTTCCT------TAGAAGGACAGGACTCGAACCCGCACCTAGAAGCTCAAAACTTCCAGCACTCCAT--TATACTACCATCTA
Oplurus           CCCTCATTTCCT------TAGGAAGACAGGATTCGAACCTGCACTTAGAAACTCAAAATTACTTGTACTCCAT-TACTACAACCTCCTA
Phrynosoma        CTCTCACTTCC-------TAAAAGAACAGGACTTGAACCTGCACACAAAGACTCAAAATCTTCAGTACTACAT--TATACTATCTTCTA
Sauromalus        CTCTCATTTCC-------TAGAAGAACAGGACTTGAACCTGCACCTAAAGACTCAAAATCTCCAGCACTTCAT--TATACTATCTTCTA
Chamaeleo         CTCTCCCTTCC------ATAGGGGGGAAGGGATTGAACCAACATCAAAAAACTCAAAATTTCACGCATTCCAT--TATACTACCCCCTA
Leiolepis         CTCTCGTCTCCC-----TTAGAAGAATGGGACTCGAACCCACAATAAAAAATCCAAAATCTTTTGTACTCCCAACTATACTATCTCCTA
Uromastix         CTCTCGATTCCC-----TTAGCAGGGAAGGAATCGAACCAACACCGAAAAACCCAAAATATTTCATACCTCCA-CTATACTACCTGCTA
Phrynocephalus    CTCTCACTTCCA-----TTAGAGAAAGAGGACTCGAACCTCCATAAAAAAATCCAAAATTTTTGCACTACCACCTATGCTACTCTCCA
Physignathus      CTCCCACTTCC------ATAGAGAAGAAGGACTCGAACCAACACCTAAAAACCCAAAATTTTTAGTACTCCCACCTATACTACTCTCTA
```

Fig. 1. Continued.

the D-stem are separated by only a single base, a condition that may have resulted from a shift in one side of the D-stem. For this reason, sequences of the D-loop, one side of the D-stem, and bases adjacent to the AA-stem of the $tRNA^{Asn}$ gene were not used (positions 1596–1610). Acrodont lizards lack a D-stem in $tRNA^{Cys}$ and instead contain a D-arm replacement loop. The interaction of bases from this D-arm replacement loop and the variable loop can cause a realignment in the T-stem. Mechanistic hypotheses for the origin of replacement loops in $tRNA^{Cys}$ genes suggest realignment of the AA-stem (Macey et al. 1997b). The absence of a D-stem and the possibility for realignment of the AA- and T-stems in acrodont lizards made all positions except the anticodon (AC) loop and stem of $tRNA^{Cys}$ difficult to assess for homologous characters. Therefore, only the AC-stem and loop were included in analyses (excluded positions 1649–1678, 1697–1717).

The origin for light-strand replication between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes appears to be missing or nonfunctional in acrodont lizards and *Gallus* (Desjardins and Morais 1990). Therefore, phylogenetic analyses excluded this region (positions 1618–1648). The criteria for inferring presence of a functional O_L in this region include presence of a possible stem-and-loop structure, a 3′-GCC-5′ heavy-strand sequence identified as the point of light-strand elongation in mouse (Brennicke and Clayton 1981), and a heavy-strand sequence related to the 3′-GGCCG-5′ sequence required for in vitro replication of human mitochondrial DNA (Hixson et al. 1986).

Sequences ranged in length from 1,704 to 1,747 bases. The phylogenetic analyses were conducted using 1,488 alignable positions (858 phylogenetically informative). The excluded regions on the longest sequence (*Liolaemus*) corresponded to 15%.

Phylogenetic Analysis. Three phylogenetic analyses were conducted. In the analysis of the DNA sequences alone, the phylogenetic tree was rooted by outgroup comparison using a bird, *Gallus gallus* (Desjardins and Morais 1990), and an anguid lizard, *Elgaria panamintina.* Another analysis was conducted on the previously published morphological data (Frost and Etheridge 1989) for the same taxa used in the analysis of DNA sequences. In this case, one ingroup taxon, *Hoplocercus,* was not sampled for morphological characters but two

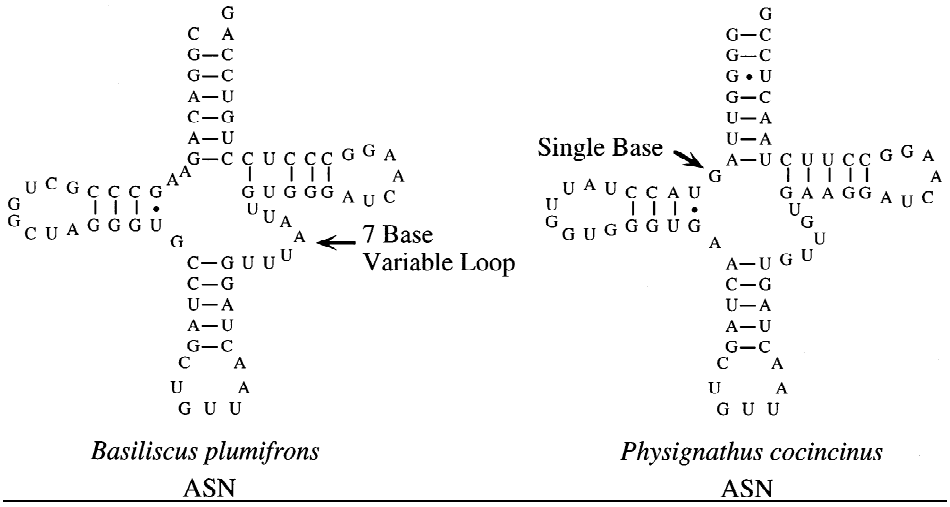

Fig. 2. Two asparagine tRNAs that deviate from standard secondary structural models. *Basiliscus plumifrons* exhibits an enlarged variable loop of seven bases, and *Physignathus cocincinus* has only one base separating the AA- and D-stems.

basal taxa from the same clade, ''*Enyalioides*'' of Frost and Etheridge (1989), were used to assess morphological character states. Three other ingroup taxa, anoles, iguanas, and agamas of Frost and Etheridge (1989), are represented by *Anolis*, *Sauromalus*, and *Phrynocephalus*, respectively, in the analysis of DNA sequences. The morphological study provided a composite outgroup, Scleroglossa, of which *Elgaria* is a member, but did not include a bird. An additional outgroup, *Sphenodon*, for which DNA sequence data are lacking, was used. Initial analyses of the morphological data with the two outgroups produced trees in which Scleroglossa was placed in the Iguanidae, which goes against other well-supported phylogenetic hypotheses (Estes et al. 1988; Gauthier et al. 1988). Therefore, all analyses using morphological data constrained the ingroup to be monophyletic. In the third analysis, the morphological and molecular data were combined. Because morphological data were not available for *Gallus* and DNA sequence data were not acquired for *Sphenodon*, the outgroup structure was constrained to well-accepted arrangements (Gauthier et al. 1988).

Phylogenetic trees were estimated using PAUP (Swofford 1993) with 100 heuristic searches using random addition of sequences. Bootstrap resampling was applied to assess support for individual nodes using 500 bootstrap replicates with 10 random additions per replicate. Decay indices (=''branch support'' of Bremer 1994) were calculated for all internal branches of the trees. To calculate decay indices, phylogenetic topologies were constrained using MacClade (Maddison and Maddison 1992) and analyzed using PAUP (Swofford 1993) with 10 heuristic searches featuring random addition of sequences (100 searches used for branches immediately ancestral to the Acrodonta and the Iguanidae).

