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

With 2 figures and 1 table

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Abstract. The genus *Leiolepis* 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 *Leiolepis* 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.

Kurzfassung. **Rezente Abspaltung der parthenogenetischen Agame *Leiolepis guentherpetersi* vom maternalen Taxon *L. guttata*: molekulare Belege (Reptilia: Squamata: Agamidae)**. – Die Gattung *Leiolepis* enthält sowohl bisexuelle als auch parthenogenetische Formen. In dieser Arbeit untersuchen wir die zwischenartlichen Beziehungen der Vertreter der Gattung *Leiolepis* in Vietnam und den angrenzenden Regionen mittels DNA-Sequenzanalysen des mitochondrialen 16S-rRNA-Gens. In allen verwendeten Analysemethoden wurde *L. guentherpetersi* als Schwesterart von *L. guttata* identifiziert. Sequenzunterschiede zwischen diesen beiden Taxa fanden sich nur in Form von zwei Deletionen bei *L. guttata*, was einer paarweisen Differenzierung von 0.372 % entspricht. Dieser extrem niedrige Wert weist auf einen sehr jungen, vermutlich pleistozänen maternalen Ursprung der parthenogenetischen *L. guentherpetersi* aus *L. guttata* hin.

Key words. Reptilia, Squamata, Agamidae, Uromastycinae, *Leiolepis*, parthenogenetic species, 16S rRNA, phylogeny, Vietnam.

1. Introduction

Butterfly lizards (genus *Leiolepis* Cuvier, 1829) consist of four bisexual species: *L. belliana* (Gray, 1827), *L. guttata* Cuvier, 1829, *L. peguensis* Peters, 1971, and *L. reevesii* (Gray, 1831), as well as of three parthenogenetic species: *L. boehmei* Darevsky & Kupriyanova, 1993, *L. guentherpetersi* Darevsky & Kupriyanova, 1993, and *L. triploida* Peters, 1971. The species *L. belliana* und *L. reevesii* are polytypic with the subspecies *L. b. belliana* (Gray, 1827), and *L. belliana ocellata* Peters, 1971, respectively *L. r. reevesii* (Gray, 1831), and *L. reevesii rubritaeniata* Mertens, 1961.

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Together with its sister genus *Uromastyx* (MOODY 1980, BÖHME 1988, JOGER 1991) *Leiolepis* is included in the family Uromastycidae (MOODY l. c., BÖHME 1982) or in a subfamily Uromastycinae in the Agamidae (BORSUK-BIALYNICKA & MOODY 1984). According to FROST & ETHERIDGE (1989) this subfamily has to be named Leiolepidinae.

MACEY et al. (2000) even proposed to rise each of the two genera to subfamily rank within the Agamidae. They also found the Chamaelonidae to be the closest sister group to the Agamidae.

Within *Leiolepis*, several hypotheses on the origin of the parthenogenetic species have been drawn. It is generally assumed that these species evolved by natural hybridisation (DAREVSKY et al. 1984). *Leiolepis triploida* is thought to have originated from individuals of the diploid parthenogenetic *Leiolepis boehmei* and *L. belliana* (BÖHME 1982) while the origin of the species *L. boehmei* itself is still unclear (DAREVSKY & KUPRIYANOVA 1993).

The parthenogenetic *L. guentherpetersi* has been considered as having originated from the geographically neighbouring bisexual species *L. reevesii* and *L. guttata* (DAREVSKY & KUPRIYANOVA l. c.). In the present paper we provide molecular data on the interspecific relationships of *Leiolepis* species occurring in Vietnam and adjacent areas, to test the maternal origin of the only parthenogenetic species in this region, *L. guentherpetersi*.

2. Material and Methods

Specimens. – Samples were taken from the following species: *Leiolepis guttata* (ZFMK 70281), Cam Ranh, Vietnam; *L. guentherpetersi* (ZFMK 70275), Thuy Phu, Vietnam; *L. belliana* (ZFMK 70297), Langkawi, Malaysia; *L. r. reevesii* (ZFMK 70250), Cua Lo, Vietnam; *Uromastyx acanthinura* (ZFMK uncatalogued), Tunisia, pet trade and *U. ocellata* (ZFMK uncatalogued), Sudan, pet trade. The chameleon *Furcifer minor* (ZFMK uncatalogued), Madagascar, and the iguanid *Oplurus cuvieri* (no voucher preserved), Kirindy, Madagascar, were used as outgroups.

