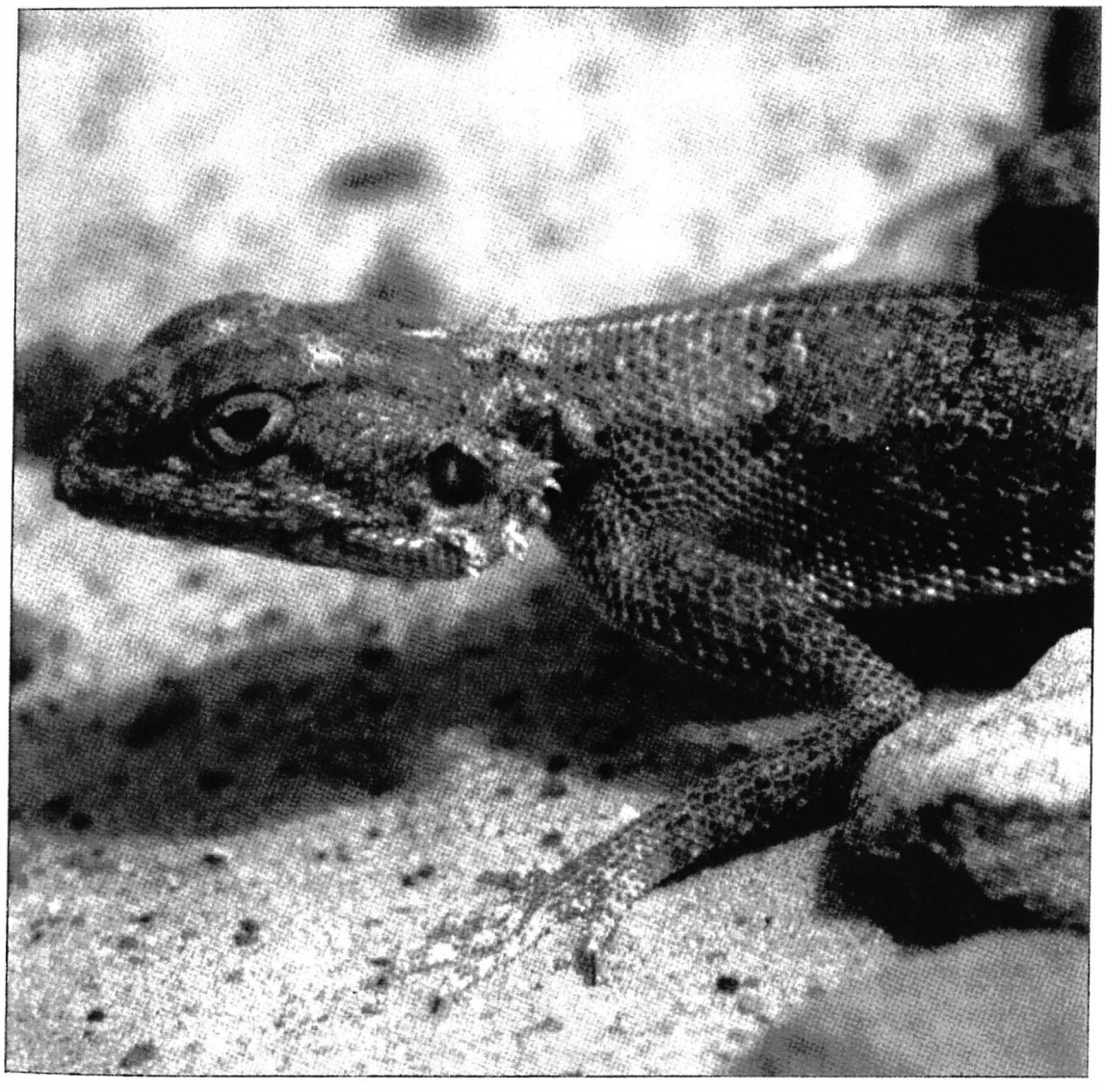
Volume 15, Number 1

January 2005
ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

The Herpetological Journal is published quarterly by the British Herpetological Society and is issued free to members. Articles are listed in *Current Awareness in Biological Sciences*, *Current Contents*, *Science Citation Index* and *Zoological Record*.

Applications to purchase copies and/or for details of membership should be made to the Hon. Secretary, British Herpetological Society, The Zoological Society of London, Regent's Park, London NW1 4RY, UK.

Instructions to authors are printed inside the back cover. All contributions should be addressed to the Scientific Editor (address below).

BRITISH HERPETOLOGICAL SOCIETY

Herpetological Journal website: http://biology.bangor.ac.uk/~bss166/HJ

FRONT COVER: Male *Agama castroviejoi* (J. M. Padial)

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 1-8 (2005)

PHYLOGENETIC RELATIONSHIPS AMONG CHINESE RANIDS INFERRED FROM SEQUENCE DATA SET OF 12S AND 16S rDNA

JIANPING JIANG[1,2] AND KAIYA ZHOU[1]

[1]*Institute of Genetic Resources, Nanjing Normal University, Nanjing 210097, China*

[2]*Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China*

Phylogenetic relationships among twenty-nine species of Ranidae representing thirteen genera were investigated on the basis of 1005 base pairs of mitochondrial DNA sequences of 12S and 16S rRNA gene fragments. Sequence data were analyzed using maximum parsimony, likelihood maximum, and neighbour joining with all indel and missing/ambiguous sites deleted. Among the twenty-nine ranids studied, two clades are well supported by the results of the three analyses, the first consists of twenty-one species in the genera *Rana*, *Glandirana*, *Rugosa*, *Pelophylax*, *Amolops*, *Odorrana*, and *Hylarana*; and the second includes eight species in the genera *Fejervarya*, *Hoplobatrachus*, *Paa*, *Nanorana*, *Altirana*, and *Limnonectes*; the six genera with multi-species samples – including *Amolops*, *Pelophylax*, *Rugosa*, *Rana*, *Odorrana*, and *Paa* – are recognized as distinct lineages with higher bootstrap and quartet puzzling supports: the phylogenetic relationships between species within each lineage are resolved well. The results testify that the traditional genus *Rana* is heterogeneous. On the basis of the phylogenetic relationships of these taxa, it is suggested that the genera *Paa*, *Nanorana*, and *Altirana* should be removed from the subfamily Raninae and to be included in the subfamily Dicroglossinae. The torrent frog of the genus *Amolops* should be retained in the subfamily Raninae rather than in a distinct subfamily Amolopinae of its own. The inclusion of *Fejervarya limnocharis* in the genus *Limnonectes* is not supported.

Key words: China, molecular systematics, mtDNA, Ranidae

INTRODUCTION

With over 700 species, the family Ranidae is one of the most species-rich amphibian families. It is distributed throughout the world, except southern South America and most of Australia (Frost, 1985, Duellman, 1993). There are a few reports on the relationships of groups from lineages or regions, based on morphological and (or) molecular data sets, such as Boulenger (1920), Liu & Hu (1961), Wallace *et al.* (1973), Emerson *et al.* (1993), Marmayou *et al.* (2000), Emerson *et al.* (2000*a*), Sumida *et al.* (2003). Nevertheless, the taxonomy of the Ranidae is still very problematic (Dubois, 1999) because the phylogenetic relationships within the family are still poorly known (Duellman & Trueb, 1985).

On the basis of a phenetic analysis, Dubois (1992) placed the species in the family into seven subfamilies, 88 genera and subgenera including some new genera, (e.g. *Paa*) and reintroduced some genera previously named (e.g. *Limnonectes*, *Hoplobatrachus*). According to the classification of Dubois (1992), Chinese ranids should be grouped into two subfamilies – Dicroglossinae and Raninae – while Fei *et al.* (1990) classed them into three subfamilies – Raninae, Amolopinae Yang, 1989, and Occidozyginae Fei, Ye *et* Huang, 1990. In the subfamily Raninae, all the new genera proposed by Fei *et al.* (1990), including *Pseudorana*, *Rugosa*, *Glandirana*, and *Odorrana*, were treated as subgenera of the genus *Rana* by Dubois (1992), so were the genera reintroduced by Fei *et al.* (1990), including *Pelophylax*, *Hylarana*, and *Rana* consisting of only brown frogs. Zhao (1994, 1995) and Inger (1996) criticised the new taxonomy proposed by Fei *et al.* (1990) and Dubois (1992). Despite this, Dubois's classification is already being adopted in influential works, for example, *Additions and Corrections to Amphibian Species of the World* (Duellman, 1993) and *Amphibian Species of the World* (ver. 2.21 online, Frost, 2002).

The purpose of the present work was therefore threefold: (1) to clarify the phylogenetic relationships between some groups of the family Ranidae; (2) to test heterogeneity of the traditional genus *Rana*; (3) to further discuss the systematic issues of some ranid groups proposed by Dubois (1992) and Fei *et al.* (1990). All the topics will be addressed here using the sequence data set of 16S and 12S mitochondrial DNA.

MATERIALS AND METHODS

SPECIES STUDIED

Twenty-nine species (Table 1) representing 13 genera were examined. Tissue samples were all derived from thigh muscle preserved in either ethanol (95%) or in refrigerator (-20°C). Specimens were kept in the herpetological collection of the Institute of Genetic Resources, Nanjing Normal University (NJNU) and

Correspondence: K. Zhou, Institute of Genetic Resources, College of Life Sciences, Nanjing Normal University, Nanjing 210097, China. *E-mail:* kyzhounj@126.com

TABLE 1. Species studied in the present study.

Family	Subfamily (Fei *et al.*, 1990)	Subfamily (Dubois, 1992)	Genus (Fei *et al.*, 1990) (Fei, 1999)	Genus (Dubois, 1992)	Traditional Genus (Frost, 1985)	Species	Locality of collection
OUTGROUP							
Bufonidae			*Bufo*		*Bufo*	*gargarizans*	Nanjing, Jiangsu
Microhylidae			*Kaloula*		*Kaloula*	*pulchra*	Zhaoan,Fujian
Hylidae			*Hyla*		*Hyla*	*chinensis*	Zhaoan, Fujian
INGROUP							
Ranidae	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*chensinensis*	Yuzhong, Gansu
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*amurensis*	Mudanjiang, Heilongjiang
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*zhenhaiensis*	Zhenhai, Zhejiang
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*omeimontis*	Emei Mt., Sichuan
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*chaochiaoensis*	Muli, Sichuan
	Raninae	Raninae	*Pelophylax*	*Rana*	*Rana*	*nigromaculata*	Huoqiu, Anhui
	Raninae	Raninae	*Pelophylax*	*Rana*	*Rana*	*hubeiensis*	Huoqiu, Anhui
	Raninae	Raninae	*Hylarana*	*Rana*	*Rana*	*adenopleura*	Nanjing, Fujian
	Raninae	Raninae	*Hylarana*	*Rana*	*Rana*	*guentheri*	Guangzhou, Guangdong
	Raninae	Raninae	*Rugosa*	*Rana*	*Rana*	*emeljanovi*	Dalian, Liaoning
	Raninae	Raninae	*Rugosa*	*Rana*	*Rana*	*tientaiensis*	Tianmushan,Zhejiang
	Raninae	Raninae	*Glandirana*	*Rana*	*Rana*	*minima*	Fuzhou, Fujian
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*margaretae*	Wawushan, Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*livida*	Hejiang, Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*schmackeri*	Emei Mt., Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*hejiangensis*	Hejiang, Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*grahami*	Muli, Sichuan
	Raninae	Raninae	*Paa*	*Paa*	*Rana*	*boulengeri*	Wawushan, Sichuan
	Raninae	Raninae	*Paa*	*Paa*	*Rana*	*spinosa*	Tianmushan, Zhejiang
	Raninae	Raninae	*Paa*	*Paa*	*Rana*	*robertingeri*	Hejiang, Sichuan
	Raninae	Dicroglossinae	*Fejervarya*	*Limnonectes*	*Rana*	*limnocharis*	Nanjing, Jiangsu
	Raninae	Dicroglossinae	*Limnonectes*	*Limnonectes*	*Rana*	*fujianensis*	Nanjing, Fujian
	Raninae	Dicroglossinae	*Hoplobatrachus*	*Hoplobatrachus*	*Rana*	*rugulosus*	Haikou, Hainan
	Raninae	Raninae	*Nanorana*	*Nanorana*	*Nanorana*	*pleskei*	Songpan, Sichuan
	Raninae	Raninae	*Altirana*	*Nanorana*	*Altirana*	*parkeri*	Lasha, Xizang
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*daiyunensis*	Nanjing, Fujian
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*ricketti*	Hejiang, Sichuan
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*wuyiensis*	Huangshan, Anhui
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*mantzorum*	Wawushan, Sichuan

Chengdu Institute of Biology, Chinese Academy of Sciences (CIB). Taxonomic assignment of examined species follows Fei *et al.* (1990) and Fei (1999), and the other two different classifications are also presented in Table 1.

CHOICE OF THE OUTGROUP

The family Ranidae is a member of the superfamily Ranoidea (Hay *et al.*, 1995; Emerson *et al.*, 2000*b*). Hay *et al.* (1995) reported that the Mantellidae was the sister group to the Ranidae, and they formed a sister group to the clade consisting of the Microhylidae and Hyperoliidae. Emerson *et al.* (2000*b*) indicated that the mantelline frogs may be nested within the Rhacophoridae; Dubois (1992) and Marmayou *et al.* (2000) suggested that the rhacophorids should be a lineage of the Ranidae. Since the relationships among the families mentioned above have not been resolved so far, three species of the three families Microhylidae, Bufonidae, and Hylidae respectively (see Table 1) were used as outgroup in this study.

DNA AMPLIFICATION AND SEQUENCING PROTOCOLS

Whole genomic DNA was extracted from alcohol-preserved or frozen tissue samples of thigh muscle using standard proteinase K/SDS digest extraction method followed by phenol-chloroform isolation and ethanol precipitation. Two regions of the mtDNA 12S and 16S rRNA genes were amplified and sequenced using the following protocols. Double stranded fragments were amplified in 35 cycles of PCR: 95°C for 30 s , 50-58°C for 30 s, 72°C for 60 s. It was pre-denatured at 95°C for

4 min before starting the cycles and elongated at 72°C for 7 min after ending the cycles. The PCRs were accomplished with the primer pairs of 12S (L2509: 5'-GCTTCAAACTGGGATTAGATACCCCACTAT-3' H2897:5'-TGACTGCAGAGGGTGACGGGCGGTGTGT-3') (Kocher *et al.*, 1989) that can amplify 388 base pairs, and 16S (L3975: 5'-CGCCTGTTTACCAAAAACAT-3', H4551: 5'-CCGGTCTGAACTCAGATCACGT-3') (Simon *et al.*, 1994) that can amplify 576 base pairs. The capital L and H indicate the amplified directions of light and heavy strand, respectively. The numbers after L and H indicate the starting position of the 3'-end of the primers in the *Xenopus laevis* mitochondrial genome (Roe *et al.*, 1985). After amplification, the PCR product was cleaned using Wizard® PCR Preps DNA Purification System (Promega) and suspended in distilled and deionized water. The cleaned DNA template was sequenced directly in both directions. The light strand was sequenced using SILVER SEQUENCE™ DNA sequencing Systems (Promega), and the heavy strand was sequenced using an ABI 310 with the BigDye kit (PE Applied Biosystems).

DNA Sequence Analysis

Sequence alignment was conducted using Clustal W (ver. 1.6; Thompson *et al.*, 1994), and minor modifications were made by eye to correct the computer-aligned sequences. The sequences from the two genes were combined as one data set for further analyses. Indel sites resulting from the alignment and missing/ambiguous data were deleted all in phylogenetic analyses. For assessing character covariance in the data set, permutation tail probability (PTP; Faith and Cranston, 1991) and the skewness test (g1 statistic; Hills & Huelsenbeck, 1992) were used.

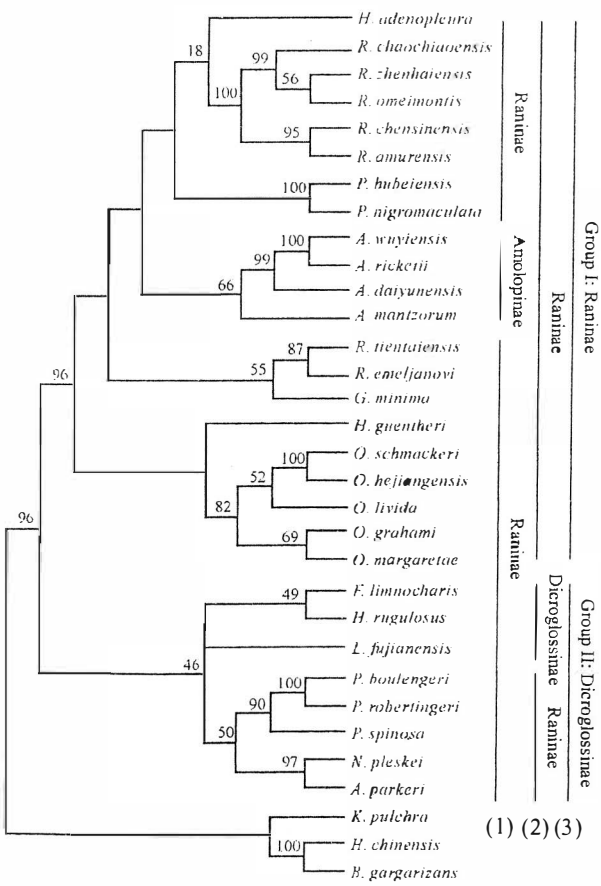


FIG. 1. Strict consensus of two trees recovered in maximum parsimony analysis (tree length=1485, CI=0.449, RI=0.561). Numbers above branches represent bootstrap support (100 replicates). (1) the subfamily classification of Fei *et al.* (1990); (2) Dubois (1992); (3) present study.

Maximum parsimony (MP) and Maximum likelihood (ML) as implemented in PAUP4.0b8a (Swofford, 1998) and Neighbour joining (NJ; Saitou & Nei, 1987) as implemented in MEGA (version 2.1, Kumar *et al.*, 2001) were employed to infer relationships among taxa. MP analyses were conducted using 100 random replicates of the heuristic search option with ACCTRAN, MULPARS, and TBR options; only minimum-length trees were retained and zero-length branches were collapsed. A sequences evolution model was chosen using Modeltest 3.06 (Posada & Crandall, 1998) and used in the ML analysis. The robustness of the phylogenetic results was tested by bootstrap proportion (BSP; Felsenstein, 1985) with 1000 replicates in NJ analysis and with 100 replicates in MP analyses, and by the quartet puzzling replicates method (Strimmer & von Haeseler, 1996) with 1000 puzzling steps for ML analysis.

RESULTS

Sequence Characteristics and Genetic Distance Between Taxa

The sequences were deposited in GenBank, Accession numbers were AF315123 to AF315130 and AF315131 to AF315162. We added 24 sequences of 12S rDNA fragment retrieved from GenBank (AF205541 to AF 205565, Jiang & Zhou, 2001). Alignment resulted in a data matrix of 1005 unambiguously aligned characters, 582 of which were variable sites and 402 parsimony informative sites. The conserved and variable sites distributed alternately but not evenly. Nucleotide compositions were A 0.302, G 0.204, C 0.258, and T 0.236, and the ratio of transitions to transversions was average 1.32 with the range of 0.72 to 3.33.

The Kimura-2-parameter distances showed that the levels of divergence ranged from 0.199 (*N. pleskei* versus *K. pulchra*) to 0.357 (*F. limnocharis* versus *H. chinensis*) between outgroup and ingroup, the average is 0.261±0.031, and those within ingroup were from 0.029 (*O. hejiangensis* versus *O. schmacheri*) to 0.311 (*F. limnocharis* versus *R. amurensis*), of which the intrageneric divergence ranged from 0.029 to 0.178 (*H. adenopleura* versus *H. guentheri*) and the average was 0.095±0.038, and the inter-generic divergence ranged from 0.113 to 0.311, the averaged was 0.199±0.046.

The sequence evolution model chosen by Modeltest and used for ML analysis is the general time-reversal model plus I and G (GTR+I+G). Base frequencies were unequal (A=0.3168; C= 0.2517; G=0.1895; T=0.2419),

Nst=6, Rmat=(2.0855 4.7154 2.0659 0.6555 9.0422), Rates=gamma, Shape=0.4939, and Pinvar=0.1530. These parameters were set in ML analysis.

PHYLOGENETIC EVALUATION

The results of both permutation tail probability test (P=0.001, with 1000 replicates) and skewness test (g1= -0.586777) indicated that there was substantial structure in the data set.

The maximum parsimony analyses resulted in two trees of 1485 length (CI=0.449, RI=0.561; Fig. 1). Variation between the two MP trees occurred at the nodes where *L. fujianensis* presented, one was that it clustered with a clade including *F. limnocharis* and *H. rugulosus*; another was that it clustered with a clade containing *N. pleskei*, *A. parkeri* and three species of *Paa.* The 29 species of ingroup unambiguously formed a clade with a very high bootstrap support proportion (BSP) 96%, and they constituted two sister groups, the first (BSP=96%) was composed of 21 species belonging to seven genera

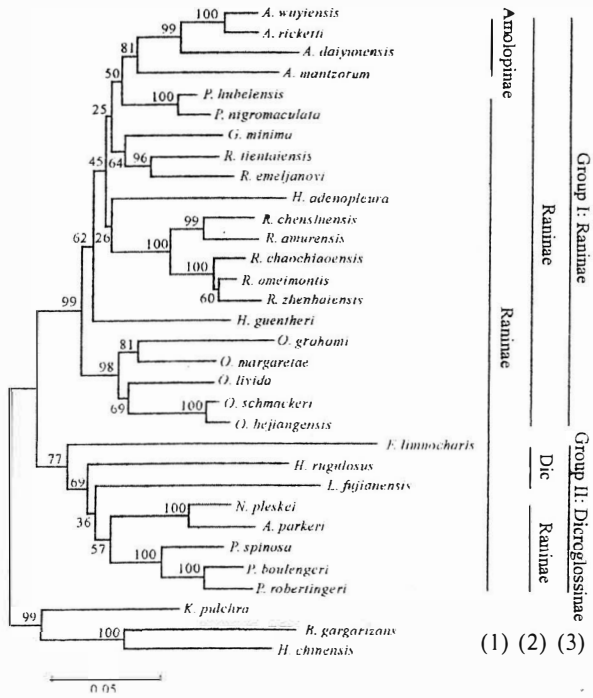


FIG. 3. The phylogenetic relationships among 29 species of ranids examined in the present study by NJ analysis. Numbers on branches are bootstrap proportions (1000 replications). (1) the subfamily classification of Fei *et al.* (1990); (2) Dubois (1992), Dic: Dicroglossinae; (3) present study.

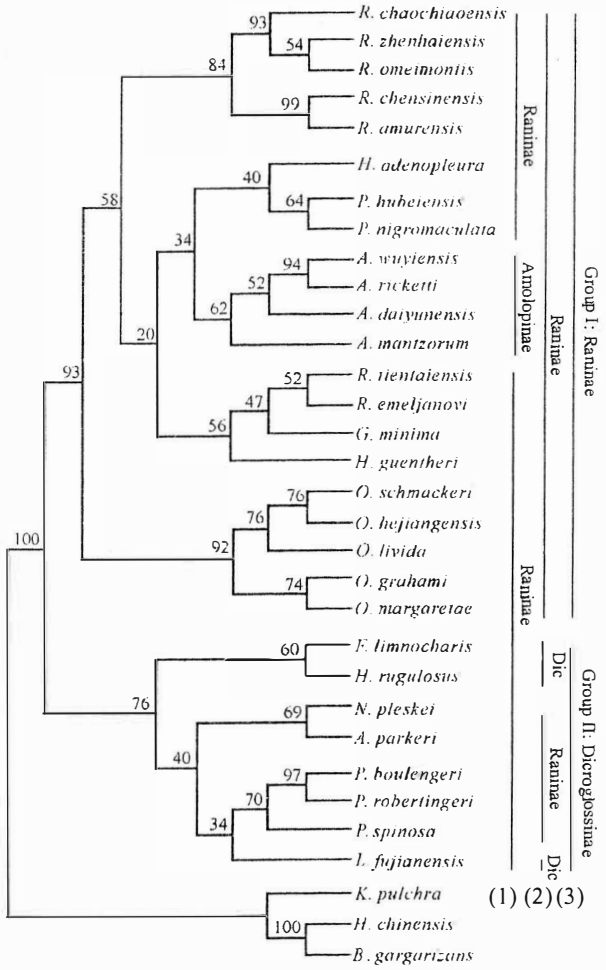


FIG. 2. The best tree derived by ML analysis. Addseq=asis, –lnL (unconstrained) = 611.50197. The number above branch is the quartet puzzling proportion with number of puzzling steps equal to 1000. (1) the subfamily classification of Fei *et al.* (1990); (2) Dubois (1992), Dic: Dicroglossinae; (3) present study.

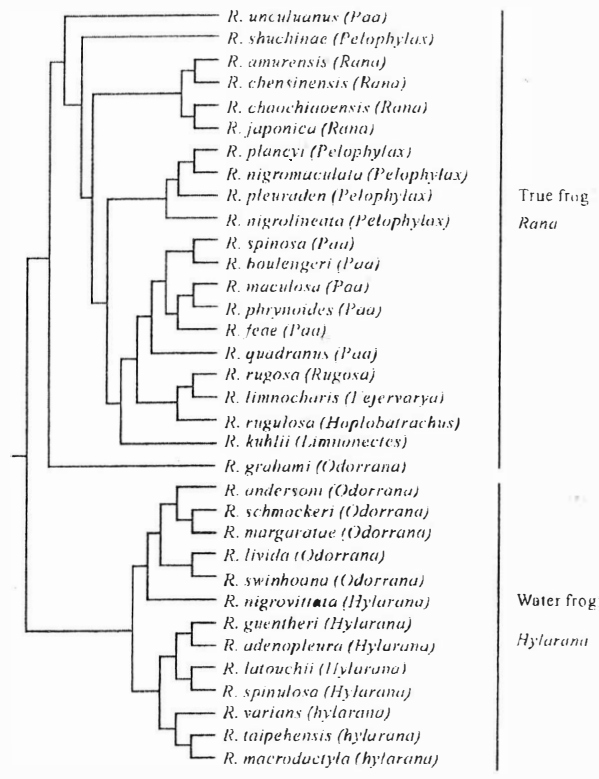


FIG. 4. The relationships between 34 species of the traditional genus *Rana* from China presented by Liu & Hu (1961, page 217). The genus name in parenthesis is proposed by Fei *et al.* (1990) & Fei (1999).

Odorrana, *Rana*, *Hylarana*, *Glandirana*, *Rugosa*, *Pelophylax*, and *Amolops*; the second (BSP=46%) consisted of eight species belonging to six genera *Paa*, *Nanorana*, *Altirana*, *Limnonectes*, *Fejervarya*, and *Hoplobatrachus*. Within the first group, monophyly of the five genera, including *Odorrana*, *Rugosa*, *Amolops*, *Pelophylax*, and *Rana*, respectively was well supported. The genus *Glandirana* (containing only one species, i.e. *G. minima*) has a sister relationship with the genus *Rugosa*. Within the second group, *Fejervarya* and *Hoplobatrachus* clustered together (BSP=49%), and the three species of the genus *Paa* clustered together as a sister group of a group containing *Altirana* and *Nanorana*.

Relationships differ somewhat under the ML analysis (Fig. 2) and NJ analysis (Fig. 3). Again, there was strong support for the two sister groups and monophyly of the six genera recognized in the MP analysis, the phylogenetic relationships among or between the species within each genus are resolved well except in the genus *Hylarana*. Within the first group, the two species of the genus *Hylarana* have no sister relationship with each other, and the relationships among genera are altered except that between the two genera *Glandirana* and *Rugosa*. Within the second group, the relationships among genera also are altered. However, the relationship between the two genera *Altirana* and *Nanorana* is similar to that of the MP tree.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

The MP, ML, and NJ analyses identified two major groups for the ranid species examined. This is consistent with the morphological data (Jiang, 1999). Group I has a smaller nasal bone and distinct space between the inner edge of the right and left nasal bones, and their sphenethmoid are visible from dorsal view; group II has a bigger nasal bone and almost no space between the inner edge of the right and left nasal bones, and the sphenethmoid is invisible from the dorsal side. The average of the Kimura-2-parameter distance between the two major groups is 0.238±0.031 (0.182~0.311), which is apparently bigger than that of inter-generic distance: 0.199±0.046 (0.113 to 0.311).

Generally, *Nanorana* and *Altirana* have a relatively close relationship with the genus *Paa* (Figs. 1 and 3). This is consistent with the results based on morphological data (Jiang, 1999). The precoracoid of the three genera is not forked at the basal end; spine patch or scattered spines can be found on the chest, fingers, belly, or lateral body, while the precoracoid of the genera *Hoplobatrachus*, *Limnonectes*, and *Fejervarya* is forked at the basal end.

The relationships between some genera studied here are not well supported by the quartet puzzling proportion or bootstrap value. Nevertheless, the results provide relatively strong evidence for resolving some taxonomic questions.

HETEROGENEITY OF THE TRADITIONAL GENUS *RANA*

As shown in Table 1, 23 of the 29 species were traditionally treated as members of the genus *Rana* since Boulenger (1920), and especially by Frost (1985). Liu & Hu (1961) presented a figure (redrawn as Fig. 4) showing a preliminary assessment of the relationships among 34 species of the genus *Rana* known in China on the basis of their own morphological study and Boulenger's monograph of 1920. They placed the 34 ranid species in two groups or subgenera, true frogs (*Rana*) and water frogs (*Hylarana*), depending on whether there are transverse grooves on the end of toes. On the basis of serum albumin data, Wallace *et al.* (1973) presented some clues for the heterogeneity of *Rana* thirty years ago. Marmayou *et al.* (2000) also provided some evidence for heterogeneity of the genus *Rana* in molecular phylogenetic relationships among ranid groups inferred from 12S rDNA fragment sequences. Our data provide further evidence that the traditional genus *Rana* is heterogeneous, and support the view of Dubois (1992) and Fei *et al.* (1990) that the traditional genus *Rana* should be split into several genera or taxa at other levels. Of course, all the split work should be based on relevant information about the phylogenetic relationships among these taxa.

CLASSIFICATION OF THE TAXA

Subfamily classification. The cladograms in figures 1, 2, and 3 support the two subfamilies, Raninae Rafinesque-Schmaltz, 1814 and Dicroglossinae Anderson, 1871, proposed by Dubois (1992). However, some modifications should be made, i.e. to move the genera *Paa*, *Nanorana* and *Altirana* from the subfamily Raninae to the subfamily Dicroglossinae and combined as tribe Paini proposed by Dubois (1992). Marmayou *et al.* (2000) introduced another subfamily name Ceratobatrachinae Boulenger, 1884 for the group at least including genera *Limnonectes*, *Taylorana*, *Sphaerotheca*, *Hoplobatrachus* and *Fejervarya* while restricting the use of the subfamily name Dicroglossinae to genera *Occidozyga* and *Phrynoglossus* (and maybe *Euphlyctis*). But the results of Bossuyt & Milinkovitch (2000) and this study do not support their suggestion.

The subfamily Amolopinae was built by Yang (1989) based mainly on the abdominal sucker of the tadpoles. This specialized character is an adaptation to a torrent stream habitat. Our data indicate that the abdominal sucker of *Amolops* tadpoles is not important enough to support building a super-generic level unit. The tadpoles of *Rana sauteri* are similar to those of *Amolops* in having abdominal suckers (Kuramoto *et al.*, 1984; Yang, 1995). Based on this morphological evidence, Fei *et al.* (2000) established a new genus *Pseudoamolops* for *Rana sauteri* and placed it in the subfamily Amolopinae. However, based on molecular data, Tanaka-Ueno *et al.* (1998) found that *R. sauteri* was closely related to *R. longicrus*, suggesting that the sucker shared by *Amolops* and *R. sauteri* tadpoles is a case of convergence and may

have evolved from different evolutionary lines. Although the four species representing the genus *Amolops* constitute a monophyletic clade (Figs. 1, 2, and 3), the results of this study do not support the establishment of the subfamily Amolopinae Yang, 1989, and support their retention in the subfamily Raninae, as in Dubois (1992).

GENERIC CLASSIFICATION

Fei *et al.* (1990) and Fei (1999) held opinions different from that of Dubois (1992) on the rank of genus or subgenus for some ranid groups. Dubois (1992) placed about 213 species in the genus *Rana* consisting of 33 subgenera, of which, *Rana* (brown frog), *Pelophylax*, *Hylarana*, *Rugosa*, *Glandirana*, *Odorrana*, and *Pseudorana* were treated as different generic units by Fei *et al.* (1990) and Fei (1999). According to the point of view of the synthetic school (Mayr, 1969, 1974, 1981; Gisin, 1964), as a unit in the evolutionary history the genus can be recognized from three aspects at least: genetic unit, phylogenetic unit, and ecological unit. Usually, the first two aspects are consistent with each other because the phylogenetic analysis is mostly based on genetics, especially the molecular phylogenetic analysis. In this study, 13 genera were included, 10 of which were split from the traditional genus *Rana*. The phylogenetic cladograms identified six genera, as well as resolved monophyletic units, or in other words different phylogenetic units, with the exception of the genus *Hylarana*. The two members of the latter were placed in different subgenera of *Rana* by Dubois (1992), *H. adenopleura* in the subgenus *Nidirana* and *H. guentheri* in *Sylvirana*. In addition, these groups, like other groups including *Amolops*, *Limnonectes*, *Nanorana*, *Altirana*, *Paa*, have adapted to particular ecological environments of their own (Fei *et al.*, 1990; Fei, 1999). As a whole, this study provides some evidence for the generic classification of ranids proposed by Fei *et al.* (1990), but more groups and genes need to be included for further analyses.

TAXONOMIC STATUS OF *ODORRANA GRAHAMI*

Liu & Hu (1961) discussed *Odorrana grahami* at length. Based on the absence of a transverse groove on the tip of the fingers, they removed the species from *Odorrana* group, and did not place it in the water frog group (Fig. 4). In the present study, *O. grahami* and *O. margaretae* represent a sister group to the clade consisting of the other three species of *Odorrana* (Figs. 1, 2, and 3). The skin of *O. grahami* can excrete poisonous liquid and produce a distinct odor, which is one of the most important characteristics of *Odorrana*. The poisonous secretion of the skin can kill other species of frogs kept temporarily in the same container during fieldwork. The evidence of both molecular phylogenetics and physiology indicate that *O. grahami* should be included in the genus *Odorrana*.

TAXONOMIC STATUS OF *FEJERVARYA LIMNOCHARIS*

Fejervarya limnocharis was placed in the genus *Limnonectes* in *Additions and Corrections to Amphibian Species of the World* (Duellman, 1993), and in the subgenus *Fejervarya* of the genus *Limnonectes* by Dubois (1987, 1992). Fei *et al.* (1990) elevated this species to its own genus *Euphlyctis*, which should be replaced by the generic name *Fejervarya* since *Euphlyctis* has applied to another group of species from the Indian region that is much more aquatic than *Fejervarya* and retains a lateral-line system in adults (see Dubois, 1992; Dubois & Ohler, 2000). Iskandar (1999), Fei (1999), Marmayou *et al.* (2000), and Dubois & Ohler (2000) considered this species should be included in the genus *Fejervarya* rather than in the genus *Limnonectes*. Their opinion was adopted in *Amphibian Species of the World* (version 2.21 online, Frost, 2002). The results of this work indicate that *Fejervarya* is a distinct genus different from the genus *Limnonectes* and do not support the suggestion of Duellman (1993) to placed *F. limnocharis* in the genus *Limnonectes*.

