Short communication

The complete mitochondrial genome of an agamid lizard from the Afro–Asian subfamily agaminae and the phylogenetic position of Bufoniceps and Xenagama

J. Robert Macey a,*, James A. Schulte II b, Jonathan J. Fong a, Indraneil Das c, Theodore J. Papenfuss a

a Museum of Vertebrate Zoology, 3101 Valley Life Science Building, University of California, Berkeley, CA 94720, USA
b Department of Biology, 177 Clarkson Science Center, MRC 5805, 8 Clarkson Avenue, Clarkson University, Potsdam, NY 13699-5805, USA
c Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia

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1. Introduction

Squamate reptiles are traditionally divided into six groups: Iguania, Anguimorpha, Scincomorpha, Gekkota (these four are lizards), Serpentes (snakes), and Amphisbaenia (the so-called worm lizards). The Iguania is recognized as having two major lineages the Iguanidae and Acrodonta (Agamidae and Chamaeleonidae). Currently, there are complete mitochondrial genomes from three Anguimorpha (Kumazawa, 2004; Kumazawa and Endo, 2004), two from the Scincomorpha (Kumazawa, 2004; Kumazawa and Nishida, 1999), one from Gekkota (Macey et al., 2005), two from Serpentes (Kumazawa, 2004; Kumazawa et al., 1998) and 12 from Amphisbaenia (Kumazawa, 2004; Kumazawa et al., 1998). In addition, two representatives of the Iguanian family Iguanidae (Janke et al., 2001; Kumazawa, 2004) have been sequenced. Its’ sister taxon, the Acrodonta, consists of seven monophyletic groups—the family Chamaeleonidae and six distantly related subfamilies of the family Agamidae (Macey et al., 2005). Currently, the only acrodont lineage sequenced for the complete mitochondrial genome is Pogona vitticeps from the Australasian agamid subfamily Amphibolurinae (Amer and Kumazawa, 2005a).

Here, we report the complete mitochondrial genome of Xenagama taylori, a North African representative of the agamid subfamily Agaminae and compare it to P. vitticeps. The agamid lizard genus Xenagama is distributed in a restricted region of the Horn of Africa in northwestern Somalia and adjacent eastern Ethiopia as shown in Fig. 1, with two species currently recognized (Moody, 1980; Wermuth, 1967). In addition, we report a segment of the mitochondrial genome of Bufoniceps laungwalanensis spanning from nad1 to cox1. The monotypic genus Bufoniceps is restricted to the Thar Desert, Jaisalmer District, Rajasthan State, India and adjacent Pakistan (Fig. 1).

Both Bufoniceps and Xenagama belong to the subfamily Agaminae and are poorly understood phylogenetically. These genera were not represented in the most recent molecular systematic study of the Agamidae (Macey et al., 2000b). Bufoniceps was originally described as a member of the West Asian genus Phrynocephalus (Sharma, 1978), and later placed in its’ own genus (Arnold, 1992) because morphological data suggested it is the sister taxon to Phrynocephalus (Arnold, 1999). Xenagama was previously considered part of the Agama complex before the allocation of its member species to several genera (see, Moody, 1980).

2. Materials and methods

2.1. Specimen information

The sample of Bufoniceps laungwalanensis from which DNA was extracted is deposited in the Raffles Museum of Biodiversity Research, National University of Singapore as ZRC 2.5681. The collection locality of this specimen is elevation 192 m, 26.50.26’N 70.32.24’E, vicinity of Sam, Rajasthan State, India. The mitochondrial segment spanning

* Corresponding author. Fax: +1 510 643 8238. E-mail address: jrobertmacey@yahoo.com (J.R. Macey).
from nad1 to cox1 is deposited in GenBank as Accession No. DQ008214. The sample of X. taylori from which DNA was extracted is deposited in the California Academy of Sciences, San Francisco as CAS 225502. The collection locality of this specimen is elevation 1140 m, 9.670000°N 44.207500°E, 21 km ENE of the center of Hargeysa on Berbera Rd., then 4 km N on dirt road, Waqooyi Galbed Region, Somalia. The complete mitochondrial genome sequence from this specimen of X. taylori is deposited in GenBank as Accession No. DQ008215.