Templeton's (1983) test, a two-tailed Wilcoxon signed ranks test (Felsenstein 1985), was applied to examine statistical significance of the shortest tree in each analysis relative to alternative hypotheses. This test asks whether the most parsimonious tree is significantly shorter than an alternative or whether their differences in length can be attributed to chance alone (Larson 1994). The test statistic T_s was compared with critical values for the Wilcoxon rank sum in table 30 of Rohlf and Sokal (1981) for n up to 50. For n over 50, a Z statistic was calculated as $Z = (\mu - T_s - 1/2)/\sigma$ where $\mu = n(n + 1)/4$ and $\sigma = \sqrt{(2n + 1)\mu/6}$ following Snedecor and Cochran (1967) and critical values were obtained from table 12 of Rohlf and Sokal (1981) using infinite degrees of freedom.

Alternative phylogenetic hypotheses were generated using trees with constraints. Phylogenetic topologies were constrained using MacClade (Maddison and Maddison 1992) and analyzed using PAUP (Swofford 1993) with 100 heuristic searches featuring random addition of sequences. Statistical tests were conducted using the ''compare trees'' option of MacClade (Maddison and Maddison 1992).

Results

Authentic Mitochondrial DNA

Several observations support our conclusion that the DNA sequences analyzed here are from the mitochondrial genome and do not represent nuclear integrated copies of mitochondrial genes (see Zhang and Hewitt 1996). All sequences reported here show strong strand bias against guanine on the light strand (G = 11–13%, T = 21–30%, A = 33–37%, and C = 24–33%), which is characteristic of the mitochondrial genome but not the nuclear genome. All genes sequenced appear functional; transfer RNA sequences form stable secondary structures and protein-coding genes have no premature stop codons. Therefore we interpret these sequences as authentic mitochondrial DNA.

Molecular Characters

tRNA Secondary Structure

Two taxa have unusual secondary structures in $tRNA^{Asn}$ (Fig. 2). *Basiliscus* has a large variable loop of seven bases which deviates from the expected three to five bases normally observed in mitochondrial tRNAs (Kumazawa and Nishida 1993). *Physignathus* has a single base separating the AA- and D-stems instead of the two normally observed.

Two secondary structural alternatives are observed for $tRNA^{Cys}$. Iguanids and *Elgaria* have a normal four-stem secondary structure, whereas acrodonts are missing the D-stem and instead have a D-arm replacement loop (Fig. 3) (Macey et al. 1997b).

Origin for Light-Strand Replication

Variation was observed for the presence of a recognizable origin for light-strand replication in the typical

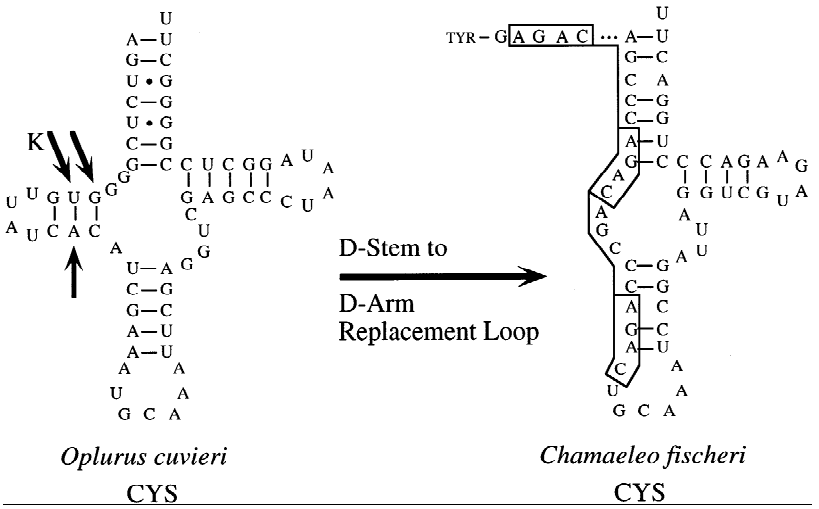

Fig. 3. Examples of alternative secondary structures for the $tRNA^{Cys}$ observed in iguanian lizards. *Oplurus cuvieri* shows a normal four-stem tRNA whereas *Chamaeleo fischeri* has a D-arm replacement loop in place of a D-stem. *Arrows* indicate D-stem positions that are invariant across all squamate reptiles that have D-stems. The *arrow with a K* has one exception of a derived substitution of G in a boiid snake (Macey et al. 1997b; Seutin et al. 1994). Note the repeats (*boxed and outlined*) in the *Chamaeleo* tRNA that have been hypothesized to result from slipped-strand mispairing of noncontiguous repeats during replication (Macey et al. 1997b). Adjacent noncoding heavy-strand sequence between the $tRNA^{Cys}$ and $tRNA^{Tyr}$ genes is separated by three dots for *Chamaeleo.*

vertebrate location between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes (Fig. 4). Iguanids show an 11–12-bp stem with the top A-T pairing absent in some taxa. The anguid lizard, *Elgaria,* has a similar 12-base stem where the bottom base pair is a T-A pair instead of a C-G pair. In contrast, only two acrodont lizards have DNA sequences that could form stem-and-loop structures: *Chamaeleo fischeri* and *Leiolepis belliana.* These structures are shorter, with 7 and 8 bp, respectively. The structures observed in the iguanids and anguid are presumably functional whereas the structures found in *Chamaeleo* and *Leiolepis* are not.

The iguanid and anguid stems show nearly complete homology and share many features with other vertebrate origins for light-strand replication. The 3′-GCC-5′ heavy-strand sequence identified as the point of light-strand elongation in mouse (Brennicke and Clayton 1981) is present. The heavy-strand sequence 3′-GGCCT-5′ or 3′-GGCCC-5′ related to the 3′-GGCCG-5′ sequence required for in vitro replication in humans (Hixson et al. 1986) is observed in a conserved position where the AA- and T-stems of $tRNA^{Cys}$ are coded. The last four bases of the O_L stem overlap the $tRNA^{Cys}$ gene.

These O_L features are mostly absent in the two acrodonts having stem regions. The two stems show almost no similarity to each other or to other vertebrate origins for replication of the light strand. Only *Leiolepis* has the 3′-GCC-5′ heavy-strand template sequence identified as the point of light-strand elongation in mouse (Brennicke and Clayton 1981). At the same position where the $tRNA^{Cys}$ AA and T-stems are coded, only *Chamaeleo fischeri* has a sequence that resembles the heavy-strand sequence 3′-GBCCB-5′ related to the 3′-GGCCG-5′ sequence shown to be required for in vitro replication in humans (Hixson et al. 1986). The two-stem structures observed in acrodonts do not overlap with the $tRNA^{Cys}$ gene. In addition, a sequence that cannot form a stem-and-loop structure between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes is observed in the acrodont, *Phrynocephalus raddei.* Two other acrodonts, *Uromastix acanthinurus* and *Physignathus cocincinus,* have little or no sequence in this position.