CLADISTIC RELATIONSHIPS OF *NANORANA* AND *ALTIRANA*

The genera *Nanorana* and *Altirana* have been treated as two distinct genera at least since Liu & Hu (1961) and Frost (1985). In this study, *N. pleskei* representing the genus *Nanorana*, and *A. parkeri* representing *Altirana* are resolved as well supported sister groups. In addition, the Kimura-2-parameter distance between them is as low as 0.062: this is within the range of intrageneric divergence. The results of this study support the view that these two genera should be combined as one, i.e. *Nanorana* (Dubois, 1992; Lu & Yang, 1995; Zhao, 1995; Fei, 1999).

ACKNOWLEDGMENTS

We thank L. Fei, M. Z. Cai, C. M. Cai, S. S. Wen, Q. W. Li, J. S. Hao, M. Q. Zheng, and X. B. Wu for their help in collecting samples that have made this study possible. We are grateful to J. Z. Fu, David B. Wake, W. Wüster, and two anonymous reviewers for their helpful comments that improved the manuscript. This work was supported by NSFC (No. 39970094 and 30470249) to ZKY, NSFC (No. 30000018) and Life Science Special Fund of Chinese Academy of Sciences (CAS) Supported by the Ministry of Finance (STZ-01-19) to JPJ.

REFFERENCES

Bossuyt, F. & Milinkovitch, M. C. (2000). Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal co-variation between larval and adult traits. *Proceedings of the National Academy of Sciences of the USA* **97**, 6585-6590.

Boulenger, G. A. (1920). A monograph of the south Asian, Papuan, Melanesian, and Australian frogs of the genus *Rana*. *Records of the Indian Museum* **20**, 1-126.

Dubois, A. (1987). Miscellanea taxonomica batrachologica (I). *Alytes* **5**, 7-95.

Dubois, A. (1992). Notes sur la classification des Ranidae (Amphibiens, Anoures). *Bulletin Mensuel de la Societe Linneenne de Lyon* **61**, 305-352.

Dubois, A. (1999). Miscellanea nomenclatorica batrachologica. 19. Notes on the nomenclature of Ranidae and related groups. *Alytes* **17**, 81-100.

Dubois, A. & Ohler, A. (2000). Systematics of *Fejervarya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 1 Nomenclatural status and type-specimens of the nominal species *Rana limnocharis* Gravenhorst, 1829. *Alytes* **18,** 15-50.

Duellman, W. E. (1993). Amphibian species of the world: additions and corrections. *Univ. Kans. Mus. Nat. Hist. Special Publ.* **21**, 1-372.

Duellman, W. E. & Trueb, L. (1985). *Biology of Amphibians*. NewYork, McGraw-Hill. 1-670.

Emerson, S. B. & Berrigan, D. (1993). Systematics of southeast Asian ranids – multiple origins of voicelessness in the subgenus *Limnonectes* (Fitzinger). *Herpetologica* **49**, 22-31.

Emerson, S. B., Inger, R. F. & Iskandar, D. (2000*a*). Molecular systematics and biogeography of the fanged frogs of southeast Asia. *Molecular Phylogenetics and Evolution* **16,** 131-142

Emerson, S. B., Richards, C., Drewes, R. C. & Kjer, K. M. (2000*b*). On the relationships among ranoid frogs: a review of the evidence. *Herpetologica* **56**, 209-230.

Faith, D. P. & Cranston, P. S. (1991). Could a cladogram this short have arisen by chance alone? On pertutation tests for cladistic structure. *Cladistics* **7,** 1-28.

Fei, L. (1999). *Atlas of Amphibians of China*. Zhengzhou: He 'nan Science and Technology Press, 1-432.

Fei, L., Ye, C. Y. & Huang, Y. Z. (1990). *Key to Chinese Amphibia*. Chongqing: Chongqing Branch, Science and Technology Literature Publishing House, pp. 1-354.

Fei, L., Ye, C. Y. & Jiang, J. P. (2000). A new genus of subfamily Amolopinae – Pseudoamolops, and its relationship to related genera. *Acta Zoologica Sinica* **46,** 19-26.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39,** 783-791

Frost, D. R. (1985). *Amphibian species of the world*. Lawrence, Kansas: Allen Press and the Society of Systematic Collections.

Frost, D. R. (2002). *Amphibian Species of the World: an online reference*. version 2.21, electronic database available at http://research.amnh.org/herpetology/amphibia/index.html.

Gisin, H. (1964). Synthetische Theorie der Systematik. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* **2,** 1-17.

Hay, J. M., Ruvinsky, I., Hedges, S. B. & Maxon, L. R. (1995). Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. *Molecular Biology and Evolution* **12,** 928-937.

Hills, D. M. & Huelsenbeck, J. P. (1992). Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83,** 189-195.

Inger, R. F. (1996). Commentary on a proposed classification of the family Ranidae. *Herpetologica* **52,** 241-246.

Iskandar, D. T. (1999). Amphibian decline monitoring in the Leuser Management Unit, Aceh, North Sumatra, Indonesia. *Froglog* **34**, 2.

Jiang, J. P. & Zhou, K. Y. (2001). Evolutionary relationships among Chinese ranid frogs inferred from mitochondrial DNA sequences of 12S rRNA gene. *Acta Zoologica Sinica* **47**, 38-44.

Jiang, J. P. (1999). *Studies on systematics of the family Ranidae in China* (PhD dissertation). Jiangsu Province, China: Nanjing Normal University.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in mammals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA* **86,** 6169-6200.

Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). *MEGA2: Molecular Evolutionary Genetics Analysis Software*. Tempe: Arizona State University.

Kuramoto, M., Wang, C. S. & Yu, H. T. (1984). Breeding, larval morphology and experimental hybridization of Taiwanese brown frogs, *Rana longicrus* and *R. sauteri*. *Japan Journal of Herpetol*ogy **8**, 387-395.

Liu, C. C. & Hu, S. Q. (1961). *Tailless Amphibians of China*. Beijing: Science Press,.

Lu, S. Q. & Yang, D. T. (1995). A study of relationships among ranid frogs of the genera *Nanorana* and *Altirana* in the Transhimalaya Mountains of China. *Asiatic Herpetological Research* **6,** 73-77.

Marmayou, J., Dubois, A., Ohler, A., Pasquet, É. & Tillier, A. *et al.* (2000). Phylogenetic relationships in the Ranidae. Independent origin of direct development in the genera *Philautus* and *Taylorana*. *Life Sciences*, **323**, 287-297.

Mayr, E. (1969). *Principles of systematic zoology*. New York: McGraw-Hill, pp. 1-428.

Mayr, E. (1974). Cladistic analysis or cladistic classification? *Zeitschrift fuer Zoologische Systematik und Evolutionsforschung* **12**, 94-128.

Mayr, E. (1981). Biological classification: towards a synthesis of opposing methodologies. *Science*, **214**, 510-516.

Posada, D. & Crandall, K. A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Roe, B. A., Ma, D. P., Wilson, R. K. & Wong, J. F. (1985). The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *Journal of Biological Chemistry* **260**, 9759-9774.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-425.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**, 651-701.

Strimmer, K. & von Haeseler, A. (1996). Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Molecular Biology and Evolution* **13**, 964-969.

Sumida, M., Ueda, H. & Nishioka, M. (2003). Reproductive isolating mechanisms and molecular phylogenetic relationships among Palearctic and Oriental brown frogs. *Zoological Sciences* **20**, 567-580.

Swofford, D. L. (1998). *PAUP*, Phylogenetic Analysis Using Parsimony (* and Other Methods)*. Version 4.0. Sinauer, Sunderland, MA.

Tanaka-Ueno, T., Matsui, M., Chen, S. L, Takenaka, O. & Ota, H. (1998). Phylogenetic relationships of brown frogs from Taiwan and Japan assessed by mitochondrial cytochrome b gene sequences (*Rana*: Ranidae). *Zoological Science* **15**, 283-288.

Thompson, J. D., Higgins, D. G. & Gibson, D. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-4680.

Wallace, D. G., King, M. C. & Wilson, A. C. (1973). Albumin differences among ranid frogs: taxonomic and phylogenetic implications. *Systematic Zoology* **22**, 1-13.

Yang, D. T. (1989). Phylogenetic systematics of the *Amolops* group of ranid frogs of southeastern Asia and the Greater Sunda Islands. In *Abstracts of Proceedings of the 55th Anniversary of the Founding of China Zoological Society*, p. 256-257.

Yang, Y. R. (1995). *Appreciating frogs visiting Xitou in night*. Taibei: Administrational Office of Agricultural College, Taiwan University, 1-81.

Zhao, E.-M. (1994). Partion of the genus *Rana* and its evaluation. *Sichuan Journal of Zoology* **13**, 111-115.

Zhao, E.-M. (1995). A checklist of Chinese amphibians and their distribution. In *Amphibian zoogeographic division of China*, 1-14. Zhao, E.-M. (Ed.) Herpetological Series No. **8**, 1-14..

Accepted: 27.8.03

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 9-13 (2005)

REPRODUCTIVE BIOLOGY OF THE "GLASS SNAKE" *OPHIODES FRAGILIS* (SQUAMATA: ANGUIDAE) IN SOUTH-EAST BRAZIL

L. PIZZATTO

Pós-graduação em Ecologia, Depto. de Zoologia, Universidade Estadual de Campinas, Campinas, SP, Brazil

The reproductive cycles of tropical lizards can be continuous or seasonal, depending on a wide variety of factors, some of which are historical. Most studies on reproductive biology of lizards have been conducted on species in the Iguanidae, Gekkonidae, Teiidae, Lacertidae, and Scincidae. The few studies on anguid lizards have been on various alligator lizards and one species of *Ophisaurus*. This study presents data on sexual maturity, adult body size, neonate body size and body mass, fecundity, and male and female reproductive cycles of a population of *Ophiodes fragilis* from São Paulo and Paraná states in south-east Brazil. Females are larger and attain sexual maturity at a larger SVL than males. The female reproductive cycle is highly seasonal with secondary vitellogenesis starting in the middle of the rainy season (February). Embryos in early stages of development occur in the dry season (June) and parturition occurs at the transition from the dry season to the rainy season (August-December). Clutch size averages 7.5 young and is not related to maternal female size as in many other lizard species. Neonates were 33-57 mm in SVL and 0.45-0.85 g in mass. The residual volume of testes does not vary throughout the year suggesting a continuous spermatogenic cycle. Diameter of the deferent duct is largest from January to March (onset of the rainy season), suggesting that even though males produce sperm continuously, they store sperm until mating season.

Key words: legless lizard, reproduction, clutch size, reproductive cycle, spermatogenic cycle

INTRODUCTION

In temperate areas lizard reproduction is seasonal with mating and egg-laying often occurring from spring to summer (Fitch 1970). Some species mate in autumn and females of some of these species can store sperm over winter (Fox, 1963; Conner & Crews, 1980; Kwait & Gist, 1987). However, tropical lizard species reproduce continuously in some areas (Inger & Greenberg, 1966; Fitch, 1982; James & Shine, 1985; Vitt, 1986; Patterson, 1990) and seasonally in some areas where rainfall is seasonal (Fitch, 1982; James & Shine, 1985; Patchell & Shine, 1986; Clerke & Alford, 1993; Vrcibradic & Rocha, 1998). Male and female reproductive cycles can differ with males producing sperm through the year whereas females may produce eggs only seasonally (Clerke & Alford, 1993; Wilhoft, 1993*a,b*; Van Sluys *et al.*, 2002). In addition, intraspecific variation in the reproductive patterns may occur across different latitudes or climatic areas (Fitch, 1982; Clerke & Alford, 1993). More recent analyses of existing squamate reproductive data demonstrate that a considerable phylogenetic signal exists in lizard reproductive strategies. Species within clades tend to be more similar to each other in their reproductive strategies than they are to species in distant clades (Dunham and Miles, 1985). The ability to discern evolutionary strategies in lizard reproduction requires reasonable data on reproductive variables of species in all major clades. One shortcoming of the study by Dunham and Miles (1985) and earlier studies (Tinkle *et al.*, 1970) has been a lack of data on several important lizard clades, such as the Anguidae.

This kind of gap is also seen in Neotropical areas. Although several studies about lizard reproduction are available in this region, they are limited to only some families: Polychrotidae (Vitt & Lacher Jr., 1981), Scincidae (Vitt & Blackburn, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999), Tropiduridae (Rocha, 1992; Van Sluys, 1993; Vieira *et al.*, 2001; Van Sluys *et al.*, 2002; Wiederhecker *et al.*, 2002) and Gekkonidae (How *et al.*, 1986; Vitt, 1986). The few reproductive studies on anguid lizards have been conducted on various alligator lizards (Vitt, 1973; Goldberg, 1972, 1975) and one species of *Ophisaurus* (Fitch, 1989), all in the northern temperate zone.

The genus *Ophiodes* (Anguidae) is exclusively Neotropical and are distributed in the east Andes, central, east and south-east South America. The genus contains about seven species of limbless, elongate lizards (Martins, 1998). *Ophiodes fragilis* Raddi, 1820 (after revision of Martins, 1998) occurs in south and south-east Brazil, in coastal south Bahia state, Minas Gerais and Mato Grosso do Sul. In Argentina it is registered in north-east of Missiones (Martins, 1998). This species is common in anthropic areas and, similar to North American *Ophisaurus*, are commonly known as glass snakes. As in other anguids, their cryptozooic habits make it difficult to conduct ecological studies on *Ophiodes*.

In the present study, I present data on the reproductive biology of *O. fragilis* in south-east Brazil, including

Correspondence: L. Pizzatto, Depto. de Zoologia, Universidade Estadual de Campinas, CP. 6109, cep. 13083-970, Campinas, SP, Brazil.
E-mail: ligia_oceanica@yahoo.com

size at sexual maturity, body size of males and females, sexual dimorphism, reproductive cycles, fecundity and neonate size.

MATERIAL AND METHODS

A total of 115 (32 females, 42 males and 40 juveniles) specimens were examined from the collections of the Museu de História Natural da Universidade Estadual de Campinas (ZUEC) and Museu de História Natural do Capão da Imbuia (MHNCI). Areas where these specimens were collected are covered mainly by open vegetation, disturbed areas and plantations in São Paulo and Paraná states, south-east Brazil (between latitudes 19.9° and 26.6°S). Climate is seasonal. The warm and rainy season extends from October to March and the cold and dry season from April to September (Nimer, 1989). During the rainy season mean temperature varies from 21 to 24°C and mean rainfall varies from 123 to 240 mm. In the cold and dry season mean temperature varies from 17 to 22°C and mean rainfall varies from 36.8 to 121 mm (Nimer, 1989).

The following data were taken for each specimen: (1) snout-vent length (SVL – to the nearest 1mm); (2) sex; (3) reproductive status: mature or immature. Females were considered mature when the diameter of their ovarian follicles was greater than 3 mm or if they had embryos; males were considered mature if the testes were large and turgid or if the deferent ducts were opaque and convoluted, indicating the presence of sperm (see Shine, 1977); (4) diameter of the largest ovarian follicles or embryos (plus yolk) in females (to the nearest 0.1 mm); (5) embryonic stage (from one to three according to the quantity of yolk, see below); (6) largest, medium and smallest diameters of testes (testicular volume –TV– was estimated using the ellipsoid volume formula $TV = 4/3ab^2$ where a = largest radius, b = smallest radius – *cf.* James and Shine, 1985); (7) deferent duct diameter near the cloaca (see Almeida-Santos et al., 2003). Development of embryos was categorized as: Stage 1 – just following ovulation (and probably fecundation) when only yolk was visible; Stage 2 – a small embryo could be observed but yolk was still very abundant, and Stage 3 – a large embryo was present and totally developed, without yolk.

Birth data were obtained from gravid females received at Instituto Butantan (IB), São Paulo, Brazil. Pregnancy was detected by palpation and these females were maintained in captivity (at room temperature 19.3-31.0°C) until birth. Food (cockroaches and lycosid spiders) and water were provided *ad libitum*. After birth, each neonate was measured in SVL (to the nearest 1 mm) and weight (to the nearest 0.1 g). Clutch size was estimated based on the number of embryos in preserved specimens. Because captivity may affect gestation and clutch size, data on number of neonate lizards from captive females were not used in fecundity estimate. Neonate SVL was recorded from individuals received at IB on birth time and individuals born in captive. An index of the degree of sexual size dimorphism (SSD) was "(mean adult SLV of the larger sex/mean adult SLV of the smaller sex) –1" (*cf.* Shine, 1994).

Analysis of volume of testes and diameter of deferent duct was made by combining data from each of four seasons: (1) onset of rainy season (October-December), (2) end of rainy season (January-March), (3) onset of dry season (April-June) and (4) end of dry season (July-September). Because only testes volumes were correlated with SVL by seasons, residuals from the regression of testes volume to SVL were used to indicate spermatogenic activity. Diameter of deferent duct was used to indicate sperm release (*cf.* Volsøe, 1944; Shine, 1977).

RESULTS

Sexual Maturity and Body Size

The smallest mature female measured 156 mm SVL and the smallest mature male measured 126 mm SVL. Females (SVL = 192.5±28 mm, range 56-300 mm) are significantly larger than males (SVL = 158.9±22.8 mm, range 126-221 mm) (t=5.87, df=1, P<0.0001). The degree of sexual size dimorphism (SSD) was 0.211.

Female Reproductive Cycle

Secondary vitellogenesis starts in February and ovulation occurs just after secondary vitellogenesis, from March until June. At this time much yolk is visible in ova (stage 1). Embryos in stage 2 are found in June and early August (Fig. 1). By the end of August, embryos in stage 3 begin to appear and continue until December (Fig. 1). In September, one female undergoing parturition was received at IB. Another gravid female gave birth in August (after five months and one week in captivity) and another one in October (after four months and two weeks; Fig. 1). Gestation lasts at least five months (Fig. 1) and all embryos within a given female were in the same developmental stage.

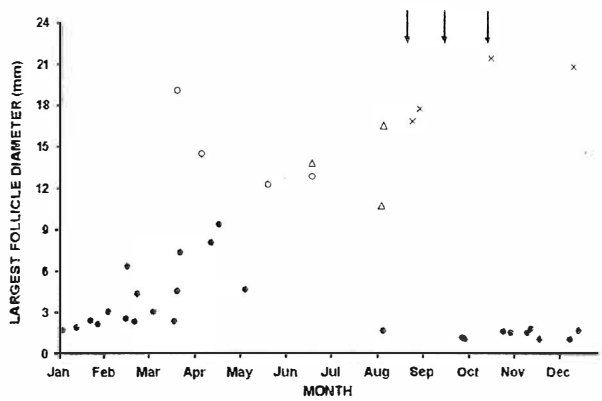


FIG 1. Seasonal variation in the diameter of the largest ovarian follicle in adult females of *Ophiodes fragilis* from south-east Brazil. Solid circles = ovarian follicle; open circles = embryo at stage one of development; triangles = embryo at stage two of development, crosses = embryo at stage three of development; arrows = birth.

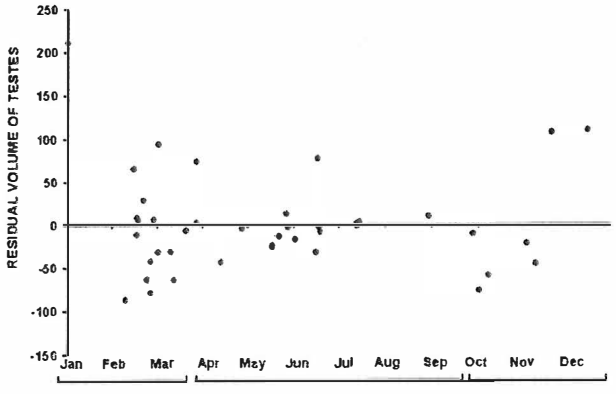


FIG 2. Seasonal variation in the residuals of volume of testes in adult male *Ophiodes fragilis* from south-east Brazil.

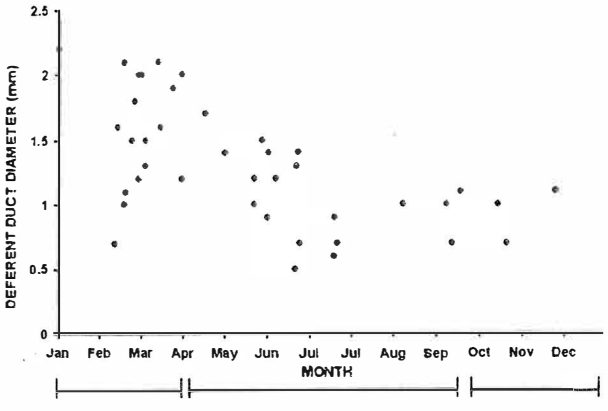


FIG 3. Seasonal variation in the diameter of deferent duct in adult male *Ophiodes fragilis* from south-east Brazil.

FECUNDITY AND NEONATE SIZE

Clutch size averaged 7.5±2.3 embryos (range 5-13, n=18) in preserved specimens. In captive specimens (females collected gravid), mean clutch size was 3.6±1.53 (range 2-5, n=3). Clutch size was not related to female SVL (r=0.23; P=0.366, n=18). Neonate SVL averaged 44.1±9.4 mm (range 33-57 mm, n=11 individuals from three clutches) and weighed 0.69±0.18 g (range 0.45-0.85 g, n=6 individuals from two clutches).

MALE CYCLE

Size-adjusted testes volume did not differ among seasons (Kruskal-Wallis test: H=1.34, df= 3, P=0.718, n=41, Fig. 2). However, diameter of deferent duct decreased significantly from July to December (Kruskal-Wallis test: H=18.98, df=3, P=0.0003, n=41) and reached maximum size from January to June (Fig. 3).

DISCUSSION

Females of *Ophiodes fragilis* are larger in SVL than males, as in some other lizard species (and also in snakes) (Patchell & Shine, 1986; Shine, 1994; Vrcibradic & Rocha, 1998). The SSD index to *O. fragilis* was higher than in viviparous skinks of the genus *Mabuya* (range 0.055-0.145; Rocha & Vrcibradic, 1999) and in many oviparous geckos (range 0.053-0.164; *cf.* How *et al.*, 1986; Vitt, 1986). However, it is similar to that of some oviparous legless Australian pygopodids (*Lialis burtonis:* 0.242; *Aprasia pulchella:* 0.236; *cf.* Patchell & Shine, 1986). SSD can be related to both ecological traits and phylogenetic lineage. As in many other lizards, including the oviparous legless anguid *Ophisaurus attenuatus*, clutch size is not significantly related to maternal SVL (How *et al.*, 1986; Patchell & Shine, 1986; Fitch, 1989; Clerke & Alford, 1993; Rocha & Vrcibradic, 1999). In many snakes the primary selective force causing larger SLV in females in relation to males is the potential increase in fecundity (clutch size) associated with increased body size (Shine, 1994). This explanation may apply to lizard species in which clutch size increases with maternal female size, but does not apply to species in which there is no clutch size-female size relationship. In these species, a tradeoff between present and future reproductive success may exist, particularly if females have a high probability of surviving to the next reproductive season Shine, 1988). As female body size increases, frequency of reproduction may increase thus driving the evolution of female-biased sexual dimorphism in the absence of a clutch size-female size relationship (*cf.* Shine, 1988).

Clutch size in *O. fragilis* is larger than that in legless oviparous pygopodid lizards but similar to the oviparous legless *Ophisaurus attenuatus* (Patchell & Shine, 1986; Fitch, 1989). Low clutch size in pygopodids probably reflects their origins within the Gekkota, a group of lizards typically having clutch sizes of one or two eggs (Fitch, 1970; Dunham and Miles, 1985; Vitt, 1986). Although relative clutch mass and clutch size may be influenced to some degree by body shape (e.g. Vitt, 1981), clutch size may be conservative in anguid lizards, even when the reproductive mode differs. In captivity, high mortality of *Ophiodes fragilis* embryos (at stage 1) is suggested by the lower clutch size in captive females than in wild ones. Circumstantial evidence suggests that early-stage ova can be reabsorbed. One gravid female (detected by palpation) collected and killed some months after being held captive, contained no vestiges of embryos, and she had regressed ova in the oviducts.

The female reproductive cycle in *Ophiodes fragilis* was highly seasonal, as is expected for viviparous reptiles. Oviparous lizards usually tend to lay eggs from late spring to early summer both in temperate (Mayhew, 1963; Fitch, 1989) and tropical areas (How *et al.*, 1986; Patchell & Shine, 1986; Rocha, 1992; Clerke & Alford, 1993; Van Sluys, 1993, Vitt, 1973). Hatching may occur in late summer or early autumn. However, in *O. fragilis* secondary vitellogenesis and ovulation starts in late summer and early autumn, pregnancy lasts at least five months (March to August) and parturition starts in late winter (August). This pattern is similar to that of some tropical viviparous skinks of the genus *Mabuya* (Vitt & Blackburn, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999) and differs significantly from that of

the viviparous anguid *Gerrhonotus coeruleus principis* in temperate areas (Vitt, 1973).

Barbosa *et al.* (1991) recorded parturition of a captive female *O.* cf. *striatus* collected in Rio de Janeiro State in October. Although this record is consistent with the present study, Barbosa *et. al.* (1991) did not describe the specimen in his work. Because *O. striatus* is a large complex of species, in which *O. fragilis* was included (Martins, 1998) it is not possible to recover its actual identification. Marques & Sazima (2003) recorded parturition of two *O. fragilis* (O.A.V. Marques, pers. com) collected in the Atlantic forest in November. Additionally, I recorded parturition, in captivity, of an individual from Minas Gerais State. This female was collected on 22 July and gave birth to six lizards on 4 October indicating that its reproductive period is similar to that of other populations close to the studied area.

Although some tropical species have seasonal spermatogenesis (Rocha, 1992; Vrcibradic & Rocha, 1998) many others produce sperm continuously (Van Sluys, 1993; Van Sluys *et al.*, 2002), similar to *O. fragilis*. However, in this species, mating appears to be seasonal, occurring at the end of the rainy season (at least from February to March) as indicated by the increase in deferent duct diameter. Mating during this season is synchronous with fecundation and thus, sperm storage by females is not essential to reproductive cycle in this species.

ACKNOWLEDGMENTS

I thank to Otavio A. V. Marques for suggesting this study. Fernanda Stender de Oliveira, João C. Ferreira, and Donizetti for help in reception of specimens at IB. Julio C. Moura-Leite for permission to access the collection of Museu de História Natural do Capão da Imbuia (MHNCI). Paulo Roberto Manzani and Fátima B. Souza for permission to access the collection of Museu de História Natural da Universidade Estadual de Campinas (ZUEC). Marcio B. Martins for confirmation on species identity. Jivanildo P. Miranda, Carlos Frederico Duarte Rocha, Monique Van Sluys and Otavio A. V. Marques for reading and commenting on this manuscript. Laurie J. Vitt and Otavio A. V. Marques for reviewing this work. I thank to FAPESP (00/13654-9 and 00/12339-2) for financial support.

REFERENCES

Almeida-Santos, S. M., Pizzatto, L. & Marques, O. A. V. (2003). Intra-sex synchrony and inter-sex coordination in the reproductive timing of the coral snake *Micrurus corallinus* (Elapidae). *Herpetological Natural History,* in press.

Barbosa O. R., Machado, C. & Fernandes, L. (1991). Notas sobre a parturição da "cobra-de-vidro", *Ophiodes striatus* (Spix, 1825) (Sauria, Anguidae). *Revista Brasileira de Biologia* **51**, 285-287.

Clerke R. B. & Alford, R. A. (1993). Reproductive biology of four species of tropical Australian lizards and comments on the factors regulating lizard reproductive cycles. *Journal of Herpetology* **27**, 400-406.

Conner, J. & Crews, D. (1980). Sperm transfer and storage in the lizard, *Anolis carolinensis*. *Journal of Morphology* **163**, 331-348.

Dunham, A. E., & Miles D. B. (1985). Patterns of covariation in life history traits of squamate reptiles: The effects of size and phylogeny reconsidered. *American Naturalist* **126**, 231-257.

Fitch, H. S. (1970). Reproductive cycles in lizards and snakes. *Miscellaneous Publications of the Museum of Natural History University of Kansas* **52**, 1-247.

Fitch, H. S. (1982). Reproductive cycles in tropical reptiles. *Occasional Papers of the Museum of Natural History University of Kansas* **96**, 1-53.

Fitch, H. S. (1989). A field study of the slender glass lizard, *Ophisaurus attenuatus*, in northern Kansas. *Occasional Papers of the Museum of Natural History University of Kansas* **125**, 1-50.

Fox, W. (1963). Special tubules for sperm storage in female lizards. *Nature* **198**, 500-501.

Goldberg, S. R. (1972). Reproduction in the southern alligator lizard, *Gerrhonotus multicarinatus*. *Herpetologica* **28**, 267-273.

Goldberg, S. R. (1975). Reproduction in the Arizona alligator lizard, *Gerrhonotus kingi*. *Southwestern Naturalist* **20**, 409-420.

How, R. A., Dell, J. & Wellington B. D. (1986). Comparative biology of eight species of *Diplodactylus* gecko in western Australia. *Herpetologica* **42**, 471-482.

Inger R. F. & Greenberg, B. (1966). Annual reproductive patterns of lizards from a Bornean rainforest. *Ecology* **47**, 1007-1021.

James C. & Shine, R. (1985). The seasonal timing of reproduction: a tropical-temperate comparison in Australian lizards. *Oecologia* **67**, 464-474.

Kwait, G. A & Gist, D. H. (1987). Annual reproductive cycle of an introduced population of European wall lizard (*Podarcis muralis*) in Ohio. *Journal of Herpetology* **21**, 205-209.

Marques, O. A. M. & Sazima, I. (2003). História Natural dos Répteis da Estação Ecológica Juréia-Itatins. In Estação Ecológica Juréia-Itatins: Ambiente Físico, Flora e Fauna, 254-274. Marques, O. A. V. & Duleba W. (Eds.). Ribeirão Preto: Editora Holos.

Martins, M. B. (1998). Revisão taxonômica e sistemática filogenética do gênero *Ophiodes* Wagler, 1828 (Sauria: Anguidae: Diploglossinae). Umpubl. PhD. Thesis, PUCRS, Porto Alegre, Brasil.

Mayhew, W. W. (1963). Reproduction in the granite spiny lizard, *Sceloporus orcutii*. *Copeia* **1963**, 144-152.

Nimer, E. (1989). Climatologia do Brasil. 2nd ED. IBGE. Departamento de Recursos Naturais e Estudos Ambientais. Rio de Janeiro, RJ.

Patchell, F. C. & Shine, R. (1986). Food habits and reproductive biology of the Australian legless lizard (Pygopodidae). *Copeia* **1**, 30-39.

Patterson, J. K. (1990). Female reproductive cycles of the tropical lizard *Mabuya striata*. *Oecologia* **44**, 168-172.

Rocha, C. F. D. (1992). Reproductive and fat body cycles of the tropical sand lizard (*Liolaemus lutzae*) of southeastern Brazil. *Journal of Herpetology* **26**, 17-23.

Rocha, C. F. D. & Vrcibradic, D. (1999). Reproductive traits of two sympatric vivivparous skinks (*Mabuya macrorhyncha* and *Mabuya agilis*) in Brazilian restinga habitat. *Herpetological Journal* **9**, 43-53.

Shine, R. (1977). Reproduction in Australian elapid snakes I – Testicular cycles and matting seasons. *Australian Journal of Zoology* **25**, 647-53.

Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's "Fecundity advantage" model. *American Naturalist* **131**, 124-131.

Shine, R. (1994). Sexual dimorphism in snakes revised. *Copeia* **1994**, 326-346.

Tinkle, D. W., Wilbur, H. M. & Tilley, S. G. (1970). Evolutionary strategies in lizard reproduction. *Evolution* **24**, 55-74.

Van Sluys, M. (1993). The reproductive cycle of *Tropidurus itambere* (Sauria: Tropiduridae) in southeastern Brazil. *Journal of Herpetology* **27**, 28-32.

Van Sluys, M, Mendes, H. M. A., Assis, V. B. & Kiefer, M. C. (2002). Reproduction of *Tropidurus montanus* Rodrigues, 1987 (Tropiduridae), a lizard from a seasonal habitat of south-eastern Brazil, and a comparison with other *Tropidurus* species. *Herpetological Journal* **12**, 89-97.

Vieira, G. H. C., Wiederhecker, H. C., Colli, G. R., Bao, S. N. (2001). Spermiogenesis and testicular cycle of the lizard *Tropidurus torquatus* (Squamata, Tropiduridae) in the Cerrado of central Brazil. *Amphibia-Reptilia* **22**, 217-233.

Vitt, L. J. (1973). Reproductive biology of the anguid lizard, *Gerrhonotus coeruleus principis*. *Herpetologica* **29**, 176-184.

Vitt, L. J. (1981). Lizard reproduction: habitat specificity and constraints on relative clutch mass. *American Naturalist* **117**, 506-514.

Vitt, L. J. (1986). Reproductive tactics of sympatric gekkonid lizards with a comment on the evolutionary and ecological consequences of invariant clutch size. *Copeia* **1986**, 773-786.

Vitt, L. J. & Blackburn, D. G. (1991). Ecology and life history of the viviparous lizard *Mabuya bistriata* (Scincidae) in Brazilian Amazon. *Copeia* **1991**, 916-927.

Vitt, L. J. & Lacher Jr., T. E. (1981). Behavior, habitat, diet and reproductive of the iguanid lizard *Polychrus acutirostris* in the caatinga of northeastern Brazil. *Herpetologica* **37**, 53-63.

Volsøe, H. (1944). Seasonal variation of the male reproductive organs of *Vipera berus* (L.). *Spolia Zoologica Musei Hauniensis* **5**, 1-157

Vrcibradic, D. & Rocha, C. F. D. (1998). Reproductive cycle and life-history traits of the viviparous skink *Mabuya frenata* in southeastern Brazil. *Copeia* **1998**, 612-619.

Wiederhecker, H. C., Pinto, A. C. S. & Colli, G. R. (2002). Reproductive ecology of *Tropidurus torquatus* (Squamata: Tropiduridae) in the highly seasonal Cerrado biome of central Brazil. *Journal of Herpetology* **36**, 82-91.

Wilhoft, D. C. (1993*a*). Reproduction in the tropical Australian skink, *Leiolopisma rhomboidalis*. *American Midland Naturalist* **70**, 442-461.

Wilhoft, D. C. (1993*b*). Gonadal histology and seasonal changes in the tropical Australian lizard, *Leiolopisma rhomboidalis*. *Journal of Morphology* **116**, 185-203.