2.2. Laboratory protocols

Genomic DNA was extracted from liver using the Qiagen QIAamp tissue kit. For B. laungwalansis, amplification of genomic DNA was conducted using a denaturation at 94 °C for 35 s, annealing at 50 °C for 35 s, and extension at 70 °C for 150 s with 4 s added to the extension per cycle, for 30 cycles. Primers used are described in Macey et al. (1997a,c, 2000b). Negative controls were run on all amplifications to check for contamination. Amplified products were sheared randomly into fragments of approximately 1.5 kb by repeated passage through a narrow aperture using a Hydroshear device (GeneMachines). After end-repair, the sheared DNA was gel purified and ligated into pUC18 vector to construct a library of random fragments, then transformed into bacterial cells. Automated colony pickers introduced single clones into bacterial broth in 384-well format. These plasmid clones were processed robotically through rolling circle amplification (Dean et al., 2001; Hawkins et al., 2002), sequencing reactions, and reaction clean up using SPRI (Elkin et al., 2002). Sequences were determined using ABI3730xl DNA sequencers, then assembled to form a deep, contiguous sequence using Phrap or Sequencher.

Fig. 1. Map showing the distribution of Bufoniceps laungwalansis and Xenagama taylori. Each taxon has a limited distribution with B. laungwalansis restricted to the Thar Desert in western India and adjacent Pakistan. The two species of Xenagama are restricted to the Horn of Africa in Somalia and adjacent Ethiopia. Other members of the Agaminae range from North Africa through Arabia, Southwest Asia to Central Asia, and Tibet (not shown).
2.3. Phylogenetic analysis

DNA sequences for protein- and tRNA-encoding genes were aligned manually as in Macey et al. (2000b). Positions encoding proteins were translated to amino acids using MacClade 4.03 (Maddison and Maddison, 2001) for confirmation of alignment. Alignments of sequences encoding tRNAs were constructed based on secondary structural models (Kumazawa and Nishida, 1993; Macey and Verma, 1997). Of the 1699 characters, unalignable regions totaling 561 positions were excluded from phylogenetic analyses as in Macey et al. (2000b).

The region analyzed in Macey et al. (2000b) from nad1 to cox1 corresponds to positions 3495–5193 of the complete mitochondrial genome of X. taylori and has a length of 1699 bases; this is the region used in phylogenetic analysis. To align the new sequences with the 72 sequences analyzed in Macey et al. (2000b) a total of 268 gaps are introduced in the B. laungwalansis sequence and 266 gaps in the X. taylori sequence. These gaps are after following positions on the B. laungwalansis sequence with the number of gaps introduced in parenthesis if more than one: 86 (18), 99, 145 (2), 162 (13), 181 (2), 218 (4), 229 (11), 246 (2), 280 (4), 295 (3), 298 (12), 319 (3), 340 (3), 1105 (3), 1318 (9), 1321 (82), 1343 (3), 1369, 1380 (4), 1393 (10), 1412, 1448, 1461 (13), 1490 (3), 1507, 1515, 1528, 1553 (13), 1570 (5), 1602 (6), 1609 (6), 1625 (3), 1634, 1661 (2), and 1674 (11). These gaps are after the following positions on the complete mitochondrial genome of X. taylori with the number of gaps introduced in parenthesis if more than one: 3579 (19), 3592, 3638 (2), 3652 (16), 3670 (3), 3706 (5), 3718 (10), 3736, 3769 (6), 3782 (4), 3785 (9), 3809 (3), 3830 (3), 4595 (3), 4808 (9), 4812 (81), 4833 (4), 4859, 4870 (4), 4883 (10), 4900 (3), 4936, 4949 (13), 4978 (3), 4995, 5003, 5016, 5041 (13), 5057 (6), 5088 (6), 5096 (6), 5111 (4), 5120, 5147 (2), and 5160 (11). Sequence divergences based on this alignment were reported as uncorrected pairwise divergences.