Gene Order

The typical vertebrate gene order of ND1, $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, ND2, $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, O_L, $tRNA^{Cys}$, $tRNA^{Tyr}$, and COI was found in all eight iguanid lineages and the anguid lizard. The five acrodont taxa show the unique vertebrate gene order where the $tRNA^{Ile}$ and $tRNA^{Gln}$ genes are switched in order.

Start Codons

Three presumptive start codons were identified for ND2: ATG, ATT, and TTG. Whereas ATG (coding for methionine) is a common start codon among mitochondrial protein-coding genes of vertebrates, ATT (usually coding for leucine) and TTG (usually coding for isoleucine) are not. Both ATT and TTG have been proposed as start codons (Seutin et al. 1994; Wolstenholme 1992), possibly being recognized by the F-met-charged tRNA when they occur in the initiation position. In COI, two start codons are observed; GTG (usually coding for valine) is found in all taxa except the acrodonts which have ATG (coding for methionine). These start codons are typical for COI among vertebrates.

Stop Codons

In iguanids and the outgroups, a stop codon for ND1 either occurs within the ND1 gene or alternatively gets formed by polyadenylation of the mRNA (Anderson et al. 1981). The ND1 transcript of *Anolis paternus* ends with a T that probably gets polyadenylated during processing, producing the stop codon TAA (Anderson et al. 1981). Construction of a stop codon by polyadenylation probably occurs also for *Liolaemus* and *Oplurus,* in which the last base of the ND1 stop codon otherwise would overlap the adjacent $tRNA^{Ile}$ gene. Because the mtDNA is transcribed as a whole followed by cleavage of the transcript to form functional RNAs (Ojala et al.

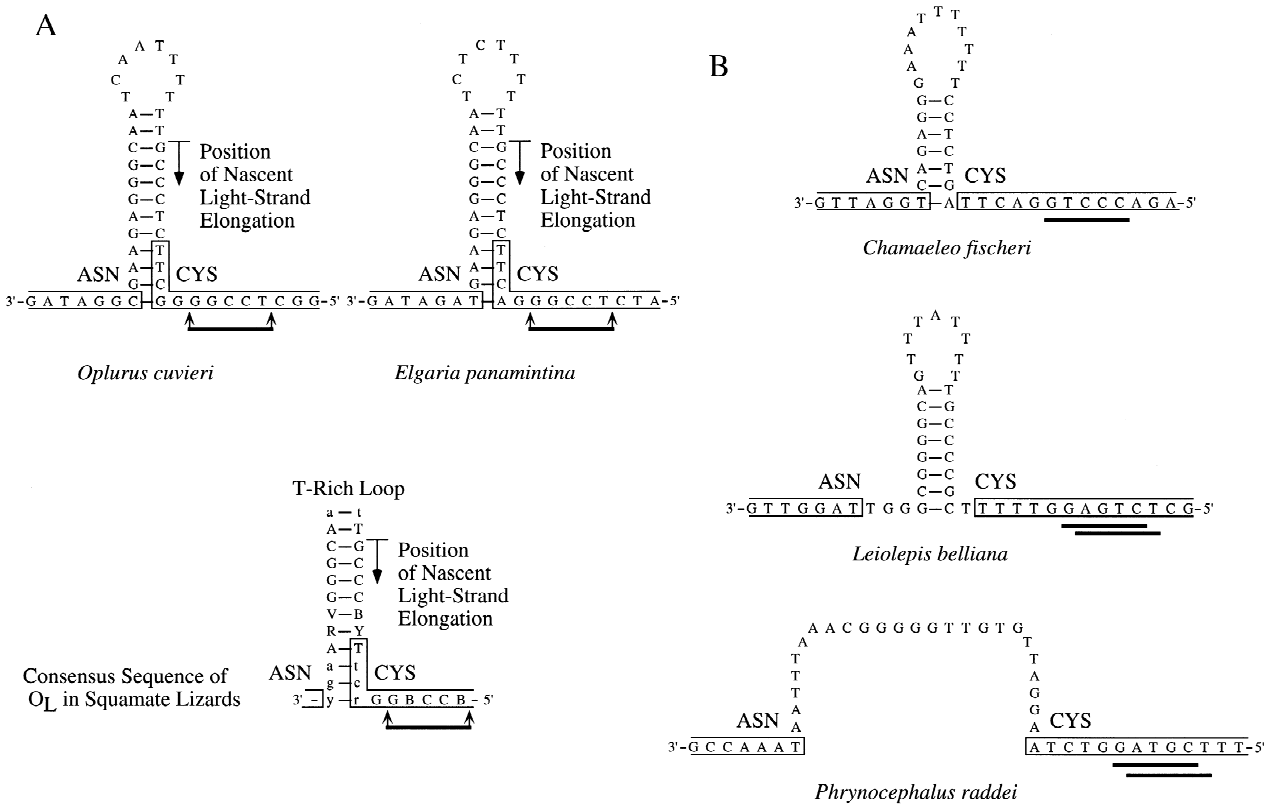

Fig. 4. Alternative stem-and-loop structures between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes. **A** Examples of presumptive functional origins for light-strand replication (O_L). All iguanids (see *Oplurus*) have an identical 11–12-bp stem with only the top A-T pairing absent in some taxa. The anguid, *Elgaria,* has a similar 12-base stem with the bottom base pair a T-A pair instead of a C-G pair. The consensus heavy-strand sequence of putative O_L from lizards is based on representatives from the Iguanidae (nine genera), Gekkonidae (*Teratoscincus*), Pygopodidae (*Lialis*), Lacertidae (*Eremias*), Teiidae (*Cnemidophorus*), Cordylidae (*Platysaurus*), Anguidae (*Elgaria*), Xenosauridae (*Xenosaurus*), and Varanidae (*Varanus*), (reported here and in Macey et al. 1997a). Bases in *capitals* are conserved pairings and downstream sequence. Bases in *lowercase* are often paired. Variable positions are labeled with their standard *one-letter codes:* R = G or A; Y = C or T; B = G, C or T; and V = G, C, or A. The 3′-GCC-5′ heavy-strand template sequence identified as the point of light-strand elongation in mouse (Brennicke and Clayton 1981) is indicated (*arrow*). The heavy-strand sequence 3′-GGCCT-5′ or 3′-GBCCB-5′ in the $tRNA^{Cys}$ gene related to the 3′-GGCCG-5′ sequence found to be required for in vitro replication of human mitochondrial DNA (Hixson et al. 1986) is underlined with *arrows.* **B** Among acrodonts, stem-and-loop structures were identified only in *Chamaeleo fischeri* and *Leiolepis belliana.* The critical features of O_L, which are observed in many lizards, are mostly absent from *Chamaeleo* and *Leiolepis,* suggesting that these acrodont sequences do not form a functional O_L. A third acrodont, *Phrynocephalus raddei,* has sequence that cannot form a stem-and-loop structure. The *underlined* sequences indicate the position where the heavy-strand sequence related to the 3′-GGCCG-5′ sequence found to be required for in vitro replication in humans (Hixson et al. 1986) should be present. There are two interpretations for the secondary structure of $tRNA^{Cys}$ in *Leiolepis* and *Phrynocephalus* (Macey et al. 1997b). The *top underline* is the segment presumed homologous to the sequences underlined for *Chamaeleo,* with the *lower underline* showing the alternative possible position for this motif.