Accepted: 22.8.03

APPENDIX 1

SPECIMENS EXAMINED

MHNCI: 198, 808, 849, 1438, 1697, 1699, 2075, 2076, 2106, 5166, 6756, 6984, 6985, 7119, 7227, 7272, 7394, 7442, 7660, 7761, 8156, 8228, 9026, 9164, -641.
ZEUC: 2511 – 2548, 2550 – 2569, 2572, 2574, 2576, 2610 – 2614, 2616 – 2618, 2807 – 2829.

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 15-20 (2005)

THE COMPLEX VOMERONASAL STRUCTURE OF *DIPSOCHELYS* GIANT TORTOISES AND ITS IDENTIFICATION AS A TRUE JACOBSON'S ORGAN

J. GERLACH

University Museum of Zoology, Department of Zoology, Cambridge UK

The nasal structure of the western Indian Ocean *Dipsochelys* giant tortoises is described. These tortoises are known to possess structures facilitating 'nasal drinking'. Additional unique nasal features include the processus dorsalis vomerinus supporting an enlarged medial nasal gland and a 'tuberculum palatinum'. The medial nasal gland can be considered homologous to the vomeronasal organ (Jacobson's organ) and is connected directly to the tuberculum palatinum in the buccal cavity through the foramina praepalatina. An homologous vomeronasal organ with a direct buccal connection is also identified in existing literature accounts of the leatherback turtle *Dermochelys coriacea* and may have been overlooked in other Chelonia.

Key words: Chelonia, olfaction, tortoise, palatine gland, pheromones

INTRODUCTION

The nasal region is of great significance in individual interactions in many tetrapod groups because of the role of the vomeronasal organ (sometimes referred to as Jacobson's organ) in pheromone detection. This structure is well developed in many reptile groups, most notably the lizards and snakes, but its presence in the Chelonia has been disputed over many years (Parsons, 1970). Tissue layers in the nasal region of several turtles have been associated with the vomeronasal organ although no discrete structure comparable to the complex Jacobson's organ of many mammals and squamate reptiles is apparent in most species examined. The view that chelonian nasal structures are highly conservative compared to other classes of tetrapods (Parsons, 1970) is not supported by some recent studies (Saito *et al.*, 2000; Murphy *et al.*, 2001). Discussion of the apparent vomeronasal structures in chelonians date from 1895 when Seydel concluded that a Jacobson's organ was present on the grounds that the vomeronasal epithelium develops ventrally and medially (as opposed to the dorsal olfactory epithelium), with innervation of the vomeronasal nerve from the accessory olfactory bulb and no Bowman's glands. This contrasts with more recent studies that have not accepted the chelonian structure as a true Jacobson's organ on the basis of not forming a distinct evaginated structure (Parsons, 1959). The presence of vomeronasal epithelium in pockets of the nasal cavity in some chelonians has obscured such classification (Parsons, 1970) and the vomeronasal epithelium is now generally referred to as a vomeronasal organ despite the absence of discrete physical structures (Murphy *et al.*, 2001).

Some of the most complex chelonian nasal structures have been described from the western Indian Ocean giant tortoise genus *Dipsochelys* (Bour, 1982; Arnold, 1979; Gerlach & Canning, 1998). The raised nasal opening and the structures within the nasal chamber have been interpreted as adaptations to facilitate drawing water up through the nasal passages during drinking through the nose (Arnold, 1979). This behaviour has been reported in wild *D. dussumieri* (also referred to as *Geochelone gigantea* or *D. elephantina*) on Aldabra (Arnold, 1979). Further discussion of the structures of the *Dipsochelys* nasal region has considered the possibility of this genus having an enhanced olfactory capability, a suggestion following from the description of a bony support for a 'vomeronasal organ' (Gerlach & Canning, 1998). The palate has also been noted as being unusual in possessing a tuberculum palatinum as described by Fritsch (1870):

"In *Testudo elephantina* [=*Dipsochelys dussumieri*] an oval whitish body 3 mm long is located ventral to the hard palate in the anterior of the upper jaw, its precise outline is obscured by the enclosing membrane. Under closer examination this body appears as only an apparent swelling in the membrane covering the partition that separates the choanae from one another, as the section is not distinguished in coloration or structure, which would allow the so called Tuberculum palatinum to be recognised as a separate organ."

The published interpretations of the nasal structures have relied on comparative osteology and dissection. These provide structural information but there have been no published accounts of the detailed morphology of the 'flap-like ridge' (Arnold, 1979) or the 'vomeronasal organ' (Gerlach & Canning, 1998) to support functional interpretations. In order to investigate these structures in more detail available dissections and microscopic preparations were examined to provide morphological and histological data on the unique features of *Dipsochelys*. The availability of freshly preserved material allowed new dissections to be prepared. The results are described below, identifying the structure as a true vomeronasal organ with a level of complexity comparable to the Jacobson's organ of mammals or squamates.

Correspondence: J. Gerlach, University Museum of Zoology, Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK. *E-mail*: jstgerlach@aol.com

MATERIAL AND METHODS

During a taxonomic revision a detailed osteological study was made on 118 specimens of all species of the genus (Gerlach & Canning, 1998). During this study, careful attention was paid to the ridges and bony processes within the nasal chamber following observation that there were differences in these structures between *D. dussumieri* and *D. grandidieri*. A dissection of an adult female head of a *Dipsochelys dussumieri* from Aldabra (preserved in 70% ethanol since 1971) has previously been figured and described (Arnold, 1979); the preserved dissection (BM(NH) R1978.772) was re-examined. Recent material of one adult *D. dussumieri* on Aldabra atoll was examined. Previously undescribed material was available in the form of serial transverse sections of a *D. dussumieri* late embryo. The 10 μ sections stained with Masson's trichrome had been prepared by A. d'A. Bellairs and are stored in the British Museum (Natural History). In 2003 five full-term embryos of *D. hololissa* were preserved and stored by the Nature Protection Trust of Seychelles. These were preserved in 4% paraformaldehyde enabling new dissections and microscope preparations to be made. All sectioned material was prepared by cutting 5 μ paraffin embedded sections followed by staining with cresyl violet.

Comparison was made with '*Geochelone*' (*Stigmochelys*) *pardalis*, which may be the closest living relative of *Dipsochelys* (Gerlach, 2001; Palkovaks *et al*., 2002) and with published accounts of the nasal anatomy of *Testudo* (Parsons, 1970) and other turtles (Bellairs & Kamal, 1981; Nick, 1912).

Due to the large size of these tortoises it is possible to observe structures in the palate when the mouth is open. One adult female *Dipsochelys hololissa* and one adult female *D. arnoldi* in the captive groups of the Nature Protection Trust of Seychelles on Silhouette island, Seychelles responded to scratching of the neck by standing upright and stretching the neck and head upwards. In this pose the lower jaw would open and could be gently levered open further to expose the palate.

RESULTS

OSTEOLOGY

The soft-tissue structure suggested to be a vomeronasal organ is supported by the processus vomerinus dorsalis, positioned on the dorsum of the vomer between the foramina praepalatina, anterior to the sulcus vomerinus (Figs. 1, 2). This bony process is a unique feature of *Dipsochelys* (Gerlach & Canning, 1998), no trace of any similar structure has been found in any other tortoise genus. The processus vomerinus dorsalis is a slightly elevated piece of bone on the dorsum of the premaxillae. In adult tortoises (straight carapace length over 45cm) it measures 2-6 mm diameter and is raised 0.5-6 mm. The height of the processus vomerinus dorsalis varies with different species, being low (0.5 mm) in *D. hololissa*, moderately elevated in *D. dussumieri* (0.5-2 mm) and *D. arnoldi* (1-2 mm) and high (6 mm) in the extinct Malagasy species *D. grandidieri*. In *D. grandidieri* the exceptionally large process bears two 1.5 mm diameter pits in the anterior surface (Fig. 2).

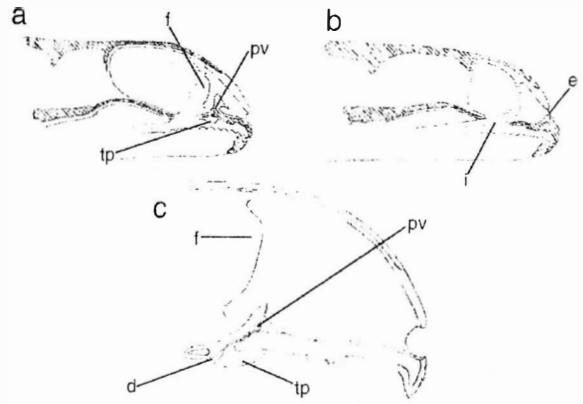


FIG 1. Nasal structures of *Dipsochelys dussumieri* and '*Geochelone*' *pardalis*; (a) medial view of right nasal passage and nasal chamber of *D. dussumieri;* (b) medial view of right nasal passage and nasal chamber of '*G.*' *pardalis*; (c). Detail of the vomeronasal structures of *D. dussumieri* in medial view; d – duct from the tuberculum palatinum; e – external naris; i – internal naris; f–nasal flap; pv – processus vomerinus dorsalis; tp – tuberculum palatinum

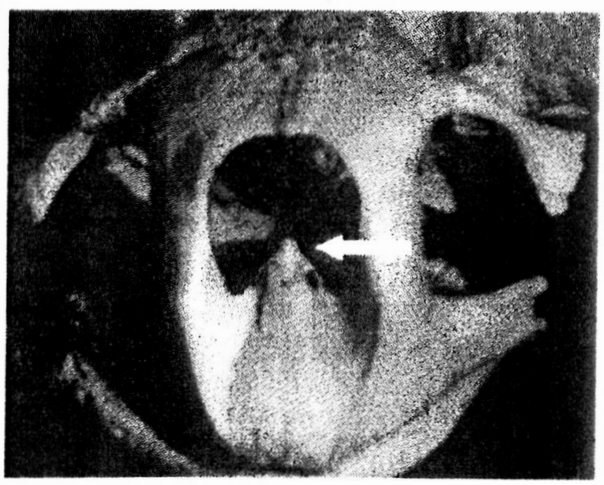


FIG. 2. Anterior view of skull of *Dipsochelys grandidieri* showing the exceptionally large processus vomerinus dorsalis (marked by arrow).

SOFT-TISSUE ANATOMY

The nasal passage in *Dipsochelys* is exceptional in tortoises in rising steeply above a vertical cartilaginous process on the floor of the nasal passage supported by the processus vomerinus dorsalis. This elongate process contains a deep pocket directed posteriorly (Fig. 1a). No distinctive features are apparent within the pocket except for a very narrow duct passing into the foramen praepalatinum. The tissues lining the processus vomerinus dorsalis are innervated by both the olfactory nerve and the accessory olfactory bulb: the nervus septi narium and the vomeronasal nerve respectively.

The nasal passage descends vertically behind the cartilaginous process before dividing into the postero-ventral nasopharyngeal duct and the postero-dorsal cavum nasi proprium. The opening to the latter is par-

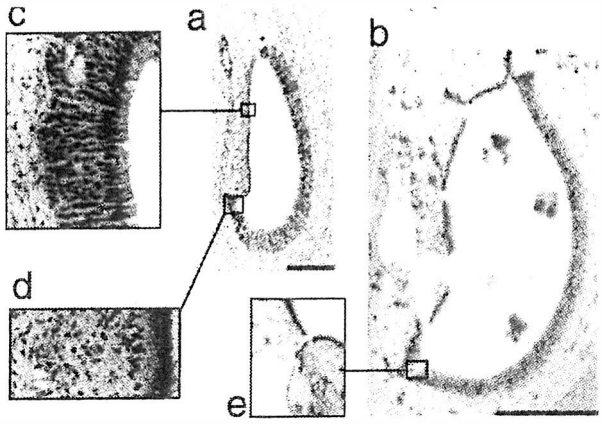


FIG. 3. Photomicrographs of the cavi nasum propium of *Dipsochelys* embryos (Scale bar 500 μ); (a) sectioned at point b on Fig. 4; (b) section posterior to point b on Fig. 4; (c) respiratory epithelium of the intermediate region, showing the absence of ciliated cells; (d) vomeronasal epithelium of the medial nasal gland showing columnar cells with cilia and thickened basal connective tissue; (e) tuberculum palatinum.

tially covered by a flap of soft tissue projecting from the septum. No muscular areas are apparent in association with the flap. Two low ridges lie along the nasopharyngeal duct, one is on the medial and one on the ventro-medial wall.

On the ventral surface of the palate; situated between the foramina praepalatina is a hemispherical structure projecting into the buccal cavity; this has previously been referred to as the 'tuberculum palatinum' (Bojanus, 1819-21; Fritsch, 1870). This structure is oval, 3 mm wide and 4 mm long in the adult tortoise, 1 × 2 mm in the full-term embryo. In live and in preserved material it is detectable as a pale bulge in the anterior of the palate (its exact extent is indistinct in the older preserved, bleached material). It is partially enclosed by the cartilage around the foramina praepalatina and the outline is indistinct.

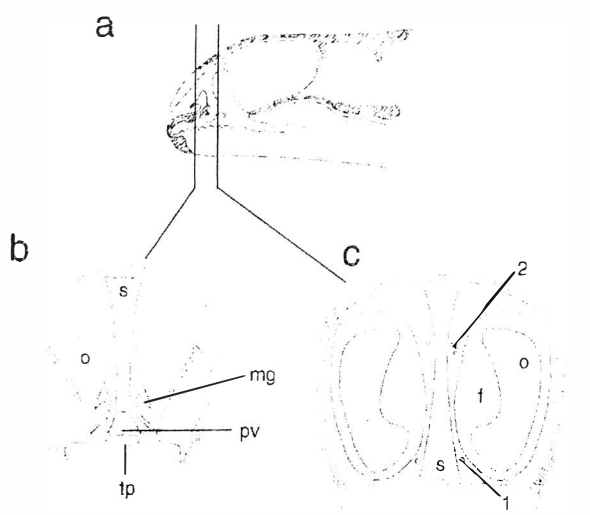


FIG. 4. Transverse sections through the nasal passages of *Dipsochelys;* (a) medial view of the skull showing the location of the sections; (b) section though the processus vomerinus; (c) section through the nasal flap; f – nasal flap; mg– medial nasal gland;o–olfactory chamber; pv–processus vomerinus dorsalis; s – septum; tp – tuberculum palatinum; 1 – nervus septi narium; 2- vomeronasal nerve.

The nasal branches of the palatine arteries pass through the foramina praepalatina and lie on either side of the tuberculum palatinum. Capillaries from the nasal arteries pass through the foramina praepalatina and enter the tuberculum palatinum, no major nerves enter the tuberculum. On either side of the tuberculum palatinum there is a duct passing from the buccal cavity into the processus vomerinus dorsalis.

HISTOLOGY

The available microscope preparations are of embryonic tortoises and the palatal region has been extensively fragmented during preparation of most sections. Consequently the description below and accompanying figures (Fig. 4-5) are based on a composite of the slides (Fig. 3).

Throughout the anterior sections the nasal passages (cavi nasi) are clearly visible as dense ovals, being closed anteriorly by the hypertrophied epithelial lining. A short distance from the external nares the nasal passages are open and the anterior portion of the cartilaginous septum is visible. At the position of the processus vomerinus dorsalis the nasal passages are dorso-ventrally elongate, broadened dorsally and with a medial fold, forming a ventro-medial pocket. Below the nasal passages the tuberculum palatinum is visible, composed of large, open cells.

The nasal passages themselves form two distinct regions; the anterior (vestibular) part and the cavum nasi proprium. The vestibulum is lined with darkly stained cuboid epithelial cells with large nuclei; a darker basal layer is also apparent. The cavum nasi proprium is lined with distinctive columnar epithelial cells above the basal layer. The medial wall is heavily stained in most sections. No elastic fibres could be detected in the basal layer.

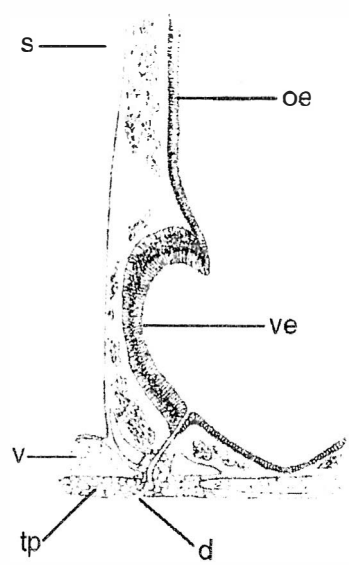


FIG. 5. Transverse section through the vomeronasal organ of *Dipsochelys*, section taken between lines b and c in Fig. 4; d – duct from the tuberculum palatinum to the vomeronasal organ; oe – olfactory epithelium; s – septum; tp – tuberculum palatinum; v – vomer; ve – vomeronasal epithelium.

Throughout the anterior sections the nasal passages (cavi nasi) are clearly visible as dense ovals, being closed anteriorly by the hypertrophied epithelial lining. A short distance from the external nares the nasal passages are open and the anterior portion of the cartilaginous septum is visible. At the position of the processus vomerinus dorsalis the nasal passages are dorso-ventrally elongate, broadened dorsally and with a medial fold, forming a ventro-medial pocket. Below the nasal passages the tuberculum palatinum is visible, composed of large, open cells.

The nasal passages themselves form two distinct regions; the anterior (vestibular) part and the cavum nasi proprium. The vestibulum is lined with darkly stained cuboid epithelial cells with large nuclei; a darker basal layer is also apparent. The cavum nasi proprium is lined with distinctive columnar epithelial cells above the basal layer. The medial wall is heavily stained in most sections. No elastic fibres could be detected in the basal layer.

Within the cavum nasi proprium the broad dorsal olfactory region and the more compressed intermediate region are distinctive. The olfactory region is characterised by the presence of olfactory epithelium with Bowman's glands. The olfactory epithelium is distinctive in its high columnar shape and the presence of long cilia projecting into the lumen. The intermediate region is covered with respiratory epithelium (lacking the cilia of the sensory olfactory cells), with the exception of the medial pocket (the medial nasal gland) that is lined by sensory epithelium, Bowman's glands were not detected (and are absent from this region in other vertebrates). The nervous evaginations of the basal layer reported for *Testudo* (Parsons, 1970) were not observed.

The sections show a large area of thickened basal connective tissue on the medial wall of the nasal chamber. The cells present are typical basal cells with a conical form and could be interpreted as an area of erectile connective tissue as conjectured by Arnold (1979). In conjunction with this a dense network of capillaries are present between the connective tissue and the septum but the connective tissue does not surround venous spaces or contain detectable elastic tissue as would be expected in an erectile tissue. In the central and posterior sections the connective tissue is generally reduced and the medial fold absent, as is the medial pocket. The posterior portion of the nasal chamber is an elongate, dorsally broadened lumen, lined by a narrow border of sensory epithelium, with only a very narrow basal layer.

Palate Observations

When the mouths of the tortoises are opened the tuberculum palatinum is visible as an oval yellowish area of the palate (Fig. 6).

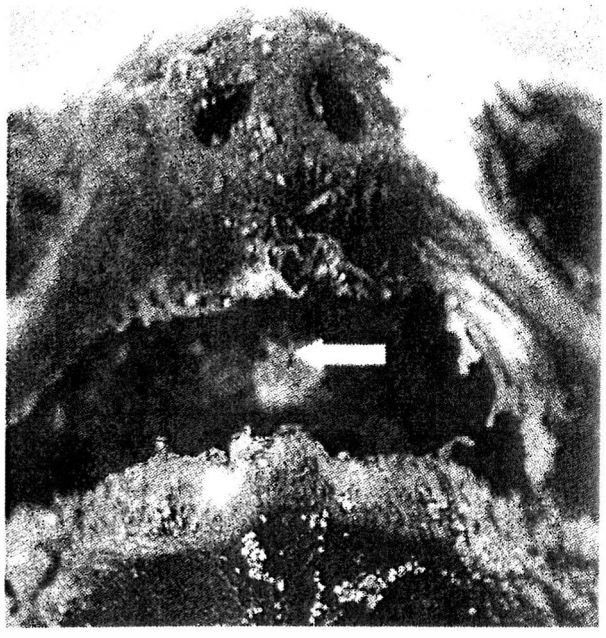


FIG. 6. Anterior view of palate of *Dipsochelys hololissa* showing the location of the tuberculum palatinum (marked by arrow).

DISCUSSION

The present study confirms earlier reports (Arnold, 1979; Gerlach & Canning, 1998) of the complexity of the nasal passages of *Dipsochelys*. The largest structure in the nasal region is the flap of soft tissue projecting from the septum across the opening to the nasal chamber. This is lined with respiratory epithelium and supported by connective tissue, suggesting that it does not have a sensory function but may serve to partially close off the nasal chamber as suggested by Arnold (1979). However, the interpretation of the thickened area of connective tissue as an erectile tissue is made doubtful by the apparent absence of venous spaces or elastic tissue and it may be a passive inhibitor to water flow, permitting 'nasal drinking' but in a less specialised form than the valve proposed by Arnold (1979).

The cartilage and soft tissue structure resting on the processus vomerinus dorsalis is lined with sensory epithelium of the chelonian vomeronasal type (non-glandular). The position of the ventro-medial pocket identifiable as the medial nasal gland where the medial sulcus would be in other testudinids and its sensory development support the suggestion that the processus vomerinus dorsalis encloses a vomeronasal organ (Gerlach & Canning, 1998). This putative vomeronasal organ is connected to the tuberculum palatinum by the open lateral ducts opening directly into the buccal cavity. In other testudinids such as *Testudo* and *Geochelone pardalis* vomeronasal-like structures are less developed, lacking extensive cartilage (the paraseptal process of the septal cartilage only partly covers the duct), are not supported by a bony process of the vomer and lack a connection to the buccal cavity. The bony support and extensive cartilaginous encapsulation in *Dipsochelys* is more reminiscent of the vomeronasal organ of mammals (Rasmussen & Hultgren, 1990).

In all testudinids examined the gland is innervated by the nervus septi narium, a branch of the opthalmic division of the trigeminal nerve. This is the case with the

vomeronasal organ of other reptiles, supporting the identification of the intermediate region of the chelonian nose as a vomeronasal organ (Tucker, 1963; Graziadei & Tucker, 1970; Parsons, 1970). There is also innervation from the accessory olfactory bulb where this has been studied (*Dipsochelys*, pers. obs.; *Caretta*, Saito *et al*., 2000) as in a true vomeronasal organ (Døving & Trotier, 1998), contrary to Gauthier *et al*.'s (1990) analysis. The pars anterior of the vomeronasal organ (equivalent to the anterior sulcus containing the medial nasal gland) is normally described as supporting sensory epithelium without Bowman's glands (olfactory regions support olfactory epithelium and Bowman's glands; Parsons, 1970). In *Testudo* the ventral, lateral walls and ventral surfaces of the olfactory ridges are non-sensory, supporting respiratory epithelium only, without Bowman's glands. The medial nasal gland duct enters the intermediate region in *Testudo* (Parsons, 1970) through the ventral surface of the medial ridge. These features are all in accordance with the observations of *Dipsochelys*. A distinct vomeronasal organ was described in the lateral wall of the nasal septum of *Gopherus polyphemus* (Tucker, 1963) but this was subsequently noted to be the duct of the medial nasal gland (Graziadei & Tucker, 1970) and, as with *Testudo*, the general region of the anterior sulcus can be considered a poorly defined vomeronasal organ.

It appears that *Dipsochelys* possesses an unusually well developed vomeronasal organ for a chelonian. This has an olfactory role and is generally associated with pheromone detection. Nose touching has been observed in genera other than *Dipsochelys*: *Gopherus agassizii* (Camp, 1916) and *Homopus areolatus* (Carpenter & Ferguson, 1977). Although it appears to be an infrequent action, it has been used to suggest that olfaction and pheromones are important in species recognition (Legler, 1960; Carpenter & Ferguson, 1977). Despite this few secretory glands have been identified in tortoises: the axillary and inguinal pores of the Rathke's glands found in many chelonians are not present in the Testudinidae (Loveridge &Williams, 1957) and mental glands (Rathke, 1848; Winokur & Legler, 1975) are only found in *Gopherus* and *Manouria*. Although it has been suggested that 'cloacal discharge of female turtles attracts or stimulates males to court during the breeding season' (Manton, 1979), only one glandular tissue has been described from this region (in *Clemmys marmoratus*; Disselhorst 1904; Whiting 1969). None of these glands has been identified in *Dipsochleys* and the 'scent-gland' described by Owen (1866) in a '*Testudo indica* of two feet long' could not be located by dissection (Gerlach, 2004) and appears to be the mental gland of a *Gopherus* specimen and not a gland of any of the giant tortoise species frequently referred to '*T. indica*' in the 19th century.

In the case of *Dipsochelys* the contact between the buccal region and the olfactory region provided by the ducts between the tuberculum palatinum and the medial nasal gland means that the vomeronasal organ closely resembles the Jacobson's organ of squamates and mammals. The lack of previous reports of such organs in chelonians may be due to the scarcity of dissections of this region in many chelonians. A review of the literature and of skull material identifies one additional taxon with a structure resembling a Jacobson's organ; the leatherback turtle *Dermochelys coriacea* possesses a direct connection between the intermediate nasal region (lined with vomeronasal epithelium; Parsons, 1970) and buccal cavities through the foramina praepalatina, a connection figured as long ago as 1912 (Nick, 1912). This connection differs from that found in *Dipsochleys* in that there is a single opening into the buccal cavity, this is through an opening between the premaxillae (Fig. 4), this may be due to modification from a common arrangement reflecting structural differences in the rostral region of these species. As with *Dipsochelys* the duct into the buccal cavity lies next to an area of loose connective tissue resembling the tuberculum palatinum. The function of this tissue remains obscure, in *Dipsochelys* it could act as a pad of tissue that would be pressed against the ducts into the medial nasal gland during chewing, effectively closing them and preventing food passing into the gland. In *Dermochelys* it appears to be positioned too far posteriorly for such a function. Similarly in *Emys* where the tuberculum palatinum was first described (Bojanus, 1819-21) no connection between the buccal and nasal cavities has been reported. The vomeronasal organ in *Dermochelys* may be associated with the location of natal nesting beaches rather than social interactions as in *Dipsochelys*. In this case a buccal connection would facilitate sensory detection underwater without flooding the nasal cavity. Other marine turtles with similar homing behaviours may also have well developed vomeronasal organs although the possibility of buccal connections is very limited due to the extensive development of the secondary palate.These findings in *Dipsochelys* and the identification of similar structures in *Dermochelys* demonstrates that much remains to be discovered in the sensory capabilities of chelonians. Although there have been detailed investigations of the anatomy and neurobiology of olfaction in some freshwater taxa (e.g. *Geoclemys reevesii,* Graziadei & Tucker, 1970; Taniguchi *et al*., 1995, 1996; *Sternotherus odoratus*, Murphy *et al*., 2001; Fadool *et al*., 2001) the Chelonia are an ecologically diverse group and there is a need for more through examination of the histology and function of this region.

ACKNOWLEDGEMENTS

I am grateful to E. N. Arnold and S. Chapman for enabling me to examine material at the British Museum (Natural History) and to R. Gerlach for careful preservation of the *Dipsochelys hololissa* embryos.

REFERENCES

Arnold, E. N. (1979). Indian Ocean giant tortoises: their systematics and island adaptations. *Philosophical Transactions of the Royal Society of London B* **286**, 127-145.

Bellairs, A. d'A. & Kamal, A. M. (1981). The chondrocranium and the development of the skull in recent reptiles. In: *Biology of the Reptilia.* Vol. 11. 1-263. Gans, C. (Ed.). London: Academic Press.

Bojanus, L. H. (1819-21). Anatome Testudinis Europeaeae. Reptinted 1970, SSAR Facsimile Reprints in Herpetology. Athens (Ohio): Society for the Study of Amphibians and Reptiles.

Bour, R. (1982). Contribution à la connaissance des Tortues terrestres des Seychelles: définition du genre endémique et description d'une espèce nouvelle probablement originaire des îles granitiques et au bord de l'extinction. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris* **295**, 117-122.

Camp, C. L. (1916). Notes on the local distribution and habits of the amphibians and reptiles of south-eastern California. *University of California Publications in Zoology* **12**, 503-544.

Carpenter, C. C. & Ferguson, G. W. (1977). Variation and evolution of stereotyped behaviour in reptiles. In: *The Biology of the Reptilia*, Vol. 7, 335-554. Gans, C., (Ed). London: Academic Press.

Disselhorst, R. (1904). Aufführapparat und Anhangsdrüsen der männlichen Geschlechtorgane. In: Lehrbuch der vergleichende mikroskopischen Anatomie der Wirbeltiere, Vol. 4, 6-89. Opel, A. (Ed). Jena: Gustav Fisher.

Døving, K. B. & Trotier, D. (1998). Structure and function of the vomeronasal organ. *Journal of Experimental Biology* **201**, 2913-2925.

Fadool, D. A., Person, D .J. & Murphy, F .A. (2000). Sexual dimorphism and developmental expression of signal transduction machinery in the vomeronasal organ. *Chemical Senses* **25**, 677.

Fritsch, A. (1870). Zur Anatomie der Elephanten-Schildkröte (*Testudo elephantina*). *Abhandlungen der Königlich Böhmischen Gesellschaft der Wissenschaften, Anatomie* (6) 4, un-numbered.

Gauthier, J., Kluge, A. G. & Rowe, T. (1988). Amniote phylogeny and the importance of fossils. *Cladistics* **4**, 105-209.

Gerlach, J. (2001). Tortoise phylogeny and the *Geochelone* problem. *Phelsuma* **9A**, 1-64.

Gerlach, J. (2004). *Giant Tortoises of the Indian Ocean.* Frankfurt: Chimaira.

Gerlach, J. & Canning, L. (1998). Taxonomy of Indian Ocean giant tortoises (*Dipsochelys*). *Chelonian Conservation & Biology* **3**, 3-19.

Graziadei, P. P. C. & Tucker, D. (1970). Vomeronasal receptors in turtles. *Zoologische Zellforschung* **105**, 498-514.

Legler, J. M. (1960). Natural history of the ornate box turtle, *Terrapene ornata ornata* Agassiz. *Publication of the Museum of Natural History, University of Kansas.* **11**, 527-699.

Loveridge, A. & Williams, E. E. (1957). Revision of the African tortoises and turtles of the suborder Cryptodira. *Bulletin of the Museum of Comparative Zoology, Harvard* **115**, 163-557.

Manton, M. L. (1979). Olfaction and behaviour. In: *Turtles: Perspectives and Research*, 289-304. Harless, M. & Marlock, H. (Eds). New York: J. Wiley & Sons.

Murphy, F. A., Tucker, K. & Fadool, D. A. (2001). Sexual dimorphism and developmental expression of signal-transduction machinery in the vomeronasal organ. *Journal of Comparative Neurology* **432**, 61-74.

Nick, L. (1912). Das Kopfskelet von *Dermochelys coriacea* L. *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere* **33**, 1-238.

Owen, R. (1866). *Anatomy of vertebrates.* p. 562. London: Longmans, Green, and Co.

Palkovacs, E. P., Gerlach, J. & Caccone, A. (2002). The evolutionary origin of Indian Ocean tortoises (*Dipsochleys*). *Molecular Phylogenetics and Evolution* **24**, 216-227.

Parsons, T. S. (1959). Nasal anatomy and phylogeny of reptiles. *Evolution* **13**, 175-187.

Parsons, T. S. (1970). The nose and Jacobson's organ. In: *The Biology of the Reptilia*, Vol. 2. 99-191. Gans, C. (Ed). London: Academic Press.

Rasmussen, L. E. L. & Hultgren, B. (1990). Gross and microscopical anatomy of the vomeronasal organ in the Asian elephant (*Elephas maximus*). In: *Chemical Signals in Vertebrates*, Vol. 5, 154-161. MacDonald, D. W., Müller-Schwarze, D. & Natynczuk, S. E. (Eds). Oxford: Oxford University Press.

Rathke, H. (1848). *Über die Entwicklung der Schildkröten.* Braunschweig: F.Veiweg.

Saito, K., Shji, T., Uchida, I. & Ueda, H. (2000). Structure of the olfactory and vomeronasal epithelia in the loggerhead turtle *Caretta caretta. Fisheries Science* **66**, 409-411.

Seydel, O. (1895). Über die Nasenhöhle und das Jacobsonsche Organ der Amphibien und Reptilien. *Morphologische Jahrbücher* **23**, 1-53.

Taniguchi, M., Kashiwayanagi, M. & Kurihara, K. (1995). Intracellular injection of inositol 1,4,5-triphosphate increases a conductance in membranes of turtle vomeronasal receptor neurons in the slice preparation. *Neuroscience Letters* **188**, 5-8.

Taniguchi, M., Kashiwayanagi, M. & Kurihara, K. (1996). Intracellular dialysis of cyclic nucleotides induces inward currents in turtle vomeronasal receptor neurons. *Chemical Senses* **25**, 67-76.

Tucker, D. (1963). Olfactory, vomeronasal and trigeminal receptor responses to odorants. In: *Olfaction and Taste*, pp. 45-70. Zotterman, Y. (Ed). Oxford: Pergamon Press.

Whiting, A. M.(1969). *Squamate Cloacal Glands: Morphology, histology and Histochemistry.* Unpublished PhD. Thesis. University Park: Pennsylvania State University.

Winokur, R. M., Legler, J. M. (1975). Chelonian mental glands. *Journal of Morphology* **147**, 275-291.

Accepted: 27.11.03

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 21-25 (2005)

CHEMICAL ASSESSMENT OF PREDATION RISK IN THE WALL LIZARD, *PODARCIS MURALIS*, IS INFLUENCED BY TIME EXPOSED TO CHEMICAL CUES OF AMBUSH SNAKES

LUISA AMO, PILAR LÓPEZ AND JOSÉ MARTÍN

Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Lizards often respond to predator presence by increasing refuge use. However, this behaviour may expose lizards to saurophagous snakes, which inhabit the same refuges to ambush their lizard prey. Snakes, which are not always visible, deposit chemical trails that can be detected by lizards. Even though there are obvious advantages of using chemical cues, chemical detection of predators might lead to very conservative estimates of risk. This is because chemical cues might indicate that an area was risky in the recent past, but not necessarily at the current time. We examined experimentally whether wall lizards (*Podarcis muralis*) avoid using refuges that contain chemical cues of smooth snakes (*Coronella austriaca*), and whether this avoidance response is maintained long term or whether it can be modified. Results suggest that wall lizards detected the chemical cues of smooth snakes inside refuges, and, in the short term, decreased the use of predator-scented refuges and increased their escape movements. However, this avoidance response seemed to decrease in the long term. By investigating the refuge again over subsequent time periods, lizards reassessed whether the snake was actually present, modified their refuge use and decreased their avoidance response. Therefore, wall lizards seem able to assess temporal variations in predation risk by snakes inside refuges and to respond accordingly.