Phylogenetic trees were inferred by parsimony using PAUP* β version 4.0b8 (Swofford, 2001) with heuristic searches featuring 100 random additions of sequences. Bootstrap resampling (Felsenstein, 1985a) was applied to assess support for individual nodes using 500 heuristic searches featuring 100 random additions of sequences. Decay indices (“branch support” of Bremer, 1994) were calculated for all internal branches using heuristic searches featuring 100 random additions of sequences in searches that retained suboptimal nodes.

To test specific, alternative phylogenetic hypotheses, we first built incompletely resolved constraint trees using MacClade (Maddison and Maddison, 2001). These were provided as input into PAUP* (Swofford, 2001) for heuristic searches featuring 100 random additions of sequences to determine the most parsimonious tree compatible with each alternative hypothesis. We then compared these to the unconstrained most parsimonious tree using Wilcoxon signed-ranks tests (Templeton, 1983). This test determines whether the most parsimonious tree is significantly shorter than each alternative or whether their differences in length are statistically indistinguishable. Wilcoxon signed-ranks tests were conducted as one-tailed tests (Felsenstein, 1985b) using PAUP* (Swofford, 2001), which incorporates a correction for tied ranks. Felsenstein (1985b) showed that one-tailed probabilities are close to the exact probabilities for this test but not always conservative. The two-tailed probabilities are simply double the one-tailed probabilities and the two-tailed test is always conservative (Felsenstein, 1985b).

3. Results

3.1. Mitochondrial genomic structure

The complete mitochondrial genome of X. taylori is 16,220 bp in length. This genome contains the same 37 genes common among animals but differs from the order as is most commonly found for vertebrates (Boore, 1999). As previously reported for Acrodonta (Agamidae and Chamaeleonidae), both B. laungwalansis and X. taylori have trnL and Q switched to yield the order nad1, trnQ, I, and M (Macey et al., 1997c, 2000a). These taxa lack any duplicated genes or secondary structure of non-coding sequences between trnQ and I as has been identified in some species of the agamid genus Uromastyx (Amer and Kumazawa, 2005b). Both taxa have trnC that encodes a transfer RNA which lacks a D-stem and instead contains a D-arm replacement loop (Macey et al., 1997b), as is typ- ical for the Acrodonta (Macey et al., 2000a). In addition, these taxa have atypical stem-loop structures between trnN and trnC where light-strand replication is thought to usually initiate for vertebrate mtDNAs, which is also observed in other members of the Agaminae clade (Macey et al., 2000a). In particular, the 3’-GCC-5’ heavy strand template sequence identified as the point of light-strand elongation in mouse (Brennicke and Clayton, 1981) is not present in these structures. B. laungwalansis has a five base stem with a 16 base loop, whereas X. taylori has a nine base stem with an eight base loop. As observed in most other vertebrates, the mt-genome of X. taylori has a large noncoding region presumed to be the Control Region (CR) of 1449 bp in length. This differs from that of the amphibolurine P. vitticeps which has a near identical second noncoding region inserted between nad5 and nad6 (Amer and Kumazawa, 2005a). Unlike previously reported mt-genomes among vertebrates (but see, McKnight and Shaffer, 1997) the noncoding region in X. taylori is between trnP and trnF and not between trnP and trnF, as is typical and observed for one CR copy in P. vitticeps (Amer and Kumazawa, 2005a). An additional 26 bases separate trnP and trnF in X. taylori. Therefore, X. taylori has the complete mt-order of trnF, rrnS, trnV, rrnL, trnL(taa), nad1, trnQ, I, M, nad2, trnW, A, N, C, stem-loop, trnY, cox1, trnS(tga), D, cox2, trnK, atp6, atp8, cox3, trnG, nad3, trnR, nad4L, nad4, trnH, S(tct), L (tag), nad5, nad6, trnE, cob, trnT, CR, and trnP.
3.2. Phylogenetic relationships