1981), adjacent genes encoded on the same strand cannot overlap and both have complete transcripts formed from the same primary transcript. Acrodonts, which have a unique vertebrate gene order, have a TAG stop codon for ND1 that is completely overlapping with the AA-stem region of the adjacent $tRNA^{Gln}$ gene. Because the $tRNA^{Gln}$ and ND1 genes are encoded on opposite strands, overlap of their coding regions is possible.

Stop codons for ND2 appear to be formed by polyadenylation during processing of the primary transcript (Anderson et al. 1981) with the exceptions of *Elgaria, Oplurus,* and *Sauromalus,* which have TAA stop codons encoded within the ND2 gene. For all remaining taxa except *Uromastix,* extension of the ND2 mRNA to include part of the adjacent $tRNA^{Trp}$ from the primary transcript would generate a stop codon. However, this mechanism would preclude production of complete ND2 mRNA and $tRNA^{Trp}$ molecules from the same primary transcript. We therefore favor the hypothesis that all taxa except *Elgaria, Oplurus,* and *Sauromalus* form the stop codons of the ND2 transcripts by polyadenylation, as presumably occurs for *Uromastix.*

tRNA Stem Pairs and Compensatory Changes

Among the tRNA genes included in the phylogenetic analyses, compensatory changes potentially could confound phylogenetic inference. Evolutionarily coupled compensatory substitutions in stem regions would result

in related events being counted as independent sources of information in a parsimony analysis. Compensatory substitutions separated in time could provide phylogenetic information on different branches. There are 21 stem pairs per tRNA but our analysis excluded the AA-, D-, and T-stems of $tRNA^{Cys}$ and half of the D-stem of $tRNA^{Asn}$. A total of 148 pairs containing 296 positions from both sides of stem regions were used. Thirty-one of these pairs are not variable and all but one are Watson-Crick pairs. The other pair is a G13-A22 pair in the D-stem of $tRNA^{Tyr}$ that appears to be conserved across vertebrates (Kumazawa and Nishida 1993). Of the 117 variable pairs, 29 show variation under Watson-Crick base pairing, and therefore may have been subjected to evolutionarily coupled compensatory changes. Among the remaining 88 variable pairs, 31 show variation under the restriction of Watson-Crick and G-U wobble pairing. These pairs were observed only in light-strand-encoded tRNAs. G-U wobble pairs can serve as one-step intermediates between alternative Watson-Crick pairings (Kumazawa and Nishida 1993), and therefore these positions are evidently not undergoing extensive compensatory substitutions. Fifty-seven pairs show no restrictions on pairing and should not be undergoing extensive compensatory mutations. These results suggest that 75% of the variable pairs used in the phylogenetic analysis are unlikely to be subjected to evolutionarily coupled compensatory changes, and stems of tRNA genes therefore should not necessarily be downweighted.

Protein-Coding Silent Transitions and Strand Bias

Irwin et al. (1991) recommended that only replacement substitutions and silent transversions be used in phylogenetic analyses of the mitochondrial cytochrome *b* gene for divergences greater than 5 million years because of expected substitutional saturation of silent transitions. Silent transitions are potentially found in all third positions and first positions of leucine codons, which have six triplets (TTR, CTR, CTY). Substitutional saturation of silent transitions appears not to be a problem in our analyses because extreme strand bias against G and T on the light strand makes silent transitions relatively uncommon. Silent sites should be subjected to strand bias more strongly than other sites (Irwin et al. 1991). This strand bias could confound phylogenetic analysis if the bias is changed between species (Irwin et al. 1991). Across all codons used in our phylogenetic analysis, the third position shows extreme strand bias and is deficient in G (2–6%) and usually T (7–18%, *Anolis* 29%) on the light strand. Across species these two bases are deficient in third positions and, therefore, because A (42–55%) and C (26–47%, *Anolis* 18%) occur in high frequency, silent transitions should be relatively infrequent. First-position leucine codons are mostly C (67–95%) across the taxa examined. Hence, silent transitions at all third positions and at first-positions of leucine codons should not show substitutional saturation.

Phylogenetic Hypotheses

Analysis of the morphological data produced a single phylogenetic estimate of 131 steps showing paraphyly of the Iguanidae (sensu lato) but with little support for individual nodes (Fig. 5A). Among iguanids, only the sister relationship of *Crotaphytus* and *Gambelia* is well supported (96% bootstrap; decay index 4). Monophyly of the Acrodonta appears well supported (98% bootstrap; decay index 8). The groupings of *Physignathus* with *Phrynocephalus* and of *Leiolepis* with *Uromastix* each have decay indices of only 1 and are therefore not well supported despite relatively high bootstrap values (82% for each branch).

Analysis of the DNA sequence data produced a single most parsimonious tree of 3,849 steps (Fig. 5B). Two major lineages, those ancestral to the Iguanidae (sensu lato) and the Acrodonta, appear well supported from the bootstrap analysis (both 100%) and show high decay indices (23 and 48, respectively), (Fig. 5B). The Iguanidae (sensu lato) and the Acrodonta appear well supported as monophyletic groups also using the following analyses (results not shown): (1) separate analyses of protein-coding and tRNA-coding subsets of the DNA sequences; (2) analysis of amino acids from the protein-coding regions; (3) analysis of the total DNA sequence data with silent transitions removed from the protein-coding sequences; and (4) use of transversion parsimony on the total DNA sequence data.

Within the Iguanidae, most relationships are not well resolved by the DNA sequence data. *Crotaphytus* and *Gambelia* are well supported as sister taxa (100% bootstrap; decay index 45), which is congruent with the morphological analysis. *Anolis* and *Basiliscus* appear as sister taxa with weak support (59% bootstrap; decay index 7). Note the inclusion of the Old World *Oplurus* from Madagascar with the New World iguanid lineages. Within the Acrodonta, the bootstrap analysis showed support only for the sister relationship of *Physignathus* and *Phrynocephalus* (81%; decay index 10), a grouping present also in the morphological analysis.

The analysis of morphological and molecular data combined produced a tree of 3,996 steps identical to the ingroup topology of the tree obtained with DNA sequences alone, and with similar bootstrap values and decay indices (Fig. 5B).