Key words: behaviour, chemoreception, *Coronella austriaca*, predator-prey interactions

INTRODUCTION

Predation is a major selective force. However, since animals must accomplish more in their lifetime than simply avoiding predation, natural selection favours individuals that minimise their individual risk of mortality while attending to other demands (Lima & Dill, 1990). Chemosensory cues may reliably reveal the presence of predators and they may also provide information on predator activity level and diet (Kats & Dill, 1998). Snakes deposit chemical trails that can be detected by lizards with their highly developed vomeronasal system (Cooper, 1990; Van Damme *et al.*, 1995; Downes & Shine, 1998*a*; Van Damme & Quick, 2001; Downes & Bauwens, 2002). Because snakes are not always visible, their chemical stimuli may be particularly important for lizards that share the same refuges (Downes & Shine, 1998*a*). For example, some geckos used their chemosensory ability to avoid entering rock crevices with snake scent (Downes & Shine, 1998*a,b*).

Prey, such as lizards, often respond to predator presence by increasing refuge use (Greene, 1988; Sih *et al.*,1992). However, refuges may have some costs that should be minimised, such as the loss of time available for other activities, or physiological costs (Dill & Fraser, 1997; Sih, 1997; Martín & López, 1999*a,b*). In addition, some types of refuges may only be useful against some particular type of predators, or may expose prey to other types of predators (Sih *et al.*, 1998). For example, lizards may face saurophagous, ambush-hunting snakes that share the same refuges (Downes & Shine, 1998*a*). The ability to detect the chemical cues of a snake may help lizards to survive an encounter with a predator (Downes, 2002). Even though there are obvious advantages of using chemical cues, especially when other cues are unavailable, chemical detection of predators may lead to very conservative estimates of risk because they indicate that a given area was risky at a certain point in time but not necessarily a current risk (Kats & Dill, 1998; Turner & Montgomery, 2003). Thus, according to the threat sensitivity hypothesis (Helfman, 1989), natural selection should favour individuals that take action appropriate to the magnitude of threat, rather than avoiding the use of refuges in response to all kinds of predator chemical cues.

The wall lizard, *Podarcis muralis*, offers an excellent opportunity to study the patterns of avoidance of hazardous refuges. Wall lizards respond to predator presence in the open by increasing refuge use (Martín & López, 1999*b*). However, by doing this, they may expose themselves to increased predation risk inside refuges by ambush-hunting smooth snakes (*Coronella austriaca*). This is a lizard specialist that hunts by ambush foraging, hidden in rock crevices (Rugiero *et al.*, 1995; Galán, 1998), and has a geographic distribution and habitat preferences that overlap frequently with those of wall lizards. Previous studies have shown that *P. muralis* is able to detect and discriminate the chemical cues of smooth snakes (Amo *et al.*, 2004). In this paper, we examined experimentally whether wall lizards avoid using refuges that contain chemical cues of smooth snakes, and whether this avoidance response is maintained long term or whether it can be modified.

Correspondence: J. Martin, Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain. *E-mail*: Jose.Martin@mncn.csic.es

MATERIALS AND METHODS

During May 2001, we captured adult *P. muralis* by noosing (9 males and 10 females; snout-vent length, SVL, ±SE = 66±2 mm) at a rock wall (120 m long , 5 m high) near Cercedilla (Madrid Province, Spain). We also captured in the same wall two adult smooth snakes to be used as source of scent of potential predators. Lizards were individually housed at "El Ventorrillo" Field Station 5 km from the capture site, in outdoor 60 × 40 cm PVC terraria containing sand substratum and rocks for cover. Every day, they were fed mealworm larvae (*Tenebrio molitor*) dusted with multivitamin powder for reptiles, and water was provided *ad libitum*. The photoperiod and ambient temperature was that of the surrounding region. Lizards were held in captivity at least one month before testing to allow acclimation to laboratory conditions. To prevent lizards from having contact with the scent stimuli before they were tested, the snakes were housed separately in glass terraria (60 × 30 × 20 cm) with sawdust on the substrate to obtain their scent. Due to its absorbent properties, the odourless sawdust is an excellent method for obtaining snake scent without disturbing the animal. All the animals were healthy during the trials and were returned to their exact capture sites at the end of experiments. The experiment was performed under licence from the "Madrid Environmental Agency" ("Consejería del Medio Ambiente de la Comunidad de Madrid").

To compare the behaviour of lizards when they found a potentially unsafe refuge (i.e. with snake chemical cues) or an unfamiliar but predator-free refuge, we used two terraria (60 × 40 × 30 cm). Terraria were divided into two halves, and had two refuges placed symmetrically on either side, one in front of the other, with a distance of 15 cm between them. The refuges were flat rocks (10 × 7 cm) placed 2 cm above the substrate, allowing lizards to hide under them. In the 'predator' treatment, the terrarium had a refuge containing chemical cues of a smooth snake and an odourless refuge. In the 'control' treatment both refuges were odourless. To add the predator scent to the refuge, we used sawdust that had been in the terrarium of the snakes for at least three days, moistened with deionized water. In the odourless refuges, we applied some deionized water to a similar quantity of odourless sawdust. In both cases, sawdust was placed on the ground, inside the refuge. We did not include a pungent control (e.g. perfume) in the experimental design because results of previous experiments showed that *P. muralis* cannot distinguish it from water and from other biologically irrelevant odours, but can distinguish it from snake scent (Amo *et al.*, 2004). Moreover, *P. muralis* does not modify the use of refuges containing a pungent odour, compared to an odorless control (Amo, López & Martín, unpublished data). Every lizard was tested in each treatment once in a randomised block design, and order of trials was counterbalanced. One trial was conducted per day for each animal. Trials were conducted under outdoor conditions during July 2001 between 1200-1700 hrs when lizards were fully active. Lizards were allowed to bask in their home terraria for at least two hours before trials. After each trial the cages and the refuges were cleaned thoroughly with water and detergent for 20 min and dried at the outdoor temperature. We used new stimuli in each trial to avoid the mixture of odours.

Experiments were recorded on videotape (Hi-8 format, 25 frames s-1) using a Sony CCD-TR810E video camera aligned perpendicularly over the terrarium. Lizards were filmed as they moved spontaneously along the terrarium during 25 min. The experimenter was not present during filming to avoid disturbing lizards. After this, we noted the location of each lizard in the terrarium every 30 min over the subsequent five hours. Later, we analysed the tapes and noted lizard behaviour in the experimental half of the terrarium (i.e. the half that contained the snake-scented refuge in the 'predator' treatment, or one of the odourless refuges in the 'control' treatment). We noted the total time spent in the experimental area, time spent in movement, motionless, or standing up trying to escape (i.e. the lizard stands in a upright position against the wall of the terrarium and performs scratching movements with the forelegs), and total time spent inside each refuge. To determine possible changes through time in the responses, we divided each 25 min period into five consecutive periods of 5 min each. We chose this interval of time because previous results of Thoen *et al.* (1986) showed that the responses of common lizards, *Lacerta vivipara*, to the scent of smooth snakes was different in the first 5 min of the trial than afterwards.

We used two-way repeated measures analysis of variance (ANOVA) to test for differences between treatments ('control' vs. 'predator') and among the five time sequences of each individual (within-subjects factor). Data of total time spent in the experimental half of the terrarium were log-transformed, which succesfully normalize the data. We used the time spent in movement, motionless, and in standing up acts in the experimental area, and the time spent in each refuge, in relation to the total time spent in the corresponding area. Angular transformations of all percentages were made to normalize the data.

Differences in the location of lizards during the subsequent five hours between treatments were analysed with one way ANOVA. We calculated the number of times that lizards were observed outside of refuges, and the number of times the lizards were seen hidden in the experimental refuge in relation to the number of times that the lizards were inside any refuge. Data were log- and arcsin-transformed, respectively, to normalize data. Tests of homogeneity of variances (Levene's test) showed that in all cases variances were not significantly heterogeneous after transformation. Pairwise comparisons of means were planned using the Tukey's honestly significant difference (HSD) test (Sokal & Rohlf, 1995).

RESULTS

Total time that lizards spent in the experimental area did not differ significantly between treatments (repeated measures two way ANOVA; $F_{1,18}$=0.52, P=0.48), although there were significant differences among sequences ($F_{4,72}$=2.47, P<0.05). The interaction was not significant ($F_{4,72}$=1.12, P=0.35). Lizards decreased the time that they spent in the experimental half of the terrarium over time, although there were only significant post-hoc differences between the first and the third sequence (Tukey's test, P=0.04).

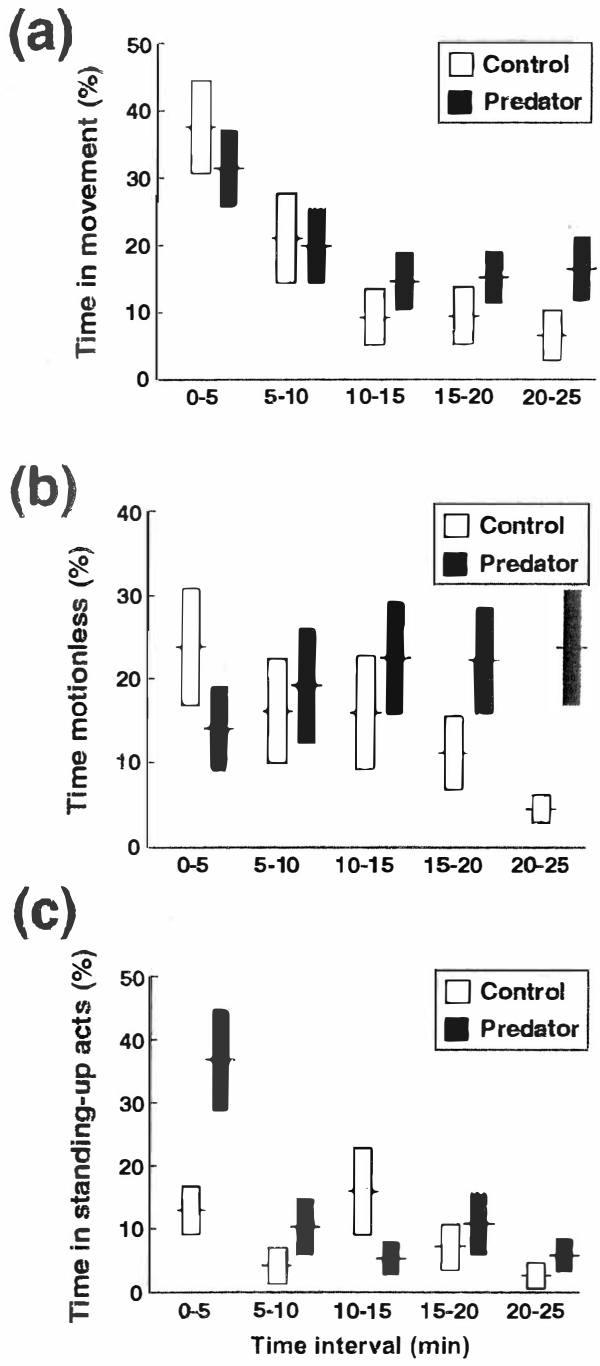


FIG. 1. Percent time (mean ±SE) spent (a) in movement, (b) motionless, and (c) in standing up acts, in relation to the total time spent in the experimental area, in the 'control' (open boxes) and 'predator' (black boxes) treatments.

Time spent moving did not differ significantly between treatments ($F_{1,18}$=0.10, P=0.75), but there were significant differences among sequences ($F_{4,72}$=11.92, P<0.0001) (Fig. 1a). The interaction was not significant ($F_{4,72}$=1.50, P=0.21). Lizards decreased their movement rate across time, especially after the first 5 min. Thus, there were significant differences between the first sequence and the subsequent four (P<0.001 in all cases), but not between the rest of sequences (P>0.21 in all cases).

Time spent motionless did not differ significantly either between treatments ($F_{1,18}$=0.58, P=0.46), or among sequences ($F_{4,72}$=0.31, P=0.87), but the interaction was significant ($F_{4,72}$=2.51, P<0.05) (Fig. 1b). Lizards increased the time spent motionless in the 'predator' treatment whereas they decreased it in the 'control' treatment in the course of time, although post hoc comparisons did not show significant differences (Tukey's test, P>0.10 in all cases).

Duration of standing up acts did not differ significantly between treatments (repeated measures two way ANOVA; $F_{1,18}$=2.14, P=0.16), although there were significant differences among sequences ($F_{4,72}$=6.07, P=0.0003) and the interaction was significant ($F_{4,72}$=4.34, P=0.003) (Fig. 1c). During the first 5 min the time spent by lizards in standing up acts was significantly higher in the 'predator' treatment than in the 'control' one (P=0.006). Whereas, later, there were no significant differences either between sequences, when considering each treatment alone, or between treatments in each sequence (P>0.57 in all cases).

Time spent inside the refuge did not differ significantly either between treatments (repeated measures two way ANOVA; $F_{1,18}$=0.001, P=0.98) or among sequences ($F_{4,72}$=2.06, P=0.09), but the interaction was significant ($F_{4,72}$=3.94, P=0.006) (Fig. 2). During the first 5 min there were no significant differences between treatments in time spent in refuges (P=0.30). However, in the course of time lizards increased the time they

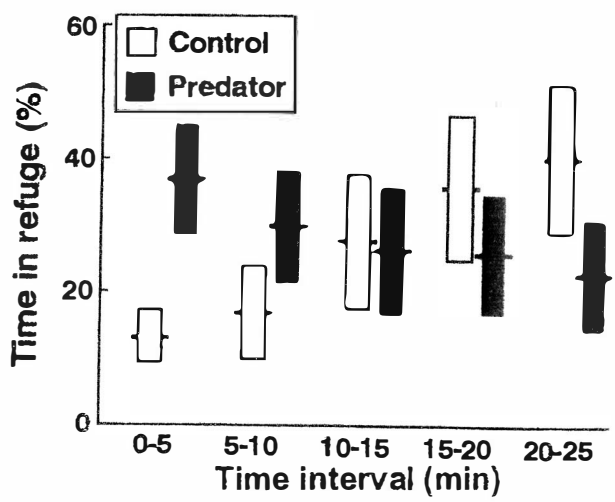


FIG. 2. Percent time (mean ±SE) spent inside the experimental refuge in relation to the total time spent in the experimental area, in the 'control' (open boxes) and 'predator' (black boxes) treatments.

spent in the control refuge (differences between the first and the other five sequences, P=0.02), but they did not increase it in the refuge containing chemical signals of a snake (P>0.97 in all cases). Nevertheless, in the long term (i.e. in the subsequent five hours), the number of times that lizards were observed out of a refuge did not significantly differ between treatments (control: 3±1 times; predator: 4±1 times; one way ANOVA, $F_{1,18}$=1.02, P=0.33). Also, there was no significant difference between treatments in the use of the experimental refuge (number of times in the experimental refuge/number of times in any of the two refuges, control: 56±7%; predator: 51±6%; $F_{1,18}$=1.26, P=0.28). Thus, in the long term lizards did not avoid to hide in the refuge soiled with snake scent.

DISCUSSION

Results of this study suggest that wall lizards were able to detect the chemical cues of smooth snakes, and to use them in the short term to assess the potential risk of predation inside a refuge, but that after some time lizards were able to reassess whether the snake was actually present and modified their response. To avoid the risk of predation by ambush snakes, lizards initially modified their behaviour and their use of potentially hazardous refuges. During the first few minutes, lizards spent the same time in both types of refuges. However, later on, lizards decreased their use of the predator-scented refuge, whereas they increased the use of the odourless refuge. This could be explained if lizards approached refuges and spent some time investigating the source of the odour, but after discriminating the snake scent, they decided to avoid using the unsafe refuge. Our results agree with previous studies that have shown that other lizard species avoid using retreats that were soiled with snake's scent (Downes & Shine, 1998*a*; Downes & Bauwens, 2002).

Lizards also modified their locomotor patterns in the predator treatment. Previous studies have shown that prey exposed to a potential predator odour often show behavioural changes such as reduced activity (Van Damme *et al.*, 1990), increased refuge use (Kiesecker *et al.*, 1996) or reduced use of the potential risky area (Downes & Shine, 1998*a*). Our results suggest that lizards increased their escape behaviour (i.e. standing up acts) when they found chemical cues of a snake inside a refuge. *Podarcis sicula* lizards also increase the time spent in standing up acts when they found chemical cues of a snake on the ground (Downes & Bauwens, 2002). Also, wall lizards showed a similar behaviour when they found chemical cues of a snake on the open ground of a terrarium (Amo, López & Martín, unpublished data). These results suggest that lizards perceived an increase in the risk of remaining near a potentially unsafe area and that they responded by trying to escape from the terrarium. A similar response to predator chemicals was observed in larval *Ambystoma* salamanders, which decreased movement only in the absence of a refuge; otherwise, increased movement in an effort to reach a refuge (Sih & Kats, 1991). Also, increased movement in larval toads in response to an alarm substance may represent refuge-seeking behaviour (Hews, 1988). Wall lizards also tended to maintain the time spent motionless in the risky area while they decreased it in the control area across the time. By standing still, lizards may try to visually detect the snake in a potentially unsafe area (Avery, 1991, 1993; McAdam & Kramer, 1998).

However, this avoidance response seemed to decrease in the long term. Chemical detection of a snake may indicate that a refuge was risky at a certain point in time but it does not necessarily indicate a current risk. Thus, chemical assessment might lead to excessively conservative estimates of risk if prey continue avoiding the refuge despite the absence of the predator. By investigating the refuge again over the subsequent minutes, lizards may assess the absence of the snake. Thus, wall lizards responded to the temporal decrease in the risk of predation inside the refuge by decreasing their avoidance response and increasing the use of such refuge. Similarly, the avoidance response to predator chemical cues diminished with time in *Physa* snails (Turner & Montgomery, 2003) and *Plethodon cinereus* salamanders (Sullivan *et al.*, 2002). In contrast, garden skinks, *Lampropholis guichenoti*, avoided the use of predator-scented areas during six months (Downes, 2001). However, in this case, the predator odour was replaced weekly. Thus, skinks probably continued avoiding the risky area because they could perceive a fresh stimulus every week. An explanation for the lack of avoidance behaviour across the time may be that wall lizards are able to assess the age of the chemical cues they found. However, our experiment did not test this effect and, thus, further research is needed to examine whether wall lizards have this ability.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for helpful comments, Kevin Pilz for checking the english, and "El Ventorrillo" MNCN Field Station for use of their facilities. Financial support was provided by the MCYT projects BOS 2002-00598 and BOS 2002-00547, and an "El Ventorrillo" CSIC grant to L. Amo.

REFERENCES

Amo, L., López, P. & Martín, J. (2004). Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. *Animal Behaviour* **67**, 647-653.

Avery, R. A. (1991). Temporal dynamics of a vigilance posture in the ruin lizard *Podarcis sicula*. *Amphibia-Reptilia* **12**, 352-356.

Avery, R. A. (1993). Experimental analysis of lizard pause-travel movement: pauses increase probability of prey capture. *Amphibia-Reptilia* **14**, 423-427.

Cooper, W. E., Jr. (1990). Chemical detection of predators by a lizard, the broad-headed skink (*Eumeces laticeps*). *Journal of Experimental Zoology* **256**, 162-167.

Dill, L. M. & Fraser, A. H. G. (1997). The worm re-turns: hiding behaviour of a tube-dwelling marine polychaete, *Serpula vermicularis*. *Behavioral Ecology* **8**, 186-193.

Downes, S. (2001). Trading heat and food for safety: costs of predator avoidance in a lizard. *Ecology* **82**, 2870-2881.

Downes, S. (2002). Does responsiveness to predator scents affect lizard survivorship? *Behavioral Ecology and Sociobiology* **52**, 38-42.

Downes, S. & Bauwens, D. (2002). Does reproductive state affect a lizard's behavior toward predator chemical cues? *Behavioral Ecology and Sociobiology* **52**, 444-450.

Downes, S. & Shine, R. (1998*a*). Sedentary snakes and gullible geckos: predator-prey coevolution in nocturnal rock-dwelling reptiles. *Animal Behaviour* **55**, 1373-1385.

Downes, S. & Shine, R. (1998*b*). Heat, safety or solitude? Using habitat selection experiments to identify lizard's priorities. *Animal Behaviour* **55**, 1387-1396.

Galán, P. (1998). *Coronella austriaca* - Laurenti 1768. In *Fauna Ibérica. Vol. 10*, 364-375. Ramos, M. A. (Ed). Madrid: Museo Nacional de Ciencias Naturales, CSIC.

Greene, H. W. (1988). Antipredator mechanisms in reptiles. In *Biology of the Reptilia. Vol. 16*, 1-152. Gans, C. and Huey, R. B. (Eds). New York: John Wiley & Sons.

Helfman, G. S. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* **24**, 47-58.

Hews, D. K. (1988). Alarm response in larval western toads, *Bufo boreas*: release of larval chemicals by a natural predator and its effects on predator capture efficiency. *Animal Behaviour* **36**,125-133.

Kats, L. B. & Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361-394.

Kiesecker, J. M., Chivers, D. P. & Blaustein, A. R. (1996). The use of chemical cues in predatory recognition by western toad tadpoles. *Animal Behaviour* **52**, 1237-1245.

Lima, S. L. & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**, 619-640.

Martín, J. & López, P. (1999*a*). When to come out from a refuge: risk-sensitive and state-dependent decisions in an alpine lizard. *Behavioral Ecology* **10**, 487-492.

Martín, J. & López, P. (1999*b*). An experimental test of the costs of antipredatory refuge use in the wall lizard, *Podarcis muralis*. *Oikos* **84**, 499-505.

McAdam, A. G. & Kramer, D. L. (1998). Vigilance as a benefit of intermittent locomotion in small mammals. *Animal Behaviour* **55**, 109-117.

Rugiero, L., Capula, M., Filippi, E. & Luiselli, L. (1995). Food habits of the Mediterranean populations of the smooth snake (*Coronella austriaca*). *Herpetological Journal* **5**, 316-318.

Sih A. (1997). To hide or not to hide? Refuge use in a fluctuating environment. *Trends in Ecology and Evolution* **12**, 375-376.

Sih, A. & Kats, L. B. (1991). Effects of refuge availability on the responses of salamander larvae to chemical cues from predatory green sunfish. *Animal Behaviour* **42**, 330-332.

Sih, A., Kats, L. B. & Moore, R. D. (1992). Effects of predatory sunfish on the density, drift and refuge use of the stream salamander larvae. *Ecology* **73**, 1418-1430.

Sih, A., Englund, G. & Wooster, D. (1998). Emergent impact of multiple predators on prey. *Trends in Ecology and Evolution* **13**, 350-355.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry, 3rd. ed.* New York: W. H. Freeman and Co.

Sullivan, A. M., Maerz, J. C. & Madison, D. M. (2002). Anti-predator response of red-backed salamanders (*Plethodon cinereus*) to chemical cues from garter snakes (*Thamnophis sirtalis*): laboratory and field experiments. *Behavioral Ecology and Sociobiology* **51**, 227-233.

Thoen, C., Bauwens, D. & Verheyen, R. (1986). Chemoreceptive and behavioural responses of the common lizard *Lacerta vivipara* to snake chemical deposits. *Animal Behaviour* **34**,1805-1813.

Turner, A. M. & Montgomery, S. L. (2003). Spatial and temporal scales of predator avoidance experiments with fish and snails. *Ecology* **84**, 616-622.

Van Damme, R. & Quick, K. (2001). Use of predator chemical cues by three species of lacertid lizards (*Lacerta bedriagae*, *Podarcis tiliguerta*, and *Podarcis sicula*). *Journal of Herpetology* **35**, 27-36.

Van Damme, R., Bauwens, D., Vanderstighelen, D. & Verheyen, R. F. (1990). Responses of the lizard *Lacerta vivipara* to predator chemical cues: the effects of temperature. *Animal Behaviour* **40**, 298-305.

Van Damme, R., Bauwens, D., Thoen, C., Vanderstighelen, D. & Verheyen, R. F. (1995). Responses of naive lizards to predator chemical cues. *Journal of Herpetology* **29**, 38-43.

Accepted: 23.1.04

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 27-35 (2005)

A NEW SPECIES OF AGAMA (SAURIA: AGAMIDAE) FROM MAURITANIA

JOSÉ M. PADIAL[1,2]

[1]*Museo de Historia Natural Noel Kempff Mercado (Area Zoologia), Santa Cruz de la Sierra, Bolivia*

[2]*Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales – CSIC, C/José Gutierrez Abascal 2, 2800 Madrid, Spain*

A new agama species of the *Agama agama* species group, is described from the Adrar Mountains of Mauritania, in the Meridional Sahara. This species is morphologically similar and genetically related to *Agama impalearis*. It is characterized by small size (snout-vent length of adults: 67.6-74.88 mm); long hind legs; gular region with brown irregular longitudinal lines; 10 preanal pores; fourth finger longer than first; small nuchal crest (composed of six spines) and absence of caudal crest; reddish eyelid in males; smooth head scales; regular keeled and mucronate dorsal scales; ventral scales smaller than dorsals; 55-63 scales around mid body; 10-12 surpralabials; 9-12 infralabials; 8-9 group of spines between the anterior margin of the ear opening and the shoulder; 12 lamellae under fourth finger; 19-21 lamellae under fourth toe. It is a solitary rock dweller inhabiting extremely dry habitats with scarce vegetation.

Key words: Africa, *Agama castroviejoi*, lizard, mtDNA, Sahara

INTRODUCTION

Information on north-west African reptiles, from the Mediterranean to the Saharan region, has increased notably in the last 20 years (Schleich *et al.*, 1996). Countries like Morocco, Western Sahara and Mali have received special attention (Joger, 1981; Böhme *et al.*, 1996; Joger & Lambert, 1996; Bons & Geniez, 1996; Geniez *et al.*, 2000), and recently also Mauritania (Ineich, 1997; Böhme, 2000; Böhme *et al.*, 2001). As a result, taxonomic work has been carried out on several complex groups and some new species have been described (Salvador, 1982; Joger, 1984; Mateo *et al.*, 1998; Wilms & Böhme, 2001). Nevertheless, new species are discovered only occasionally (Joger, 1980; Joger & Lambert, 1996; Geniez & Foucart, 1995; Brown *et al.*, 2002), indicating that we have still not reached a complete knowledge of the reptile diversity in north-western Africa, above all in the Saharan region, where vast areas still remain unexplored.

Four *Agama* species have been cited for Mauritania: *Agama agama* (Linnaeus, 1758), *Agama boueti* Chabanaud, 1917, *Agama boulengeri* Lataste, 1886 and *Agama impalearis* (Duméril & Duméril, 1851) (Chabanaud, 1917; Dekeyser & Villiers, 1956; Ineich, 1997; Lambert & Mullié, 1998; Böhme *et al.*, 2001). During field-work carried out in Mauritania, only the first three species were found. *A. agama* is a typical species from Sahel savannah, but it also inhabits human settlements (Joger, 1979) and is common in Southern Mauritania (pers. obs.). *A. boueti* is also a typical inhabitant of the sandy savannahs of southern Mauritania. Although Chabanaud (1917) reported *A. impalearis* for Mauritania ("Mauritanie Saharienne"), Joger (1979) stated that this species did not occur there and that the nearest record would be that of Seguia el Hamra (Western Sahara). Despite *A. agama* being cited for the Village Chinguetti in the Adrar region (Dekeyser & Villiers, 1956) it was not found by the author. Only *A. boulengeri* and five specimens of an unidentified *Agama* species could be collected in this area. These five specimen were discovered on a tableland ("Dahr") from the Adrar Mountains, in the Sahara Region. They are morphologically similar to *A. impalearis*, but some morphological differences in scalation count characters where found. Nevertheless, some works have demonstrated great morphological and genetic variation among populations of *A. impalearis* (Brown & Znari, 1998; Brown *et al.*, 1999; Brown *et al.*, 2002) and have recognized a vicariant linage as a putative new species (Brown *et al.*, 2002). In fact, the diversity of the group is higher than previously supposed. Therefore, a genetic comparison among the aforementioned species was also necessary to clarify the identity of Adrar population. The results indicate the specimens collected in the Adrar Mountains do not correspond to any of the known North African agamid species. The aim of this paper is to describe these specimens and to clarify their possible relationship with *A. impalearis*.

Correspondence: J. M. Padial, Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales – CSIC, C/José Gutierrez Abascal 2, 2800 Madrid, Spain. *E-mail*: jmpadial@yahoo.com

MATERIAL AND METHODS

Specimens of the new species are deposited in Museo Nacional de Ciencias Naturales (MNCN), Madrid (Spain). Other specimens examined are listed in Appendix 1. Geographical localities were obtained with a Garmin E-Trek GPS receiver. Colour slides of the specimens were taken in the field. All measurements of the specimens are in millimetres and were taken with a digital calliper (to the nearest 0.01mm). Specimens were collected the 20 May 2002 during a field-trip to the Adrar plateau of Mauritania. They were sacrificed by injection of a dilution of nicotine, fixed with 10 % formalin and preserved in 70% ethanol. Tissue samples for

DNA studies were obtained from four specimens of the new species (MNCN 41776-79) and preserved in absolute ethanol.

The following 27 morphological characters were obtained from each specimen: (1) 16 linear body dimensions: SVL, snout-vent length; TAL, tail length; TAH, tail height (at the base of the tail); TAW, tail width (at the base of the tail); RTAL, relative tail length (% of SVL); HL, head length; HW, maximum head width; HH, maximum head height; FOOT, foot length (from heel to the tip of the longest toe, including the claw); HAND, hand length (from wrist to the tip of the longest finger, including the claw); FINGER, length of the fourth finger (from the joint with the hand to the tip, including the claw); TOE, fourth toe length (from the joint with the foot to the tip, including the claw); TL, tibia length; FL, femur length (shank length); EN, eye-nostril distance (from the posterior margin of the nostril to the anterior corner of the eye); EE, distance ear opening-eye (from the posterior corner of the eye to the anterior margin of the ear opening); (2) 11 scalation counts characters: VE, number of ventral scales (from the inguinal region to the level of the axilla); EMB, number of scales around midbody; SL, number of supralabials scales; IL, number of infralabial scales; SO, number of supraocular scales; RSLE, rows of scales between supralabials and eye; GNS, groups of neck spines; IP, number of inguinal pores; LFF, number of lamellae under fourth finger; LFT, number of lamellae under fourth toe; SC, number of spines of the nuchal crest.

Scalation count characters of males of the two forms of *A. impalearis* were provided by Brown (*in litt.*). Comparisons with *A. boueti* and *A. boulengeri* were based on data from Joger (1979) and personal observations; and on *A. agama* from personal observations.

Partial DNA sequences of the 16S rRNA mitochondrial gene were obtained for four specimens of the new species and compared with previously published 16S rRNA sequences of *A. impalearis* from Morocco (Brown *et al.*, 2002). *Laudakia atricollis* from Tanzania was used as outgroup (Brown *et al.*, 2002).

Accession numbers for the MNCN specimens sequenced are as follows: MNCN 41776=AY522926; MNCN 41777= AY522927; MNCN 41778= AY522928; MNCN 41779= AY522929. Total genomic DNA extraction followed standard protocols described elsewhere (Carranza *et al.*, 1999, 2000). Primers used in both amplification and sequencing were L2510 (5'-CGCCTGTTTATCAAAAACAT-3') and H3062 (5'-CCGGTTTGAACTCAGATCA-3') from Lenk *et al.* (2001).

Mitochondrial sequences were aligned in ClustalX (Thompson *et al.*, 1997) with default parameters. Only two gaps had to be postulated to align the new species with *A. impalearis*. In total, 464 base pairs of 16S rRNA mitochondrial DNA were included in the phylogenetic analysis, of which 84 were variable and 27 parsimony-informative. The results of the alignment are available from the author upon request. The aligned data set was analyzed using maximum-parsimony (MP) in PAUP* 4.0B10 (Swofford, 2002), and include heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 100 random stepwise additions of taxa. Gaps were included as fifth state. Nodal support for the MP tree was assessed using bootstrap analysis (Felsenstein, 1985) involving 1000 pseudo-replications.

RESULTS

AGAMA CASTROVIEJOI SP. NOV. FIGS. 1-8

Types. Holotype: MNCN-41779 an adult male from Dahr Chinguettti, on the road between Atar and Tidjikja (20° 26.547'N/12° 49.407'W), Wilaya of Adrar. Collected by José M. Padial, 20 May 2002.

Paratypes. MNCN-41778 an adult female, MNCN-41780 a subadult female and MNCN-41776, 41777 two young females (same data as the holotype).

Etymology. This species is a patronym for Dr Javier Castroviejo (Galicia, Spain), for his help and encouragement and in recognition of his great effort in funding the study and conservation of biodiversity in Spain, Africa and Latin America.

Diagnosis. A species morphologically similar to *Agama impalearis* with the following combination of

FIG. 1. Male (holotype, MNCN 41779) of *Agama castroviejoi* sp. nov. Picture taken in the Adrar Mountains, Mauritania.

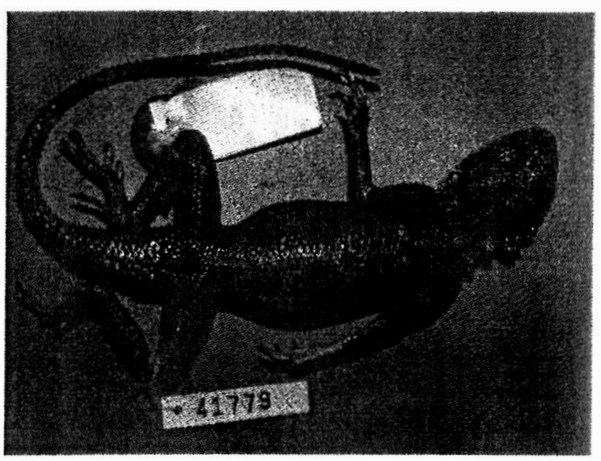


FIG. 2. Dorsal view of the male MNCN 41779 (holotype) of *Agama castroviejoi*.

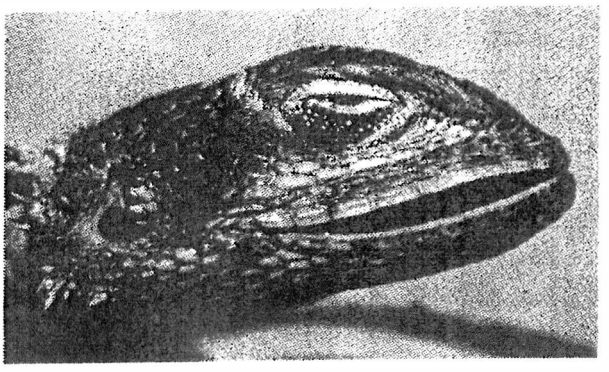


FIG. 3. Lateral view of the head of the male MNCN 41779(holotype) of *Agama castroviejoi*.

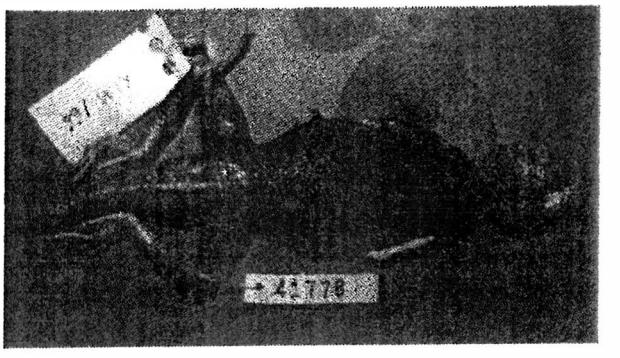


FIG. 6. Dorsal view of an adult female MNCN 41778 (paratype) of *Agama castroviejoi*.