Genetic analysis. – Extraction methodology and PCR procedures and subsequent alignment steps followed MAUSFELD et al. (2000). We used the primers 16SA (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16SB (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3') of PALUMBI et al. (1991) to amplify a section of the mitochondrial 16S ribosomal RNA gene. The obtained sequences (lengths referring to the aligned sequences including gaps) comprised 537 bp, homologous to positions 4005-4588 of the *Xenopus laevis* mitochondrial genome (ROE et al. 1985). Five short sections (together 96 bp) from the original 16S data set were too variable to be reliably aligned and were therefore excluded from analysis. Sequences have been submitted to GenBank (accession numbers AF215260, AF378374 –AF378380).

The presence of a significant phylogenetic signal was estimated using the g1 statistic (HILLIS & HUELSENBECK 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*4.0b8 (SWOFFORD 1998) we applied parsimony and maximum likelihood phylogenetic reconstruction methods which are two of the most consistent and accurate methods available (HUELSENBECK & HILLIS 1993). For maximum parsimony (MP) we used the branch-and-bound search option with parsimony uninformative sites included. The maximum likelihood (ML) analysis employed the HKY+G model which uses nucleotide frequencies estimated from the data, corrects for unequal base frequencies and allows for variation in rates of substitution among sites (HASEGAWA et al. 1995). The values were estimated using MODELTEST 3.04 (POSADA & CRANDALL 1998); the following parameters were used: transition/transversion ratio = 2.1947; base frequencies A = 0.3524, C = 0.2358, G = 0.1886, T = 0.2232, with a gamma distribution shape parameter of 0.3588. The ML tree was obtained using the heuristic search option, random addition sequences with 10 replications, and tree bisection reconnection (TBR) branch-swapping. Relative branch support in each phylogenetic analysis was evaluated with 2000 bootstrap pseudoreplicates for MP and 2000 pseudoreplicates for ML analysis. We considered bootstrap values of 80% as giving strong support to the respective node (HILLIS & BULL 1993).