The morphological and molecular data analyzed independently produced very different phylogenetic hypotheses, with the morphological data suggesting a paraphyletic Iguanidae and the sequence data supporting a monophyletic Iguanidae. When Templeton's test was applied to the morphological data set, the hypothesis produced from the sequence data was rejected as an alter-

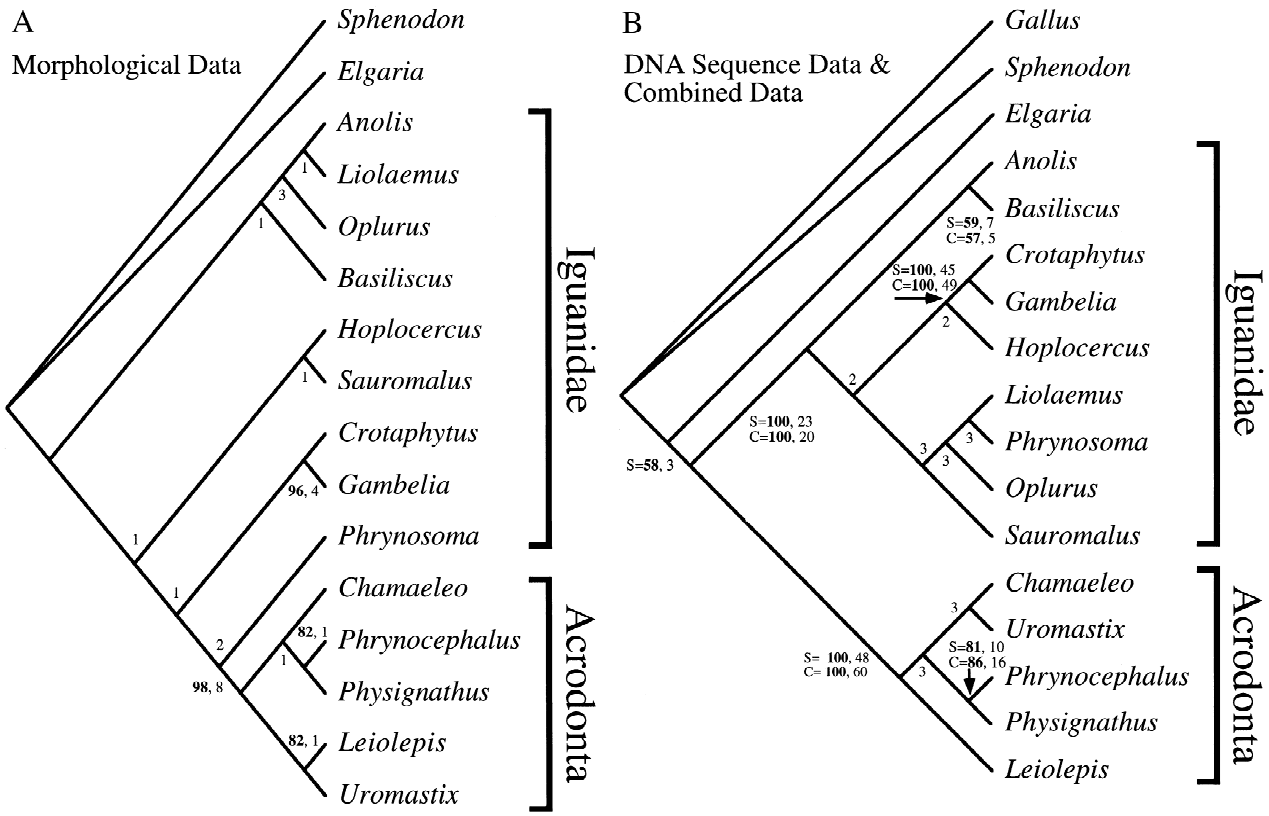

Fig. 5. **A** Phylogenetic hypothesis generated from the morphological data of Frost and Etheridge (1989) where outgroups were constrained [131 steps, consistency index (CI) 0.550]. Bootstrap values acquired from 500 replicates with 10 random addition of taxa per replicate are plotted on nodes in *bold type.* Decay indices are plotted on nodes in *plain type.* **B** Phylogenetic hypothesis generated from the DNA sequence data (3,849 steps, CI 0.471) and the combined morphological and molecular data (3,996 steps, CI 0.471). *Sphenodon* was not included in the analysis of DNA sequences and the outgroup structure was constrained in the combined analysis. Bootstrap values acquired from 500 replicates with 10 random additions of taxa per replicate are plotted on nodes in *bold type* and decay indices in *plain type* where S = DNA sequence data and C = combined morphological and molecular data when different. Note the presence of two well-supported clades corresponding to the Iguanidae and Acrodonta. For the following genera, morphological data were reported by Frost and Etheridge (1989) for more inclusive taxa containing the genera as follows: *Phrynocephalus* = Agamas, *Anolis* = Anoles, *Hoplocercus* = ''*Enyalioides*,'' and *Sauromalus* = Iguanas. Molecular data include 858 phylogenetically informative characters (parsimony criterion).

native to the shortest estimate of phylogeny obtained from the morphological data (n = 19, T_s = 30, P < 0.01). When this test was applied to the DNA-sequence data, the sequence-based topology was significantly more parsimonious than the morphologically based topology (n = 295, T_s = 14,900, P < 0.001). Hence the two data sets appear to contain conflicts.

Templeton tests using the morphological data showed that the six shortest phylogenetic trees constrained to contain a monophyletic Iguanidae (each 134 steps, Appendix 2) were not significantly longer than the overall shortest tree obtained from the morphological data (for two trees, n = 9, T_s = 15, n.s.; for four trees, n = 7, T_s = 8, n.s.). For the DNA sequence data, the shortest tree constrained to have a nonmonophyletic Iguanidae (3,872 steps, Appendix 2) was not significantly longer than the overall shortest tree obtained from the DNA sequence data (Templeton test: n = 203, T_s = 9,203, n.s.).

Monophyly of the Acrodonta was supported in all analyses. A Templeton test of the morphological data showed that the shortest trees constrained to contain a nonmonophyletic Acrodonta (139 steps, Appendix 2) were not significantly longer than the overall shortest tree obtained from the morphological data (tree 1, n = 20, T_s = 63, n.s.; tree 2, n = 18, T_s = 47.5, n.s.). Analysis of the DNA sequence data showed that the shortest tree constrained to have a nonmonophyletic Acrodonta (3,897 steps, Appendix 2) was significantly longer than the overall shortest tree obtained from the DNA sequence data (Templeton test: n = 334, T_s = 24,154.5, P < 0.05). Analysis of the combined morphological and molecular data showed that the two shortest trees constrained to have a nonmonophyletic Acrodonta (both 4,056 steps, Appendix 2) were significantly longer than the overall shortest tree obtained from the combined data (Templeton test: tree 1, n = 269, T_s = 14,260, P < 0.005; tree 2, n = 278, T_s = 15,458, P < 0.005).

Monophyly of the Acrodonta is supported further by the unique vertebrate mitochondrial gene order of ND1, $tRNA^{Gln}$, $tRNA^{Ile}$, $tRNA^{Met}$. Gene order appears to be a

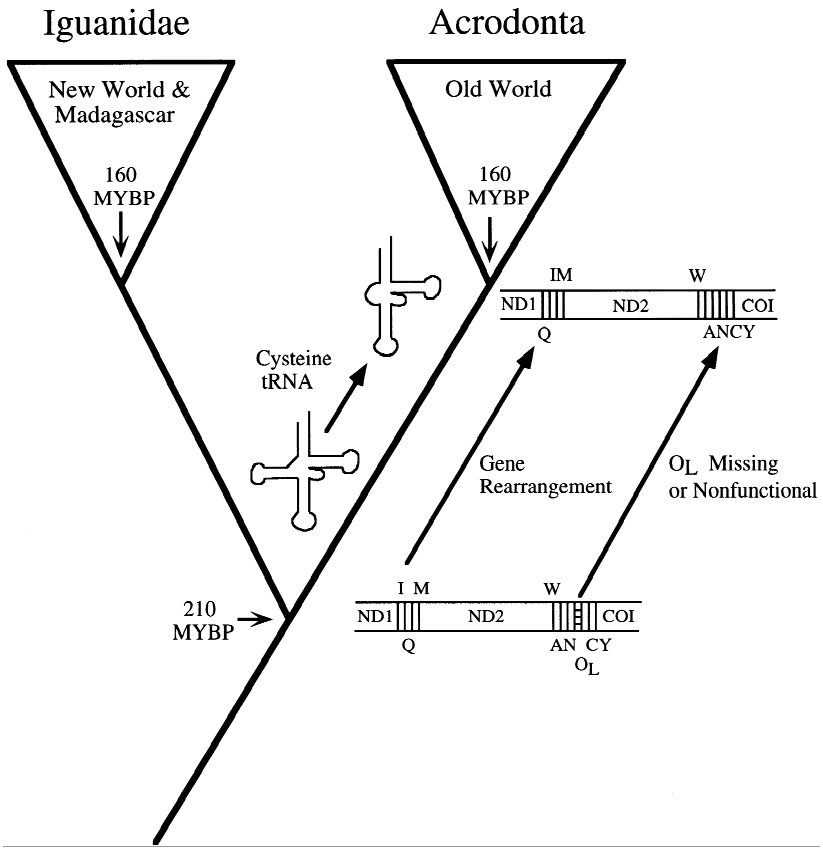

Fig. 6. Phylogenetic position for shifts in gene order, position of a recognizable O_L, and secondary structure of $tRNA^{Cys}$. Branch lengths are not shown to scale with time.