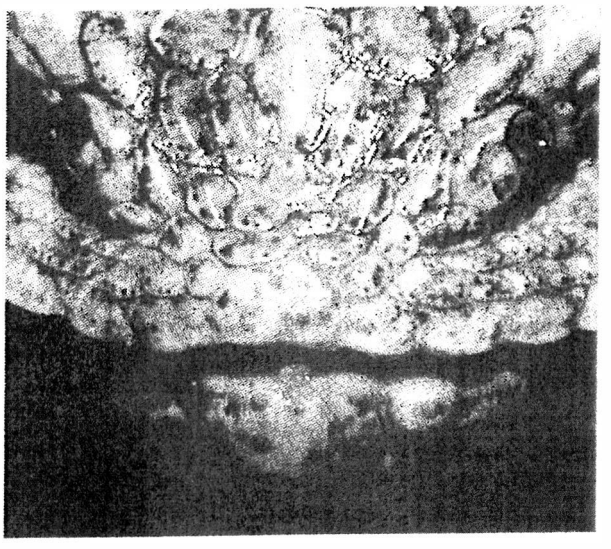


FIG. 4. Frontal view of the snout of the male MNCN 41779 (holotype) of *Agama castroviejoi* showing the orientation of the nostrils.

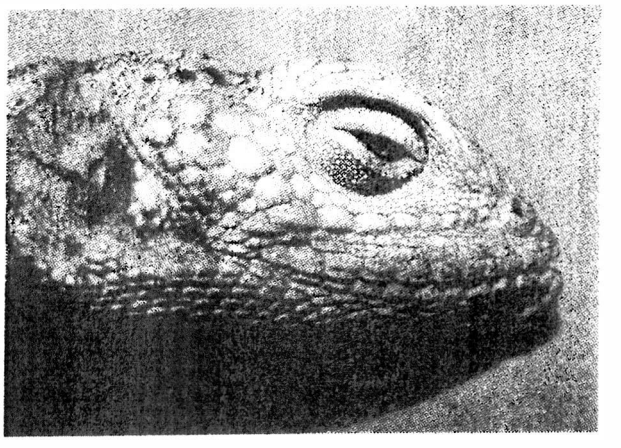


FIG. 7. Lateral view of the head of an adult female MNCN 41778 (paratype) of *Agama castroviejoi*.

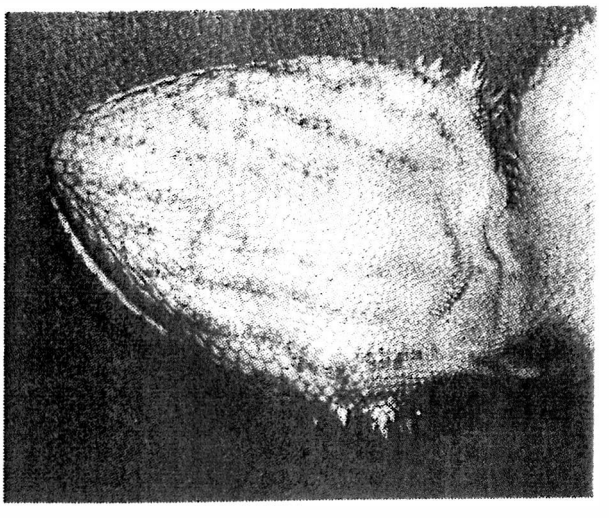


FIG. 5. Ventral view of the gular region of the male MNCN 41779 (holotype) of *Agama castroviejoi*.

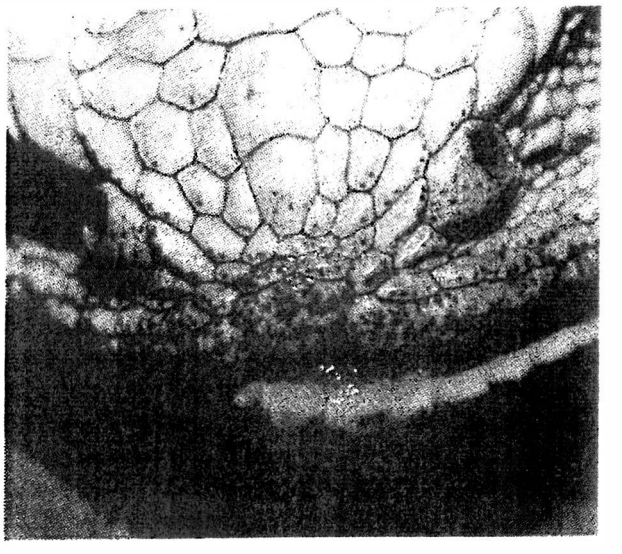


FIG. 8. Frontal view of the snout of an adult female MNCN 41778 (paratype) of *Agama castroviejoi*, showing the orientation of the nostrils.

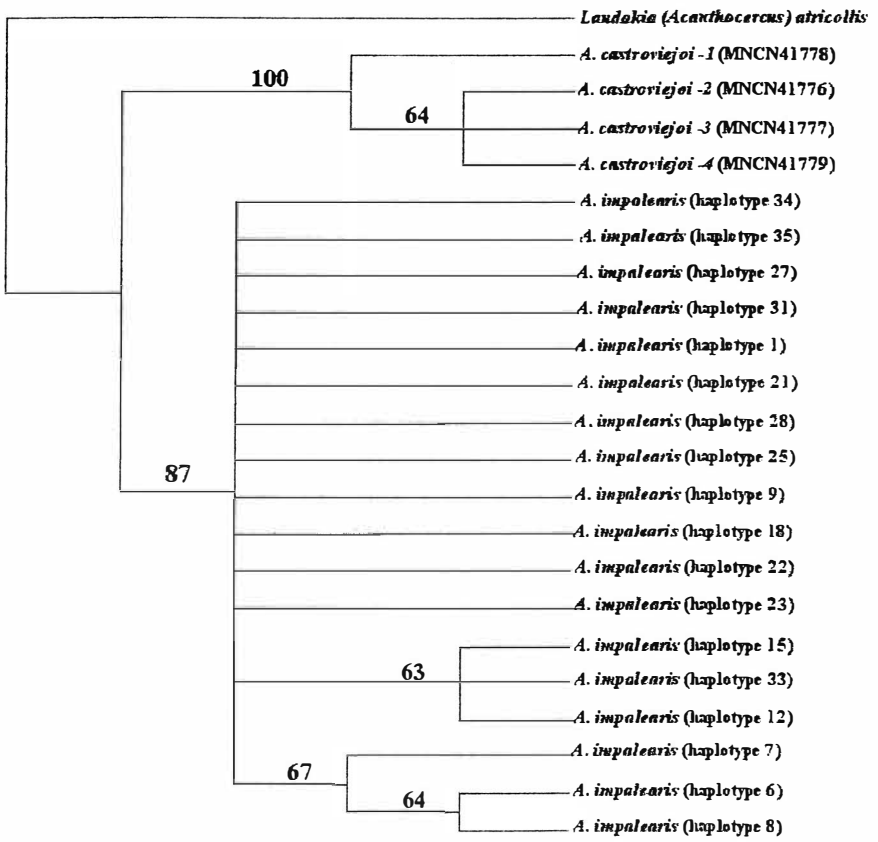


FIG. 9. Strict consensus of 19 equally parsimonious trees showing the phylogenetic relationships between *A. impalearis and A. castroviejoi*. Tree-length= 97 steps, CI= 0.750 and RI= 0.922 (both under exclusion of uninformative sites). Bootstrap support values are shown above the nodes. Haplotype numbers refer to Brown *et al.* (2002).

characters (see Figs. 2-8): long, narrow and low head; scales on head smooth; small nuchal crest; nine groups of spines on each side of the head; nostrils oriented dorsolaterally; plicate throat, with two gular folds and one mite pocket on each side of the neck; regular keeled and mucronate scales in dorsum, keels converging toward midbody; less keeled and mucronate dorsal scales in females; relative length of fingers 3>4>5=2>1; relatively long hind legs (always reaching the anterior margin of the ear opening, some reaching the eye); base of the tail weakly compressed; tail scales keeled and mucronate; absence of caudal crest; tail one and 3/4 to twice as long as SVL; gular region with brown irregular longitudinal lines; ten preanal pores; live male with reddish eyelid; ventral regions of the body, tail and extremities immaculate white; dorsal surfaces of female light (sandy) brown with dark brown subocular and labial bars and some transverse dark brown bands on back; males with dorsal surfaces grey-blue with light brown spots. Distinguished from the north-west African species (characters of the other species in parentheses) (see also Table 2): from *Agama impalearis* by relative length of finger and toes; smaller nuchal crest and absence of caudal crest; reddish eyelid in males and different colour pattern; some scalation count characters (Table 3), and 4.3% difference in the 16S rRNA mitochondrial region sequenced for this study. The phylogenetic analysis presented in Fig. 9 shows that *A. castroviejoi* and *A. impalearis* form two reciprocally monophyletic groups supported by relatively high bootstrap values. Distinguished from *Agama boueti* by gular region with brown irregular longitudinal lines (immaculate); red eyelid in males (brown); 8-9 groups of neck spines (4-7); 10 preanal pores (12); 12 scales on fourth finger (9-10); 19-21 scales on fourth toe (15-17); different relative length of fingers and toes; longer hind legs. Distinguished from *Agama agama* by very smaller size and very different colour pattern and sexual dimorphism (metallic blue body and bright orange on heads of adult males). Distinguished from *Agama boulengeri* by its smaller size; very different colour pattern (specially sexual dimorphism); fewer number of scales around midbody; smooth head scales; higher number of group of spines on the sides of the head and shoulder and by absence of caudal crest.

DESCRIPTION OF THE HOLOTYPE

An adult male with head scales smooth, occipital scale big; dorsal scales homogeneous, large rhomboidal, keeled and mucronate, all keels converging towards the vertebral line; keels on arms and limbs mucronate; throat plicate, with two gular folds and one mite pocket on each side of the neck; ventral scales smooth; relative finger length 3>4>5=2>1; tail twice as long as SVL; base of the tail weakly compressed; tail scales keeled and mucronate; nostrils oriented upwards and backwards.

Measurements (mm): Snout-vent length 74.88, tail length 116.70, head length 19.84, head width 15.99,

TABLE 1. Linear body measurements (mm) and scalation count characters of the type series of *Agama castroviejoi* sp. nov. See methodology for abbreviations.

Linear body measurement and dimension	MNCN 41779 (male)	MNCN 41777 (juv. female)	MNCN 41778 (female)	MNCN 41776 (juv. female)	MNCN 41780 (female)	Range
SVL	74.21	59.91	74.88	59.31	67.60	59.31-74.88
TAL	116.40	105.1	-	-	90.17	90.17-116.4
TAH	8.81	6.92	7.25	6.75	6.87	6.92-8.81
TAW	11.46	8.20	10.55	8.13	9.84	8.13-11.46
RTAL	63.75	57.0	-	-	74.97	57.0-74.97
HL	19.84	17.16	19.65	15.91	18.40	15.91-19.84
HW	15.99	13.58	15.67	13.21	15.11	13.21-15.99
HH	9.69	8.88	9.67	8.67	9.86	8.67-9.69
EN	4.76	3.0	3.98	3.11	4.35	3.0-4.76
EE	6.01	4.37	4.80	4.61	5.33	4.37-6.01
HAND	14.55	12.04	12.80	11.53	14.14	11.53-14.55
FOOT	23.64	19.69	21.04	18.82	22.61	18.82-23.64
FINGER	6.77	6.23	5.75	5.19	6.67	5.19-6.77
TOE	12.02	9.64	9.75	9.20	11.74	9.20-12.02
TL	21.21	20.31	21.75	19.77	21.34	19.77-21.75
FL	19.36	17.58	19.86	15.44	20.29	15.44-20.29
HL/SVL	0.27	0.29	0.26	0.27	0.27	0.26-0.29
HB/SVL	0.22	0.23	0.21	0.22	0.22	0.21-0.23
HL/HB	1.24	1.26	1.25	1.20	1.22	1.20-1.26
TL/SVL	0.29	0.34	0.29	0.33	0.32	0.29-0.34
FL/SVL	0.26	0.29	0.27	0.26	0.30	0.26-0.30
EN/SVL	0.24	0.17	0.20	0.20	0.24	0.17-0.24
EE/SVL	0.30	0.25	0.24	0.29	0.29	0.24-0.30
TOE/SVL	0.16	0.16	0.13	0.16	0.17	0.13-0.17
FOOT/SVL	0.32	0.33	0.28	0.32	0.33	0.28-0.33
Scalation count characters						
VE	63	59	61	58	59	58-63
EMB	61	58	55	60	63	55-63
SL	10	11	11	11	12	10-12
IL	9	11	11	11	12	9-12
SO	6	7	6	7	7	6-7
RSLE	4	3	4	4	4	3-4
GNS	9	8	8	8	9	8-9
IP	10	-	-	-	-	-
LFF	12	12	12	12	12	-
LFT	19	19	21	19	20	19-21
SC	6	6	7	7	7	6-7

head height 9.69, hand length 14.55 (from posterior end of wrist to top of longest finger), foot length 23.64 (from posterior end of heel to top of longest toe), limb length 19.36, tibia length 21.21, length of fourth finger (to join with third) 6.77; length of fourth toe 12.02; eye-nostril length (from the anterior border of the eye orbit to the nostril) 4.76; ear-eye length (from the anterior border of the tympanum to the posterior border of the eye orbit) 6.01; tail width 11.46; tail height (at the base) 8.81.

Pholidosis. 63 ventral scales between inguinal fold and the beginning of the arm; 61 scales around midbody; 11 supralabials; 9 infralabials; 6 rows of supraoculars; 4 rows of scales between supralabials and suboculars (including the subocular row); 9 groups of spines between the anterior margin of the ear opening and the shoulder. In each group, the central spines are largest; 10 anal pores; 12 lamellae under fourth finger; 19 lamellae under fourth toe; nuchal crest composed of 6 spines.

Colour in life. Ventral regions of the body, tail and extremities immaculate white; dorsal surfaces grey-blue with dark brown subocular and labial bars; gular region with brown irregular longitudinal lines; some transverse dark brown bands on the back; a perpendicular thin beige band in the anterior margin of the back

TABLE 2. Diagnostic characters for *Agama castroviejoi* sp. nov., *Agama impalearis* and *A. boueti*.

	A. castroviejoi	*A. impalearis*	*A. boueti*
SVL	67.6-74.88	to 120	to 99
TL	90.17-116.4	150	to 160
EMB	55-63	?	50-62
Dorsal scales	keeled	keeled	keeled
Head scales	smooth	smooth	smooth
Nuchal crest	small	strong	small
GNS	8-9	3-9	4-8
Caudal crest	absent	present	absent
Relative length of fingers	3>4>5≥2>1	3≥4>5≥2>1	3>4>2>5≥1
Relative length of toes	4>3≥5>2>1	3=4>5≥2>1	3≥4>5>2>1
Gular region	brown lines	brown lines	white to yellow
Sexual dicromatism	low	low	low
Sociability	solitary	solitary	solitary
Habitat	desert rocky areas	semidesert rocky areas	sandy savannah

and one in the posterior margin; two beige irregular circumferences in the middle of the back.

Colour in alcohol. Ventral regions of the body, tail and extremities immaculate white; gular region with brown irregular longitudinal lines; all dorsal and dorsolateral regions grey-blue (slate grey) with some light brown spot on the head, back and forearms; hands yellow; a perpendicular thin beige band in the anterior margin of the back and one in the posterior margin; two beige irregular circumferences in the middle of the back.

VARIATION

For variation in morphometrics see Table 1. The only males has more ventral scales and fewer labial scales than females. The dorsal scales of the male are more mucronate than in females. There is colour difference between sexes, females being paler than males. In life, females have almost no colour differences: they have light brown head with dark brown subocular stripes; the dorsal region is beige to light brown with transverse dark brown bands from the scapular region to the middle of the tail; each dark band is composed of 3-7 scales; they have a thin longitudinal light stripe. The ventral region is immaculate white, with subgular longitudinal brown stripes. In alcohol, the colour becomes faded but the pattern is retained; the feet and hands become light yellow.

From the genetic point of view, there is a single base pair difference (and A-G transition) between specimen MNCN 41778 and specimens MNCN 41776, MNCN 41777, MNCN 41779 in the 464 of the 16S rRNA mitochondrial region sequenced for this study (Fig. 9).

DISTRIBUTION AND ECOLOGY

Specimens are only known from the type locality. They were found on a tableland (*dahr*) 679 m above sea level, in the Adrar region in Mauritania. This region is part of the "Sahara Meridional Occidental", and is characterized by annual rainfall of about 100 mm and average annual temperatures of 30°C, with minimum temperatures in January rarely descending below 10°C (Le Houérou, 1990). The habitat is a rocky plain with little vegetation, composed of sparse bushes and some small trees (*Acacia* spp.). All specimens were found alone and separate by few kilometres from each other. The first specimen encountered was active at 09.20 hr (air temperature of 28.6°C). The other specimens were found perching around the middle of the day, and were easily caught when they moved under solitary stones.

DISCUSSION

Among the four *Agama* species (*A. agama*, *A. boulengeri*, *A. boueti*, *A. impalearis*) inhabiting the arid and semi-arid regions of NW Africa, the last two show

TABLE 3. Some scalation counts characters of male (holotype) of *Agama castroviejoi* and two geographical forms of *A. impalearis*. (a) southern form (Tan Tan, Morocco); (b) northern form (Chefchauen, Morocco). See methodology for abbreviations.

	SL	IL	GNS	LFF	LFT	IP
Holotype	10	9	9	12	19	10
a (n=6)	11-13	10-15	10-11	14-16	16-18	12-14
b (n=4)	11-13	11-13	10-11	15	16-19	12-13

superficial resemblance with *A. castroviejoi*. Nevertheless, morphological quantitative and meristic characters distinguish *A. castroviejoi* well from the other species recorded for Mauritania (*A. boulengeri*, *A. agama* and *A. boueti*) and also from the two Moroccan forms of *A. impalearis*. MtDNA analyses demonstrate the identity of *A. castroviejoi* and its close relationship to *A. impalearis* and the vicariant form from south and east of the Atlas mountains.

Furthermore, distributional, social and ecological factors also allow a clear separation among species. *A. castroviejoi* is solitary and inhabits extremely dry rocky areas of the Adrar plateau. *Agama boueti* is solitary and inhabits the semi-desert sandy plains and savannahs of the Sahel region of southern Mauritania and the Atlantic Coast. *Agama impalearis* is a typical solitary rock dweller (Schleich *et al.*, 1996). Its distribution ranges from the Mediterranean coast (Bons & Geniez, 1996) to Seguia el Hamra, in Western Sahara (Geniez *et al.*, 2000). Although it was cited for "Mauritanie Saharienne" (Chabanaud, 1917) and despite appearing widely distributed across the country in Le Berre's (1989) distribution map for this species, there are actually no confirmed records for Mauritania. Moreover, Joger (1979) noted that this species did not occur in the Adrar and that the nearest record corresponded to Seguia el Hamra. The presence of this species in the country is still plausible in rocky areas similar to those of the Zemmour Mountains (Geniez *et al.*, 2000), but this requires confirmation. *A. boulengeri* is a species from the Sahel Savannah that inhabits rocky areas and forms social groups. We found this species as far north as Oued Choûm (21° 22.6'N/12° 58.6'W), near the border with Western Sahara. At this latitude, it is always associated to wet rocky gorges (*Gueltas*) in the mountains. *A. agama* is another social species, reported to be associated with human settlements in the Adrar region (Dekeyser & Villiers, 1956). In the south of the country, this species prefers trees from the Sahel savannahs but it shows high habitat plasticity (pers. obs.).

Miocene vicariance events have been found to be responsible of the variation and differentiation in *A. impalearis* in NW Africa (Brown & Znari, 1998; Brown *et al.*, 2002). The uplift of the Atlas mountains constituted a great geographical barrier causing allopatric fragmentation of the populations. *A. castroviejoi* is isolated in a rocky tableland surrounded by lowland sandy plains of the Sahara that could serve as a barrier for a rock dweller. Also *in situ* selection-mediated responses to the ecological current conditions of the isolated populations are responsible of the morphological variation of *A. impalearis* (Brown & Znari, 1998). Present ecological conditions of the Adrar are very extreme, and is probably one of the hottest areas of the Sahara (Le Houérou, 1990). As demonstrated by Brown & Znari (1998), variation in scalation is significantly associated with different temperature conditions. Therefore, both isolation and ecological differentiation could be implicated in the differentiation of *A. castroviejoi*.

Although the Adrar belongs to the Meridional Sahara (Le Houérou, 1990), this area shows a complex history, with its faunas and floras comprising a mixture of Mediterranean, Saharan and Sahelian species (Dekeyser & Villiers, 1956). Probably *A. castroviejoi* constitutes an example of a Saharan endemic of the Adrar Mountains, as some invertebrate species and also vertebrate subspecies (Dekeyser & Villiers, 1956). Nevertheless, the phylogeographic affinities of *A. castroviejoi* remain unclear, since it could have a Sahelian or Saharan origin. A more thorough phylogenetic comparison among North African agamas will help to resolve this question.

ACKNOWLEDGEMENTS

This work could not have been carried out without the effort of J. Castroviejo and the people of Asociación Amigos de Doñana. I am very gratefully to S. Carranza (BMNH), who did the genetic work and helped me to improve the manuscript. To I. de la Riva (MNCN) for his help in many aspects of this work. To Dr N. Anderson (BMNH), who revised the draft version and the English of the manuscript. To R. García, M. Urcera, C. Carballo, M. O. Deida and E. Mohamed O. Saleh for their help and companion in Mauritania and J. Pérez, E. Avila, S. Castroviejo-Fisher and A. Quintana for their friendship and companion in the first trip to Mauritania. P. Geniez, J. A. Mateo, H. Nickel and W. Böhme all helped us in some aspect of this work. W. Wuster and two anonymous reviewers critically commented an earlier draft of the manuscript. R. Brown kindly provided raw scalation data for *A. impalearis* from Morocco and contributed to the improvement of the manuscript.

REFERENCES

Böhme, W. (2000). Die wechselvolle Geschichte der Sahara: Untersuchung von Reptilienzönosen entlang eines westsaharischen Transektes, mit einem überraschenden Fund im Südosten Mauritaniens. *Tier und Museum* **7**, 11-21.

Böhme, W., Meinig, H. & Rödel, M. A. (1996). New records of amphibians and reptiles from Burkina Faso and Mali. *British Herpetological Society Bulletin* **56**, 7-26.

Böhme, W., Wilms, T., Nickel, H. & Merz, M. (2001). Bericht über eine herpetologische Forschungsreise in die westliche Sahara und ihre Randgebiete. *Zeitschrift des Kölner Zoo* **44**, 117-131.

Bons, J. & Geniez, P. (1996). *Anfibios y reptiles de Marruecos (Incluido Sáhara Occidental). Atlas biogeográfico*. Barcelona: Asociación Herpetológica Española.

Brown, R. P. & Znari, M. (1998). Morphological variation in *Agama impalearis* from Morocco: evidence for historical population vicariance and current climatic effects. *Ecography* **21**, 605-612.

Brown, R. P., Znari, M., El Mouden, E. H. & Harris, P. (1999). Estimating asymptotic size and geographic variation in *Agama impalearis* from Morocco. *Ecography* **22**, 277-284.

Brown, R. P., Pestano, J. & Suárez, N. M. (2002). The Atlas mountains as a biogeographical divide in North-West Africa: evidence from mtDNA evolution in the Agamid lizard *Agama impalearis*. *Molecular Phylogenetics and Evolution* **24**, 324-332.

Carranza, S., Arnold, E. N., Thomas, R. H., Mateo, J. & Lopez-Jurado, L. F. (1999). Status of the extinct giant lacertid lizard *Gallotia simonyi simonyi* (Reptilia: Lacertidae) assessed using mtDNA sequences from museum specimens. *Herpetological Journal* **19**, 83-86.

Carranza, S., Arnold, E. N., Mateo, J. & Lopez-Jurado, L. F. (2000). Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society, Series B* **267**, 637-649.

Chabanaud, P. (1917). Enumération des reptiles non encore étudiés de l'Afrique occidentale, appartenant aux collections du Muséum, avec la description des espéces nouvelles. *Bulletin du Muséum National d'Histoire Naturelle de Paris, Serie Zoologique* **23**, 83-105.

Dekeyser, P. L. & Villiers, A. (1956). Contribution a l'étude du peuplement de la Mauritanie. Notations écologiques et biogéographiques sur la faune de l'Adrar. *Memoires de l'Institut Français de l'Afrique Noire* **44**. 222 p, 25 pl.

Felsenstein, J. (1985). Confidence-limits on phylogenies – an approach using the bootstrap. *Evolution* **39**, 783-791.

Geniez, Ph. & Foucart, A. (1995). Un nouvel Acanthodactyle en Algérie: *Acanthodactylus taghitensis* n. sp. (Reptilia, Sauria, Lacertidae). *Bulletin du Muséum National d'Histoire Naturelle, Paris 4° série* **17**, 3-9.

Geniez, P., Mateo, J. A. & Bons, J. (2000). A checklist of the amphibians and reptiles of Western Sahara (Amphibia, Reptilia). *Herpetozoa* **13**, 149-163.

Ineich, I. (1997). Les amphibiens et reptiles du littoral mauritanien. In *Environnement et littoral mauritanien*, 93-99. Colas, P. (Ed.). Montpellier: CIRAD.

Joger, U. (1979). Zur Ökologie und Verbreitung wenig bekannter Agamen Westafrikas (Reptilia: Sauria: Agamidae). *Salamandra* **15**, 31-52.

Joger, U. (1980). Eine neue Art der Gattung *Tarentola* (Reptilia: Sauria: Gekkonidae) aus Westafrika. *Amphibia-Reptilia* **1**, 137-147.

Joger, U. (1981). Zur Herpetofaunistik Westafrikas. *Bonner zoologische Beiträge* **32**, 297-340.

Joger U. (1984). Taxonomische Revision der Gattung *Tarentola* (Reptilia: Gekkonidae). *Bonner zoologische Beiträge* **35**, 129-174.

Joger, U. & Lambert, M. R. K. (1996). Analysis of the herpetofauna of the Republic of Mali, I. Annotated inventory, with description of a new *Uromastyx* (Sauria: Agamidae). *Journal of African Zoology* **110**, 21-51.

Lambert, M. R. K. & Mullié, W. C. (1998). Sexual dichromatism of *Agama boulengeri* observed in southern Mauritania. *British Herpetological Society Bulletin* **65**, 42-44.

Le Berre, M. (1989). Faune du Sahara. I. Poissons-Amphibiens-Reptiles. Paris: Lechevalier-R. Chabaud.

Le Houérou, H. N. (1990). Définition et limites bioclimatiques du Sahara. *Sècheresse* **1**, 246-259.

Lenk, P., Kalyabina, S., Wink, M. & Joger, U. (2001). Evolutionary relationships among the true vipers (Reptilia: Viperidae) inferred from mitochondrial DNA sequences. *Molecular Biology and Evolution* **19**, 94-104.

Mateo, J. A., Geniez, Ph., López-Jurado, J. L. & Bons, J. (1998). Chorological analysis and morphological variation of saurians of the genus *Uromastyx* (Reptilia, Agamidae) in western Sahara. Description of two new taxa. Revista *Española de Herpetologia* **12**, 97-109.

Salvador, A. (1982). A revision of the lizards of the genus *Acanthodactylus* (Sauria: Lacertidae). *Bonner zoologische Beiträge Monographien* **16**,167 p.

Schleich, H. H., Kästle, W. & Kabisch, K. (1996). *Amphibians and reptiles of North Africa*. Koenigstein: Koeltz Scientific Publications.

Swofford, D. L. (2002). *PAUP*: phylogenetic analysis using parsimony (and others methods), v4.0b10*. Sinauer Associates, Sunderland, MA.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**, 4876-4882.

Wilms, T. & Böhme, W. (2001). Revision der *Uromastyx acanthinura* – Artengruppe, mit Beschreibung einer neuen Art aus der Zentralsahara (Reptilia: Sauria: Agamidae). *Zoologische Abhandlungen, Staatliches Museum für Tierkunde Dresden* **51**, 73-104.

Accepted: 22.3.04

APPENDIX 1

Additional specimens of *Agama* species examined for this work. EBD: Estación Biológica de Doñana, Seville, Spain; CET: Centro de Estudios Tropicales, Sevilla, Spain. Locality and coordinates in parentheses.

Agama agama. CET(RIM)-028 (near Bouli, 15° 25.803' N/11° 55.562' W, Mauritania); CET(RIM)-328-329 (El Wad-Zoueina,15° 42.689' N/ 9° 39.906' W, Mauritania); CET(RIM)-331-335 (El Wad-Foulania, 15° 31.682' N/9° 48.896' W, Mauritania).

Agama boueti. CET(RIM)-287-291, 336 (Ayoûn el Atroûs, 16° 37.412' N/9° 37.441' W, Mauritania).

Agama boulengeri. CET(RIM)-016-018 (Bougari,16° 32.034' N/10° 47.892' W, Mauritania); CET(RIM)-021-022 (between Timbedgha and Ayoûn El Atroûs, 16° 26.890' N/9° 14.690' W, Mauritania); CET(RIM)-066 (Terjît, 20° 15.578' N/13 ° 05.854' W, Mauritania); CET(RIM)-094-96 (Guelta Molomhar, 20° 34.873' N/13° 07.630' W, Mauritania); CET(RIM)-116-117 (Oued Choûm, 21° 22.654' N/12° 58.581' W, Mauritania); CET(RIM)-174-175 (Zig, between Lekhcheb and Tîchît, 18° 34.487' N/9° 48.379' W, Mauritania); CET(RIM)-256-261 (Guelta Oumm Lebare, 16° 29.472' N/10° 49.822' W, Mauritania); CET(RIM)-338 (Ayoûn el Atroûs, 16° 37.412' N/9° 37.441' W, Mauritania); CET(RIM)-344 (Guelta Matmata,17° 53.571' N/12° 07.467' W, Mauritania).

Agama impalearis. EBD-11352 (Mount Marcan-Kbir, Tetuán, Morocco); EBD-20222 (Taklim, Morocco); EBD-22084 (Yassinen, Morocco); EBD-24515 (Bab Bou Ichir-Fritissa, Morocco); EBD-6552 (El Aioum, Western Sahara).

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 37-44 (2005)

A NEW SPECIES OF *MANTIDACTYLUS* FROM THE EAST COAST OF MADAGASCAR AND ITS MOLECULAR PHYLOGENETIC RELATIONSHIPS WITHIN THE SUBGENUS *GUIBEMANTIS*

MIGUEL VENCES[1] AND FRANK GLAW[2]

[1]*Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Amsterdam, the Netherlands*

[2]*Zoologische Staatssammlung, Münchhausenstr, München, Germany*

We describe a new species of arboreal frog of the genus *Mantidactylus* from low altitude sites on the eastern coast of Madagascar. *Mantidactylus timidus* sp. n. has hitherto been considered as *Mantidactylus tornieri*, but some of the characters that distinguish it from typical populations of this species from mid-altitude localities had already been detected. The new species mainly differs from *M. tornieri* and all related species in the subgenus *Guibemantis* by a shorter relative hand length. Furthermore, it has shorter hind limbs, a smaller tympanum diameter and – at least in some populations – green egg pigmentation (as opposed to brown or white). Vocalizations of *M. timidus* were irregular and unstructured blasts and moans but the available recordings may not represent the real advertisement calls. A molecular phylogeny based on analysis of 539 nucleotides of the mitochondrial 16S rRNA gene placed the new species sister to a clade containing *M. tornieri, M. depressiceps* and *M. kathrinae*. Genetic differentiation from these related species was large, with uncorrected pairwise divergences of more than 8% in all cases. We discuss the recently increasing use of mitochondrial genetic markers to draw taxonomic conclusions and suggest that mitochondrial differentiation should not be used as an exclusive character to describe new amphibian taxa. Instead, phylogenetic placement of populations and morphological, ecological and behavioural arguments need to be carefully evaluated in each case to understand whether a population merits the status of a separate species.

Key words: Amphibia, Anura, Mantellidae, *Mantidactylus timidus* sp. n., *Mantidactylus tornieri*, cryptic species

INTRODUCTION

Frogs of the genus *Mantidactylus* belong to the family Mantellidae and are endemic to Madagascar and the Comoro island of Mayotte (Blommers-Schlösser & Blanc, 1991; Vences & Glaw, 2001). *Mantidactylus* is paraphyletic because the genus *Mantella* is nested within one *Mantidactylus* lineage (Richards *et al.*, 2000; Vences *et al.*, 2003). The large species diversity of currently about 85 scientifically named species is reflected by the current division of *Mantidactylus* into 12 subgenera (Dubois, 1992; Glaw & Vences, 1994). One of these, *Guibemantis,* had previously been considered as the *Mantidactylus depressiceps* group (Blommers-Schlösser, 1979; Blommers-Schlösser & Blanc, 1991). It has recently been reviewed by Glaw *et al.* (2000) who described a new species of these medium-sized, largely arboreal frogs. According to this account, the following species are currently assigned to *Guibemantis: Mantidactylus depressiceps, M. kathrinae, M. liber* and *M. tornieri.* Males of these species are characterized by a largely undifferentiated state of their femoral glands (Glaw *et al.*, 2000). These glands are typical for males of *Mantidactylus* and in some subgenera are also present in females (Blommers-Schlösser & Blanc, 1991). *Guibemantis* mainly reproduce in stagnant water. Males deposit their eggs on leaves or stones above ponds, the hatching tadpoles drop into the water and complete development in the ponds. Two species of *Guibemantis* (*Mantidactylus depressiceps* and *M. kathrinae*) are unique in having a white, non-transparent colour of the jelly of their clutches (Blommers-Schlösser, 1979; Glaw *et al.*, 2000). One species initially considered to belong to this group (Blommers-Schlösser & Blanc, 1991), *Mantidactylus elegans*, has been hypothesized to be possibly related to *Mantidactylus brunae* and *M. peraccae* instead, within the subgenus *Spinomantis* (Andreone *et al.*, 1998), but a thorough test of this hypothesis is so far lacking.

In this paper we report on our discovery that populations assigned to *Mantidactylus* (*Guibemantis*) *tornieri* are in fact a complex of at least two species, one of which is described herein. We furthermore present a molecular phylogeny that includes most species belonging to *Guibemantis* or previously believed to be closely related to this subgenus, in order to assess monophyly and relationships among these frogs.

