Recent maternal divergence of the parthenogenetic lizard
Leiopelis guentherpetersi from L. guttata: molecular evidence
(Reptilia: Squamata: Agamidae)

With 2 figures and 1 table

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Abstract. The genus Leiopelis consists of bisexual as well as parthenogenetic forms. In this study we use DNA sequences of the mitochondrial 16S rRNA gene to examine the interspecific relationships of the Leiopelis species occurring in Vietnam and adjacent areas. L. guentherpetersi was consistently placed as the sister species of L. guttata. Differentiation between these two taxa consisted of only two indels, corresponding to 0.372 % pairwise sequence divergence. This extremely low differentiation supports a rather recent maternal origin of the parthenogenetic L. guentherpetersi from L. guttata in the Pleistocene.


Key words. Reptilia, Squamata, Agamidae, Uromastyclinae, Leiopelis, parthenogenetic species, 16S rRNA, phylogeny, Vietnam.

1. Introduction

Butterfly lizards (genus Leiopelis Clavigero, 1820) consist of four bisexual species: L. belliana (Gray, 1827), L. guttata Clavigero, 1820, L. pygmaea Peters, 1871, and L. reinerti (Gray, 1821), as well as of three parthenogenetic species: L. belliana Dobrovsky & Kuznetzova, 1963, L. guentherpetersi Dobrovsky & Kuznetzova, 1963, and L. guentherpetersi Dobrovsky & Kuznetzova, 1963. The species L. belliana and L. reinerti are polytypic with the subspecies L. b. belliana (Gray, 1827), and L. belliana olivaceata Peters, 1871, respectively L. r. reinerti (Gray, 1821), and L. r. reinerti olivaceata Peters, 1871.

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Together with its sister genus *Unimastix* (MOORE 1898, BOUHIN 1888, JEGER 1961) *Leleupis* is included in the family *Unimastictidae* (MOORE 1872, EICHHORN 1864) or in a subfamily *Unimastictinae* in the Agamidae (LAM.BEUKEMA & MOORE 1994). According to PRUD' HOMME & KUPINIAKOVA (1990) this subfamily has to be named *Leleupini*.

MACKEY et al. (1998) even proposed to raise each of the two genera to subfamily rank within the Agamidae. They also found the Characophylidae to be the closest sister group to the Agamidae.

Within *Leleupis*, several hypotheses on the origin of the parthenogenetic species have been drawn. It is generally assumed that these species evolved by natural hybridization (DUESEY et al. 1984). *Leleupis leleupis* is thought to have originated from individuals of the diplaid parthenogenetic *Leleupis bohu Midwest. L. bellara (BOUHIN 1882) while the origin of the species *L. bohu* itself is still unclear (DUESEY & KUPINIAKOVA 1990).

The parthenogenetic *L. guentheri* has been considered as having originated from the geographically neighboring bisexual species *L. boucheti* and *L. guentheri* (DUESEY & KUPINIAKOVA 1990). In the present paper we provide molecular data on the interspecific relationships of *Leleupis* species occurring in Vietnam and adjacent areas, to test the maternal origin of the only parthenogenetic species in this region, *L. guentheri*.

2. Material and Methods

**Specimens.** - Samples were taken from the following species: *Leleupis leleupis* (ZFMK 70230), Chinh Rinh, Vietnam; *L. guentheri* (ZFMK 70231), Thu Duc, Vietnam; *L. boucheti* (ZFMK 70237), Langvand, Malaysia; *L. bellara* (ZFMK 70235), Cua Leo, Vietnam; *Unimastix neomurphii* (ZFMK unregistered), Tanzania; pet trade; *L. occulta* (ZFMK unregistered), Madagascar; and the runway *Oplurus curviset* (no voucher preserved). Kimnyu Madagasgabe, were used as outgroups.

**Genetic analysis.** - Extraction methodology and PCR procedures and subsequent alignment steps followed MAVLESD et al. (2000). We used the primers 165A (forward: 5'-CCAGTATCGATACGAAACTTTGTA-3') and 165B (reverse: 5'-AACTCCATGATTCTTACAAG-3') of PULLUM et al. (2000) to amplify a section of the mitochondial 16S tRNA gene. The obtained sequences (lengths referring to the aligned sequences including gaps) comprised 357 bp, homologous to positions 985–1336 of the *Noboa porsani* (NCBI accession numbers RUCM652365). Five short sections (together 96 bp) from the original 357 bp data set were too variable to be reliably aligned and were therefore excluded from analysis. Sequences have been submitted to GenBank (accession numbers AP252968, AF370574, AP370554).