highly robust character in which parallelism and reversal are unlikely because of the complex evolutionary processes required to cause a genomic rearrangement (for details see Macey et al. 1997a).

These results suggest that the Iguania consists of two major subgroups. One group, the Acrodonta, receives statistical support from the DNA sequence data using Templeton's test. The other group, the Iguanidae, appears monophyletic as indicated by the high bootstrap value (100%) and decay index (23) from the analysis of DNA sequences, although it does not receive statistical support using Templeton's test.

Discussion

Shifts in Major Structural Characters

The two monophyletic groups identified within iguanian lizards each have different states for three major structural characters of the mitochondrial genome (secondary structure of $tRNA^{Cys}$, position of a recognizable replication origin for the light strand, and gene order; Fig. 6).

Lepidosaurian reptiles show considerable variation for the presence of the D-stem in $tRNA^{Cys}$ (Fig. 3) (Macey et al. 1997b). *Sphenodon punctatus,* in a sister position to all other lepidosaurian reptiles (Gauthier et al. 1988), lacks a D-stem in $tRNA^{Cys}$ (Seutin et al. 1994), yet iguanian lizards, the sister group to all other lepidosaurian reptiles except *Sphenodon* (Estes et al. 1988; Macey et al. 1997a; Schwenk 1988), show variation for the presence of the $tRNA^{Cys}$ D-stem (Macey et al. 1997b). Two interpretations are possible for the evolution of this character among reptiles: a single loss with multiple parallel gains, or multiple parallel losses. Parallel losses of D-stems seem more likely because $tRNA^{Cys}$ D-stems when present have invariant sites that suggest a shared history (Macey et al. 1997b).

Variation has been observed among reptiles for the presence of a recognizable O_L between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes. Two basal taxa, crocodilians/avians and *Sphenodon,* lack sequence between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes, suggesting the absence of O_L from the typical vertebrate position. Three additional squamate taxa, Amphisbaenia, Scincidae and Xantusiidae, lack a recognizable O_L in this position (Macey et al. 1997b). Snakes (Kumazawa and Nishida 1995; Kumazawa et al. 1996; Seutin et al. 1994) and iguanian lizards each show variation for this character. The evolution of O_L among reptiles is subject to two interpretations: a single loss with multiple gains, or multiple losses (Macey et al. 1997a). Parallel losses seem more likely because of tremendous similarity of stem pairs among reptiles and other vertebrates that have the stems corresponding to the O_L located between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes (Macey et al. 1997a). Within acrodont lizards, two taxa

lack sequence between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes, another two taxa have strange O_L stems, and a third has sequences that cannot form a stem. This extreme variation suggests that the O_L has shifted position in the Acrodonta.

Among animal mitochondrial DNAs, the gene order observed in acrodont lizards is unique and therefore may be unambiguously polarized. A single switch of tRNA order must have occurred in the common ancestor of acrodont lizards exclusive of iguanids.

Unusual secondary structures observed in $tRNA^{Asn}$ (Fig. 2) appear as autapomorphies in *Basiliscus* and *Physignathus* because no other taxa show these features.

All protein-coding regions of the vertebrate mitochondrial genome are encoded on the heavy strand except ND6. The two tRNA genes rearranged in iguanian lizards are encoded on opposite strands, with the $tRNA^{Ile}$ gene on the heavy strand and the $tRNA^{Gln}$ gene on the light strand. When the $tRNA^{Ile}$ gene is in the standard vertebrate position it directly follows ND1, and processing of the primary heavy-strand transcript does not allow these two genes to overlap. In this case the stop codon for ND1 either must be encoded within the ND1 gene or get formed by polyadenylation during processing of the primary heavy-strand transcript (Anderson et al. 1981). Because ND1 is encoded on the heavy strand and $tRNA^{Gln}$ on the light strand, these two genes can overlap when rearranged to be adjacent.

Acrodont lizards appear to utilize a conserved sequence in $tRNA^{Gln}$ as a stop codon for ND1. This triplet is conserved in the $tRNA^{Gln}$ gene as either TAG or TAA even among taxa whose ND1 and $tRNA^{Gln}$ genes are not adjacent and cannot overlap (Macey et al. 1997a). This genomic rearrangement increases the compactness of the animal mitochondrial genome by permitting a conserved sequence in the $tRNA^{Gln}$ gene to double as the stop codon of the ND1 gene.

Timing of Evolutionary Shifts

Iguanian diversification has been considered a result of fragmentation of Gondwanaland plates beginning in the Jurassic (Estes 1983; Moody 1980). Both iguanids and acrodonts occur in Madagascar. Recent dispersal to Madagascar from Africa seems improbable for Chamaeleonidae because of a large radiation there. Oplurines in Madagascar almost certainly did not disperse from South America, where the nearest iguanids are found. The separation of Madagascar from Africa occurred in the Middle to Late Jurassic (160 million years before present, MYBP) (Chatterjee 1992; Rabinowitz et al. 1983), predating the formation of the South Atlantic Ocean (80 MYBP). These observations suggest that both groups are at least 160 million years old. The major lineages of squamate reptiles (lizards) are recorded in the fossil record at this time (Estes 1983). Because fossil squamate reptiles that precede splitting of the Iguania from other squamates occur in the Upper Triassic–Lower Jurassic, the Iguania should be no older than 210 million years (Estes 1983). Hence, the shift in the three major structural characters of the mitochondrial genome occurred in iguanians during the Jurassic between 210 and 160 MYBP.