MATERIALS AND METHODS

In this study we focus on the medium- to large-sized species of *Guibemantis* and do not consider the small-sized and easily recognized *Mantidactylus liber*. Hence, morphological and bioacoustic comparisons refer to

Correspondence: M. Vences, Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Mauritskade 61, PO Box 94766, 1090 GTAmsterdam, the Netherlands.
E-mail: vences@science.uva.nl

specimens and call data of *M. depressiceps, M. kathrinae* and *M. tornieri*. To assess the molecular phylogenetic relationships of these species, we further studied DNA sequences of other species that currently or in the past were considered to be related to them: *Mantidactylus liber*, three representatives of the subgenus *Blommersia* (*M. wittei, M. domerguei, M. blommersae*), *M. elegans*, and two species of the subgenus *Spinomantis* (*M.* aff. *peraccae* and *M. aglavei*) to which *M. elegans* has been hypothesized to belong (Andreone *et al.*, 1998). *Boophis tephraeomystax* was used as the outgroup.

Specimens were captured by locating calling males during the night. They were euthanised using chlorobutanol solution, fixed either in 5% formalin or 95% ethanol, preserved in 70% ethanol, and included in the herpetological collections of the Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK) and Zoölogisch Museum Amsterdam (ZMA). Further museum acronyms used herein are BMNH (The Natural History Museum, London), and Zoologisches Museum der Universität Berlin (ZMB).

The following morphometric measurements were taken by the senior author to the nearest tenth of a millimetre using a calliper: snout-vent length, SVL; maximum head width (HW); head length from tip of snout to posterior edge of snout opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe (HIL); foot length (FOL). The webbing formula is given according to Blommers-Schlösser (1979).

Muscle tissue samples were taken from freshly killed specimens in the field and preserved in 98% ethanol. DNA was extracted using different standard protocols and a fragment of the mitochondrial 16S rRNA gene amplified using the primers 16Sa-L and 16Sb-H of Palumbi *et al.* (1991). After purification with Qiaquick kits (Qiagen), the fragments were resolved on automated DNA sequencers (ABI 377 and ABI 3100). Sequences were validated and aligned with the software Sequence Navigator (Applied Biosystems), and deposited in Genbank (accession numbers of newly obtained sequences: AY684185-AY684191). The alignment required inclusion of gaps to account for indels in only three cases; one of these was a 1-3-gap interval in a hypervariable region, the others were single gaps and could unambiguously be aligned. We therefore included the complete dataset, after exclusion of gaps, in the phylogenetic analysis after assessing that exploratory analyses excluding ca. 70 b.p of hypervariable regions resulted in identical topologies.

Phylogenetic analysis was carried out using PAUP, version 4b10 (Swofford, 2002). We performed unweighted maximum parsimony heuristic searches, with tree-bisection reconnection branch swapping, and random sequence addition with 100 replicates. Furthermore, a maximum likelihood tree was constructed after determining the substitution model that best fits our data through hierarchical likelihood ratio tests as implemented in Modeltest (Posada & Crandall, 1998). Robustness of nodes was tested by full heuristic bootstrapping, with 2000 replicates (and 10 random addition sequence replicates) under maximum parsimony and 500 replicates under maximum likelihood.

RESULTS

VARIATION IN *MANTIDACTYLUS TORNIERI*

Blommers-Schlösser (1979) was the first to recognize, after thorough observations in the field, that the taxon previously considered as *Mantidactylus depressiceps* was heterogeneous. She revalidated *Mantidactylus tornieri* (Ahl, 1928) for a species with transparent jelly around the clutches, occurring sympatrically with *M. depressiceps*, with white clutches. The name *M. tornieri* was applied both to specimens from near sea level at the east coast of Madagascar (Foulpointe) and from the mid-altitude locality Andasibe, located at ca. 900 m above sea level. However, Blommers-Schlösser (1979) already noted distinct morphological differences between specimens from these two localities: the disk of the third finger was found to be larger than the tympanum in specimens from Andasibe and equal to the tympanum in specimens from Foulpointe; furthermore, the latter had comparatively shorter hind limbs and smaller eyes. Blommers-Schlösser (1979) concluded: "It is possible, that the specimens of Foulpointe represent a subspecies, but the material is not sufficient to decide this for the moment".

Glaw & Vences (1994) noted that clutches of *M. tornieri* from Andasibe contained brownish eggs, while eggs in clutches from an east coast locality (Nosy Boraha) were greenish. In addition, calls recorded from Nosy Boraha were unstructured single blasts whereas calls from Andasibe were always series of several short notes.

During recent fieldwork in 2003, at a locality north of Toamasina at the east coast, we again collected *M. tornieri*-like specimens and again only heard short unstructured calls. This prompted us to analyse the morphological differentiation between coastal and mid-altitude populations currently attributed to *M. tornieri*.

Original measurements of specimens assignable to *M. tornieri* sensu lato from the localities Andasibe, Ranomafana, Voloina, Foulpointe and Toamasina, as well as comparative data of all relevant type specimens and of the morphologically similar species *M. kathrinae* and *M. depressiceps*, are shown in Table 1. Comparing the east coast *M. tornieri*-like specimens from Foulpointe, Voloina and Toamasina to those of mid-altitude localities (Andasibe and Ranomafana) reveals a distinct morphological differentiation. East coast specimens have much smaller hands, which is reflected by the

TABLE 1. Morphometric measurements of specimens of *Mantidactylus timidus*, *M. tornieri*, *M. depressiceps* and *M. kathrinae* (all in mm). See Materials and Methods for abbreviations; additional abbreviations used: HT, holotype; PT, paratype; LT, lectotype; PLT, paralectotype. Asterisks mark types of two junior synonyms of *Mantidactylus depressiceps*: * holotype of *Mantidactylus acuticeps* Ahl, 1929; ** holotype of *Rhacophorus mocquardii* Boulenger, 1896.

Collection no.	Locality	Sex	Status	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOL
M. timidus sp. n.															
ZMA 19466	Toamasina	M	HT	35.8	12.3	13.5	2.2	4.0	3.4	1.9	3.3	21.1	10.4	54.0	16.7
ZMA 19492	Toamasina	M	PT	33.7	11.3	12.6	2.0	4.0	3.3	1.8	3.1	20.6	9.3	50.4	15.7
ZMA 7109 (814)	Foulpointe	M	PT	42.1	14.1	16.1	2.7	4.9	4.4	2.2	4.0	23.4	11.4	62.7	19.1
ZMA 7110 (706)	Foulpointe	M	PT	44.7	14.7	16.1	2.3	4.5	4.2	2.0	3.8	26.7	12.5	67.7	19.4
ZMA 7110 (707)	Foulpointe	M	PT	42.8	13.1	15.5	2.6	4.9	4.2	2.2	4.1	24.8	11.3	58.9	18.7
ZMA 7110 (708)	Foulpointe	F	PT	44.1	13.1	16.7	2.6	5.3	4.6	2.1	4.0	25.0	12.2	62.9	19.5
ZMA 7110 (558)	Foulpointe	M	PT	41.9	14.0	15.4	2.5	4.9	4.1	2.9	4.0	24.5	12.3	61.1	19.5
ZMA 7112 (686)	Foulpointe	M	PT	43.7	14.0	15.8	2.6	4.3	4.5	2.0	4.0	28.1	13.2	63.0	20.0
ZMA 7112 (475)	Foulpointe	M	PT	39.9	13.5	15.6	2.4	4.9	-	-	-	25.2	11.5	60.5	18.3
ZFMK 52698	Voloina	M	PT	55.4	19.8	21.0	3.2	6.4	5.7	3.1	4.9	36.2	16.7	86.4	26.9
M. tornieri															
ZMB 30533	Ankoraka	?	HT	47.9	16.7	18.6	2.5	5.3	5.2	3.3	4.9	30.2	16.0	75.9	23.4
ZFMK 52700	Andasibe	M	-	45.0	15.3	16.0	2.4	4.9	4.3	2.7	3.9	30.0	14.8	74.4	22.8
ZFMK 52699	Andasibe	M	-	42.0	15.0	16.0	2.4	4.5	4.2	2.2	4.0	27.6	14.7	64.8	21.6
ZMA 6986 (683)	Andasibe	M	-	43.1	14.8	16.4	2.0	5.5	4.4	2.4	4.5	30.5	14.6	68.8	22.2
ZMA 6987 (684)	Andasibe	M	-	47.9	16.8	17.3	2.0	5.4	4.7	2.9	5.1	30.4	15.9	75.3	25.5
ZMA 6988 (685)	Andasibe	M	-	47.5	16.0	17.7	2.5	5.0	4.6	3.0	5.0	30.6	15.8	74.7	23.6
ZMA 6989 (950)	Andasibe	M	-	45.7	16.0	17.4	2.2	5.4	4.7	2.4	4.2	31.1	15.2	75.7	22.7
ZMA 19402	Ranomafana	M	-	50.6	16.7	18.0	2.6	5.6	5.2	2.6	5.0	34.0	16.1	78.1	24.9
M. depressiceps															
BMNH 1947.2.27.50	East Betsileo	M	LT	39.6	13.2	13.7	2.4	4.2	3.7	2.4	3.9	27.4	13.2	69.6	20.9
BMNH 1947.2.27.51	East Betsileo	M?	PLT	36.7	11.8	12.2	2.2	4.0	3.2	2.2	4.1	22.0	12.16	1.0	19.0
BMNH 1947.2.27.52	Ankafana	M?	PLT	34.5	11.9	12.7	2.0	4.0	3.3	2.2	3.7	22.0	11.16	0.7	17.7
BMNH 1947.2.27.53	Ankafana	?	PLT	32.5	10.7	11.2	2.2	3.6	3.0	2.3	3.5	22.0	10.45	6.6	16.6
ZMB 30496*	Central Madagascar	M?	HT	35.1	11.8	12.6	2.0	4.0	3.2	2.4	3.8	23.9	11.76	3.7	19.3
BMNH 1947.2.8.62**	Sahembendrana	?	HT	33.0	12.3	13.6	1.8	3.7	3.4	2.1	4.0	21.9	10.45	4.9	15.6
ZMA 6973 (228)	Moramanga-Andasibe	M	-	32.2	10.8	12.7	2.0	4.1	3.0	1.7	3.6	19.9	9.6	50.8	15.0
ZMA 6974 (1154)	Mandraka	M	-	41.1	13.2	15.1	2.6	4.3	3.7	2.4	3.9	27.2	13.1	70.5	21.1
ZMA 6875 (120)	Ranomafana	F	-	35.0	11.8	11.6	2.3	4.4	3.5	2.2	4.0	21.4	11.3	57.4	17.3
ZMA 6976 (1072)	Mandraka	M	-	41.4	13.3	15.4	2.5	5.0	3.4	2.3	4.0	28.1	13.6	66.3	21.4
ZMA 6976 (1073)	Mandraka	M	-	42.4	13.6	15.7	2.4	4.8	3.9	2.3	4.0	29.3	14.4	73.0	23.4
ZMA 6976 (1076)	Mandraka	M	-	43.1	13.6	15.7	2.6	4.8	4.0	2.0	4.0	31.1	14.8	72.9	23.9
ZMA 6976 (1077)	Mandraka	M	-	37.4	12.3	14.8	2.3	4.1	3.5	2.3	3.7	25.4	12.6	64.6	19.7
ZMA 6976 (1078)	Mandraka	M	-	40.5	12.8	15.6	2.5	5.0	3.5	2.4	4.1	27.5	13.6	67.7	21.1
ZMA 6976 (1079)	Mandraka	M	-	44.9	14.7	16.5	2.6	4.8	3.9	2.7	4.7	27.5	13.5	69.9	21.5
ZMA 6982 (995)	Mandraka	M	-	39.7	13.0	14.7	2.3	4.5	3.3	2.0	4.0	28.0	13.2	68.0	20.8
ZMA 6979 (600)	Andasibe	M	-	41.1	13.7	15.1	2.6	5.0	4.0	1.9	4.2	28.0	12.7	67.7	21.2
ZMA 6983 (1050)	Mandraka	M	-	39.9	12.4	14.9	2.3	4.5	3.9	1.7	4.0	28.7	14.0	69.8	21.8
ZMA 19474	Ranomafana	M	-	39.1	12.6	15.0	2.3	3.9	3.7	2.2	3.9	23.8	12.9	59.1	18.2
M. kathrinae															
ZFMK 62264	An'Ala	M	HT	56.7	20.0	22.4	3.0	5.6	6.1	4.1	6.3	37.0	19.7	92.9	29.7
ZFMK 62263	An'Ala	M	PT	58.6	20.6	23.3	3.3	5.8	6.8	4.2	6.6	37.5	19.1	94.2	30.6
ZFMK 62266	An'Ala	M	PT	57.4	21.0	23.4	3.4	6.5	6.7	4.0	6.3	37.5	19.7	97.8	30.2

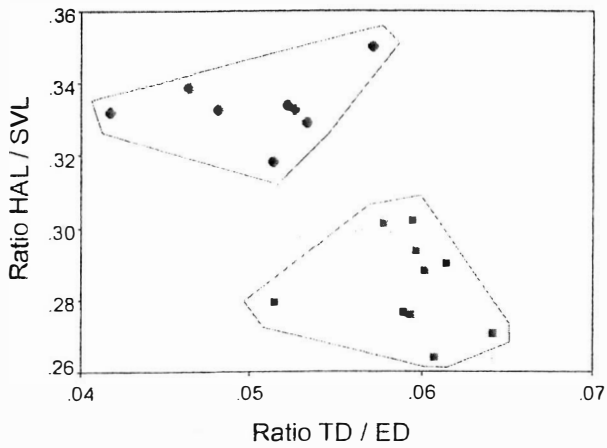


FIG. 1. Scatterplot of relative hand length (ratio HAL/SVL) vs. relative tympanum diameter (ratio TD/ED), showing the differentiation between *Mantidactylus timidus* (east-coast specimens previously attributed to *M. tornieri*; squares) to *M. tornieri* (circles).

absence of overlap of relative hand length values: ratio HAL/SVL, 0.28±0.01 (0.26-0.30) in east coast specimens vs. 0.33±0.01 (0.32-0.35) in mid-altitude specimens (Mann-Whitney *U*-test, $P<0.001$). Furthermore, the relative tympanum size is significantly larger in the east-coast specimens (*U*-test, $P<0.005$), although the values of the two forms do widely overlap (Fig. 1). The hind limb never reaches the anterior eye corner in the east coast specimens but it does sometimes in mid-altitude specimens, indicating a shorter relative hind limb length; the differences in the ratio HIL/SVL are highly significant (*U*-test; $P<0.001$). We did not detect a distinct difference in relative eye diameter as mentioned by Blommers-Schlösser (1979), but this might be due to the fact that eye diameter is difficult to measure reliably, especially among specimens in a different state of fixation. The holotype of *M. tornieri* agreed in relative hand length and other morphological characters with specimens from mid-altitudes.

Together with the observations provided by Blommers-Schlösser (1979), the available morphological evidence strongly suggests the east coast form to be differentiated from typical *M. tornieri* on the species level.

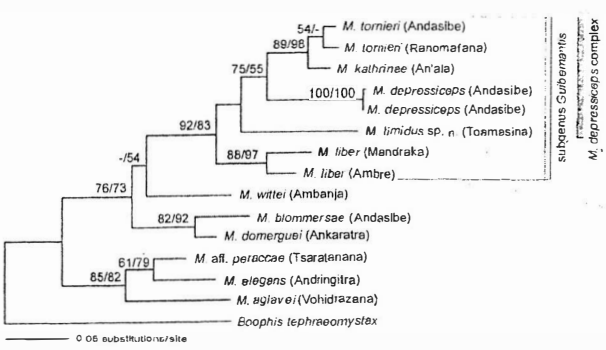


FIG. 2. Maximum likelihood phylogram based on 539 b.p of the mitochondrial 16S rRNA gene in species of the subgenus *Guibemantis*, and other *Mantidactylus* species previously thought to be related to these. The values show bootstrap support of nodes in percent (maximum likelihood, 500 replicates / maximum parsimony, 2000 replicates). Only values >50% are shown.

MOLECULAR PHYLOGENY

After exclusion of 10 gapped characters, the dataset comprised 539 characters, of which 386 were invariable and 104 were parsimony-informative. The molecular analyses produced well-resolved phylogenetic trees. The maximum likelihood topology (Fig. 2) agreed largely with the most parsimonious tree (not shown) that received a consistency index of 0.595 and a retention index of 0.604, and required 378 steps. Bootstrap support was high for most nodes (Fig. 2). Within *Guibemantis*, *M. liber* was most basal and the larger-sized *Guibemantis* species were arranged in one clade. The east-coast specimen previously attributed to *M. tornieri* was most basal in this clade, and *M. depressiceps* was sister to a group containing *M. tornieri* and *M. kathrinae*. Whereas the clade with *M. depressiceps, M. kathrinae* and mid-altitude *M. tornieri* was moderately well supported by bootstrap analysis, this was not the case for the placement of the east-coast *M. tornieri* as their sister group, which only received bootstrap supports <50%. *M. peraccae* was very clearly grouped with *M. aglavei*, corroborating its placement in the subgenus *Spinomantis* (Glaw & Vences, 1994; Andreone *et al.*, 1998), and *M. elegans* resulted to belong into the same clade and subgenus rather than into *Guibemantis*.

Uncorrected pairwise sequence divergence was 1.9% between the Ranomafana and Andasibe specimens of *M. tornieri*, 2.9-3.0% between these and *M. kathrinae*, 7.5-8.0% between *M. kathrinae* and *M. depressiceps*, and 8.0% comparing the *M. tornieri* individuals from Ranomafana and Andasibe to the specimen from Toamasina at the east coast. This high genetic differentiation of the east coast specimen, and its basal placement in the phylogeny, corroborate the status of these populations as separate species as indicated by the morphological analysis. We therefore describe the east coast populations in the following as:

MANTIDACTYLUS TIMIDUS SP. N. (FIG. 3-4)

Holotype. ZMA 19466, male, collected by M. Vences on 10 February 2003, less than 10 km north of Toamasina, eastern Madagascar (18°03'51"S, 49°22'39"E, 8 m above sea level).

Paratypes. ZMA 19492, one male, same locality and collecting data as holotype; ZMA 7109, one male (field number 814), collected by R. Blommers-Schlösser on 2 August 1972 at Foulpointe; ZMA 7110, three males (field numbers 556, 706, 707) and one female (field number 708), collected by R. Blommers-Schlösser on 13 February 1972 at Foulpointe; ZMA 7112, two males (field numbers 475 and 686), collected by R. Blommers-Schlösser on 13 October 1971 at Foulpointe; ZFMK 52698, collected by F. Glaw and M. Vences on 19 March 1991 at Voloina in the Antongil bay south of Maroantsetra.

Diagnosis and comparisons. The new species is considered as *Mantidactylus* based on the presence of an intercalary element between ultimate and penultimate phalanges between fingers and toes (as assessed by external examination), and molecular phylogenetic data (Fig. 2). Within *Mantidactylus,* the new species is allocated to the subgenus *Guibemantis* based on the structure of femoral glands in males (of type 1 sensu Glaw *et al.,* 2000), arboreal habits, molecular phylogeny (Fig. 2), white single subgular vocal sac, smooth dorsal skin without dorsolateral ridges, enlarged terminal disks of fingers and toes and complete separation of lateral metatarsalia. Within *Guibemantis*, it is distinguished from *M. depressiceps* and *M. kathrinae* by the transparent jelly of its clutches (versus white jelly) and by green eggs (versus white eggs), from *M. liber* by its distinctly larger size, and from *M. liber, M. depressiceps*, *M. kathrinae* and *M. tornieri* by its shorter relative hand length. From all these species it differs by its large genetic differentiation (Fig. 2).

Description of the holotype. Adult male in excellent state of preservation. A small amount of muscle tissue from left tibia removed for molecular analysis. For measurements see Table 1. Body relatively slender; head longer than wide, slightly wider than body; snout slightly pointed in dorsal and relatively truncate in lateral view; nostrils directed laterally, not protuberant; canthus rostralis distinct, straight; loreal region concave; tympanum distinct, relatively small, its diameter 55% of eye diameter; distinct and almost straight supratympanic fold; tongue ovoid, distinctly bifid posteriorly; vomerine teeth as one distinct oblong group posterolateral of each choana; choanae medium-sized, rounded. Forelimbs slender; subarticular tubercles single; inner and outer metacarpal tubercles present; a small prepollex (length ca. 1.4 mm) of about one fourth of the length of the first finger. Fingers with rudiments of webbing; relative finger length 1<2<4<3; finger disks strongly enlarged; nuptial pads absent. Legs slender, when legs are adpressed along body, the tibiotarsal articulation reaches the centre of the eye; lateral metatarsalia separated; inner metatarsal tubercle small, of same size of outer metatarsal tubercle which is distinctly recognizable; webbing formula of the foot 1(1), 2i(1.5-2); 2e(0.75); 3i(2); 3e(1); 4i/e(2.25), 5(0.75) (because only one subarticular tubercle is recognizable on the second toe, the relative extension of web on this toe can only be estimated); relative toe length 1<2<3<5<4. Skin on the dorsum smooth; ventral skin smooth on throat and chest, slightly granular on belly. Femoral glands indistinct, of type 1 as defined by Glaw *et al.* (2000).

FIG. 3. Holotype of *Mantidactylus timidus* sp. n. in life. Photographed on 10 February 2003, ca. 10 km north of Toamasina, eastern Madagascar.

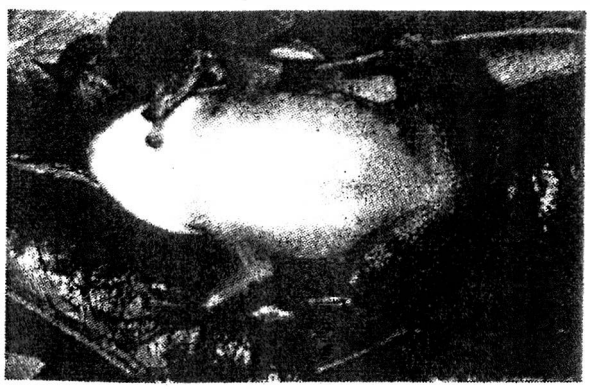


FIG. 4. Holotype of *Mantidactylus timidus* sp. n. in life, ventral view. Photographed on 10 February 2003, ca. 10 km north of Toamasina, eastern Madagascar.

Coloration of the holotype. General dorsal colour grey-brown, with a relatively diffuse grey-beige symmetrical longitudinal dorsolateral markings, and a distinct beige patch on the anterior head, between the eyes and the nostrils, bordered anteriorly and posteriorly by dark brown. A distinct dark brown tympanic patch, and a poorly contrasted trace of a light frenal stripe. Flanks marked with several small beige spots. Hind limbs light grey-brown with brown crossbands. Ventral side fading from bright white on the throat and dirty white on the chest to light brown on the limbs.

Variation. Morphometric measurements of all paratypes of *M. timidus* are found in Table 1. The size range is remarkable, from 33.7 mm SVL in a paratype from Toamasina to 55.4 mm in the single available specimen from Voloina. The mean SVL of males was 42.2 mm (± 6.1 mm standard deviation), thus being slightly smaller than that of the single female specimen (44.1 mm). Besides the morphometric characters highlighted in the diagnosis, it is conspicuous that from all localities of *M. timidus* there are specimens with a beige patch on the anterior head, running between the eyes, similar to that of the holotype (Fig. 3). This applies to ZFMK 52698 from Voloina, and very distinctly to ZMA 7109 from Foulpointe. We never observed such patches in other *Guibemantis*, except for one specimen of *M. tornieri* from Andasibe (ZMA 6987). When the hind limb is adpressed along the body, the tibiotarsal articulation reaches at most to the eye center, often only to the posterior eye corner or to the tympanum.

Etymology. The specific name is derived from the Latin adjective timidus = timid, and refers to the shy and inconspicuous calling behaviour of this species of which we only heard very few vocalizations on the two occa-

sions that we encountered it in the wild during its breeding season.

Advertisement call. We heard advertisement calls of this species on Nosy Boraha and near Toamasina. These were irregular short blasts and moaning sounds. They were often emitted from hidden position in dense vegetation, and after long silent intervals, which stands in contrast to the loud, regular and exposed calling behaviour of *Mantidactylus depressiceps, M. tornieri* and *M. kathrinae*. We therefore hypothesize that these vocalizations may not be the real advertisement calls as emitted by fully motivated males, and here refrain from a detailed description. Also Blommers-Schlösser (1979) did not refer to the calls of this species but only to those of *M. tornieri* at Andasibe.

Natural history. Specimens near Toamasina, on Nosy Boraha and Voloina were found in secondary vegetation and heavily degraded forest, sitting on leaves at heights of 1-3 m. The Toamasina site is far from any primary forest in an area of lowland swamps and rice fields. Egg masses are attached on leaves over water. According to Blommers-Schlösser (1979) and to our observations on Nosy Boraha (Glaw & Vences, 1994), the jelly is transparent and the eggs are greenish. Tadpoles of *M. timidus* have been described by Blommers-Schlösser (1979) under the name *Mantidactylus tornieri*.

Distribution. Voucher specimens of *M. timidus* are available from three localities, (1) the type locality N of Toamasina, (2) Foulpointe and (3) Voloina. We also assign specimens observed but not collected by us on the eastern offshore island (4) Nosy Boraha (Ile Ste. Marie) as belonging to this species.

Available older names. Rhacophorus mocquardii Boulenger, 1896, and *Mantidactylus acuticeps* Ahl, 1929, both considered to be junior synonyms of *Mantidactylus depressiceps*, could potentially be available as earlier names for *M. timidus*. Table 1 provides measurements of the holotypes of these taxa, and of the types of *M. depressiceps, M. tornieri* and *M. kathrinae*. All of these have large relative hand lengths, demonstrating their distinctness from *M. timidus*.

DISCUSSION

Recent studies have shown that many amphibian species that previously were thought to occur over wide geographic and altitudinal ranges of Madagascar actually are complexes of well differentiated species. Some of the newly recognized taxa appear to have an elevated degree of elevational endemism. Examples encompass the genus *Boophis* (B. *schuboeae* vs. *B. ankaratra;* Glaw & Vences, 2002*b*) as well as *Mantidactylus* (*M. sarotra* vs. *M. kely*; Glaw & Vences, 2002*a*). The data herein provide another example, showing that lowland populations previously considered as *M. tornieri* actually belong to another species, *M. timidus*.

Originally, the subgenus *Guibemantis* had been conceived as *Mantidactylus depressiceps* group to include only the two phenetically similar species *M. tornieri* and *M. depressiceps* (Blommers-Schlösser, 1979). Several other species were later added to the group (Blommers-Schlösser & Blanc, 1991), namely *M. peraccae, M. elegans* and *M. guibei*. While the relationships of *M. guibei* remain unstudied, the molecular results herein confirm that *M. peraccae* and also *M. elegans* belong into the subgenus *Spinomantis* (see Andreone *et al.*, 1998). *Mantidactylus liber*, which was added to *Guibemantis* by Glaw & Vences (1994) because of its femoral gland morphology and large relative hand length, is the sister taxon of other *Guibemantis* according to the results herein but may also be related to the leaf-axil breeding subgenus *Pandanusicola* (Vences *et al.*, 2003). Indeed, our study did not include any *Pandanusicola* species, but recent molecular studies by Lehtinen & Nussbaum (2003) suggested that *M. liber* was nested within that subgenus rather than in a clade with the larger-sized species of *Guibemantis*. Furthermore, not even the phylogenetic placement of *M. timidus* as sister group of the *M. depressiceps-kathrinae-tornieri* clade was sufficiently supported by the bootstrap analyses, emphasizing the need for more comprehensive molecular studies to fully clarify the phylogenetic relationships of these taxa.

An interesting aspect is the high intraspecific variability of body size in this lineage. According to the data presented herein, this regards both *M. timidus* (SVL of adult males 34-55 mm) and *M. tornieri* (42-51 mm). It also coincides with the rather large differences found between specimens of *M. kathrinae* from the type locality An'Ala (57-59 mm) and those from Andapa in north-eastern Madagascar (44-46 mm; Glaw *et al.*, 2000). In contrast, several other mantellids seem to have quite constant body sizes, and low differences in SVL can be used to distinguish among species (e.g., Glaw & Vences, 2002*a*). Our results suggest that body size differences in frogs must be studied carefully if they are to be used for taxonomic purposes (see also Andreone *et al.*, 2002).

Molecular data, more specifically the comparison of mitochondrial rRNA sequences, have shown to be a valuable tool to elucidate the taxonomic status and identity of certain frog populations, especially in taxa of inconspicuous advertisement calls (e.g., Glaw & Vences, 2004). Haplotype sharing between populations of mantellid frogs can exist (Vences *et al.*, 2004), but has so far only been observed among very closely related species or variants. The high divergence values found herein between *M. timidus* and other species of *Guibemantis* would be unprecedented if considered as within-species differentiation, and therefore alone already provide a strong indication of specific distinctness.

However, we discourage the uncritical use of mitochondrial divergences as only marker to draw taxonomic conclusions. Strongly divergent haplotypes are known to be shared among conspecific populations and individuals of some organisms (e.g. snails; Thomaz *et al.*, 1996). Threshold values of mitochondrial divergence have been proposed to distinguish species of

mammals in terms of a genetic species concept (Bradley & Baker, 2001). The identification of such thresholds in amphibians, above which two taxa can be regarded as not conspecific with a defined statistical probability, is a promising endeavour in terms of DNA taxonomy (Tautz *et al.*, 2003; Blaxter, 2004) or DNA barcoding (Hebert *et al.*, 2003). However, it needs to be stressed that this technique is based on genetic distances among individuals and therefore is prone to error whenever these are not representative of their populations, e.g. caused by phenomena of introgression, haplotype sharing, or non-monophyletic species. DNA barcoding holds a great potential for quick exploratory studies but should not be used as exclusive basis for the formal descriptions of new species. In our data set, also the rather strong divergences between individuals of *Mantidactylus liber* (from Mandraka and Montagne d'Ambre; uncorrected pairwise distance 4.6%) could be seen as indications for specific distinctness, but more detailed studies on their morphology and bioacoustics are necessary before formalizing such conclusions by the description of new taxa. Of course, in the case of *Mantidactylus timidus,* not only its strong molecular divergence but also its well corroborated isolated phylogenetic position supports a specific distinctness from *M. tornieri.* But in light of the surprisingly high number of non-monophyletic species that have been identified (Funk & Omland, 2003), also this evidence should not be used as exclusive taxonomic argument.

If we consider the subgeneric assignation of *Mantidactylus liber* and *M. guibei* as in need of confirmation and regard only the *M. depressiceps* complex (see Fig. 2), then the discovery of two new species, *M. kathrinae* and *M. timidus,* has led to a doubling of the species diversity of this clade. Considering the genetically divergent *M. tornieri* population from Ranomafana, it would not be surprising if future studies result in the discovery and identification of further new species in this lineage.

ACKNOWLEDGEMENTS

This work would not have been possible without the help of many friends and colleagues who contributed especially to the completeness of the molecular sampling. We want to thank especially Franco Andreone, Marta Puente, Liliane Raharivololoniaina, Meike Thomas and David R. Vieites for their assistance in the field. We are also grateful to Axel Meyer who made the sequencing of most samples possible. This work was carried out in the framework of cooperation accord of the Université d'Antananarivo (Département de Biologie Animale) and the Association Nationale pour la Gestion des Aires Protégées (ANGAP) with the institutions of the authors. We are indebted to the Malagasy authorities who issued permits for collection and export of specimens, and to the Deutscher Akademischer Austauschdienst (DAAD), the Deutsche Forschungsgemeinschaft (DFG) and the Volkswagen-Stiftung for financial support.

REFERENCES

Andreone, F., Glaw, F., Vences, M. & Vallan, D. (1998). A new *Mantidactylus* from south-eastern Madagascar, with a review of *Mantidactylus peraccae* (Ranidae: Mantellinae). *Herpetological Journal* **8**, 149-159.

Andreone, F., Vences, M., Guarino, F. M., Glaw, F. & Randrianirina, J. E. (2002). Natural history and larval morphology of *Boophis occidentalis* (Anura: Mantellidae: Boophinae) provide new insights into the phylogeny and adaptive radiation of endemic Malagasy frogs. *Journal of Zoology, London* **257**, 425-438.

Blaxter, M. L. (2004). The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London, Biological Sciences* **359**, 669-679.

Blommers-Schlösser, R.M.A. (1979). Biosystematics of the Malagasy frogs. I. Mantellinae (Ranidae). *Beaufortia* **352**, 1-77.

Blommers-Schlösser, R. M. A. & Blanc, C. P. (1991). Amphibiens (première partie). *Faune de Madagascar* **75**, 1-379.

Bradley, R. D. & Baker, R. J. (2001). A test of the genetic species concept: cytochrome *b* sequences and mammals. *Journal of Mammalogy* **82**, 960-973.

Dubois, A. (1992). Notes sur la classification des Ranidae (Amphibiens Anoures). *Bulletin ménsuel de la Societé Linnéenne de Lyon* **61**, 305-352.

Funk, D. J. & Omland, K. E. (2003). Species-level paraphyly and polyphyly: frequency, causes and consequences, with insights from animal mitochondrial DNA. *Annual Reviews of Ecology and Systematics* **34**, 397-423.

Glaw, F. & Vences, M. (1994). *A fieldguide to the amphibians and reptiles of Madagascar. Second edition, including mammals and freshwaterfish.* Köln: Vences & Glaw Verlag.

Glaw, F. & Vences, M. (2002*a*). A new sibling species of the anuran subgenus *Blommersia* from Madagascar (Amphibia: Mantellidae: *Mantidactylus*) and its molecular phylogenetic relationships. *Herpetological Journal* **12**, 11-20.

Glaw, F. & Vences, M. (2002*b*). A new cryptic treefrog species of the *Boophis luteus* group from Madagascar: bioacoustic and genetic evidence. *Spixiana* **25**, 173-181.

Glaw, F. & Vences, M. (2004). A preliminary review of cryptic diversity in the subgenus *Ochthomantis* based on mtDNA sequence data and morphology (Anura, Mantellidae, *Mantidactylus*). *Spixiana* **27**, 83-91

Glaw, F., Vences, M. & Gossmann, V. (2000). A new species of *Mantidactylus* from Madagascar, with a comparative survey of internal femoral gland structure in the genus (Amphibia: Ranidae: Mantellinae). *Journal of Natural History* **34**, 1135-1154.

Hebert, P. D. N., Cywinska, A., Ball, S. L. & deWaard, J. R. (2003). Biological identification through DNA barcodes. *Proceedings of the Royal Society, Biological Sciences* **270**, 313-321.

Lehtinen, R. M. & Nussbaum, R. A. (2003). Parental care: a phylogenetic perspective. In *Reproductive biology*

and phylogeny of Anura, 343-386. Jamieson, B. G. M. (Ed.). Enfield, NH: Science Publishers Inc..

Palumbi, S.R., Martin, A., Romano, S., McMillan, W. O., Stice, L. & Grabowski, G. (1991). *The Simple Fool's Guide to PCR, Version 2.0*. Privately published document compiled by S. Palumbi, Dept. Zoology. Honululu: Univ. Hawaii.

Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Richards, C. M., Nussbaum, R. A. & Raxworthy, C. R. (2000). Phylogenetic relationships within the Madagascan boophids and mantellids as elucidated by mitochondrial ribosomal genes. *African Journal of Herpetology* **49**, 23-32.