The presence of a significant phylogenetic signal was estimated using the g1 statistic (Hillis & HILGARDER 1992) estimated from 100,000 randomly generated parsimony trees, and the permutation-tailed probability (PTP) test with 100 replicates, both implemented in PAUP*.

Using PAUP* 4.0a (SWOFFORD 1998) we applied parsimony and maximum likelihood phylogenetic reconstruction methods which are two of the most consistent and accurate methods available (HILLIS & HILGARDER 1992). For maximum parsimony (MP) we used the branch-and-bound search option with parsimony informative sites included. The maximum likelihood (ML) analysis employed the HKY + G model which uses nucleotide frequencies estimated from the data, codes for unequal base frequencies and allows for variation in rates of substitution among sites (HARLOW et al. 1996). The values were estimated using MODELTEST 3.04 (Posada & CRANDALL 1998); the following parameters were used: transition/transversion ratio = 2.1947; base frequencies A = 0.3252, C = 0.2576, G = 0.1862, T = 0.2323; with a gamma distribution shape parameter of 0.5806. The ML tree was obtained using the heuristic search option with random addition sequences with 10 replicates, and the tree bisection-reconnection (TBR) branch-swapping. Relative branch support in each phylogenetic analysis was evaluated with 1000 bootstrap pseudoreplicates for ML and 2000 pseudoreplicates for PAUP* analysis. We considered bootstrap values of 80% as giving strong support to the respective node (Hillis & Bull 1999).

3. Results and Discussion

Four hundred and forty-one aligned sites in the studied 16S data set for the 8 species were used in the phylogenetic analysis. Of these, 155 characters were variable, and 96 (21.77%) were parsimony-informative. The data set was strongly left-skewed (g1 = -1.824916) which indicates strong phylogenetic signal. The PTP test resulted in a significant difference (p = 0.00) between the most parsimonious tree and trees generated from random permutations of the data matrix, demonstrating presence of significant phylogenetic signal.
A second monophyletic group included all species of Leptosephus (100 % and 99 %). L. guentheri (100%) was consistently placed as the sister species of L. guttata with maximum bootstrap support (100 % and 99 %). Leptosephus bellus appeared as sister species of L. retusus with only weak support in the MP tree (62 %) while in the ML tree L. bellus was placed in a position basal to all other Leptosephus, with even lower bootstrap support (< 50 %), and is therefore collapsed into a polytomy in the bootstrap consensus tree in Fig. 2.

While the pairwise sequence divergence in the analyzed fragment (after exclusion of ambiguous alignment sites; Table 1) between most Leptosephus species was in the same order of magnitude as between both Uromastax (3.0–6.5 % uncorrected ‘d’ distance, corresponding to 13–26 substitutions), the divergence between L. guentheri and L. guttata was extremely low (not a single substitution when indels are not considered). In fact, the sequence of L. guentheri differed from that of L. guttata only by the presence of two additional adenine nucleotides (rendering a total difference of only 0.372 %). As these nucleotides were also present in the possible parental taxon L. retusus, we consider their deletion as an autapomorphy of L. guttata. Even taking the complete sequences into account (including the variable regions excluded in the above analysis), no further differences between L. guttata and L. guentheri could be detected.

Although the rRNA genes are considered to evolve rather slowly as compared with protein-coding mitochondrial genes (Avise 2000), all reptile calibrations available to us assume rates of at least 0.3 % total pairwise sequence divergence per million years (my). More often, the calibrations used result in rates of about 0.4–0.5 % (e.g., those of Rasnitsyn 1997). The reliable calibration of this molecular clock is at present available for species of Leptosephus, and a conservative approach, there are no reasons to assume that it proceeds much slower than 0.3 %/my. Applying this rate, the divergence between L. guentheri and L. guttata was placed at 1.24 million years. However, actual rates in Leptosephus may be faster, and the divergence therefore significantly younger. In any case, the extremely low differentiation between both taxa suggests a young divergence, which most probably occurred in the Pleistocene. This hypothesis is also in accordance with the geographical proximity of the known distribution areas of the two species.

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