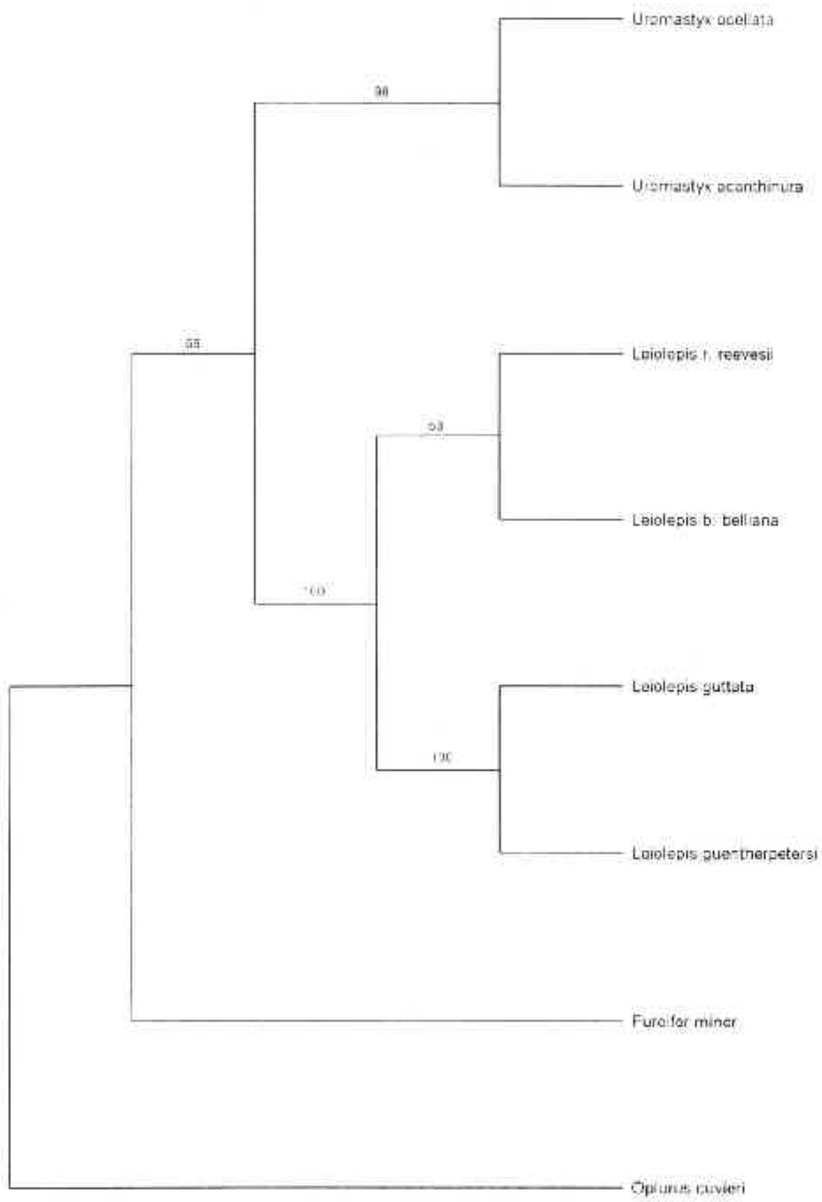

Fig. 1: Maximum parsimony cladogram (MP) obtained from PAUP* searches using *Furcifer minor* and *Oplurus cuvieri* as outgroups. Numbers at the nodes represent bootstrap proportions in percent for 2000 pseudoreplicates. Bootstrap proportions lower than 50 % are not shown.

3. Results and Discussion

Four hundred and fourty-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 = –1.824919) which indicates strong phylogenetic signal. The PTP test resulted in a significant difference (p = 0.01) between the most parsimonious tree and trees generated from random permutations of the data matrix, demonstrating presence of significant phylogenetic signal.