Independence of Evolutionary Shifts

The evolutionary shifts in gene order, position of O_L, and the loss of a D-arm in $tRNA^{Cys}$ may be related events. These three major changes in genomic structure occur on the same branch of the phylogeny. A concentrated-changes test (Maddison 1990) can be used to determine whether changes in one character coincide with changes in another character more often than would be expected by chance. The probability of two characters changing together on any branch in our phylogenetic hypothesis is less than would be expected by chance ($P < 0.038$) and therefore the probability of a third character changing on the same branch is even smaller ($P < 0.001$). The concentrated-changes test will be misleading when changes are occurring on a branch that is relatively long compared to other branches in the phylogenetic tree (Maddison 1990). The branch in which the three characters shift is maximally 50 million years in length. The two major iguanian clades are at least 160 million years old. The major clades of acrodonts are thought to have formed in response to the separation of Africa, Madagascar, India, Australia, and southeast Asia approximately 160–120 MYBP (Estes 1983; Moody 1980). The major lineages of iguanids probably had formed by the beginning of the Tertiary (65 MYBP) (Estes 1983), and oplurines should be at least 80 million years old because the South Atlantic, which separates oplurines from New World iguanids, formed in the Late Cretaceous (80 MYBP). The branch leading to the Acrodonta is therefore not a particularly long branch, making the results of our concentrated-changes test conservative.

Cascade Model for Evolutionary Shifts in Genomic Structure

Evolutionary shifts in these genomic structural characters may have involved a cascade process where changes in $tRNA^{Cys}$ secondary structure may destroy O_L function, which then causes a shift in gene order. The $tRNA^{Cys}$ gene is in a position adjacent to the typical vertebrate position of O_L. The O_L stem overlaps with the portion of the $tRNA^{Cys}$ gene encoding the AA-stem, and regions of the $tRNA^{Cys}$ gene encoding the AA- and T-stems are used to initiate light-strand synthesis (Fig. 7). D-arm replacement loops of $tRNA^{Cys}$ have been found associated with directly repeated sequences (Fig. 3). These repeats may result from replication slippage that

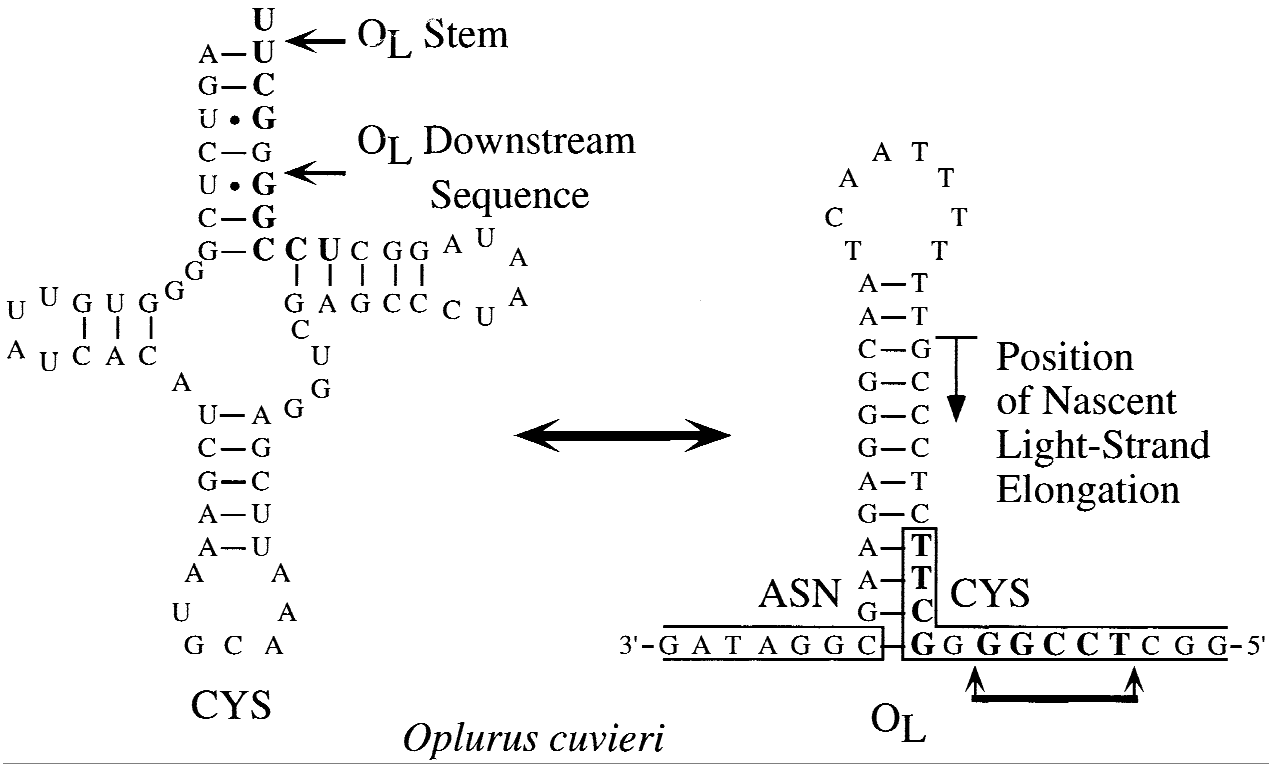

Fig. 7. Overlap between the tRNA^{Cys} gene and O_L. O_L is depicted as template heavy-strand sequence and the tRNA^{Cys} gene, which is encoded on the light strand, is depicted as the presumptive transcribed tRNA. *Boldface* bases are used in the function of light-strand replication and also tRNA^{Cys}. Three bases of the tRNA^{Cys} gene encoding the AA-stem are also used in the O_L stem. Another three bases of the tRNA^{Cys} gene encoding the AA-stem are also used in initiation of light-strand replication (required for in vitro replication in humans, Hixson et al. 1986). Hence, six out of seven bases encoding one side of the tRNA^{Cys} AA-stem are also used in initiation of light-strand replication. Note that an insertion to the D-loop/stem region resulting in a realigned AA-stem on the opposite side could cause compensatory mutation to the O_L functional regions that would disrupt the initiation of light-strand replication.

produces duplication of sequence in the D-stem/loop region (Macey et al. 1997b). We have hypothesized that the adjacent AA-stem may realign to accommodate the addition of sequence in the D-stem/loop region (Macey et al. 1997b). Such a realignment could cause compensatory mutation of bases on the other side of the AA-stem, which overlaps with O_L and is used in the initiation of light-strand replication (Fig. 7). Across vertebrates, shifts in gene order are evolutionarily coupled with loss of a recognizable O_L from the typical vertebrate position (Macey et al. 1997a). Hence, the formation of a D-arm replacement loop in the tRNA^{Cys} gene could cause the loss of a functional O_L which could facilitate a shift in gene order (Macey et al. 1997a).

D-arm replacement loops of tRNA^{Cys} have been identified only in lepidosaurian reptiles (Macey et al. 1997b; Seutin et al. 1994). If this model is accurate, lepidosaurian reptiles should have relatively high incidences of an O_L displaced from the typical vertebrate position and unique gene orders.

The cascade model may be tested with the knowledge of gene order, position for replication of the light strand, and the secondary structural alternatives for tRNA^{Cys} for numerous reptilian groups. Ideally, complete genomes should be sampled, but even sampling of gene junctions could be used to assess gene order. An understanding of the evolutionary processes involved in genomic rearrangements will help to evaluate the likelihood of homoplasy and the resulting phylogenetic utility of major structural features of the mitochondrial genome.