Swofford, D. L. (2002). PAUP*. *Phylogenetic Analysis Using Parsimony (* and other methods), Version 4*. Sunderland, Massachusets: Sinauer Associates.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H. & Vogler, A. P. (2003). A plea for DNA taxonomy. *Trends in Ecology and Evolution* **18**, 70-74.

Thomaz, D., Guiller, A. & Clarke, B. (1996). Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proceedings of the Royal Society of London, Biological Sciences* **263**, 363-368.

Vences, M. & Glaw, F. (2001). When molecules claim for taxonomic change: new proposals on the classification of Old World treefrogs. *Spixiana* **24**, 85-92.

Vences, M., Vieites, D. R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M. & Meyer, A. (2003). Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society of London, Biological Sciences* **270**, 2435-2442.

Vences, M., Y. Chiari, L. Raharivololoniaina & A. Meyer (2004). High mitochondrial diversity within and among populations of Malagasy poison frogs. *Molecular Phylogenetics and Evolution* **30**, 295-307.

Accepted: 26.7.04

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 45-50 (2005)

CANALIZATION OF SIZE AT METAMORPHOSIS DESPITE TEMPERATURE AND DENSITY VARIATIONS IN *PELODYTES PUNCTATUS*

PIERRE JOLY, ALAIN MORAND, SANDRINE PLÉNET AND ODILE GROLET

UMR CNRS 5023 Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1, France

We investigated variation in length of larval period and size at metamorphosis in *Pelodytes punctatus* in response to temperature and density. Experiments followed a 3 × 2 factorial design, with density and temperature as fixed factors. Length of the larval period was very sensitive to temperature. Although significant, size at metamorphosis showed less variation. Density did not strongly affect the studied variables. No trade-off between length of larval period and size at metamorphosis was detected, even when a trend was perceptible at low density. *Pelodytes punctatus* differs from most other European species of tailless amphibian with regard to these traits, but is similar to *Bombina variegata*. These two species both belong to taxa of ancient origin, but also use habitats in the early stages of ecological succession (e.g. those following physical disturbance). Further studies are required to distinguish the influence of phylogenetic factors from those ecological factors that drive the evolution of such biological traits.

Key words: larval period, plasticity, size at metamorphosis, tadpole development

INTRODUCTION

Phenotypic plasticity is a response to environmental variability. Plasticity may be adaptive when an animal is faced with immediate constraints, but it can also respond to habitat unpredictability by producing phenotypic variation within the progeny (Kaplan, 1987; Scheiner, 1993; Schlichting & Pigliucci, 1995). In animals with complex life cycles, the timing of the transition from one biological stage to another through metamorphosis would ideally be optimized by an accurate assessment of the conditions in the premetamorphic and postmetamorphic habitats respectively (Wilbur & Collins, 1973; Wilbur, 1980; Werner, 1986; Alford & Harris, 1988). However, only information on the immediate habitat is available to the individual. As a consequence, the timing of metamorphosis depends on information gathered in the premetamorphic habitat and putative performance objectives in the postmetamorphic habitat that have been forged by natural selection. Such performances are mainly influenced by body size, and consequently metamorphosis is expected to be delayed until body size reaches a specific threshold that optimizes the trade-off between growth in the premetamorphic and postmetamorphic habitats (Wilbur & Collins, 1973; Werner, 1986). As a consequence, body size at metamorphosis, length of the larval period, and survival may vary according to premetamorphic ecological conditions. However, such a trade-off (negative correlation) between the shortness of the larval period and the size at metamorphosis is expected to follow species-specific reaction norms, as it results from specific interactions between habitat and genotype.

Correspondence: P. Joly, UMR CNRS 5023 Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1, 69622 Villeurbanne Cedex, France.
E-mail: pjoly@biomserv.univ-lyon1.fr

In tailless amphibians, variation in the timing of metamorphosis has been well documented. Most studies have shown that growth rate and body size determine the timing of metamorphosis according to threshold effects (Wilbur & Collins, 1973; Smith-Gill & Berven, 1979; Alford & Harris, 1988; Hensley, 1993). The factors that most strongly influence size at metamorphosis are density, food availability, temperature and stress (Travis, 1984; Berven, 1987; Tejedo & Reques, 1994; Newman, 1994; Denver, 1997; Denver *et al.*, 1998). Moreover, comparisons of tadpole development under standardized rearing conditions among species have revealed variation in the reaction norm of the trade-off between size at metamorphosis and shortness of larval period in response to temperature treatments (Blouin, 1992 ; Morand *et al.*, 1997). In the study of Morand *et al.* (1997) that compared development of species belonging to the anuran assemblage of the Rhone floodplain, most species exhibited such a trade-off. *Bombina variegata* was an exception, and showed a canalization of size at metamorphosis (absence of trade-off as the slope of the bivariate reaction norm did not differ from zero). This result was surprising because this species mainly occupies unstable habitats within floodplains (Morand, 1996). Such habitat use would be expected to promote plasticity.

The aim of the present study was to firstly investigate reaction norms to temperature and density in *Pelodytes punctatus*, a species that also inhabits unstable habitats in the early stages of ecological succession (i.e. 'young' habitats that appeared recently or that experience frequent physical disturbances that maintain them at early successional stages). These habitats are often located within floodplains (Diaz Paniagua, 1988; Toxopeus *et al.*, 1993; Morand, 1996). Secondly, the life history of this species is poorly documented and the present study also aimed to provide precise estimations of length of larval period and size at metamorphosis under controlled conditions.

TABLE 1. Mean survival, length of larval period (±SE), body length (±SE), and dry mass (±SE) of *P. punctatus* for each replicate according to temperature and density.

		Survival (% metamorphs)		Length of larval period (days)		Dry mass (mg)			
Density	Replicate	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C
5	1	100	80	100±15	31±3	18.8±0.8	16.5±1.2	98.1±22.4	55.1±14.4
5	2	100	60	89±10	46±6	18.5±1.0	16.3±0.6	107.6±17.0	55.4±6.1
5	3	80	80	103±19	41±7	17.3±0.9	16.5±1.1	70.1±16.5	64.3±8.8
	Mean	93	73	97.3	39.3	18.2	16.4	91.9	58.3
15	1	73	60	76±5	54±9	17.3±1.2	16.7±1.3	72.2±9.6	69.6±14.1
15	2	67	80	89±18	58±12	15.9±1.4	15.5±1.1	54.7±19.1	47.8±11.2
15	3	60	100	104±22	65±11	15.8±0.9	15.3±0.9	48.8±7.5	45.9±12.9
	Mean	67	80	89.7	59.0	16.3	15.8	58.6	54.4
30	1	50	33	106±13	61±16	15.8±1.3	16.1±0.6	50.4±14.9	54.3±5.0
30	2	53	33	93±16	72±24	15.6±1.2	15.7±0.8	47.3±13.5	50.0±7.5
30	3	77	60	115±10	58±19	16.5±0.8	14.5±0.8	56.5±9.5	37.6±6.4
	Mean	60	42	104.7	63.7	16.0	15.4	51.4	47.3

MATERIAL AND METHODS

Three clutches of eggs were collected in April 1996 in the Jons sector of the Rhone floodplain (see Joly, 1992). After hatching, tadpoles were distributed randomly among 18 tanks according to a 3 × 2 factorial design. Density and temperature were fixed factors with three levels of density (5, 15 and 30 tadpoles per tank) and two levels of temperature (15 and 25 °C). Each treatment (density × temperature) was replicated three times. Replicates were distributed randomly in the experimental room. Each tank was 40 × 40 cm long and 10 cm deep, and contained 4 l of continuously aerated water. The lower temperature (15°C) was the ambient temperature in the cooled room, whereas the higher temperature (25°C) was maintained by using water heaters in the aquaria. Temperature was checked in each tank three times a week, and tadpoles were counted and the tanks were cleaned. Boiled lettuce was provided *ad libitum* as food.

When the froglets reached metamorphosis, they were humanely euthinased, then dried on filter paper, measured to the nearest 0.1 mm (snout-urostyle length) under a stereoscopic microscope and weighed to the nearest 1 mg. Dry mass was measured after heating for 48 hr at 80°C. Survivorship was estimated by the number of tadpoles that successfully reached metamorphosis. After experimental groups were selected, surplus tadpoles were carefully reared in large tanks and released at the sampling site when they reached stages 35-40 (Gosner 1960) to compensate for sampling impact on the source population.

Survivorship, size and mass at metamorphosis, and length of the larval period were compared by a two-way ANOVA. Before performing ANOVAs, data were checked for homogeneity of variance and normality. Survival data were transformed from simple proportions (p) to arcsin ($\sqrt{p}$) before analysis. Pairwise comparisons of treatment means were performed using Scheffé's tests.

RESULTS

Survival to metamorphosis, mean length of larval period, mean body length and mean dry mass in each replicate are listed in Table 1. Mean survival to metamorphosis varied with density ($F_{2,12}$=6.40, P=0.013), declining as density increased, but it did not differ significantly between temperature treatments (Fig. 1a, Table 2).

The mean length of larval period was significantly greater at 15°C than at 25°C at all densities (Fig. 1b). Length of larval period also increased with increasing density; this trend was more apparent at 25°C than at 15°C ($F_{2,12}$=3.43, P=0.066, Table 2).

Both body length and dry mass at metamorphosis were affected by the temperature and density treatments (Figs 1c and 1d ; Table 2). Body length and dry mass differed significantly between temperature treatments, but only at low density. On day 5, mean body length and mean dry mass were higher at 15°C than at 25°C (Table 1; Fig. 1) (Scheffé tests; P=0.032 and P=0.014, respectively; for the other densities: P>0.05). Density affected body length and dry mass only at low temperature, with tadpoles larger at lower density (Scheffé tests on 15°C data; for body length: days 5-15, P =0.023 ; days 5-30, P=0.008; for dry mass: days 5-15, P=0.015; days 5-30, P=0.004). No difference was detected between day 15 and day 30 (Scheffé test; P>0.05). At 25°C, size at metamorphosis was unaffected by density (Scheffé tests; P>0.05).

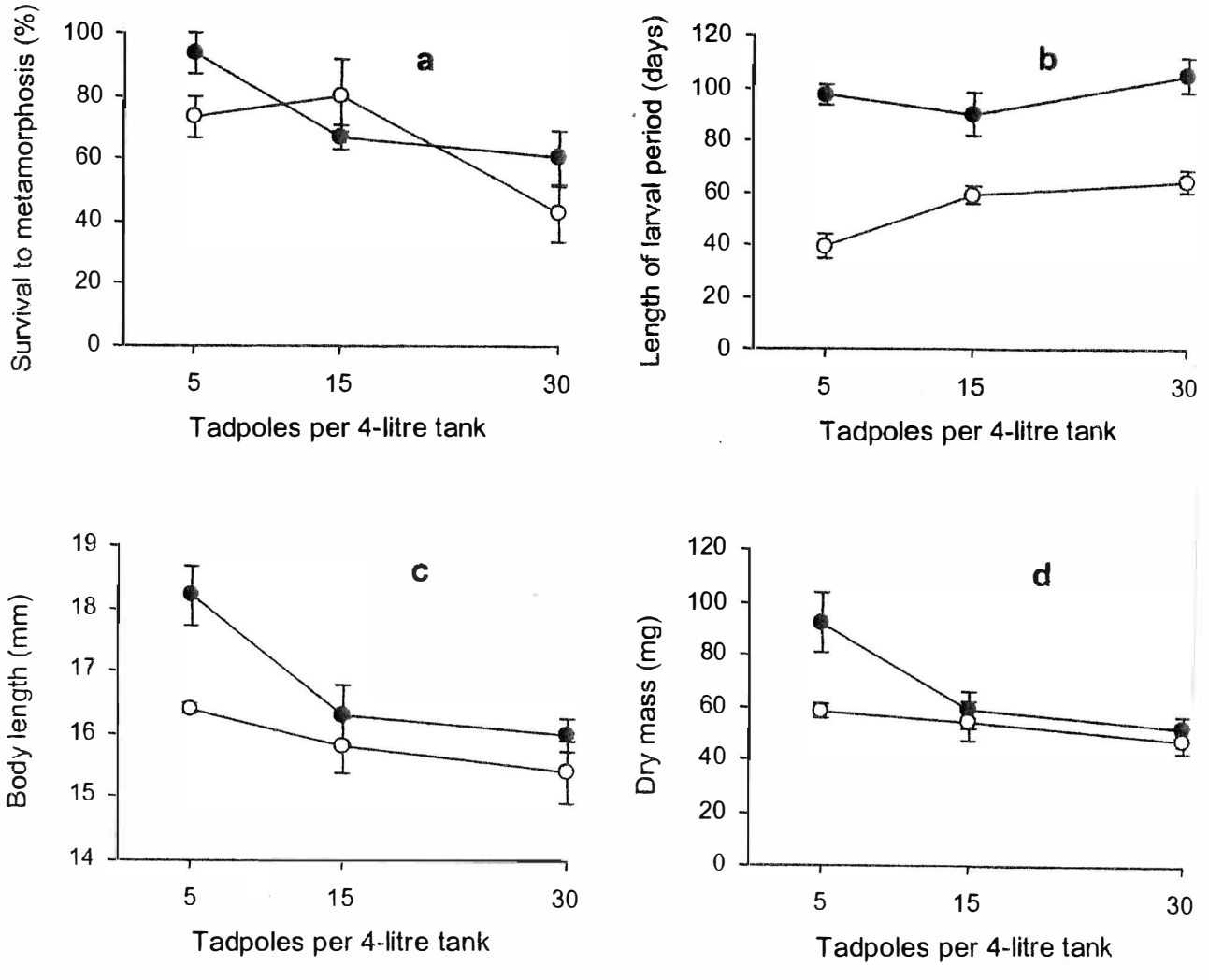


FIG. 1. Influence of temperature and density on (a) survival; (b) length of larval period; (c) body length and (d) dry mass. Solid squares: low temperature (15°C); open squares: high temperature (25°C).

TABLE 2. ANOVAs for length of larval period, body length and dry mass of *P. punctatus* tadpoles at metamorphosis. ***$P<0.0001$, **$P<0.001$, *$P<0.05$.

	Source of variation	df	MS	*P*
Length of larval period	Temperature	1	8390.76	98.59***
	Density	2	380.28	4.47*
	Temp. × Density	2	292.15	3.43
	Error	12	85.11	1.85
Body length	Temperature	1	3.80	7.82*
	Density	2	4.23	8.68*
	Temp. × Density	2	0.76	1.55
	Error	12	0.49	
Dry mass	Temperature	1	877.90	6.38*
	Density	2	1057.89	7.69*
	Temp. × Density	2	435.49	3.16
	Error	12	137.62	

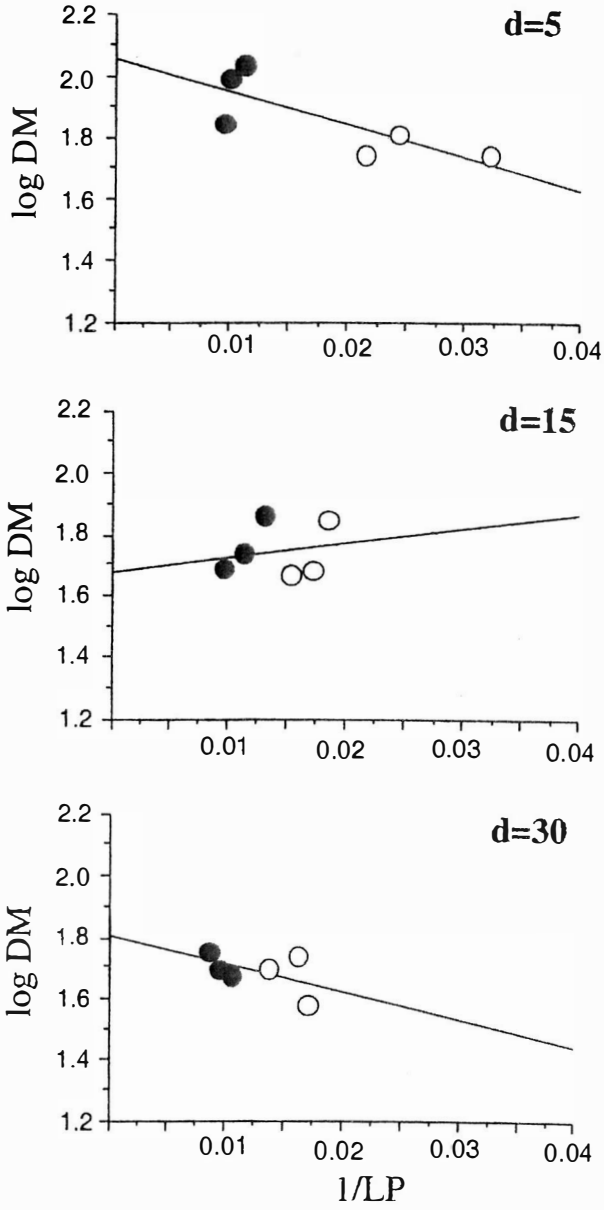


FIG. 2. Bivariate reaction norms of size at metamorphosis (log (dry mass)) and length of larval period (1/LP) to temperature for each density. Full dots: individual data at 15°C; open dots: individual data at 25°C. DM = dry mass; LP, larval period; d, density. Dry mass appeared to be influenced by temperature only at low density.

The bivariate reaction norms of dry mass in relation to length of larval period (1/length of larval period) highlighted the absence of trade-offs (negative correlations) at each density (P=0.06 for day 5; P=0.71 for day 15; P=0.27 for day 30; Fig. 2). However, the P-value for day 5 was close to significance, suggesting a trend toward a trade-off at this density level.

DISCUSSION

Our data can be compared with those from other species belonging to the anuran assemblage of the Rhone floodplain obtained by Morand *et al.* (1997) with the same rearing equipment (Table 3). As density was 30 tadpoles per 4 l in these previous experiments, comparisons are possible at this density. Length of larval period of *Pelodytes punctatus* was one of the longest of the species studied, and was very close to that of *Bombina variegata* at both low and high temperatures. This result differs from that of Diaz Paniagua (1988) who considered that development of *P. punctatus* (approximately 80 days in the field) was as short as that of other species. However, the latter study concerned length of larval period under field conditions where *P. punctatus* did not spawn at the same time of the year as most other species, making comparisons among species less straightforward because of seasonal variation in temperature, food availability and competition. In the north of France, Toxopeus *et al.* (1993) also found the duration of the larval period varied from 67 to 102 days in the field, depending on the year. From other studies, the length of the larval period in the field ranges from 73 to 97 days (Balcells, 1955; Girard, 1989). All these data are within the range we observed (from 31 to 115 days depending on both temperature and density). We are confident in our comparisons with other species studied by Morand *et al.* (1997) because of similar controlled conditions. When compared to the data of Morand *et al.* (1997), size at metamorphosis of *P. punctatus* reached high values (Table 3). This result is consistent with that of Diaz Paniagua (1988), who also showed that premetamorphic tadploles of *P. punctatus* reached a larger size than in most other species, except for *Pelobates cultripes*, the tadpoles of which are exceptionally large.

TABLE 3. Comparison of larval traits of *Pelodytes punctatus* with those of other European species reared at two temperature levels and at a density of 30 tadpoles per 4 litres. Data from Morand *et al.*, 1997.

	Dry mass (mg)		Length larval period (days)	
Temperature	15	25	15	25
Pelodytes punctatus	51±4	47±8.7	104±11	63±7
Bombina variegata	25±2	25±1	106±4	65±4
Bufo calamita	14±1	7±0.4	73±1	44±2
Bufo bufo	30±1	9±0.3	73±1	29±0.3
Rana temporaria	41±2	24±3	64±1	48±3
Rana dalmatina	60±3	25±2	106±2	49±7

Long larval period and large size at metamorphosis often correspond to the use of permanent water habitats (Pough & Kamel, 1984). However, *Pelodytes punctatus* is not a typical inhabitant of permanent waters, as most studies state that this species avoids permanent waters where fish are present. However, it can be abundant in permanent waters where fish are absent, such as gravel pits (Morand, 1996) or dune ponds (Toxopeus, *et al.* 1993). It also seems to avoid competition with other species by occurring in mesotrophic and early succession habitats that can be temporary (Diaz Paniagua, 1988; Morand, 1996; Piégay *et al.*, 1997). Morand *et al.* (1997) also noted a long larval period in *Bombina variegata* which occupies early succession habitats that can be temporary (e.g. water-filled ruts and small ponds that are frequently flooded).

In comparing different temperature conditions, a slight trade-off between shortness of larval period and size at metamorphosis was only detected at low density. High temperatures are usually expected to induce a shortening of larval period and hence a lower size at metamorphosis (Blouin, 1992 ; Morand *et al.*, 1997). However, canalization of size at metamorphosis with respect to temperature variation has already been shown in a few species such as *Hyla squirella* (Blouin, 1992) and *Bombina variegata* (Morand *et al.* 1997). As for long larval periods and large size at metamorphosis, low plasticity of size in response to temperature variation is a biological trait shared by both *P. punctatus* and *B. variegata*, but not by other European anurans. Demonstrating the adaptive value of this trait is not an easy task because confounding factors such as phylogeny can influence the expression of biological traits (Harvey & Pagel, 1991). In this context, both species belong to ancient groups of tailless amphibians (i.e. Archeobatrachia for *B. variegata* and Mesobatrachia for *P. punctatus*). Other species belonging to these old groups, such as *Alytes obstetricans* (Guyétant, 1975) and *Pelobates cultripes* (Knoepffler, 1961; Diaz Paniagua, 1988), also show large size at metamorphosis and long larval periods.

According to Werner (1986), the evolution of size at metamorphosis results from a trade-off between performance in aquatic habitats and performance in terrestrial habitats, before and after metamorphosis, respectively. Canalization of size at metamorphosis suggests that success in the terrestrial habitat is constrained by selective pressures for a given minimum size. Such pressures remain to be identified since our knowledge of the ecology of juvenile anurans is still scanty. However, it is surprising that the species that present the highest canalization of size at metamorphosis are also those that inhabit unstable (i.e. temporary, frequently flooded) habitats where they appear as pioneer species.

Our work investigated plasticity by varying temperature, which is an important ecological factor because of its influence on development rate and on species distribution. Other factors have also to be investigated, such as water duration or presence of predators that can appear more relevant with respect to performance in the field. Other experiments in more complex environments (mesocosms) are needed to confirm our conclusions as variation in tadpole growth may be an artefact of the rearing conditions in small tanks (Richards, 1958; Biesterfeldt *et al.*, 1993). Moreover, we also need field studies that can quantitatively estimate the respective impacts of different factors on tadpole growth and survival in various habitats. Understanding the determinants of the trade-off between length of larval period and size at metamorphosis needs more observations on other species to estimate the respective influences of phylogeny (improving our knowledge of larval traits of both Archeobatrachia and Mesobatrachia) and ecology (species inhabiting unstable *vs* stable habitats).

ACKNOWLEDGEMENTS

This paper has been revised by Eric Pattee. The statistical analyses benefited from the help of Peter Rothery. We thank Clive Cummins and an anonymous referee for their comments.

REFERENCES

Alford, R. A. & Harris, R. N. (1988). Effects of larval growth history on anuran metamorphosis. *American Naturalist* **131**, 91-106.

Balcells, E. R. (1955). Contributions to the study of the life cycle of Spanish amphibians. *British Journal of Herpetology* **2**, 1-6.

Berven, K. A. (1987). The heritable basis of variation in larval developmental patterns within populations of the wood frog (*Rana sylvatica*). *Evolution* **41**, 1088-1097.

Biesterfeldt, J. M., Petranka, J. W. & Sherbondy, S. (1993). Prevalence of chemical interference competition in natural populations of wood frogs, *Rana sylvatica*. *Copeia* **1993**, 688-695.

Blouin, M. S. (1992). Comparing bivariate reaction norms among species: time and size at metamorphosis in three species of *Hyla* (Anura Hylidae). *Oecologia* **90**, 288-293.

Denver, R. J. (1997). Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *American Zoologist* **37**, 172-184.

Denver, R. J., Mirhadi, N. & Phillips, M. (1998). Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology* **79**, 1859-1872.

Diaz Paniagua, C. (1988). Temporal segregation in larval amphibian communities in temporary ponds at a locality in SW Spain. *Amphibia-Reptilia* **9**, 15-26.

Girard, F. (1989). Observations sur l'activité de divers batraciens dans une dune littorale de Loire atlantique. *Bulletin Societe Herpetologica France* **51**, 27-32.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on idenfication. *Herpetologica* **16**, 183-190.

Harvey, P. H. & Pagel, M. (1991). *The comparative method in evolutionary biology.* Oxford University Press, Oxford.

Hensley, F. R. (1993). Ontogenetic loss of phenotypic plasticity of age at metamorphosis in tadpoles. *Ecology* **74**, 2405-2412.

Joly, P. (1992). The amphibian fauna of the Upper Rhone floodplain. *Alytes* **10**, 117-129.

Morand, A. (1996). *Dynamique de la coexistence des espèces: de la théorie des perturbations à la théorie des traits d'histoire de vie. L'exemple du modèle amphibien dans l'espace alluvial du Haut-Rhône.* Thèse de Doctorat, Université Lyon1.

Morand, A., Joly, P. & Grolet, O. (1997). Phenotypic variation in metamorphosis in five anuran species along a gradient of stream influence. *Comptes Rendus Acadamie Sciences Paris, Life Sciences* **320**, 645-652.

Newman, R. A. (1994). Genetic variation for phenotypic plasticity in the larval life history of the spadefoot toads (*Scaphiopus couchii*). *Evolution* **48**, 1773-1785

Piégay, H., Joly, P., Foussadier, R., Mourier, V. & Pautou, G. (1997). Principes de réhabilitation des marges du Rhône à partir d'indicateurs géomorphologiques, phyto-écologiques et batrachologiques. *Revue de Géographie de Lyon* **72**, 7-22

Pough, F. H. & Kamel, S. (1984). Post-metamorphic change in activity metabolism of anurans in relation to life history. *Oecologia* **65**, 138-144.

Richards, C. M. (1958). The inhibition of growth in crowded *Rana pipiens* tadpoles. *Physiological Zoology* **31**, 138-151.

Scheiner, S. M. (1993). Genetics and the evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**, 35-68.

Schlichting, C. D. & Pigliucci, M. (1995). Gene regulation, quantitative genetics and the evolution of reaction norms. *Evolutionary Ecology* **9**, 154-168.

Smith-Gill, S. J. & Berven, K. A. (1979). Predicting amphibian metamorphosis. *American Naturalist* **113**, 563-585.

Tejedo, M. & Reques, R. (1994). Plasticity in metamorphic traits of natterjack tadpoles: the interactive effects of density and pond duration. *Oikos* **71**, 295-304.

Toxopeus, A. G., Ohm, M. & Arntzen, J. W. (1993). Reproductive biology of the parsley frog, *Pelodytes punctatus*, at the northernmost part of its range. *Amphibia-Reptilia* **14**, 131-147.

Travis, J. (1984). Anuran size at metamorphosis : experimental test of a model based on intraspecific competition. *Ecology* **65**, 1155-1160.

Werner, E. E. (1986). Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist* **128**, 319-341.

Wilbur, H. R., (1980). Complex life cycles. *Annual Review of Ecology and Systematics* **11**, 67-93.

Wilbur, H. R. & Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis. *Science* **182**, 1305-1314.

Accepted: 1.11.04

SHORT NOTE

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 51-55 (2005)

VARIATION IN PREFERRED BODY TEMPERATURE IN AN OVIPAROUS POPULATION OF *LACERTA (ZOOTOCA) VIVIPARA*

M. A. CARRETERO[1], J. M. ROIG[2] AND G. A. LLORENTE[2]

[1]*Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), Campus Agrário de Vairão, Portugal*

[2]*Dpt. Biologia Animal (Vertebrats). Fac. Biologia. University of Barcelona, Barcelona, Spain*

The intraspecific variation of preferred temperatures (Tp) was analysed in an oviparous population of *Lacerta vivipara* and compared with viviparous populations. Lizards collected in central Pyrenees were exposed to a thermal gradient and Tp was measured at four time intervals. Tp was strongly dependent on lizard condition (males > non-pregnant females > pregnant females = immatures) and more weakly with time of day (early morning > mid-day). Individual females increased their Tp after egg-laying. Class-by-class comparisons did not reveal substantial differences with viviparous populations as expected for the thermal rigidity hypothesis. Nevertheless, on a short time scale, Tp should be interpreted as a compromise between different selective pressures including not only thermal environment but also reproductive condition and energy allocation.

Key words: behaviour, lizard, thermoregulation

The temperature selected in the absence of thermoregulatory constraints (preferred body temperature, Tp) is a relevant trait highly correlated with optima for many physiological processes in lizards (Huey & Bennet, 1987; Bauwens *et al*., 1995). Two kinds of variability in Tp should be considered: among populations/species and within a population. At the population/species level, evidence for both rigidity and flexibility on an evolutionary scale has been found in many lizard groups (i.e. Hertz *et al*., 1983; Bennet & John-Alder, 1986; Christian & Weavers, 1996; Castilla *et al*., 1999). These discrepancies probably stem from different rates of response to directional selection for this trait across evolutionary lineages (Labra, 1998). Studies on lacertids seem to support the thermal rigidity hypothesis within this family (Van Damme *et al*., 1989*a*, 1990; Gvozdík & Castilla, 2001; but see Scheers & Van Damme, 2002). Independently of this, Tp can also change within a population on a short time scale in response to temporal variation within an individual lizard's life (e.g. seasonal changes, reproductive condition and feeding status, see Castilla *et al*., 1999 for a revision in lacertids).

Correspondence: M. A. Carretero, Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO/UP), Campus Agrário de Vairão.4485-661 Vairão, Portugal.
E-mail: carretero@mail.icav.up.pt

The common lizard *Lacerta* (*Zootoca*) *vivipara* (for taxonomic aspects, see Harris & Carretero, 2003) is the only lacertid exhibiting reproductive bimodality. Whereas viviparism is the reproductive mode throughout most of its range, oviparous populations have been recorded in two disjunct areas: the Cantabrian Mountains, the Pyrenees and Aquitaine, and Austria, Slovenia and N Italy (Bea, 1978; Braña & Bea, 1987; Heulin *et al*., 2000; Mayer *et al*., 2000; Suget-Groba *et al*., 2002). Oviparism is considered plesiomorphic within this species but oviparous populations of SW Europe and those from Central Europe, described as subespecies *carniolica* (Mayer *et al*., 2000), are not directly related (Surget-Groba *et al*., 2001). Recently, a study carried out on viviparous populations (Gvozdík & Castilla, 2001) confirmed thermal rigidity of Tp in populations living under different climate regimes but also detected intraspecific variation within the same population (see also Patterson & Davies, 1978, Van Damme *et al*., 1986, 1987). These findings suggest that, although Tp is evolutionary conservative at population/species level, within one particular population physiological optima may conflict and selective pressures vary with the lizard's condition (i.e. sex, reproductive status, size). Thus, overall Tp for one species would be an oversimplification. On the other hand, viviparity in squamates is considered a response to cold since embryo development can be completed under more favourable thermal environment inside the mother (see for instance Shine, 1985; Andrews & Rose, 1994; Qualls *et al*., 1997). If this is the case in *L* (*Z*.) *vivipara*, a shift in Tp would be expected (at least in females) between oviparous and viviparous forms.

This note aims to determine the extent of intraspecific variation for Tp using an oviparous population of *L*. (*Z*.) *vivipara* as a model. Furthermore, the results are compared with those reported for some viviparous populations.

A total of 31 common lizards (6 adult males, 19 adult females and 6 subadults) belonging to an oviparous Pyrenean population were collected by hand in a subalpine meadow at 1800 m (Plan de Beret, Naut Aran UTM grid 31T CH3434: see Roig *et al*. (2000) for a detailed description of the study site) between the second half of June and the first half of July in 1997 and 1998. This interval corresponds to the egg-laying period of this population (Roig *et al*., 2000). Lizards were kept in individual 0.5 × 0.4 × 0.3 m terraria for less than two weeks with food and water provided *ad libitum*, and then released after the experiments. Each lizard was individually exposed to a photothermal gradient (~ 20-45°C, 0.5 × 0.5 × 1.5 m length) produced by a 100 W reflector bulb fixed 15 cm above the substrate maintaining natural photoperiod. Humidity (90%) was kept uniform along the gradient by using a pebble base covered by moss which was periodically sprayed and provided sufficient shelter. Tp was measured by insert-

TABLE 1. Descriptive statistics of Tp considering the different classes and time intervals. In order to prevent pseudoreplication, totals are calculated with the means of each individual for the four time intervals (8:45-14:45).

Class	Time	*n*	Mean	SE	Min.	Max.
MALES						
	8:45	6	33.90	0.86	30.2	36.5
	10:45	6	33.12	0.30	32.3	34.3
	12:45	6	33.37	0.66	30.5	35.0
	14:45	6	33.22	0.59	31.8	35.5
	total	6	33.40	0.90	30.2	36.5
PREGNANT FEMALES	8:45	12	29.60	0.46	26.7	32.2
	10:45	12	27.98	0.39	26.4	30.0
	12:45	12	28.19	0.55	26.1	31.9
	14:45	12	28.18	0.38	25.7	30.2
	total	12	28.49	0.45	25.7	32.2
NON-PREG. FEMALES	8:45	18	31.92	0.35	28.7	34.6
	10:45	18	31.77	0.33	28.9	34.2
	12:45	18	32.31	0.40	29.7	35.5
	14:45	18	31.61	0.51	27.3	34.8
	total	18	31.90	0.40	27.3	35.5
SUBADULTS						
	8:45	6	29.90	0.21	29.2	30.5
	10:45	6	29.67	0.70	27.7	32.3
	12:45	6	29.40	0.49	27.8	31.4
	14:45	6	28.12	0.83	25.5	30.8
	total	6	29.27	0.56	25.5	32.3

TABLE 2. Results of the general ANOVAR of Tp considering class and time, and separate ANOVAR for females considering time. When significant, results of *post-hoc* Schffée's test are provided.

2-WAY ANOVAR	*F*	df	*P*
Class	43.73	3, 38	2.11×10^{-12}
Time	3.14	3, 114	0.03
Class x time	1.07	9, 114	0.39

SCHEFFÉ TESTS Class	Mean	Males	Non-preg. fem.	Preg. fem.
Males	33.40			
Non-preg. fem.	31.90	0.04		
Preg. fem.	28.49	4×10^{-10}	2×10^{-9}	
Subadults	29.27	4×10^{-7}	7×10^{-5}	0.52

Time	Mean	8:45	10:45	12:45
08:45	31.33			
10:45	30.63	0.18		
12:45	30.82	0.45	0.95	
14:45	30.28	0.01	0.73	0.40

NESTED ANOVAR FOR FEMALES	*F*	df	*P*
Pregnancy	51.34	1, 10	3.05×10^{-5}
Time [pregnancy]	1.73	6, 60	0.12

ing a k-thermocouple probe associated with a digital thermometer (Digitron 3208K, accuracy 0.01°C) in the cloaca. Body temperatures were recorded only for active lizards during a single day at four consecutive intervals of 2 hrs (8:45-12:45, Table 1) distributed throughout the period of diel activity observed in the field; the photothermal gradient was connected one hour before the first measurement. When possible (n=11), individual females were analysed before and after egg-laying in terraria.