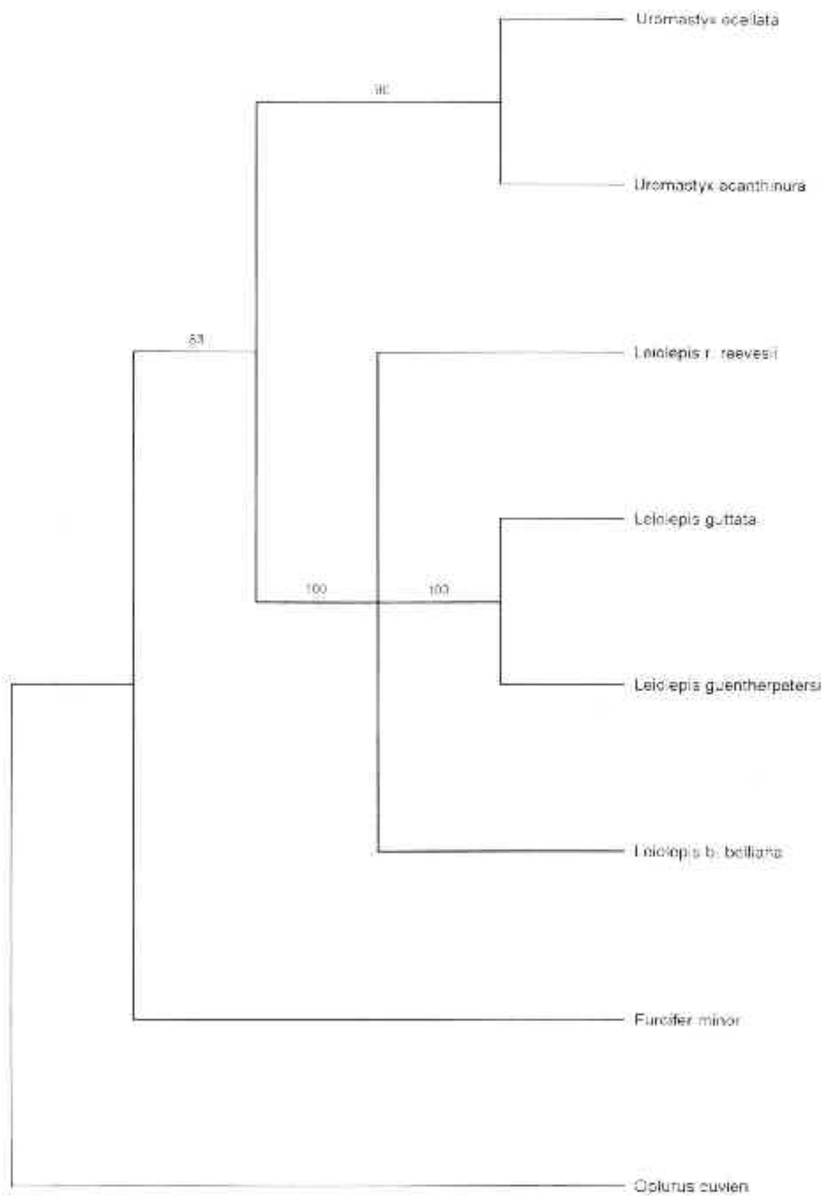

Fig. 2: Maximum likelihood bootstrap consensus cladogram (ML) obtained from PAUP* searches using *Furcifer minor* and *Oplurus cuvieri* as outgroups. Numbers at the nodes represent bootstrap proportions in percent for 2000 pseudoreplicates. Bootstrap proportions lower than 50 % are not shown.

Both tree building methods recovered very similar topologies. In the MP analysis only one most parsimonious tree (fig. 1) was found with a tree length of 232 steps. Consistency index, rescaled consistency index and retention index (FARRIS 1989) were 0.8276, 0.6274 and 0.7695, respectively.

The ingroup (consisting of both *Uromastyx* and *Leiolepis*) received an only moderate bootstrap support in the MP tree (69 %) while in the ML tree the bootstrap support was strong (83 %). The two *Uromastyx* species resulted as a highly supported monophylum (98 % and

Table 1: Summary of the absolute number of DNA-sequence differences (above the diagonal) and HKY'85 corrected genetic distances below.

		1	2	3	4	5	6	7	8
1	*Uromastyx ocellata*	-	26	80	76	84	84	76	86
2	*Uromastyx acanthinura*	0.06345	-	81	69	74	74	70	84
3	*Oplurus cuvieri*	0.22913	0.22736	-	81	90	90	86	87
4	*Leiolepis r. reevesii*	0.20565	0.17434	0.22569	-	22	22	18	100
5	*Leiolepis guttata*	0.23325	0.20029	0.25719	0.05377		0	28	104
6	*Leiolepis guentherpetersi*	0.23325	0.20029	0.25719	0.05377	0.00000	-	28	104
7	*Leiolepis b. belliana*	0.21624	0.18613	0.23862	0.03086	0.06881	0.06881	-	100
8	*Furcifer minor*	0.23769	0.23129	0.24508	0.28583	0.30037	0.30037	0.28498	-

90 %). A second monophyletic group included all species of *Leiolepis* (100 % and 100 %). *L. guentherpetersi* was consistently placed as the sister species of *L. guttata* with maximum bootstrap support (100 % and 100 %). *Leiolepis belliana* appeared as sister species of *L. reevesii* with only weak support in the MP tree (63 %) while in the ML tree *L. belliana* was placed in a position basal to all other *Leiolepis* included 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 analysed fragment (after exclusion of ambiguous alignment sites; table 1) between most *Leiolepis* species was in the same order of magnitude as between both *Uromastyx* (3.0–6.5 % uncorrected "p"-distance, corresponding to 13–28 substitutions), the divergence between *L. guentherpetersi* and *L. guttata* was extremely low (not a single substitution when indels are not considered). In fact, the sequence of *L. guentherpetersi* differed from that of *L. guttata* only by the presence of two additional adenine nucleotides (meaning a total difference of only 0.372 %). As these nucleotides were also present in the possible parental taxon *L. reevesii*, 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. guentherpetersi* 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 RASSMANN 1997). No reliable calibration of the molecular clock is at present available for species of *Leiolepis*, but in a rough and conservative approach, there are no reasons to assume that it proceeds much slower than 0.3 % / my. Applying this rate, the divergence between *L. guentherpetersi* and *L. guttata* would be placed at 1.24 million years. However, actual rates in *Leiolepis* 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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