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Appendix 1: A Suggested Taxonomy for Iguanian Lizards

Frost and Etheridge (1989) proposed a new taxonomy for the Iguania in an attempt to maintain monophyly of taxa. Three-fourths of the approximately 650 iguanian species were placed in alternative families. The Iguanidae (sensu lato) was divided into eight families corresponding to the monophyletic groups of Etheridge and de Queiroz (1988): Corytophanidae, Crotaphytidae, Hoplocercidae, Iguanidae (sensu stricto), Opluridae, Phrynosomatidae, Polychridae, and Tropiduridae. All acrodonts were placed in the Chamaeleonidae with three subfamilies, the Chamaeleoninae, Leiolepidinae, and Agaminae. This taxonomic revision was done because no evidence for monophyly of the Iguanidae (sensu lato) or Agamidae could be found from an analysis of 67 morphological characters. This taxonomy has been highly controversial (for a review see Schwenk 1994).

We suggest that the Iguanidae be restored to its previous content based on our results indicating its monophyly. The eight monophyletic groups of Etheridge and de Queiroz (1988) therefore should be considered subfamilies (taxa in parentheses are representatives sampled here): Corytophaninae (*Basiliscus*), Crotaphytinae (*Crotaphytus, Gambelia*), Hoplocercinae (*Hoplocercus*), Iguaninae (*Sauromalus*), Oplurinae (*Oplurus*), Phrynosomatinae (*Phrynosoma*), Polychrinae (*Anolis*), and Tropidurinae (*Liolaemus*).

We suggest that the Acrodonta, Chamaeleonidae, and Agamidae also be restored to their original content following suggestions of Schwenk (1994). Monophyly of the Acrodonta and Chamaeleonidae has never been disputed but monophyly of the Agamidae with respect to the Chamaeleonidae has been questioned (Frost and Etheridge 1989). No evidence convincingly rejects monophyly of the Agamidae. Therefore, the Acrodonta may be recognized to contain two families (taxa in parentheses are representatives sampled here): Chamaeleonidae (*Chamaeleo*) and Agamidae* (*Leiolepis, Uromastix, Phrynocephalus*, and

Physignathus). Agamidae* may be recognized as a *metataxon* (Estes et al. 1988; Gauthier et al. 1988), denoted by an asterisk, in which monophyly has not been demonstrated. Two subfamilies in the Agamidae* may be recognized, the Agaminae (*Phrynocephalus* and *Physignathus*) and the Leiolepidinae* (*Leiolepis* and *Uromastix*), with the latter also representing a *metataxon* in which monophyly has not been demonstrated. Note that monophyly of the Leiolepidinae* (*Leiolepis* and *Uromastix*) is a point of disagreement between the morphological and molecular data sets analyzed here.

Appendix 2: Trees Used as Alternative Hypotheses in Templeton Tests Derived Using Constraints

Taxon names appear as in Fig. 5. Lengths of trees and consistency indices (Swofford 1993) are given in parentheses.

Six shortest trees (134 steps, CI 0.537) constrained to show monophyly of the Iguanidae from analysis of the morphological data:

Tree 1 = *(Sphenodon, (Elgaria, (((((((Anolis, Liolaemus), Oplurus), Basiliscus), Phrynosoma), (Crotaphytus, Gambelia)), (Hoplocercus, Sauromalus)), (Chamaeleo, ((Leiolepis, Uromastix), (Phrynocephalus, Physignathus)))))))*

Tree 2 = *(Sphenodon, (Elgaria, (((((Anolis, Liolaemus), Oplurus), Basiliscus), (((Crotaphytus, Gambelia), Phrynosoma), (Hoplocercus, Sauromalus))), (Chamaeleo, ((Leiolepis, Uromastix), (Phrynocephalus, Physignathus)))))))*

Tree 3 = *(Sphenodon, (Elgaria, (((((((Anolis, Liolaemus), Oplurus), Basiliscus), Phrynosoma), (Crotaphytus, Gambelia)), (Hoplocercus, Sauromalus)), ((Chamaeleo, (Phrynocephalus, Physignathus)), (Leiolepis, Uromastix)))))*

Tree 4 = *(Sphenodon, (Elgaria, (((((Anolis, Liolaemus), Oplurus), Basiliscus), (((Crotaphytus, Gambelia), Phrynosoma), (Hoplocercus, Sauromalus))), ((Chamaeleo, (Phrynocephalus, Physignathus)), (Leiolepis, Uromastix)))))*

Tree 5 = *(Sphenodon, (Elgaria, ((((((Anolis, Liolaemus), Oplurus), Basiliscus), Phrynosoma), (Crotaphytus, Gambelia), (Hoplocercus, Sauromalus)), ((Chamaeleo, (Phrynocephalus, Physignathus)), (Leiolepis, Uromastix)))))*

Tree 6 = *(Sphenodon, (Elgaria, ((((((Anolis, Liolaemus), Oplurus), Basiliscus), Phrynosoma), ((Crotaphytus, Gambelia), (Hoplocercus, Sauromalus))), ((Chamaeleo, (Phrynocephalus, Physignathus)), (Leiolepis, Uromastix)))))*

Shortest tree (3,872 steps, CI 0.468) constrained to show nonmonophyly of the Iguanidae from analysis of the DNA sequence data: *(Gallus, (Elgaria, (((Anolis, Basiliscus), Hoplocercus), (((Crotaphytus, Gambelia), ((Liolaemus, (Oplurus, Phrynosoma)), Sauromalus)), ((Chamaeleo, (Leiolepis, (Phrynocephalus, Physignathus))), Uromastix)))))).*

Shortest trees (139 steps, CI 0.518) constrained to show nonmonophyly of the Acrodonta from analysis of the morphological data:

Tree 1 *(Gallus, (Sphenodon, ((((((Anolis, Chamaeleo), ((Leiolepis, Uromastix), (Phrynocephalus, Physignathus))), Phrynosoma), (Crotaphytus, Gambelia)), (Hoplocercus, Sauromalus)), (Basiliscus, (Liolaemus, Oplurus)))))*

Tree 2 *(Gallus, (Sphenodon, (((((((Anolis, Chamaeleo), (Phrynocephalus, Physignathus)), (Leiolepis, Uromastix)), Phrynosoma), (Crotaphytus, Gambelia)), (Hoplocercus, Sauromalus)), (Basiliscus, (Liolaemus, Oplurus)))))*

Shortest tree (3,897 steps, CI 0.465) constrained to show nonmonophyly of the Acrodonta from analysis of the DNA sequence data: *(Gallus, ((Elgaria, ((Anolis, Basiliscus), (((((Crotaphytus, Gambelia), Liolaemus), (Oplurus, Phrynosoma)), Sauromalus), Hoplocercus))), (Chamaeleo, (Leiolepis, (Phrynocephalus, Physignathus)))), Uromastix)).*

Shortest trees (4,056 steps, CI 0.464) constrained to show nonmonophyly of the Acrodonta from analysis of the combined morphological and molecular data:

Tree 1 *(Gallus, (Sphenodon, (Elgaria, ((((Anolis, Basiliscus), (((((Crotaphytus, Gambelia), Liolaemus), (Oplurus, Phrynosoma)), Sauromalus), Hoplocercus)), ((Leiolepis, Uromastix), (Phrynocephalus, Physignathus))), Chamaeleo))))*

Tree 2 *(Gallus, (Sphenodon, (Elgaria, ((((Anolis, Basiliscus), (((((Crotaphytus, Gambelia), Liolaemus), (Oplurus, Phrynosoma)), Sauromalus), Hoplocercus)), ((Leiolepis, (Phrynocephalus, Physignathus)), Uromastix)), Chamaeleo))))*