Phylogenetic analysis of the 1434 aligned positions (1046 informative) for the 72 taxa in Macey et al. (2000b) and the two newly reported sequences from nad1 to cox1 produces seven equally most parsimonious trees (Fig. 2). The tree is largely the same as that reported by Macey et al. (2000b) with the exception of a few weak nodes that are collapsed in this analysis because of the four additional equally parsimonious trees. Here, we concentrate on the phylogenetic relationships of the Agaminae, which is monophyletic with a bootstrap of 100% and decay index of 27. The strict consensus tree yields nine lineages in the Agaminae labeled A–I in Fig. 2. The African genus Agama (sensu stricto) appears monophyletic with strong support (A in Fig. 2, bootstrap 100%, decay index 97). Pseudotrapelus sinaitus of Arabia, Egypt, and Libya groups with X. taylori from the Horn of Africa and is well-supported (B in Fig. 2, bootstrap 99%, decay index 17). B. laungwalansis, restricted to the Indian Subcontinent, forms the sister taxon to the wide-ranging genus Trapelus with considerable support (C in Fig. 2, bootstrap 100%, decay index 22). Laudakia sacra of Tibet forms its own deep-lineage (D in Fig. 2). Laudakia nupta of the Iranian Plateau groups weakly with Laudakia tuberculata of the Himalaya (E and F in Fig. 2, bootstrap 57%, decay index 2). Laudakia stellio of Anatolia and the Levant forms its own deep-lineage (G in Fig. 2). The Asian genus Phrynocephalus is well supported (H in Fig. 2, bootstrap 100%, decay index 41). A well supported clade of Laudakia ranging from the Iranian Plateau to Mongolia is present (I in Fig. 2, bootstrap 100%, decay index 20).

The Wilcoxon-signed-rank test (Felsenstein, 1985b; Templeton, 1983) is applied to compare the most parsimonious tree from these nucleotide sequences with alternative hypotheses. The genus Bufoniceps has been previously suggested to be either in the genus Phrynocephalus or the sister taxon to Phrynocephalus. The seven shortest alternative trees that unite Bufoniceps with Phrynocephalus require 55 extra steps and are rejected in favor of the unconstrained shortest trees ($P < 0.0046$). The genus Xenagama has been previously suggested to be related to Agama. The two shortest alternative trees that unite Xenagama with Agama require 30 extra steps and are not rejected in favor of the unconstrained shortest trees ($P < 0.0861$).

4. Discussion

4.1. Biogeography and the breakup of Gondwana

Acrodont lizards (Agamidae and Chamaeleonidae) are of Gondwanan origin (Macey et al., 2000b). Clades of agamid lizards rafted with alternative fragments of Gondwana which collided with the southern margin of Asia. The subfamily Agaminae arrived in Asia either with the Indian Subcontinent 50 MYBP (million years before present) or with Afro-Arabia 18 MYBP.

Bufoniceps is found to be the sister taxon to Trapelus and is statistically rejected as the sister taxon to Phrynocephalus as previously suggested (Arnold, 1999). Note that Bufoniceps and Trapelus share the morphological character of an open ear, and it is Phrynocephalus that has the derived feature of a closed ear (Arnold, 1999). The genus Trapelus ranges from North Africa across Arabia, through the Iranian Plateau and Caspian Basin to the western edge of the Indian Subcontinent in the vicinity of the range of Bufoniceps. Bufoniceps is restricted to a small region of the Thar Desert on the western edge of the Indian Subcontinent. The tree presented in Macey et al. (2000b) was suggestive of an Afro-Arabian origin for the Agaminae but the alternative of an origin in the Indian Subcontinent could not be rejected. The analysis presented here is equivocal for an origin in either region because of a basal polytomy. The fact that the sister taxon to a major clade of the Agaminae is an Indian endemic raises the question of a possible origin of the Agaminae in the Indian Subcontinent. Further work is needed to resolve this issue, perhaps with phylogenetic analysis of complete mitochondrial genomes.
**Table 1**

Sequence divergences across taxa

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* Values above the dashed line are uncorrected pairwise distances and those below are uncorrected transversional distances. Note genera are abbreviated as P., Pseudotrapelus; X., Xenagama; B., Bufoniceps; and T., Trapelus.

**Acknowledgments**

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**References**


