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SCIENCE DIRECT
Gene 346 (2005) 249–256
GENE
SECTION
EVOLUTIONARY GENOMICS
www.elsevier.com/locate/gene

# Mitochondrial genome of *Pogona vitticepes* (Reptilia; Agamidae): control region duplication and the origin of Australasian agamids

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Received 29 July 2004; received in revised form 30 October 2004; accepted 17 November 2004
Available online 1 February 2005
Received by C. Saccone

**Abstract**

The complete mitochondrial DNA sequence for an Australian agamid *Pogona vitticepes* was determined. Twenty-two tRNA genes, two rRNA genes, thirteen protein-coding genes, and two control regions were identified in this mitochondrial genome. The second control region was inserted between NADH dehydrogenase subunits 5 and 6 genes. The duplication of the control region was found in all Australasian agamids examined and was not found in other Asian or African taxa. The two control regions had nearly identical sequences within species but they were divergent among species, suggesting their concerted sequence evolution. Phylogenetic analyses including divergence time estimation without assuming the molecular clock suggested that the duplication of the control region occurred on a lineage leading to the Australasian agamids 25–45 million years ago after their divergence from a Southeast Asian *Physignathus cocincinus*. Our finding thus supports the recent dispersal origin of Australasian agamids in connection with plate tectonic movement of Australia to the proximity of Southeast Asia.


*Keywords:* Bearded dragon; Sauria; Mitochondrial DNA; Divergence time; Historical biogeography; Plate tectonics

## 1. Introduction

Mitochondrial DNAs (mtDNAs) of vertebrates are 16–18 kbp double-stranded circular molecules, which encode genes for 2 rRNAs, 22 tRNAs and 13 respiratory proteins together with the control region (CR) (Boore, 1999). The control region is a major noncoding region that was shown to include controlling signals for replication and transcription of mtDNAs in mammals (Clayton, 1992). Structural and functional characterization of the mitochondrial genomes has revealed a variety of intriguing phenomena, such as gene rearrangements, genetic code changes, simplification of tRNA and rRNA structures, peculiar mechanisms in replication and transcription, and base compositional bias (Clayton, 1992; Boore, 1999; Saccone et al., 2002). Since many of these variations occur in a lineage-specific manner, characterization of more mitochondrial genomes from underrepresented animal groups (e.g., reptiles) is expected to add new findings from the molecular evolutionary standpoint. In addition, the complete mtDNA sequences have recently been applied successfully to phylogenetic questions of various animal groups (see, e.g., Janke et al., 2001; Rest et al., 2003; Kumazawa, 2004).

Agamidae is one of the lizard families, members of which are widely distributed in the tropical and subtropical regions of the Old World (Africa, Asia and Australia). There

*Abbreviations:* 12S, 12S rRNA; 16S, 16S rRNA; AT6 and AT8, ATPase subunits 6 and 8; COI–III, cytochrome oxidase subunits I–III; CR, control region; CSB, conserved sequence block; cytb, cytochrome *b*; LA-PCR, long-and-accurate polymerase chain reaction; ML, maximum-likelihood; MP, maximum-parsimony; mtDNA, mitochondrial DNA; MYA, million years ago; ND1–6 and 4L, NADH dehydrogenase subunits 1–6 and 4L; PCR, polymerase chain reaction.

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doi:10.1016/j.gene.2004.11.014

are extensive debates on the phylogenetic relationships and evolutionary origin of agamid groups, especially on the origin of Australasian taxa (Honda et al., 2000; Macey et al., 2000a; Schulte et al., 2003). Two major hypotheses were proposed on the origin of Australasian agamids. First, they may have originated in Early Cretaceous Gondwanaland and vicariantly separated from the Asian groups in response to the continental breakup (Moody, 1980; Macey et al., 2000a; Schulte et al., 2003). Second, they may have more recently (e.g., in the Miocene) originated from Asian ancestors through transmarine dispersal across Wallace's Line (Baverstock and Donnellan, 1990; Honda et al., 2000).

Molecular phylogenetic approaches are expected to provide a clue to answer this kind of question. However, molecular evolutionary rates usually differ from lineage to lineage and conventional methods for divergence time estimation were restricted to the data to which the molecular clock approximation can be applied. Recent advancement in the Bayesian estimation of divergence times without assuming the molecular clock (Thorne et al., 1998) has provided a tool to solve this problem.