Data were not transformed since distributions did not deviate from normality (Komogorov-Smirnov tests, P>0.05 in all cases), were homocedastic (univariate Levene tests and multivariate Box M, P>0.05 in all cases) and variances and means were uncorrelated. Since measurements were repeated for the same individual (for each interval and, in most females, before and after egg-laying), statistical analysis was based on Analysis of Variance for Repeated Measures (ANOVAR) of Tp with class (males, pregnant females, non-pregnant females, subadults) as between subject factor and time interval as within subject factor. Sphericity assumption was not rejected prior to non-nested analysis (Mauchley's sphericity tests W=0.89, χ^2=4.34, P=0.50 and W=0.89, χ^2=4.34, P=0.50). Sequential Bonferroni correction (Rice, 1989) was used when multiple tests were evaluated simultaneously.

Table 1 shows the descriptive statistics of Tp for the four classes and the four time intervals. Data have not been pooled due to the high degree of heterogeneity in the results. In fact, ANOVAR revealed variation of Tp with time and class (Table 2). Lizards tended to select higher temperatures in the early morning and lower in the early afternoon. Moreover, significant class variation was detected. Males attained higher Tp than non-pregnant females; both classes exhibited higher values than pregnant females and subadults which did not show significant differences between them. A separate analysis performed for those females analysed before and after

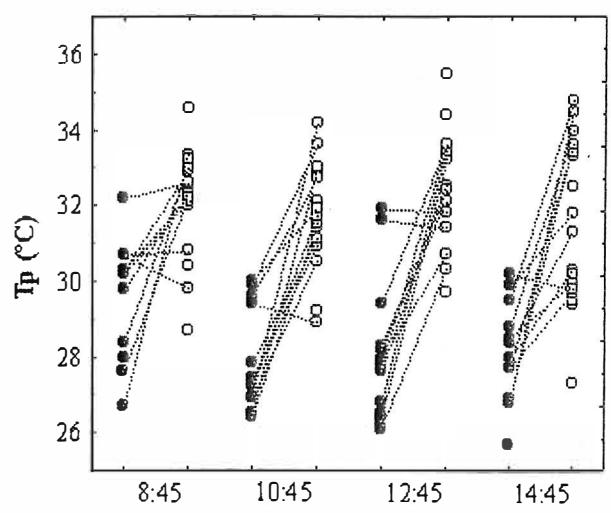


FIG. 1. Diel variation of Tp in females showing individual variation after egg-laying. Lines connect dots belonging to the same individual. Black circles, pregnant females; open circles, non-pregnant females.

TABLE 3. Comparative statistics of Tp in adult *Lacerta (Zootoca) vivipara* from different European populations. Homologous comparisons are performed between Pyrenees and the other localities (samples from the literature) by means of *t*-tests. Pooled calculations excluded pregnant females. n.p., non-pregnant; p., pregnant. * significant after sequential Bonferroni correction.

Area (groups compared)	Reproductive mode	Reference	*n*	Tp mean±S.D.	Compared to Pyrenees		
					t	df	*P*
Great Britain (pooled)	viviparous	Patterson & Davies (1978)	12	31.0±0.9	2.34	34	0.02
Austrian Alps (pooled)	viviparous	Van Damme *et al.* (1990)	92	32.1±2.9	0.32	114	0.75
Belgium (pooled)	viviparous	Van Damme *et al.* (1990)	151	32.3±2.5	<0.0001	173	>0.99
Czech Republic (pooled)	viviparous	Gvozdík & Castilla (2001)	24	31.4±1.3	1.99	46	0.05
Pyrenees (pooled)	oviparous	This study	24	32.3±1.8	-	-	-
Belgium (males, June)	viviparous	Van Damme *et al.* (1986)	100	32.3±2.0	1.32	104	0.19
Belgium (males, July)	viviparous	Van Damme *et al.* (1986)	128	33.3±1.1	0.21	132	0.83
Czech Republic (males)	viviparous	Gvozdík & Castilla (2001)	12	31.5±1.4	2.63	16	0.02
Pyrenees (males)	oviparous	This study	6	33.4±1.5	-	-	-
Belgium (n.p. females, June)	viviparous	Van Damme *et al.* (1986)	19	34.0±1.3	4.23	35	0.0001*
Belgium (n.p. females, July)	viviparous	Van Damme *et al.* (1986)	60	32.7±2.3	1.39	76	0.16
Czech Republic (n.p. females)	viviparous	Gvozdík and Castilla (2001)	12	31.3±1.4	1.01	28	0.32
Pyrenees (n.p. females)	oviparous	This study	18	31.9±1.7	-	-	-
Belgium (p. females June)	viviparous	Van Damme *et al.* (1986)	74	30.3±2.6	2.33	84	0.02
Belgium (p. females July)	viviparous	Van Damme *et al.* (1986)	10	30.0±1.3	4.79	20	0.0001*
Czech Republic (p. females)	viviparous	Gvozdík & Castilla (2001)	7	29.5±0.5	1.61	17	0.10
Pyrenees (p. females)	oviparous	This study	12	28.5±1.5	-	-	-

egg-laying (Table 2) showed a clear increase of Tp for the same individuals (Fig. 1). Time differences were not significant when this group was analysed separately.

Comparative Tp values for different populations of *L. (Z.) vivipara* are shown in Table 3. Overall differences found with the viviparous populations of Britain and the Czech Republic were not significant after sequential Bofferoni correction. Available results allowed separate comparisons with Belgium and the Czech Republic. Pyrenean pregnant and non-pregnant females showed higher temperatures than their Belgian equivalents in June and in July, respectively. No differences were found for males when results were Bonferroni-corrected.

The results indicate that, within the population studied, Tp is strongly dependent on lizard condition and more weakly on time of day (Castilla *et al.*, 1999 and references therein, Rismiller & Heldmaier, 1982). Interpopulation analysis should then avoid using pooled data but perform at least class-by-class comparisons. When comparing in such a way, however, present results mostly agree with the results obtained for viviparous populations.

During the long gestation period, selected body temperatures of females seem to reflect the optimum for embryonic development (~27°C for *in vitro* development in viviparous populations, Maderson & Bellairs, 1962), rather than the optimum for physiological processes of the female itself. This seems to be true for both reproductive modes (Heulin, 1987; Van Damme *et al.*, 1987; Gvozdík & Castilla, 2001; this study). Alternatively, lower Tp in gravid females could reflect decreased basking intensity, associated with changes in antipredatory behaviour. Gravid (viviparous) females of this species tend to be slower (Van Damme *et al.*, 1989*b*) and therefore may be more sensitive to predation when basking in the open (Bauwens & Thoen, 1981). In any case, individual monitoring of females in this study demonstrates that, once released from embryos after egg-laying, females immediately raise their Tp. This confirms that selective pressure for decreasing Tp is linked (directly or indirectly) to pregnancy. Nevertheless, differences between males and non-pregnant females still persist. This result, not detected for the viviparous populations (Gvozdík & Castilla, 2001), needs additional testing with pre-reproductive females in order to distinguish between possible residual effects of pregnancy in females and active selection of higher temperatures by males due to other selective pressures, especially the spermatogenic cycle (Patterson & Davies, 1978; Van Damme *et al.*, 1986).

Subadult lizards preferred lower temperatures than adults (except pregnant females). It has been suggested (Carretero & Llorente, 1995) that this opportunistic thermal behaviour allows immature lacertids to remain active for a longer period (both daily and annually) than adults, thus increasing their opportunities for food con-

sumption and, hence, for growing. Alternatively or additionally, evaporative loss is important in this species (Reichling, 1957) and probably more acute for small lizards due to their high surface/volume ratio (Bowker, 1993; Lorenzon *et al.*, 1999).

Diel variation of Tp observed was weak but significant. In general, lacertids tend to raise their Tp when environmental temperatures are low, i.e. when the thermal environment is more unfavourable and thermoregulation become a priority in relation to other requirements (Rismiller & Heldmaier, 1982; Tosini & Avery, 1994). Excluding the effect of reproductive activity, the seasonal variation reported for this species seems to follow a similar pattern (Patterson & Davies, 1978; Rismiller & Heldmaier, 1982; Van Damme *et al.*, 1987).

Finally, concerning the Tp variation found between populations, marginal differences between oviparous (Pyrenean) and viviparous populations can be easily attributed to differences in laboratory methods (see Gvozdík & Castilla, 2001) and/or in the numbers of lizards of each class composing the total samples. However, procedures were essentially the same for Pyrenean, Belgian and Czech populations and Tp variation between members of the same class was still recorded in some cases. However, such differences do not show a clear pattern of divergence between oviparous and viviparous forms, but may well arise secondarily from seasonal variation (Van Damme *et al.*, 1986) since experiments were not simultaneous and the reproductive cycle changes with environmental conditions. For instance, the strong increase of Tp observed in Pyrenean males after the breeding season may be tentatively associated with rapid spermatogenesis observed in this high mountain population due to climatic constraints (Roig *et al.*, 2000).

In conclusion, the present results did not differ from those expected for thermal rigidity at species/population level, extending the confirmation of this hypothesis to both reproductive modes within *L. (Z.) vivipara*. Nevertheless, within a population, Tp is a complex trait that should be interpreted as a compromise between different selective pressures including not only thermal environment but also energy allocation and, obviously, reproductive condition. Other possible factors should also not be excluded, among them social behaviour and parasites (Castilla *et al.*, 1999). Future research should try to generalize these findings to other lacertid species (i.e. Tosini & Avery, 1996).

Acknowledgements. Thanks are due to Conselh Generau d'Aran for funding, and to the Invertebrate section of the Departament de Biologia Animal and Centre de Recerca d'Alta Muntanya (Universitat de Barcelona) for logistic support. Collecting permits were provided by the Subdirecció General de Conservació de la Natura, Departament d'Agricultura, Ramaderia i Pesca (now Departament de Medi Ambient) of the Generalitat de Catalunya.

REFERENCES

Andrews, R. M. & Rose, B. R. (1994). Evolution of viviparity: constraints on egg retention. *Physiological Zoology* **67**, 1006-1024.

Bauwens, D., Garland T. Jr., Castilla, A. M. & Van Damme, A. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. *Evolution* **49**, 848-863.

Bauwens, D. & Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology* **50**, 733-743.

Bea, A. (1978). Nota sobre *Lacerta vivipara* Jaquin, 1787, en la Península Ibérica. *Bulletí de la Institució Catalana de Història Natural* **42** (Sec. Zool. 2), 123-126.

Bennet, A. F. & John-Alder, H. (1986). Thermal relations of some Australian skinks (Sauria: Scincidae). *Copeia* **1986**, 57-64.

Bowker, R. G. (1993). The thermoregulation of the lizards *Cnemidophorus exanguis* and *C. velox*: some consequences of high body temperature. In *Biology of Whiptail Lizards (genus* Cnemidophorus*)*, 117-132. Wright, J. W. & Vitt, L. J. (Eds.). Oklahoma Mus. Nat. Hist. Norman, Oklahoma.

Braña, F. & Bea, A. (1987). Bimodalité de la reproduction chez *Lacerta vivipara (Reptilia, Lacertidae)*. *Bulletin de la Société Herpétologique de France* **44**, 1-5.

Carretero, M.A . & Llorente, G. A. (1995). Thermal and temporal patterns of two Mediterranean Lacertidae. In *Scientia Herpetologica*, 213-223. Llorente, G. A., Montori, A., Santos, X. & Carretero, M. A. (Eds). AHE. Barcelona.

Castilla, A. M., Van Damme, R. & Bauwens, D. (1999). Field body temperatures, mechanisms of thermoregulation, and evolution of thermal characteristics in lacertid lizards. *Natura Croatica* **8**, 253-274.

Christian, K. A. & Weavers, B. W. (1996). Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. *Ecological Monographs* **66**, 139-157.

Gvozdík, L. & Castilla, A. M. (2001). A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient in the Czech Republic. *Journal of Herpetology* **35**, 486-492.

Harris, D. J. & Carretero, M. A. (2003). Comments on the taxonomic value of (sub)genera within the family Lacertidae (Reptilia). *Amphibia-Reptilia* **24**, 119-122.

Hertz, P., Huey, R. B. & Stevenson, R. D. (1983). Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. *Evolution* **37**, 1075-1084.

Heulin, B. (1987). Température diurne d'activité des mâles et des femelles de *Lacerta vivipara*. *Amphibia-Reptilia* **8**, 393-400.

Heulin, B., Guillaume, C. P., Vogrin, N., Surguet-Groba, Y. & Tadic, Z. (2000). Further evidence of the existence of oviparous populations of *Lacerta (Zootoca) vivipara*. *Comptes Rendus de l'Académie des Sciences Série III - Sciences de la Vie / Life Sciencees* **323**, 461-468.

Huey, R. B. & Bennett, A. F. (1987). Phylogenetic studies of coadaptation: preferred temperatures versus optimal

performance temperatures of lizards. *Evolution* **41**, 1098-1115.

Labra, A. (1998). Selected body temperatures of seven species of Chilean *Liolaemus* lizards. *Revista Chilena de Historia Natural* **71**, 349-358.

Lorenzon, P., Clobert, J., Oppiger, A. & John-Alder, H. B. (1999). Effect of water constraint on growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*). *Oecologia* **118**, 423-430.

Maderson, P. F. A. & Bellairs, A. D. (1962). Culture methods as an aid to experiment on reptile embryos. *Nature* **195**, 401-402.

Mayer, W., Böhme, W., Tiedemann, F. & Bischoff, W. (2000). On the oviparous populations of *Zootoca vivipara* (Jacquin, 1787) in south-eastern Central Europe and their phylogenetic relationship to neighbouring viviparous and south-west European oviparous populations. *Herpetozoa* **13**, 59-69.

Patterson, J. W. & Davies, P. M. C. (1978). Preferred body temperature: seasonal and sexual differences in the lizard *Lacerta vivipara*. *Journal of Thermal Biology* **3**, 39-41.

Qualls, C. P., Andrews, R. M. & Mathies, T. (1997). The evolution of viviparity and placentation revisited. *Journal of Theoretical Biology* **185**, 129-135.

Reichling, H. (1957). Transpiration und Vorzugstemperatur mitteleuropäischer Reptilien und Amphibien. *Zoologische Jahrbücher Systematik* **67**, 1-64.

Rice, W. C. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Rismiller, P. D. & Heldmaier, G. (1982). The effect of photoperiod on temperature selection in the European green lizard, *Lacerta viridis*. *Oecologia* **53**, 222-226.

Roig, J. M., Carretero, M. A. & Llorente, G. A. (2000). Reproductive cycle in a Pyrenean oviparous population of the common lizard (*Zootoca vivipara*). *Netherlands Journal of Zoology* **50**, 15-27.

Scheers, H. & Van Damme, R. (2002). Micro-scale differences in thermal habiat quality and a possible case of evolutionary flexibility in the thermal physiology of lacertid lizards. *Oecologia* **132**, 323-331.

Shine, R. (1985). The evolution of viviparity in reptiles: an ecological analysis. In *Biology of the Reptilia Vol. 15* 603-694. Gans, C. & Billet, F. (Eds.). John Wiley, New York.

Surget-Groba Y., Heulin B., Ghiehmi, S., Guillaume, C. P. & Vogrin, N. (2002). Phylogeography and conservation of the populations of *Zootoca vivipara carniolica*. *Biological Conservation* **106**, 365-372.

Surget-Groba Y., Heulin B., Guillaume C. P., Thorpe, R. S., Maslak, R., Mazzotti, S., Venczel, M., Ghira, I., Odierna, G., Leontyeva, O., Monney, J. C. & Smith, N. (2001). Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution* **18**, 449-459.

Tosini, G. & Avery, R. (1994). Diel variation in thermoregulatory set points of the lizard *Podarcis muralis*. *Amphibia-Reptilia* **15**, 93-96.

Tosini, G. & Avery, R. (1996). Pregnancy decreases set point temperatures for behavioural thermoregulation in the wall lizard *Podarcis muralis*. *Herpetological Journal* **6**, 84-96.

Van Damme, R, Bauwens, D., Castilla, A. M. & Verheyen, R. F. (1989*a*). Altitudinal variation of thermal biology and running performance in the lizard *Podarcis tiliguerta*. *Oecologia* **80**, 516-524.

Van Damme, R, Bauwens, D., Castilla, A. M. & Verheyen, R. F. (1989*b*). Effect of relative clutch mass on sprint speed in the lizard *Lacerta vivipara*. *Journal of Herpetology* **35**,459-461.

Van Damme, R.; Bauwens, D. & Verheyen, R. (1986). Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. *Journal of Thermal Biology* **11**, 219-222.

Van Damme, R.; Bauwens, D. & Verheyen, R. (1987). Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*. *Herpetologica* **43**, 405-425.

Van Damme, R.; Bauwens, D. & Verheyen, R. (1990). Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. *Oikos* **57**, 61-67.

Accepted: 14.5.03

BOOK REVIEWS

Amphibians and Reptiles of the Hashemite Kingdom of Jordan: An Atlas and Field Guide. Ahmad M. Disi, David Modrý, Petr Nečas and Lina Rifai (2001). 408 pp. (215 x 150 mm). Edition Chimaira, Frankfurt am Main, Germany. £37.50 (cloth).

The Hashemite Kingdom of Jordan (al-Mamlakah al-Urdunniya al-Hashimiya) is in everyday life known simply as Jordan. Geographically, Jordan bridges the gap between Iraq in the east and the Jordan River in the west. Faunistically, the book fills the gap between Leviton *et al.*'s (1992) *Handbook to Middle East Amphibians and Reptiles* (covering only a fraction of the Middle East, i.e. the triangle Greece-Iran-Sudan, according to British WW2 usage) and assorted texts on Israel's herpetofauna, all in Hebrew (recently: Werner, 1995; Bouskila & Amitai, 2001). Herpetologically, Disi *et al.*'s work – although not flawless – surpasses all of these neighbouring books put together.

The book's 408 pages (the count begins with the half title page) are allocated as follows: *Opening*, *Contents*, *Preface* (by Wolfgang Böhme) and *Acknowledgements* (10 pp.); *General part* (60 pp.); *Identification keys* (20 pp.); *Species accounts* (260 pp.); *Species of possible occurrence and/or questionable status* (16 pp.); and "Appendix" (38 pp.).

When I see, as in this case, a full page of warm *Acknowledgements* in the wake of a half page of guest *Preface*, I sense a spirit of empathy emanating from the authors. Reading on, this forecast holds water, as the book is formulated and produced very much "with the face to the reader".

The *General part* begins with *Introduction and technical notes* (3 pp.) explaining, as did Böhme's preface, that the book occupies a previously free literary ecological niche, and the book's structure and function. Of special interest here is the explanation of the distribution maps, one for each non-marine species, which are based on the system of presence versus presence-unknown in quarter-degree squares [occupying here approx. 27.5 (S-N) × 23.75 (W-E) km]. This biocartographic method produces neat standardized maps well suited for the biogeographical level, though less so for the ecological level (as intimated by the authors). The evidence for regarding a square "occupied" comprised (and is planned to comprise in a hoped-for future edition) museum specimens, literature records, photo-documentation but also observations, even of readers. From experience in neighbouring Israel, I regard the last as an unsafe source because, to cut a long story short, even the best observer will not distinguish sibling species before they are described and publicized. But probably more potential readers will be interested in the explanation of the fine set of original animal photographs, nearly all of which originate from the relevant area.

Next comes a very short list of *Used abbreviations* (0.5 p.), obviously not in the sense of second-hand abbreviations, but simply meaning abbreviations used. I shall return later to the mini-flaw of a bit of slight linguistic roughness. The list is unique in catering to the non-academic in explaining that, for example, N means north, northern; and to the non-European in explaining that I ... XII mean January ... December.

The biological content begins with *Geography and climate of the Hashemite Kingdom of Jordan* (7 pp.) and the following *Biogeography of Jordan* (4 pp.), well illustrated with maps and landscape photographs [in Fig. 7, "Rough distribution of the sandy areas of Jordan (dots)", the dots must have been too fine as the map looks as if all sand had been blown away]. As rightly explained in Böhme's Preface, the location of Jordan between the mesic Mediterranean, the semi-arid Irano-Turanian, the arid Arabian desert and the tropical Sudanian ecozones, increases the appeal of the book in two ways. On the one hand, the Kingdom's herpetofauna is enriched by contributions from all of these directions; on the other hand, most of the book is relevant to adjacent areas. The text brings numerous examples of trees and shrubs, and of amphibians and reptiles, representing within Jordan these diverse biogeographical and ecological regions. No formal statistics are presented to show how much of the herpetofauna is derived from each region, and indeed such statistics would neither improve our understanding nor serve any other real purpose. However, many readers may prefer to see some summary statement of the number of species in the herpetofauna and in the book. I counted 89 (of certain occurrence), of which four are anurans and four are marine turtles.

Herpetological research in Jordan, history and present state (3 pp.) gives an interesting account of the involvement of persons and institutions in local herpetological research. Naturally this is not an account of the development of the understanding of various aspects of the herpetofauna. *Field observation and collecting of amphibians and reptiles* (10 pp.) gives expert practical advice (signed separately by coauthors Modrý and Nečas) intended to advance the knowledge of the herpetofauna. *The main threats and conservation of herpetofauna* (4 pp.) and *Conservation of Jordanian nature, protected areas* (5 pp.), respectively, explain the threats to the herpetofauna and describe Jordan's nature reserves and national parks, seven locations spread across the country and representing its diverse ecology. The section on *Reptiles and amphibians in the cultural heritage of Jordan* (4 pp.) is not something necessarily expected in a field guide but is uniquely enlightening to the culture-minded. Would you expect that whereas "he is a snake" implies a sly and treacherous person both in English and in Israeli Hebrew (next door to Jordan), in Jordanian Arabic this phrase means an old person, because snakes are believed to live long? This section ends with a recipe for "Dabb Mansaf", a rice and meat dish

based on *Uromastyx* (Moslems are prohibited from eating carnivorous animals) and enriched (excuse the pun) with pine nuts. The authors gently refrain from explaining why they emphasize "Eat with right hand only."

The general part ends with a relatively thorough section on *Venomous snakes and snakebite* (17 pp.), including general principles, species by species presentations of Jordan's venomous species (recognition, behaviour, toxicity, symptoms, treatment etc.) and of course prevention and (up to date) first aid. Particularly dramatic is a lateral close up of the head of *Atractaspis engaddensis* with the long fang protruding sideways and backwards from the nearly closed mouth. Indeed, the species was described in 1950 by G. Haas, after H. Mendelssohn discovered it at the En Gedi oasis (Israel) on the western shore of the Dead Sea, picking the unknown but obviously non-viperid snake carefully up by the neck, and being envenomated by the usual backward stab. A short paragraph describes some bizarre practices of traditional folk medicine, such as burying the bitten person in sand up to his neck for a day and giving him dried desert monitor meat to eat. The authors reasonably indicate that statistically all these treatments appear to be effective, for three reasons: (1) they have a positive psychological effect; (2) in Jordan most venomous snake bite victims survive anyway, even without treatment; and (3), as always many of the apparent victims were really bitten by non-venomous snakes [including rear fanged ones].

The *Identification keys to the amphibians and reptiles of Jordan* (20 pp.) are generally good, well explained and effectively illustrated. They prudently include also the species of doubted occurrence or uncertain status presented at the end of the book. These should have been marked as such in the keys. The keys do contain in small quantities all the typical errors to which keys are prone: invalid characters – tadpole size (p. 75) is informative in only one way, for how do I know that a small tadpole won't grow further? Erroneous information on a character state – the colour of *Hyla savygnyi* is defined as "back uniform" (p. 75) although this chameleon-like species can assume a blotched pattern (at least, in other countries); vague character-state definitions, meaningful only if both alternatives are available for immediate comparison – "tail relatively short" versus "tail moderately long" (p. 81); and even a black-out-type confusion – *Sphenops sepsoides* is characterized as having "Hindlimbs reduced, small ... about half the forelimbs" (p. 83) while the species is quite normal, as also depicted (p. 234), with forelimbs much smaller than hindlimbs.

The *Species accounts* (263 pp., or 64.5% of the book) of course constitute the core of the book and fulfil its main purposes – atlas and field guide. They have a uniform structure, well considered and considerate of the user. Each starts with the currently accepted *species name*, including author and year, followed by the name under which it was originally described and terra typica, and common (vernacular) names in English, German and French (for some reason in this order). Thereafter, *Systematics*: very wisely and efficiently, the units of treatment are the species, and this short section discusses any subspeciation, with appropriate brevity. *General distribution* defines in words the world distribution of the species and *Local distribution* defines its distribution within Jordan, exemplified by a list of localities, and illustrated by a map as discussed above. The description of the *Habitat* recognizes that this can vary between parts of a wide world distribution. The *Description* is usually the longest section within the account. Each description starts with a general definition of the habitus of the animal, including size ("small", "medium-sized" etc.), covers morphology and pholidosis, then coloration, and winds up with a more exact definition of size, sometimes made vague by a statement of sexual size difference (an issue ignored in most accounts, probably rightly so in a field guide). For example "The species reaches up to 130 mm total length (SVL up to 60 mm). Males are larger and more robust than females" (p. 145). So, is 130 mm the average adult size (with the males larger and the females smaller), or a rare maximum size (possessed by a male)? *Notes on biology* naturally vary extremely in extent and types of information presented (available). Unfortunately there is usually no indication of the source of data for biological parameters likely to be affected by conditions of captivity (such as the number of eggs). Finally the *Remarks*, if any, often contain particularly interesting information, and the *Pertinent references* are an almost unique asset of this field guide. The species accounts strictly exclude anything that is not hard-core information. Thus, for example, the piquant fact that the presumably relict population of the large venomous *Macrovipera lebetina* was discovered in Jordan just recently, can be learned by the reader only through following the references at the end of the accounts (in this case, Al-Oran *et al.*, 1998).

The species accounts include photographs of all species (on average 2.2 photographs, each of half-page size, per species). These are carefully selected for relevance. The most frequent combination is a whole-animal photograph and another showing a close-up of the head, but there are some presentations of sex differences, juveniles, postures, and habitats. The vast majority is in colour, in almost all photographs the animals are on the correct substratum, and the quality of almost all is excellent or very good. Besides the full scientific name, the legend under each photograph also describes (unlike the abbreviation of the genus name in the photographs accompanying the keys) the geographical source of the animal (mostly in Jordan; otherwise closely adjacent areas) and the name of the photographer (most are by coauthor Modrý).

The species accounts are followed by *Species of possible occurrence and/or questionable status* (16 pp.), consisting of another nine species presented in the same format. The reasons for these species to be included in the book but banished from the main series of species

accounts vary greatly. The commonest reason is that the species has been encountered in Jordan only once or twice, in circumstances not above suspicion of introduction. This series of accounts is somewhat inferior to the main series. First, the order of presentation is unclear. But mainly the selection of the photographs, which should help readers to identify any additional finds of these species, is not ideal for the purpose. Thus, for *Triturus vittatus*, a male in the aquatic phase is shown, doubly impressive because, although this is not stated, it clearly is the spectacular *T. v. ophryticus* from northern Turkey (Özeti & Yilmaz, 1994; Baran &Atatür, 1998). It would have been more useful to portray a *T. v. vittatus* from Israel or southern Turkey, and especially the terrestrial phase, which can be encountered under stones. For *Acanthodactylus* cf. *pardalis* a typically orange-coloured Egyptian male of *A. pardalis* is shown, although geographically the gap between these two populations is filled by the grey-coloured Israeli *A. beershebensis* (Moravec *et al.*, 1999). The impressive close up of *Elaphe quatuorlineata* (without stating subspecies and locality) gives no clear impression of the colour pattern.

The Appendix begins with *Amphibians and Reptiles in the Holy Quran* and *Amphibians and Reptiles in the Holy Bible* (together 7 pp.), again perhaps not necessary in a field guide. This section might have been more appropriately included in "Reptiles and amphibians in the cultural heritage of Jordan", earlier in the book. The Bible translation used is not specified, although that may explain the omission of Proverbs, Chapter 30 (28) "The gecko (in some translations: spider) taketh hold with her hands, and is in kings' palaces". Next, the *Gazetteer* (2 pp.) is most welcome, though it quotes no source to the English spelling, which differs a little from that in the Times Atlas (1997) (e.g., Al 'Aqabah in the former, 'Aqaba in the latter), although one would expect both to follow the governmental map. I am exempt from commenting on *Antivenoms* (2 pp.), due to perfect ignorance. *Common Arabic names* (5 pp.) (meaning of course Arabic common names) is a major disappointment. First, one would have wanted these in the species accounts, despite the problematic situation explained by the authors: the names vary regionally, and the "official" ones are not in wide use. Second, the authors say, "This is the reason why we included also a table with official Arabic names." However, present is only one list, which looks more popular than official, with, for example, all lacertids plus the skinks *Mabuya* and *Ophiomorus* pooled as "sahliya", and most non-viperid snakes being "hayeh" while both *Typhlops* and *Cerastes* are called "Dudah". Finally the *References* (20 pp., >400 references) are a distinct asset although some that bear on field identification could be added, such as those on the mimicry-like behaviour of some snakes (Werner & Frankenberg, 1982; Werner, 1983). Indeed, the authors erroneously describe *Coluber nummifer* "The head is flattened, wide" (p. 259) whereas this only happens during the defensive behavioural head triangulation.

Throughout the book there are occasional linguistic mini-slip-ups, never jeopardizing the communication with the reader, yet, in a book, a little irritating. Some are independent of the language used, e.g., "... the number of eggs ... varies between 6-14." (=varies between 6 and 14, or ranges 6-14, p. 142), or "An adult, basking male of..." (=a basking adult male, p. 168). Others concern the use of English, e.g., "Snakes are the most hardly predictable animals" (=Snakes are the least predictable, p.37), "The exact number of clutches ... probably vary between one and three" (varies, p. 148), or "The nostrils lay on an entire nasal shield" (lie, p. 225). Some involve terminology, e.g., "*Coluber jugularis* ... A polymorphic species with two ... subspecies" (= polytypic species, p. 255).

The authors are, according to Böhme's preface (no dust jacket introduces them), a somewhat diverse team: "Prof. Ahmad M. Disi may be termed the influential founder of modern herpetology in Jordan. Dr. David Modrý and Petr Nečas, Czech Republic, two dedicated Moravian researchers, combine the facets of professionalism and knowledgeable amateur herpetology. Lina Rifai represents the next generation of students in herpetology in Jordan that will further develop the study of amphibians and reptiles in this country." The book produced by this team is, in summary, not only the best book on Middle East herpetology but also is – despite assorted mini-flaws awaiting correction in the next edition – a good and enjoyable book already in its present version.

REFERENCES

Al-Oran, R. M., Rostum, S., Joger, U. & Amr, Z. S. (1998). First record of the Levantine viper (*Macrovipera lebtina*) from Jordan. *Zoology in the Middle East* **16**, 65-70.

Baran, I. & Atatür, M. (1998). *Turkish herpetofauna (amphibians and reptiles)*. Ankara: Ministry of Environment.

Bouskila, A. & Amitai, P. (2001). *Handbook of amphibians and reptiles of Israel*. Jerusalem: Keter (in Hebrew. The Hebrew title page reads "P. Amitai & A. Bouskila, *Guide to reptiles and amphibians in Israel*").

Haas, G. (1950). A new *Atractaspis* (mole viper) from Palestine. *Copeia* **1950**, 52-53.

Leviton, A. E., Anderson, S. C., Adler, K. & Minton, S. A. (1992). *Handbook to Middle East amphibians and reptiles*. Ithaca, New York: SSAR.

Moravec, J., Baha El Din, S., Seligmann, H., Sivan, N. & Werner, Y. L. (1999). Systematics and zoogeography of the *Acanthodactylus pardalis* group (Reptilia: Sauria: Lacertidae) in Egypt and Israel. *Zoology in the Middle East* **17**, 21-50.

Özeti, N. & Yilmaz, I. (1994). *Türkiye amfibileri*. Bornova-Izmir: Ege Üniversitesi Fen Fakültesi Kitaplar Serisi No. 151.

Times Atlas of the World, Comprehensive Edition (1997). London: Times Books.

Werner, Y. L. (1983). Behavioral triangulation of the head in three boigine snakes: possible cases of mimicry. *Israel Journal of Zoology* **32**, 205-228.

Werner, Y. L. (1995). *A guide to the reptiles and amphibians of Israel*. Jerusalem: Nature Reserves Authority (in Hebrew. The Hebrew title reads "A guide to the reptiles of our country").

Werner, Y. L. & Frankenberg, E. (1982). Head triangulation in two colubrine snakes: probable behavioral reinforcement of Batesian mimicry. *Israel Journal of Zoology* **31**, 137-150.

Yehudah L. Werner
The Hebrew University of Jerusalem

THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS
(revised January 2004)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: *Full Papers* (no length limit); *Reviews* and *Mini-reviews* (generally solicited by a member of the editorial board); *Short Notes*; controversies, under *Forum* (details available from the Editor); and *Book Reviews*. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the *Herpetological Journal* is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

2. *Two* copies of all submissions, and illustrations, should be sent to the Scientific Editor, together with a computer diskette (IBM formatted) containing the text and, if possible, figures. Alternatively, submission by e-mail is possible - please contact the Scientific Editor for information. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigor, and the degree of general interest in the subject matter. The Editor's decision will be final.

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer, and double-spaced with wide margins all round. The journal is typeset direct from the author's electronic text, so all manuscripts should be prepared using a word processor (preferably on a PC-compatible microcomputer). If figures are prepared using computer graphics, they should be supplied separately and NOT embedded in the text of the word processor file. Preferred formats are MS Word for Windows (text) and MS Excel, Bitmap, TIFF, Windows Metafiles (.wmf, .emf) or JPEG files (graphics).

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of five words or less, and the name and address of the corresponding author with (if available) an email address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted. *Short Notes* (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text, preceded by an abstract of no more than 100 words. A *sans serif* font (e.g. Universe or Helvetica) is preferred.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. TABLE 1; they should be typed double spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. FIG. 1. Colour photographs can only be included at cost to the author (quotes can be obtained from the Managing Editor). If an illustration has more than one part, each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. Illustrations produced using other types of computer printer are not usually of suitable quality. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated —"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the first author's surname followed by *et al.* should be used (Smith *et al.*, 1972). In the list of references the full title of the journal should be given. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal:

Bellairs, A. d'A. (1957). *Reptiles*. London: Hutchinson.

Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* **2**, 206–210.

Dunson, W. A. (1969*a*). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

Dunson, W. A. (1969*b*). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American Journal of Physiology* **216**, 995–1002.

9. Final acceptance of a paper will depend upon the production by the author of a typescript, illustrations and computer file(s) ready for the press.

10. Proofs are usually prepared as PDF files and corrections should be returned to the Managing Editor by return of post or email. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

11. Twenty-five offprints OR a PDF file of the paper are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.

12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

THE HERPETOLOGICAL JOURNAL

Volume 15, Number 1 2005

CONTENTS

Herpetological Journal vol. 14, no. 4 was published on 30 November 2004