To the best of our knowledge, complete mtDNA sequences have been reported from relatively few saurian (lizard) species (Kumazawa and Nishida, 1999; Janke et al., 2001; Kumazawa, 2004; Kumazawa and Endo, 2004), including no representative from the Acrodonta (Agamidae and Chamaeleonidae). In order to gain insights into the agamid phylogeny, as well as the mitogenomic evolution in reptiles, we set out to sequence an entire mitochondrial genome of a representative agamid species; the Australian inland bearded dragon (*Pogona vitticepes*). Unexpectedly, the *P. vitticepes* mtDNA contained two CRs that were very similar to each other in nucleotide sequence. It was also found that this feature is shared by the mtDNAs of several other Australasian agamids. Construction of molecular phylogeny, followed by divergence time estimation, allowed us to suggest the evolutionary origin of Australasian agamids, as well as the timing of the CR duplication.

## 2. Materials and methods

### 2.1. DNA Extraction, amplification and sequencing

Complete sequencing of the *P. vitticepes* mtDNA was carried out as described by Kumazawa and Endo (2004). Briefly, with the total DNA extracted from animal tissues as a template, parts of the genes for cytochrome *b* (cytb) and NADH dehydrogenase subunit 2 (ND2) were amplified and sequenced using some conserved primers. These sequences were used for designing taxon-specific primers: Pvit-L1, Pvit-L2, Pvit-H1 and Pvit-H2 (see Table 1 for their sequences and Fig. 1 for their locations) for the long-and-accurate polymerase chain reaction (LA-PCR) amplifications of spacing regions between them. The condition for the LA-PCR using an LA-Taq (Takara Shuzo) was 32 cycles of denaturation at 94 °C for 30 s and simultaneous annealing and extension at 68 °C for 15 min. Because the two CRs were not included in the same LA-PCR product, we could avoid a situation that may potentially induce the jumping PCR.

The amplified products (11.0 kbp by Pvit-L1 and Pvit-H2 and 5.7 kbp by Pvit-L2 and Pvit-H1) were purified by agarose gel electrophoresis and used as templates for nested PCRs (product length: 0.5–1.5 kb). Nested PCRs were conducted with a set of reptile-oriented primers, which had been

Table 1
Primers designed for sequencing *P. vitticepes* mtDNA in this study

| Name | Sequence (5′ to 3′) | Matching gene |
|---|---|---|
| Pvit-L1 | CCACTGACTAACAGCCTGACTAGGCCT | ND2 |
| Pvit-H1 | GTGCCGAGGATAACTCCTAGTGTGACT | ND2 |
| Pvit-L2 | CGGCCGAGGACTCTACTACGGCTCTTA | cytb |
| Pvit-H2 | CCCGTAGAAAACTTCTCGGGAGATGTAT | cytb |
| Pvit-CO1-1L | GTCAGCGCTCTTACCA | CO1 |
| Pvit-Gly-1L | GTGGCTTCCAACCACA | Gly |
| Pvit-His-1L | AGGTCGTGACCCTAAAG | His |
| Pvit-ND5-1L | GGCTCACCCGGACTAGCTA | ND5 |
| Pvit-ND5-2L | GGCCCTAAAACTAAGCCAGG | ND5 |
| Pvit-ND6-1H | TGGGGTTGCATCTAAC | ND6 |
| Pvit-Cont-1H | CCATCAACCATAGGTCTTGG | CR |
| Pvit-Cont-3H | TGAGCCTGGGGAATAT | CR |
| rAT6-4H | TAGGCTTGRATTATKGCTAC | AT6 |
| rND4-1L | TACTAATAATYGCCCAYGG | ND4 |
| rND4-2L | TTTGAAGCAACMYTAATYCCAAC | ND4 |
| rPro-1L | CATCTCTAGYCCCCAAAAC | Pro |
| rCont-1L | ATCGCACATCCCACGTGARAYCA | CR |
| rCont-2L | TTTTCCAAGGCCTCTGG | CR |
| rCont-2H | ATAACAACCAGAGGCCTTGGA | CR |
| rPhe-2H | GCATYTTCAGTGCCGTGCTT | Phe |

Primers starting with 'Pvit' are taxon-specific primers, while those starting with 'r' were designed for diverse groups of reptiles.